MBO-04

Vardhman Mahaveer Open University, Kota

Plant Physiology & Metabolism

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Production : June 2015

Printed and Published on behalf of the Registrar, V.M. Open University, Kota.
Printed by : Pragya Publications Pvt. Ltd., Mathura

Vardhman Mahaveer Open University, Kota

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Preface

The present book entitled "**Plant Physiology and Metabolism**" has been designed so as to cover the unit-wise syllabus of MBO-04 course for M.Sc. Botany (Previous) students of Vardhman Mahaveer Open University, Kota. The basic principles and theory have been explained in simple, concise and lucid manner. Adequate examples, diagrammes, photographs and self-learning exercises have also been included to enable the students to grasp the subject easily. The unit writers have consulted various standard books on the subject and they are thankful to the authors of these reference books.

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Unit – 1

Bioenergetics

NOTES

Structure of the Unit

1.0 Objective

After going through this unit you will able to understand-

- The principles of bioenergetics and flow of energy in ecosystem.
- The application of laws of thermodynamics in energy transfer processes in biological world.
- The basic concepts of laws of thermodynamics.

- The structure of ATP and its role in a cell as energy currency and as coenzyme.
- The Redox reactions, their examples and chemistry of oxidation and reduction in cell

NOTES

1.1 Introduction

Biological world are acts as an open reacting system where energy transfer in a cell, an organism, or an ecosystem follows the laws of thermodynamics. Energy can neither be created nor destroyed, energy keep changing from one form to another. None of the energy transfer process is 100% efficient; there is a loss of useful energy (free energy) and an inevitable increase in the amount of unusable energy (heat and entropy). All living things needs continuous input of energy so as to maintain order. This is taken from the sun directly or indirectly. The energy that cells can use is free energy, described as Gibbs free energy. In cells all transform energy (free energy) store in the form of ATP the energy currency of cell. It provides energy to the cell after its hydrolysis. The energy released by cleaving a phosphate (p1) from ATP at standard state of is -30.5 KJ/Mol (-7.3Kcal/Mol).

Redox (**reduction** and **oxidation**) reactions include all chemical reactions in which atoms have their oxidation state changed; in general, redox reactions involve the transfer of electrons between species.

1.2 Principle of Bioenergetics

Principle of Bioenergetics –The total energy of the universe is constant the total entropy is continuously increasing.

To understand principle of bioenergetics we have to know the energy transfer processes.

1.2.1 Energy Cycle

Energy is the ability to do work; all living things contain energy in some or the other forms. Energy and its transformation are governed by two basic laws of thermodynamics.

The carbon, oxygen and water are constantly cycled between the heterotrophic and autotrophic worlds, with solar energy as the driving force for all global process; the cycling depends on a proper balance between the activities of producers (autotrophs) and consumers (heterotrophs) in our biosphere.

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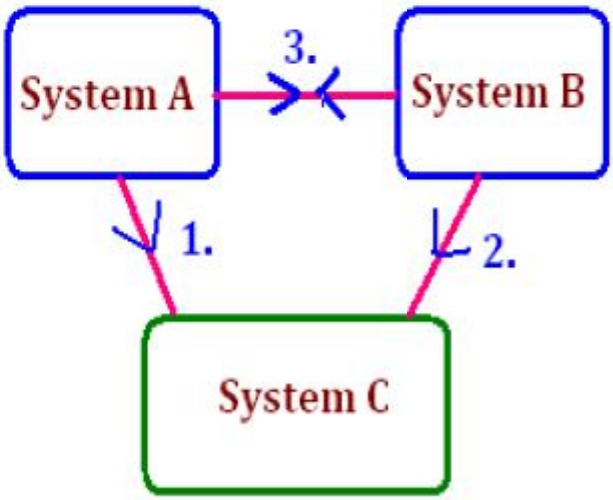

Fig-1.1 Energy cycle

These cycles of matter are driven by an enormous flow of energy in to and through the biosphere beginning with the capture of solar energy by photosynthetic organisms and use of this energy to generate energy rich carbohydrates and other organic nutrients, these nutrients are then used as energy source by heterotrophic organisms. In contrast to the cycling of matter, energy flows in one way through the biosphere, organisms cannot regenerate useful energy from energy dissipated as heat and entropy. Matters recycle continuously, but energy is constantly transformed in to unusable forms such as heat.

In metabolic processes, and in all energy transformations, there is a loss of useful energy (free energy) and an inevitable increase in the amount of unusable energy (heat and entropy).

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1.2.2 Energy transformation from one to another

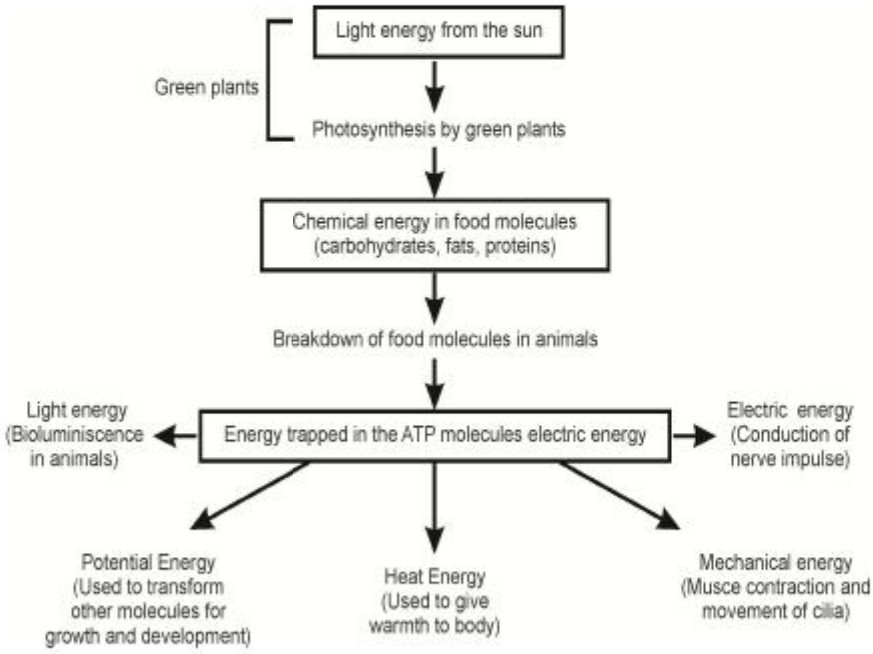


Fig-1.2 Energy Flow through an Ecosystem

1.3 Laws of Thermodynamics

Bioenergetics is the quantitative study of the energy transductions that occur in living cells, and the nature and function of the chemical processes underlying there transductions.

Biological energy transformation obey the laws of thermodynamics –

1.3.1 FLOT- 'First Law of Thermodynamics' Energy can neither be created nor destroyed, energy keep changing from one form to another.

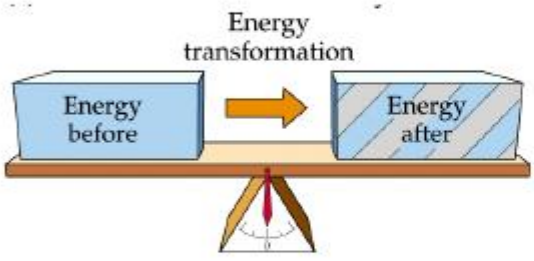


Fig-1.3 First law of thermodynamics

The first law is the principle of the conservation of energy for any physical or chemical change, the total amount of energy in the universe

remains constant, energy may change form or it may be transported from on region to another, but it cannot be created or destroyed.

1.3.2 SLOT - Second Law of Tthermodynamics

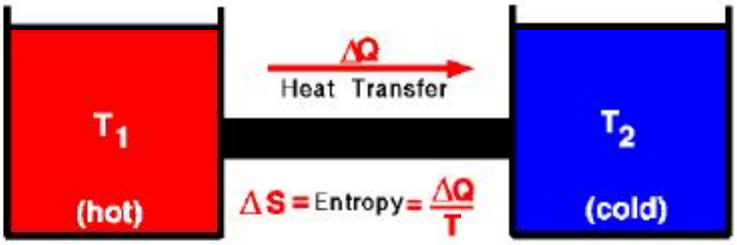
'Law at entropy (randomness)

NOTES

The second law of thermodynamics which can be stated in several forms says that the universe always tends towards increasing disorder, in all natural processes the entropy of the universe increases. No process that involves energy transformation can occur spontaneously without the degradation of energy in to dispersed form, e.g. the heat from a hot object will spontaneously get dispersed to cooler surroundings.

As energy is always dispersed into unavailable heat energy – no transformation of energy is 100% efficient.

Law of entropy means that any system including universe when left to itself tends to increase entropy i.e. disorder or randomness



S- Entropy, Q-Heat, T-Temperature

Fig-1.4 Second Law of Thermodynamics

As energy keeps flowing from higher to lower energy area the loss of energy occurs repeatedly in these energy changing reactions, this leads to increased entropy, free energy from sun minimizes entropy. That's why all living things needs continuous input of energy so as to maintain order. This is taken from the sun directly or indirectly.

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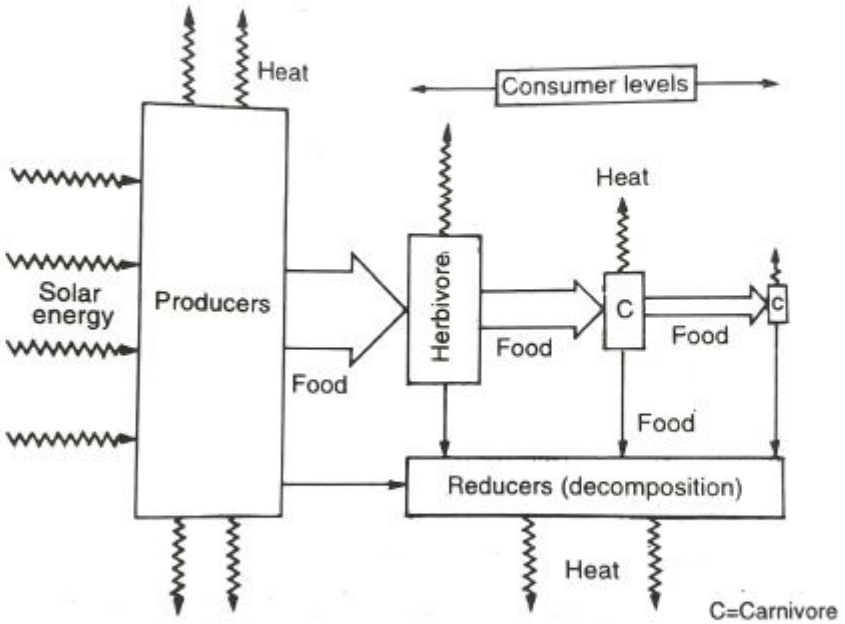

Fig-1.5 Energy flow in an ecosystem

1.3.3 ZLOT- Zeroth Law of Thermodynamics

If two bodies A and B are in thermal equilibrium with the third body C then will also be in thermal equilibrium with each other.

1.3.4 TLOT -Third Law of Thermodynamics

At absolute zero (0° K) entropy of perfect crystalline solid is zero.

First and second laws of thermodynamics are more relevant then third and fourth in biological systems.

1.3.5 Definitions of three thermodynamic quantities

A. Gibbs free energy G

B. Enthalpy H

C. Entropy S

1. Gibbs free energy 'G' expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure, when a reaction proceeds with the release of free energy, the free energy change to be exergonic. In endergonic reactions the system gains free energy and ΔG is positive.

2. Enthalpy '*H*' is the heat content of the reacting system, it reflects the number and kinds of chemical bonds in the reactant and products, when a chemical reaction release heat, it is said to be exothermic, the heat content of the products 10 time less than that of reactants and ΔH has by convention, a negative value. Reacting systems that take up heat from their surrounding are endothermic and have positive value of ΔH.

NOTES

3. Entropy, S is a quantitative expression for the randomness or disorder in a system, when the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

The units of ΔG *and* ΔH are in joules. Mole (J/Mol) or Calories/Mole (Cal/Mol), unit of entropy is Joules/Mole. Kelvin (J/Mol ΔK). (1Cal=4.184J)

Relation between ΔG, ΔH *and* ΔS

Under the conditions existing in biological systems (including constant temperature and pressure) changes in free energy, enthalpy and entropy are related to each other quantitatively by the equation –

$$\Delta G = \Delta H - \Delta T \Delta S$$

In which

ΔG = Change in Gibbs free energy of the reaching system

ΔH = Change in enthalpy of the system

ΔT = Absolute temperature

ΔS = Change in entropy of the system

Δ *G* of a spontaneously reacting system is always negative. By convention ΔS has a positive sign when entropy increases and ΔH, has a negative sign when heat is released by system to its surrounding, either of these conditions which are typical of favorable processes, tends to make ΔG negative.

NOTES

1.4 Free Energy: Sources of free energy of the cells

Cells are isothermal system i.e. function at essentially constant temperature and pressure. The energy that cells can use is free energy, described by the Gibbs free energy function G, which allows prediction of direction of chemical reactions, there exact equilibrium position and the amount of work they can perform at constant temperature and pressure. Heterotrophic cells acquire free energy from nutrient molecules, and photosynthesis cells acquire it from absorbed solar radiations. Both kinds of cells transform this free energy in to ATP and other energy rich compounds capable of providing energy for biological work at constant temperature.

The standard free energy change is directly related to the equilibrium constant.

The composition of a reacting system (a mixture of chemical reactants and products) tends to continue changing until equilibrium is reached.

At the equilibrium concentration of reactants and products, the rates of the forward and reverse reactions are exactly equal and no further net change occurs in the systems.

The concentration of reactions and products at equilibrium define the equilibrium constant:-

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

ABCD = Molar Concentration of reaction components

abcd = No. of moles

When a reacting system is not at equilibrium the tendency to move towards equilibrium represents a driving force, the magnitude of which can be expressed as the free energy change for the reaction ΔG.

When ΔG is large and negative, the reaction tends to go in the forward direction, when ΔG is large positive, the reaction tends to go in the reverse direction, and when $\Delta G=0$, the system is at equilibrium.

1.5 Living world as reacting systems

NOTES

Living organism consist of collections of molecules, more highly organized than the surrounding, called as reacting system, the reacting system may be an organism, a cell, or two reacting compounds. The reacting system undergoes particular chemical or physical processes.

Reacting system + Surrounding = Universe

Living organisms are open reacting systems, that exchanging both material and energy with there surroundings but living system are never at equilibrium with their surroundings, and the constant transactions between system and surroundings takes place. This explains how organisms can create order within themselves while operating within the second law of thermodynamics.

In short living organizations preserve their internal order by taking free energy of surroundings in the form of nutrients or sun light and returning to their surrounding an equal energy as heat and entropy.

Open system and Homeostasis

An organism that has access to environment for matter and energy is regarded as an open system and when input and output of energy are balanced it is called a steady state

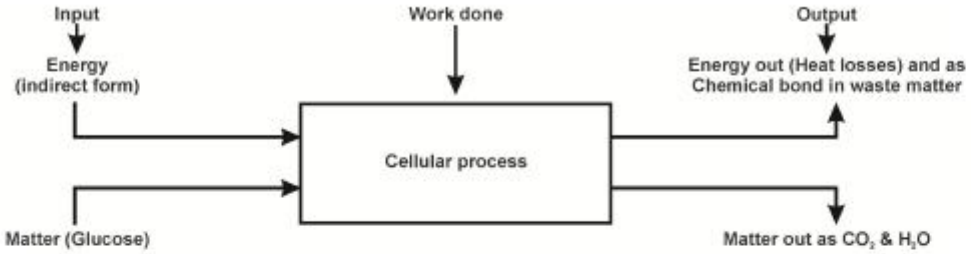

Fig-1.6 Open system is a system in steady state

- Homeostasis is the most important adaptation of all organisms, all ecosystems are capable of self maintenance and self regulation, it is a tendency for biological systems to resist change and to remain in a state of equilibrium.
- All living cells exist in a carefully regulated internal environment that is stable and an organism survives as long as the fitness of internal environment is maintained, the regulatory controls are found in all the

system of the body, they work on the feedback mechanism; any deviation from normal condition brings an opposite effect to restore the original condition.

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1.6 ATP Structure & Function

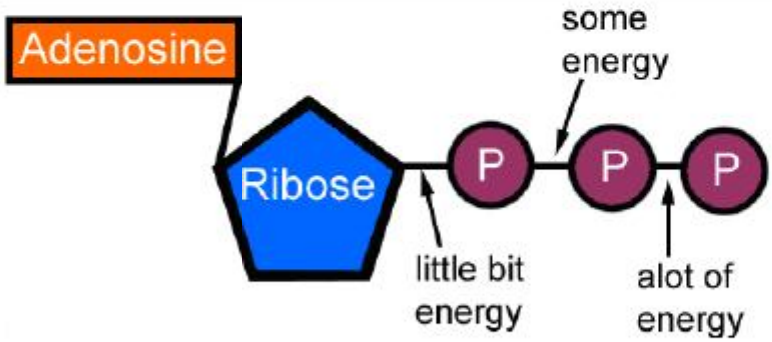

Fig-1.7 Structure of ATP

Adenosine triphasphate (ATP) is a nucleoside tri-phosphate used in cells as a coenzyme. It is often called the molecular unit of currency of intracellular energy transfer, ATP transports chemical energy within cells for metabolism.

It is one of the end products of phosphorylation, cellular respiration and fermentation and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions.

One molecule of ATP contains three phosphate groups, and it is produced by a wide variety of enzymes, including ATP synthatase from adenosine di-phosphate and adenosine mono-phosphates and various phosphate group donors,

Mechanism of ATP biosynthesis – At least three types of phosphorylation are known

1. Substrate level phosphorylation
2. Oxidative phosphorylation (respiration)
3. Photo-phosphorylation (photosynthesis)

In the substrate level phosphorylation a phosphate group having high transfer potential is transferred from a phosphorylated compound to ADP, this type of phosphorylation takes place in respiration. In oxidative phosphorylation reduce coenzymes ($NADH_2$) ($FADH_2$) or succinic acid are oxidized. The electrons

obtained from these compounds pass over a series of electron carriers called respiratory chain. It is chiefly made up of cytochromes. Electron transport and phosphorylation occur at the same time. Photo-phosphorylation is similar to oxidative phosphorylation. The ATP concentration inside the cell is 1-10 mm; ATP can be produced by redox reaction using simple sugar or lipid as energy source.

NOTES

Structure

ATP was discovered in 1929 by Karl Lahmann and Y Subbarrow of Harvard Medical school, it was proposed to be the main energy transfer molecule in the cell by fritz Albert Lipmann in 1941, that is being the intermediary molecule between energy yielding and energy requiring (exogonic & endergonic) reactions it was first artificially synthesized by Alexander Todd in 1948.

The structure consist of a purine base (adenine) attached to the 1 carbon atom of a pentose sugar (ribose), the phosphate groups are attached at the 5' carbon of pentose sugar, it is the addition and removal of these phosphate groups that inter convent ATP, ADP and AMP, when ATP is used in DNA synthesis the ribose sugar is first convert to deoxyribose by ribonucleotide reductase.

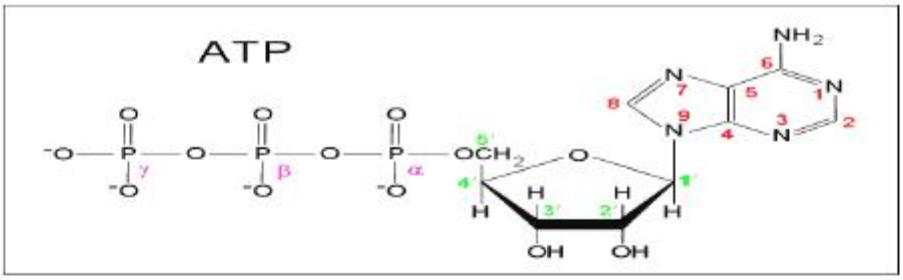

Fig-1.8 Position of bonds in ATP

Physical and chemical properties

ATP consists of adenosine-composed of an adenine ring and a ribose sugar and the three phosphate groups (tri-phosphates) the phosphate group, starting with the group closed to ribose, are referred as $\alpha\ \beta\ \gamma$

ATP is highly soluble in water and is quite stable in solution between PH 6.8-7.4.

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Two phosphoanhydride bond (P~P) in an ATP molecule are responsible for the high energy contents of this molecule, (referred as high energy bonds, despite the fact it take energy to break bonds).

Energy stored in ATP may be released upon hydrolysis of the anhydride bonds, the primary phosphate group on the ATP molecule that is hydrolyzed when energy is needed to drive anabolic reactions is the γ phosphate group located the farthest from the ribose sugar. It has a higher energy of hydrolysis than either the α or β phosphate. The bonds formed after hydrolysis or the phosphorylation of a residue by ATP are lower in energy than the phosphoanhydride bonds of ATP, during energy catalyzed hydrolysis of ATP or phosphorylation by ATP the available free energy can be harnessed by a living system to do work.

Functions

1. ATP is used as a substrate in signal transduction pathways by kinases that phosphorylated proteins and lipids.
2. It is also used by adenylate cyclase, which uses ATP to produce the second messenger molecule cyclic AMP.
3. The ratio between ATP & AMP is used as a way for a cell to sense how much energy is available and control the metabolic pathway that produce & consume ATP.
4. Apart from its roles in signaling and energy metabolism, ATP is also incorporated in to nucleic acids by polymerases in the process of transcriptions.
5. ATP is the neurotransmitter believed to signal the sense of taste.

Metabolic processes that use ATP as an energy source converts it back in to its precursors; ATP is therefore continuously recycled in living beings.

However, in most polymeric biomolecules, the breakdown of RNA, DNA and ATP in to simpler monomers is driven by both energy release and entropy increase considerations, in both standard concentrations, and also in concentrations within the cell.

The standard amount of energy released from hydrolysis of ATP can be calculated from the changes in energy under non natural (standard) conditions, The energy released by cleaving either a phosphate (p1) or pyrophosphate (pp1) unit from ATP at standard state of 1M is

NOTES

$ATP + H_2O \rightarrow ADP + P_i$ G = -30.5 KJ/Mol (-7.3Kcal/Mol)

$ATP + H_2O \rightarrow AMP + P_i$ G = -45.6 KJ/Mol (-10.9Kcal/Mol)

$ADP + H_2O \rightarrow AMP + P_i$ G = -32.8 KJ/Mol (-7.8Kcal/Mol)

$AMP + H_2O \rightarrow Adeno\,sin\,e + P_i$ G = -14.2 KJ/Mol (-3.4Kcal/Mol)

Standard free energies of hydrolysis of some phosphorylated compounds

These values can be used to calculate the change under physiological conditions of cell and the cellular ATP/ADP ratio.

1.7 Redox Reactions

Redox reactions are important class of reaction in which oxidation and reduction occurs simultaneously.

Every oxidation must be accompanied by a reduction, in which an electrons acceptor acquires the electrons removed by oxidation. Oxidation reaction generally release energy e.g.-(camp fire = wood + air), most living cells obtain energy needed for cellular work by oxidizing metabolic fuels such as carbohydrates fats, photosynthetic organism can also trap energy of sunlight, the metabolic pathways are oxidative reaction sequences that result in transfer of electrons from fuel molecules through a series of electron carrier, to oxygen, the high affinity of O_2 for electrons makes the overall electron transfer process highly exergonic providing the energy that drivers ATP synthesis – the central goal of catabolism.

A number of phenomena, both physical as well as biological, are concerned with redox reactions these reactions find extensive use in pharmaceutical, biological, industrial, metallurgical and agricultural areas.

The importance of these reactions is apparent from the fact that burning of different types of fuels for obtaining energy for domestic, transport and other commercial purposes, electrochemical processes for extraction of highly

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reactive metals and non metals, manufacturing of chemical compound like caustic soda operation of batteries, falls within the preview of redox processes and environmental issues like hydrogen economy (use of liquid hydrogen as fuel.) and development of ozone hole have started figuring under redox phenomenon.

Definition: Redox (reduction and oxidation) reactions include all chemical reactions in which atoms have their oxidation state changed; in general, redox reactions involve the transfer of electrons between species.

This can be either a simple redox process, such as the oxidation of carbon to yield carbon dioxide (CO_2) or the reduction of carbon by hydrogen to yield methane (CH_4), or a complex process such as the oxidation of glucose ($C_6H_{12}O_6$) in the human body through a series of complex electron transfer processes.

The term "redox" comes from two concepts involved with electron transfer: reduction and oxidation. It can be explained in simple terms:

- **Oxidation** is the *loss* of electrons or an *increase* in oxidation state by a molecule, atom, or ion.
- Reduction is the *gain* of electrons or a *decrease* in oxidation state by a molecule, atom, or ion.

Although oxidation reactions are commonly associated with the formation of oxides from oxygen molecules, these are only specific examples of a more general concept of reactions involving electron transfer.

Redox reactions, or oxidation-reduction reactions, have a number of similarities to acid–base reactions. Like acid–base reactions, redox reactions are a matched set, that is, there cannot be an oxidation reaction without a reduction reaction happening simultaneously. The oxidation alone and the reduction alone are each called a *half-reaction*, because two half-reactions always occur together to form a whole reaction. When writing half-reactions, the gained or lost electrons are typically included explicitly in order that the half-reaction be balanced with respect to electric charge.

Though sufficient for many purposes, these descriptions are not precisely correct. Oxidation and reduction properly refer to *a change in oxidation*

state — the actual transfer of electrons may never occur. Thus, oxidation is better defined as an *increase in oxidation state*, and reduction as a *decrease in oxidation state*. In practice, the transfer of electrons will always cause a change in oxidation state, but there are many reactions that are classed as "redox" even though no electron transfer occurs (such as those involving covalent bonds).

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Fig-1.9: Rusting iron

Fig-1.10 : A bonfire

Oxidizing and reducing agents

In redox processes, the reductant transfers electrons to the oxidant. Thus, in the reaction, the reductant or *reducing agent* loses electrons and is oxidized, and the oxidant or *oxidizing agent* gains electrons and is reduced. The pair of an oxidizing and reducing agent that are involved in a particular reaction is called a **redox pair**. A **redox couple** is a reducing species and its corresponding oxidized form, e.g., Fe^{2+}/Fe^{3+}.

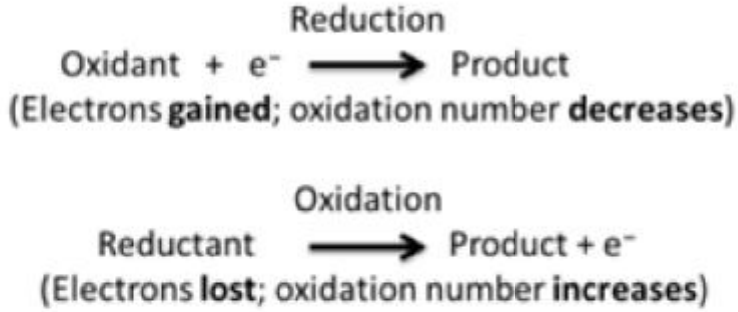

Fig-1.11; Redox reaction

Oxidizers; *Oxidizing agent*

Substances that have the ability to oxidize other substances (cause them to lose electrons) are said to be oxidative or oxidizing and are known as oxidizing agents, oxidants, or oxidizers. That is, the oxidant (oxidizing agent), because it

"accepts" electrons, the oxidizing agent is also called an electron acceptor, hence the name. Oxygen is the quintessential oxidizer.

NOTES

Oxidants are usually chemical substances with elements in high oxidation states e.g., H_2O_2 , MnO_4, CrO_3, Cr_2O_7, or else highly electronegative elements (O_2, F_2, Cl_2, Br_2) that can gain extra electrons by oxidizing another substance.

Reducers; ***Reducing agent***

Substances that have the ability to reduce other substances (cause them to gain electrons) are said to be reductive or reducing and are known as reducing agents, reductants, or reducers. The reductant (reducing agent) transfers electrons to another substance, and is thus itself oxidized. And, because it "donates" electrons, the reducing agent is also called an electron donor. Electron donors can also form charge transfer complexes with electron acceptors.

Reductants in chemistry are very diverse. Electropositive elemental metals, such as lithium, sodium, magnesium, iron, zinc, and aluminium, are good reducing agents. These metals donate or *give away* electrons readily. *Hydride transfer reagents*, such as $NaBH_4$ and $LiAlH_4$, are widely used in organic chemistry, primarily in the reduction of carbonyl compounds to alcohols. Another method of reduction involves the use of hydrogen gas (H_2) with a palladium, platinum, or nickel catalyst. These *catalytic reductions* are used primarily in the reduction of carbon-carbon double or triple bonds.

Redox reaction in biology

Many important biological processes involve redox reactions.

Cellular respiration, for instance, is the oxidation of glucose ($C_6H_{12}O_6$) to CO_2 and the reduction of oxygen to water. The summary equation for cell respiration is:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

The process of cell respiration also depends heavily on the reduction of NAD^+ to NADH and the reverse reaction (the oxidation of NADH to NAD^+). Photosynthesis and cellular respiration are complementary, but photosynthesis is not the reverse of the redox reaction in cell respiration:

$$6\,CO_2 + 6\,H_2O + light\ energy \rightarrow C_6H_{12}O_6 + 6\,O_2$$

Biological energy is frequently stored and released by means of redox reactions. Photosynthesis involves the reduction of carbon dioxide into sugars and the oxidation of water into molecular oxygen. The reverse reaction, respiration, oxidizes sugars to produce carbon dioxide and water. As intermediate steps, the reduced carbon compounds are used to reduce nicotinamide adenine dinucleotide (NAD^+), which then contributes to the creation of a proton gradient, which drives the synthesis of adenosine triphosphate (ATP) and is maintained by the reduction of oxygen. In animal cells, mitochondria perform similar functions.

NOTES

Free radical reactions are redox reactions that occur as a part of homeostasis and killing microorganisms, where an electron detaches from a molecule and then reattaches almost instantaneously. Free radicals are a part of redox molecules and can become harmful to the human body if they do not reattach to the redox molecule or an antioxidant. Unsatisfied free radicals can spur the mutation of cells they encounter and are, thus, causes of cancer.

The term **redox state** is often used to describe the balance of NAD^+/NADH and $NADP^+$/NADPH in a biological system such as a cell or organ. The redox state is reflected in the balance of several sets of metabolites (e.g., lactate and pyruvate, beta-hydroxybutyrate, and acetoacetate), whose interconversion is dependent on these ratios. An abnormal redox state can develop in a variety of deleterious situations, such as hypoxia, shock, and sepsis. Redox mechanism also controls some cellular processes. Redox proteins and their genes must be co-located for redox regulation according to the CoRR hypothesis for the function of DNA in mitochondria and chloroplasts.

1.8 Summary

This unit involves principles of bioenergetics (bio+energy), energy transfer and related conditions in reacting systems that may be a cell, an organism or an ecosystem or even in the universe. It explains how the input and output of energy is balanced in an open reacting system for maintaining the steady state by feedback mechanism and homeostasis.

It also explains energy transfer processes in biological world involving laws of thermodynamics. All four laws of thermodynamics discussed in detail including with concept of 'Gibbs free energy', and enthalpy of heat, and quantitative relation between them.

NOTES

Structure and function of ATP and **Redox reactions** and there applications in biological world are also dicussed in this unit.

1.9 Glossary

- **ATP :** Adenosine triphosphate, A ribonucleotide 5'triphosphate functioning as a phosphate group donar in the cell.
- **ATP synthatase :** ATP synthatase an enzyme complex that form ATP from ADP and phosphate.
- **ATPase :** An enzyme that hydrolyzes ATP to ADP and phosphate.
- **Autotroph :** An organism that can synthesize its own molecule from simple carbon, hydrogen and oxygen.
- **Catabolism :** The phase metabolism, concern with the degradation of nutrients.
- **Endothermic reaction :** A chemical reaction that take up heat(that is for which delta H is positive).
- **Endergonic reaction :** A chemical reaction that consume energy (that is for which delta G is positive).
- **Exergonic reaction :** A chemical reaction that proceed the release of free energy (that is for which delta G is negative).
- **Exothermic reaction :** A chemical reaction that release heat(that is for which delta H is negative).
- **Enthalpy :** The heat content of the system.
- **Enthalpy change (ΔH) :** Difference between energy used to break bonds and energy gained by the formation of new ones.

- **Entropy :** The extent of randomness or disorder in a system.
- **Feed back inhibition :** End product inhibition.
- **Free energy :** The component of total energy of a system that can do work at constant T and P.
- **Heterotrophs :** An organism that require complex food as energy source.
- **NAD, NADP :** Nicotinamide adenine di nucleotide and Nicotinamide adenine di nucleotide phosphate.
- **Neurotransmitter :** Low molecular weight compound, serve to transmit a nerve impulse.
- **Open system :** A system that exchange energy and matter to its surroundings.
- **Redox reaction :** Oxidation and Reduction reactions
- **Redox pair :** An Electron donar and its corresponding oxidized form.

NOTES

1.10 Self-Learning Exercise

Section A (Very Short Answer Type Question)

1. What is the name of second laws of thermodynamics?
2. Which term is used for heat change in energy transfer?
3. Redox reaction involves which reactions?
4. Entropy, is a quantitative expression for the which things?
5. What is the full name of ATP?
6. Which is the Energy currency of cell?
7. What is the full form of FLOT?
8. Gibbs free energy is denoted by which sign?

Section B (Short Answer Type Questions)

1. Explain Gibbs free energy?
2. What is homeostasis?
3. Explain the function of ATP?
4. Draw flow chart of energy transfer in ecosystem?

Section C (Long Answer Type Questions)

NOTES

1. Write short notes on—
   a. Redox reactions
   b. ATP structure and function
2. Explain laws of thermodynamics and its relevance to biological world?

Answer key of section A

1. Law of entropy
2. Enthalpy
3. Oxidation reduction
4. Randomness.
5. Adenosine tri phosphate
6. ATP
7. First law of thermodynamics
8. ΔG

1.11 References

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Unit - 2

Fundamentals of Enzymology

NOTES

Structure of the Unit

NOTES

2.0 Objective

After going through this unit you will able to understand -

- About the enzyme and their role as biological catalysts.
- Types of enzymes, and the pattern of nomenclature and classification.
- Properties and physiochemical nature of enzymes.
- The mechanism of enzyme action, and concept of activation energy in enzyme action.
- To understand the enzyme kinetics, and there related terminology, as V_{max} and K_m.
- The quantitative relationship between substrate concentration and enzyme action by Michaelis- Menten equation, and the applications of transformed versions of this reaction.
- About allosteric enzyme there action, isozyme and abzymes.

2.1 Introduction

Enzymes are biological catalysts and are proteinaceous in nature. Enzyme act as catalyst, basically they lower the amount of activation energy leads to faster the reaction rate. Enzymes are extremely sensitive to the changes in pH and temperature, and work best at optimum temperature. Enzymes show absolute specificity in terms of substrate in which they act and in types of reaction being performed. The specificity of the enzyme is due to its definite three dimensional structures and to its active site. In a chemical reaction enzyme binds reversibly with the substrate through its active site and form enzyme substrate complex. This enzyme substrate complex is more stable than either free enzyme or substrate. These interactions involve vanderwall's forces, Hydrogen bonds and ionic interactions. By lowering of activation energy, transition enzyme substrate can convert in to product. The relationship between substrate concentration and reaction rate can be expressed quantitatively by Michaelis – Menten equation. Many enzymes have molecular multiplicity are called as isozymes. Abzyme also called cat mob, is a monoclonal antibody with

catalytic activity, Abzymes are usually artificial constructed. This kind of interaction where an allosteric modulator modifies the activity of an enzyme is called allosteric mechanism.

2.2 Enzyme

NOTES

2.2.1 Enzyme a general account

Enzymes are biological catalysts required for almost every reaction in the body. Enzymes are proteinaceous molecules, and are able to accelerate the rate of chemical reaction in a living cell. Like catalysts, they are not used up in the reaction, but unlike catalysts they are produced by living cell only.

Kuhne 1878, firstly used the term enzyme (meaning is yeast) for the soluble 'ferment' of bacteria or yeast.

J.B. Summer 1926 firstly obtained pure crystallin form of 'urease' from Jack bean meal)

2.2.2 Non catalyzed and catalyzed reaction

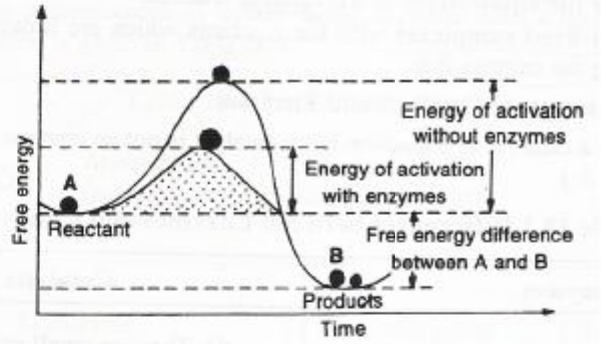


Fig- 2.1 Non catalyzed and catalyzed reaction

In the coordinate diagram, free energy of a system is plotted against the progress of the reaction.

- Ground state – The starting point for either the forward or reverse reaction is called ground state.
- Transition state – At the top of the energy hill is a point at which decay to the S & P state is equally probable (It is down hill either way) called transition state.

NOTES

2.2.3 Activation energy

The activation energy is the energy required to break the potential energy barrier in a chemical reaction to convert substrate in to product e.g. H_2O_2 decomposition require 18 Kcal $Mo1^{-1}$ but in the presence of enzyme catalases require only $\frac{1}{3}$ energy i.e. 6.4 Kcal $Mo1^{-1}$.

Definition of Activation energy –The difference between the energy levels of ground state and the transition state is called activation energy.

Higher activation energy represent slow reaction rate, low activation energy faster the reaction rate.

2.2.4 Role of enzyme and activation energy

Chemical reaction takes place only when molecules collides, the energy which is required to produce a collision that is powerful enough to bring about a chemical reaction is called "Activation energy. The reaction occurs at room temperature has sufficient activation energy, while in many other reaction outer source of activation energy is necessary. A high temperature also provides activation energy, but it is not good for living cells, on the other side, in the presence of certain chemical, molecules can interact without heating, these are called as catalyst, basically they lower the amount of activation energy. Catalyst act in living organisms called as enzymes.

2.2.5 Enzyme effect reaction rates, but not the equilibrium

The function of catalyst is to increase the rate of reaction, it do not affect reaction equilibrium.

The equilibrium between S and P reflects the difference in the free energies of their ground state, e.g. – If the free energy of the ground state of P is lower than that of S so ΔG for the reaction is negative and the equilibrium favors P. So the position and direction of equilibrium are not affected by any catalyst.

The rate of reaction reflect the activation energy, a higher activation energy corresponds to slower reaction, Reaction rates can be increased by raising the temperature, increase the number of molecules with sufficient energy to overcome the energy barrier, Alternatively the activation energy can be lowered by adding a catalyst because enzyme enhance reaction rates by

lowering activation energies and reaction reaches much faster when the appropriate enzyme is present because the rate of reaction is increased.

2.3 Structure

NOTES

All enzymes are proteinaceous in nature; some enzymes need a non proteinaceous part as well. Most of the enzymes made up of two portions i.e. Apoenzyme and Cofactor; both portions are enzymatically inactive when separated from each other.

Holoenzyme	-	Apoenzyme + cofactor
(Conjugated enzyme)		protein part + nonprotein part

Apoenzyme - composed of alpha amino acid units. This is the specificportion of the enzyme.

Cofactor- It is the nonspecific portion and it can participate in many reactions. It can be differentiated into these categories; 1. Coenzyme 2. Prosthetic group 3. In-organic ions.

Coenzyme - organic molecules only loosely arranged to the enzyme. e.g. NAD, NADP, ATP, coenzyme A.

Prosthetic group - organic molecules tightly bound to enzyme. e.g. FMN, biotin, Heam.

Inorganic ions - also known as enzyme activators like chloride ions (Cl^-).

2.4 Properties

- Enzyme remains unchanged after the reached they can be used over and over again.
- Required in minute quantities compared to the substrate.
- Enzyme does not initiate a reaction, but only lower the activation energy and increase the speed of reaction.
- It does not alter the equilibrium of a reversible reaction.
- It makes short lived complexes with the reactants, which are broken down to give the product with releasing the enzyme free.

NOTES

- Enzymes are proteinaceous in nature and have a complex three dimensional organization.
- It can catalyze only specific reactions (specific to substrate)
- Enzymes are extremely sensitive to the changes in pH and temperature,
- Work best at optimum temperature (low temp.-inactive, high temp-denature)

Substrate Specificity (Active sites)

Enzymes show absolute specificity in terms of substrate in which they act and in types of reaction being performed. The binding energy that provides energy to a catalyst also gives an enzyme its specificity, the ability to discriminate between subtract and competing molecules.

Enzymes divided in two categories –

1. Absolute specific
2. Broader specificity range

Eg. – Glucokinase specific to glucose only, while Hexokinase catalyses phosphorylation of glucose, mannose, fructose, glucosamine.

Active site – The specificity of the enzyme is due to its definite three dimensional structures and it's the active site or active center of the enzyme.

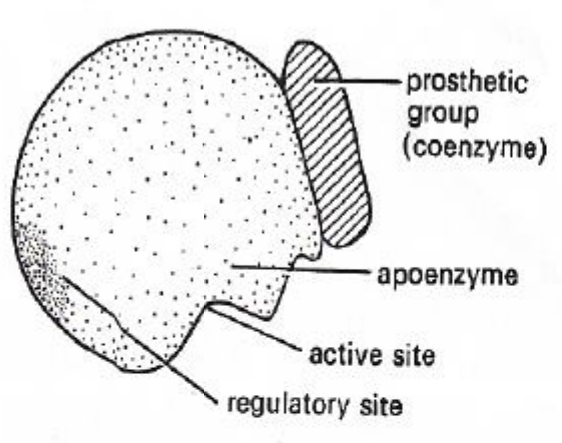


Fig- 2.2 Active site

The tertiary structure of the enzymic protein is folded in such a way as to create a region called active site, Active site has the correct molecular dimensions and appropriate topology to accommodate and bind with a specific substrate.

Active site may be either at the surface or in a cleft in 'Apoenzyme'. Active site made up of few (3-12) amino acids and has a certain degree of flexibility in structural organization which is helped in accommodate substrate molecules.

NOTES

Regulatory site is an addition site present in an enzyme near the active site, at these site regulatory molecules may bind to activate or deactivate the enzyme.

2.5 Classification

1. **Basis of nomenclature -** According to International Union of Biochemistry IUB a systematic has been adopted. The enzymes have been placed into six groups according to the reaction they catalyse.

Each enzyme has been given a 'trivial' name also-

(i) First is the name of substrate they act on

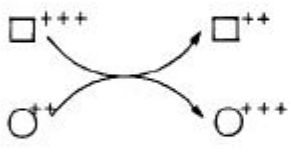
(ii) Second part is the type of reaction they undergo.

(iii) Suffix-ase (at the end)

e.g. Glucose-6-phosphatase

2. Classes of enzymes - Enzymes have been grouped into six major classes on the basis of the activity of enzymes –

Table - 1 various classes of enzymes and their catalytic reaction

S.No.	Classes of Enzyme	Reaction Catalyzed	Kind of reaction
1.	Oxidoreductases	Catalyse oxidation by removing electrons or adding O atom and reduction by adding electrons or adding H atom	

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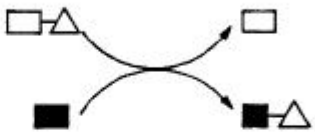
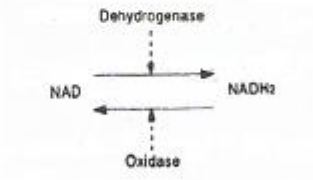
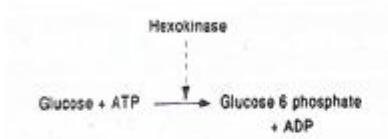
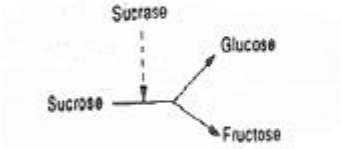
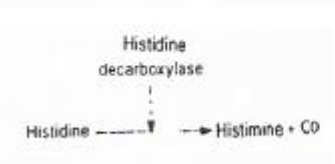
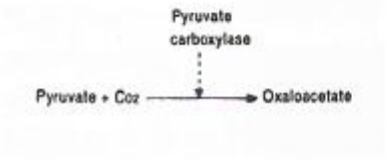
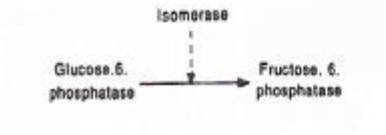
2.	Tranferases	Transfer specific group from one substrate to another	
3.	Hydrolases	Break a complex molecule into two products by hydrolysis(addition of H_2O)	
4.	Lyases	Breaking of covalent bonds and removal of groups without hydrolysis	
5.	Ligases	Join together two molecules by synthesis of new covalant bonds	
6.	Isomerases	Catalyse rearrangement of molecular structure to from isomers	

Table -2 Example of enzymes

S.No.	Group of Enzyme	Common enzyme	Specific Example
1.	Oxido reductases	Oxidase Dehydrognase	

2.	Transferases	Transaminase	
3.	Hydrolases	Lipase Amylase	
4.	Lyases	Decarboxylase Fumerase	
5.	Ligases	Synthetase	
6.	Isomerases	Isomerase Mutase	

NOTES

2.6 Mechanism of Enzyme Action

Firstly enzyme binds reversibly with the substrate through its active site and form enzyme substrate complex.

$$E+S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E+P$$

where E-enzyme, S- substrate, P- Product, ES & EP transient complex

a. $E+S \rightleftharpoons ES$

This enzyme substrate complex is more stable than either free enzyme or substrate. These interactions involve vanderwall's forces, Hydrogen bonds and ionic interactions.

NOTES

Transient chemical species – Any reaction may have several steps involving the formation and decay of transient chemical species called reaction intermediate. A reaction intermediate is a species on the reaction pathway that was a finite chemical lifetime. The ES & EP complexes can be considered intermediates ever through S & P are stable chemical species ES & EP complexes occupy valleys in the reaction coordinate diagram.

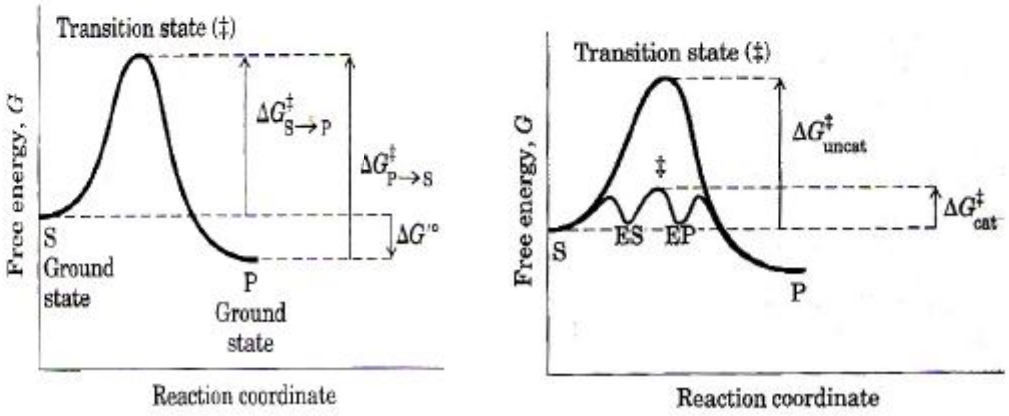


Fig- 2.3 A&B Mechanism of Enzyme Action

b. $E+S \rightleftharpoons ES \longrightarrow P$

By lowering of activation energy, transition enzyme substrate complex (ES) can convert in to product.

Two models have been proposed to explain the type if complexing between enzyme and substrate-

1. **Lock and Key model** – It was proposed by German chemist Emil Fischer in 1890's. According to this model the enzyme-substrate complex formed is analogous to the fitting of lock and key, where both have strictly complementary structures, so the substrate fit exactly with active site of enzyme as key fits in to a lock.

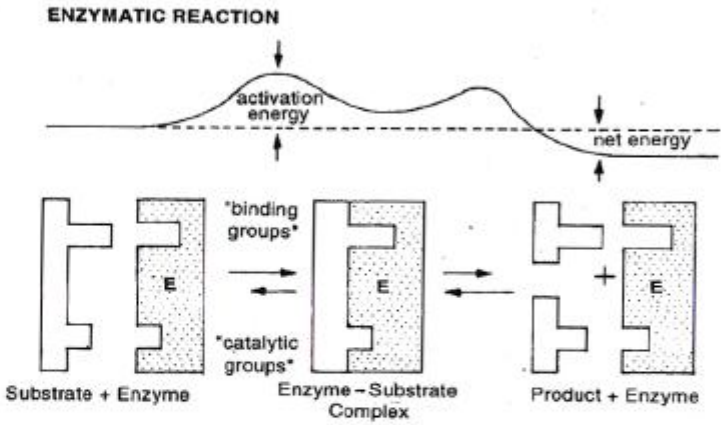


Fig-2.4 Lock and Key Model

2. **Induced fit model** – This model was proposed by D Koshland in 1966, it envisages a flexible active site structure, enzyme and substrate do not have strictly complimentary active site, but active site is flexible and can change the configuration according to substrate, so substrate induced the active site to fit called induced fit mechanism.

2.7 Enzyme Kinetics

The central approach to studying the mechanism of an enzyme – catalyzed reaction is to determine the rate of reaction and how it changes in response to changes in experimental parameters, is known as enzyme kinetics.

Enzyme kinetic deals with the rates of enzyme catalyzed reaction, such studies provides information about the - i.) Insight into the specificity of enzyme. ii.) Mechanism of enzyme action. iii.) Several parameters that characterize the physical properties of enzyme

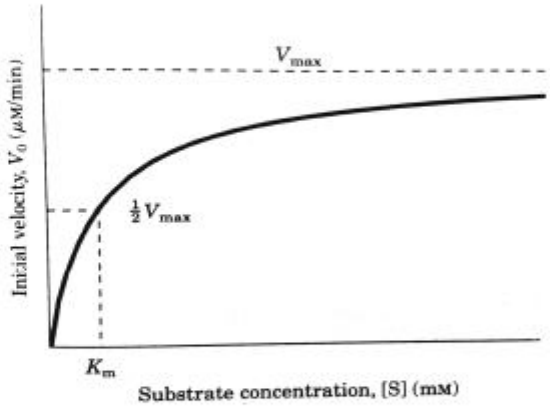


Fig- 2.5 Reaction Rate of Enzyme at increasing Substrate Concentrations

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Substrate concentration affect the rate of enzyme- catalyzed reaction – A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate [s].

Initial rate or initial velocity – Vo when [S] is much greater than the concentration of enzyme [E]. (Beginning of reaction i.e. first 60 sec)

The effect on Vo of varying [S] where enzyme concentration is held constant, is shown in fig.-5 At relatively low concentrations of substrate, Vo increases almost linearly with an increase in [S], finally a point is reached beyond which increases in Vo are vanishingly small as [S] increases, This plateau like Vo region is close to the maximum velocity, V_{max}.

The kinetic pattern is that the combination of an enzyme with its substrate molecular to form an ES complex is a necessary step in enzymatic catalysis; this idea was expanded in to a general theory of enzyme action, particularly by Leonor Michaelis and Maud Menten in 1913. They postulated the enzyme first combines reversibly with its substrate to form an enzyme complex in a relatively fast reversible step

$$E+S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES$$

The ES complex than break down in a slower second step to yield the free enzyme and the reaction product

$$ES \overset{K3}{\rightleftharpoons} E+P$$

Because the slower second reaction must limit the rate of the overall reaction. The overall rate must be proportional to the concentration of the species that reacts in the second step that is ES. The maximum initial rate of the catalyzed reaction (V_{max}) is observed when virtually all the enzyme is present as the ES complexes and [E] is vanishingly small, under then condition the enzyme is saturated with its substrate, so that further increases in [S] have no effect on rate. The saturation effect is a distinguishing characteristic of enzymatic catalysts and is responsible for the plateau observed in fig-5

The relationship between substrate concentration and reaction rate can be expressed quantitatively by Michaelis – Menten equation.

Michaelis and Menten derived this equation starting from their basic Hypothesis that the rate limiting step in enzymatic reaction is the breakdown of the ES complex to product and free enzyme, The equation is

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{.....(1)}$$

NOTES

Km – Michaelis Constant

This is the Michaelis-Menten equation, the rate equation for a one 'substrate enzyme' catalyzed reaction. It is a statement of the quantitative relationship between the initial velocity Vo, the maximum velocity V_{max} and the initial substrate concentration[S], all related through the Michaelis constant Km (unit of concentration). The experimental values of Michaelis constant for most of the enzyme have been determined and it has been found to be that **concentration of substrate at which the rate of enzymic reaction is at half of the maximum rate**.

An important numerical relationship emerges from the Michaelis-Menten equation is the special case when Vo is exactly one half V_{max},

$$V = \frac{1}{2} V_{max} \qquad \text{.....(2)}$$

Than

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{..... (3)}$$

On dividing by V_{max} either side we get

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \qquad \text{..... (4)}$$

Or

$$K_m = 2[S] - [S] = [S] \qquad \text{..... (5)}$$

$$K_m = [S] \text{ when } V_0 = \frac{1}{2} V_{max} \qquad \text{..... (6)}$$

Same transformation of Michaelis Menten equation:-

The double reciprocal plot

NOTES

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Reciprocal

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

Separating the components of the numerator on the right side of the equation given

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

Which simplifies to

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

This form of Michaelis Menten equation is called the line weaver, Burk equation plot of $\frac{1}{V_0}$ verses $\frac{1}{[S]}$ (double reciprocal) yield a straight line.

Significance of Michaelis constant

Michaelis constant is an important kinetic parameter of the enzyme, it reflects the efficiency of enzyme, a lower Km means more efficient enzyme and higher Km means less efficient, a larger terms Km means that a high substrate concentration is required to achieve half of the maximum rate. It means the enzyme has low affinity for the substrate. If an enzyme is able to catalyze the conversion of more than one type of substrate its relative efficiency for different substance can be expressed in terms of its Michaelis constant for different substances, unlike most other constants Michaelis substrate. Thus kinetic behavior of a enzyme is defined by Michaelis Menten Equation.

2.8 Isozyme

Isoenzymes or isoezymes are multiple forms of an enzyme that differ by minor variation in amino acid composition and sometimes in regulation. Many enzymes have molecular multiplicity which is characteristic of many cells, tissues and organs. Such enzymes which have differences in their molecular structure but are similar in function are called isozymes.

Prior it was believed that one gene coded for one enzyme, which was entirely responsible for a particular biochemical reaction.

One gene – One enzyme – One catalytic reaction.

This reaction is no more correct, because. It is well known that multiple varieties of an enzyme are needed to catalyze the same reaction under different metabolic conditions or in different places in the same cell, or in different cells.

NOTES

The appearance of the molecular multiplicity of an enzyme may caused by various mechanism. The gene may undergo mutation to form modified version of original enzyme, or isozyme and their controlling genes subjected to evolutionary selection that results in tailoring the enzyme to fit in the specialized metabolic requirements of cell.

Today isozyme is known to be more than 100 in number eg. Peroxidase, Phasphatases, Amylase, Catalase, lactic acid dehydrogenase (LDH). LDH is the most well known example of isozyme act on pyruvic acid to convert it in to lactic acid. There are five LDH isoezymes that differ in their electrophoretic mobility in starch gels, it is a tetramer that can be formed by two types of subunits i.e. M and H, each subunit is the product of a different gene; the five isoezymes result from the five possible combinations of these subunits.

Importance- Isozyme studies can be fruitfully employed for identifying difference between taxa.

2.9 Enzyme Inhibition

The activity of enzyme can be inhibited by compounds called enzyme inhibitors; it could be reversible or irreversible.

Some inhibitor can be removed easily from the enzyme while the other blocks the enzymes causing a damage in its structure making it non functional.

The inhabitation can be studied under following types:-

2.9.1 Competitive Inhibition

The inhibitor molecule competes with the substrate molecule as the inhibitor has a similar structure and hence the rate of reaction declines.

NOTES

2.9.2 Non-Competitive Inhibition

The inhibitor molecule does not have any structural resemblance to the substrate and hence there is no competition but by altering the structure of the enzyme it declines the rate of reaction.

Table -3

Difference between Competitive and Non-Competitive Inhibition

S.No.	Competitive Inhibition	Non-competitive Inhibition
1.	The Structure of the inhibitor molecule is similar to the substrate	The Structure of the inhibitor is entirely different from the substrate.
2.	The inhibitor gets attached to the enzyme's active site.	The inhibitor forms a complex at a point other then the active site.
1.	The substrate competes with the inhibitor for the position of the active site.	The substrate does not compete with the inhibitor and hence the name non-competitive
2.	The inhibitor does not alter the structure of the enzyme	The inhibitor alters the structure of the enzyme in such a way that even if the substrate gets attached, the products will not be formed.
3.	The reaction can be reversed at any stage by increasing the substrate concentration.	The reaction will keep on decreasing till the inhibitor saturation has reached.
4.	Using Lineweaver-Burk plot it is observed that Vmax is not changed in compititive inhibition, but the Km increases ie apparent affinity of the enzyme for the substrate decreases.	Competitive inhibition cannot be reversed by high concentration of the substrate therefore, the Km remains unchanged, but the V_{max} is decreases.

.	Example : It is used as a cure in medicine also enzymes + P amino benzoic acid. Folic acid synthesis from P amino acid is a normal path way in bacteria causing the disease. Sulpha drugs which are given as a medicine compete and substitute for P amino henzoic acid. This inhibits he reaction and the acid synthesis this controlling the bacterial multiplication.	Example : Cyaninde combines with the prosthetic group of cytochrome oxidase and inhibits the electron transport chain.

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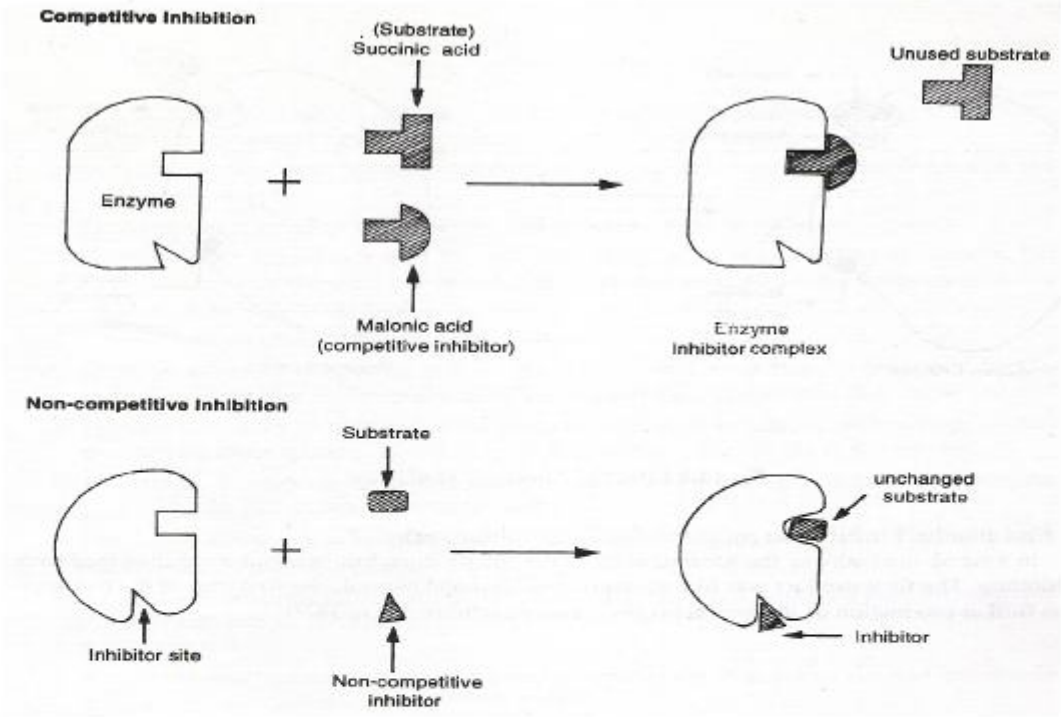

Fig-2.6 Competitive and Non competitive inhibition

2.9.3 Allosteric Mechanism

NOTES

At times the activity of the enzyme is regulated by the compounds which are the products of some other enzyme in a metabolic chain. These molecules (modulator) bind at a specific site far away from active site and bring about a change in the shape of an enzyme. It is called allosteric effect. It can increase or decrease the reaction rate. It is a reversible kind of a change. This kind of interaction where an allosteric modulator modifies the activity of an enzyme is called allosteric modulation.

Allosteric enzyme-- Some enzymes do not obey the normal Michaelis Menten kinetics and exhibit a hyperbolic curve when there initial velocity is plotted as function of substrate concentration. Instead they show sigmoid curve. The rate of reaction catalyzed by these enzymes at a given (s) is enhanced by the addition of a specific activator and decreased by the addition of a specific inhibitor, and accordingly the curve tends to become hyperbola and sigmoid under the two situation respectively. The enzyme which show this behavior are called allosteric enzyme

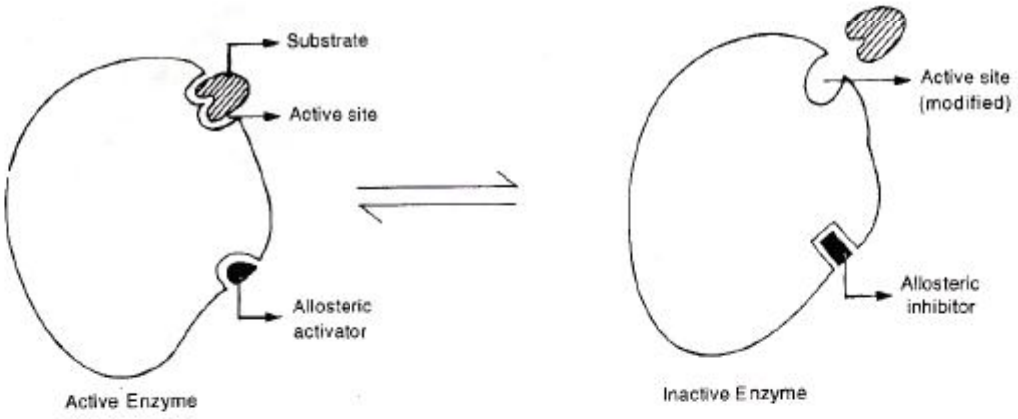

Fig-2.7 Allosteric Mechanism

E.g. – The allosteric enzymes are the first enzymes in a series of enzymatic reactions of metabolic pathway. The end products of the pathway combine with the allosteric site of first enzyme and block its catalytic activity, and inhabit the reaction. The end product act as effecter is believed to bring about conformational change in the active sites of enzyme resulting in the reduction of catalytic activity. The phenomenon has been thrived as allosteric transition by Jacab & Monad.

Most allosteric enzyme posses' sits other than catalytic site called allosteric site, on these additional sites that allosteric effecters are bound and influence catalytic events.

Allosteric enzymes have

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1. The kinetic behavior of an allosteric enzyme suggests the presence of multiple binding sites when identical molecules are bound at allosteric as well as active sites; the resulting interaction between these sites is called a homotropic effect which is generally co-operative.
2. When molecules which are structurally quite different from the substrate such as the end product the resulting interaction between allosteric and active site is called heterotropic effect.
3. Allosteric enzymes are oligomaric enzyme, aggregates of many subunits which are separately inactive, but exhibit cooperativty as a whole.
4. In allosteric enzyme there is a deviation in the usual Michaelis menten kinatics due to cooperative interactions between the different enzyme subunits. Eg ATCase gives a sigmoidal type of curve instead of a hyperbola.

If the interaction is cooperative i.e. the catalytic activity of the enzymes is stimulated, it is positive feedback and if their interaction is antagonistic i.e. the catalytic activity of enzyme is inhibited it is termed as negative feedback. It is interesting to note that certain structural analogs activate the enzymes at low concentration by perhaps acting as allosteric effectors but acts as competitive inhibitor at high concentrations.

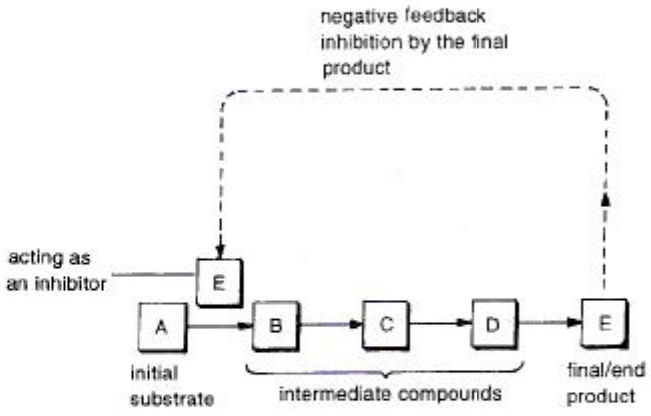

Fig- 2.8 End Product Inhibition

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2.9.4 Feed Back Mechanism (End Product Inhibition)

Negative feedback inhibition in a metabolic pathway the acumination of end product brings about a negative feed back inhibition. The final product act like allosteric inhibitor and control the first step of the path way, the further production of the product is decreased or inhibitor.

2.10 Abzyme

Abzyme : An antibody that express catalytic activity

Abzymes (Antibody and enzyme), also called cat-mob (catalytic monoclonal antibody) is a monoclonal antibody with catalytic activity,

Antibodies and enzymes share the ability to bind with compounds with great specificity and high affinity. This property has been exploited in the development of antibodies with catalytic activity. One basic difference between antibodies and enzymes is that the former binds the complementary structure in its ground state, while enzymes bind in high energy state.

Abzymes are usually artificial constructs, there are potential tool in biotechnology. They also obtained from human and animal serum.

They also found in normal humans and in patients with autoimmune diseases.

These are capable of hydrolyzing proteins, DNA, RNA, polysaccharides etc e.g. To perform specific actions on DNA, they are also useful in hydrolysis of esters, rate of hydrolysis was increased 1000 times.

Enzymes function by lowering the activation energy of the transition state intermediate between reactant and products. If an antibody is developed to stabilize a molecule that's similar to an unstable intermediate of another reaction, the developed antibody will enzymatically bind to and stabilize the intermediate state, thus catalyzing the reaction; a new and unique type of enzyme is produced.

Applications-

Synthesis of simple organic molecules

Drug development

Treat Cancer

Treat allergy

Treat viral and bacterial infection

Abzyme are currently being researched for the possible use against HIV infection the abzyme could target a specific site on the HIV infected cells that do not mutate and then make the virus inert.

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The abzyme are discovered nearly three decades ago and now with the advancement in the area of protein engineering they show tremendous possibilities.

2.11 Summary

This unit involves all the basic concepts about the enzymes, and related terms, as activation energy, transient chemical species, substrate specificity etc.

In this unit, we discussed about the enzymes, there structure, properties, classification and mechanism of enzyme action in detail, and simultaneously kinetics of enzymatic catalysis with the help of quantitative expression of michaelis- menten equation discussed with the help of graphical representation. Allosteric modulation mechanism also discussed in this unit along with special categories of two enzymes that are isozymes (multiple molecular form of enzyme for a reaction) and abzymes (antibody+enzyme).

2.12 Glossary

- **Activation energy** (ΔG^0) : The amount of energy (in joules) required to convert all the molecules in 1 mole of a reacting substance from the ground state to the transition state.
- **Active site :** The region of an enzyme surface that binds the substrate molecule and catalytically transforms it; also known as the catalytic site.
- **Allosteric enzyme :** A regulatory enzyme, with catalytic activity modulated by the no covalent binding of a specific metabolic at a site other than the active site.
- **Allosteric site :** The specific site of the surface of an allosteric enzyme molecule to which the molulator or effector molecule is bound.

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- **Apoenzyme :** The protein portion of an enzyme, exclusive of any organic or inorganic cofactors or prosthetic groups that might be required for catalytic activity.
- **Coenzyme :** An organic cofactor required for the action of certain enzymes; often contains a vitamin as a component.
- **Cofactor :** An inorganic ion or a coenzyme required for enzyme activity.
- **Competitive inhibition :** A type of enzyme inhibition reversed by increasing the substrate concentration; a competitive inhibitor generally competes with the normal substrate or ligand for a protein's binding site.
- **Enhancers :** DNA sequences that facilitate the expression of a given gene; may be located a few hundred, or even thousand, base pairs away from the gene.
- **Enzyme :** A biomolecule, either protein or RNA, that catalyzes a specific chemical reaction. It does not effect the equilibrium of the catalyzed reaction; it enhances the rate of a reaction by providing a reaction path with a lower activation energy.
- **Enzyme cascade :** A series of reactions, often involved in regulatory events, in which one enzyme activates another.
- **Equilibrium :** The state of a system in which no further net change is occurring; the free energy is at a minimum.
- **Equilibrium constant (K_{eq}) :** A constant. Characteristic for each chemial reaction; relates the specific concentrations of all reactants and products at equilibruim at a given temperature and pressure.
- **Free energy (G) :** The component of the total energy of a system that can do work at constant temperature and pressure.
- **Ground state :** The normal, stable from of an atom or molecule; as distinct from the excited state.
- **Heterotropic enzyme :** An allosteric enzyme requiring a modulator other than its substrate.

- **Inducted fit :** A change in the confirmation of an enzyme in response to substrate binding that renders the enzyme catalytically active.
- **Inducer :** A single molecule that, when bound to a regulatory protein, produces an increase in the expression of a given gene.
- **Isozymes :** Multiple forms of an enzyme that catalyze the same reaction but differ from each other in their amino acid sequence, substrate affinity, V_{max}, and/or regulatory properties; also called isoenzymes.
- **Lineweaver-Burk equation :** An algebraic transform of the Michaelis-Menten equation, allowing determination of V_{max} and K_m by extrapolation of [S] to infinity.
- **Michaelis-constant (K_M) :** The substrate concentration at which an enzyme-catalequation reaction proceeds at one-half its maximum velocity.
- **Michaelis-Menten equation :** The equation describing the hyperboloc dependence of the initial reaction velocity, V_o, on subtrate concentration , [S], in many enzyme-catalyzed reactions: $V_o = \frac{V_{max}[S]}{K_m + [S]}$
- **Michaelis-Menten kinetics :** A kinetic pattern is which the initial rate of an enzyme-catalyzed reaction exhibits a hyperbolic dependence on substrate concentration.
- **Modulator :** a metabolite that, when bound to the allosteric site of an enzyme, alters its kinetic characteristics.
- **Prosthetic group :** a metal ion or an organic compound (other than an amino acid) that is covalently bound to a protein and is essential to its activity.
- **Rate constant :** The proportionality constant that relates the velocity of a chemical reaction to the concentration(s) of the reaction(s).
- **Rate-limiting step :** (1) Generally, the step in an enzymatic reaction with the greatest activation energy or the transition state of highest free energy. (2) The slowest step in a metabolic pathway.

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- **Reaction intermediate :** Any chemical species in a reaction pathway that has a finite chemical lifetime.
- **Repressor :** The protein that binds to the regulatory sequence or operator for a gene, blocking its transcription.
- **Ribozymes :** Ribonucleic acid molecules with catalytic activities; RNA enzymes.
- **Steady state :** A nonequilibrium state of a system through which matter is flowing and in which all components remain at a constant concentration.
- **Uncompetitive inhibition :** The reversible inhibition pattern resulting when an inhibitor molecule can bind to the enzyme-substrate complex but not to the free enzyme.

2.13 Self-Learning Exercise

Section A (Very Short Answer Type Question)

1. Which act as an electron donor?
2. Holoenzyme consists of what components?
3. What will the nature of enzyme at high temperature?
4. What is the usual nature of enzyme?
5. Lock and key model of enzyme substrate complex given byWhom?
6. Define abzyme?
7. Who use the term enzyme firstly?
8. Which process deals with rates of enzyme catalyzed reaction?

Section B (Short Answer Type Questions)

1. Write short notes on
   a. Michaelis constant
   b. Abzyme
   c. Lowering of energy of activation
   d. Allosteric modulation

Section C (Long Answer Type Questions)

1. Describe the effect of substrate and enzyme concentrations on the rate of enzymic reactions.
2. Describe briefly the molecular mechanism of enzyme action.

Answer key of Section A

1. NADH,NADPH
2. Apoenzyme.+ Cofector,
3. Denatured
4. Protienaceous,
5. Emil Fischer,
6. An antibody that expresses catalytic activity is called Abzyme,
7. Kuhne
8. Enzyme kinetics

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Unit - 3

Membrane Transport and Translocation of Water and Solutes

Structure of the Unit

3.0 Objective

After going through this unit you will be able to understand:

- Importance of water
- Plant water relations
- Mechanism driving water movement in Plants
- Phloem loading and unloading
- Passive and Active solute transport
- Membrane transport proteins

3.1 Introduction

Water being considered as universal solvent, occupies 75% of our planet in the form of oceans. Added to this water is also found in the atmosphere in the form

of Hydrospheric mantle. The evaporation of water from the surface of ocean, formation clouds and raining, is a natural cycle evolved during course of Evolution of this planet. Nearly 3.8 billion years ago, life took its origin as a speck of protoplasm in the churning oceanic water which was not salty as it is today. In the course of Chemical Evolution, the birth of life has chosen H2O as the medium of biochemical activities. Thus water has become mother of life or "Solvent of Life". Cells of all organisms are made up 90% or more of water. And all other components are either dissolved or suspended in water to form protoplasm, which is often referred to as physical basis of life. In this context one is tempted to know why water is so important and how water is useful to life forms.

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3.2 Plant Water Relations

Importance of water: Water is the major component of living cells and constitutes more than 90% of protoplasm by volume and weight. It acts as medium for all biochemical reaction that takes place in the cell, and also acts a medium of transportation from one region to another region. Water is a remarkable compounded made up of Hydrogen and oxygen (2:1) and it has high specific heat, high heat of vaporization, high heat of fusion and expansion (colligative properties). Water because of its bipolar nature acts as universal solvent for it dissolves more substances than any other solvent. Electrolytes and non-electrolytes like sugars, and proteins dissolve very well. Even some hydrophobic lipid molecules show some solubility in water. Water acts as a good buffer against changes in the Hydrogen ion concentration (pH). This is because of its ionization property. Certain xerophytes use water as buffer system against high temperature. Water also exhibits viscosity and adhesive properties. Because of hydrogen bonds, water molecules are attracted towards each other, they are held to each other with considerable force. This force of attraction is called cohesive force. Thus water possesses a high tensile strength. If this water is confined in very narrow columns of dimensions of xylem vessels, its tensile and cohesive forces reach very high values (1000-1200 Gms). And this force is very helpful in ascent of sap. Water is of great importance in osmoregulation, particularly in the maintenance of turgidity of cells, opening and closing of stomata and growth of the plant body. Water is an

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important substrate in photosynthesis, for it provides reducing power in CO_2 fixation; water is also used in breaking or making chemical bonds of polypeptides, poly-nucleotides, carbohydrates etc.All the above features clearly indicate that water plays an important role in the regulation of life processes.

(a) Diffusion

If the scent is sprayed in one corner of the room, the smell spreads to all part of the house in no time. If a fire wood is burnt, the black soot goes up and spreads. If a pinch of solid potassium permanganate if dropped into water contained in a beaker, pink color slowly diffuses and spreads throughout. The above said spreading phenomenon is due to movement of molecules. Having their own kinetic energy, water molecules will be in constant motion randomly.

(b) Osmosis

If two solutions of different concentrations are separated by a plasma membrane, which is semi permeable as well as selectively permeable, the solvent (in this case it is H_2O) moves through the membrane from higher concentration towards lower concentration. Here the plasma membrane has a differential permeability, where it allows the diffusion of water molecules to move from higher concentration to lower concentration but it prevents the movement of solute molecules from higher concentration to lower concentration. Such differential diffusion through a semi permeable membrane is called Osmosis

(c) Osmotic Potential

Osmosis is always referred to living cells. The movement of water into the cell (endosmosis or plasmolysis) mostly depends upon the concentration of solutes which are of varied types like mineral salts, proteins, carbohydrates, fatty acids etc., and these contribute to the osmotic potential or osmotic concentration, which contributes to the osmotic pressure. Higher the concentration of solutes, higher is the osmotic pressure and vice-versa.

(d)Exosmosis, Endosmosis and Turgidity

$w.\psi = \pi \psi$ When a normal cell is placed in hypertonic solution(solution with high concentration of solutes than the solute concentration of the cells) a water potential or DPD gradient is created between the cell and external

solution.Hence the water diffuses out of the cell the process is called Exosmosis or plasmolysis. As a consequence the cell collapses and the plasma membrane withdraws from the cell wall and the whole cytoplasm gets concentrated in a corner of the cell. Such a cell is called Flaccid cell.In this state turgor pressure(TP) is zero and osmotic pressure(OP) is very high,but if such a cell is transferred to hypotonic solution i.e.solute concentration is less than that of a cell. If the solute concentration of the solution is equal to cell concentration then it is called Isotonic or pure water again an osmotic gradient is created.Hence water from external solution enters into the cell.This process is called Endosmosis or deplsmolysis. As a result, the concentration of water or water potential within the cell increases. Increases in the water concentration create its own molecular pressure within the cell and it is called turgour pressure. With the increase in turgour pressure, the cytoplasm swells and gradually plasma membrane is pushed towards the cell walls. As more and more water enters, more and more of turgour pressure builds up and the cell goes on increasing in the size. The water potential of the cell increases towards zero value becomes equal to wall pressure, the water potential within the cell and outside the cell reaches an equilibrium state. Such a cell is called turgid cell and ψAs turgour pressure exerts its impact outwardly i.e., on to the cell wall, the cell wall being plastic, exerts counter pressure; this is called wall pressure.

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The relation can be expressed in the following formulae:

$$\mathbf{DPD = OP - TP} \qquad \mathbf{\psi W = \psi S + \psi p}$$

(e) Diffusion Pressure Deficit (DPD)

In a pure solvent, all molecules will be moving freely by virtue of their chemical potential. This random movement is called diffusion. It further depends upon the concentration of diffusing molecules, which in turn exert a pressure termed diffusion pressure. The direction and rate of diffusion in a pure solvent is random but equal and opposite. Hence the diffusion pressure exerted in such a system can be taken as zero. To such a system, if solute is added, it undergoes solubility, where some freely moving solvent molecules get bound to solute molecules and in fact in some cases they form a shell around such salts. This results in the loss of considerable number of solvent molecules for free diffusion. This loss is called diffusion pressure deficit. In this process

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there is loss of chemical free energy of water because of the binding of solvent to solutes. Thus the DPD is governed by the relative concentration of solute in a given volume of a solution. Increase in the concentration of solute in a known volume of solution increases the DPD of the system. Furthermore increase in solute's concentration also increases OP; hence DPD and OP are related to each other. The water or solvent always moves from lower DPD to higher DPD.

All the above mentioned phenomenon like Osmosis, OP, DP, DPD are governed by simple physical forces. Nevertheless, they play a very important role in living systems. Cell can be considered as an osmotic bag for it is bounded by cell membrane, enclosing a semi liquid cytoplasm which is made up of more than 90% of water and the rest are inorganic salts and organic molecules and cell organelles. While the cell membrane acts as osmotic membrane (semi permeable), the other organic and inorganic component of the cell act as solute. Creation of differential osmotic potentials between the cells or between the environment and the cell, acts as the motive force in performing various functions like absorption of water, ascent of sap, root pressure, turgour pressure, plasmolysis, absorption of water translocation, transpiration etc.

(f) Water Potential (ψW)

water potential (ψW) which refers to the chemical free energy of water. The chemical free energy of pure water or solutes is always expressed in terms pressure units such as bars.1 atmosphere = 14.7 pounds per square inch, = 760mm Hg at sea level, =1.013 bar, = 0.1013 Mpa, = 1.013 x 10^5 Pa (1 bar = 0.987 atmospheric units, 10 bars = 1 Mega Pascal (Mpa). 1 mpa = 106dynes / cm2, under standard conditions).

The chemical free energy of water in its purest form is also called water potential (ψW). Purest form means there are no other molecules in it. The chemical energy is maximum and its value is given as 0 bars. Addition of solutes to pure solvent decreases the chemical free energy of pure water, because certain amount of energy of a number of water molecules is used for binding to the surface of solutes. So the total value of water potential of a solution is less than zero; it is always expressed in negative pressure values.

Here it is equal to DPD; if the water potential of pure water is zero and DPD is also zero. But the water potential of solution is less than zero expressed in negative value, but DPD of the solution is expressed in positive value.

These energy relations are governed by the said equations, understanding of it is very important.

NOTES

1. $\Psi W = \Psi s + \Psi p = \Psi g$

Ψw = water

Ψs = solutes-solutes potential or osmotic potential.

Ψp = pressure-hydrostatic pressure of the solution, it is often called turgour pressure, which can be negative or positive.

Ψg = gravity- will not be considered for normal calculations.

Pure water:

$\Psi p = 0$ Mpa

$\Psi s = 0$ Mpa

$\Psi w = \Psi p + \Psi s = 0$ Mpa

DPD of pure water = 0 bars; DPD of a solution = (+) bars

Ψ w of pure water = 0 bars; Ψ w of a solution = (-) bars

3.3 Mechanism of Water Transport through Xylem

Transport in plants occurs on three levels: the uptake and release of water and solutes by individual cells absorption of water and minerals from the soil by root cells short-distance transport of substances from cell to cell loading of sucrose from photosynthetic cells into the sieve tube cells of the phloem long-distance transport of sap within the xylem and phloem this is a whole plant phenomena - transport of photosynthate from leaf to root.

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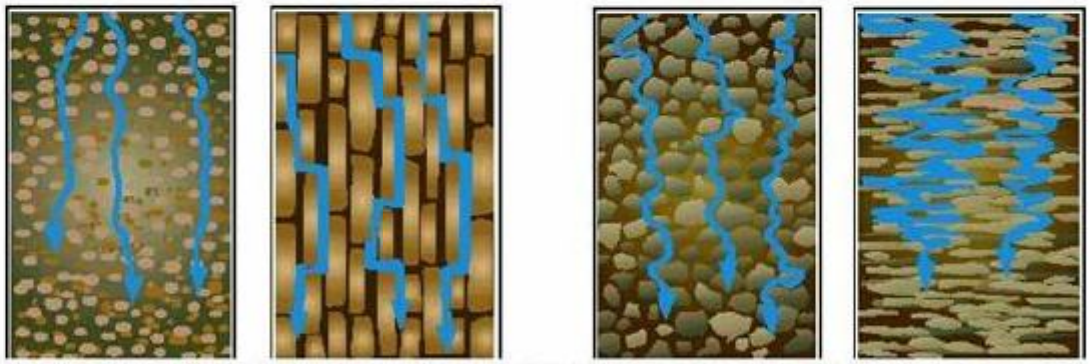
A. Absorption of Water

Water in the soil is mostly and abundantly, under normal conditions, is available in the form of Capillary water. In the soil the space in between soil particle forms a network of spaces, which normally is filled with water. The water that is present in such spaces is called capillary water.



Preferentail flow - gravitational water movement through granular, prismatic, subangular blocky, and platy soils © (left to right).

Fig 3.1 : Water Movements

B. Structures involved in Absorption

The root terminal region is made up various structures such as; from the tip towards base, apical meristem, zone of elongation, root hair zone and zone of maturation. The root hair zone is studded with root hairs; they are the extensions of epidermal cells in the form of tubular structures.

Root Hairs Absorb Water and Nutrients from the Soil

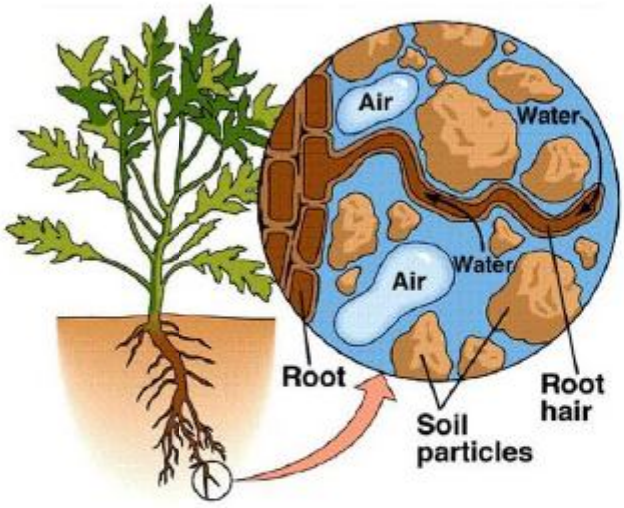


Fig 3.2 Absorption of Water and Nutrients

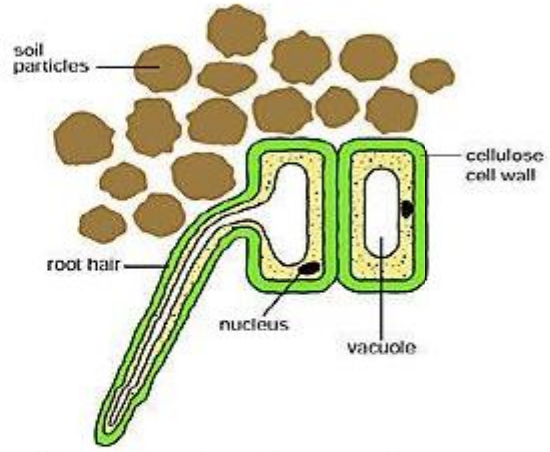


Fig. 3.3 Absorption of Water from Root Hair

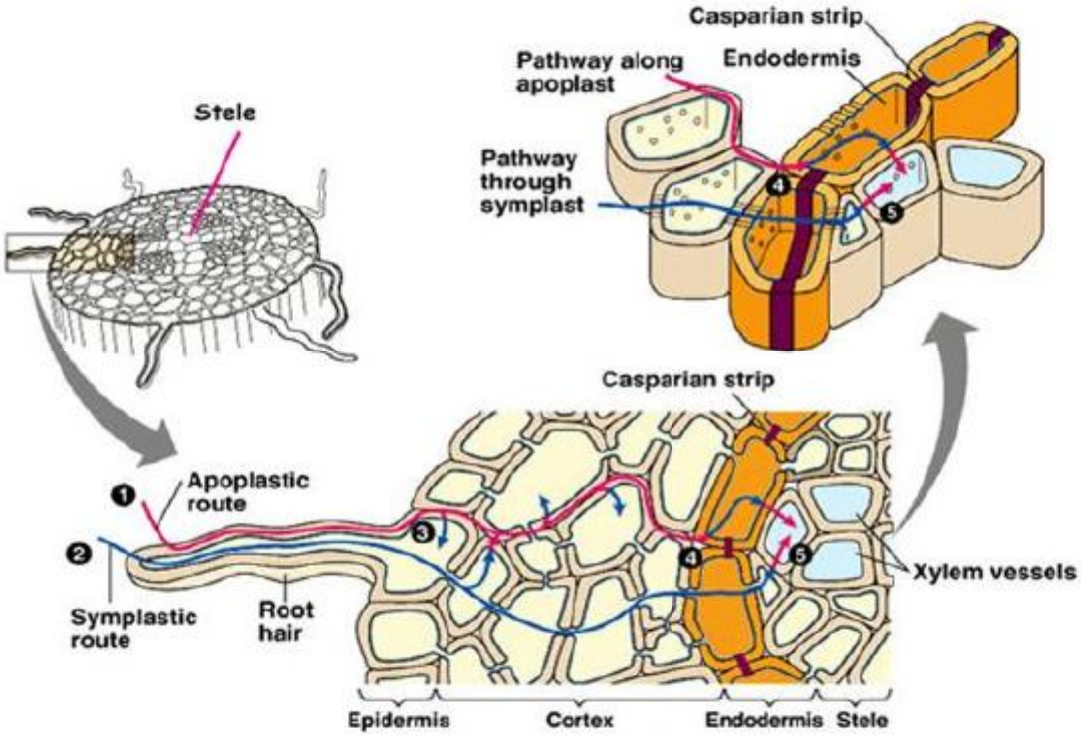


Fig 3.4 The Route of Water Absorption through Xylem

C. Cellular-level Transport

A key component of cellular-level transport is the movement of solutes and ions across the plasma membrane. Survival of the plant depends on balancing water uptake and water loss.In an animal cell, water flows from hypotonic to hypertonic solutions, but in a plant cell, there is the added presence of the pressure created by the cell wall. The combination of solute concentration differences and physical pressure are incorporated into water potential,

abbreviated with the Greek letter psi (Ψ) Water will flow through a membrane from a solution of high water potential to a solution of low water potential.

Water potential is measured in units of megapascals (MPa)

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Pure water has a water potential of 0 MPa (Ψ = 0 MPa)

The addition of solutes lowers water potential (Ψ = -0.2 MPa for instance)

An increase in pressure (by lowering a piston for example) will raise water potential

These two forces combine to form the following equation:

$\Psi = \Psi p + \Psi s$

Ψ = total water potential

Ψp = water potential due to pressure

May be positive or negative

Ψs = water potential due solute concentration (also known as Osmotic Potential)

Always negative or zero

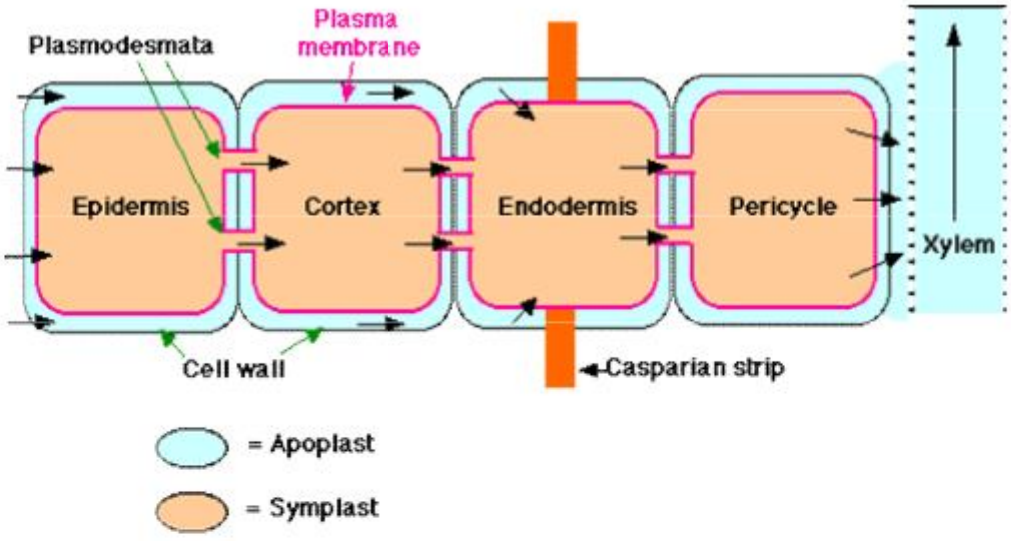

Fig 3.5 Movement of water through cell

D. Transport of Water and Minerals in Plants

Movement of Water through Cells occurs by - Two Routes, the Symplast and the Apoplast

a) Symplastic Movement is the movement of water and solutes through the continuous connection of cytoplasm (though plasmodesmata).There is no crossing of the plasma membrane

b) Apoplastic Movement is the movement of water and solutes through the cell walls and the intercellular spaces.There is no crossing of the plasma membrane.It is more rapid - less resistance to the flow of water

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Soil water enters the root through its epidermis. It appears that water then travels in both the cytoplasm of root cells — called the symplast — that is, it crosses the plasma membrane and then passes from cell to cell through plasmodesmata in the nonliving parts of the root — called the apoplast — that is, in the spaces between the cells and in the cells walls themselves. This water has not crossed a plasma membrane.However, the inner boundary of the cortex, the endodermis, is impervious to water because of a band of lignified matrix called the casparian strip. Therefore, to enter the stele, apoplastic water must enter the symplasm of the endodermal cells. From here it can pass by plasmodesmata into the cells of the stele. Once inside the stele, water is again free to move between cells as well as through them. In young roots, water enters directly into the xylem vessels and/or tracheids.

These are nonliving conduits so are part of the apoplast.Once in the xylem, water with the minerals that have been deposited in it (as well as occasional organic molecules supplied by the root tissue) move up in the vessels and tracheids.At any level, the water can leave the xylem and pass laterally to supply the needs of other tissues.At the leaves, the xylem passes into the petiole and then into the veins of the leaf. Water leaves the finest veins and enters the cells of the spongy and palisade layers.Here some of the water may be used in metabolism, but most is lost in transpiration.

3.4 Phloem loading and unloading

In angiosperms, the specialized cells that transport food in the plant are called sieve-tube members, arranged end to end to form large sieve

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tubes.Phloem sap is very different from xylem sap.sugar (sucrose) can be concentrated up to 30% by weight.Phloem transport is bidirectional.Phloem moves from a sugar source (a place where sugar is produce by photosynthesis or by the breakdown of sugars) to a sugar sink (an organ which consumes or stores sugar)

Phloem Loading and Unloading

Sucrose manufactured in the mesophyll cells can travel via the symplast to sieve-tube members.In some species, sugar can leave the symplast and enter the apoplast, where is it pumped back into the sieve-tube members and the companion cells.Some companion cells have cell wall ingrowths that facilitate apoplatic transport of sucrose into the symplast.Sucrose is loaded into the phloem via a chemiosmotic ATPase mechanism coupled with a H+/sucrose symport.

Other Active and Transport Mechanisms - The H+ / Sucrose Pump

H+ is actively pumped out by hydrolyzing ATP.Sucrose H+ accumulated outside the membrane, generating a concentration and electrochemical gradient.

The H+ cannot cross the membrane, but there is a carrier protein.H+ binds to carrier protein, but sucrose must also bind. When both are bound, the configuration changes and the protein opens to the membrane interior.Downstream, sucrose must be unloaded, again utilizing an H+ / pump

The Mechanism of Translocation in Angiosperms

- Phloem loading results in a high solute concentration at the source end
- This creates hypnotic conditions in the phloem, causing water to flow into the phloem
- Hydrostatic pressure builds in the sieve tube, but it is greatest in the source
- At the sink, osmosis occurs with the unloading of sugar - water flows out of the phloem

- The buildup of pressure at the source and the reduction of that pressure at the sink cause water to flow from source to sink, carrying the sugar along with it.
- Water is recycled via transport in the xylem
- This explanation is very simplified - scientists are just now discovering the subtle details of phloem movement in plants.

NOTES

3.5 Active and Passive solute transport

Active transport: In active transport a solute is moved against a concentration or electrochemical gradient, in doing so the transport proteins involved, consume metabolic energy, usually ATP. In primary active transport the hydrolysis of the energy provider (e.g. ATP) takes place directly in order to transport the solute in question, for instance, when the transport proteins are ATPase enzymes. ATP-powered pumps (or simply pumps) are ATPases membrane that uses the energy of ATP hydrolysis to move ions or small molecules across against a chemical concentration gradient or electric potential. This process, referred to as active transport, is an example of a coupled chemical reaction. In this case, transport of ions or small molecules "uphill" against a concentration gradient or electric potential across a membrane, which requires energy, is coupled to the hydrolysis of ATP to ADP and Pi, which releases energy. The overall reaction — ATP hydrolysis and the "uphill" movement of ions or small molecules — is energetically favorable. Such pumps maintain the low calcium (Ca2+) and sodium (Na+) ion concentrations inside virtually all animal cells relative to that in the medium, and generate the low pH inside animal-cell lysosomes, plant-cell vacuoles, and the lumen of the stomach.

Passive transport: Passive diffusion is a spontaneous phenomenon that increases the entropy of a system and decreases the free energy.The transport process is influenced by the characteristics of the transport substance and the nature of the bilayer. Membrane proteins (with the exception of channels - facilitated diffusion) are not involved in passive diffusion. The diffusion velocity of a pure phospholipid membrane will depend on: concentration gradient, hydrophobicity, size, charge, if the molecule has a net charge.

3.6 Membrane Transport Proteins

NOTES

A membrane transport protein (or simply transporter) is a membrane protein involved in the movement of ions, small molecules or macromolecules, such as another protein, across a biological membrane. Transport proteins are integral transmembrane proteins; that is they exist permanently within and span the membrane across which they transport substances. The proteins may assist in the movement of substances by facilitated diffusion or active transA port. These mechanisms of action are known as carrier-mediated transport. In cellular biology the term **membrane transport** refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes which are lipid bilayers that contain proteins embedded in them. The regulation of passage through the membrane is due to selective membrane permeability - a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. In other words, they can be permeable to certain substances but not to others.

The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules.

As the diversity and physiology of the distinct cells is highly related to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage. In primary active transport the hydrolysis of the energy provider (e.g. ATP) takes place directly. In secondary active transport, the energy is stored in an electrochemical gradient. Secondary active transporter proteins move two molecules at the same time: one against a gradient and the other with its gradient. They are distinguished according to the directionality of the two molecules:

Antiporter: (also called exchanger or counter-transporter) move a molecule against its gradient and at the same time displaces one or more ions along its gradient. The molecules move in opposite directions.

Symporter: move a molecule against its gradient while displacing one or more different ions along their gradient. The molecules move in the same direction.

Both can be referred to as co-transporters.

Uniporters transport one molecule at a time down a concentration gradient. This type of transporter, for example, moves glucose or amino acids across

the plasma membrane into mammalian cells. In contrast, antiporters and symporters couple the movement of one type of ion or molecule *against* its concentration gradient to the movement of a different ion or molecule *down* its concentration gradient.

Like ATP pumps, antiporters and symporters mediate coupled reactions in which an energetically unfavorable reaction is coupled to an energetically favorable reaction. Because symporters and antiporters catalyze "uphill" movement of certain molecules, they are often referred to as "active transporters," but unlike pumps, they do not hydrolyze ATP (or any other molecule) during transport. A better term for these proteins is cotransporters, referring to their ability to transport two different solutes simultaneously.

NOTES

3.7 Summary

Water being considered as universal solvent, occupies 75% of our planet in the form of oceans. Cells of all organisms are made up 90% or more of water. And all other components are either dissolved or suspended in water to form protoplasm, which is often referred to as physical basis of life. Water in the soil is mostly available in the form of Capillary water. Survival of the plant depends on balancing water uptake and water loss. Soil water enters the root through its epidermis. It appears that water then travels in both the cytoplasm of root cells — called the symplast — that is, it crosses the plasma membrane and then passes from cell to cell through plasmodesmata in the nonliving parts of the root — called the apoplast — that is, in the spaces between the cells and in the cells walls themselves. This water has not crossed a plasma membrane. Phloem transport is bidirectional. In active transport a solute is moved against a concentration or electrochemical gradient, in doing so they consume metabolic energy, usually ATP. Passive transport is a spontaneous phenomenon that increases the entropy of a system. A membrane transport protein is involved in the movement of ions, small molecules or macromolecules across a biological membrane. They exist permanently within and span the membrane across which they transport substances.

3.8 Glossary

- **Diffusion :** Diffusion is the movement of molecules from a region of high concentration to a region of low concentration.

NOTES

- **Osmosis :** Osmosis is the movement of solvent molecules from a region of high concentration to a region of low concentration through a semi permeable membrane.
- **Osmotic potential :** The concentration of solutes in an enclosed system having a constant volume exerts a pressure because of the kinetic movement and collision of the solute molecules.The pressure that is exerted by the solute in a system either separated or enclosed in a semi permeable membrane is called osmotic pressure
- **Exosmosis or Plasmolysis :** When a normal cell is put in hypertonic solution water potential or DPD gradient is created between the cell and the external solution. Hence the water diffuses out of the cell; the process is called Exosmosis or Plasmolysis.
- **Flaccid cell :** As a result of exosmosis the cell collapses and the plasma membrane withdraws from the cell wall and the whole cytoplasm gets concentrated in a corner of the cell. Such a cell is called flaccid cell.
- **Isotonic :** If the solute concentration of the solution is equal to the cell concentration then it is called Isotonic
- **Endosmosis or Deplasmolysis :** water from external solution enters into the cell. This process is called Endosmosis or Deplasmolysis.
- **Diffusion Pressure Deficit (DPD) :** Diffusion pressure of pure solvent is zero. To such a system if solute is added there is loss in the number of solvent molecules for free diffusion .This loss is called Diffusion Pressure Deficit.
- **Water potential (φW) :** which refers to the chemical free energy of water.
- **Symplastic Movement :** is the movement of water and solutes through the continuous connection of cytoplasm (though plasmodesmata).
- **Apoplastic Movement :** is the movement of water and solutes through the cell walls and the intercellular spaces
- **Membrane transport proteins :** are proteins involved in the movement of ions, small molecules or macromolecules, such as another protein, across a biological membrane.

- **Antiporter** : (also called exchanger or counter-transporter) move a molecule against its gradient and at the same time displaces one or more ions along its gradient. The molecules move in opposite directions.
- **Symporter :** move a molecule against its gradient while displacing one or more different ions along their gradient. The molecules move in the same direction.
- **Uniporters :** transport one molecule at a time down a concentration gradient.

NOTES

3.9 Self-Learning Exercise

Section A (Very Short Answer Type Question)

1. Define Diffusion?
2. Define osmosis and osmotic potential?
3. Define Active and Passive transport?

Section B (Short Answer Type Questions)

1. Differentiate between Antiport and Symport?
2. Mention the importance of water?
3. Define diffusion pressure deficit?

Section C (Long Answer Type Questions)

1. Explain in detail the mechanism of water transport through xylem?
2. Write a note on phloem loading and unloading?
3. Discuss membrane transport proteins?

3.10 References

- Jain J.L.,Fundamentals of Biochemistry,S.Chand and Company, New Delhi, 2013
- Verma S.K. A Text book of Plant Physiology and Biochemistry, S.Chand and Company,NewDelhi, 2008

Unit - 4

Signal Transduction

NOTES

Structure of the Unit

4.0 Objective

After going through this unit you will be able to understand:

- Receptors and G-proteins
- Phospholipid Signaling
- Role of cyclic nucleotides
- Calcium- Calmodulin cascade
- Diversity in protein kinases and phosphotases
- Specific signaling mechanisms

4.1 Introduction

Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. It involves the binding of extracellular signalling molecules and ligands to cell-surface receptors that trigger events inside the cell. The combination of messenger with receptor causes a change in the conformation of the receptor, known as receptor activation. This activation is

always the initial step (the cause) leading to the cell's ultimate responses (effect) to the messenger.

Signal transduction occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell.

NOTES

In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response. Depending on the cell, the response alters the cell's metabolism, shape, gene expression, or ability to divide. The signal can be amplified at any step. Thus, one signaling molecule can cause many responses.

4.2 Receptors and G-proteins

Signal transducing receptors are of three general classes:

1. Extracellular receptors

Extracellular receptors are integral transmembrane proteins and make up most receptors. They span the plasma membraneof the cell, with one part of the receptor on the outside of the cell and the other on the inside. Signal transduction occurs as a result of a ligand binding to the outside; the molecule does not pass through the membrane. This binding stimulates a series of events inside the cell; different types of receptor stimulate different responses and receptors typically respond to only the binding of a specific ligand. Upon binding, the ligand induces a change in the conformation of the inside part of the receptor. These result in either the activation of an enzyme in the receptor or the exposure of a binding site for other intracellular signaling proteins within the cell, eventually propagating the signal through the cytoplasm; examples include tyrosine kinase and phosphatases. Some of them create second messengers such as cyclic AMP and IP3, the latter controlling the release of intracellular calcium stores into the cytoplasm. These receptors penetrate the plasma membrane and have intrinsic enzymatic activity.

Receptors include tyrosine kinases (e.g. PDGF, insulin, EGF and FGF receptors), tyrosine phosphatases (e.g. CD45 [cluster determinant-45] protein of T cells and macrophages), guanylate cyclases (e.g. natriuretic peptide receptors) and serine/threonine kinases (e.g. activin and TGF-β receptors).These receptors are capable of autophosphorylation as well as phosphorylation of other substrates.

NOTES

2. G protein-coupled receptors (GPCRs)

G protein-coupled receptors are a family of integral transmembrane proteins that possess seven transmembrane domains and are linked to a heterotrimeric G protein. Many receptors are in this family, including adrenergic receptors andchemokine receptors. These receptors that are coupled, inside the cell, to GTP-binding and hydrolyzing proteins (termed G-proteins). These receptors are termed **serpentine** receptors. Examples of this class are the adrenergic receptors, odorant receptors, and certain hormone receptors (e.g. glucagon, angiotensin, vasopressin and bradykinin).

3. Intracellular receptors

Receptors that are found intracellularly and upon ligand binding migrate to the nucleus where the ligand-receptor complex directly affects gene transcription. Because this class of receptors is intracellular and functions in the nucleus as transcription factors they are commonly referred to as the nuclear receptors. Receptors of this class include the large family of steroid and thyroid hormone receptors. Receptors in this class have a ligand-binding domain, a DNA-binding domain and a transcriptional activator domain.

To initiate signal transduction, the ligand must pass through the plasma membrane by passive diffusion. On binding with the receptor, the ligands pass through the nuclear membrane into the nucleus, enabling gene transcription and protein production. Intracellular receptors, such as nuclear receptors and cytoplasmic receptors, are soluble proteins localized within their respective areas. The typical ligands for nuclear receptors are lipophilic hormones like the steroid hormones testosteroneand progesterone and derivatives of vitamins A and D.

G-proteins

G-proteins are so-called because their activities are regulated by binding and hydrolyzing GTP. When a G-protein is bound to GTP it is in the active ("on") state and when the GTP is hydrolyzed to GDP the protein is in the inactive ("off") state. The G-proteins possess intrinsic GTPase activity that is regulated in conjunction with interaction with membrane-associated signal transducing receptors (termed G-protein coupled receptors, GPCRs) or with intracellular effector proteins.

There are two major classes of G-protein: those that are composed of three distinct subunits (α, β and y) and the monomeric class that are related to the archetypal member Ras (originally identified as an oncogene causing sarcomas in rats). The monomeric class of G-protein is also referred to as the Ras superfamily or the small GTPase family of G-proteins. The structure and function of the monomeric G-proteins is similar to that of the α-subunit of the trimeric G-proteins.

NOTES

All known cell surface receptors that are of the G-protein coupled receptor class interact with trimeric G-proteins. The α -subunit of the trimeric class of G-proteins is responsible for the binding of GDP/GTP. When G-proteins are activated by receptors or intracellular effector proteins there is an exchange of GDP for GTP turning on the G-protein which enables it to transmit the original activating signal to downstream effector proteins. In the trimeric class of G-protein, when associated receptor activation stimulates the GDP/GTP exchange in the α -subunit, the protein complex dissociates into separate α and βy activated complexes. The released and activated βy complex serves as a docking site for interaction with downstream effectors of the signal transduction cascade. Once the α-subunit hydrolyzes the bound GTP to GDP it re-associates with the βy complex thereby terminating its activity.

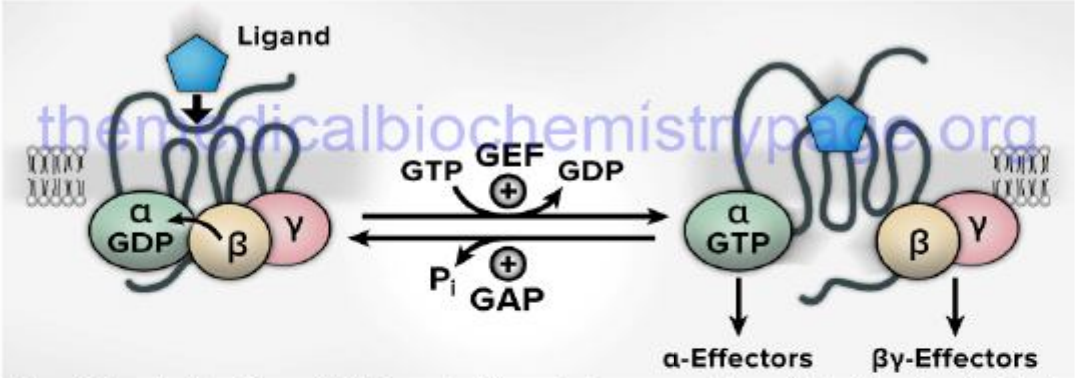

Fig.- 4.1 : **Activation of Trimeric G-proteins upon ligand binding to typical G-ProteinCoupled Receptors**

4.3 Phospholipid Signaling

Phospholipases and phospholipids are involved in the processes of transmitting ligand-receptor induced signals from the plasma membrane to intracellular

proteins. The enzymes whose activities are modulated as a consequence of plasma membrane receptor activation are the members of the phospholipase C (PLC) family.

NOTES

Once a PLC enzyme is activated a chain of events occurs leading to subsequent activation of the kinase, PKC. Proteinkinase C (PKC) is maximally active in the presence of calcium ion and DAG (diacylglycerol). Activation of PLC results in the hydrolysis of membrane phospholipids, primarily phosphatidylinositol-4,5-bisphosphate (PIP_2) leading to an increase in intracellular DAG and inositol trisphosphate (IP_3). The released IP_3 interacts with intracellular membrane receptors leading to an increased release of stored calcium ions. Together, the increased DAG and intracellular free calcium ion concentrations lead to increased activity of PKC.

Phospholipases D and A_2 (PLD and PLA_2) also are involved in the sustained activation of PKC through their hydrolysis of membrane phosphatidylcholine (PC). PLD action on PC leads to the release of phosphatidic acid which in turn is converted to DAG by a specific phosphatidic acid phosphomonoesterase. PLA_2 hydrolyzes PC to yield free fatty acids and lysoPC.This leads to neoplasia.

Phospholipase C (PLC) Family

Members of the phospholipase C (PLC) family play crucial roles in the regulation of signal transduction in a wide array of systems. The members of the PLC family hydrolyze membrane-associated phosphatidylinositol-4,5-bis-phosphate PIP_2 resulting in the generation of two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), in response to activation of receptors by hormones, growth factors, and neurotransmitters. As indicated above, these second messengers in turn activate the kinase, PKC.

Thus far, a total of 13 PLC genes have been identified in the human genome. The proteins encoded by these 13 genes have been assigned to six subclasses of enzyme defined on the basis of structure and regulatory activation mechanisms. These six subfamilies are referred to as PLC-beta (PLCβ1–β4), PLC-gamma (PLCy1 and PLCy2), PLC-delta (PLCdelta1,delta3, and delta4), PLC-epsilon (PLCw), PLC-zeta (PLCζ), and PLC-eta (PLCη1 and PLCη2)..

Phospholipase D (PLD) Family α ββ

Humans express two major PLD isoforms identified as PLD1 and PLD2.. Both PLD1 and PLD2 are capable of hydrolyzing PC, PE, PS, lysophosphatidylcholine (LPC), and lysophosphatidylserine (LPS). However, these two isoforms are not capable of hydrolyzing PI, PG, or cardiolipin. When the substrate is LPC or LPS the product of PLD action is lysophosphatidic acid (LPA).LysoPLD is an enzyme which hydrolyzes lysophospholipids (lysoPL) to produce lysophosphatidic acid (LPA).

NOTES

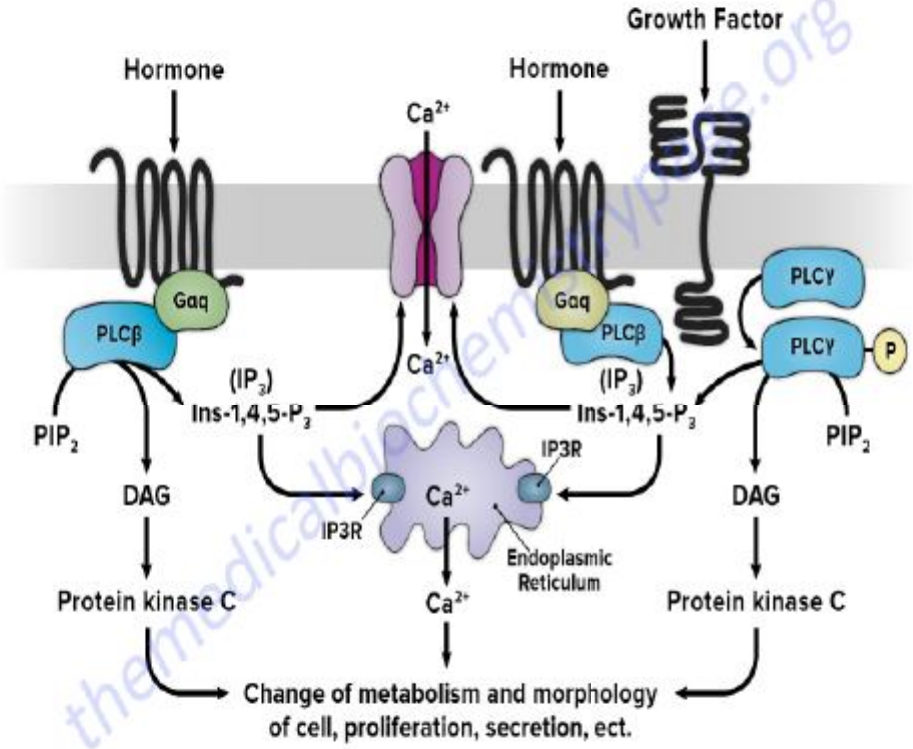

Fig-4.2 : Receptor-mediated Activation of PLC

There are 13 members of the PLC family of phospholipases with the PLCβ and PLCγ members being the most well characterized with respect to their role in signal transduction cascades. The PLCβ enzymes are activated by GPCRs coupled to G_q-type G-proteins while the PLCγ enzymes are activated with intrinsic tyrosine kinase activity or receptors that activate tyrosine kinases. Both pathways ultimately activate the kinase PKC, leading to numerous changes within the activated cell.

NOTES

Phospholipase A (PLA) Family

The PLA family of lipases consists of the PLA_1 and PLA_2 subfamilies. PLA_1 enzymes catalyze hydrolysis of fatty acids from the *sn*-1 position of glycerophospholipids generating 2-acyl-lysophospholipids and free fatty acids. PLA_2 enzymes catalyze hydrolysis of the *sn*-2 position of glycerophospholipids releasing free fatty acids and 1-acyl-lysophospholipids. PLA_1 activity belongs to the pancreatic lipase gene family. These enzymes include phosphatidylserine (PS)-specific PLA_1 (PS-PLA_1), two membrane-associated phosphatidic acid (PA)-selective PLA_1 (mPA-$PLA_1\alpha$ and mPA-$PLA_1\beta$), hepatic lipase (HL, encoded by the LIPC gene, also commonly called hepatic triglyceride lipase, HTGL), endothelial cell-derived lipase (EDL, encoded by the LIPG gene) and pancreatic lipase-related protein 2 (PLRP2). PS-PLA_1 preferentially hydrolyzes phosphatidylserine (PS) hence the naming of this enzyme. The products of PS-PLA_1 are a fatty acid and lysoPS. LysoPS has been implicated in several biological processes that include suppression of T-cell proliferation, activation of mast cells, induction of fibroblast and glioma cell chemotaxis, and the promotion of neurite outgrowth.

Phosphatidylinositol-3-Kinase (PI3K)

PI3K is a heterodimeric protein containing an 85 kDa and 110 kDa subunits.. The 85 kDa subunit is non-catalytic, however, it does contain a domain homologous to GTPase activating (GAP) proteins. It is the 110 kDa subunit that is enzymatically active. PI3K phosphorylates various phosphatidylinositols at the 3 position of the inositol ring. This activity generates additional substrates for PLCγ allowing a cascade of DAG and IP_3 to be generated by a single activated RTK or other protein tyrosine kinases.

Lysophospholipids

Lysophospholipids (LPLs) are minor lipid components compared to the major membrane phospholipids such as phosphatidylcholline (PC), phosphatidylethanolamine (PE), and sphingomyelin. The LPLs were originally presumed to be simple metabolic intermediates in the *de novo* biosynthesis of phospholipids. The most biologically significant LPLs are lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI),

sphingosine 1-phosphate (S1P), and sphingosylphosphorylcholine (SPC). Each of these LPLs functions via interaction with specific G-protein coupled receptors (GPCRs) leading to autocrine or paracrine effects. The first LPL receptor identified was called LPA_1because it bound LPA. The first GPCR shown to bind S1P was called $S1P_1$.

NOTES

Endothelial differentiation genes (EDGs) were found to be the same as LPL receptors. Thus LPA_1 is also known as EDG-2, LPA_2 as EDG-4, and LPA_3 as EDG-7. $S1P_1$ is also known as EDG-1, $S1P_2$ as EDG-5, $S1P_3$ as EDG-3, $S1P_4$ as EDG-6, and $S1P_5$ as EDG-8. Activation of the LPA receptors triggers several different downstream signaling cascades. These include activation of MAP kinase (MAPK), activation of PLC, Akt/PKB activation, calcium moblization, release of arachidonic acid, inhibition or activation of adenylate cyclase, and activation of several small GTPases such as Ras, Rho, and Rac. The LPs exert a wide-range of biochemical and physiological responses including platelet activation, smooth muscle contraction, cell growth, and fibroblast proliferation.

4.4 Role of Cyclic Nucleotides

A **cyclic nucleotide** (cNMP) is a single-phosphate nucleotide with a cyclic bond arrangement between the sugar and phosphate groups.Cyclic nucleotides are composed of three functional groups: a sugar, a nitrogenous base, and a single phosphate group. As can be seen in the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) images, the 'cyclic' portion consists of two bonds between the phosphate group and the 3' and 5'hydroxyl groups of the sugar, very often a ribose.Their biological significance includes a broad range of protein-ligand interactions. They have been identified as secondary messengers in both hormone and ion-channel signalling in eukaryotic cells, as well as allosteric effector compounds of DNA binding proteins in prokaryotic cells. cAMP and cGMP are currently the most well documented cyclic nucleotides, however there is evidence that cCMP (cytosine) is also involved in eukaryotic cellular messaging. The role of cyclic uridine monophosphate (cUMP) is even less well known.Discovery of cyclic nucleotides has contributed greatly to the understanding of kinase and phosphatase mechanisms, as well as protein regulation in general.

NOTES

Cyclic nucleotides are found in both prokaryotic and eukaryotic cells. Cyclic nucleotides are produced from the generic reaction NTP $\longrightarrow$ cNMP + PP_i, where N represents a nitrogenous base. The reaction is catalyzed by specific nucleotidyl cyclases, such that production of cAMP is catalyzed by adenylyl cyclase and production of cGMP is catalyzed by guanylyl cyclase.

Both cAMP and cGMP are degraded by hydrolysis of the 3' phosphodiester bond, resulting in a 5'NMP. Degradation is carried out primarily by a class of enzymes known as phosphodiesterases (PDEs). Some phosphodiesterases are cNMP-specific, while others are non-specific. However, the cAMP and cGMP degradation pathways are much more understood than those for either cCMP or cUMP. A highly conserved cyclic nucleotide binding domain (CNB) is present in all proteins that bind cNMPs, regardless of their biological function. The domain consists of a beta sandwich architecture, with the cyclic nucleotide binding pocket between the betasheets. The binding of cNMP causes a conformational change that affects the protein's activity

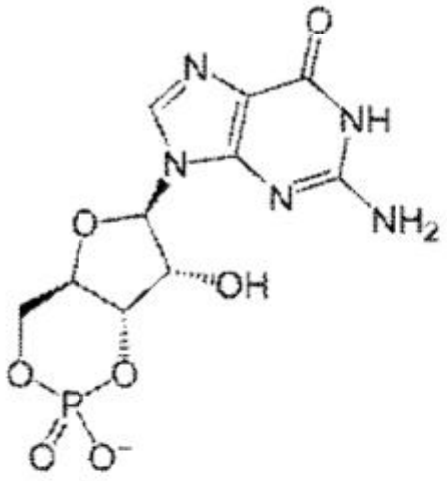

Fig 4.3 A Cyclic Nnucleotide

Cyclic guanosine monophosphate. The cyclic portion refers to the two single bonds between the phosphategroup and the ribose

Cyclic nucleotides are integral to a communication system that acts within cells. They act as "second messengers" by relaying the signals of many first messengers, such as hormones and neurotransmitters, to their physiological destinations. Cyclic nucleotides participate in many physiological responses,

including receptor-effector coupling, down-regulation of drug responsiveness, protein-kinase cascades, and transmembrane signal transduction. Cyclic nucleotides act as second messengers when first messengers, which cannot enter the cell, instead bind to receptors in the cellular membrane. The receptor changes conformation and transmits a signal that activates an enzyme in the cell membrane interior called adenylyl cyclase. This releases cAMP into the cell interior, where it stimulates a protein kinase called cyclic AMP-dependent protein kinase. By phosphorylating proteins, cyclic AMP-dependent protein kinase alters protein activity. cAMP's role in this process terminates upon hydrolysis to AMP by phosphodiesterase.

NOTES

Table-1 Biological Role of Cyclic Nucleotide

S.No.	Cyclic Nucleotide	Known binding proteins	Pathway/ Biological association
1.	cAMP	protein kinase A cyclic nucleotide-gated ion channels Epac Catabolite Activator Protein (CAP)	smooth muscle relaxation photo/olfactory receptors glucagon production in pancreatic beta cells lac operon regulation in E. coli
2.	cGMP	cGMP-dependent protein kinase (PKG) cyclic nucleotide-gated ion channels	smooth muscle relaxation photo/olfactory receptors
3.	cCMP	cGMP kinase I protein kinase A	smooth muscle relaxation

Cyclic nucleotides are well-suited to act as second messengers for several reasons. Their synthesis is energetically favorable, and they are derived from common metabolic components (ATP and GTP). When they break down into AMP/GMP and inorganic phosphate, these components are non-toxic. Finally, cyclic nucleotides can be distinguished from non-cyclic nucleotides because they are smaller and less polar.

4.5 Calcium- Calmodulin Cascade

NOTES

Calmodulin the predominant calcium receptor, is one of the best-characterized calcium sensors in eukaryotes.In recent years, advances in functional genomics have helped to identify and characterize numerous calmodulin-binding proteins in plants. Some of these proteins are likely to act as 'hubs'during calcium signal transduction. Hence, a better understanding of the function of these calmodulin target proteins should help in deciphering the Ca21/calmodulin-mediated signal network and its role in plant growth, development and response to environmental stimuli.

Types

There are two types of CaM kinase:

a)Specialized CaM kinases

Specialized CaM kinases such as the myosin light chain kinase that phosphorylates myosin, causing smoothmuscles to contract.

b)Multifunctional CaM kinases

Multifunctional CaM kinases also collectively called CaM kinase II, which play a role in neurotransmitter secretion,transcription factor regulation,and glycogen metabolism. Itis a serine/threonine-specific protein kinase that is regulated by the Ca2+/calmodulin complex.

Structural Domain

All of the isoforms of CaMKII have: a catalytic domain, an autoinhibitory domain, a variable segment, and a self-association domain. The catalytic domain has several binding sites for ATP and is responsible for the transfer of phosphate from ATP to Ser or Thr residues in substrates. The autoinhibitory domain features a pseudosubstrate site, which binds to the catalytic domain and blocks its ability to phosphorylate proteins. The structural feature that governs this autoinhibition is the Threonine 286 residue. Phosphorylation of this site will permanently activate the CaMKII enzyme. Once the Threonine 286 residue has been phosphorylated, the inhibitory domain is blocked from the pseudosubstrate site. This effectively blocks autoinhibition, allowing for permanent activation of the CaMKII enzyme. This enables CamKII to be

active, even in the absence of calcium and calmodulin.The other two domains in CaMKII are the variable and self-association domains. Differences in these domains contribute to the various CaMKII isoforms. The self-association domain (CaMKII AD) is found at the C terminus, the function of this domain is the assembly of the single proteins into large (8 to 14 subunits) multimers.

NOTES

Calcium and calmodulin dependence

The sensitivity of the CaMKII enzyme to calcium and calmodulin is governed by the variable and self-associative domains. Initially, the enzyme is activated; however, autophosphorylation does not occur because there is not enough Calcium or calmodulin present to bind to neighboring subunits.

As greater amounts of calcium and calmodulin accumulate, autophosphorylation occurs leading to persistent activation of the CaMKII enzyme for a short period of time.. Autophosphorylation is the process in which a kinase attaches a phosphate group to itself. However, the Threonine 286 residue eventually becomes dephosphorylated, leading to inactivation of CaMKII when CaMKII autophosphorylates, it becomes persistently active. Phosphorylation of the Threonine 286 site allows for the activation of the catalytic domain.

Currently, three CaM-activation mechanisms have been observed in animal systems. The first activation mechanism is relieving autoinhibition: the CaM binding site is adjacent to or within an autoinhibitory domain of the enzyme such as the CaM kinases. CaM binding to the target induces a conformational rearrangement that displaces the pseudosubstrate inhibitory domain and allows full enzyme activity. The second activation mechanism is active site remodeling: in the case of activation of anthrax adenylyl cyclase (oedema factor), four discrete regions of the oedema factor form a surface that recognizes an extended conformation of CaM. Upon CaM binding, a helical domain of the oedema factor undergoes a 308 rotation away from the catalytic core, which stabilizes a disordered loop and leads to enzyme activation. The third activation mechanism is CaMinduced dimerization: two CaM molecules tightly interact with two Kþ channel domains of the Ca2þ-activated potassium channels upon Ca2þ-binding. The C-terminal EF hands mediate tethering to the

channel and the N-terminal EF hands are responsible for Ca2þ-induced dimerization leading to channel gating.

Recently, it has been reported that a single CaM molecule interacts with two peptides derived from the C-terminal CaMbinding domain of petunia glutamate decarboxylase (GAD). This provides evidence of the conformational flexibility of plant CaMs. This is understandable because plants are sessile organisms and must therefore adapt to a changing environment to survive.

NOTES

4.5 Diversity in Protein Kinases and Phosphatases

A protein kinase transfers the terminal phosphate of ATP to a hydroxyl group on a protein.A protein phosphatase catalyzes removal of the phosphate by hydrolysis.

Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:1.Some protein kinases are activated by Ca^{++}-calmodulin.

2.Protein Kinase A is activated by cyclic-AMP (cAMP).

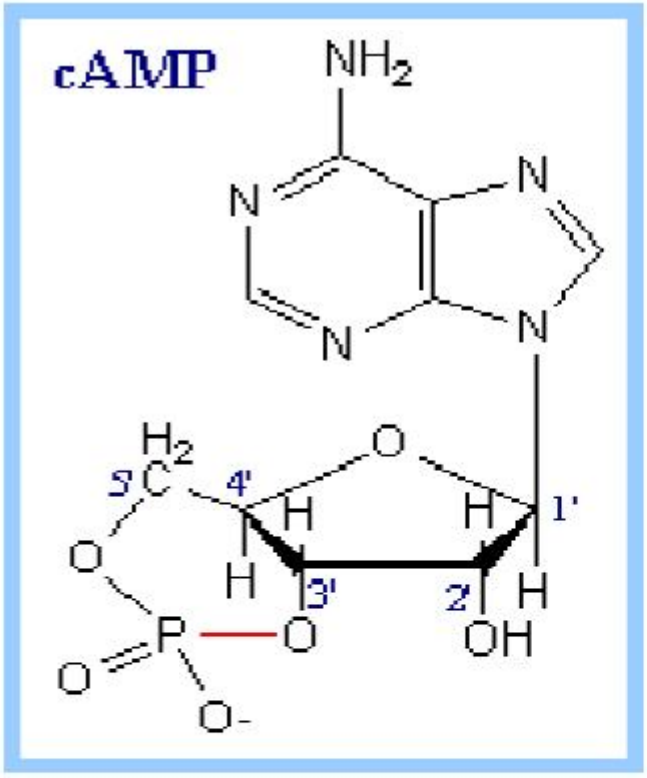

Fig. 4.4 cAMP Structure

AdenylateCyclase (AdenylylCyclase) catalyzes: **ATP: cAMP + PP_i**

Binding of certain **hormones** (e.g., epinephrine) to the outer surface of a cell activates Adenylate Cyclase to form cAMP within the cell. Cyclic AMP is thus considered to be a **second messenger**.

NOTES

Phosphodiesterase enzymes catalyze: **cAMP + H_2O: AMP**

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A. Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.

Protein Kinase A exists in the resting state as a complex of:

2 regulatory subunits (R)

2 catalytic subunits (C)

Each regulatory subunit **(R)** of Protein Kinase A contains a **pseudosubstrate** sequence omparable to the substrate domain of a target protein for Protein Kinase A, but with alanine substituting for the serine or threonine. The pseudosubstrate domain of the regulatory subunit, which **lacks a hydroxyl** that can be phosphorylated, binds to the active site of the catalytic subunit, blocking its activity.

When each regulatory subunit binds 2 cAMP, a conformational change causes the regulatory subunits to release the catalytic subunits. The catalytic subunits (C) can then catalyze phosphorylation of serine or threonine residues on target proteins.

R2C2 + 4 cAMP R2cAMP4 + 2 C

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (**C**).

Signal amplification is an important feature of signal cascades.

One hormone molecule can lead to formation of many cAMP molecules.

Each catalytic subunit of Protein Kinase A catalyzes phosphorylation of many proteins during the life-time of the cAMP.

Different **isoforms of G_α** have different signal roles. For example: While the **stimulatory $G_{s\alpha}$**, when it binds GTP, **activates** Adenylate Cyclase, an

inhibitory$G_{i\alpha}$, when it binds GTP, **inhibits** Adenylate Cyclase. Different effectors and their receptors induce $G_{i\alpha}$ to exchange GDP for GTP than those that activate $G_{s\alpha}$

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The complex of **G$\beta\gamma$** that is released when $G_{\square}$ binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins. For example, **G $\beta\gamma$** inhibits one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme. **Protein Kinase B** (also called **Akt**) becomes activated when it is recruited from the cytosol to the plasma membrane surface by **binding** to products of PI-3 Kinase, such as **PI-3,4,5-P_3**. Other kinases at the cytosolic surface of the plasma membrane then catalyze phosphorylation of Protein Kinase B, activating it.The activated **Protein Kinase B** catalyzes **phosphorylation** of serine or threonine residues of many proteins, with diverse effects on metabolism, cell growth, and apoptosis. **Downstream metabolic effects** of Protein Kinase B activity include stimulation of glycogen synthesis, stimulation of glycolysis, and inhibition of gluconeogenesis.

Protein kinases are targetted by pharmaceuticals because PKs play a variety of roles in disease states. **Kinase inhibitors** bind to the kinase in at least four different binding modes:

(1) Direct competition with ATP at the ATP binding site;
(2) Engagement of an adjacent allosteric binding site in the ATP pocket, which is usually accessible when the activation loop is in the inactive conformation; and
(3) binding at sites remote from the ATP site (but still close to the ATP) that impact kinaseactivity;

(4) Binding outside of the ATP binding pocket (truly allosteric). Kinases can escape inhibition by mutating key residues in their catalytic domain, thus becoming resistant to the kinase inhibitors Removal of the incorporated phosphates must be a necessary event in order to turn off the proliferative signals.

This suggests that phosphatases may function as anti-oncogenes or growth suppressor genes. The loss of a functional phosphatase involved in regulating

growth promoting signals could lead to neoplasia. There are two broad classes of protein tyrosine phosphatases (PTPs). One class is transmembrane enzymes which contain the phosphatase activity domain in the intracellular portion of the protein. This class of PTP is commonly called the receptor (R) class of PTP. The other class is intracellularly localized enzymes and are referred to as NT PTPs (for non-transmembrane). Currently over 40 genes have been characterized as encoding one or the other class of PTP. The first transmembrane PTP characterized was the leukocyte common antigen protein, CD45. This protein was shown to have homology to the intracellular PTP, PTP1B. There are at least eight sub-classes of the transmembrane PTPs and ten sub-classes of the NT PTPs.The clearest studies of a role for transmembrane PTPs in signal transduction have involved the CD45 protein. These studies have shown that CD45 is involved in the regulation of the tyrosine kinase activity of LCK in T cells. As indicated above LCK is associated with T cell antigens CD4 and CD8 generating a split-RTK involved in T cell activation. It is suspected that CD45 dephosphorylates a regulatory tyrosine phosphorylation site in the C-terminus of LCK, thereby, increasing the activity of LCK towards its substrate(s).

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The second class of PTPs is the intracellular proteins. The C-terminal residues of most if not all intracellular PTPs are very hydrophobic and suggest these sites are membrane attachment domains of these proteins. One role of intracellular PTPs is in the maturation of *Xenopus* oocytes in response to hormone.

Over expression of PTP1B in oocytes resulted in a marked retardation in the rate of insulin- and progesterone-induced maturation. These results suggest a role for PTP1B in countering the signals leading to cellular activation other phosphatases that recognize serine and/or threonine phosphorylated proteins also exist in cells. These are referred to as protein serine phosphatases (PSPs).

The PSPs are grouped into three major families: phosphoprotein phosphatases (PPPs), metal-dependent protein phosphatases (PPMs), and the aspartate-based phosphatases represented by FCP/SCP. This latter family name is derived from transcription factor IIF (TFIIF)-associating component of RNA polymerase II C-terminal domain (CTD) phosphatase/small CTD phosphatase.The broad

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spectrum of activity associated with the members of the PPP family stems from the ability of the catalytic subunit to associate with a large variety of different regulatory subunits. The representative members of the PPP family include protein phosphatase 1 (PP1), PP2A, PP2B (commonly known as calcineurin), PP4, PP5, PP6, and PP7.The signaling pathway also includes a phosphatase that dephosphorylates the response regulator, returning it to a responsive state. The phosphatase may be the histidine kinase, the response regulator, or a separate protein.

Histidine kinases are often located in the membrane, though they may be found in the cytoplasm. Response regulators are located in the cytoplasm. The histidine kinase need not be the first protein in the signal transduction pathway to respond to the signal. In many systems, signals first interact with protein proteins other than the histidine kinase, then the stimulus is relayed to the histidine kinase.

Most of the known phosphorylated response regulators stimulate or repress thetranscription of specific targetted genes. (Exceptions to this include P-CheB and P-CheY, which affect the chemotaxis machinery).The rate at which the aspartyl-phosphate is released as inorganic phosphate – returning the response regulator to its basal state – is fine tuned to meet the needs of the specific regulation system. Thus, half lives of the phospho-intermediate vary from seconds to hours.

The two domains, transmitter and receiver, are usually found in separate polypeptides. A single transmitter may communicate with more than one receiver domain, and rarely, a single receiver domain may become phosphorylated by more than one transmitter domain. Phosphorylation may even be achieved by metabolic organic phosphates such as acetyl-phosphate or carbamoyl-phosphate.Most gene regulation proteins are single proteins, often homodimers or homotetramers, which bound to two ligands: a. a metabolic intermediate, and b. a cis-acting gene regulation element.

4.7 Specific Signaling Mechanisms

Two-component systems

The **two-component signaling system** comprises a histidine kinase protein that receives a signal and transmits it, via phosphorelay, to a partner response regulator protein. Such systems are found within all kingdoms of life, and more than 500 two component signal transduction systems have been identified. *E. coli* contains more than 30 two-component systems, controlling various aspects of cellular physiology.

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The two components of these signaling systems are:

1. a sensor, and
2. a response regulator.

1. The first (sensory) component is called the **transmitter domain.** This and is a kinase function that phosphorylates a histidine usually located in the same protein, and so is considered an autokinase. The transmitter domain becomes a substrate for dephosphorylation by one or more "second" components.
2. The second (response regulator) component is called the **receiver domain.** This is a phosphatase that removes the histidyl-phosphate from the sensor by a mechanism that involves an aspartyl-phosphate intermediate in the receiver domain. The phospho-intermediate of the receiver domain induces a conformational change that regulates the functional state of an output domain, which is usually covalently linked to the receiver domain.

In response to a signal, the histidine kinase of transmitter domain autophosphorylates (employing ATP as the phosphoryl donor) a histidine residue in the carboxyl-terminal region of receiver domain (comprising approximately 240 amino acids), and then transfers the phosphoryl group to an aspartate residue in the amino -terminal region (comprising about 120 amino acids) of the partner response regulator protein.

Thus activated, the response regulator transmits the signal to its target. The signaling pathway also includes a phosphatase that dephosphorylates the response regulator, returning it to a responsive state. The phosphatase may be the histidine kinase, the response regulator, or a separate protein.Histidine kinases are often located in the membrane, though they may be found in the cytoplasm. Response regulators are located in the cytoplasm. The histidine

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kinase need not be the first protein in the signal transduction pathway to respond to the signal. In many systems, signals first interact with protein proteins other than the histidine kinase, then the stimulus is relayed to the histidine kinase. Most of the known phosphorylated response regulators stimulate or repress thetranscription of specific targetted genes. (Exceptions to this include P-CheB and P-CheY, which affect the chemotaxis machinery). The rate at which the aspartyl-phosphate is released as inorganic phosphate – returning the response regulator to its basal state – is fine tuned to meet the needs of the specific regulation system. Thus, half lives of the phospho-intermediate vary from seconds to hours.The two domains, transmitter and receiver, are usually found in separate polypeptides. A single transmitter may communicate with more than one receiver domain, and rarely, a single receiver domain may become phosphorylated by more than one transmitter domain. Phosphorylation may even be achieved by metabolic organic phosphates such as acetyl-phosphate or carbamoyl-phosphate.Most gene regulation proteins are single proteins, often homodimers or homotetramers, which bound to two ligands: a. a metabolic intermediate, and b. a cis-acting gene regulation element.

Physiological Functions :

Two-component systems regulate diverse responses including

a) nutrient acquisition : nitrogen, phosphorus, carbon

b) energy metabolism : electron transport systems, uptake and catabolic machinery

c) adaptation to physical or chemical aspects of the environment : chemotaxis, pH, osmolarity, light quality

d) complex developmental pathways : sporulation, fruiting body development, swarmer cell production

e) virulence : plasmid transfer (conjugation), degredative secretions, toxin production

Sensor regulator system in bacteria

Some eukaryotic two-component systems with more than one protein component:

DctB/DctD - dicarboxylate transport in *Rhizobium leguminosarum*

EnvZ/OmpR - osmoregulation in *E. coli*
NtrB/NtrC - nitrogen assimilation in a variety of bacteria
PhoR/PhoB - phosphate scavenging in *E. coli*
VirA/VirG - virulence by *Agrobacterium tumefaciens*
A 125 amino acid peptide segment is "conserved" in one subset of these gene products: OmpR, PhoB, NtrC, DctD, VirG
A "homologous" segment is present in these regulatory proteins:
Spo0A - sporulation
Spo0F - sporulation
CheY - chemotaxis
CheB – chemotaxis

A second, but different, "homologous" segment is present in these proteins: EnvZ, PhoR, NtrB, DctB, VirA, and probably CheA - chemotaxis in enteric bacteria (Che system).
NRII protein –bifunctional kinase/phosphatase regulated by PII – phosphorylates and dephosphorylates NRI, and controls the rate of transcription initiation from nitrogen-regulatedpromoters.
Escherichia coli BarA-UvrY two-component system is needed for efficient switching between glycolytic and gluconeogenic carbon sources.

***H. pylori* two-component systems**: HP0703-HP0244 is involved in flagellar regulation; HP0166-HP0165 activates the transcription of H. pylori-specific genes in response to environmental stimuli; a set of essential target genes is regulated by HP0166. The expression of the HP0166-HP0165 two-component system is tightly balanced by a negative autoregulatory mechanism exerted by the phosphorylated response regulator.Cyanobacterial phytochrome Cph is a light-regulated histidine kinase that mediates red, far-red reversible phosphorylation of a small response regulator, Rcp1 (response regulator for cyanobacterial phytochrome), encoded by the adjacent gene, thus implicating protein phosphorylation-dephosphorylation in the initial step of light signal transduction by phytochrome. Protein kinases act as regulator switches and modify their target proteins by adding a phosphate group to them. This process, called 'phosphorylation,' results in altered activity of the phosphorylated protein. It is estimated that 30% of all proteins are regulated by this process.

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Sucrose sensing mechanism

In addition to their essential roles as substrates in carbon and energy metabolism and in polymer biosynthesis, sugars have important hormone-like functions as primary messengers in signal transduction. The pivotal role of sugars as signaling molecules is well illustrated by the variety of sugarsensing and signaling mechanisms discovered in free-living microorganisms such as bacteria and yeast. For such unicellular organisms, nutrient availability is the main extracellular factor controlling growth and metabolism. In plants, sugar production through photosynthesis is a vital process, and sugar status modulates and coordinates internal regulators and environmental cues that govern growth and development. Although the regulatory effect of sugars on photosynthetic activity and plant metabolism has long been recognized, the concept of sugars as central signaling molecules is relatively novel. Recent progress has begun to reveal the molecular mechanisms underlying sugar sensing and signaling in plants, including the demonstration of hexokinase (HXK) as a Glc sensor that modulates gene expression and multiple plant hormone-signaling pathways. Analyses of HXK mutants will provide new evidence for distinct signaling and metabolic activities. Diverse roles of Snf1-related protein kinases (SnRKs) in carbon metabolism and sugar signaling also are emerging.

Sugar Signals in Plants

Sugar regulation is necessarily far more complex in plants. First, multicellular organisms need both long-distance and tissue- or even cell-type-specific signaling mechanisms and coordination with both development and physiological and environmental changes. As autotrophic, photosynthetic organisms,plants are made up of sugar exporting (source)and sugar importing (sink) tissues and organs,and sugar signals are generated from different sources at different locations Suc is transported from photosynthesizing source leaves to sink organs such as roots, meristems, young leaves, flowers, fruit, and developing seed. Lowered (L) sugar levels can increase source activities, including photosynthesis, nutrient mobilization, and export. In contrast, higher (H) sugar levels in sink tissues stimulate growth and storage. Accumulation of higher (H) sugar levels in source tissues, however, is believed to downregulate

photosynthesis, ensuring the maintenance of sugar homeostasis. The differential source-sink effects allow the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients. In photosynthetic (source) cells photosynthate generated in the Calvin cycle is exported, mainly as triose-phosphates, from the chloroplast to the cytosol, where it is used in glycolysis or converted to sucrose for local use or export to sink tissues. Net export or import of sucrose depends on the source or sink status of the leaf cells.

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Excess photosynthate is transiently stored as starch in the chloroplast during the day. ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch synthesis, is highly regulated by sugars. A major source for glucose signals is transitory starch breakdown from chloroplasts in leaf cells during the night (mainly via maltose and glucose export and from plastids (amyloplasts) in starch-storing organs .In sink tissues sucrose can be imported into cells through plasmodesmata(symplastic transport) or the cell wall (apoplastic transport). Intracellular sucrose is cleaved by cytoplasmic INV (C-INV), generatingglucose and fructose, or by sucrose synthase (SUS) producing fructose and UDPglucose. Sucrose can also be imported and stored in the vacuole, and vacuolar INV (V-INV) is a major intracellular source of hexoses in expanding tissues. In the apoplast, extracellular sucrose is hydrolysed by CWINV, a major driving force in sugar unloading and gradient maintenance and therefore sinks strength. These enzymes generate high levels of extracellular glucose and fructose that are taken up by hexose transporters, which are coexpressed and coordinatedly regulated withCW-INV (cell wall Invertase) It is clear that sucrose transport and hydrolysis play key regulatory roles in carbon allocation and sugar signal generation. The extensive feedback regulation of the INVs and SUS by sugar signaling generates a very sensitive self-regulatory system

a)

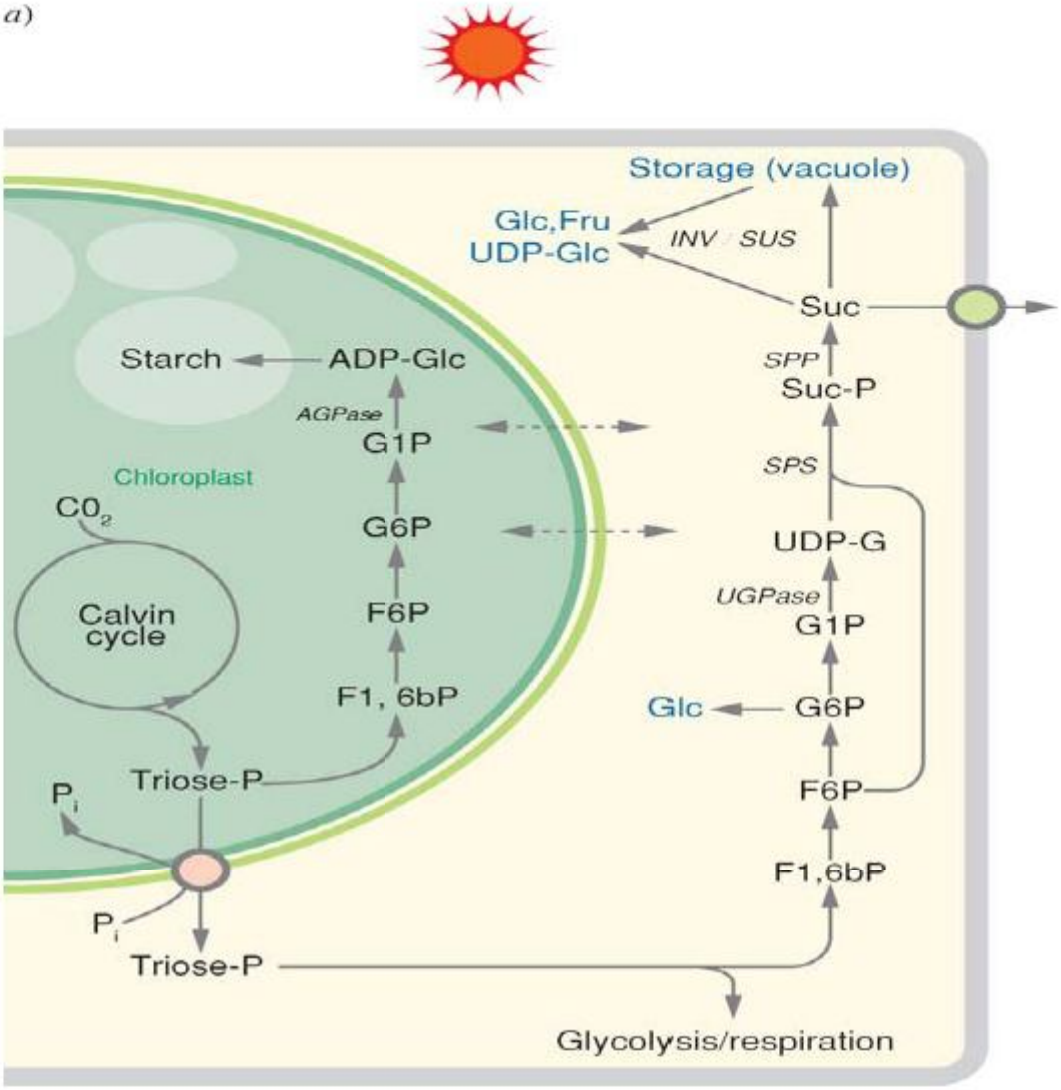

Fig 4.2 : Sugar Signals in Source and Sink cells.

Simplified model of the major carbon fluxes and sugar signal generation by photosynthesis, transport and hydrolysis in photosynthetic source cells represented in Fig. 4.2.

SPS, sucrose-P synthase; SPP, sucrose-P phosphatase; AGPase, ADP-glc pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; INV, invertase; C-INV, cytosolic INV; CW-INV, cell wall INV; V-INV, vacuolar INV; SUS, sucrose synthase

T6P: trehalose-6-P

TPS: trehalose-6-P .

In general, source activities like photosynthesis, nutrient mobilization, and export are upregulated under low sugar conditions, Synthase whereas sink activities like growth and storage are upregulated when carbon sources are abundantly available. Photosynthesis and sink demand need to be rigorously coordinated, and this coordination involves both metabolic (substrate and allosteric) regulation and specific sugar-signaling mechanisms. Although sucrose is the major photosynthetic product and transport sugar in plants, many sugarsignaling effects on growth and metabolism

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Model of sugar-sensing mechanisms in plants

(*a*) The HXK1 glucose sensor is mainly associated with mitochondria, possibly as part of a glycolytic metabolon. In addition, HXK1 is found in high-molecular-weight complexes in the nucleus where it controls transcription and proteasome-mediated degradation of the EIN3 TF. Other HXK and HKL proteins are also associated with the outer membrane of plastids, including chloroplasts, or cytosol. HXK can also be found in the chloroplast stroma.

(*b*) Sucrose (and other disaccharides) appears to be sensed at the plasma membrane, possibly by transporter homologs. Monosaccharide transporters might have similar functions as membrane sensors.

(*c*) G-protein coupled receptor signaling by RGS1 and GPA1 is involved in glucose control of seed germination and seedling development, possibly in a hexokinase-independent way.

(*d*) SnRK1 proteins play an important role in plant sugar and starvation signaling, although the significance of the regulation of these proteins by sucrose (*Suc*) and G6P is still unclear.

(*e*) Important regulatory effects are reported for trehalose (*Tre*) and T6P, apparently downstream of SnRK1.

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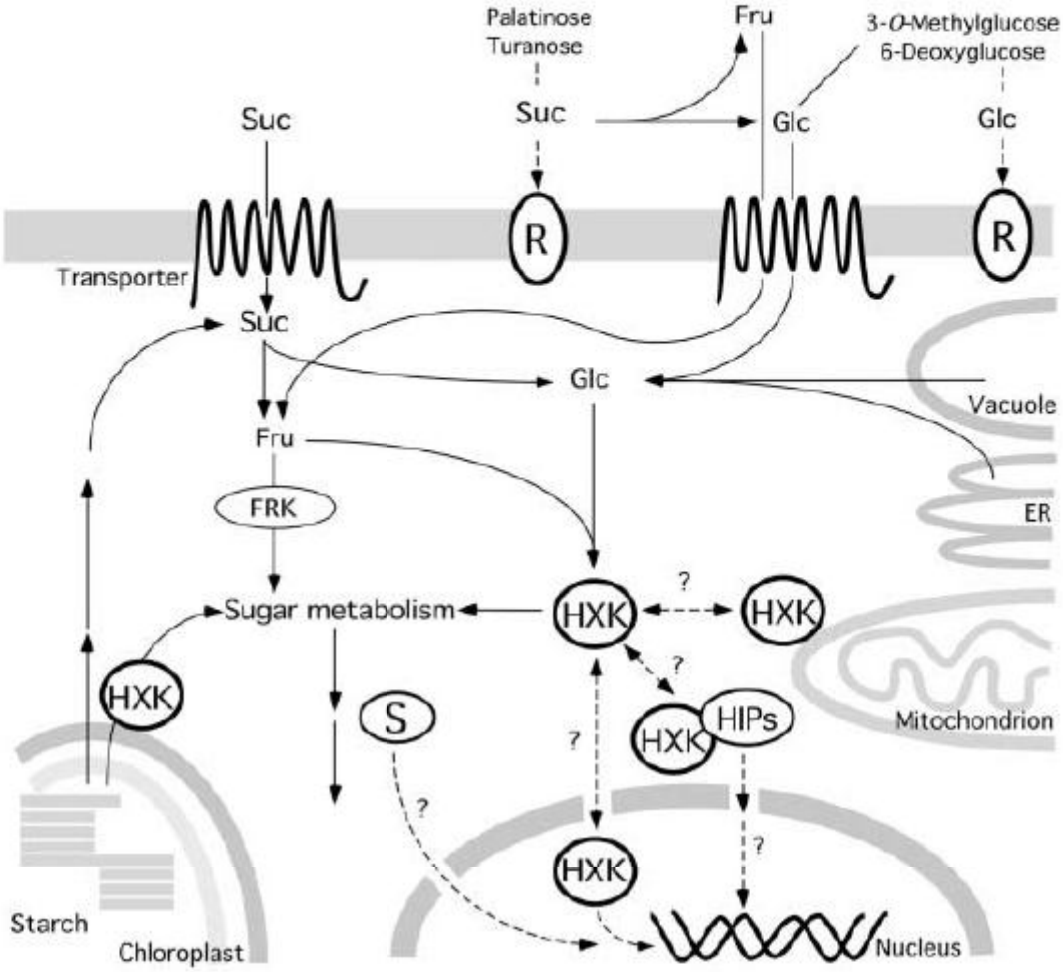

Fig 4.3 : Possible Sugar Signals and Sensing Sites in Plant Cells

In the nucleus, several types of transcription factors are involved in sugar-regulated transcription Glc (and Fru) can be transported into the cell by hexose transporters or mobilized from cytosolic and vacuolar Suc and plastid starch. Glc then enters metabolism after HXK-catalyzed phosphorylation.

The HXK sugar sensor, as a cytosolic protein or associated with mitochondria or other organelles, then could activate a signaling cascade through HXK-interacting proteins (HIPs) or affect transcription directly after nuclear translocation. Possibly, different HXK (and fructokinase [FRK]) isoforms and HXK-like proteins have distinct metabolic and signaling functions.

Metabolic intermediates could trigger signal transduction by activating metabolite sensors (S). Negative regulation of SnRK activity by Glc-6-phosphate, for example, suggests that SnRKs might act as sensors of metabolic activity. Finally, sugars, including Suc and hexoses and nonmetabolizable

sugars and sugar analogs, also could be sensed at the plasma membrane by sugar transporters or transporter-like proteins or by specific sugar receptors (R). Solid lines represent transport and enzymatic reactions involved in sugar sensing and signaling, and dashed lines represent putative interactions and translocations. ER, endoplasmic reticulum.

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4.8 Summary

Signal transduction occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response.Depending on the cell; the response alters the cell's metabolism, shape, gene expression, or ability to divide. The signal can be amplified at any step. Thus, one signaling molecule can cause many responses. Signal transduction involves the binding of extracellular *signalling molecules* and ligands to cell-surface receptors that trigger events inside the cell.

The combination of messenger with receptor causes a change in the conformation of the receptor, known as *receptor activation*. This activation is always the initial step (the cause) leading to the cell's ultimate responses (effect) to the messenger.Intracellular signaling cascades can be started through cell-substratum interactions; examples are the integrin that binds ligands in the extracellular matrix and steroids. Most steroid hormones have receptors within the cytoplasm and act by stimulating the binding of their receptors to the promoter region of steroid-responsive genes.

Examples of signaling molecules include the hormone melatonin; the neurotransmitter acetylcholine and the cytokine interferon γ with single-celled organisms, the variety of signal transduction processes influence its reaction to its environment. With multicellular organisms, numerous processes are required for coordinating individual cells to support the organism as a whole; the Extracellular receptors are integral transmembrane proteins and make up most receptors. G protein-coupled receptors (GPCRs) are a family of integral transmembrane proteins that possess seven transmembrane domains and are linked to a heterotrimeric G protein.

Many receptors are in this family, including adrenergic receptors andchemokine receptors. Intracellular receptors, such as nuclear receptors and cytoplasmic receptors, are soluble proteins localized within their respective areas.

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Thetypical ligands for nuclear receptors are lipophilic hormones like the steroid hormones testosteroneand progesterone and derivatives of vitamins A and D. To initiate signal transduction, the ligand must pass through the plasma membrane by passive diffusion. On binding with the receptor, the ligands pass through the nuclear membrane into the nucleus, enabling gene transcription and protein production.

First messengers are the intercellular chemical messengers (hormones, neurotransmitters, and paracrine/autocrine agents) that reach the cell from the extracellular fluid and bind to their specific receptors. Second messengers are the substances that enter the cytoplasm and act within the cell to trigger a response. In essence, second messengers serve as chemical relays from the plasma membrane to the cytoplasm, thus carrying out intracellular signal transduction.

Principles of Signal Transduction: An environmental signal, such as a hormone, is first received by a cell-surface receptor. *Membrane receptors transfer information from the environment to the cell's interior. the information that signal molecules are present must be transmitted across the cell membrane without the molecules themselves entering the cell.* A membrane-associated receptor protein often performs the function of information transfer across the membrane.

Such a receptor is an intrinsic membrane protein that has both extracellular and intracellular domains.A binding site on the extracellular domain specifically recognizes the signal molecule (often referred to as the *ligand.*The information embodied by the presence of the ligand, often called the*primary messenger,* must be transduced into other forms that can alter the biochemistry of the cell.

Second messengers relay information from the receptor-ligand complex.

Changes in the concentration of small molecules, called *second messengers,* constitute the next step in the molecular information circuit. Particularly important second messengers include cyclic AMP and cyclic GMP, calcium ion, inositol 1,4,5-trisphosphate, (IP_3), and diacylglycerol (DAG *Protein phosphorylation is a common means of information transfer.* Many second messengers elicit responses by activating *protein kinases.* These enzymes transfer phosphoryl groups from ATPto specific serine, threonine, and tyrosine residues in proteins. , protein phosphorylation is not irreversible. Indeed,

protein phosphatases are enzymes that hydrolytically remove specific phosphoryl groups from modified proteins.

The signal is terminated. Protein phosphatases are one mechanism for the termination of a signaling process.

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4.9 Glossary

- **Ligands :** A binding site on the extracellular domain specifically recognizes the signal molecule often referred to as the *ligand.* Chemical signals **(ligands)** include :
  1. neurotransmitters : acetylcholine, dopamine, epinephrine, GABA, gly cine, norepinephrine,serotonin (5HT), etc.
  2. hormones
  3. phospholipids
  4.growthfactors.
  5. nutrients
- **Protein-coupled receptors :** (GPCRs) are a family of integral transmembrane proteins that possess seven transmembrane domains and are linked to a heterotrimeric G protein. Many receptors are in this family, including adrenergic receptors andchemokine receptors.
- **First Messengers :** First messengers are the intercellular chemical messengers (hormones, neurotransmitters, and paracrine/autocrine agents) that reach the cell from the extracellular fluid and bind to their specific receptors.
- **Second messengers :** Second messengers are the substances that enter the cytoplasm and act within the cell to trigger a response. In essence, second messengers serve as chemical relays from the plasma membrane to the cytoplasm, thus carrying out intracellular signal transduction.
- **cyclic nucleotide :** (cNMP) is a single-phosphate nucleotide with a cyclic bond arrangement between the sugar and phosphate groups.
- **Kinase** : A protein kinase transfers the terminal phosphate of ATP to a hydroxyl group on a protein.
- **Phosphatase :** A protein phosphatase catalyzes removal of the phosphate by hydrolysis.

4.10 Self -Learning Exercise

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Section A (Very Short Answer Type Question)

1. Define G-proteins?
2. Define protein kinases and phosphatases?
3. Define cyclicnucleotides?

Section B (Short Answer Type Questions)

1. Write the difference between extracellular and intracellular recepters?
2. Mention the importance of Calcium- Calmodulin cascade?
3. Define two component sensor regulator systems in bacteria?

Section C (Long Answer Type Questions)

1. Explain in detail the mechanism of signal transduction?
2. Write a note on diversity in protein kinases and phosphotases?
3. Discuss sucrose sensing mechanism?

4.12 References

- Sugar sensing and signaling in plants-Rolland and Sheen
- Lewin B.,GENE VII, oxford University press, 2004
- Calmodulin mediated signal network in plants-yang

Unit –5

Photochemistry and Photosynthesis-I

NOTES

Structure of the Unit

5.0 Objective

After studying this unit you should be able to understand the-

- Mechanism of conversion of solar energy into chemical energy.
- Basic structure and functional organization of chloroplast, grana, thylakoids & quantasome.
- Photosynthesis process as dark reaction and light reaction
- Important intermediate compounds and enzymes involved in the process.
- About electron transport chain and its work.
- Role of water as reducing agent

5.1 General concepts and Historical background

Life on earth would be impossible without photosynthesis.Every oxygen atom in the air we breathe was once part of a water molecule, liberated by

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photosynthesis.The energy released by the burning of coal, firewood, gasoline, and natural gas, and by our bodies' burning of all the food we eat all, directly or indirectly, has been captured from sunlight by photosynthesis. It is vitally important that we understand photosynthesis.

The sole source of energy on this earth is the Sun, where from the energy is received in the form of light and heat. The ability to convert this light energy into chemical energy by living system is an important phenomenon.

The process of trapping this light energy and ultimately fixing it into carbon bond energy (the energy of carbohydrates) is known as photosynthesis.

Photosynthesis can be defined as,"*formation of carbon containing compounds from carbon di oxide and water by green cells,water and oxygen will be by – product"*

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

Photosynthesis proceeds in two parts; first, the light trapping where the light energy is trapped by chlorophyll molecules, transferred into ATP and reducing power of the system in the form of NADPH and the second, where the carbon of carbon dioxide is reduced to carbohydrates(i.e. fixation of carbon). The energy for the carbon bond formation or we can say fixation of carbon comes from the ATP and the hydrogen for reducing it comes from NADPH (both generated in first part of photosynthesis).

Thus, the whole process of photosynthesis could be divided into two distinct processes, the first which is involved in absorption of light and synthesis of ATP and NADPH (it is a light requiring/dependent step) and the second involving reduction of CO_2 to carbohydrates (it is light independent and can easily proceed even in the absence of light if ATP and NADPH are available). These two processes are, therefore, often referred to as:

(1) The Light reaction,

(2) The Dark reaction/Light independent reaction.

Historical background

Sir Isac **Newton** (1727) believed that green plants get some part of their nourishment through their leaves and sun-light had some role to play.

About 50 year later Jan lngeahousz in 1779 suggested that light converted CO_2 into oxygen and that CO_2 taken by the plans through the plants.

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Senebier J. (1782) first reported that the CO_2 was taken by the leaves from atmosphere and not through the roots as suggested by lngenhousz.

Robert Mayer (1843-48) proclaimed the idea at organic synthesis suggesting that CO_2 + water + light energy was converted to organic matter + oxygen + chemical energy by plants.

The source of oxygen had remained debated till Hill first demonstrated that it came from water.

Engelmann T.W. (1880) was the first to have performed some interesting experiments and concluded that the process occurred within the chloroplast. He also studied absorption spectra and said that the reaction responded only to the blue and red light.

The term photosynthesis was first proposed by Barnes C. in 1893. He also proposed alternative term photosyntax' and preferred to use this but the term photosynthesis was later accepted by all.

In 1905, F.F. **Blackman** (Blackman's law of limiting factor fame) and G. Matthaei observed that light induced reaction was independent of temperature.

This information and some more related findings made Otto Warburg *(1925)* to suggest that the process of photosynthesis comprised of the following two reactions: (i) Light reaction, (ii) Dark reaction.

1939 **Kamen** and **S. Ruben** and co-workers established the decomposition of H_2O and the resulting liberation of O_2 by using $^{18}O_2$. Furthermore, they were the first to use the tracer technology for work with $^{14}CO_2$

Studies on light reaction progressed with the experiments of R. **Hill** (1937-39). He could demonstrate that oxygen comes from water and not from CO_2 because oxygen could be released even in absence of CO_2. He also reported that isolated chloroplasts could perform partial photosynthesis. This was supported and

convincingly proved by S.M. Ruben M. Randall, M. **Randall** and **J.L. Hyde** in 1941. They used radioactive O^{18} to prove it. The experiment also became the first to use a tracer element in solving some problem.

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R. Emerson1957confirmed the existence of two photosystems in the thylakoid membrane which was later verified by **Döring et al**. in 1967

Use of radioactive tracers was also made by M. Calvin (Nobel Prize, 1961) who studied the course of carbon ($C0_2$) in a cyclic pathway leading to the synthesis of glucose. The cycle is popularly known as Calvin cycle.The cycle demonstrated that CO_2 was fixed and the first product formed was a three carbon compound.

Later in 1966, **M.D. Hatch and C.R.** Slack reported that in sugar cane the first product was a four carbon compound (C-4 cycle).

During l950s Robert Emerson and his associates, while studying the effect of different wavelengths of light on photosynthesis, discovered the possibility of two pigment systems involved in trapping light energy.

Making use of this finding **Robert Hill and Fay Bendall** (1960) proposed a scheme of electron transport from one pigment to the other involving a series of electron acceptors which were earlier reported to be associated with the light reaction. The scheme, because of its Z-shape is popularly known as Z-scheme.

Arnon and his associates (1954) discovered that ATP is generated from ADP and phosphorus during photosynthetic electron transport in isolated chloroplasts. It was later supported by studies of Frenkel *et al.*

P. Mitchell (1961-66) published his work on "*Coupling of phosphorylation to electron transfer by a chemiosmotic type of mechanism*".

In 1966 **Andre Jagendorf** reported that a pH gradient across the thylakoid membrane was responsible for ATP synthesis.

Paul **Boyer** (Nobel Prize, 1997) making use of the findings of **Arnon,** D., Jagendorf, A. and also Mitchell, P. (for chemiosmosis) and several others suggested a mechanism of ATP synthesis by the enzyme ATP synthase

5.2 Evolution of Photosynthetic Apparatus

NOTES

Light absorption

Light is a form of electromagnetic radiation. Like other forms of electromagnetic energy, light travels in rhythmic waves.

The distance between crests of electromagnetic waves is called the wavelength.Wavelengths of electromagnetic radiation range from less than a nanometer (gamma rays) to more than a kilometer (radio waves).The entire range of electromagnetic radiation is the electromagnetic spectrum.

The most important segment for life is a narrow band between 380 to 750 nm, the band of visible light.While light travels as a wave, many of its properties are those of a discrete particle, the photon.Photons are not tangible objects, but they do have fixed quantities of energy.The amount of energy packaged in a photon is inversely related to its wavelength.Photons with shorter wavelengths pack more energy.While the sun radiates a full electromagnetic spectrum, the atmosphere selectively screens out most wavelengths, permitting only visible light to pass in significant quantities.Visible light is the radiation that drives photosynthesis.When light meets matter, it may be reflected, transmitted, or absorbed.Different pigments absorb photons of different wavelengths, and the wavelengths that are absorbed disappear.

A leaf looks green because chlorophyll, the dominant pigment, absorbs red and blue light, while transmitting and reflecting green light.

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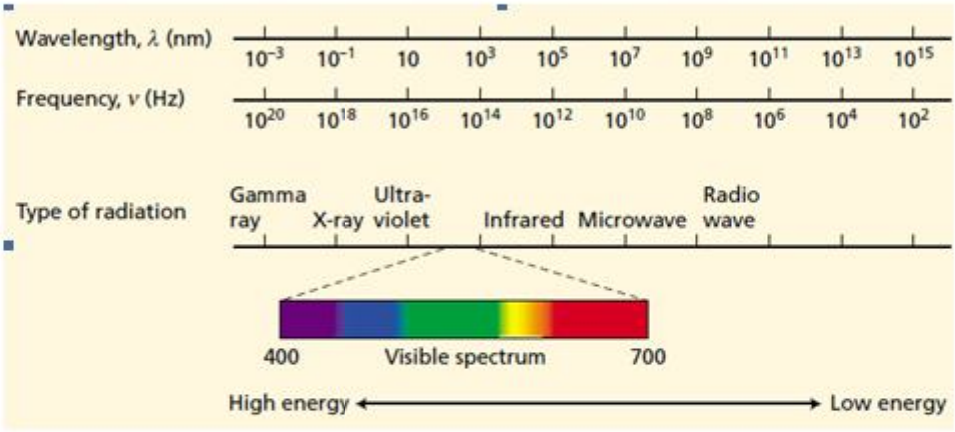

Fig.5.1-Electromagnetic Spectrum showing Visible Spectrum

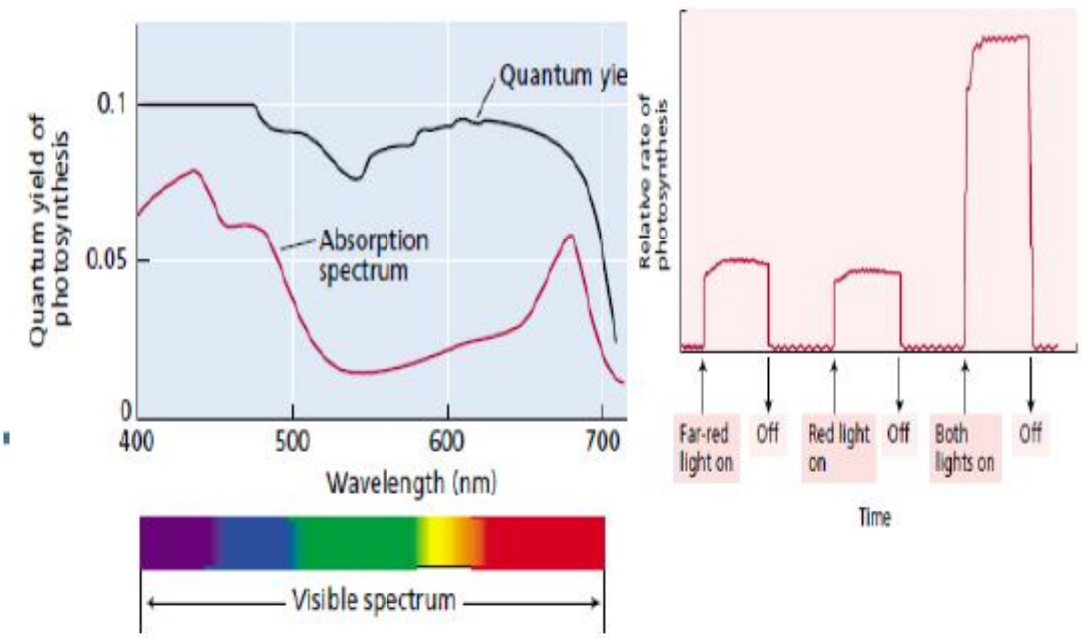

Fig.5.2 Absorption Spectra

A spectrophotometer measures the ability of a pigment to absorb various wavelengths of light.It beams narrow wavelengths of light through a solution containing the pigment and measures the fraction of light transmitted at each wavelength. An absorption spectrum plots a pigment's light absorption versus wavelength.The light reaction can perform work with those wavelengths of light that are absorbed.There are several pigments in the thylakoid that differ in their absorption spectra.Chlorophyll *a*, the dominant pigment, absorbs best in the red and violet-blue wavelengths and least in the green.Other pigments with different structures have different absorption spectra.Collectively, these photosynthetic pigments determine an overall action spectrum for photosynthesis.An action spectrum measures changes in some measure of photosynthetic activity (for example, O_2 release) as the wavelength is varied.

NOTES

The action spectrum of photosynthesis was first demonstrated in 1883 in an elegant experiment performed by Thomas Engelmann.In this experiment, different segments of a filamentous alga were exposed to different wavelengths of light.

Areas receiving wavelengths favorable to photosynthesis produced excess O_2.Engelmann used the abundance of aerobic bacteria that clustered along the alga at different segments as a measure of O_2 production. The action spectrum of photosynthesis does not match exactly the absorption spectrum of any one photosynthetic pigment, including chlorophyll *a*.

Only chlorophyll *a* participates directly in the light reaction, but accessory photosynthetic pigments absorb light and transfer energy to chlorophyll *a*.

Chlorophyll *b*, with a slightly different structure than chlorophyll *a*, has a slightly different absorption spectrum and funnels the energy from these wavelengths to chlorophyll *a*.

Carotenoids can funnel the energy from other wavelengths to chlorophyll *a* and also participate in *photoprotection* against excessive light.

These compounds absorb and dissipate excessive light energy that would otherwise damage chlorophyll.They also interact with oxygen to form reactive oxidative molecules that could damage the cell.When a molecule absorbs a photon, one of that molecule's electrons is elevated to an orbital with more potential energy.The electron moves from its ground state to an excited

state.The only photons that a molecule can absorb are those whose energy matches exactly the energy difference between the ground state and excited state of this electron.

NOTES

Because this energy difference varies among atoms and molecules, a particular compound absorbs only photons corresponding to specific wavelengths.

Thus, each pigment has a unique absorption spectrum.Excited electrons are unstable.

Generally, they drop to their ground state in a billionth of a second, releasing heat energy.

Some pigments, including chlorophyll, can also release a photon of light in a process called fluorescence.

If a solution of chlorophyll isolated from chloroplasts is illuminated, it will fluoresce and give off heat.Chlorophyll excited by absorption of light energy produces very different results in an intact chloroplast than it does in isolation.In the thylakoid membrane, chlorophyll is organized along with proteins and smaller organic molecules into photosystems.

Photosynthetic Pigments

The energy of sunlight is first absorbed by the pigments of the plant. All pigments active in photosynthesis are found in the chloroplast. The chlorophylls and bacterio-chlorophylls (pigments found in certain bacteria) are the typical pigments of photosynthetic organisms, but all organisms contain a mixture of more than one kind of pigment, each serving a specific function.

Chlorophylls a and b are abundant in green plants, and c and d are found in some protists and cyanobacteria. A number of different types of bacteriochlorophyll have been found; type a is the most distributed.

All chlorophylls have a complex ring structure that is chemically related to the porphyrin-like groups found in hemoglobin and cytochromes. In addition, a long hydrocarbon tail is almost always attached to the ring structure. The tail anchors the chlorophyll to the hydrophobic portion of its environment. The ring structure contains some loosely bound electrons and is the part of the molecule involved in electron transitions and redox reactions.

The different types of carotenoids found in photosynthetic organisms are all linear molecules with multiple conjugated double bonds. Absorption bands in the 400 to 500 nm region give carotenoids their characteristic orange color. The color of carrots, for example, is due to the carotenoid β-carotene, whose structure and absorption are shown.

NOTES

Carotenoids are found in all photosynthetic organisms. except for mutants incapable of living outside the laboratory. Carotenoids are integral constituents of the thylakoid membrane and are usually associated intimately with both antenna and reaction center pigment proteins. The light absorbed by the carotenoids is transferred to chlorophyll for photosynthesis; because of this role they are called accessory pigments.

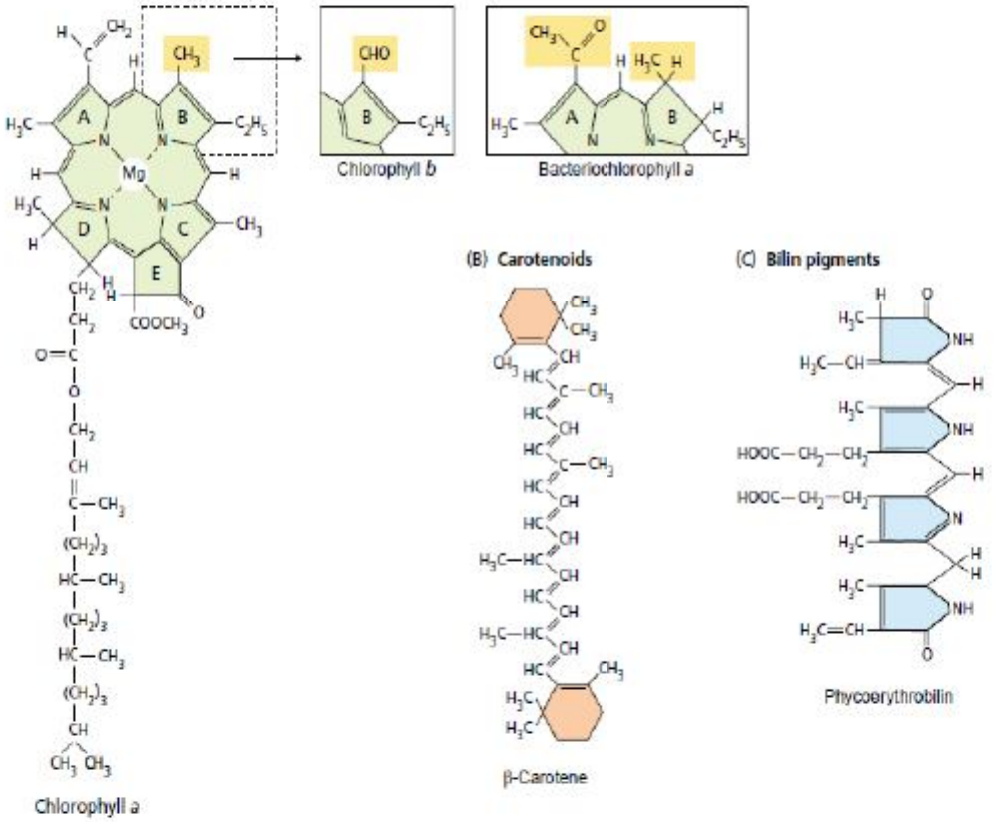

Fig 5.3 Structure of various type of Pigments found in Plants &Bacteria

Site of Photosynthesis

The site of photosynthesis in eukaryotes is the chloroplast where both the processes-the light reaction and the CO_2 assimilation take place. The

chloroplast is a double membrane structure with several membrane bound vesicles or sacs-the thylakoids.

Photosynthetic Apparatus

NOTES

The thylakoid membrane in most plants can be differentiated into the stacked and unstacked regions which differ in their nature of photosynthetic assemblies. The photosystem I and ATP synthase are mostly located in the unstacked region whereas photosystem II lies mostly in the stacked region. The cytochrome b6-f complex is distributed in both regions. These have a common thylakoid lumen and hence the ATP synthase could utilize protons in the lumen even from the unstacked regions. The functional significance of this arrangement is:

The positioning of PS-I in the unstacked region places the PS-I system directly in contact with the stroma so that it may easily synthesize NADPH.

ATP synthase is a large and complex system which could be adjusted with difficulty in the stacked region. In the unstacked region it finds sufficient space in the stroma for its CF region. In the stroma, it can also have free availability of ADP which is synthesized to ATP.

Stacking increases the amount of thylakoid membrane in small space.

The stacking, which could adversely affect PS-I and ATP synthase poses no problem for PS-ll because it needs water as proton donor and small lipid soluble electron acceptors. The water occupies almost no space and the plastoquinones being lipid soluble move within the thylakoid membrane.

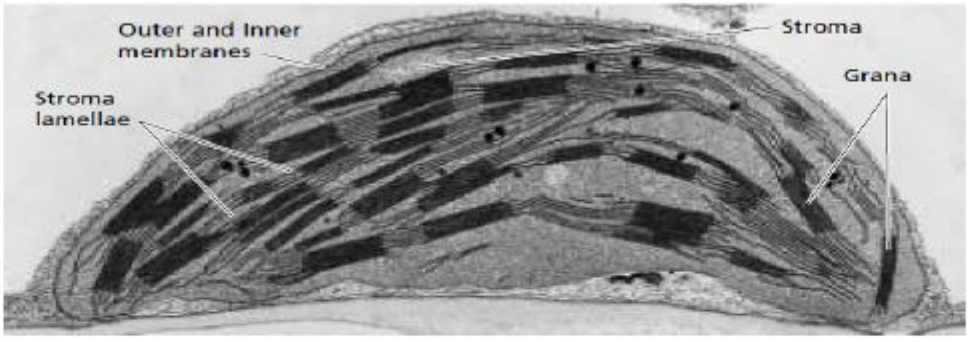

Fig 5.4---Structure of Chloroplast

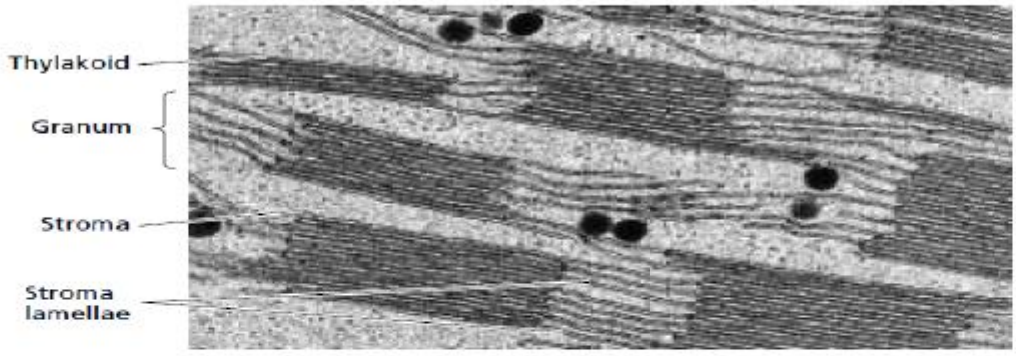

Fig 5.5---Ultra-Structure of Chloroplast

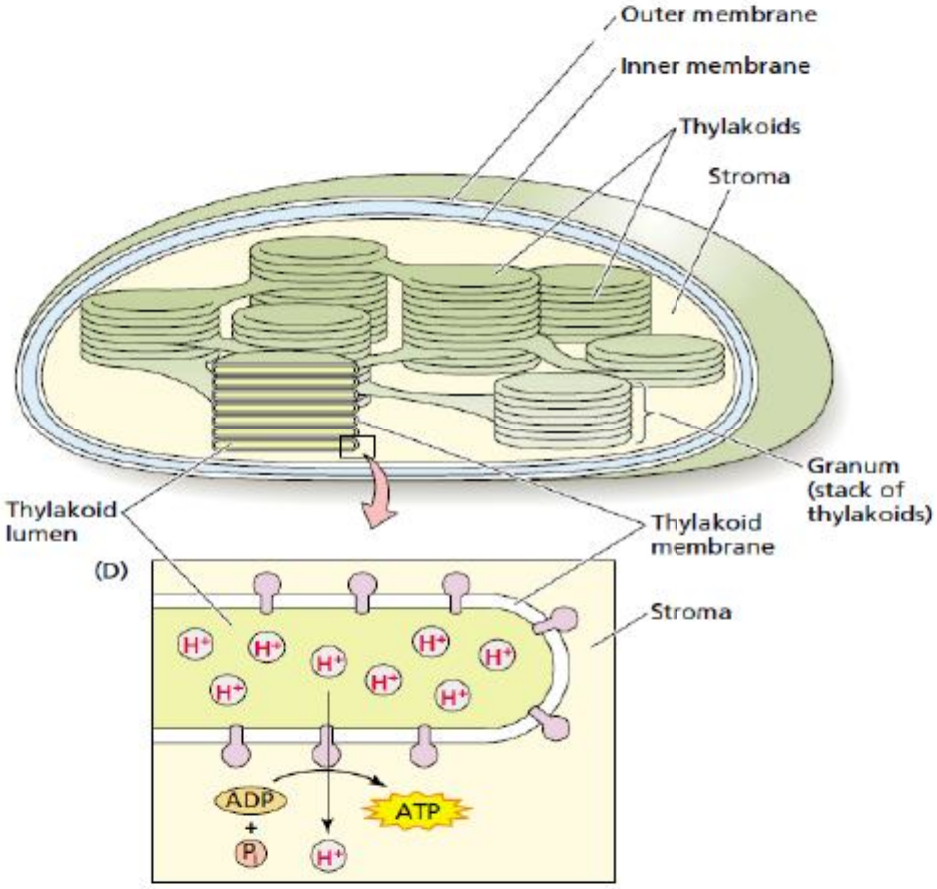

Fig 5.6- Structure of Chloroplast: H^+ -ATPase location on thylakoid membrane

Evolution of Photosynthetic Apparatus

In photosynthetic eukaryaotes, photosynthesis takes place in the sub-cellular organelle known as the chloroplast. The most striking aspect of the structure of the chloroplast is the extensive system of internal membranes known as

thylakoids. All the chlorophyll is contained within this membrane system, which is the site of the light reactions of photosynthesis.

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The carbon reduction reaction, which are catalyzed by water-soluble enzymes, take place in the stroma (plural stromata), the region of the chloroplast outside the thylakoids. Most of the lakoids appear to be very closely associated with each other. These stacked membranes are known as grana lamellae (singular lamella each stack is called a granum), and the exposed membranes in which stacking is absent are known as stroma lamellae.

Two separate membranes, each composed of a lipid bilayer and together known as the envelope, surround most types of chloroplasts. This double-membrane system contains a variety of metabolite transport systems.

The chloroplast also contains its own DNA, RNA, and ribosomes. Many of the chloroplast proteins are products of transcription and translation within the chloroplast itself, whereas others are encoded by nuclear DNA, synthesized on cytoplasmic ribosomes, and then imported into the chloroplast. This remarkable division of labor, extending in many cases to different subunits of the same enzyme complex.

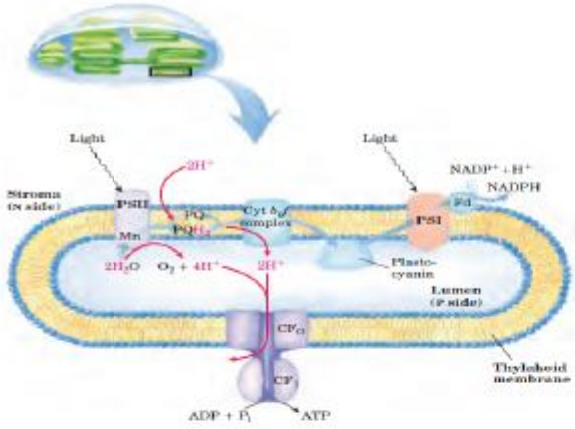

Fig 5.7 --Thylakoid Membrane and its various components

Photosystems I and II are spatially separated in the Thylakoid Membrane

The PS-II reaction center, along with its antenna chlorophylls and associated electron transport proteins, is located predominantly in the grana lamellae.

The PS-I reaction center and its associated antenna pigments and electron transfer proteins, as well as the coupling-factor enzyme that catalyzes the

formation of ATP,are found almost exclusively in the stroma lamellae and at the edges of the grana lamellae. The cytochrome $b_6 f$ complex of the electron transport chain that connects the two photosystems is evenly distributed between stroma and grana.

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Thus the two photochemical events that take place in O_2-evolving photosynthesis are spatially separated. This separation implies that one or more of the electron carriers that function between the photosystems diffuses from the grana region of the membrane to the stroma region, where electrons are delivered to photosystem I.

In PS-II, the oxidation of two water molecules produces four electrons, four protons, and a single O_2 .The protons produced by this oxidation of water must also be able to diffuse to the stroma region, where ATP is synthesized. The functional role of this large separation (many tens of nanometers) between photosystems I and II is not entirely clear but is thought the two photosystems (Trissl and Wilhelm 1993; Allen and Forsberg 2001).

The spatial separation between photosystems I and II indicates that a strict one-to-one stoichiometry between the two photosystems is not required. Instead, PS-II reaction centers feed reducing equivalents into a common intermediate pool of soluble electron carries (plastoquinone). The PS-I reaction centers remove the reducing equivalents from the common pool, rather than from any specific PS-II reaction center complex.

Most measurements of the relative quantities of photosystems I and II have shown that there is an excess of photosystem II in chloroplasts. Most commonly, the ratio of PS-II to PS-I is about 1.5:1, but it can change when plants are grown in different light conditions.

Non-O_2-evolving (anoxygenic) organisms, such as the purple photosynthetic bacteria of the genera Rhodubacter and Rhodopseudomonas, contain only a single photosystem. These simple organisms have been very useful for detailed structural and functional studies that have contributed to a better understanding of oxygenic photosynthesis.

Hartmut Michel, Johann Deisenhofer, Robert Huber, and coworkers in Munich resolved the three-dimensional structure of the reaction center from the purple photosynthetic bacterium Rhodopseudomonas viridis (Deisenhpfer and Michel

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1989). This landmark achievement, for which a Nobel Prize was awarded in 1988, was the first high-resolution, X-ray structural determination for an integral membrane protein, and the first structural determination for a reaction center complex. Detailed analysis of these structures, along with the characterization of numerous mutants, has revealed many of the principles involved in the energy storage processes carried out all reaction centers.

The structure of the bacterial reaction center is thought to be similar in many ways to that found in photosystem II from oxygen-evolving organisms, especially in the electron acceptor portion of the chain. The proteins that make up the core of the bacterial reaction center are relatively similar, in sequence to their photosystem II counterparts, implying an evolutionary relatedness.

5.3 Photosynthetic Pigments and Light harvesting complexes

Light Harvesting Complexes

In all eukaryotic photosynthetic organisms that contain both chlorophyll a and chlorophyll b, the most abundant antenna proteins are members of a large family of structurally related proteins. Some of these proteins are associated primarily with photosystem II and are called light-harvesting complex II (LHC-II) proteins; others are associated with photosystem I and called LHC-I proteins. These antenna complexex are also known as chlorophyll a/b antenna proteins

The structure of one of the LHC-II proteins has been determined by a combination of electron microscopy and electron crystallography. The protein contains contains three $\alpha -$ helical regions and binds about 15 chlorophy II a and b molecules, as well as a few carotenoids. Only some of these pigments are visible in the resolved structure. The structure of the LHC-I proteins has not yet been determined but is probably similar to that of the LHC-II proteins. All of these proteins have significant sequence similarity and are almost certainly descendants of a common ancestral protein.

Light absorbed by carotenoids or chlorophyll b in the LHC proteins is rapidly transferred to chlorophyll a and then to other antenna pigments that are

intimately associated with the reaction center. The LHC-II complex is also involved in regulatory processes.

The location of the two photosystems at different sites on the thylakoid membranes requires that at least one component be capable of moving along or within the membrane in order to deliver electrons produced by photosystem II to photosystem I. The cytochrome b_6f complex is distributed equally between the grana and the stroma regions of the membranes, but its large size makes it unlikely that it is the mobile carrier. Instead, plastoquinone or plastocyanin or possibly both are thought to serve as mobile carriers to connect the two photosystem.

NOTES

Plastocyanin is a small (10.5 kDa), water-soluble, copper-containing protein that transfer electrons between the cytochrome b_6f complex and P700. This protein is found in the luminal space. In certain green algae and cyanobacteria, a c-type cytochrome is sometimes found instead of plastocyanin; which of these two proteins is synthesized depends on the amount of copper available to the organism.

The PS-I reaction center complex is a large multisubunit complex. In contrast to PS-II, a core antenna consisting of about 100 chlorophylls is a part of the PS-I reaction center, P700. The core antenna and P700 are bound to two proteins, PsaA and PsaB, with molecular masses in the range of 66 to 70 kDa (Brettel 1997; Chitnis 2001.

The antenna pigments form a bowl surrounding the electron transfer cofactors, which are in the center of the complex. In their reduced form, the electron carriers that function in the acceptor region of photosystem I are all extremely strong reducing agents. These reduced species are very unstable and thus difficult to identify. Evidence indicates that one of these early acceptors is a chlorophyll molecule, and another is a quinine species, phylloquinone, also known as vitamin K_1.

Additional electron acceptors include a series of three membrane-associated iron-sulfur proteins, or bound ferredoxins, also known as Fe-S centers FeS_X, FeS_A and FeS_B . Fe-S center X is part of the P700-binding protein; centers A and B reside on an 8 kDa protein that is part of the PS-I reaction center complex. Electrons are transferred through centers A and B to ferredoxin (Fd),

a small, water-soluble iron-sulfur protein. The membrane-associated flavoprotein ferredoxin-NADP reductase (FNR) reduces $NADP^+$ to NADPH, thus completing the sequence of noncyclic electron transport that begins with the oxidation of water.

NOTES

In addition to the reduction of $NADP^+$, reduced ferredoxin produced by photosystem I has several other functions in the chloroplast, such as the supply of reductants to reduce nitrate and the regulation of some of the carbon fixation enzymes .

Some of the cytochrome b_6f complexes are found in the stroma region of the membrane, where photosystem I is located. Under certain conditions cycle electron flow from the reducing side of photosystem I, through the b_6f complex and back to P700, is known to occur. This cyclic electron flow is coupled to proton pumping into the lumen, which can be utilized for ATP synthesis but does not oxidize water or reduce $NADP^+$. Cyclic electron flow is especially important as an ATP source in the bundle sheath chloroplasts of some plants.

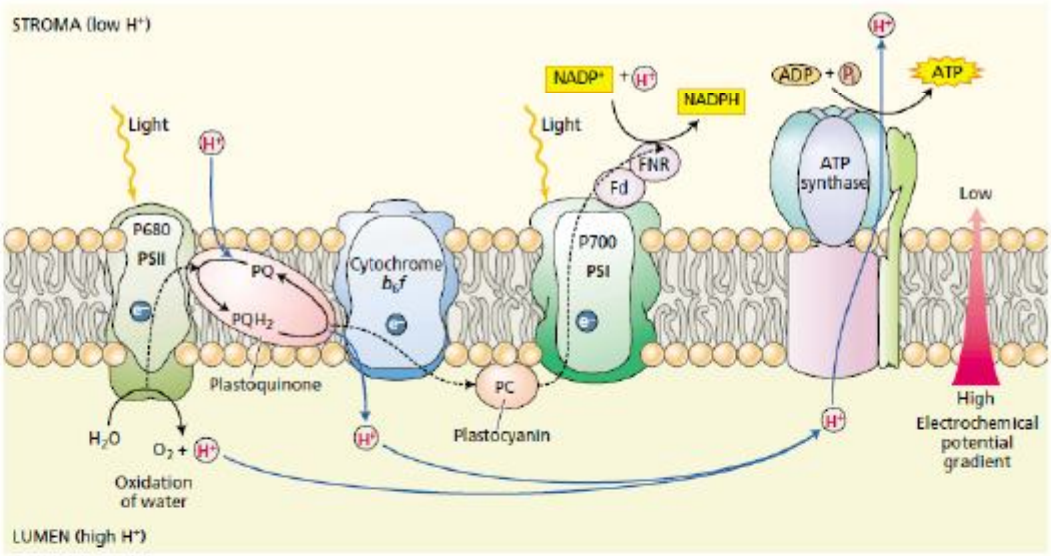

Fig 5.8 Transfer of Electron and Proton in the Thylakoid Membrane

Two pigment systems

Organisms with oxygenic photosynthesis have two distinct photosystems. Photosystem I responds to light of wavelength nearly 700 nm and photosystem II responds to a wavelength of 680 nm or less. The two photosystems are linked by a number of redox substances including cytochrome bf complex' and

plastocyanin. These pigment systems are, therefore, known by the wavelength they respond to. Thus photosystem I has a pigment system (P700) and photosystem II has a pigment system (P680).

Photosystem II. The photosystem II of green plants is very similar to bacterial photosystem. Like the bacterial photosystem this also contains two central transmembrane polypeptides I and II each 32 kd subunits formed by five a-helices. It also has a special pair of chlorophyll a molecules which absorb light of 680 nm and is, therefore, called P 680. These chlorophyll molecules are placed close to thylakoid lumen. Close to this and projecting in the thylakoid lumen is a small structure which includes four manganese ions, a calcium ion, and a chloride ion. This is the site for water oxidation. A phaeophytin molecule is present slightly above the special pair. At the top, and close to the stroma side are present the second and third electron acceptors -the plastoquinones. In addition to polypeptides I and II (Fig. 8) there are a number of other transmembrane polypeptides subunits (ex-helices) which bind additional chlorophyll molecules which absorb light energy and transfer it to P680. The subunits I and II are also associated with cytochrome molecules.

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Thus, although the ground plan of photosystem II in oxygenic photosynthesis and the bacterial photosystem is the same, they differ in several respects.

(1) The special pair in bacterial system absorbs light at 960 nm whereas the photosystem If has special pair which absorbs light at 680 nm.

(2) The electron hole in the special pair of bacterial system is filled by cytochrome, but in photosystem II the electron is taken from water which is oxidized by a system of four manganese atoms releasing molecular oxygen .

(3) The photosystem II has a number of additional polypeptides around the central polypeptide I and II which carry a large number of chlorophyll molecules. These also absorb light and transfer the energy to special pair. This makes the photosystem II more efficient.

(4) In bacterial system the reduced QB2- takes a proton to get neutralized and thus create a proton gradient. In photosystem II the electrons are transferred to P700 through a series of acceptors. The proton gradient is created by Mn system which draws out oxygen and electron from water

leaving only proton in the thylakoid lumen thus creating a gradient across membrane.

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Photosystem I: The core of the photosystem I also is formed by about 13 polypeptides in two subunits of 83 kd and 82 kd. A special pair of) chlorophyll a lies in the centre which absorbs light at 700 nm. This is therefore, known as P700. Surrounding these core subunits are a number of polypeptides carrying chlorophyll molecul~s as in) PS-II. The special pair lies towards the thylakoid lumen; in the centre of the complex. A little above P700, is another chlorophyll molecule and quinone A. To the stroma side and projecting into the stroma there is a set of 4 Fe - 4S protein. The inorganic iron and sulfur cluster in the cubic form is held by four cysteine residues present in core polypeptides of the reaction centre.

The two other Fe-S complexes are bound to separate proteins. The photosystem I is located mainly in the unstacked lamellae (stroma lamellae).

The last Fe-S protein ultimately transfers electrons to ferredoxin, a protein with 2F-2S complex, where again it is supported by cysteine residues of the protein.

Electron flow through the photosystems

The light strikes the special pair in PS-II. The energy is absorbed to the extent that an electron is highly chergized and moves out of the orbits (the energy levels) The electron is immediately accepted by phaeophytin which now acquires negative charge Phe- and the chlorophyll of the s pair attains positive charge Chl+. This is known as charge separation. Four Mn atoms extract electron from water liberating molecular oxygen and releasing protons in the thylakoid lumen. This electron is transferred to Chl+ to fill the electron hole. Phaeophytin now transfers the electron to one of the plastoquinones. It then passes to the second plastoquinone. When the· second plastoquinone is fully reduced (Q^{2-}), it picks up two protons ($2H^{+}$) from the stromal side of thylakoid membrane and ·dissociates from the reaction centre to join pool of plastoquinoncs. The plastoquinone transfers electron to cytochromc b6f which then transfers it to phycocyanin wherefrom it is finally transferred to P700 in PS-I (Fig 12).

The PS-I is also excited by another flash, of light. The special pair P700 here absorbs this wavelength and emits electron which is immecliately transferred to another chlorophyll a present nearby which ultitnately transfers it to quinone. From quinone the electron is transferred to a series of Fe-S proteins. 4Fe-4 S to 2Fe-2 S to 2Fe-2 S and then to a ferredoxin in a complex carrying ferredoxin flavoprotein, and NADP+. This complex is associated with an enzyme ferredoxin NADP+ reductase and FAD. This enzyme accepts one electron at' a time from ferredoxin to form FADH2 through semiquinone intermediate. This enzyme then transfers a hydride ion to NADP+ to form NADPH $^{+}$ H^{+} and is changed to FAD.

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The whole process appears to increase H+ protons in the thylakoid lumen. Thus, a proton gradient is created across e membrane. This excess proton is pumped out through ATP synthase, synthesizing ATP from ADP and the energy is fixed in phosphoanhydride bonds of ATP. The detailed mechanism is discussed in A TP synthesis (see oxidative phosphorylation).

5.4 Photo-oxidation of Water

Oxidation of Water: The manganese centre close to the thylakoid lumen and attached to core subunits of PS-II is responsible for oxidation of water. It breaks two molecules of water releasing four protons (H^{+}) to the thylakoid lumen and a molecule of oxygen which is liberated in photosynthesis. The corresponding four electrons are carried by quinones which are reduced to QH_2 by drawing protons from the matrix. The manganese centre, as mentioned earlier, carries four manganese atoms forming a complex with chloride and calcium and a protein that holds the whole complex. Selection of manganese for this centre appears to be due to its property of forming complexes and existing in several oxidized forms (Mn^{2+}, Mn^{3+}, Mn^{4+} and Mn^{5+}). A possible structure of the manganese complex is presented in the figure 13 (a). The entire oxidation process takes place in five steps named, S_0, S_1, S_2, S_3 an S_4· In the S_0 state two molecules of water appear attached to the complex, one at the first Mn^{2+} and the other with Ca. A hydrogen is also attached to oxygen between Mn atoms Mn^{1} and Mn^{2}. When it passes from S_0 to S_1 a proton and

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an electron are liberated from this hydrogen The Mn^{1} acquires one more positive charge to change to Mn^{3+}. In the next step when it passes from S_1 to S_2 state one more electron is released changing Mn_2 from Mn^{3+} to Mn^{4+}.

In between S_2 and S_3 a hydrogen from the water molecule attached to Mn_1 is released liberating a proton and an electron. The Mn_1 now acquires one more +ive charge and change to Mn^{4+}. Between S_3 and S_4 the other hydrogen of the water attached to Mn1, also is freed in the form of a proton and an electron adding one more +ive charge to Mnl, which changes to Mn^{5+}. Between S_4 and S_o' the hydrogen atoms of water attached to calcium are rearranged. One of it is released as proton and the other is transferred to oxygen between Mn_1 and Mn_2. The oxygen of both the water molecules get together and attached to Mn_1. This molecular oxygen is released. The Ca and Mn_1 again attach to one water molecule each. This completes the cycle and it has once again reached S_0 with Mn_1 and M_2 loosing charges. It can be seen from the figure that each oxidation state (S_o-S_1, S_I-S_2, S_2-S_3 and S_3-S_4) looses electron. Thus a total of 4 electrons and 4 protons are liberated in one cycle. The S_4 decays spontaneously releasing O_2. It is also important to note that the onus of releasing electron does not lie with Mn. It is the PS-II which looses electron after excitation. The P_{680} has acquired positive change and hence it actually draws the electron from Mn system. Since the PS-II looses onIy one electron at each excitation it draws only one electron from Mn centre at one time and a molecular oxygen is released only after four such flashes.

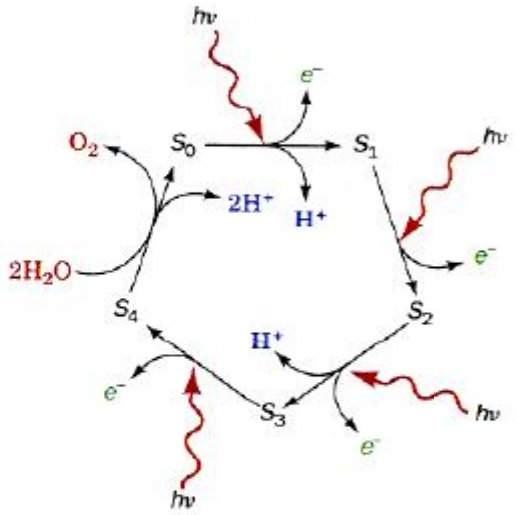

Fig 5.9: Mechanism of O_2 generation in Chloroplast

5.5 Mechanisms of Electron and Proton transport

The electron expels from P 680 and P 700 after travelling through Electron Transport System (E.T.S) of photosynthesis, are either assumed in reducing $NADP^+$ to NADPH + H^+ or cycled back. The extra light energy is used in the formation of ATP molecule s at different place during its transport. It is called as **photo- phosphorylation.**

According to **Arnon and associates**, photophosphorylation or E.T.C. involves the following two processes :

1. Non-cyclic photophosphorylation,
2. Cyclic Photophosphorylation.

In **cyclic photophosphorylation** the electrons lost by PS-I is cycled back to it, whereas in **non-cyclic photophosphorylation**, one electron is lost it doesn't enter into PS-II, thus it involves both PS-I and PS-II.

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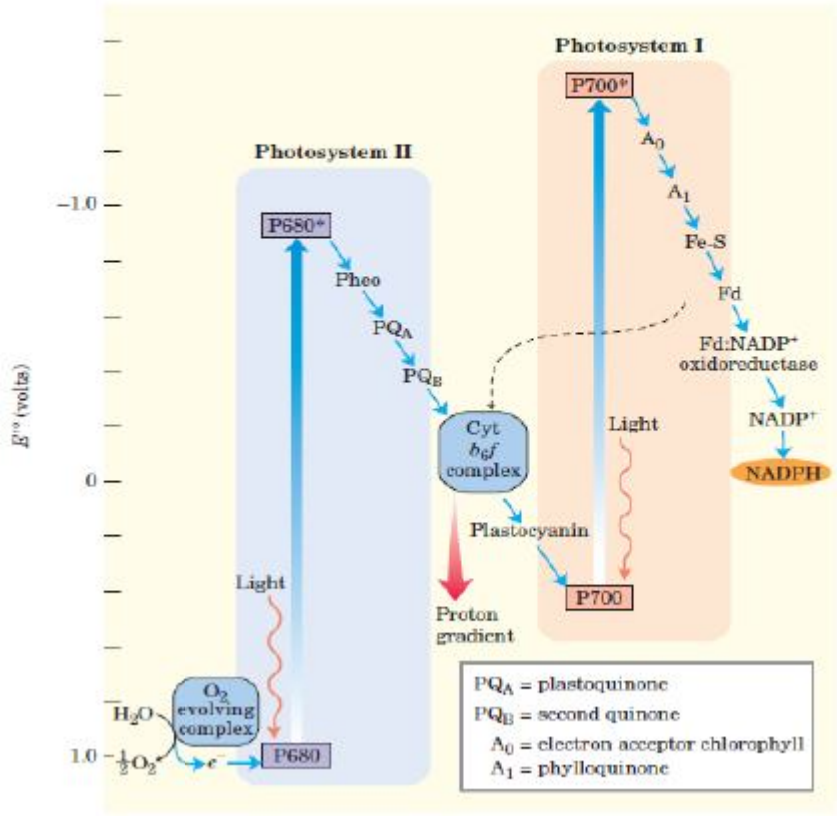

Fig 5.10: Z-scheme of Photo-Phosphorylation

(i) Non-cyclic photophosphorylation

Hill and **Bendal** (1960) and **Robinowitch** and **Govindjee** (1965) have proposed **Z- scheme** to explain the process of photophosphorylation. In this type of photophosphorylation, both the photochemical processes (PS-I and PS-II) takes place in a series and the product of one reaction is used in the second reaction.

When a quantum of light of wavelength above 680nm is received by a molecule of PS-I the energy is transferred to a chain of other chlorophyll molecules by induction resonance, until finally it is transferred to a molecule of P 700, which becomes excited and releases an electron. These electrons are accepted by 'X'$(_{OX))oxidised}$ due to which it become reduced (X_{red}). The electron is then transferred to **ferredoxin reducing substance (FRS)**. FRS further reduces an iron containing protein called **ferredoxin**.

The electron from reduced ferredoxin then reduces NADP to NADPH with the help of H^+ released from H_2O. When a quantum of wave length of light of

lower wavelength is received by PS-II its reaction canter P 680 loses electron to a substance which is probably a quinone. The electrons then travel downhill and fall back to +4eV in a dark reaction through a series of PS-I. The carriers are **cytochrome-b (Cyt-b), plastoquinone (PQ), cytochrome-f.(Cyt-f)** and **plastocyanin (PC).**

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The electron thus does not complete the cycle as it starts from PS-II and is drained off in the carbohydrates produced by CO_2 reduction. The energy released in the transfer of electron from PQ to Cytochrome-f is utilized to convert ADP and inorganic phosphate into ATP. The ATP synthesis resulting from this type of non-cyclic electron transport chain is known as **non-cyclic photophosphorylation.** Water molecule is utilized as a source of electron (H_2 donor) in this system at the same time water molecules become dissociated into H^+ and OH^- ions.

$$4H_2O \longrightarrow 4H^+ + 4OH^-$$

$$4OH^- \longrightarrow 4OH + 4e^-$$

OH^- ions transfer their electrons (e^-) to 'Z' (an unknown substance) and OH radical is formed. These electrons are then transferred to PS-II and OH radical become dissociated form H_2O and O_2

$$4OH \longrightarrow 2H_2O + O_2$$

H^+ ions originated from hydrolysis of water reduces $NADP^+$ into NADPH $+H^+$. This NADP + H^+ functions as reducing agent. Thus, we observe that the electrons released from PS-II does not again enter to PS-II hence, it is called non-cyclic photophosphorylation.

$$2NADP^+ 4H^+ + 4e^- \longrightarrow 2NADPH + 2H^+$$

In this process, two molecules of ATP are formed per two molecules of NADP reduced or one more molecule of oxygen evolved or two molecules of water oxidized.

$$2ADP + 2Pi + 2NADP^+ + 2H_2O \longrightarrow 2ATP + 2NADPH + 2H^+ + O_2$$

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(ii) Cyclic Photophosphorylation

The **cyclic photophosphorylation** take place under certain condition *e.g.*, when the amount of available NADP is low or PS-II is absent. It involves PS-I and therefore, photolysis of water and the consequent evolution of O_2 does not take place. Non-cyclic electron transfer does not take place and NADPH is not formed. The electron lost by P 700 is cycled back to it through **X, FRS, FD** and **cytochrome-b_6, cytochrome –f** and **plastocynanin**. 2ATP molecules are synthesized from 2ADP and inorganic phosphate when electron is transferred from **cytochrome–b_6** to **PQ** and from **cytochrome-b** to **cytochrome-f .**

Thus, from the light reaction results:

a. Photolysis of water and release of O_2

b. Formation of 3 ATP

c. Formation of $2NADPH_2$

ATP and NADPH are used in the reduction of CO_2 during dark reaction. Similarly ATP and $NADPH_2$ function as carrier of energy of sunlight and transfer it up to dark reaction. ATP together with $NADPH_2$, called as **assimilatory power** and $NADPH_2$ is called as **reducing power**.

5.6 Summary

Photosynthesis is the important process carried out by plants, algae, and photosynthetic bacteria. Absorbed photons excite chlorophyll molecules, and these excited chlorophylls can dispose of this energy as heat, fluorescence, energy transfer etc. Light is absorbed mainly in the antenna complexes, which comprise chlorophylls, accessory pigments, and proteins and are located at the thylakoid membranes of the chloroplast. Photosynthetic antenna pigments transfer the energy to a specialized chlorophyll–protein complex known as a reaction center. The reaction center contains multisubunit protein complexes and hundreds or, in some organisms, thousands of chlorophylls.

The reaction center initiates a complex series of chemical reactions that capture energy in the form of chemical bonds.The relationship between the amount of absorbed quanta and the yield of a photochemical product made in a light-dependent reaction is given by the quantum yield.

Plants and some photosynthetic prokaryotes have two reaction centers, photosystem I and photosystem II, that function in series. The two photosystems are spatially separated: PSI is found exclusively in the nonstacked stroma membranes, PSII largely in the stacked grana membranes. The reaction center chlorophylls of PSI absorb maximally at 700 nm, those of PSII at 680 nm. Photosystems II and I carry out noncyclic electron transport, oxidize water to molecular oxygen, and reduce NADP+ to NADPH. It is energetically very difficult to oxidize water to form molecular oxygen, and the photosynthetic oxygen-evolving system is the only known biochemical system that can oxidize water, thus providing almost all the oxygen in Earth's atmosphere. The photooxidation of water is modeled by

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the five-step S state mechanism. Manganese is an essential cofactor in the water-oxidizing process, and the five S states appear to represent successive oxidized states of a manganese- containing enzyme. The electron flow ends with the reduction of NADP+ to NADPH by a membrane-bound, ferrodoxin–NADP reductase. A portion of the energy of photons is also initially stored as chemical-potential energy, largely in the form of a pH difference across the thylakoid membrane. This energy is quickly converted into chemical energy during ATP formation by action of an enzyme complex known as the ATP synthase. The photophosphorylation of ADP by the ATP synthase is driven by a chemiosmotic mechanism.

Some of the proteins that are essential for photosynthesis.Additional proteins are encoded by nuclear DNA, synthesized in the cytosol, and imported into the chloroplast. Chlorophylls are synthesized in a biosynthetic pathway involving more than a dozen steps, each of which is very carefully regulated. Once synthesized, proteins and pigments are assembled into the thylakoid membrane

5.7 Glossary

- **ATP :** Adenosine triphosphate, a small water soluble molecule that acts as an energy currency in cells.
- **ATP Synthase :** A membrane bound protein complex that uses the energy stored across the photosynthetic membrane to add inorganic phosphate to ADP, thus creating ATP. (Also known as coupling factor.)
- **Autotroph :** Photosynthetic organisms which convert light energy into the chemical energy they need to develop, grow, and reproduce.

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- **Calvin Cycle :** The biochemical reactions, initiated by Rubisco, that result in the reduction of CO2 to a carbohydrate (also known as the photosynthetic carbon reduction cycle).
- **Carbon fixation :** ATP and NADPH are used to fix CO_2 into carbohydrates. Carbon fixation takes place in the chloroplast stroma.
- **Cytochrome :** Heme containing protein.
- **Cytochrome bc Complex :** A membrane bound electron transfer protein complex found in all anoxygenic photosynthetic organisms that oxidizes reduced quinone and reduces a c-type cytochrome. The complex contains a c-type cytochrome, two b-type cytochromes and an FeS center.
- **Cytochrome bf Complex :** A membrane bound electron transfer protein complex found in all oxygenic photosynthetic organisms that oxidizes reduced plastoquinone and reduces plastocyanin (or cytochrome c). The complex contains a c-type cytochrome, two b-type cytochromes and an FeS center.
- **Free Energy :** The amount of energy in a reaction available to do work. Because most biochemical reactions occur at a constant temperature and pressure, the free energy is frequently the Gibbs Energy.
- **Light :** Electromagnetic radiation; the shorter the wavelength the greater amount of energy. Light supplies the energy for the light reactions of photosynthesis.
- **Light Harvesting Complex :** A protein complex that harvests light energy and converts it to exciton energy that can migrate to a reaction center. The light is absorbed by pigment molecules (*e.g.*, chlorophyll, bacteriochlorophyll, carotenoids, phycobilin) that are attached to the protein.
- **Lumen :** Region within the thylakoid membrane where water is split to obtain oxygen. The oxygen diffuses out of the cell, while the protons remain inside to build positive electrical charge inside the thylakoid.
- **NADPH :** Reduced form of nicotinomide adenine dinucleotide phosphate, a small water soluble molecule that acts as a hydrogen carrier in biochemical reactions.

- **NADP+** : oxidized form of nicotinomide adenine dinucleotide phosphate.
- **Phosphorylation :** The covalent attachment of a phosphate group to a molecule.
- **Photorespiration :** The removal of O2 from the atmosphere by Rubisco and the subsequent biochemical reactions that serve to recycle some of the reduced carbon.
- **Photosynthesis** : The physical-chemical process by which certain chlorophyll (or bacteriochlorophyll) containing organisms use light energy for the biosynthesis of organic molecules.
- **Photosynthetic Membrane :** A bilayer of lipid molecules in which are embedded proteins that transform light energy into chemical free energy. (Also known as the thylakoid membrane.)
- **Photosystem** : a cluster of chlorophyll and other molecules in a thylakoid that harvest the energy of light for photosynthesis
- **Photosystem I** : A protein complex located in the photosynthetic membrane. Photosystem I is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem I reaction center uses light energy to transfer an electron from a mobile electron transfer protein (plastocyanin or a cytochrome c) on one side of the photosynthetic membrane to a mobile electron transfer protein (ferredoxin) on the opposite side of the photosynthetic membrane.
- **Photosystem II** : A protein complex found in the photosynthetic membrane. Photosystem II is one of two types of reaction centers found in higher plants, algae and cyanobacteria. The photosystem II reaction center uses light energy to transfer electrons from water to plastoquinone. Photosystem II is the source of the molecular oxygen in the atmosphere.
- **Plastoquinone** : A small organic molecule involved in electron and proton transfer in photosynthesis.
- **Protein :** A chemical structure composed of one or more polypeptides. In photosynthesis proteins serve as the scaffolding that hold the cofactors that gather light energy, transfer electrons, and catalyze biochemical reactions.

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- **Reaction Center :** A protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center.
- **Reduction :** The addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added.
- **Rubisco :** (D-ribulose 1,5-bisphosphate carboxylase/oxygenase) A water soluble protein complex responsible for the removal of CO2 from the atmosphere. The enzyme works by attaching CO2 to a five-carbon compound (1,5 ribulose bisphosphate) that is split into two identical three-carbon compounds (phosphoglycerate). In addition to catalyzing the removal of CO2 from the atmosphere, Rubisco also catalyzes the removal of O2 from the atmosphere (less efficiently). The removal of O2 is thought to be a consequence of poor design and leads to a complex set of compensatory reactions known as photorespiration.
- **Thylakoid : D**isc-shaped portion of chloroplast, found in stacks

5.8 Self-Learning Exercise

Section A (Very Short Answer Type Question)

1. What is vision region?
2. What is other name of dark reaction?
3. Who discovered that O_2 come from water?
4. What is *LHC*?
5. What do you mean by grana?
6. What is ATP?

Section B (Short Answer Type Questions)

1. Write about structure of chloroplast.
2. Write a note on photo-oxidation of water.
3. Write a note on red drop effect.
4. Write a note on emmerssion enhancement effect.
5. Briefly explain about Z-scheme.

Section C (Long Answer Type Questions)

1. Give a general account on mechanism of electron transport.
2. Give a general account on photosynthetic pigments.
3. Describe the structure of thylakoid membrane.
4. Describe LHC.

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Answer Key of Section - A

1. The region between 400 nm to 700 nm.
2 Blackman reaction
3 Robert Hill
4 Light Harvesting Complex
5 Stacked arrangement of thylakoids
6 Adenosine tri Phosphate

5.9 References

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Unit – 6

Photochemistry and Photosynthesis-II

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Structure of the Unit

6.0 Objective

After studing this unit you will be able to understand the-

- Mechanism of path of carbon in C_3 plants.
- Basic structure and functional organization of C4 plant their anatomical peculiarities,physiological specialization
- Basic structure and functional organization of CAM plant their anatomical peculiarities,physiological specialization and evolutionary significance
- Basic mechanism of photorespiration their evolutionary significance.

6.1 Carbon Assimilation

Carbon Fixation

This forms the second part of the photosynthesis mechanism, the dark reaction, where CO_2 is reduced to carbohydrates utilizing hydrogen from NADPH and

energy from ATP that are obtained in light reaction. This part of the process, therefore, does not require any light for itself and can proceed even without light if a continuous supply of NADPH and ATP is assured. This is, therefore, also known as dark reaction. The details of the process were worked out by Melvin Calvin during 1950s using tracer technique. Initially it was believe that there is only one type of carbon fixation process butt later on various form are also found like C_4 cycle, CAM cycle etc.All variation form shows some significance in plant life.

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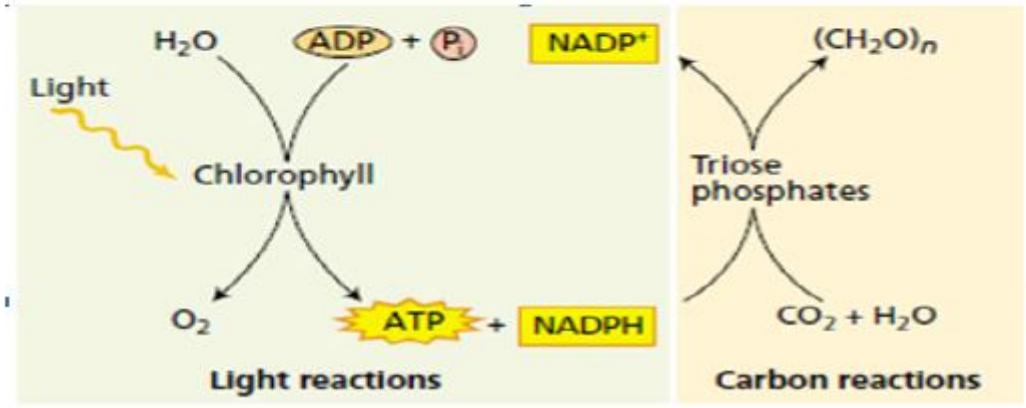

Fig 6.1 Relationship between Light and Dark Reaction

6.1.1 The Calvin cycle

The C 3 carbon fixation pathway was firstly discovered by Melvin Calvin and others in 1950.They applying $^{14}CO_2$ used green alga like *Chlorella* and *Scenedesmus*. The algal cells from the culture were drawn, killed instantaneously in boiling ethanol, homogenized and the extract was put to paper chromatography for soluble carbohydrates. Different spots on the paper were tested for the presence of radioactivity. Thus, the movement of radioactivity through different spots could be traced by analysing the chromatographs at different time intervals. Spots showing radioactivity in shorter time were thought to have been formed earlier than those showing activity at a later period. The time interval was less than 5 seconds was found that the first compound to be radioactive was phosphoglyceric acid(3C). This compound was then isolated and analysed. It was found that the radioactivity was limited to carboxylic carbon. Further study gave other compounds in series which led Calvin to propose a cycle for the conversion/interconversion of these

molecules. The cycle is popularly known as Benson - Calvin cycle. The cycle was later slightly modified but the basic pathway is still the same.

A careful observation of the cycle shows that the whole cycle has three different sets of reactions.

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(1) The carbon fixation stage: In this stage a molecule of CO_2 reacts with ribulose 1, 5-bisphosphate to produce phosphoglyceric acid.

(2) The reduction stage: This includes reduction of phosphoglyceric acid to phosphoglyceraldehyde through 1, 3-bisphosphoglyceric acid.

(3) The regeneration stage: All other reactions are simply an attempt to convert the remaining molecules back to ribulose 1, 5-bisphosphate to make it ready for accepting another CO_2, This completes the· cycle.

(4) A fourth stage, the storage stage could also be recognised when a molecule of fructose 6- phosphate is converted to sucrose and is then diverted to storage side. This, however, is not a part of the cycle.

The details of the reactions can be seen in diagram

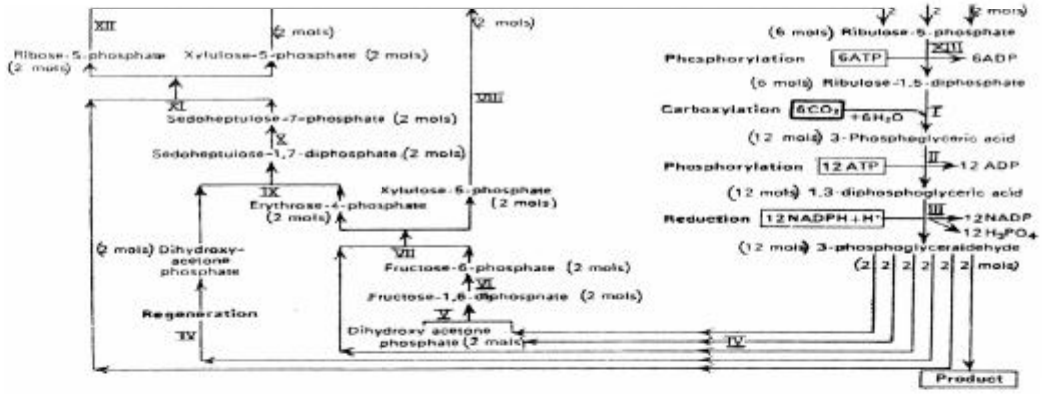

Fig 6.2 - Calvin Cycle

It can be seen from that if six ribulose 1, 5-bisphosphate molecules are taken to start with, they incorporate 6 CO_2 to form 12 molecules of triosephosphate utilizing 12 NADPH and 12 ATP. Ten molecules of these triosephosphates appear to regenerate six molecules of 5 carbon ribulose 1, 5-bisphosphate and utilize 6 ATP. Two molecules of the trioses are gained which may combine to

form one molecule of glucose. Thus for every molecule of glucose synthesized, the energy consumed in the cycle is 12 NADPH and 18 ATP.

All the enzymes required in Calvin cycle are present in the stroma of the chloroplast where these reactions take place. These enzymes are also found in the cytosol and catalyze same reactions. These are synthesized from different genes and are called isozymes. Animal systems do not have the enzyme ribulose 1, 5-bisphosphate carboxylase/oxygenase (RUBISCO), sedoheptulose 1, 7 - bisphosphatase and ribose - 5-phosphate kinase. It is the absence of these enzymes and non-availability of abundant of ATP and NADPH that the animals can not fix carbon dioxide.

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The fixation of CO_2 and synthesis of simpler carbohydrate molecules takes place only in the stroma of the chloroplast.

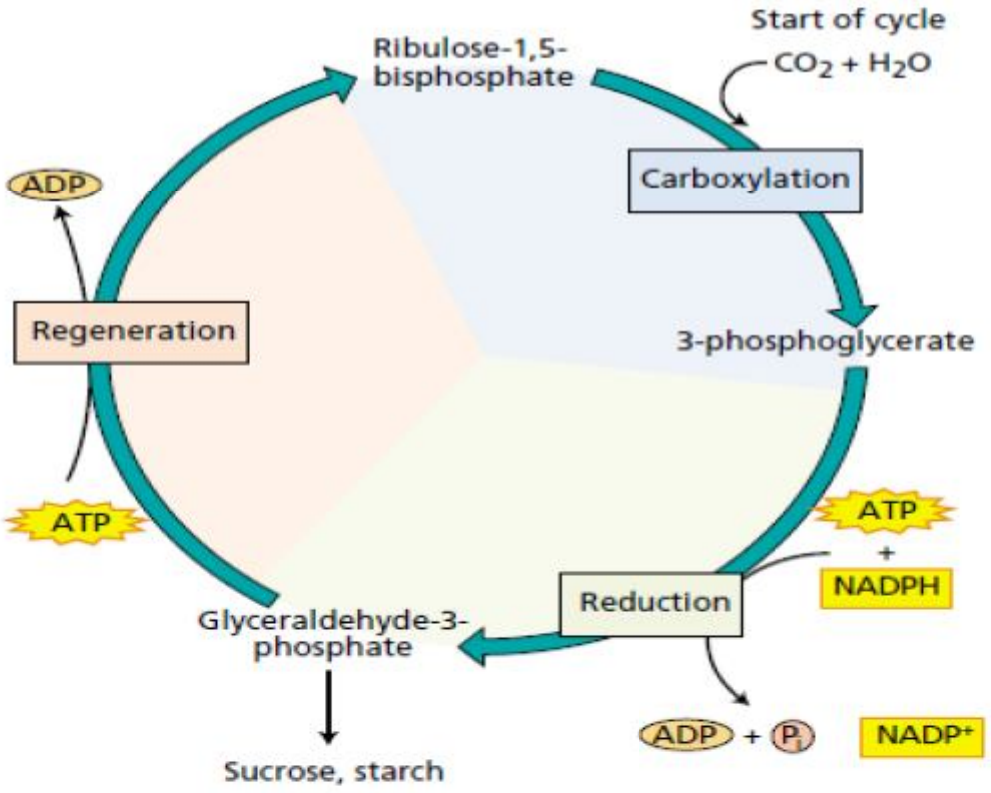

Fig 6.3—Calvin Cycle

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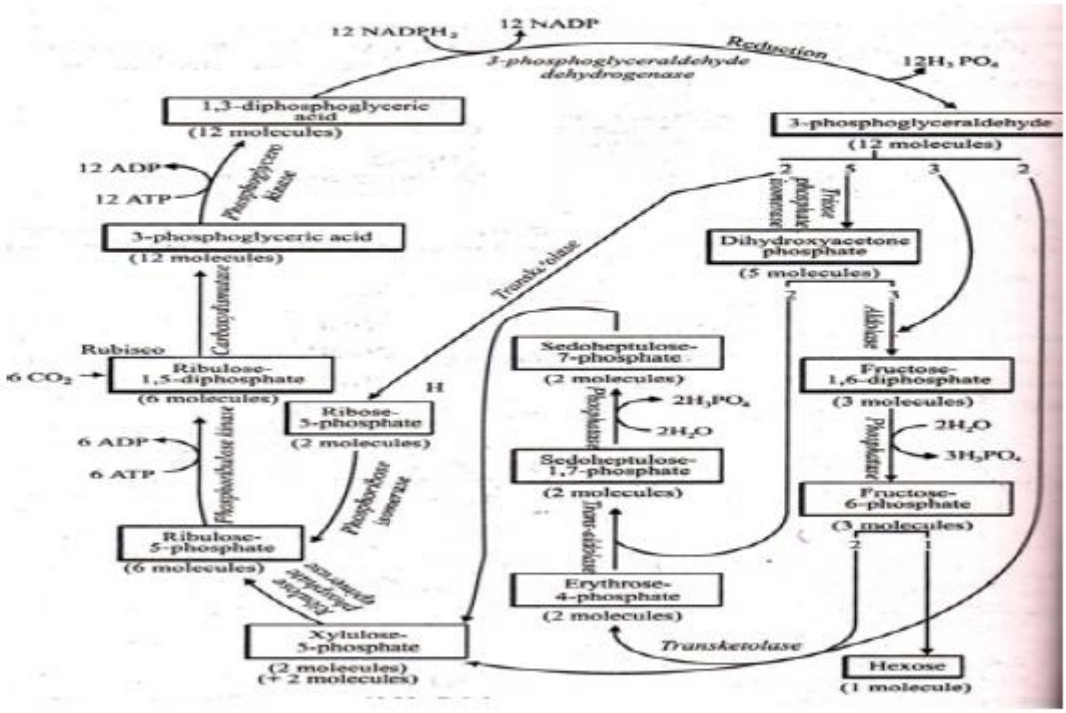

Fig 6.4 -Calvin Cycle in Step-manner

Table -1 Reactions of the Calvin Cycle

Reactions of the Calvin cycle

Enzyme	Reaction
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase	6 Ribulose-1,5-bisphosphate + 6 CO_2 + 6 H_2O → 12 (3-phosphoglycerate) + 12 H^+
2. 3-Phosphoglycerate kinase	12 (3-Phosphoglycerate) + 12 ATP → 12 (1,3-bisphosphoglycerate) + 12 ADP
3. NADP:glyceraldehyde-3-phosphate dehydrogenase	12 (1,3-Bisphosphoglycerate) + 12 NADPH + 12 H^+ → 12 glyceraldehye-3-phosphate + 12 $NADP^+$ + 12 P_i
4. Triose phosphate isomerase	5 Glyceraldehyde-3-phosphate → 5 dihydroxyacetone-3-phosphate
5. Aldolase	3 Glyceraldehyde-3-phosphate + 3 dihydroxyacetone-3-phosphate → 3 fructose-1,6-bisphosphate
6. Fructose-1,6-bisphosphatase	3 Fructose-1,6-bisphosphate + 3 H_2O → 3 fructose-6-phosphate + 3 P_i
7. Transketolase	2 Fructose-6-phosphate + 2 glyceraldehyde-3-phosphate → 2 erythrose-4-phosphate + 2 xylulose-5-phosphate
8. Aldolase	2 Erythrose-4-phosphate + 2 dihydroxyacetone-3-phosphate → 2 sedoheptulose-1,7-bisphosphate
9. Sedoheptulose-1,7,bisphosphatase	2 Sedoheptulose-1,7-bisphosphate + 2 H_2O → 2 sedoheptulose-7-phosphate + 2 P_i
10. Transketolase	2 Sedoheptulose-7-phosphate + 2 glyceraldehyde-3-phosphate → 2 ribose-5-phosphate + 2 xylulose-5-phosphate
11a. Ribulose-5-phosphate epimerase	4 Xylulose-5-phosphate → 4 ribulose-5-phosphate
11b. Ribose-5-phosphate isomerase	2 Ribose-5-phosphate → 2 ribulose-5-phosphate
12. Ribulose-5-phosphate kinase	6 Ribulose-5-phosphate + 6 ATP → 6 ribulose-1,5-bisphosphate + 6 ADP + 6 H^+
Net: 6 CO_2 + 11 H_2O + 12 NADPH + 18 ATP → Fructose-6-phosphate + 12 $NADP^+$ + 6 H^+ + 18 ADP + 17 P_i	

6.1.2 The C_4 cycle Or Hatch and Slack cycle

In Calvin cycle we have seen that ribulose 1, 5-bisphosphate fixes CO_2 and forms phosphoglyceric acid which is a 3-carbon compound. This cycle is, therefore, also known as C_3 cycle against another C_4 cycle where the first product of CO_2 fixation is a 4-carbon compound. This cycle was first reported by Kortschak and his co-workers in 1954 in Sugar cane plant later on such type of study confirmed on other plants by M.D.Hatch and C.R.Slak (1966) of Australia.

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Kortschak and his associates (1965) first observe 10 sugar cane leaves that the first product of photosynthesis was malate. Hatch and Slack confirmed this observation and also traced the whole series of reactions involved in carbon dioxide assimilation in sugar cane leaves. Since then it has been reported in a number of plants which constitute the C_4 species.

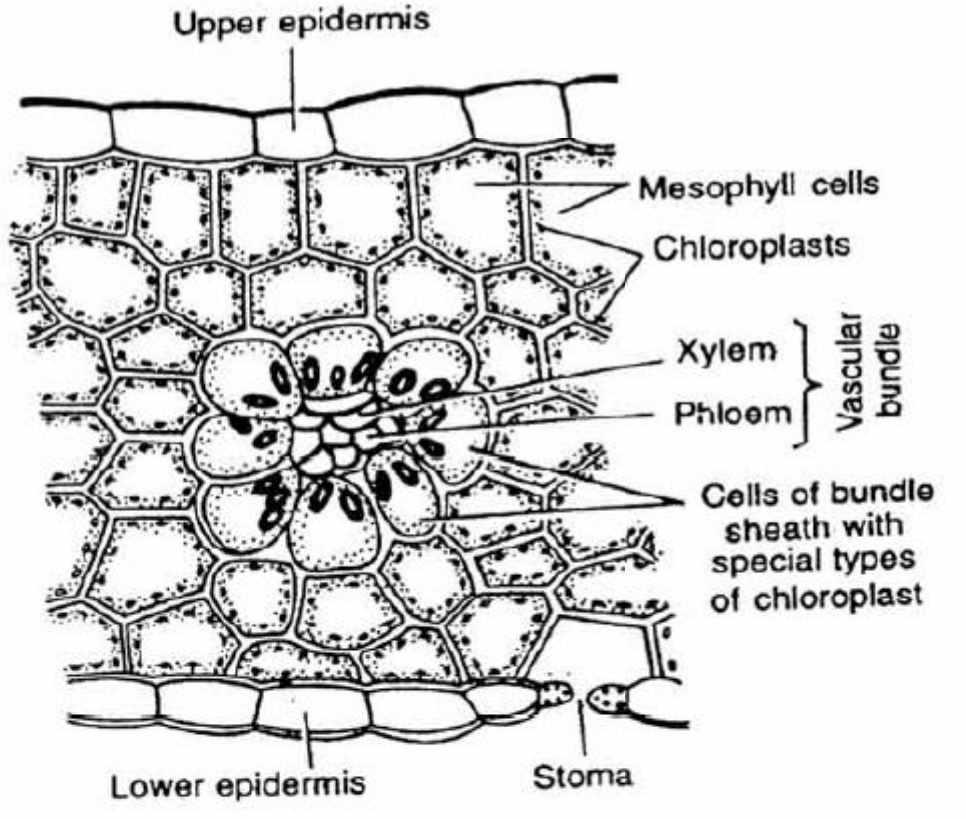

Fig 6.5 - Kranz Anatomy in C_4 Plant Leaf

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The C_4 plants are now known to occur in at least 10 families of angiosperms viz. Aizoaceae, Amaranthaceae, Asteraceae, Chenopodiaceae, Cyperaceae, Euphorbiaceae, Poaceae, Nyctaginaceae, Portulacaceae and Zygophyllaceae. Besides differing in the carbon fixation pathway from C_3 plants, the C_4 plants differ in a number of ways:

(1) The C_4 plants differ from C_3 plants in a peculiar leaf anatomy known as *Kranz anatomy*. In plants showing Kranz anatomy the vascular bundles in the leaf are bound by bundle sheath cells which contain large chloroplasts. An outer ring of mesophyll cells also surrounds the vascular bundles but they resemble mesophyll cells.

(2) The bundle sheath cells in C_4 plants have larger chloroplasts as compared to those found in mesophyll cells and in bundle sheath and mesophyll cells of C_3 plants. These chloroplasts lack grana and when mature store starch.

(3) The mesophyll cells of C_4 plants lack the key enzyme ribulose bisphosphate carboxylase although this activity is present in bundle sheath cells. This activity is present in all the cells of C_3 plants.

(4) The absence of enzyme ribulose bisphosphate carboxylase in the mesophyll cells of C_4 plants is compensated by the presence of another enzyme phospho enol pyruvate carboxylase which can fix CO_2. The enzyme fixes CO_2 with phospho enol pyruvate which is converted to oxaloacetic acid.

(5) Bundle sheath cells in the chloroplasts of C_4 plants have all the enzymes of C_3 cycle and the ultimate incorporation of carbon into carbohydrates takes place only in these cells. ' The mesophyll cells transfer the fixed CO_2 to bundle sheath cells where it is released by a process of decarboxylation and utilized in C_3 cycle. Thus, the presence of these decarboxylating enzyme is another important difference

between C_4 and C_3 species. The details of this mechanism are discussed with the C_4 cycle.

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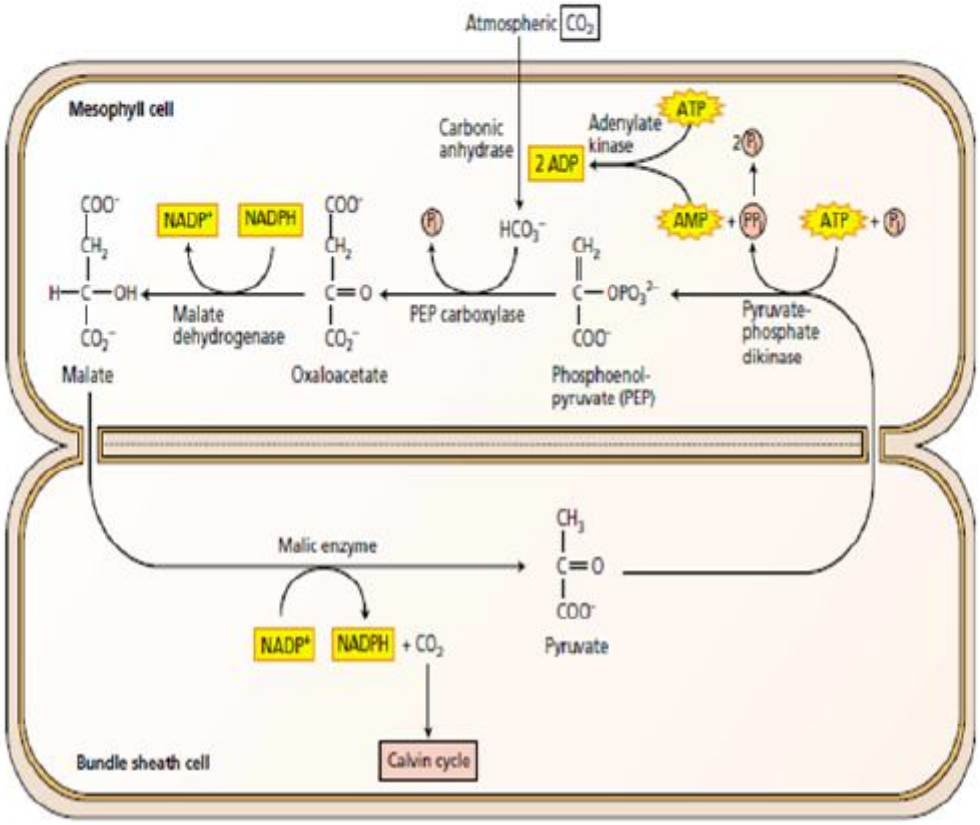

Fig 6.6 - C_4 Photosynthetic Pathway

C_4 Pathways

The C_4 plants have developed a special enzyme system by which it can fix CO_2 in the cytosol itself. In C_3 plants it is fixed in the stroma of the chloroplast.

The C_4 carbon assimilation starh with the condensatlon of CO_2 with phosphoeno pyruvate in the cytosol of the mesophyll cells. The reaction is catalyzed by the enzyme *phosphoenol pyruvate carboxylase(PEPCase).* It yields oxaloacetic acid releasing inorganic phosphate.

The oxaloacetic acid may next be converted to malic acid or aspartic acid. Thus at least two types of C_4 pathways can be recognised as shown in. The two types of pathways are discussed separately as:

(l)Malate pathway

(2) Aspartate pathway

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I. Malate pathway: The whole pathway is discussed in the following steps--

i. The Oxaloacetic acid (OAA) formed in the cytosol of mesophyll cells is transported to the chloroplast in the mesophyll cells. Here the OAA is reduced to malic acid (MA) by the enzyme malate dehydrogenase utilizing the reducing power of $NADPH^{+}H^{+}$

ii. The malic acid is transported to the bundle sheath cells through the plasmodesmata and ultimately reaches the chloroplast stroma. Here the malic enzyme catalyzes the conversion of malic acid to pyruvic acid and CO_2. It utilizes NADP and regenerates $NADPH^{+}H^{+}$ This pyruvic acid is transported back to the chloroplast in the mesophyll cells and the CO_2 condenses with ribulose 1,5 bisphosphate in the Calvin cycle operating in the stroma of bundle sheath chloroplast.

iii. The pyruvic acid is converted to phosphoenol pyruvic acid by the enzyme pyruvate phosphate dikinase at the expense of ATP and inorganic phosphate. It releases AMP and pyrophosphate.

This enzyme in the chloroplasts of C_4 plants is a unique enzyme in the sense that it is light activated and yet utilizes ATP. The pyrophosphate is converted to inorganic phosphate by the enzyme pyrophosphatase. .

Thus, the cycle is complete. The phosphoenol pyruvic acid has transported CO_2 from the mesophyll to the bundle sheath chloroplast and is available again for the next cycle.

II. Aspartate –Pathway: The whole pathway is discussed in the following steps--

i. In this pathway oxaloacetic acid is not transported to the chloro plast of mesophyll cells as malate. Here it is aminated to aspartic acid. The reaction is catalyzed by aspartate aminotransferase. The amino donor is glutamic acid.

ii. The aspartic acid in this pathway is the first stable 4-carbon compound. It is transported to the mitochondria of the bundle sheath cells and not to the chloroplast. In the mitochondria it is deaminated giving back oxaloacetic acid. The enzyme involved is amino transferase and the amino acceptor is ketoglutaric acid which forms glutamic acid. The enzyme is again aspartate amino transferase.

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iii. In the next step oxaloacetic acid is converted to malic acid by the enzyme malate dehydrogenase. The reaction is the same that occurred in of malate type but the site was chloroplast. This reaction in the aspartate type is taking place in mitochondria and hence the reducing agent will be NADH and not NADPH.

iv. The malic acid is now decarboxylated releasing CO_2 and forming pyruvic acid. The reaction is catalyzed by the enzyme malic enzyme.

v. The carbon dioxide goes to the Calvin cycle in the stroma of chloroplast and condenses with ribulose 1, 5-bisphosphate.

vi. In the next step pyruvic acid is aminated to alanine by the enzyme alanine amino transferase. The amino donor is glutamic acid which changes to ketoglutaric acid. It seems to be shuttling between steps.

vii. The alanine is transported out into the mesophyll cell where it is deaminated to release pyruvic acid to the chloroplast matrix. Deamination is catalysed again by the enzyme alanine amino transferase. a-ketoglutaric acid is the amino acceptor.

viii. In the mesophyll chloroplast, the pyruvic acid is phosphorylated to phosphoenol pyruvic acid. It is catalysed by the enzyme pyruvate dikinase and uses ATP. It also requires Mg^{2+}.

The phosphoenol pyruvic acid just formed is transported out into the cytosol of mesophyll cells where it is ready to accept and fix CO_2 for another cycle.

In some of the aspartate type C_4 pathways the oxaloacetic acid in the mitochondria of the bundle sheath cells is directly transported to the cytosol without being converted to malic acid. The oxalo-acetate is decarboxylated

producing phosphoenol pyruvate by the enzyme phosphoenol pyruvate carboxy kinase. It utilizes GTP.

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This phosphoenol pyruvate is processed normally as in the aspartate pathway. Thus, it is only a short cut to get phosphoenol pyruvate. The CO_2 enters the Calvin cycle in chloroplast.

C_4 is an expensive pathway .The C_4 pathway, discussed above; do not seem to be different from C_3. Instead, in all such cycles, ultimately it is the C_3 cycle which is being used to fix CO_2. The only difference is that in C_4 species, the C_3 enzymes particularly the RUBISCO is not present in the mesophyll cells. This enzyme is present only in the bundle sheath cells and hence completes carbon fixation via C_3 cycle only in those tissues. The C_4 pathway enzymes appear to be only supporting the transport of CO2 from the mesophyll cells to the chloroplast of bundle sheath cells. In this process it appears to consume 2 ATP molecules per CO_2 transpor1ed. Thus the process in true sense appears to be a process, of active transport where the transport of CO_2 molecules is driven by the hydrolysis of one molecule of ATP forming AMP and pyrophosphate.

This comes to an energy equivalent of two A TP molecules

The total energy required to synthesize one molecule of glucose (6 carbon) will be :

C_4 - 6.CO_2 mesophyll ---. 6CO_2 bundle sheath (consumed energy 12 ATP)

C_3 - 6.CO_2 bundle sheath 1 hexose

(consumed energy 18 ATP + 12 NADPH) The combined reaction will be

$$\mathbf{6\,CO_2 + 30\,ATP + 12\,NADPH + 12\,H_2O \rightarrow C_6H_{12}O_6 + 30\,ADP + 30\,Pi + 12\,NADP^+ + 18\,H^+}$$

Thus, completing CO_2 fixation via the C_4 cycle consumes 30 ATP molecules per hexose synthesized. Now if we look at only the C_3 cycle, it consumes only 18 ATP.

Thus C_4 cycle is much expensive than C_3.

Table 1 Comparison of C_3 and C_4 plants

Characters	C_3 Plants	C_4 Plants
Cell type	One (mesophyll)	Two (mesophyll and bundle-sheath)
Kranz anatomy	No	Yes
Chloroplasts	One type (granal only)	Two types (granal and agranal)
CO_2 acceptor	RuBP	PEP
First CO_2 fixation product	3-PGA (3C compound)	Oxaloacetic acid (4C compound)
Carboxylase enzyme	Rubisco	PEPcase; Rubisco
CO_2 fixation rate	Low	High
O_2 inhibition of photosynthesis	Yes	No
Photorespiration	High	Negligible
Productivity	Low	High
CO_2 compensation point	High (25-100 μ $CO_2.l^{-1}$)	Low (0-10 μl co_2. l^{-1})
Temperature optimum	20°C - 25°C	30°C - 45°C
Examples	Rice, wheat, potato	Maize, pearl millet, *Amaranthus*

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6.1.3 The CAM pathway

Crassulacean Acid Metabolism

There is yet another photosynthetic system which involves the operation of C_3 (Ribulose 1-') bisphosphate carboxylase/oxygenase i.e. RUBISCO) and C_4 (Phosphoenol pyruvate carboxylase, PEPC) in the same cell, though the two enzymc activities are temporally separated not spatially separated. Such a system is found usually in succulents initially found in family crassulaceae. This system is, therefore, known as crassulacean acid metabolism or simply CAM pathway.

Table - 2

Examples of C4 and CAM plants

Family	Common examples
C4 plants	
Amaranthaceae	Amaranths
Asteraceae	Asters and daisies
Euphorbiaceae	Euphorbias
Poaceae	Grasses including corn (maize), sugarcane and sorghum
Nyctaginaceae	Bougainvillea
CAM plants	
Agavaceae	Agaves
Asteraceae	Asters and daisies
Cactaceae	Cacti
Crassulaceae	Crassulas
Euphorbiaceae	Euphorbias
Liliaceae	Lilies
Orchidaceae	Orchids
Vitaceae	Grape vines

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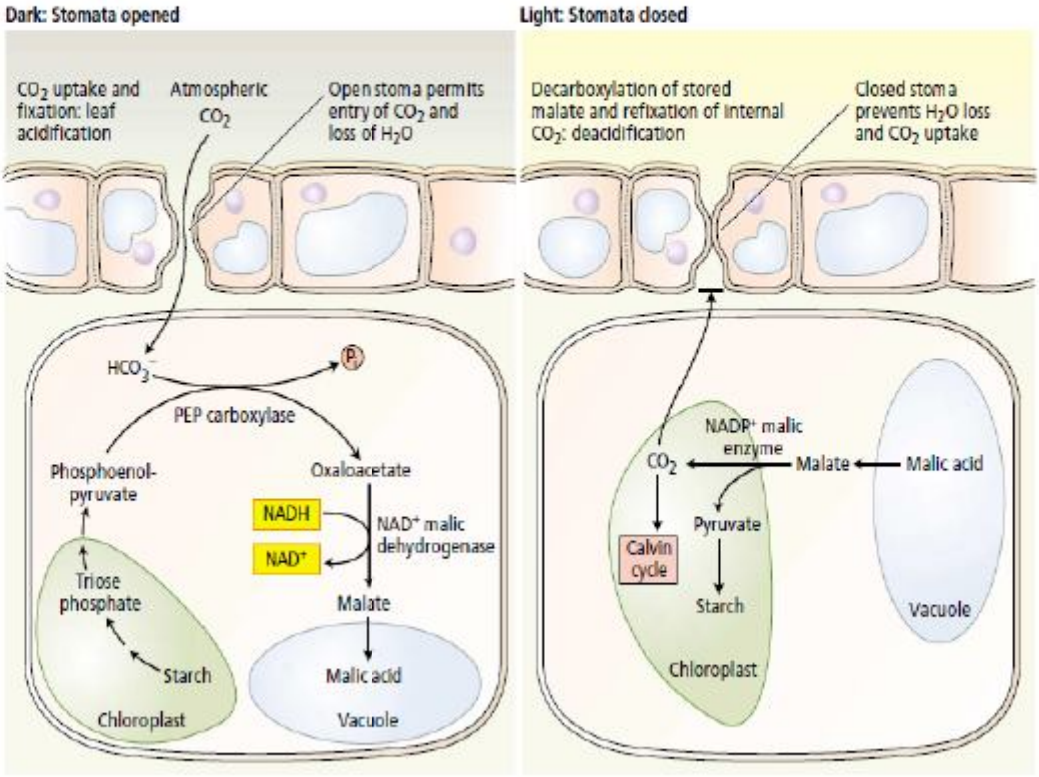

Fig 6.7 - The CAM Pathway

The CAM plants have their stomata closed in the day time and open in night. Therefore, these plants take CO_2 in the night and store it in the form of malate in the cell vacuole. The malatc is broken down and sugars are synthesized during the following day. This pathway, thus is similar to C_4 pathway but the plants do not have difference in bundle sheath and mesophyll cells. The CL\M-pathway has been detected in about 1000 plants belonging to 17 different families. This pathway appears to be an ecological adaptation.

The diurnal changes in photosynthetic process of CAM plants can be divided into 4 stages.

(1) Night hours-the nocturnal uptake of CO_2 through open stomata and its fixation by phosphoenol pyruvate carboxylase (PEPC) in the fonn of malate. Thus, CO_2 in this form (malate) is stored in vacuoles.

(2) Morning hours-the early light period when stomata remain open for CO_2 uptake for a shon period.

(3) Day time - when the stomata are closed at high temperature. The vacuolar organic acid (usually malate) stored in previous night is remobilized. It is decarboxylated to pyruvate and the CO_2 released is fixed via Calvin cycle behind closed stomata.

(4) Evening hours-the stomata reopen during late light period when the temperature is moderate. A direct CO_2 uptake takes place at this stage when malate has exhausted.

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The first reaction of the process is the fixation of CO_2. The CO_2 from the atmosphere enters the mesophyll through the stomata which are open in night. Since the light energy is not available in night, the photosynthetic fixation of CO_2 is not possible and when light is available (day time) the stomata remain closed due to intense heat and CO_2 can not enter the mesophyll.

The plants are, therefore, adapted to keep the stomata open in night and harvest CO_2 and store in the form of malate. The reaction is catalysed by the enzyme phosphoenol pyruvate N, carboxylase (PEPC). The enzyme condenses CO_2 m (as bicarbonate) with phosphoenol pyruvate which is available in the cytosol by glycolytic breakdown of starch or sugars and forms oxaloacetate.

In the second step oxaloacetate is reduced to malate by the enzyme malate dehydrogenase and the coenzyme $NADH^+H^+$.

The malate, so synthosized, is transported into the central vacuole through specific transporters. This transport usually is passive and is associated with active transport of proton via ATPase pump.

In the following day hours the reverse reaction takes place. The malate is transported out of the vacuole. This export of malate (or the impOlt), at least one of the two processes needs ATP. To meet this ATP requirement some of the malate is channeled through mitochondria through TCA cycle to produce $NADH^+H^+$ which ultimately goes to synthesize ATP. The malate is decarboxylated to pyruvate and CO_2 by malic enzyme and $NADP^+$.

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In the early light hours, the stomata remain open and CO_2 is taken up, but is directed to C_3 pathway (Calvin cycle) instead of forming malate. This switch also lies with the enzyme PEPC. The affinity of PEPC for CO_2 is much higher than rubisco and it can utilize even traces of CO_2. Hence, in the presence of PEPC, rubisco remains at a low state of activation. With the beginning of the dawn the PEPC is deactivated by dephosphorylation (a switch controlled by PEPC-kinase). This makes rubisco active, which then picks up the CO_2 released from malate into the Calvin cycle. Since, in the morning hours when the temperature and light both are moderate, the stomata remain open. This favours direct CO_2 fixation via C_3 pathway.

The malate is synthesized from phosphoenol pyruvate which comes from stored carbohydrates. On the basis of carbohydrate source utilized two types of CAM plants may be recognized, those which utilize starch from the chloroplast and those which utilize sugars in the cytosol. In either case, however, this results in the loss of some stored carbon but when compared to C_3 plants, it is more economical. C_3 plants loose about 20% of fixed CO_2 in photorespiration. CAM plants do not have photorespiration. Further, in much higher light intensities the net gain in CO_2 fixation is much higher.

6.2 Photorespiration and its significance

Photorespiration is also known as C_2 cycle, basically it involves the light dependent uptake of O_2 and evolution of CO_2. RUBISCO is a bifunctional enzyme i.e, it has both the carboxylase activity and the oxygenase activity. It is believed to have appeared early in evolution when the atmosphere was reducing and there was almost no oxygen.

It was thus selected to fix CO_2 in an atmosphere devoid of oxygen. Gradually when oxygen concentration became higher in the atmosphere it also developed oxygenase activity. Thus, the present day enzyme is not specific for CO_2 as substrate and oxygen seems to be competing with CO_2. As a result RUBISCO catalyzes condensation of oxygen with ribulose 1, 5-bishosphate and forms 3-phosphoglyceric acid and phosphoglycolate. The enzyme at the same time also

performs carboxylase activity and condenses CO_2 with ribulose 1, 5-bisphosphate. The carbon fixation rate is about 4-5 times that ofoxidation. Since RUBIS CO is activated only in the presence of CO_2 and light and since it is involved in condensation of oxygen with the same substrate (ribulose 1,5-bisphosphate) with release of CO_2 at a later stage, the process is known as photorespiration). This mechanism of oxygen utilization and release of CO_2 can not take place in dark. When light is available CO_2 is consumed in the internal atmosphere of leaf tissues with a simultaneous increase in O_2. This increased O_2 concentration effectively competes with the CO_2.

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This is more pronounced at higher temperatures when affinity of RUBISCO for CO_2 decreases. It is however, a wasteful process since it ultimately looses a carbon atom as CO_2.

One of the oxidation products of ribulose 1, 5-biphosphate, the 3-phosphoglyceric acid, reenters the Calvin cycle while the phosphoglycolate is channeled through a pathway known as the glycolate pathway. It involves three important cell organelles, the chloroplast, the peroxisomes and the mitochondria. The pathway is shown in figure below.

The reaction takes place in the following steps:

(1) Rubisco- ribulose 1, 5-bisphosphate oxygenase catalyzes the condensation of O_2 with ribulose 1, 5-bisphosphate from the Calvin cycle and leads to the formation of 3-phosphoglyceric acid and phosphoglycolate. 3-phosphoglyceric acid enters again into the Calvin cycle.

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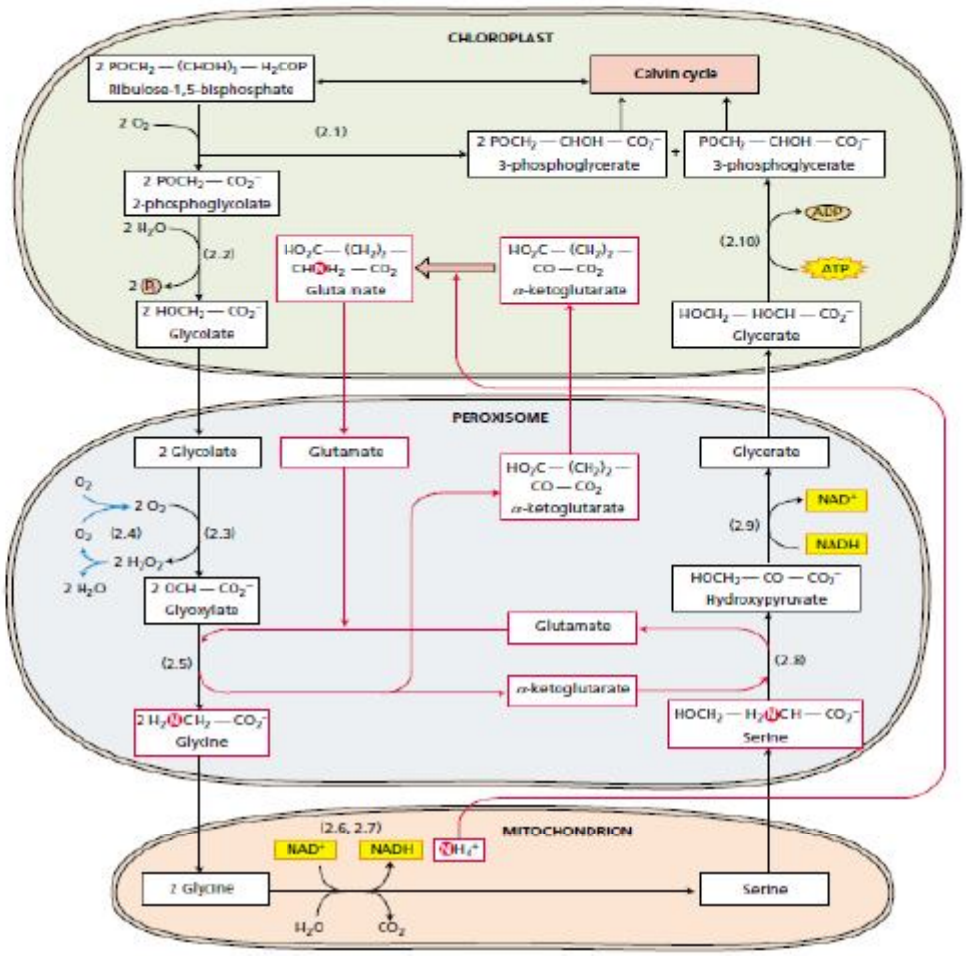

Fig 6.8 - Photorespiration involves three organelles

The reaction proceeds through an enzyme bound unstable intermediate.

(2) This step, takes place in the chloroplast stroma. A specific phosphatase catalyses it and converts glycolate phosphate to glycolate releasing an inorganic phosphate.

(3) Glycolate is now transported to peroxisome; a cell organelle intermediated between chloroplast on one hand and mitochondria on the other. In peroxisomes glycolate is oxidized to glyoxylate by an enzyme glycolate oxidase. A molecule of H_2O_2 is also formed utilizing O_2.

This H_2O_2 is immediate converted to water and molecular oxygen by the enzyme catalase present in peroxisomes.

(4) Glyoxylate now reacts with serine within the peroxisome to form glycine and hydroxypyruvate. This is a reaction involving transfer of amino group from serine to glyoxylate. It is catalyzed by the enzyme serine - glyoxylate amino transferase.

(5) In the next step glycine is transported to mitochondria where two glycine molecules condense to form serine utilizing oxygen and releasing NH_3 and CO_2.The ammonia is utilized in synthesis of glutamine. It is at this stage that an organic carbon is lost as CO_2 without production of any ATP. This is the reason why photorespiration is called a wasteful process.The serine synthesized in this step is transported to peroxisomes, where it will be ready to transfer amino group to glyoxylate again and the cycle continues.

(6) The hydroxy pyruvate formed in peroxisomes as a result of transamination in step 4 is now -reduced to glycerate. The reaction is catalyzed by the enzyme hydroxypyruvate reductase and utilizes $NADH^+H^+$ .The glyceric acid (glycerate) just formed is transported from the peroxisomes to the chloroplast matrix.

(7) The glycerate in chloroplast matrix is phosphorylated to 3-phosphoglyceric acid by the enzyme glycerate kinase using phosphate from ATP. This 3-phosphoglyceric acid now enters the Calvin cycle.

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Significance of Photorespiration

Although the C_2 oxidative photosynthetic carbon cycle recovers 75% of the carbon originally lost from the Calvin cycle as 2-phosphoglycolate, why does 2-phosphoglycolate form at all? One possible explanation is that the formation of 2-phosphoglycolate is a consequence of the chemistry of the carboxylation reaction, which requires an intermediate that can react with both CO_2 and O_2.

Such a reaction would have had little consequence in early evolutionary times if the ratio of CO_2 to O_2 in air were higher than it is today. However, the low CO_2: O_2 ratios prevalent in modern times are conducive to photorespirat ion, with no other function than the recovery of some of the carbon present in 2-phosphoglycolate.

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Another possible explanation is that photorespiration is important, especially under conditions of high light intensity and low intercellular CO_2 concentration (e.g., when stomata are closed because of water stress), to dissipate excess ATP and reducing power from the light reactions, thus preventing damage to the photosynthetic apparatus. ArabidoPS-Is mutants that are unable to photorespire grow normally under 2% CO_2, but they die rapidly if transferred to normal air. There is evidence from work with transgenic plants that photorespiration protects C_3 plants from photooxidation and photoinhibition (Kozaki and Takeba 1996). Further work is needed to improve our understanding of the function of Photorespiration.

6.3 Physiological and Ecological considerations

The impact of the environment on photosynthesis is of interest to both plant physiologists and agronomists. From a physiological stand-point, we wish to understand how photosynthesis responds to environmental factors such as light, ambient CO_2 concentrations, and temperature. In studying the environmental dependence of photosynthesis, a central question arises. How many environmental factors can limit photosynthesis at one time? The British plant physiologist F. F. Blackman hypothesized in 1905 that, under any particular conditions, the rate of photosynthesis is limited by the slowest step, the so-called *limiting factor*.

Three major metabolic steps have been identified as important for optimal photosynthetic performance:

(i) Rubisco activity

(ii) Regeneration of ribulose bisphosphate (RuBP)

(iii) Metabolism of the triose phosphates.

In the following sections, biophysical, biochemical, and environmental aspects of photosynthesis in leaves are discussed in detail.

Leaves also adapt to high light conditions, illustrating that plants are physiologically flexible and that they adapt to their immediate environment. Both the amount of light and the amount of CO2 determine the photosynthetic response of leaves. In some situations, photosynthesis is limited by an inadequate supply of light or CO2. In other situations, absorption of too much

light can cause severe problems, and special mechanisms protect the photosynthetic system from excessive light. Multiple levels of control over photosynthesis allow plants to grow successfully in a constantly changing environment and different habitats.

Role of Light

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Three light parameters are especially important in the measurement of light: (1) spectral quality, (2) amount, and (3) direction. Question arise that how to quantify light, it is important to match sensor geometry and spectral response with that of the plant. Flat, cosine-corrected sensors are ideally suited to measure the amount of light that strikes the surface of a leaf; spherical sensors are more appropriate in other situations, such as in studies of a chloroplast suspension or a branch from a tree.

The anatomy of the leaf is also highly specialized for light Absorption. The outermost cell layer, the epidermis, is typically transparent to visible light, and the individual cells are often convex. Convex epidermal cells can act as lenses and can focus light so that the amount reaching some of the chloroplasts can be many times greater than the amount of ambient light. Epidermal focusing is common among herbaceous plants and is especially prominent among tropical plants that grow in the forest understory, where light levels are very low. Some environments, such as deserts, have so much light that it is potentially harmful to leaves. In these environments leaves often have special anatomic features, such as hairs, salt glands, and epicuticular wax that increase the reflection of light from the leaf surface, thereby reducing light absorption. Such adaptations can decrease light absorption by as much as 40%, minimizing heating and other problems associated with the absorption of too much light.

Leaf and dissipitation of heat

When exposed to excess light, leaves must dissipate the surplus absorbed light energy so that it does not harm the photosynthetic apparatus. The heat load on a leaf exposed to full sunlight is very high. In fact, a leaf with an effective thickness of water of 300 m would warm up by 100°C every minute if all available solar energy were absorbed and no heat were lost. However, this enormous heat load is dissipated by the emission of longwave radiation, by sensible (i.e., perceptible) heat loss, and by evaporative (or latent) heat loss.

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Effect of Cabon di oxide on Photosynthesis

CO2 diffuses from the atmosphere into leaves—first through stomata, then through the intercellular air spaces, and ultimately into cells and chloroplasts. In the presence of adequate amounts of light, higher CO2 concentrations support higher photosynthetic rates. The reverse is also true; that is, low CO2 concentration can limit the amount of photosynthesis.

Carbon dioxide is a trace gas in the atmosphere, presently accounting for about 0.037%, or 370 parts per million (ppm), of air. Certain gases in the atmosphere, particularly CO_2 and methane, play the same role as the glass roof in a greenhouse. The increased CO_2 concentration and temperature associated with the greenhouse effect can influence photosynthesis. At current atmospheric CO_2 concentrations, photosynthesis in C_3 plants is CO_2 limited (as we will discuss later in the chapter), but this situation could change as atmospheric CO_2 concentrations continue to rise. Under laboratory conditions, most C3 plants grow 30 to 60% faster when CO_2 concentration is doubled (to 600–700 ppm), and the growth rate changes depend on nutrient status (Bowes 1993). In some plants the enhanced growth is only temporary. For many crops, such as tomatoes, lettuce, cucumbers, and roses growing in greenhouses under optimal nutrition, carbon dioxide enrichment in the greenhouse environment results in increased productivity. The photosynthetic performance of C_3 plants under elevated CO_2 is enhanced because photorespiration decreases.

Effect of Temparature on Photosynthesis

Temperature affects all biochemical reactions of photosynthesis, so it is not surprising that the responses to temperature are complex. Respiration rates also increase as a function of temperature, and the interaction between photorespiration and photosynthesis becomes apparent in temperature responses. These changes in photosynthetic properties in response to temperature play an important role in plant adaptations to different environments. Plants are remarkably plastic in their adaptations to temperature. In the lower temperature range, plants growing in alpine areas are capable of net CO_2uptake at temperatures close to 0°C; at the other extreme, plants living in Death Valley, California, have optimal rates of photosynthesis at temperatures approaching 50°C.

6.4 Summary

The reduction of CO_2 to carbohydrate is coupled to the consumption of NADPH and ATP synthesized by the light reactions of thylakoid membranes. Photosynthetic eukaryotes reduce CO_2 via the Calvin cycle that takes place in the stroma, of chloroplasts. Here, CO_2 and water are combined with ribulose-1,5-bisphosphate to form two molecules of 3-phosphoglycerate, which are reduced and converted to carbohydrate.The continued operation of the cycle is success by the regeneration of ribulose-1,5-bisphosphate. The Calvin cycle consumes two molecules of NADPH and three molecules of ATP for every CO_2 fixed and, provided these substrates, has a thermodynamic efficiency close to 90%.

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Rubisco, the enzyme that catalyzes the carboxylation of ribulose-1,5-bisphosphate, also acts as an oxygenase. In both cases the enzyme must be carbamylated to be fully active. The carboxylation and oxygenation reactions take place at the active site of rubisco. When reacting with oxygen, rubisco produces 2-phosphoglycolate and 3-phosphoglycerate from ribulose-1,5-bisphosphate rather than two 3-phosphoglycerates as with CO_2, thereby decreasing the efficiency of photosynthesis.

The C_2 oxidative photosynthetic carbon cycle rescues the carbon lost as 2-phosphoglycolate by rubisco oxygenase activity. The dissipative effects of photorespiration are avoided in some plants by mechanisms that concentrate CO_2 at the carboxylation sites in the chloroplast. These mechanisms include a C_4 photosynthetic carbon cycle, CAM metabolism, and "CO_2 pumps" of algae and cyanobacteria.

6.5 Glossary

- **ATP :** Adenosine triphosphate, a small water soluble molecule that acts as an energy currency in cells.
- **ATP Synthase :** A membrane bound protein complex that uses the energy stored across the photosynthetic membrane to add inorganic phosphate to ADP, thus creating ATP. (Also known as coupling factor.)

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- **Autotroph :** photosynthetic organisms which convert light energy into the chemical energy they need to develop, grow, and reproduce.
- **Calvin Cycle :** The biochemical reactions, initiated by Rubisco, that result in the reduction of CO_2 to a carbohydrate (also known as the photosynthetic carbon reduction cycle).
- **Carbon fixation :** ATP and NADPH are used to fix CO_2 into carbohydrates. Carbon fixation takes place in the chloroplast stroma.
- **Cytochrome :** Heme containing protein.
- **Cytochrome b/c Complex :** A membrane bound electron transfer protein complex found in all anoxygenic photosynthetic organisms that oxidizes reduced quinone and reduces a c-type cytochrome. The complex contains a c-type cytochrome, two b-type cytochromes and a FeS center.
- **Cytochrome b/f Complex :** A membrane bound electron transfer protein complex found in all oxygenic photosynthetic organisms that oxidizes reduced plastoquinone and reduces plastocyanin (or cytochrome c). The complex contains a c-type cytochrome, two b-type cytochromes and an FeS center.
- **Free Energy :** The amount of energy in a reaction available to do work. Because most biochemical reactions occur at a constant temperature and pressure, the free energy is frequently the Gibbs Energy.
- **Light :** Electromagnetic radiation; the shorter the wavelength the greater amount of energy. Light supplies the energy for the light reactions of photosynthesis.
- **Light Harvesting Complex :** A protein complex that harvests light energy and converts it to exciton energy that can migrate to a reaction center. The light is absorbed by pigment molecules (*e.g.*, chlorophyll, bacteriochlorophyll, carotenoids, phycobilin) that are attached to the protein.

- **Lumen : R**egion within the thylakoid membrane where water is split to obtain oxygen. The oxygen diffuses out of the cell, while the protons remain inside to build positive electrical charge inside the thylakoid.
- **NADPH : R**educed form of nicotinomide adenine dinucleotide phosphate, a small water soluble molecule that acts as a hydrogen carrier in biochemical reactions.
- **NADP+ :** Oxidized form of nicotinomide adenine dinucleotide phosphate.
- **Phosphorylation :** The covalent attachment of a phosphate group to a molecule.
- **Photorespiration :** The removal of O_2 from the atmosphere by Rubisco and the subsequent biochemical reactions that serve to recycle some of the reduced carbon.
- **Photosynthesis :** The physical-chemical process by which certain chlorophyll (or bacteriochlorophyll) containing organisms use light energy for the biosynthesis of organic molecules.
- **Photosynthetic Membrane :** A bilayer of lipid molecules in which are embedded proteins that transform light energy into chemical free energy. (Also known as the thylakoid membrane.)
- **Photosystem :** a cluster of chlorophyll and other molecules in a thylakoid that harvest the energy of light for photosynthesis
- **Photosystem I :** A protein complex located in the photosynthetic membrane. Photosystem I is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem I reaction center uses light energy to transfer an electron from a mobile electron transfer protein (plastocyanin or a cytochrome c) on one side of the photosynthetic membrane to a mobile electron transfer protein (ferredoxin) on the opposite side of the photosynthetic membrane.

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- **Photosystem II :** A protein complex found in the photosynthetic membrane. Photosystem II is one of two types of reaction centers found in higher plants, algae and cyanobacteria. The photosystem II reaction center uses light energy to transfer electrons from water to plastoquinone. Photosystem II is the source of the molecular oxygen in the atmosphere.
- **Plastoquinone :** A small organic molecule involved in electron and proton transfer in photosynthesis.
- **Protein :** A chemical structure composed of one or more polypeptides. In photosynthesis proteins serve as the scaffolding that holds the cofactors that gather light energy, transfer electrons, and catalyze biochemical reactions.
- **Reaction Center :** A protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center.
- **Reduction :** The addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added.
- **Rubisco :** (D-ribulose 1,5-bisphosphate carboxylase/oxygenase) A water soluble protein complex responsible for the removal of CO_2 from the atmosphere. The enzyme works by attaching CO_2 to a five-carbon compound (1,5 ribulose bisphosphate) that is split into two identical three-carbon compounds (phosphoglycerate). In addition to catalyzing the removal of CO2 from the atmosphere, Rubisco also catalyzes the removal of O2 from the atmosphere (less efficiently). The removal of O_2 is thought to be a consequence of poor design and leads to a complex set of compensatory reactions known as photorespiration.
- **Thylakoid : D**isc-shaped portion of chloroplast, found in stacks.

6.6 Self-Learning Excercise

NOTES

Section A (Very Short Answer Type Question)

1. What is Kranz anatomy?
2. Name three plants of CAM type.
3. Define photorespiration?
4. What is *RUBISCO*?
5. What do you mean by PEP case?
6. What is compensation point?
7. What is Warburg effect?

Section B (Short Answer Type Questions)

1. Write general characters of C_4 plants.
2. Differentiate between C_3 and C4 plants.
3. Write a note on anatomy of C_4 plants.
4. Describe CAM cycle and its significance.
5. Briefly explain C_2 cycle.

Section C (Long Answer Type Questions)

1 Give a general account on various path of carbon fixation.

2 Describe C_4 cycle and its significance.

3 Describe the ecological consideration of photosynthesis.

4 Describe Calvin cycle.

Answer Key of Section A

1. In C_4 plants bundle sheath cells surrounded by one or more wreath like layers of mesophyll cells such type of arrangement is called *Kranz anatomy*
2. Grape wine, orchids
3 Release of carbon di oxide in presence of light

4 Ribulose 1,5-bi phosphate Carboxylase

5 Phospho enol pyruvate Carboxylase

6 It is the amount of light intensity on which rate of photosynthesis is exactly matches the rate .of respiration

NOTES

7 It is decrease of photosynthesis by high oxygen concentration

6.7 References

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Unit - 7

Carbohydrates and Lipids

NOTES

Structure of the Unit

7.0 Objective

After studying, Carbohydrates & lipid you will be able to know

- Different types of carbohydrates & lipids structure
- Process of biological oxidation of Carbohydrates.

7.1 Introduction

Carbohydrates are synthesized from CO_2 and H_2O in Chlorophyl containing plants during Photosynthesis. They are made up of Carban, hydrogen and Oxygon and were originally represented as hydrates of carbon with empirical formula e.g. deoxyribose ($C_5H_{10}O_4$) and glycosamine ($C_6H_{13}O_5N$). Laeticacid ($C_3H_6O_3$) with this general formula is not a carbohydrate Carbohydrates are defined as the compounds having either an aldehyde or a ketone group are modified aldehyde or ketone group and other carban atoms with Lialcoholic (-OH) groups.

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The name carbohydrate indicates that they are hydrates of carbon and contain carbon, hydrogen and oxygen. Most of them contain hydrogen and oxygen in the ratio of 2:1. For that reason, the general empirical formula of carbohydrates is given as $[C(H_2O)]_n$ *e.g.*, glucose ($C_6H_{12}O_6$), fructose ($C_6H_{12}O_6$) and sucrose ($C_{12}H_{22}O_{11}$) have the ratio of hydrogen to oxygen as 2:1 as in water. There are certain other sugars like rhamnose ($C_6H_{12}O_5$) and sorbitol ($C_6H_{14}O_6$) where this ratio of hydrogen to oxygen is not like water. There are certain other organic compounds like formaldehyde (H.CHO), acetaldehyde (CH_3.CHO), and lactic acid (CH_3.CHOH.COOH) which contain C,H and O and the ratio of H:O is also the same as in water, but are not carbohydrates.

Thus, *carbohydrates are substances which are either polyhydroxy aldehydes or ketones or are substances that yield polyhydroxy aldehydes or ketones on hydrolysis*, *e.g.* glucose is a polyhydroxy aldehyde and fructose a polyhydroxy ketone.

The carbohydrates formed in photosynthesis plays an important role in the life of plants and animals. These molecules are store and transport energy that is utilized in various biochemical and physiological processes in the cell. The carbohydrates in a living cell are in a constant flux participating in many enzyme catalyzed reactions. This is necessary to convert bond energy into chemical energy for the growth and development of the cell.

A large number of carbohydrates isolated from the plants are components of the cell wall, protoplasm and cell-sap while others accumulate as insoluble storage products.

Carbohydrates occur in grains, tubers, roots, flowers, fruits and in certain other secretions. In grains, tubers and roots, the carbohydrates are starch and cellulose and form the staple food for men. The wood produced by plants is cellulose. The nectar contains cane-sugar and glucose. Fruits and juices of various plants also contain cane-sugar and glucose. The seed husk, corn-cobs, plant-gums and mucilages contain large amount of pentosans.

7.2 Classification of Carbohydrates

The naturally occurring carbohydrates may be classified into four main groups, particularly on the basis of their behaviour towards hydrolysis.

1. *Monosaccharides* (Gk: Mono = one; Sakcharon = Sugar).

   They are the simplest sugars and can not be hydrolyzed into simpler compounds. Their general formula is $C_nH_{2n}O_n$.

2. *Oligosaccharides* (Gk: Oligo = few; Sakcharon = Sugar).

   On hydrolysis they generally yield 2 to 9 molecules of monosaccharides which are sugars and include di-, tri-, tetrasaccharides etc.

3. *Polysaccharides* (Gk: Poly = many; Sakcharon = Sugar).

   On hydrolysis they yield many monosaccharides (hundreds or even thousands) and are non-sugars. Their general formula is $(C_6H_{10}O_5)_x$.

4. *Glycosides*

   They are a kind of oligosaccharides which on hydrolysis yield a carbohydrate and a non-carbohydrate fragment, which may be a hydroxy-compound or a nitrogen base *e.g.*, Arbutin and Salicin are o-glycosides.

$$\underset{\text{Arbutin}}{C_{12}H_{16}O_7} + H_2O = \underset{\substack{\text{Glucose}\\(\text{Carbohydrate})}}{C_6H_{12}O_6} + \underset{\substack{\text{Hydroquinone}\\(\text{non-carbohydrate})}}{C_6H_6O_2}$$

   Adenosine gives a pentose sugar and adenine – a nitrogenous base.

1. Monosaccharides:- They are further classified on the basis of number of carbon atoms present in a molecule e.g.

(*i*) Trioses contain 3-carbon atoms *e.g.* Glyceraldehyde and Di-hydroxy acetone.

(*ii*) Tetroses contain 4-carbon atoms *e.g.* Erythrose and threose.

(*iii*) Pentoses contain 5-carbon atoms *e.g.* Ribose, Ribulose, De-oxyribose, Arabinose, Xylose and Xylulose.

(*iv*) Hexoses contain 6-carbon atoms *e.g.* Glucose, Fructose, Mannose and Galactose.

(*v*) Heptoses contain 7-carbon atoms *e.g.* Sedoheptulose.

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Others are Octoses and Nonoses, containing eight and nine carbon atoms respectively.

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2. **Oligosaccharides**:- May be classified into:

(*i*) *Disaccharides*. Yield 2-molecules of monosaccharides on hydrolysis. *e.g.* Sucrose, Maltose, Lactose, Melibiose, Cellobiose, Gentiobiose, Trehalose.

(*ii*) *Trisaccharides*. Yield 3-molecules of monosaccharides on hydrolysis. *e.g.* Gentianose, Reffinose & Melezitose.

(*iii*) *Tetrasaccharides*. Yield 4-molecules of monosaccharides on hydrolysis *e.g.* Stachyose.

Others are penta-and hexasaccharides which yield 5 and 6-molecules of monosaccharides respectively.

3. **Polysaccharides:-** Include the following non-sugars.

(*i*) *Pentosans. e.g.* Araban and Xylan.

(*ii*) *Hexosans* – They are further classified into-

(a) Glucosans. *e.g.* Starch, Cellulose and Glycogen.

(b) Fructosans. *e.g.* Inulin, Synanthrin, Graminin, etc.

(c) Mannans. *e.g.* Mannane, Mannocellulose.

(d) Galactans. *e.g.* Galactane, Paragalactane.

(*iii*) *Pectic Compounds. e.g.* Pectic acid, Pectin & Protopectin.

(*iv*) *Gums.*

(*v*) *Amino-hexosans. e.g.* Chitin.

(*vi*) *Mucilages*.

7.3 Chemistry of Monosaccharides

In the chemistry of monosaccharides the following two facts are important:

(1) Isomerism, and

(2) Ring Structure

Isomerism: The term isomer (Gk: *isos* = equal; *meros* = part) was first used by **J.J. Berzelius** (1827) to different compounds with same molecular formula. The isomerisms are of two types.

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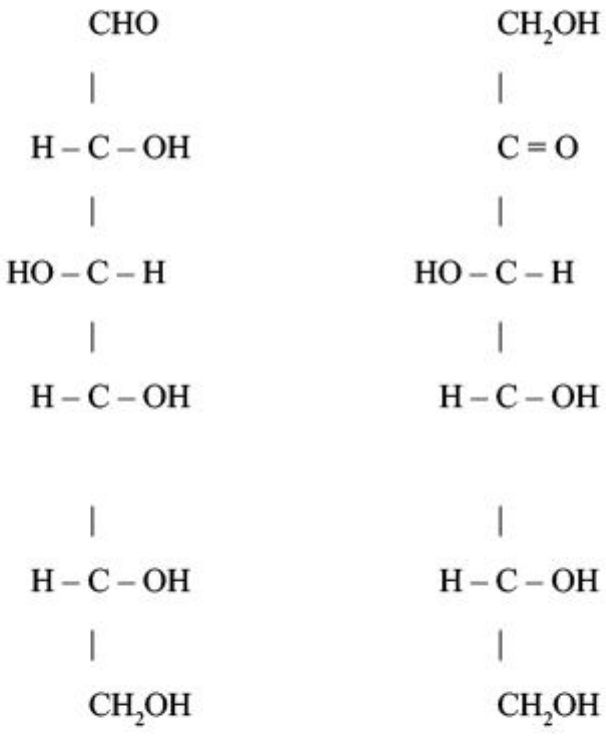
(*i*) Structural isomerism,

(*ii*) Stereoisomerism.

Structural Isomerism

The structural isomers have similar molecular formulae, but different structural formulae *e.g.* D-glucose and D-fructose have the same molecular formula *i.e.*, $C_6H_{12}O_6$, but differ in their structural formulae.



D-Glucose *D-Fructose*

The structural isomers are again of three types-

(*a*) *Chain isomers*:- They have different arrangement of carbon atoms generally in the form of a chain or link.

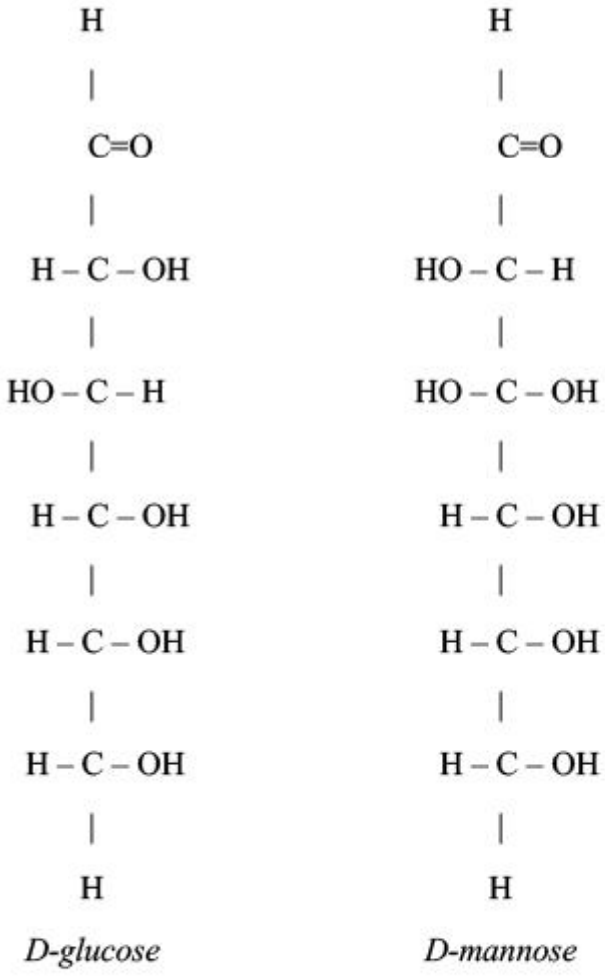
(*b*) *Positional isomers*:- A substituent group in two compounds is at different positions, but their chain is the same.

(*c*) *Functional isomers*:- Isomers in which the compounds have different functional groups, *e.g.* compounds having formula (C_2H_6O) may be an ethyl alcohol (CH_3-CH_2OH) or a dimethyl-ether (CH_3-O-CH_3) and D-Glucose and D-Fructose.

NOTES

Stereoisomerism

Stereoisomers have the same structural and molecular formula, but differ in spatial arrangement of atoms or groups in the molecule. The arrangement of the groups in different patterns always takes place around the **asymmetric carbon** atoms i.e. carbon atoms in which their 4 valencies are completely satisfied by different kinds of atoms, *e.g.* D-glucose and D-mannose have change in spatial arrangement of hydrogen and hydroxyl groups.



D-glucose *D-mannose*

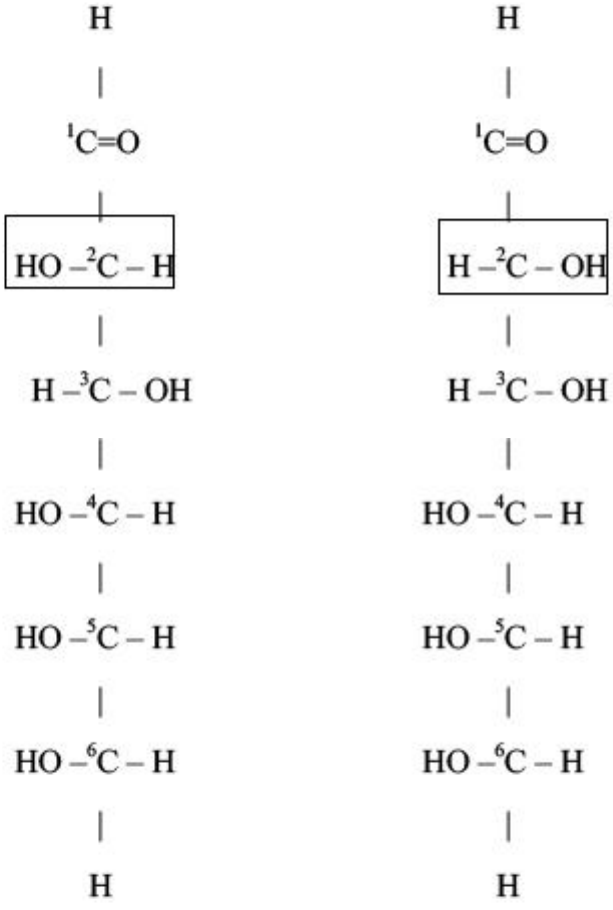
'D' molecule is one in which the

(*i*) Asymmetric carbon atom is farthest from aldehyde or keto-group and

(*ii*) Adjacent to the terminal $-CH_2OH$ group, the hydroxyl or –OH group is projected to the right side.

NOTES

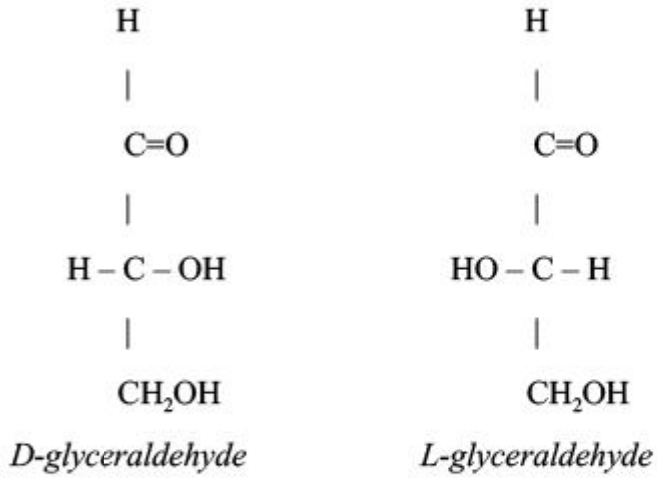
In L-forms the –OH group of the same carbon is shown on the left. The L-form of glucose and mannose would be-



L-glucose *L-mannose*

The structural formulae of L-glucose and L-mannose would be the mirror image of D-glucose and D-mannose respectively. This is also known as **optical isomerism** which is a kind of stereoisomerism.

In the same way the D-glyceraldehyde and L-glyceraldehyde would be-



D-glyceraldehyde *L-glyceraldehyde*

Ring structure:The molecules of sugars may exist in two different ring forms. The two forms differ from each other in their stability and reactivity. The two ring forms are-

Pyranose ring

It is more stable than furanose ring. Here C-1 and C-5 are linked by an oxygen atom, thus forming a large sized stable ring. This ring structure is exhibited by pentose and hexose sugars which are generally written as closed hexagons or pentagons. The ring may be written either in the form of a straight carbon chain with oxygen atom shared between appropriate carbon atoms or in a closed form. Thus, pyranose ring for the glucose may be written in either way.

Furanose ring

It is less stable. Here C-1 and C-4 are linked by an oxygen atom, thus forming a small sized less stable ring. This ring structure is exhibited by pentose and hexose sugars. They may be written as closed carbon rings or as chain-carbon rings.

Each of the hexoses exists in α-and β-forms depending upon the position of the –H and –OH groups to C-1 which is also asymmetric in pyranose and furanose rings. In β-form the –OH group is attached to C-1 on the left side, while in α-form towards the right side.

Hemiacetal Formula

Generally it has been seen that the aldehyde can add hydroxyl compounds to the carbonyl (C=0) bond. When water is added, the hydrate of aldehyde is formed, but if an alcohol is added in place of water, the *hemiacetal* is formed. Further, when other molecules of alcohol are added in the hemiacetal, they form the **full acetals** and the elimination of water takes place. This reaction is the basis of glycoside formation by carbohydrates.

NOTES

A ring is produced with the formation of hemiacetal. A five membered ring with one oxygen atom forms **furan** and a six membered ring with one oxygen atom forms **pyran**. The sugars containing these forms are correspondingly known as *Furanose* or *Pyranose* sugars.

On the basis of this ring, the β-D-glucose would be a *glucopyranose* while α-D glucose would be a *glucofuranose*.

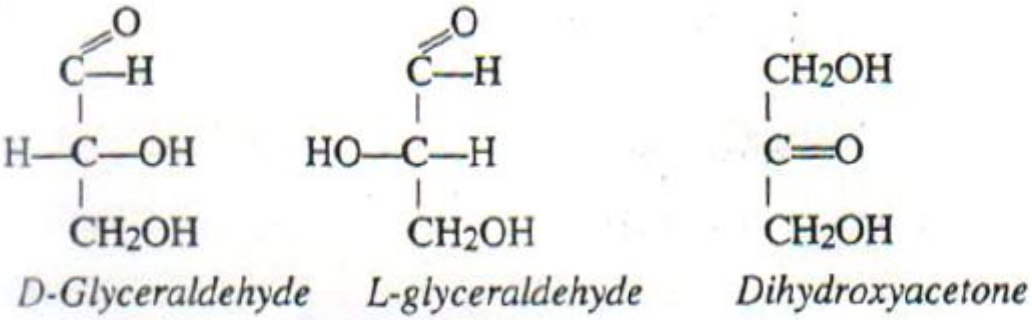
Fischer's Projection and Haworth's Projection

The formula written in the chain form with carbon-atoms, along with oxygen atom forming a ring was given by *Fischer*. This formula does not show the proper arrangement of molecules in the ring form *e.g.*, in the Fischer's formula the ring form of glucopyranose does not show that-

(*i*) C_6 and its attached groups are trans i.e., they are alternately up and down with respect to hydroxyl groups on carbons 1, 2 and 4.

(*ii*) It does not show the proximity of carbon 1 and 5. Both these things are clear in *Haworth's Projection*.

7.4 Classification of Monosaccharides



D-Glyceraldehyde *L-glyceraldehyde* *Dihydroxyacetone*

NOTES

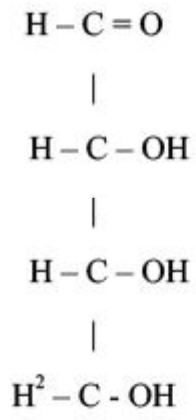
Trioses

The simplest compounds having three carbon atoms are known as **trioses** *e.g.*, glyceraldehydes and dihydroxyacetone. Glyceraldehyde is an aldotriose (containing-CHO gp), while the dihydroxyacetone is a ketotriose (containing C = Ogp).

Both are colourless, sweet, crystalline and soluble in water, but insoluble in ether. Both show the properties of an aldehyde and a ketone and cannot be hydrolyzed. They are formed in plants during glycolysis.

Tetroses

Erythrose: This sugar has four carbon atoms and properties like that of a triose. It is produced in plants in photosynthesis in presence of transketolase from fructose-6-phosphate.



Erythrose

Pentoses

They contain 5-carbon atoms. The general formula used for them is $C_5H_{10}O_5$. In plants they are found in combined state. Pentoses reduce Fehling's solution and give Molisch's test. They are not fermentable.

Ribose

It is an aldopentose (containing an aldehyde group) and occurs in the furanose form. Its ketonic form is ribulose. When the hydroxyl group at carbon-2 of ribose is replaced by a hydrogen atom, it forms deoxyribose sugar which is

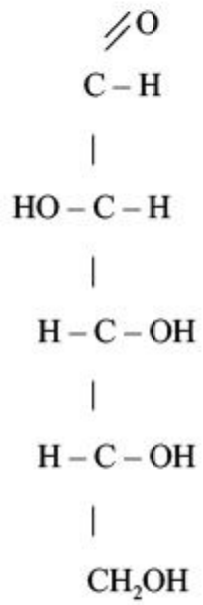
very important in the formation of nucleic acid DNA, while ribose is used in the formation of RNA.

(Here oxygen atom at Carbon no. 2 is lacking)

Arabinose

NOTES

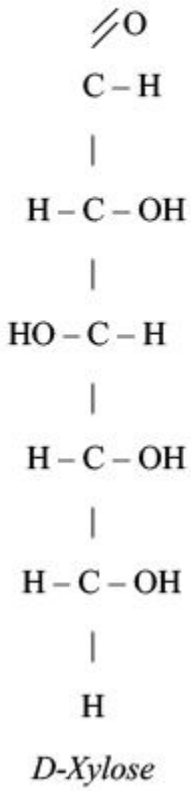
It is colourless, crystalline and sweet in taste. It can be obtained by the hydrolysis of gum Arabic, peach gum and cherry gum. It reduces Fehling's solution and with diphenyl hydrazine forms a characteristic diphenylhydrazone. The melting point of arabinose is 160^0C.



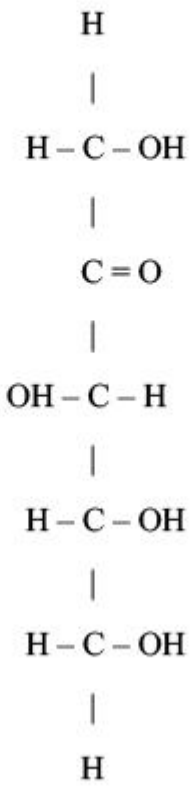
D-arabinose

Xylose

It is an aldose sugar. Its ketonic form is xylulose and is formed in photosynthesis. It is colourless, crystalline optically inactive having melting point of 144-145^0C. It yields xylonic acid when oxidized with bromine. It is obtained by the hydrolysis of wood gum or xylane and also from maize fruits, straw or from other forms of celluloses.



D-Xylose



D-Xylulose

Hexoses

These sugars have 6-carbon atoms and have the same properties as pentoses. They may be present either as aldoses or as ketoses. They cannot be hydrolyzed. Examples are:

Glucose

It is also known as dextrose. It is formed by the hydrolysis of cane-sugar glucosides and many polysaccharides, such as starch, cellulose etc. It has needle shaped crystals, which are anhydrous. It crystallizes in the form of plates with one molecule of water. It has an aldehyde group and thus shows the properties of aldehyde.

Glucose differs from an aldehyde in:

(*a*) It shows no addition reaction with NH_3 and $NaHSO_3$, (*b*) It has properties of –OH groups, (*c*) It does not give any colour with schiff's reagen (*d*) It shows mutarotation.

Fructose

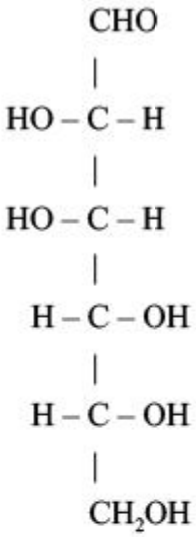
It is a ketose sugar having 6-carbon atoms. It is formed in equal quantity with glucose by the hydrolysis of cane-sugar. It is soluble in hot absolute alcohol and ether.

NOTES

Fructose differs from ketones because, (*a*) It shows the properties of alcohols or –OH gp, (*b*) It shows mutarotation, (*c*) It chars in cold with conc. H_2SO_4, (*d*) It does not form additional product with $NaHSO_3$, (*e*) It shows reducing properties.

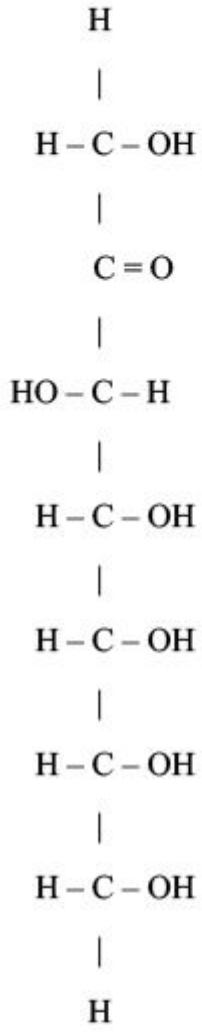
Mannose

It is prepared by hydrolyzing Mannane, which is a polysaccharide and found in Ivory nuts, the fruits of *Phytelephas macrocarpa* and in salep mucilage (Orchid *Morio*). It is also prepared by hydrolysis of hemi-celluloses contained in peas, coffee beans and date stones. In dry state it is a hard crumbling substance, readily soluble in water, slightly soluble in hot alcohol and is insoluble in ether. It is readily fermentable by yeast and is dextrorotatory. It is detected by phenyl hydrazones and reduces fehling's solution.



Mannose

Heptoses. They are 7-C sugars. Sedoheptulose is a ketose sugar and is formed is photosynthesis.



Sedoheptulose

7.5 Some Important Reactions of Monosaccharides

1. Due to Aldehyde and Ketone groups

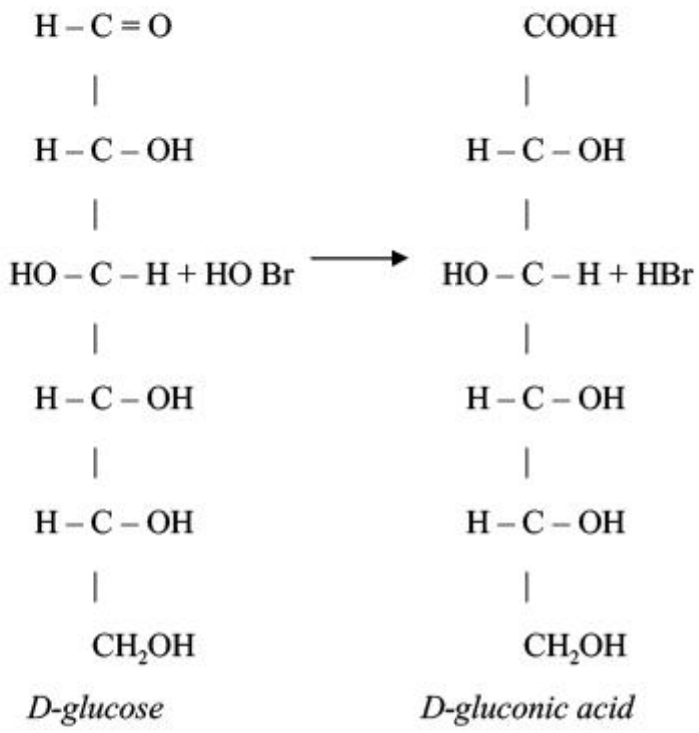
(*i*) Oxidation to produce sugar acids. Monosaccharides on oxidation under proper conditions form different products *e.g.*, aldoses may form monobasic aldonic acids or dibasic saccharic acids or monobasic uronic acids containing the aldehyde group.

(*a*) *Production of Aldonic acid*. Aldoses when oxidized in presence of bromine water the aldehyde group is converted into carboxyl group and forms the corresponding acids. The hypobromous acid, an oxidizing

agent is formed by the reaction of bromine with water and it oxidizes glucose into gluconic acid.

$$Br_2 + HOH \rightarrow HOBr + HBr$$

Hypobromous acid



D-glucose *D-gluconic acid*

Similarly, galactose, mannose and arabinose give galactonic, mannonic and arabonic acid respectively. The Ketoses are not readily oxidized by bromine water.

Monosaccharides with Fehling's solution and Tollen's reagent give rise to red precipitate of cuprous oxide and silver mirror respectively and are oxidized into gluconic acid.

2. Reactions of Sugars Due to hydroxyl (-OH) gp

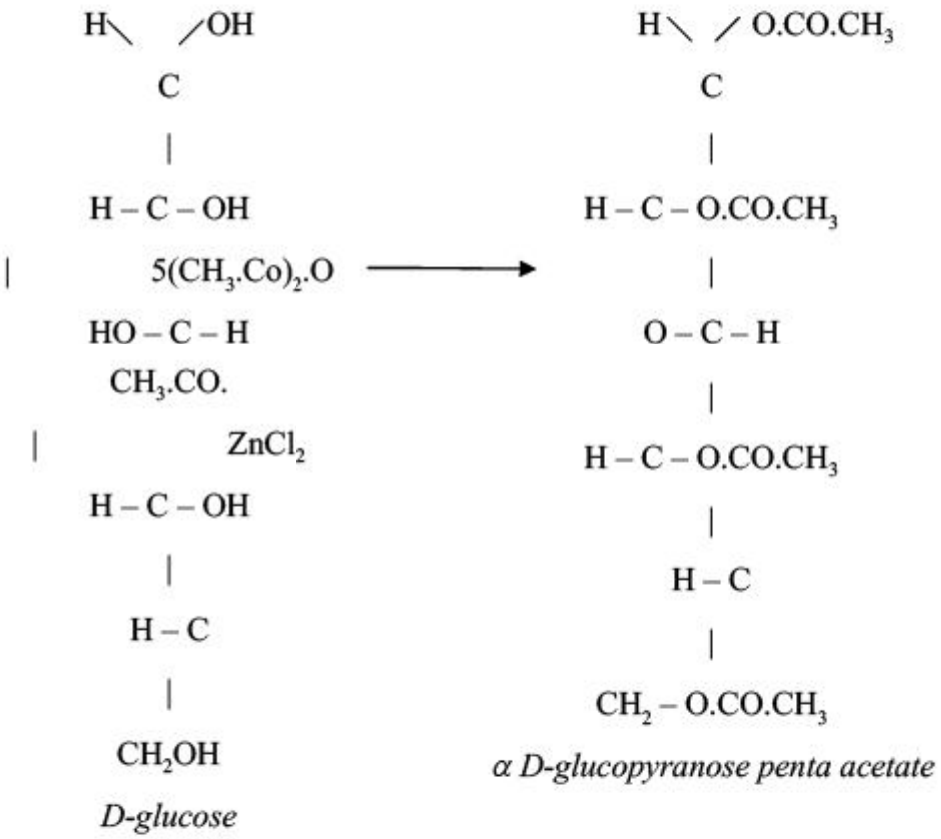
(*i*) *Formation of Glycosides*. The simple sugars react with alcohols in presence of hydrogen chloride as catalyst and form glycosides. Generally, the hydroxyl group of carbon-1 of sugar undergoes reaction under these conditions *e.g.*, glucose or fructose forms glucosides or fructosides respectively.

NOTES

$$C_6H_{11}O_5 . OH + HOCH_3 \xrightarrow{\text{dry HCl}} C_6H_{11}O_5 . OCH_3 + H_2O$$

glucose or fructose → methyl glucoside or fructoside

(*ii*) *Formation of Esters*. When the sugars are treated with an appropriate acid anhydride or chloride under proper conditions, their hydroxyl groups are esterified to give esters, such as Sugar acetates, benzoates, propionates etc. *e.g.*, glucose and fructose with acetic-anhydride form penta-acetyl derivative in presence of fused $ZnCl_2$.



7.6 Chemistry of Oligosaccharides

This group is composed of disaccharides, trisaccharides, tetrasacchrides, pentasaccharides and hexasaccharides. Di-, tri-, tetra- and so on indicate the number of monosaccharides present in a molecule. In this case also, the

carbohydrates which contain hemiacetal (free sugar group) possess 2 α - and β -forms, like those of monosaccharides.

Disaccharides

The general molecular formula of a disaccharide is $C_{12}H_{22}O_{11}$. They are mostly sugars and may be reducing or non-reducing. According to the classification some of the important disaccharides are as follows-

NOTES

i. Non-reducing sugars-

(a) Sucrose consists of glucose, fructose.

(b) Trehalose consists of glucose, glucose.

ii. Reducing sugars-

(a) Maltose consists of glucose, glucose.

(b) Lactose consist of glucose, galactose.

(c) Melibiose consists of glucose, galactose.

(d) Cellobiose consists of glucose, glucose.

(e) Gentiobiose consists of glucose, glucose.

Sucrose

Sucrose is found widely distributed in plants. It occurs especially in plants such as sugar-cane, sugar maple, sugar beet, pine apple, sorghum and in little quantities in wheat, Barley, Carrots, maize and in mostly sweet fruits. It is a non-reducing sugar and on hydrolysis yields one molecule of glucose and one molecule of fructose.

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose Glucose Fructose

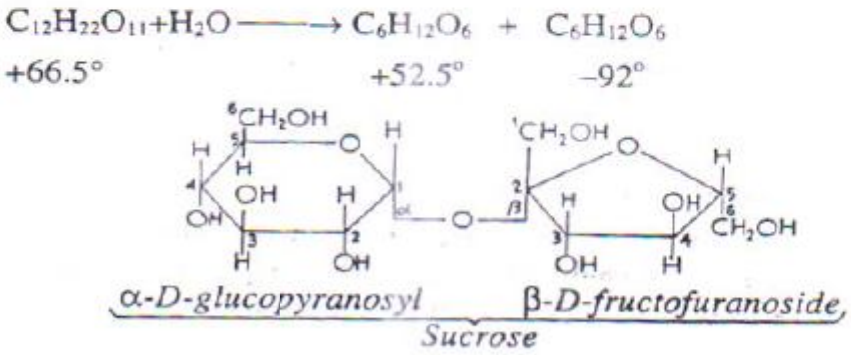
It does not give the reactions of the sugar group (Hemiacetal group). The linkage of glucose to fructose in the molecule involves –OH of C-1 of glucose and OH of C-2 of fructose.

Hydrolysis of sucrose is done by *enzyme invertase or sucrase* or by dilute acids. The glucose and fructose molecules are produced with a change in optical rotation from positive to negative because D-fructose is more

levorotatory than D-glucose which is dextrorotatory. This is known as **inversion**.

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$
$$+66.5^0 \qquad +52.5^0 \qquad -92^0$$

NOTES



7.7 Classification and Chemistry of Polysaccharides

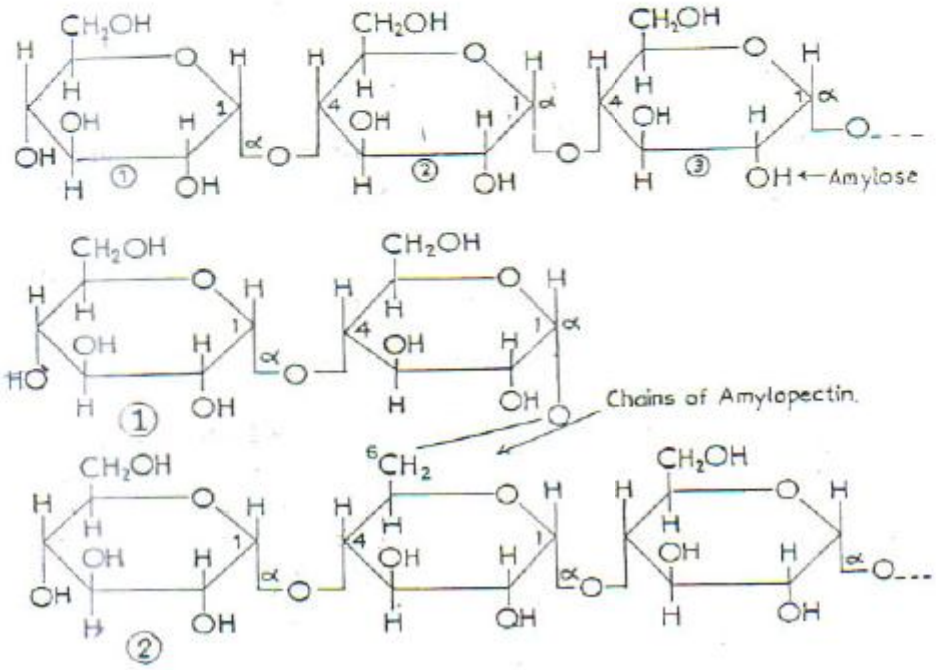
Polysaccharides are formed by the combination of many monosaccharides joined together by glycosidic linkages, like oligosaccharides. They are also known as "Glycans" and are again classified into: (1) *Homoglycans*, and (2) *Heteroglycans*. Those polysaccharides which are made up of only one kind of monosaccharides are known on as **Homoglycans**, while those which are made up of two or more kinds of monosaccharides are known as **Heteroglycans**. Polysaccharides are hydrolyzed either by enzymes or by mineral acids and are resistant to alkaline hydrolysis. The most common examples of polysaccharides are starches, celluloses, glycogen, chitin and

Inulin.

Starch

The structure of starch includes two chemical substances, viz., amylase and amylopectin and these two substances are again made up of a number of glucose units joined through the glycosidic linkages like maltose i.e., amylase consists of glucose residue with repeating maltose units, while amylopectin consists of glucose units in α-glycosidic linkage. In its structure amylase shows α-1, 4 glucoside linkage, while amylopectin in addition to α-1, 4

glucoside linkage has α-1, 6 glucoside linkage in side chains. Thus the chain of amylopectine is branched.



The starches are found as reserve food material in potatoes, in seeds, in rhizomes, in gerams, in fruits and in pith of plants. The shape of starch grains differ in plants. The general structural formula used for starch is $(C_6H_{10}O_5, O_2O)_n$. Starch is slightly soluble in hot water, but insoluble in cold water. It generally gives a characteristic test i.e. ***Starch-iodide test***.

In presence of enzyme ***amylase***, it is hydrolyzed and in presence of enzyme ***diastase***, starch is digested i.e. it is converted into sugars.

Table 7.1

Distinction between Mono-, Oligo- and Polysacchaides

S.No.	Monosaccharides	Oligosaccharides	Polysaccharides
1.	Monosaccharides are simplest sugars *e.g.*, glucose and fructose and are generally reducing sugars.	Oligosaccharides are also sugars, may be reducing or non-reducing *e.g.*, Sucrose, and maltose.	Polysaccharides are non-sugars *e.g.*, Starch, glycogen, cellulose inulin & pectin.

NOTES

NOTES

2.	Monosaccharides contain generally upto 9-Carbon atoms.	Oligosaccharides contain generally 12 to 36-carbon atoms.	Polysaccharides contain more number of carbon atoms.
3.	They contain a carbonyl group (C = O) and show the properties of aldehyde or ketones.	Do not contain the carbonyl group and fail to give the reactions of aldehydic or ketonic group.	Do not contain the carbonyl group and so fail to give the reactions of aldehydic or ketonic group.
4.	They are colourless, crystalline and sweet.	They are also generally colourless, crystalline and sweet.	These are colourless amorphous and tasteless.
5.	Soluble in water.	Soluble in water.	Insoluble in water.
6.	These are optically active.	Same	These are optically inactive.
7.	They have free or potential aldehyde or ketonic group.	No	No
8.	Monosaccharides cannot be hydrolyzed.	Oligosaccharides can be hydrolyzed & generally yield 2 to 6 molecules of monosaccharides.	Can be hydrolyzed and upon hydrolysis yield many number of monosaccharides.

7.8 Significance of Carbohydrates

The carbohydrates are of great importance to plants as well as to animals and human beings.

In Plants and animals

(*i*) Carbohydrates are the structural materials of the plants for example, cellulose is found in plant fibers and in wood.

(*ii*) They are wide spread and act as a reserve food material as starch in tubers, grains and roots.

(*iii*) Sucrose is present in the nectar of flowers, in roots and in fruits. Glucose, fructose and simple sugars are also found in small amount in plants as reserve food material.

(*iv*) The carbohydrates on oxidation release energy which is utilized by plants for various physiological processes.

NOTES

For Human beings

They are of great significance for human beings both from biochemical and industrial point of view.

(*i*) The carbohydrates such as starches and sugars are the main food for human beings. They are easily digestible and are easily oxidized to provide energy for various physiological processes. These are present in cereals.

(*ii*) Various carbohydrates which are present in seeds such as rice, maize, rye, barley and also in fruits are utilized in the production of alcoholic beverages.

(*iii*) The carbohydrate derivatives such as glucosides form important drugs and other medicines for various diseases.

(*iv*) The carbohydrates particularly cellulose and its derivatives are used in the production of artificial silk, paper, plastics, cinema films and explosives.

(*v*) Carbohydrates form other derivatives which are of practical use and are used in the detection of certain chemicals.

(*vi*) All animal tissues, blood, milk and tissue fluids contain carbohydrates and their derivatives as important constituents *e.g.* Blood contains glucose as sugar.

(*vii*) *Importance of Blood glucose*.

(a) Muscles and other tissues remove glucose from blood and form glycogen which provides energy on oxidation.

(b) Mammary glands form milk sugar i.e. lactose from blood glucose.

(c) Many tissues are formed by combinations of sugars or sugar derivatives and `Proteins.

7.9 Summary

Carbohydrates are involved in the structural organization of many tissues both in plants and animals. Cellulose, hemicelluloses (Xylans and Mannans) and

Peetic substances (galactaurananes, arabars and cellulose) are common structural carbohydractes in plants. The primary cell wall in plants contains about 43% of cellulose. Lignin is also present in the cell walls of the older tissues.

NOTES

Carbohydrates are stored as reserve products in many tissues. The most important role of Carbohydretes is The Production of Energy in the Form of ATP both In Plants and Animals.

7.10 Glossary

- **Carbohydrates :** Synthesized during photosynthesis and are the compounds having either an aldehyde or a Ketone group.
- **Sugars :** simpler carbohydrates.
- **Monosaccharide :** Sugars which are made up of only one unit
- **Oligosaccharides :** Sugars composed of few monosaccharide units.
- **Polysaccharides :** Sugars composed of several monosaccharide units joined together by glucosidic bonds.
- **Lipids :** are heterogenous group of naturally occurring compounds insoluble in water and soluble in organic solvents
- **Simple Lipids :** Esters of fatty acid
- **Compound Lipids :** Lipids containing some additional groups or elements besides fatty acids and alcohol.

7.11 Self -Learning Exercise

Section A (Very Short Answer Type Question)

1. What is the other name of Glycolysis?
2. What is the molecular structure of Insulin?

Section B (Short Answer Type Questions)

Write short notes on-

(a) Triglycerides (b) Insulin

(c) Electron Transport system in mitochondria

Section C (Long Answer Type Questions)

1. Write an account of physical and chemical properties of carbohydrates
2. Give an account of thee structure and biological significance of at learn two PD by sallharides.

NOTES

Answer key of Section A

1 EMP Pathway 2. $C_6H_{12}O_0$

7.12 References

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Unit - 8

Respiration

NOTES

Structure of the Unit

8.0 Objective

After going through this unit you will be able to understand

- Mechanism of respiration process,
- Definition & Terminology involved in the process

8.1 Introduction

The term respiration was first used by animal physiologists to describe the breathing movements of animals, but was subsequently extended to include the chemical reactions by which complex organic substances like carbohydrates, fats and proteins are broken down to release carbon dioxide, water and energy. In plants the problem of definition is slightly different because (*i*) breathing movements are not performed, (*ii*) the gaseous exchange typical of animals is often masked by photosynthesis in the day-time, (*iii*) oxygen need not be utilized, and (*iv*) CO_2 may not be released in some cases. For these reasons plant physiologists use the term 'respiration' for the process of oxidation of foods only in living cells.

NOTES

8.2 The Respiratory Ratio

The ratio of the volume of CO_2 released to the volume of O_2 absorbed in the respiratory process is termed the *respiratory ratio* or *quotient*.

The R.Q. of a plant organ depends upon the nature of the substrate which is oxidized in respiration. Respiratory quotient can be equal to unity, less than unity or more than unity. An account of the respiratory substrates and conditions which give different R.Q. values is given below:

(1) R.Q. Equal to Unity

In the oxidation of a carbohydrate, a hexose, the amount of CO_2 given out is equal to the amount O_2 absorbed as indicated in the reaction given below:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ kcal}$$

$$\text{R.Q.} = CO_2/O_2 = 6/6 = 1.$$

(2) R.Q. Less than Unity

(*a*) Respiration of fats and proteins: Respiratory ratio of seeds in which the stored food is mostly in the form of oils (liquid fat), has been found to be less than one. The following summary equation represents the complete oxidation of tri-palmitin, a fat:

$$2C_{51}H_{98}O_6 + 145O_2 \rightarrow 102CO_2 + 98H_2O$$

$$\text{R.Q.} = CO_2/O_2 = 102/145 = 0.7$$

NOTES

The proportion of oxygen to carbon is invariably less in fats than in carbohydrates, i.e. fats are poorer in oxygen. Such compounds require comparatively more oxygen for complete oxidation. Actually fats, being insoluble compounds, are not oxidized directly as indicated in the above equation. Fats are oxidized only after they are hydrolyzed to fatty acids and glycerol. A fraction of oxygen absorbed from outside is used in this transformation without any corresponding evolution of carbon dioxide taking place.

Similarly oxidation of the hydrolytic products of the proteins results in a respiratory ratio of less than one (usually 0.8 – 0.9) since the proportion of oxygen to carbon in such compounds is less than that of the carbohydrates.

(*b*) Respiration of succulents and red leaves (incomplete oxidation of carbohydrates): In some plants like *Opuntia* the carbohydrates are not completely oxidized to CO_2 and water. Instead they are incompletely oxidized to certain organic acid without any accompanied evolution of CO_2 as shown in the following equation:

$$2C_6H_{12}O_6 + 3O_2 \rightarrow 3C_4H_6O_5 + 3H_2O + 386 \text{ Kcal}$$

Malic acid

$$\text{R.Q.} = CO_2/O_2 = 0/3 = 0.$$

A somewhat similar case to that of succulents is that of plants whose leaves are coloured (red) by the presence of anthocyanin in their cells. These leaves show greater accumulation of organic acids.

An interesting feature in the case of certain succulents like *Bryophyllum*, is that their leaves can make direct use of carbon dioxide, liberated in the respiratory process, for snythesising organic acids in the dark. The peculiar metabolism of these succulents results in a respiratory ratio of less than one.

(3) R.Q. More than Unity

(*a*) Respiration of maturing fatty seeds: During the maturation of fatty seeds, simple carbohydrates are converted into fats. Oxygen is eliminated internally in the process. This is utilized in respiration with a corresponding liberation of carbon dioxide, for which there was no absorption of oxygen from outside. This results in a respiratory quotient of more than one.

8.3 Types of Respiration

Normally respiratory process consists in the oxidation of organic substances with the help of oxygen. This type of respiration which requires oxygen is called *aerobic respiration*. Animal respiration is essentially aerobic process and ceases altogether if oxygen is not available. Many plants, on the other hand, continue to respire and give off carbon dioxide for some time even in the complete absence of oxygen. This kind of respiration is called *anaerobic respiration*. While the end products of aerobic respiration of carbohydrate are CO_2 and water, those of anaerobic respiration are ethyl alcohol and carbon dioxide. *Fermentation* is the form of anaerobic respiration which is carried on by some fungi and bacteria. A feature often peculiar to fermentation is that the substrate is presents outside the cell and is in a liquid medium.

NOTES

8.4 Mechanism of Respiration

During cell respiration food materials are oxidized to carbon dioxide and water in the presence of oxygen. One of the most important fuels is glucose. The overall equation for cell respiration in the presence of oxygen is:

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

Under aerobic conditions glucose metabolism takes place in four stages, glycolysis, pyruvic acid oxidation, Krebs citric acid cycle and oxidative phosphorylation in the hydrogen/electron transfer system.

(1) **Glycolysis**. The breakdown of glucose to pyruvic acid is called glycolysis. Glycolysis can take place in the absence of oxygen (*anaerobic condition*) or in the presence of oxygen (*aerobic condition*). The enzymes for glycolysis are found in the soluble fraction of the cell, outside the mitochondria.

(2) **Pyruvic acid oxidation**. Under aerobic conditions the pyruvic acid molecule is metabolized to a molecule of *acetyl coenzyme A* (acetyl CoA).

(3) **Krebs citric acid cycle**. Each acetyl CoA molecule condenses with a molecule of oxaloacetic acid to produce a molecule of citric acid. After several steps oxaloacetic acid is regenerated. Krebs cycle takes place

under aerobic conditions. The enzymes for the Krebs cycle are located in the matrix of the mitochondrion.

NOTES

(4) **Oxidative phosphorylation in the hydrogen transfer system**. Several pairs of hydrogen atoms are released during glycolysis, pyruvic acid oxidation and the Krebs cycle. These are passed through the election transfer system, and are finally accepted by molecular oxygen. During this process ADP is phosphorylated to ATP with inorganic phosphate. The enzymes of the hydrogen transport system are located in the inner membrane of the mitochondrion.

8.5 Glycolysis

Glycolysis is the breakdown of glucose upto the formation of pyruvic acid. Other substances like glycogen and fructose may also be the starting compounds in glycolysis. The most common pathway in glycolysis is the *Embden Meyerhof-Parnas* (EMP scheme). Among the other pathways are the *Entner-Duodoroff* scheme (ED scheme) and the *Hexose Monophosphate* scheme (HMP scheme). As mentioned previously, glycolysis can take place both in the absence of oxygen (*anaerobic* condition) or in the presence of oxygen (*aerobic* condition).

Fate of pyruvic acid: The fate of pyruvic acid differs in the different types of respiration.

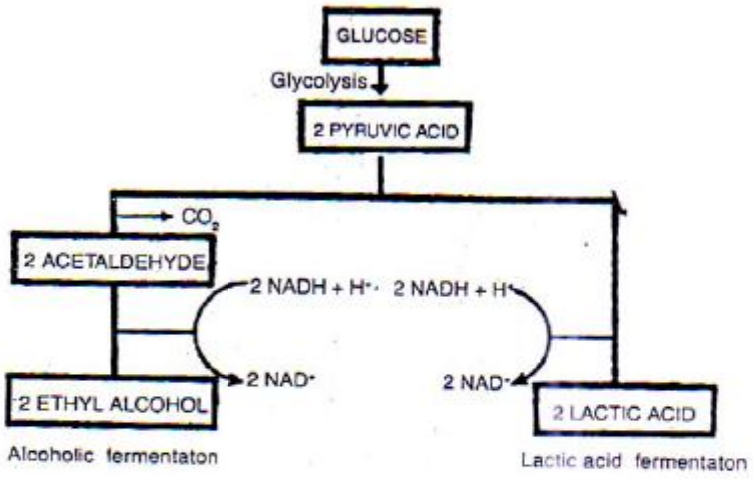

Fig. 8.1 Alcoholic and Lactic Acid Fermentation

1. In yeast (*Saccharomyces* species) glucose is metabolized to pyruvic acid through the EMP pathway. Each glucose molecule yields two molecules of pyruvic acid. During glycolysis, two molecules of ATP are used and four are generated. Thus there is a net gain of two molecules of ATP per molecule of glucose metabolized. Pyruvic acid is decarboxylated to form *acetaldehyde* and CO_2. Acetaldehyde serves as the final hydrogen acceptor. Reduction of acetaldehyde by $NADH_2$ produces *ethanol* (ethyl alcohol), and NAD^+ is regenerated.

2. **Homolactic fermentation**. In the lactic acid bacteria (*e.g., bacilli, lactobacilli, streptococci* and *clostridium* a molecule of glucose yields two molecules of pyruvic acid through the EMP pathway. As in yeast, there is a net gain of two ATP molecules. Pyruvic acid itself is the final hydrogen acceptor. It is reduced by $NADH_2$ to yield lactic acid and NAD^+ is regenerated. This type of fermentation is called *homolactic fermentation* because lactic acid is the sole product. The bacteria are called homo fermentative lactic acid bacteria.

3. In the bacterium *Pseudomonas*, glucose gives rise to pyruvic acid through the ED pathway. In this bacterium a molecule of glucose yields only one molecule of pyruvic acid. One molecule of ATP is used and two molecules generated, resulting in a net gain of one molecule of ATP.Pyruvic acid is decarboxylated to *acetaldehyde*, which is then reduced to ethanol, as in yeast.

4. **In hetero fermentative lactic acid bacteria** glucose is metabolized through the HMP pathway to *pyruvic acid* and acetic acid.

(1) Phosphorylation

The first step of glycolysis consists in the combination of hexose sugars with phosphates forming various types of hexose phosphates and the process is called *phosphorylation* in hexose molecules. In these reactions and many others that follow later, *adenosine diphosphate* (ADP) and *adenosine triphosphate* (ATP) play important roles in the transfer of phosphate from one molecule of carbohydrate to another. ATP acts as a donor of phosphate group and is converted into ADP. These reactions are catalyzed by enzymes of the *phosphorylase* and transphosphorylase groups.

NOTES

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An important phosphate ester formed is *fructose 1, 6-diphosphate*, which serves as the respiratory substrate. The first step in its formation is the reaction between glucose and ATP resulting in the formation of *glucose-6-phosphate* and ADP. The resulting is catalyzed by an enzyme of the transphosphorylase group called *hexokinase*. *Glucose-6-phosphate* may also be formed from *glucose-1-phosphate* under the influence of the enzyme *phosphogylcomutase*. *Glucose-1-phosphate* arises from starch under the influence of enzyme phosphorylase in the presence of inorganic phosphate. It may also be formed from *galactose* via *galactose-6-phosphate*. Glucose-6-phosphate may also arise from *mannose* via *mononose-6-phosphate*. Glucose-6-phosphate then changes into its isomer, *fructose-phosphate* under the influence of the enzyme *phosphohexose isomerase*. This reaction is reversible and is the only one known to occur in plants by which glucose and fructose are inter-converted. Fructose-6-phosphate is also formed directly from *fructose* and ATP under the influence of hexokinase. Fructose may arise from sucrose, when the latter reacts with *uridine diphosphate* (UDP). Glucose liberated from sucrose reacts with UDP to form UDPG. In the next stage further phosphorylation of fructose-6-phosphate occurs with the help of ADP results in the formation of fructose 1, 6-diphosphate. This reaction is catalyzed by the enzyme *phosphor-fructokinase*, and appears to be irreversible.

8.6 Anaerobic Oxidation of Pyruvicacid

In the absence of oxygen and under certain other conditions anaerobic oxidation of pyruvic acid usually occurs but the course of reaction differs in different tissues and organisms. In general the products of anaerobic respiration of pyruvic acid are incompletely oxidized compounds such as alcohols and organic acids.

The pyruvic acid is broken down by the action of the enzyme carboxylase, the products being acetaldehyde and carbon dioxide. The latter is one of the final products of fermentation of anaerobic respiration ethyl alcohol is formed by the reduction of acetaldehyde under the influence of the enzyme dehydrogenase and in the presence of reduced coenzyme 1 ($NADH_2$). The $NADH_2$ may be the one which is formed in the previous glycolytic reaction. The two reactions are as follows:

Table 8.6. Enzymes of glycolysis

Step No.	*Enzyme*	*Enzyme Commission number*	*Coenzyme(s) and Cofactor(s)*	*Activator (s)*	*Inhibitor (s)*	*Kind of reaction catalyzed*	*$*G^{0}$, kcal/mol*
1.	Hexokinase	2.7.1.1	Mg^{2+}	ATP^{4-}, Pi	Glucose 6-phosphate	Phosphoryl transfer	- 4.0
2.	Phosphogluco-isomerase	5.3.1.9	Mg^{2+}	-	2-dioxyglucose 6-phosphate	Isomerization	+ 0.4
3.	Phosphofructo-kinase	2.7.11.	Mg^{2+}	Fructose 2, 6-di-phosphate, AMP, ADP, CAMP, K^{+}	ATP^{4-}, citrate	Phosphoryl transfer	- 3.4
4.	Aldolase	4.1.2.7	Zn^{2+} (in microbes)	-	Chelating Agents	Aldol Cleavage	+ 5.73
5.	Phosphotriose	5.3.1.1	Mg^{2+}	-	-	Isomerization	+ 1.83

NOTES

	isomerase						
6.	Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	NAD	-	Iodoacetate	Phosphorylation coupled to oxidation	+ 1.5
7.	Phosphoglycerate kinase	2.7.2.3	Mg^{2+}	-	-	Phosphoryl transfer	- 4.5
8.	Phosphoglycerate mutase	5.4.2.1	Mg^{2+} 2, 3-diphos phoglycerate	-	-	Phosphoryl Shift	+ 1.06
9.	Enolase	4.2.1.11	Mg2+, Mn^{2+}, Zn^{2+}, Cd^{2+}	-	Fluoride + Phosphate	Dehydration	+ 0.44
10.	Pyruvate kinase	2.7.1.40	Mg^{2+}, K^{+}	-	Acetyl CoA, analine, Ca^{2+}	Phosphoryl transfer	-7.5

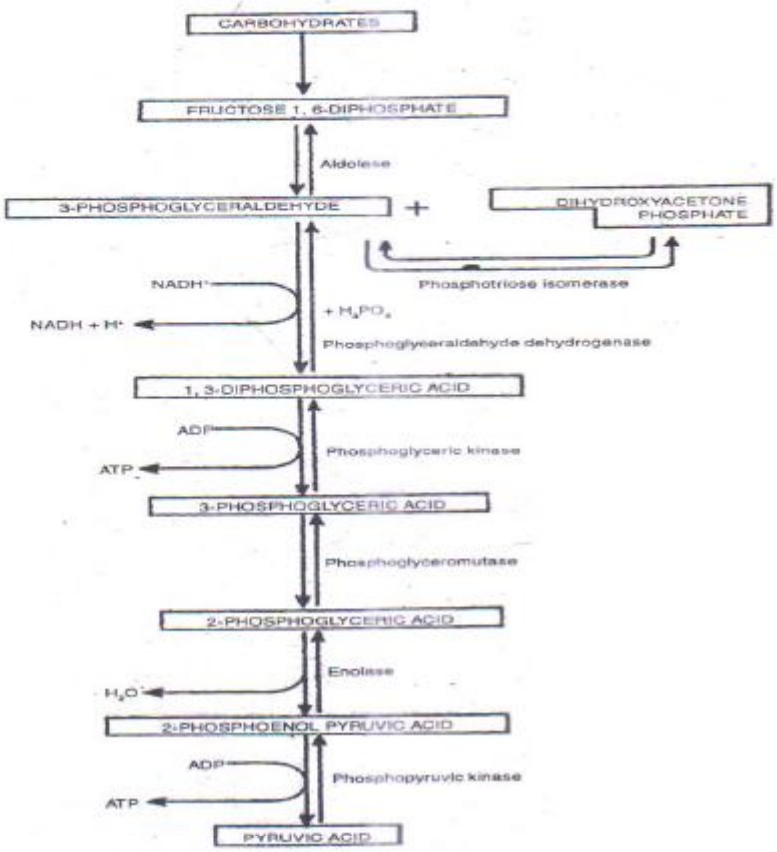

Fig. 8.2 - The Glycolytic Pathway

$$CH_3COCOOH \xrightarrow[Mg^{2+}]{\text{Pyruvic carboxylase}} CH_3CHO + CO_2$$

$$CH_3CHO + NADH_2 \xrightarrow{\text{Alcohol dehydrogenase}} CH_3CH_2OH + NAD$$

Acetaldehyde Alcohol

In the muscle tissue of animals and in some bacteria anaerobic respiration results in the conversion of pyruvic acid into lactic acid.

8.7 Aerobic Oxidation of Pyruvic Acid

NOTES

There are three stages in the aerobic oxidation of pyruvic acid:

I. First stage. *Oxidative decarboxylation of pyruvate to acetyle CoA and* CO_2

II. Second stage. *Krebs cycle*

III. Third stage. *Electron transport and oxidative phosphorylation*

Formation of acetyl coenzyme A

Under aerobic conditions pyruvic acid is oxidized through a '**tricarboxylic acid cycle**' given by **Wood et al**. (1942) and **Krebs** (1943). Before entering the Krebs cycle pyruvic acid which is a 3-carbon compound loses a molecule of carbon dioxide under the influence of the enzyme carboxylase and a 2-carbon compound acetyle CoA is formed in the following manner:

Pyruvic acid enters the matrix of the mitochondria. The oxidative decarboxylation of pyruvic acid to form acetyl co-enzyme A is a very complex reaction, requiring the presence of atleast five essential cofactors and a complex enzyme called pyruvic acide dehydrogenase. The complex enzyme includes three enzymes – pyruvic acid decarboxylase, dihydroxylipoyl transacetylase and dihydrolipoyl dehydrogenase. The six cofctors necessary for the successful formation of acetyl coenzyme A are Mg ions, thiamine pyrophosphate (TPP), NAD^+, coenzyme A (CoA), FAD and lipoic acid.

In the first step pyruvate loses CO_2 under the influence of pyruvate dehydrogenase to become *a*-hydroxyethly derivative of the coenzyme thiamine pyrophate. The second step involves *dihydrolipoyl transacetylase* the hydroxyethly group is dehydrogenated to an acyl group. The latter is transferred to the sulphur atom of C-6 of lipoic acid (prosthetic group of transacetylase). Lipoic acid is contained in the *lipoyllsyl* side chain of transacetylase which is acts as a *"swinging arm"*. In the third reaction the acetyl group of lipoyllysyl arm is transferred to the third group of thiol group of CoA, producing *acetyl CoA* and *dihydrolipoic acid* (reduced form of the oxidized state with an associated reduction of FAD, the prosthetic group. The 4^{th} step restores lipoid acid to its oxidized form by action of *dihydrolipoyl dehydrogenase*. The $FADH_2$ reduces NAD in the last step.

The reactions taking place in the oxidative decarboxylation of pyruvic acid are summarized below:

$$\text{Pyruvic} + \text{TPP} \xrightarrow{Mg^{2+}} \text{TPP complex} + CO_2$$

$$\text{TPP complex} + \text{Lipoic acid} \rightarrow \underset{\text{(oxidized form)}}{\text{Acetyl-lipoic acid complex}} + \text{TPP}$$

$$\underset{\text{(complex)}}{\text{Acetyl-lipoic acid}} + \text{CoA} \rightarrow \underset{\text{(reduced form)}}{\text{Acetyl-CoA}} + \text{Lipoic acid}$$

$$\underset{\text{(reduced form)}}{\text{Lipoic acid}} + \text{NAD} \rightarrow \underset{\text{(oxidized form)}}{\text{Lipoic acid}} + NADH_2$$

The net result of the above reaction is:

$$\text{Pyruvate} + \text{CoA} + \text{NAD} \rightarrow \text{Acetyl CoA} + Co_2 + NADH_2$$

NOTES

8.8 Krebs Cycle

The citric acid cycle is the common pathway for the oxidation of not only carbohydrates but also of the fatty acids and the amino acids.

It was in 1948 that **Kennedy** and **Lehninger** discovered that pyruvate and all the intermediates of citric acid cycle were oxidized by mitochondria of rat liver. It was also found that Mg^{2+} and AMP, ADP or ATP is also needed besides oxygen for this. The different enzymes which participate in the citric acid cycle are present within the matrix or the inner membrane of the mitochondria.

Acetyl-CoA is the 'connecting link' between glycolysis and the Krebs cycle. Its complete oxidation to CO_2 and H_2O involves the reactions of the Krebs cycle and the electron transport system.

Acetyl coenzyme A combines with oxaloacetic acid a four carbon dicarboxylic acid, under the influence of a condensing enzyme, *citrate synthese* to form a six carbon tricarboxylic acid called citric acid. A molecule of water is used up in the reaction and coenzyme A is liberated. The large molecule of citric acid then undergoes stepwise degradation until oxaloacetic acid is regenerated.

NOTES

Citric acid then loses a molecule of water to form cis-aconitic acid which takes back a molecule of water to yield isocitric acid. Both these reactions are catalyzed by the same *enzyme*, *aconitase*.

In the first oxidation step of the Krebs cycle isocitric acid is converted to oxalosuccinic acid in presence of *isocitric acid dehydrogenase*. The oxalosuccinic acid is then decarboxylated to a ketoglutaric acid under the influence of the same enzyme, Mn^{2+} is an important requirement of the decarboxylation reaction.

The oxidation of *a*-ketoglutaric acid is somewhat similar to that of pyruvic acid. The *oxidative decarboxylation* of a-ketoglutaric acid results in the formation of succinyl CoA. This reaction is mediated by a complex enzyme called *a-ketoglutaricacid dehydrogenase*.

Succinyl CoA then loses coenzyme A in a reaction which is catalyzed by *succinate thiokinase* and in which guanosine diphosphate (GDP) reacts with inorganic phosphate to form guanosine triphosphate (GTP) by a process, which is called substrate phosphorylation. This GTP can enzymatically react with ADP to form GDP and a molecule of ATP according to the following reaction:

$$GTP + ADP \rightarrow GDP + ATP$$

A molecule of water is also used up in the conversion of succinyl CoA to succinic acid.

In the third oxidation step of Krebs cycle succinic acid is oxidized to **fumaric acid**. The reaction is catalyzed by the enzyme *succinic acid dehydrogenase* (flavoprotein). The reaction is interesting since this is the only oxidation reaction of Krebs cycle, which does not utilize coenzymes I or II (NAD or NADP). Instead, the flavin prosthetic group, *flavin adenine dinucleotide* (FAD) of the enzyme takes up the two hydrogen ions and two electrons from succinic acid and gets reduced. The fumaric acid reacts with a molecule of water to form **malic acid** in the presence of the enzyme *fumarase*.

In the fourth and the last oxidation step of the Krebs cycle, malic acid is dehydrogenated in the presence of malic acid *dehydrogenase* to yield **oxaloacetic acid**.

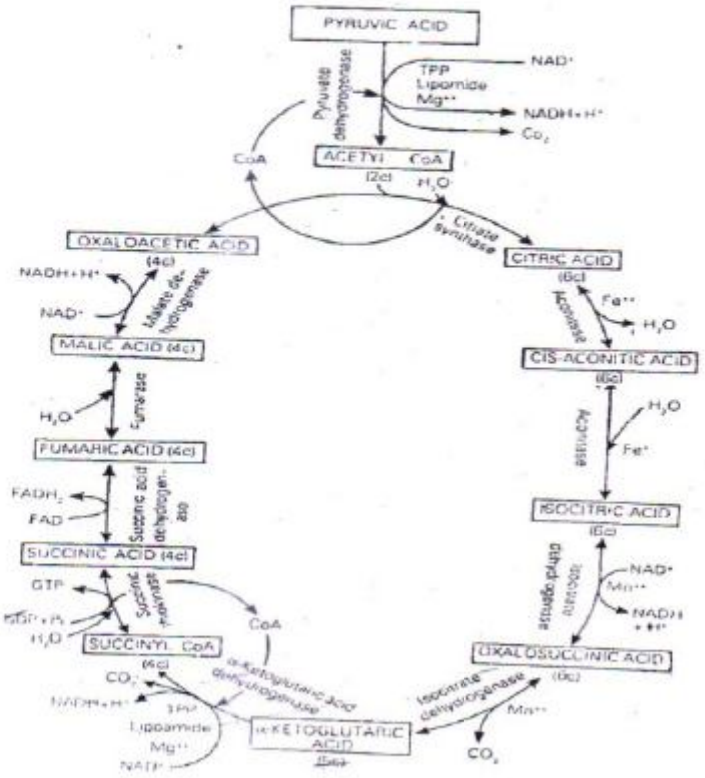

Fig 8.3: The Kreb's Cycle

It will be noticed that in the oxidation of pyruvic acid, there are five oxidation steps. In each step a pair of H ions and a pair of electrons are removed. A total of four pairs of H ions and electrons are utilized in the reduction of pyridine nucleotides (NAD). In the case of succinic acid a pair of H ions and electrons are taken up by FAD prosthetic group of succinic dehydrogenase. The total amount of oxygen consumed in the five oxidation steps of pyruvic acid is 2-½ molecules. Water is produced in six of these reactions but is a reactant in four other so that the net amount of water liberated is 2 molecules. Carbon dioxide is released in three different reactions. The summary equation for the oxidation of pyruvic acid is therefore,

$$CH_3COCOOH + 2½O_2 \rightarrow 3CO_2 + 2H_2O + \text{Energy}$$

If the $½O_2$ required to oxidize $NADH_2$ formed in one of the reaction of glycolysis is added to the left hand side and H_2O thus formed on the right hand side of the equation given above, the total amount of oxygen in the reaction will be $3O_2$ and that of water will be $3H_2O$. This reaction will be for the complete oxidation of a triose sugar such as glyceraldehydes. The overall

reaction for the complete oxidation of hexose can be obtained by doubling the equation arrived at for a triose sugar. The equation will be as follows:

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

NOTES

According to Blackman, all the intermediate products of glycolysis are not oxidized to carbon dioxide and water in aerobic respiration. A fraction of them is built back into the carbohydrates. This anabolic resynthesis has been termed oxidative anabolism.

It is interesting to note that the integrated group of enzymes-the dehydragenases, the carboxylases, and so forth-which engineer the complex series of the reactions of the Krebs cycle are localized in the mitochondria of the cell. Bound to the structure in some unknown way are the various coenzymes which include coenzyme A, coenzyme 1 (NAD), the flavin coenzymes, as well as the cytochromes.

The mitochondria have been isolated from several kinds of cells and have been shown to be capable of carrying on the final oxidative stages of cell metabolism by themselves.

The enzyme concerned in the electron transfer and consequently in the transformation of energy of the substrate into ATP molecules are located with in the mitochondria. It is no wonder, therefore, that the mitochondria have been called the 'power houses' of the cell.

ATP produced during complete oxidation of one molecule of glucose to carbon dioxide and water under aerobic conditions

(I) Glycolysis

(i) ATP produced by oxidation at substrate level

1.3 diphophoglyceric acid to	
3, phosphogylceric acid (1 x 2 = 2)	2 ATP
Phosphoenol pyruvic acid to pyruvic acid to	
pyruvic acid (1 x 2 = 2)	2 ATP
	4 ATP

	ATP consumed	
	Glycose to glycose-6-phosphate	1 ATP
	Fructose-6-phosphate to	
	Fructose 1-6 diphosphate	1 ATP
		2 ATP
	Net Gain of ATP (4 minus 2)	2 ATP
(ii)	ATP from hydrogens produced and sent down	
	the hydrogen transport system	
	PGAL to 1.3 diphosphoglyceric	
	acid (3 x 2 = 6)	6 ATP
	TOTAL ATP PRODUCED DURING AEROBIC	
	GYCOLYSIS	8 ATP
	(II) Pyruvic Acid Oxidation	
	ATP from hydrogen produced and sent down	
	the hydrogen transport system	
	Pyruvic acid to acetyle CoA (3 x 2 = 6)	6 ATP
	(III) Krebs Citric Acid Cycle	
	ATP produced by oxidation at substrate level	
	Succinyl CoA to succinic acid (1 x 2 = 2)	2 ATP
	ATP from hydrogen produced and sent down	
	the hydrogen transport system	
	Isocitric acid to oxalosuccinic acid,	
	NAD acceptor (3 x 2 = 6)	6 ATP
	a-ketoglutaric acid to syccinyl CoA.	
	NAD acceptor (3 x 2 = 6)	6 ATP
	Succinic acid to fumaric acid,	

NOTES

FAD acceptor (2 x 2 = 4)	4 ATP
Malic acid to oxaloacetic acid,	
NAD acceptor (3 x 2 = 6)	6 ATP
TOTAL ATP PRODUCED	24 ATP
	38 ATP

NOTES

8.10 Pentose Phosphate Pathway

An alternative pathway for the breakdown of glucose also exists in many organisms. This scheme, which involves a number of pentose sugars, a 7-c sugar, and a 4-c sugar has been called *pentose phosphate pathway* (PPP) or *hexose monophosphate shunt*.

Ever since the works of **Warburg et al.** (1935) and Dickens it has been recognized that glucose monophosphate could be oxidized by a path which is independent of the EMP pathway reactions of the pentose phosphate pathway were elucidated through the efforts of **Horecker** *et. al.* (1951) and **Racker**.

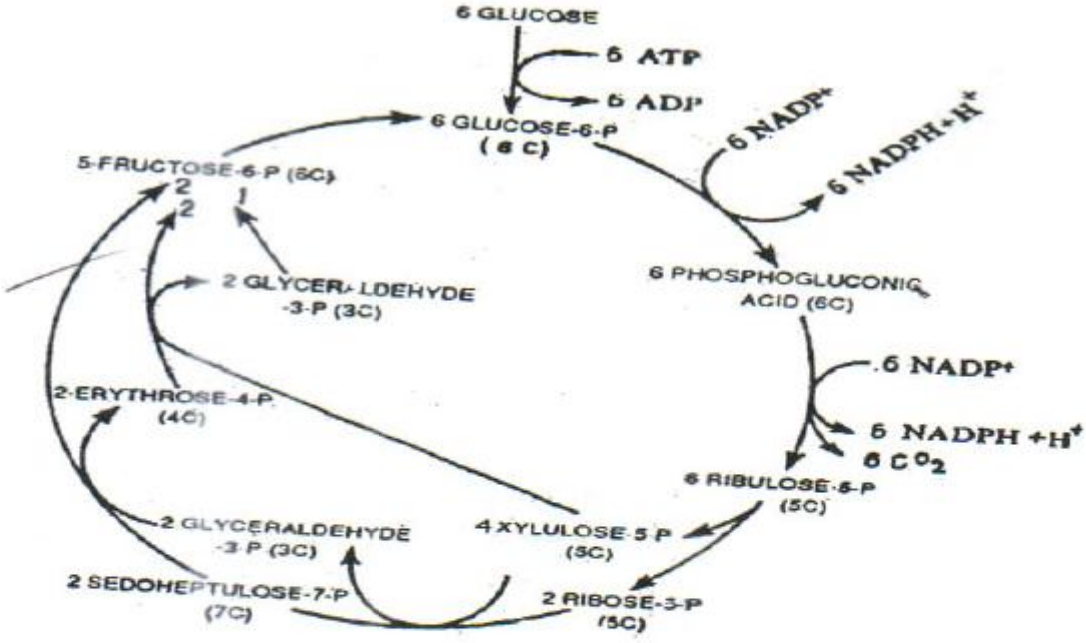

Fig 8.4 Pentose Phosphate Pathway

The reactions of the pentose phosphate pathway are as follows:

Twelve molecules of $NADPH_2$ formed in the reactions oxidized back to 12 NADP with the help of the cytochrome and oxygen of the air.

cytochrome

$$12\ NADPH_2 + 6O_2 \rightarrow 12H_2O + 12\ NADP$$

System

NOTES

In this electron transfer process, 36 molecules of ATP synthesized. The capture of energy released in the oxidation molecule of glucose via this pathway is almost as efficient as the glycolytic-Krebs cycle pathway.

There are two types of evidences in support of the existed such an alternative pathway-works on the inhibiting action of acid on the Krebs cycle and studies with the radioactive carbon.

The reactions can be summarized as follows:

(1) 6 Glucose + 6 ATP $\rightarrow$ 6 Glucose-6-phosphate

Hexokinase

(2) 6 Glucose-6-phosphate $\rightarrow$ 6 phosphogluconic

Dehydrogenase

+ 6 NADP + $6H_2O$ acid + $6NADPH_2$

oxidative

(3) 6 Phosphogluconic $\rightarrow$ 6 ribulose-5-phosphate

acid + 6 NADP decarboxylation 6 CO_2 + 6 $NADPH_2$

dehydrogenase

(4) 2 Ribulose-5-phosphate $\rightarrow$ 2 ribose-5-phosphate

isomerase

isomerase

(5) 4 Ribulose-5-phosphate $\rightarrow$ 4 xylulose-5-phosphate

Isomerase

NOTES

(6) 2 Xylulose-5-phosphate → 2 sedoheptulose-7

+ 2 ribose-5-phosphate phosphate + 2 glyceraldehyde-3-phosphate

Transketolase

(7) 2-Sedoheptulose-7 phosphate → 2 fructose-6-phophate

+ 2 glyceraldehyde + 2 erythrose-4-phosphate

Transketolase

(8) 2 Erythose-4-phosphate + 2 → 2 fructose-6-phosphate + 2 glyceraldehyde

Xylulose-5phospate + 3-phosphate

Aldolase

(9) 2 Glyceraldehyde-3-phosphoate + H_2O → fructose-6-phosphate + H_3PO_4

Sum total of the reactions:

6 Glucose-phosphate + 12 NADP + $7H_2O$ →

5 Fructose-6-phosphate + $6CO_2$ + 12 $NADPH_2$ + H_3PO_4

Metabolic Significance of PPP

In extra-mitochondrial regions the need of reduced $NADPH_2$ for some synthetic actions *viz.*, synthesis of fatty acids and amino acids, and the need of ribose for nucleic acid synthesis, erythrose for the synthesis of lignin and other aromatic compounds is met by hexose monophosphate shunt.

8.11 Electron Transport Chain and Oxidative Phosphorylation

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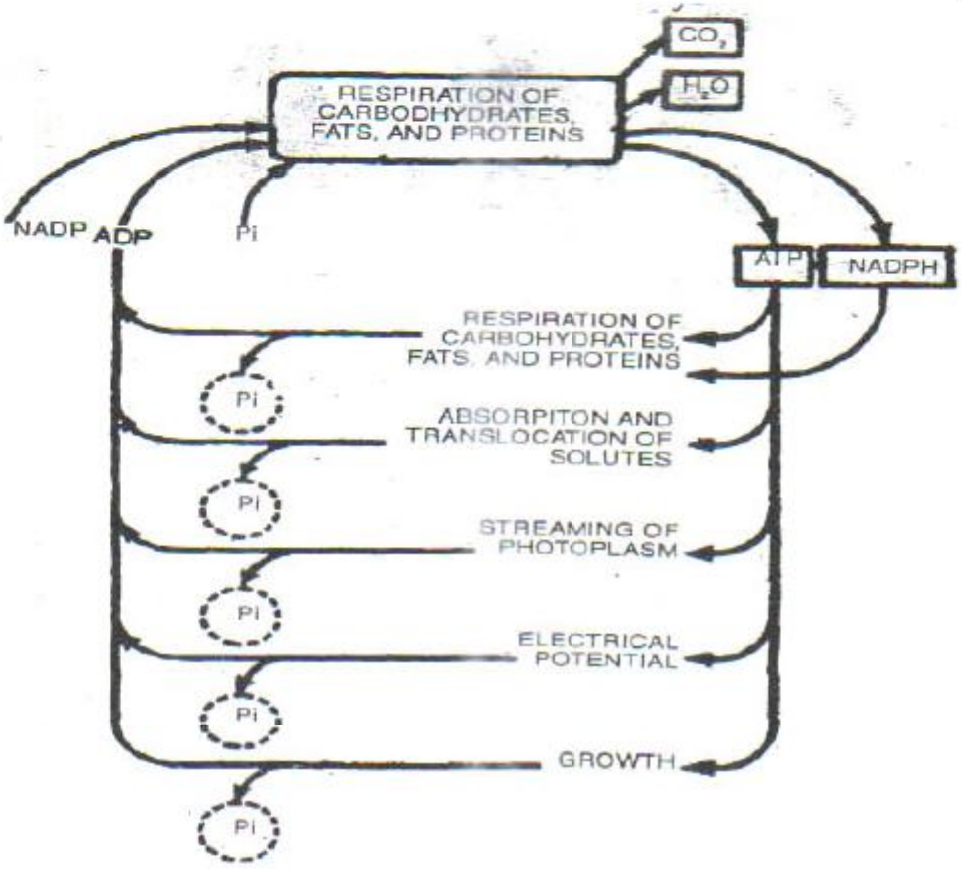

Fig 8.5: Role of ATP

In the respiratory breakdown of simple carbohydrates intermediates like phosphoglyceraldehyde, pyruvic acid, isocitric acid, *a*-ketoglutaric acid, succinic acid and malic acid are oxidized. The oxidation in all these is brought about by the removal of a pair of hydrogen atoms (2H) from each one of them.

Since the hydrogen pair can dissociate into two protons and two electrons, *i.e.*, $2H = 2H^+ + 2e^-$, the oxidation process is usually equated to the removal and transport of electrons to molecular oxygen.

The pairs of hydrogen ($2H^+ + 2e^-$) removed in the oxidative steps of the Krebs cycle do not combine directly with O_2 but pass through an assembly of enzymes of the electron transport system before reacting with O_2 to form water.

NOTES

Electrons flow through the chain in a stepwise manner from the more electronegative compounds to the more electropositive oxygen. Thus, the redox potential of a component of the respiratory chain contributes to the information necessary to assign it a tentative position in the chain. Several other techniques have been employed by a number of investigators to identify components and their relative positions.

The main respiratory chain in mitochondria proceeds from the NAD-linked dehydrogenase systems on the one hand, through flavoproteins and cytochromes, to molecular oxygen. The reducing equivalents are transported either as H^+ or a covalent hydrogen. Because of their more positive redox potentials (*e.g.* succinate) some substrates are linked directly to flavoproteins dehydrogenases.

An additional component found in respiratory chain is *non-heme iron* (NHI) which is combined with protein and is associated with the flavoproteins (metalloflavoproteins) and with cytochrome *b*.

The reduced NAD of the respiratory chain is in turn oxidized by a *metalloflavoprotein* enzyme which contains non-heme iron and the prosthetic group FMN.

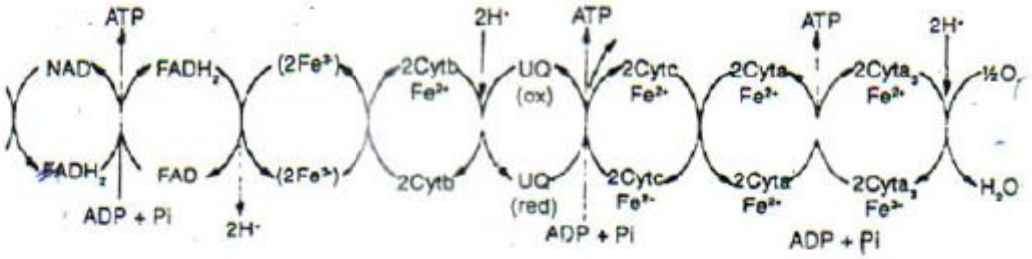

Fig 8.6: Electron Transport Chain

It would be noted that in actual reduction of a molecule of O_2 to water, four electrons and four protons i.e. two pairs of hydrogen atoms will be needed.

$$O_2 + 4e^- \rightarrow 2(O^{2-})$$

$$2(O^{2-}) + 4H^+ \rightarrow 2H_2O$$

From the stand-point of cellular energies the number of ATP molecules generated per molecule of hexose oxidized is very significant. In the electron

transport scheme the energy of the substrate is captured in the form of ATP. For capturing the large difference in energy potential between the substrate and oxygen, it is rather fitting that the energy is released in many small steps. In the electron transport system, in each step the electron flows from a high energy level to a low energy level, the energy difference being transformed into a phosphate bond (ADP – ATP). This synthesis of ATP during oxidation of the cofactors is called oxidative phosphorylation.

There is evidence to suggest that the mitochondrial membrane is not very permeable to ATP or ADP. It is, therefore, believed that the phosphate transfer and transfer of energy occur at the membrane surface itself i.e., the ADP present just outside the membrane is phosphorylated to ATP.

Lipids

(i) Simple Lipids- Simple lipids are often referred to as fats examples are netural fats and waxes. Fats are easten of glycerol and faty acids whereas waxes are afters of fatly acids with long chain alcohol.

Fats- Are tryiglycerides as three makcules of fatly acids condensed with are moleurle of glycerol. The propertcs of Fats differ according to the nature of fatly acids which are present in them.

Fatly acids- Saturated fatly acids containing more than 8 corbon atoms are solid whereas fatly acids, with shorter chain and more unsaturation are liquids. There are about 50 naturally occurninngg fatly acids found in bound state.

The unsaturated fatly acids have one or more double bonds, ranging between 1to 6 and occur after 9, 12, 15 and 18 carbon atoms. Okicacid is simplest are widely occurning fatly acid.

Waxes are efters of fatly acid with complex monohydric alcohol other than glycerol. Wax is an ester of high molecular weight alcohol and a high molecular weight fatly acid. They have high melting point. They are chemically inert and used in furniture polishing.

Chemical Properties of Fats:-

(1) Saponification : The hydrolysis of fat is called saponification.

(2) Hydrogenation : Unsaturated plant Fats are converted into more saturated and solid fats by catalytic hydrogenation.

(3) Oxidation – It occurs in the air which is accompanied with hydrolysis. The process of oxidation occurs at double bonds of the fatly acids and produces aldehydes and ketones. Oxidation and hydrolysis of fats induces rancidity (Odour)

NOTES

Compound Lipids- or Conjugated lipids are characterized by conjugation with other groups other than glycerol and fatly acids. The other groups may cartain phosphorus, nitrogen, sulphur or even protein. There comprise 5 members-

(i) Phospholipids (Phosphatides)

(ii) Glycolipids (Cerebrofids)

(iii) Gargliolipids (Gargliosides)

(iv) Aminolipids (Liporotein)

(v) Sulpholipids

Derived Lipids- Derived lipids are products of hydrolysis of the first two groups of lipids, which are insoluble in water and include fatty acids alcohols, hydrocarbons, fat soluble vitamins and sterols.

8.12 Oxidative Phosphorylation

There are two main hypothesis to explain the mechanism of oxidative phosphorylation.Out of these **Mitchell's Chemiosmotic theory** has found under acceptance. The theory believes that oxidation in the respiratory chain causes translocation of protons (H^+) to the exterior of a coupling membrane (*i.e.*, the inner membrane of the mitochondrion) resulting in their accumulation there. This creates an electrochemical potential (difference in pH) as well as an electrical potential. The enzyme ATP synthetase located in the membrane synthesizes ATP from ADP and Pi.

The electrochemical gradient is shown to be disturbed by uncoupling agents which allow leakage of H^+ across the membrane and inhibitors like oligomycin which prevent the passage of protons through F_0 protein submit.

It is assumed that the respiratory chain is folded into 3 oxidation/reduction loops in the membrane, each loop corresponding to the respiratory complexes I, II and IV. According to this scheme each electron pair transferred from $NADH_2$ to oxygen causes a total of 6 protons to be translocated from inside to the

outside of the mitochondrial membrane. NADH provides one proton and two electrons, which together with another proton from the internal medium reduces the large FMN molecule to $FMNH_2$. FMN releases two protons to the outside. It returns two electrons to the inside via FeS protein. Ubiquinone (Q) which is a small lipidsoluble membrane receives a proton from inside the membrane and becomes QH_2. It releases a pair of protons to the outside and two electrons to cytochrome *b* (cyt b_{566} and b_{562}). The large cytochrome b molecule helps another molecule of ubiquinone to pick from them two electrons as well as two protons from the internal medium. The QH_2 now moves to the outer surface and releases two protons there. Two electrons are passed on to two molecules of cytochrome C, which transfers them to cytochrome a_3 present on the inner side of the membrane.

NOTES

The enzymes of the respiratory chain and the F_1 subunits of oxisomes are situated on the inner membrane. For every proton pair passing through $F_0 - F_1$ complex one molecule of ATP is synthesized from ADP and Pi

Some of the evidences in support of the chemiosmotic theory are as follows:

(a) Addition of protons (acid) to the external medium of mitochondria results in the formation of ATP.

(b) The members of the respiratory chain are arranged in a sided manner which explains the working of the mechanism.

(c) Closed membranes alone can synthesis ATP since ATP is never synthesized in soluble systems.

(d) The $P:H^+$ ratio is also according to requirement.

Amount of ATP

In the oxidation of one molecule of reduced NAD three molecules of ATP are generally generated, one each in the oxidation of $NADH_2$, reduced cytochrome *b*, and reduced cytochrome *a*. In the case of **succinic acid** oxidation, the NAD step is by-passed and therefore, only *two* ATP molecules are formed. On the other hand during the oxidation of *a*-ketoglutaric acid, there is also a phosphorylation step in the formation of GTP or ATP. This is an example of the so-called **substrate phosphorylation**, the production of energy in the form

of ATP by the direct oxidation of substrate. *a*-Ketoglutaric acid oxidation, therefore, results in the formation of 4 molecules of ATP.

The complete oxidation of one molecule of glucose leads to the net synthesis of 38 molecules of ATP as shown in the Table or 36 molecules only.

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When the ATP molecule is hydrolyzed, the energy released is about 7,600 calories per mole of terminal phosphate group in ATP. Thus the total usable energy obtainable from 38 ATP molecules will be 38 × 7,600 = 288,800 calories. The total energy in a molecule of glucose is however, 686,000 calories and therefore, the process is 686,000/288,800 i.e., 40% efficient.

Table – 2 : Number of ATP formed in the various reactions of Respiration

Compounds to be oxidized	*Compounds after Oxidation*	*Acceptor*	*Number of ATP formed*
Pyruvic acid	Acetyl CoA	NAD+	2 × 3 = 6
Isocitric acid	Oxalosuccinic acid	NAD+	2 × 3 = 6
a-Ketoglutaric acid	Succinic acid	NAD+	2 × 4 = 8
Succinic acid	Fumaric acid	FAD	2 × 2 = 4
Malic acid	Oxaloacetic acid	NAD+	2 × 3 = 6
Net gain of ATP in glycolysis = 8			
Total = 38 ATP molecules			

8.13 Oxidation of Extramitochondrial NADH

NADH is produced in plenty in the cytoplasm by the enzyme phosphoglyceraldehyde dehydrogenase is glycolysis. NADH, however, cannot enter into the mitochondria.

Under aerobic conditions this extra-mitochondrial NADH, however, does not accumulate in the cytoplasm and enters the mitochondria for oxidation in the respiratory chain. It can enter the mitochondria *via* substrate pairs linked by

suitable dehydrogenases. There are two possible mechanisms for transferring the NADH to the mitochondria.

(*a*) **Malate – Oxaloacetate – Aspartate Shuttle**: It is comparatively of more universal occurrence. NADH is oxidized to oxaloacetate which is reduced to malic acid. The malate is transferred to mitochondria by *a*-ketoglutarate transporter (1). Malate is oxidized by the respiratory chain of the inner membrane. The oxaloacetate is converted to aspartate by a transminase. The aspartate diffuses out of the mitochondria into the cytosol by a glutamate – aspartate transporter (2). The aspartate is converted to oxaloacetate by a transaminase.

NOTES

(*b*) **Glycerophosphate – Dihydroxyacetone Phosphate shuttle**: In this mechanism the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein (FAD) rather than NAD. Thus only 2 rather 3 ATP are generated per atom of oxygen consumed, as in the case of succinate oxidation. One ATP is consumed in causing the entry of cytoplasmic NADH into the mitochondria against NADH concentration gradient. The net gain of ATP per hexose is thus 36 and not 38.

The external NADH reduces dihydroxyacetone phosphate to glycerol phosphate. The mitochondrial membrane is permeable to glycerol phosphate, which enters the mitochondrion where it is reoxidized by the respiratory chain to form DHAP, which is free to diffuse to the outside to react again with NADH.

Protein Oxidation

Protein is hydrolyzed into free amino acids. Of all the amino acids of plant cells only *glutamic acid* is believed to be oxidized directly by the enzyme *glutamic acid dehydrogenase* into *a*-Ketoglutaric acid and ammonia in the presence of NAD *a*-Ketoglutaric acid enters the Krebs cycle to undergo cyclic degradation and oxidation.

Special Types Of Respiration

Unlike the normal respiration process in which organic substrates are oxidized, there is a type of bacterial respiration in which several inorganic substances are

oxidized with the help of atmospheric oxygen. Such bacteria are called *chemosynthetic* and aerobic.

8.14 Regulation of Respiration

NOTES

According to Chance and **Williams** (1956) the rate of respiration occurring within the mitochondria is controlled by 5 conditions or states.

Table – 3 : States of Respiratory conditions limiting the Rate of Respiration

State 1.	Availability of ADP and substrate.
State 2.	Availability of substrate only.
State 3.	The capacity of the respiratory chain itself when all substrates and components are present in adequate amounts.
State 4.	Availability of ADP only.
State 5.	Availability of oxygen only

Metabolic Pool

The oxidation of carbohydrates, fats, as well as amino acids takes place through the Krebs cycle. The cycle is not only a degradation process. Some of the intermediate compounds of the cycle lead to the synthesis of large molecules. The whole complex of intermeshing systems can be regarded as a "metabolic pool" a universal melting pot or a roundabout form which eradiate the roads leading to the synthesis of protein, fats, carbohydrates and other compounds as well as the roads on which materials are brought in form carbohydrates and proteins.

8.15 Respiratory Enzymes the Non-Oxidative Enzymes

(1) Transphosphorylases

Although these enzymes are not involved in the oxidation and reduction reactions of respiration, they plan an important role in some of the reactions of glycolysis. They catalyse transfer of phosphate either from one kind of molecule to another or from one position to another position in the same

molecule. The presence of magnesium ions seems to be necessary for their activity. Some of the enzymes of this group and the reactions that they catalyze are given below-

Table – 4 : Enzymes of Transphosphorylases

S.No.	*Enzyme*	*Substrate*	*End Product*
1	Hexokinase	Glucose or fructgose + ATP	Glucose or fructose-6-phosphate + ADP
2	Phosphorylase	Starch + Phosphate	Glucose-1-phosphate
3	Phosphoglucomutase	Glucose-1-phosphate	Glucose-6-phosphate
4	Phosphohexose isomerase	Glucose-6-phosphate	Fructose-6-phosphate
5	Phosphoto fructokinase	Fructose-6-phosphate + ATP	Fructose-1, 6-diphosphate + ADP
6	Phosphoglyceric kinase	1, 3, Phosphoglyceric acid + ADP	2, Phosphoglyceric acid + ATP
7	Phosphoglyceromutase	3, Phosphoglyceric acid	2, Phosphoglyceric acid
8	Phosphopyruvate kinase	2, Phosphopyruvic acid + ADP	Pyruvic acid + ATP

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(2) Desmolases

These are enzymes which catalyze reactions in which carbon chains are broken or lengthened. The best known example is aldolase. This enzyme splits up

fructose 1, 6-diphosphate into 3-phosphoglyceraldehyde and dihydroxyacetone phosphate during glycolysis.

(3) Carboxylases

NOTES

They catalyze reactions in which carbon dioxide is removed from a compound. The decarboxylation of oxalosuccinic acid is catalyzed by a *carboxylase* enzyme.

(4) Hydrases

These are enzymes which catalyze the addition or subtraction of water to or from molecules without causing their splitting. One of the hydrases, *enolase*, removes water from 2-phosphoglyceric acid to produce 2-phosphopyruvic acid. Other examples of hydrases are *aconitase* and *fumarase*.

8.16 The Oxidative Enzymes

(1) Dehydrogenases

In respiration, the oxidation reactions involve the removal of hydrogen atoms and electrons from the substrates which, therefore, get oxidized. The hydrogen atoms and the electrons are taken up by certain acceptors which get reduced. Such reactions are catalyzed by *dehydrogenases*.

The hydrogen acceptors are normally the coenzymes, NAD or NADP. The reduced coenzymes then transfer the hydrogen to FAD of the flavoproteins which get reduced.

(2) Flavoproteins

Flavoproteins are another important enzyme (dehydrogenases) concerned in the transfer of hydrogen and electrons. They are natural hydrogen acceptors and are alternatively reduced and oxidized. As with the dehydrogenases it is their prosthetic group, flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) which is active in oxidation. The prosthetic group contains the yellow riboflaving.

In respiratory metabolism they play an important role in as much as they accept the hydrogen from the reduced dehydrogenases and get reduced. They are reoxidized by the enzymes of the cytochrome group.

(3) Cytochromes

The pioneering work of **Mac Munn** (1886) regarding the existence of an oxidized and a reduced form of *cytochromes* remained unaccepted for about 40 years. **Keilin** (1925) elaborated **Mac Munn's** finding and pointed out that cytochromes were a link between the flavoproteins and oxygen (Fig. 15.30). He discovered three cytochromes which he called cytochromes *a*, *b* and *c*. These are *iron-porphyrins* combined with protein (hemeproteins). They contain ferric (Fe^{3+}) in the oxidized and ferrous (Fe^{2+}) iron in the reduced form. These cytochromes are the enzymes which function as intermediate carriers between flavoproteins and the *cytochrome oxidase*.

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The cytochromes *b*, *c* and c_1 get reduced and oxidized alternatively in that order after receiving the electrons from the flavoprotein.

(4) Terminal Oxidases

(*a*) **Cytochrome Oxidase**: It was only in 1939 when **Keilin** and **Hartee** made the remarkable separation of *cytochrome oxidase* (Cyt. a_3), which reacted directly with oxygen. This new cytochrome proved to be the ultimate step in the transport of electrons and the hydrogen ions to oxygen to form water.

(*b*) **Polyphenol Oxidase**: The phenol (catechol) oxidases are very important copper containing enzymes. They are involved in the oxidation of phenols to the corresponding quinines. The quinines react with the cell contents to produce characteristic brown pigments which can be seen after injury of plant tissues.

(*c*) **Ascorbic acid Oxidase**: Ascorbic acid oxidase is another copper protein which is also considered to be a possible terminal oxidase in plant respiration. It catalyzed the oxidation of ascorbic acid to dehydroascorbic acid.

(*d*) **Glycolic acid Oxidase**: Glycolic acid oxidase is another enzyme which catalyzes the direct oxidation of its substrate by molecular O_2. It oxidizes glycolate to glyoxylate, which is further oxidized.

(*e*) **Peroxidase and Catalase**: Peroxidase is of universal occurrence in higher plants. It oxidizes a variety of substrates (phenols and amines) when H_2O_2 is available as the electron acceptor.

The hydrogen peroxide on receiving the hydrogen atoms and the electrons gets converted into water.

Peroxidase

$$AH_2 + H_2O_2 \rightarrow 2H_2O + A$$

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The reaction is analogous to that of oxidases, except that instead of oxygen the hydrogen perioxide serves as the hydrogen acceptor.

Catalase brings about the decomposition of hydrogen peroxide into water and oxygen.

catalase

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

As the oxygen is given off in the molecular state, catalase is generally not regarded as an oxidizing enzyme. Its function is supposed to be the destruction of hydrogen peroxide, which may be produced as a by-product of metabolism.

8.17 Factors Affecting the Aerobic Respiration

The rate of respiration varies with a number of factors, both external as well as internal. Normally the rate of respiration would increase with increase in respirable material or oxygen (in aerobic respiration). However, there are many cases where oxygen reduces the rate of sugar break down and even conserves it. This is known as the **Pasteur Effect**. The rate of sugar loss also decreases with the accumulation of the end products and carbon dioxide. Temperature has a marked effect on the rate of respiration. Recent studies have shown that light also affects the rate of respiration – a phenomenon which is called **photorespiration**.

Respiration rate varies greatly with age. In the growing stage the rate of respiration is some what steady. In a maturing fruit there is rise in the rate of respiration. This is called **Climacteric** and is associated with the production of ethylene. In the old age – the period of *senescence*, the respiration rate declines. Ethyl alcohol acetaldehyde accumulates due to less availability of oxygen to the cells.

There are a number of factors, some internal, others external which influence the rate of aerobic respiration. The internal factors are the protoplasmic factors

and the concentration of the respirable material. The external factors are temperature, light, oxygen concentration, CO_2 concentration, water, injury, mechanical effects and effects of certain chemicals.

Internal Factors

(1) Protoplasmic Factors

(2) Concentration of the Respirable Material

External Factors

(1) Temperature

(2) Light

(3) Oxygen concentration of the atmosphere

(4) Carbon dioxide concentration

(5) Water

(6) Injury

(7) Mechanical effect

(8) Effect of certain chemical compounds on respiration

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8.18 Summary

Aerobic (oxygen-requiring) respiration is common to nearly all eukaryotic organisms and in its broad outlines, the respiratory process in plants is similar to that found in animals and lower eukaryotes. However, some differences exist in plant respiration that distinguish it from animal respiration (1) the plants lack respiratory system and respiratory movements (2) The exchange of gases in plants is also different because during day light respiration is slightly suppressed due to photosynthesis. (3) Sometimes the plants do not use oxygen during respiration (anqerobic) (4) In some cases CO2 is not liberated outside the cells.

The term Respiration is only used for the "Oxidation of food" only

8.19 Glossary

- **Respiration :** Oxidation of food materials inside the cells.

NOTES

- **ATP :** Adenosine Triphosphate
- **Aerobic Respiration :** Respiration occuming in presence of oxygen
- **Respiratory Substrate :** Substrates which are used as fuel in respiration\
- **ETS :** Electron Transport System
- **Respiratory Quotient (R.Q.) :** The ratio of volumes of CO_2 liberated and O_2 used during respiration.

8.20 Self -Learning Excercise

Section A (Very Short Answer Type Question)

1. Respiration is
   (a) Exothermic process (b) Endotherrmic process
   (b) Endergonic process (d) Anabolic process
2. Common immediate source of (highest) in cellular is
   (a) NAD (b) ATP (c) DNA (d) RNA
3. Enzymes taking Part in glycolysis are present in-
   (a) Mito Chandria (b) Cytoplasm
   (c) Vacuole (d) Both mitochondria and cytoplasm
4. The value of R.Q. at comensation point is
   (a) Unity (b) Infinity (c) 71 (d) Zero

Section B (Short Answer Type Questions)

1. How many ATP motecules are gained during glycolysis.
2. Where is ETS located in prokaryotes?
3. Which compound links glycolysis with TCA Cycle?

Section C (Long Answer Type Questions)

1. Describe briefly (a) Glycolysis (b) Pentose phosphate pathway.
2. Discuss the election transport system (ETS) in mitochondria.

Answer of Section -A

1 (a) 2 (b) 3 (b) 4 (d)

8.21 References

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NOTES

Unit – 9

Nitrogen Fixation, Nitrogen and Sulphur Metabolism

Structure of the Unit

9.0 Objective

After going through this unit you will be able to understand:

- Biological Nitrogen Fixation and its Mechanism
- Nodule formation in leguminous and non - legumes
- Mechanism of nitrate uptake and reduction

- Ammonia assimilation
- Nitrogen and Sulphur Metabolism in Plants
- Sulphate Assimilation

9.1 Introduction

NOTES

Nitrogen is one of the chief and important constituent of protein and nucleic acids which helps in plant growth and production. It is also a major component of chlorophyll, the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. It is also found in other important biomolecules, such as ATP and nucleic acids. Even though it is one of the most abundant elements, nitrogen gas (N_2) in the Earth's atmosphere, plants can only utilize reduced forms of this element. Plants acquire these forms of "combined" nitrogen by the addition of ammonia and/or nitrate fertilizer or manure to soil, release of these compounds during organic matter decomposition, the conversion of atmospheric nitrogen into the compounds by natural processes, such as lightning, and) biological nitrogen fixation .

Sulphur is also a crucial element for growth and development of plants. Plants utilize it in the form of sulphides. Sulphur is a essential nutrient for plants as it incorporate into a wide range of secondary compounds that have an impact, in varied and subtle ways, on our use of plants and on the way that plants influence the environment.

9.2 Nitrogen fixation

The growth of all organisms depends on the availability of mineral nutrients, and none is more important than nitrogen, which is required in large amounts as an essential component of proteins, nucleic acids and other cellular constituents. There is an abundant supply of nitrogen in the earth's atmosphere - nearly 79% in the form of N_2 gas. However, N_2 is unavailable for use by most organisms because there is a triple bond between the two nitrogen atoms, making the molecule almost inert.

Nitrogen fixation is the natural process, either biological or abiotic, by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). This process is essential for life because fixed nitrogen is required to biosynthesize the basic

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building blocks of life, e.g., nucleotides for DNA and RNA and amino acids for proteins. Nitrogen fixation also refers to other abiological conversions of nitrogen, such as its conversion to nitrogen dioxide. Similarly this process is utilized by numerous prokaryotes, including bacteria, actinobacteria, and certain types of anaerobic bacteria. Microorganisms that fix nitrogen are called diazotrophs. Some higher plants, and some animals (termites), have formed associations (symbioses) with diazotrophs. Nitrogen fixation also occurs as a result of non-biological processes. These include lightning, industrially through the Haber - Bosch process, and combustion. Atmospheric nitrogen or molecular nitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. Nitrogen fixation is essential for agriculture and the manufacture of fertilizer. It is also an important process in the manufacture of explosives (e.g. gunpowder, dynamite, TNT, etc.). Nitrogen fixation occurs naturally in the air by means of lightning.

9.2.1 Types of Nitrogen fixation

Nitrogen fixation is of two types- Physical and biological. In Physical Nitrogen fixation it is carried out by many steps without any biological agent and involve in combination of atmospheric nitrogen with oxygen to form Nitric oxide. This nitric acid is then oxidized by O_2 to form nitrogen peroxide. It further combines with water to form nitric acd or nitrous acid. This acid is then combining with alkali radicle which comes from rains to ground water and then they are absorbed by the roots of plants.

Whereas in Biological nitrogen fixation is the process that changes inert N_2 to biologically useful NH_3. This process is mediated in nature only by bacteria. Other plants benefit from nitrogen-fixing bacteria when the bacteria die and release nitrogen to the environment or when the bacteria live in close association with the plant. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules. Within these nodules, nitrogen fixation is done by the bacteria, and the NH_3 produced is absorbed by the plant. Nitrogen fixation by legumes is a partnership between a bacterium and a plant. Biological nitrogen fixation can take many forms in nature, including blue green algae (a bacterium), lichens and free-living soil bacteria. These types of nitrogen fixation contribute significant quantities of NH_3 to natural ecosystems.

9.2.2 Biological Nitrogen fixation and its Mechanism

Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901, is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can readily assimilate NH_3 to produce the nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as Azotobacter, bacteria that form associative relationships with plants, such as Azospirillum, and most importantly, bacteria, such as Rhizobium and Brady rhizobium that form symbioses with legumes and other plants

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In Biological nitrogen fixation (BNF) nitrogen is converted to ammonia by an enzyme called nitrogenase. The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway. Nitrogenase consists of two different proteins both of which are required for enzyme activity. The large protein (Mo Fe) contains 2 Mo and 20 Fe atom and 20 sulphides and the smaller (Fe) protein contains only 4 Fe and 4 acid labile sulphides. The Mo Fe protein has mol.wt. 180-230 K Dalton whereas protein has mol.wt. 70 K Dalton. Ferrodoxin and flavodoxin serves as electron source and ATP is used as energy source.

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

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase. This enzyme consists of two proteins - an iron protein and a molybdenum-iron protein, as shown below.

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism,

the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation.

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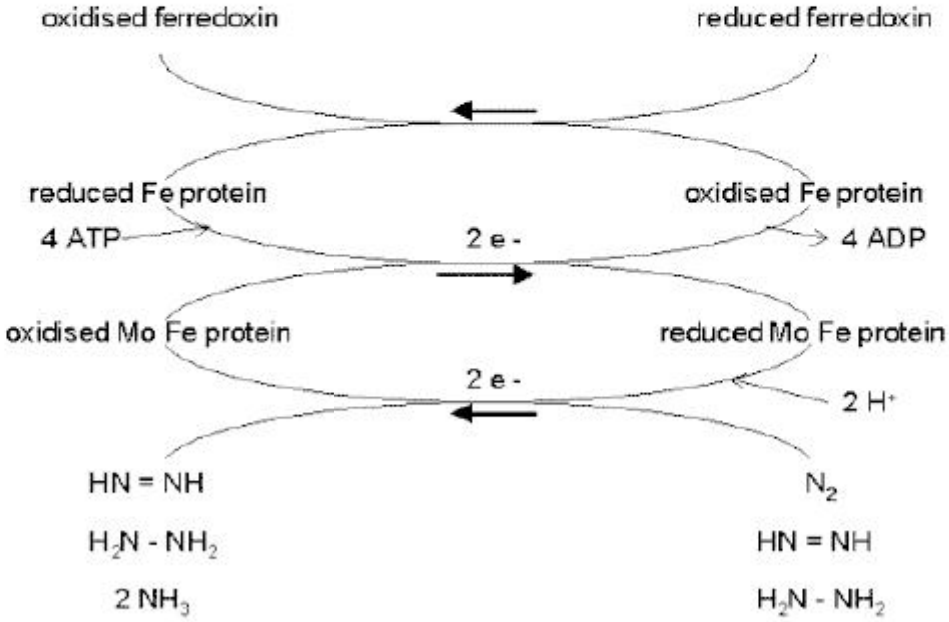

Fig. 9.1: Biological Nitrogen fixation by Nitrogenase Enzyme

Site of Nitrogen fixation

According to Bergersen 1969, the membrane envelops surrounded the group of bacteroids are the site of nitrogen fixation, however, recently it has been proved that bacteroids are the site of nitrogen fixation.

The nitrogen-fixing organisms

All the nitrogen-fixing organisms are prokaryotes (bacteria). Some of them live independently of other organisms - the so-called free-living nitrogen-fixing bacteria. Others live in intimate symbiotic associations with plants or with other organisms (e.g. protozoa). Examples are shown in the table below.

Table :1 -Nitrogen fixing Bacteria

Free living		Symbiotic with plants	
Aerobic	Anaerobic	Legumes	Other plants-non legumes

Azotobacter Beijerinckia Klebsiella (some) Cyanobacteria (some)*	Clostridium (some) Desulfovibrio Purple sulphur bacteria* Purple non-sulphur bacteria* Green sulphur bacteria*	Rhizobium, Bradyrhizobium sps.	Frankia Azospirillum Anabaena,Nostoc
(*denotes a photosynthetic bacterium)			

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Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. Many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as leghemoglobin. Diazotrophs are cyanobacteria, e.g. the highly significant trichodesmium , green sulfur bacteria, azotobacteraceae, rhizobia and Frankia.Cyanobacteria inhabit nearly all illuminated environments on Earth and play key roles in the carbon and nitrogen cycle of the biosphere. In general, cyanobacteria are able to utilize a variety of inorganic and organic sources of combined nitrogen, like nitrate, nitrite, ammonium, urea, or some amino acids.

Plants that contribute to nitrogen fixation include the legume family – Fabaceae – with taxa such as kudzu, clovers, soybeans, alfalfa, lupines, peanuts, and rooibos. They contain symbiotic bacteria called Rhizobia within nodules in their root systems, producing nitrogen compounds that help the plant to grow and compete with other plants. When the plant dies, the fixed nitrogen is released; making it available to other plants and this helps to fertilize the soil. The great majority of legumes have this association, but a few genera (e.g., Styphnolobium) do not. In many traditional and organic farming practices, fields are rotated through various types of crops, which usually includes one consisting mainly or entirely of clover or buckwheat (non-legume family Polygonaceae), which are often referred to as "green manure" .

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Non-legume

Although by far the majority of plants able to form nitrogen-fixing root nodules are in the legume family Fabaceae, there are a few exceptions: Parasponia, a tropical genus in the Cannabaceae also able to interact with rhizobia and form nitrogen-fixing nodules Actinorhizal plants such as alder and bayberry can also form nitrogen-fixing nodules, thanks to a symbiotic association with Frankia bacteria. These plants belong to 25 genera distributed among 8 plant families.

The ability to fix nitrogen is far from universally present in these families. For instance, of 122 genera in the Rosaceae, only 4 genera are capable of fixing nitrogen. All these families belong to the orders Cucurbitales, Fagales, and Rosales.

Nitrogen Fixation by Free-Living Heterotrophs

Many heterotrophic bacteria live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of Azotobacter, Bacillus, Clostridium, and Klebsiella. These organisms must find their own source of energy, typically by oxidizing organic molecules released by other organisms or from decomposition. There are some free-living organisms that have chemolithotrophic capabilities and can thereby utilize inorganic compounds as a source of energy

Artificial or Chemical Method

The possibility that atmospheric nitrogen reacts with certain chemicals was first observed by Desfosses in 1828. He observed that mixtures of alkali metal oxides and carbon react at high temperatures with nitrogen. With the use of barium carbonate as starting material the first commercially used process became available in the 1860s developed by Margueritte and Sourdeval. The resulting barium cyanide could be reacted with steam yielding ammonia. In 1898 Adolph Frank and Nikodem Caro decoupled the process and first produced calcium carbide and in a subsequent step reacted it with nitrogen to calcium cyanamide. The Ostwald process for the production of nitric acid was discovered in 1902. Frank-Caro process and Ostwald process dominated the industrial fixation of nitrogen until the discovery of the Haber process in 1909.

The reduction of atmospheric nitrogen is a complex process that requires a large input of energy to proceed. The nitrogen molecule is composed of two nitrogen atoms joined by a triple covalent bond, thus making the molecule highly inert and nonreactive. Nitrogenase catalyzes the breaking of this bond and the addition of three hydrogen atoms to each nitrogen atom.

Industries use the Haber-Bosch process to reduce nitrogen essentially in the same way. Conventional agriculture has depended upon this process to produce the commercial fertilizer needed to grow most of the world's hybrid crops. But this approach comes with many consequences, including using fossil fuels for the energy needed to produce this fertilizer, the resulting carbon dioxide emissions and pollution from burning these fuels, and adverse affects on human health.

Haber process

Artificial fertilizer production is now the largest source of human-produced fixed nitrogen in the Earth's ecosystem. Ammonia is a required precursor to fertilizers, explosives, and other products. The most common method is the Haber process. The Haber process requires high pressures (around 200 atm.) and high temperatures (at least 400 °C), routine conditions for industrial catalysis. This highly efficient process uses natural gas as a hydrogen source and air as a nitrogen source. Many compounds react with atmospheric nitrogen to give dinitrogen complexes. The first dinitrogen complex to be reported was based on ruthenium, $[Ru(NH_3)^5(N_2)]^{2+}$.

Ambient nitrogen reduction

Catalytic chemical nitrogen fixation at temperatures considerably lower than the Haber process is an ongoing scientific endeavour. Nitrogen was converted to ammonia and hydrazine by Alexander E. Shilov in 1970. Few compounds will cleave the N_2 molecule. Under an atmosphere of nitrogen, lithium metal converts to lithium nitride. Treatment of the resulting nitride gives ammonia. Another example of homolytic cleavage of dinitrogen under mild conditions where two equivalents of a molybdenum complex reacted with one equivalent of dinitrogen, creating a triple bonded MoN complex. Since then, this triple bonded complex has been used to make nitriles. Trimethylsilyl chloride,

lithium, and nitrogen molecule react to give tris (trimethylsilyl) amine, under catalysis by nichrome wire or chromium trichloride in tetrahydrofuran.

$3\ Me_3SiCl + 3\ Li + 1/2\ N_2 \longrightarrow (Me_3Si)_3N + 3\ LiCl$

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Tris (trimethylsilyl) amine can then be used for reaction with α,δ,ω-triketones to give tricyclic pyrroles.

Catalytic systems for converting nitrogen to ammonia have been developed since the 1980s. In 2003 another was reported based on molybdenum compound, a proton source, and a strong reducing agent. However, this catalytic reduction fixates only a few nitrogen molecules.

9.3 Legume Nodule Formation and Nod factor

Legume nitrogen fixation starts with the formation of a nodule. A common soil bacterium, Rhizobium, invades the root and multiplies within the cortex cells. The plant supplies all the necessary nutrients and energy for the bacteria. Within a week after infection, small nodules are visible with the naked eye. In the field, small nodules can be seen 2-3 weeks after planting, depending on legume species and germination conditions. When nodules are young and not yet fixing nitrogen, they are usually white or gray inside. As nodules grow in size, they gradually turn pink or reddish in colour, indicating nitrogen fixation has started. The pink or red colour is caused by leghemoglobin (similar to hemoglobin in blood) that controls oxygen flow to the bacteria.

The main functions of leghemoglobin are to bind and regulate the levels of oxygen in the nodule. Because the enzyme nitrogenase is sensitive to oxygen, free oxygen in nodule cell cytoplasm would inhibit the levels of nitrogen fixation in the nodule. Leghemoglobin seems to transport enough oxygen to allow the rhizobia to carry out cellular respiration, but not too much to inhibit the action of nitrogenase. This heme protein is jointly produced by the legume and bacterium; the legume produces the apoprotein while the bacterium produces the heme (porphyrin ring bound to an iron atom.

Specific signal molecules secreted by Rhizobium, named Nod factors, play a pivotal role in the induction of all early responses. For example, they are

required for gene activation in the epidermis and pericycle cells, for the mitotic reactivation of the cortical cells, and for the formation of pre–infection threads.

9.3.1 Nodule Formation

Major research on the legume–Rhizobium interaction is common in two model legume species, *Lotus japonicus* and *Medicago truncatula*. Two model legumes have been selected because most legumes form nodules that belong either to the determinate or to the indeterminate nodule type and *L. japonicus* and *M. truncatula*, respectively, represent these two major legume groups .Although the morphology and ontology of these two nodule types is different, the mechanisms underlying the formation of them are probably very similar

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The formation of a nodule requires the reprogramming of differentiated root cells to form a primordium, from which a nodule can develop. Furthermore, the bacteria must infect the root before the nitrogen-fixing root nodule can be formed. These steps in nodule formation involve changes in three root tissues, namely epidermis, cortex, and pericycle. When rhizobia have colonized the root surface of their host, they induce morphological changes in the epidermis. These morphological changes are preceded by the induction of certain genes in a broad region of the epidermis. The examples of early-nodulin genes are ENOD12 and ENOD11, which are homologous and belong to a gene family encoding proline-rich proteins. ENOD12 and ENOD11 have a similar expression pattern and have been used as molecular markers to monitor signal transduction events in the epidermis.

Upon inoculation with rhizobia, root hairs will deform. This is caused by a reinitiating of tip growth in these cells, but with a changed growth direction. These morphological changes are preceded by changes in the actin skeleton .In some root hairs, the rhizobia induce deformations that resemble a so-called shepherd's crook, and such curled root hairs play an important role in the infection process. Root hair curling is probably caused by a gradual and constant reorientation of the growth direction of the hair by which a curl with a turn of 360° is formed .During the curling process, the bacteria become entrapped in the pocket of the curl. There the plant cell wall is modified in a very local manner, the plasma membrane invaginates, and new plant material is deposited. In this way a tube-like structure, the infection thread, is formed that

contains the bacteria. The infection thread will grow toward the base of the root hair cell and subsequently to the nodule primordium.

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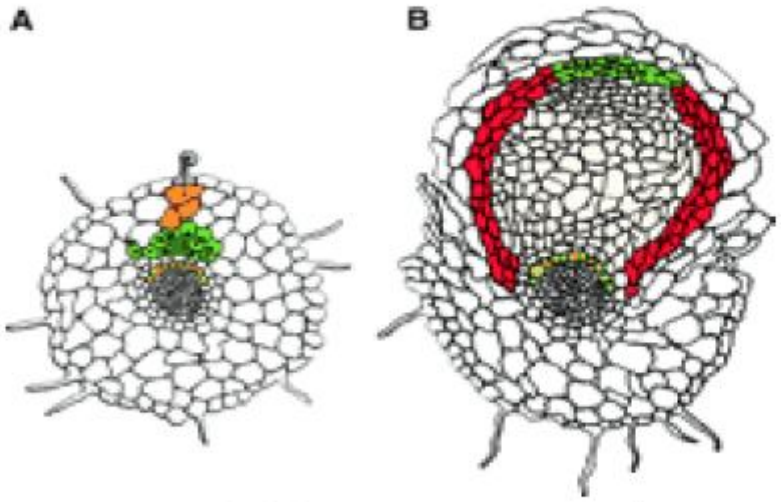

Fig. 9.2 : Root Nodule Formation

(A) **Nodule primordium formation.**

The bacteria induce root hair curling and infection thread formation in epidermal cells. Concomitantly, the inner cell layers are activated. Pericycle cells opposite a proto-xyleme pole express the ENOD40 gene and eventually will undergo a limited number of cell divisions. Cells in the cortex will loose their cell identity and enter the cell cycle. This results in the formation of a nodule primordium in the inner cortex. In these cells, ENOD12 and ENOD40 are expressed. Outer cortical cells also enter the cell cycle, but are arrested and will form preinfection threads.

(B) **Root nodule differentiation**

Cells of the nodule primordium obtain their final identity after rhizobial infection. At the base of the primordium, a radial pattern of a central tissue surrounded by peripheral tissues is established. Similarly, cells at the apex of the primordium form a meristem. These cells differ in their identity compared with primordium cells, in that they do not express ENOD12. The central tissue contains the cells that host the rhizobia.

When the infection thread has crossed the epidermis, cortical and pericycle cells respond in a local manner to the rhizobia. In pericycle cells, this is reflected by the rapid induction of ENOD40 in a zone opposite a protoxylem

pole and by rearrangements of the microtubles. Eventually, these cells will undergo a limited number of cell divisions. Following the activation of the pericycle, cells in the inner cortex dedifferentiate by entering the cell cycle. The group of dividing cortical cells is named the nodule primordium.

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Outer cortical cells, through which the infection threads will grow, form radially oriented, conical cytoplasmic columns. This organization of the cytoplasm takes place before the infection thread enters these cells; these therefore are named pre–infection threads. The pre–infection thread forming outer cortical cells also enter the cell cycle but are arrested before they can enter the M phase. Therefore, it is assumed that pre–infection threads are in some way related to phargmoplasts .The pre–infection threads will facilitate infection thread growth and direct it toward the nodule primordium. There the infection thread ramifies, followed by the release of bacteria into the primordial cells. Upon release, the bacteria remain surrounded by a membrane of plant origin and subsequently will differentiate into their symbiotic form and will start to fix nitrogen.

In general, the transition from nodule primordium to young developing nodule occurs after infection of primordial cells. During this transition, cells at the base of the primordium establish a radial pattern consisting of a central tissue surrounded by peripheral tissues. At the same time, cells at the apex of the primordium form a meristem that, by division, maintains itself and adds new cells to the different tissues according to the pattern established at the base of the primordium (Figure 1B). The identity of nodule primordium cells and nodule meristematic cell is different. For example, a meristematic cell is never infected by rhizobia, and genes that are activated in the primordium, e.g., ENOD12, are not transcribed in the nodule meristem.

9.3.2 Nod Factors

Proteins encoded by the rhizobial nodulation genes (nod, nol, and noe genes) are involved in the synthesis and secretion of Nod factors. The expression of these genes is activated when the bacteria perceive specific molecules, flavonoids which are secreted by the plant root. Flavonoids activate the bacterial transcriptional regulator NodD that in turn induces the transcription of the other bacterial nodulation genes involved in the synthesis of Nod factors.

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The basic structure of Nod factors produced by different rhizobial species is very similar. Generally, they consist of a β-1,4–linked N-acetyl-d-glucosamine backbone with 4 or 5 residues of which the non-reducing terminal residue is substituted at the C2 position with an acyl chain. Depending on the rhizobial species, the structure of the acyl chain can vary, and substitutions at the reducing and nonreducing terminal glucosamine residues can be present. The structure of Nod factors of different rhizobia and their function in nodulation has been reviewed in Sinorhizobium meliloti, the bacterium interacting with the Medicago species..

The major Nod factor produced by Sinorhizobium meliloti contains four glucosamine units, an acyl chain of 16 C-atoms in length with two unsaturated bonds (determined by NodE and NodF), an acetyl group at the non-reducing terminal sugar residue (determined by NodL), and a sulfate group at the reducing terminal sugar residue (determined by NodH, NodP and NodQ) .

The differences in structure of Nod factors made by different rhizobial species are determined by the presence of species-specific nodulation genes or are due to allelic variation resulting in a different activity of the encoded enzymes. Nod factor is a major determinant of host specificity because it is required for the induction of almost all symbiotic responses. In contrast, the O-acetate group as well as the structure of the acyl chain is especially required for efficient infection.

Nod Factor Binding

Nod factors are recognized by a high affinity receptor. Further, the amphiphylic nature of Nod factors, with their hydrophobic lipid tail and -hydrophilic sugar backbone, suggests that Nod factor receptors are located in the plasma membrane.

To identify putative Nod factor receptors, two approaches have been used: a direct approach to identify Nod factor binding sites in protein extracts, and a "candidate gene" approach to determine whether proteins encoded by known genes are able to bind Nod factors. Both approaches have led to the identification of Nod factor binding proteins. Two Nod factor binding proteins, NFBS1 and NFBS2, have been identified using binding studies with protein extracts. By using a "candidate gene" approach, it was shown that Nod factors

bind to a lectin–nucleotide phosphohydrolase (LNP) named Db-LNP isolated from the roots of the legume Dolichos biflorus.

Moreover, two other lectins have been shown to play a role in the early steps of nodulation. These are pea seed lectin PSL1 and Le1 of soybean. When these genes are expressed in heterologous plants, the host range of these plants is extended, but they also become more susceptible to their normal host rhizobial species. However, it is unlikely that the mode of action of these lectins depends on Nod factor binding. Nod factors may have a dual function, first as a ligand for a Nod factor receptor activating signal transduction cascades and second as a positional cue to redirect root hair growth.

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9.4 Nitrogen Metabolism

9. 4 .1 Nitrogen cycle

The diagram shows an overview of the nitrogen cycle in soil or aquatic environments.

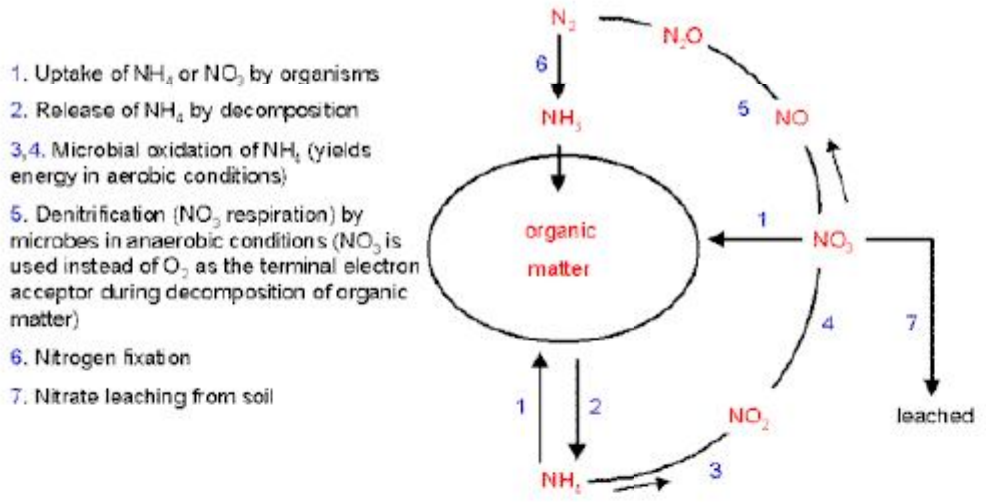

Fig. 9.2 : Nitrogen cycle

Steps of Nitrogen cycle

(i) Nitrification

The term nitrification refers to the conversion of ammonium to nitrate. This is brought about by the nitrifying bacteria, which are specialised to gain their energy by oxidising ammonium, while using CO_2 as their source of carbon to synthesise organic compounds are called chemoautotrophs - they gain their

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energy by chemical oxidations (chemo-) and they are autotrophs (self-feeders) because they do not depend on pre-formed organic matter. In principle the oxidation of ammonium by these bacteria is no different from the way in which humans gain energy by oxidising sugars. Their use of CO_2 to produce organic matter is no different in principle from the behaviour of plants.

The nitrifying bacteria are found in most soils and waters of moderate pH, but are not active in highly acidic soils. They almost always are found as mixed-species communities (termed consortia) because some of them - e.g. *Nitrosomonas* species - are specialised to convert ammonium to nitrite (NO^{2-}) while others - e.g. *Nitrobacter* species - convert nitrite to nitrate (NO^{3-}). In fact, the accumulation of nitrite inhibits Nitrosomonas, so it depends on Nitrobacter to convert this to nitrate, whereas *Nitrobacter* depends on *Nitrosomonas* to generate nitrite.

The nitrifying bacteria have some important environmental consequences, because they are so common that most of the ammonium in oxygenated soil or natural waters is readily converted to nitrate. Most plants and microorganisms can take up either nitrate or ammonium. However, process of nitrification has some undesirable consequences. The ammonium ion (NH^{4+}) has a positive charge and so is readily adsorbed onto the negatively charged clay colloids and soil organic matter, preventing it from being washed out of the soil by rainfall. In contrast, the negatively charged nitrate ion is not held on soil particles and so can be washed down the soil profile - the process termed leaching. In this way, valuable nitrogen can be lost from the soil, reducing the soil fertility. The nitrates can then accumulate in groundwater, and ultimately in drinking water.

(ii) Denitrification

Denitrification refers to the process in which nitrate is converted to gaseous compounds (nitric oxide, nitrous oxide and N_2) by micro-organisms. The sequence usually involves the production of nitrite (NO^{2-}) as an intermediate. Several types of bacteria perform this conversion when growing on organic matter in anaerobic conditions. Because of the lack of oxygen for normal aerobic respiration, they use nitrate in place of oxygen as the terminal electron acceptor. This is termed anaerobic respiration.In aerobic respiration (as in

humans), organic molecules are oxidised to obtain energy, while oxygen is reduced to water:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

(iii) Nitrogen Assimilation

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Primary products of assimilated ammonia are glutamine and glutamate, which constitute the central reservoir of nitrogen for many biosynthetic pathways. Glutamate is the principal source of nitrogen for the production of N-amine and is involved in transanimation reactions at the core of amino acid metabolism. Three key enzymes in ammonia assimilation: glutamine synthetase (GS-ase), glutamate synthase (GOGAT-ase), and glutamate dehydrogenase (GDH-ase), GS-ase catalyzes the incorporation of ammonia into glutamate to produce glutamine, at the expense of one ATP molecule. Glutamine plus α-ketoglutarate can then be converted to glutamate by the action of GOGAT-ase. This is the major pathway at low ammonia concentrations. In contrast, in energy-limiting conditions, glutamate may also be synthesized directly by α-ketoglutarate amination, catalyzed by GDH-ase. This reaction is reversible: glutamate is therefore synthesized by this enzyme principally when ammonium concentration is high, whereas GS-ase is still necessary for glutamine synthesis. Owing to the central role of GS-ase in nitrogen assimilation in E. coli, the expression of the corresponding gene and its activity are highly regulated.

9. 4. 2 Nitrate uptake and reduction

Nitrogen is one of the most expensive nutrients to supply and commercial fertilizers represent the major cost in plant production. Furthermore, there is serious concern regarding nitrogen loss in the field, giving rise to soil and water pollution. Incomplete capture and poor conversion of nitrogen fertilizer also causes global warming through emissions of nitrous oxide. The ability of a plant to capture nitrogen from the soil depends on soil type, environment and species. It has been estimated that 50–70 % of the nitrogen provided to the soil is lost .The use of nitrogen by plants involves several steps, including uptake, assimilation, translocation and, when the plant is ageing, recycling and remobilization.

The form of nitrogen also plays a key role in cation–anion relationships in plants and hence in net proton release by roots. Ammonium nutrition and N_2 fixation are accompanied by the release of H^+, whereas NO^{3-} uptake involves OH^- excretion.

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Nitrate is generally considered to be the major form of inorganic nitrogen in most agricultural soils leaching of nitrate, which is produced during N transformations, is a major cause of topsoil acidification. Nitrate uptake occurs at the root level and two nitrate transport systems have been shown to coexist in plants and to act co-ordinately to take up nitrate from the soil solution and distribute it within the whole plant. Nitrate reduction is carried out in two steps. Nitrate is first reduced to nitrite (NO_2^-) in the cytosol by nitrate reductase using NADH or NADPH Nitrite is then reduced to ammonia in the chloroplasts (plastids in roots) by a ferredoxin dependent nitrite reductase. In photosynthesizing tissues, it uses an isoform of ferredoxin (Fd^{1+}) that is reduced by PSI while in the root it uses a form of ferredoxin (Fd^{3+}) that has a less negative midpoint potential and can be reduced easily by NADPH. In non photosynthesizing tissues, NADPH is generated by glycolysis and the pentose phosphate pathway.

Nitrate absorbed by plants must be reduced to ammonium before incorporation into amino acids. Nitrate reduction is catalysed by nitrate reductase and nitrite reductase. There are large differences among species and genotypes in the role of shoot and root systems in the reduction of nitrate. Plants can be categorized into three groups according to the major site of nitrate reduction: (1) root; (2) root and shoot; and (3) shoot. However, it is not known whether the site of nitrate reduction is related to the ability of plants to take up nitrate and the release net acid by the root. Nitrate is reported to improve tolerance of plants towards oxygen deficiency enabled by waterlogging of the root system, but the mechanism underlying the phenomenon remains poorly understood. Nitrate depletion from the medium was more intense under hypoxia than normoxia, but in the presence of chloramphenicol, consumption under hypoxia was significantly reduced. Nitrite accumulated in the medium in the state of hypoxia and this effect was partially eliminated by chloramphenicol. Nitrate consumption sensitive to chloramphenicol was attributed to bacterial activity. Endogenous root nitrate was strongly reduced under hypoxia indicating

mobilization. Although the transport of nitrate to the shoot via the xylem was also reduced under hypoxia, the severity of this reduction was dependent on the concentration of nitrate in the medium, suggesting that at least some of the nitrate in the xylem came from the medium. Root nitrate reductase was also strongly reduced under hypoxia, but recovered rapidly on return to normoxia.

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9.4.3 Ammonia assimilation

Ammonia assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment. Organisms like plants, fungi and certain bacteria that cannot fix nitrogen gas (N_2) depend on the ability to assimilate nitrate or ammonia for their needs. Other organisms, like animals, depend entirely on organic nitrogen from their food.

Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonia (NH_3). In aerobic soils where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed. Plant roots themselves can affect the abundance of various forms of nitrogen by changing the pH and secreting organic compounds or oxygen. This influences microbial activities like the inter-conversion of various nitrogen species, the release of ammonia from organic matter in the soil and the fixation of nitrogen by non-nodule-forming bacteria. Nitrate reduction takes place in both roots and shoots but is spatially separated between the cytoplasm where the reduction takes place and plastids/chloroplasts where nitrite reduction occurs. Nitrate reduction into nitrite is catalysed in the cytosol by the enzyme nitrate reductase (NR). This enzyme is a homodimer, each monomer being associated with three prosthetic groups: flavin adenine dinucleotide (FAD), a haem and a molybdenum cofactor (MoCo). After nitrate reduction, nitrite is translocated to the chloroplast where it is reduced to ammonium by the second enzyme of the pathway, the nitrite reductase (NiR). The Nii genes encoding the NiR enzyme have been cloned from various species, the number of genes varying from one to two copies.

Ammonium, originating from nitrate reduction, and also from photorespiration or amino acid recycling, is mainly assimilated in the plastid/chloroplast by the so-called GS/GOGAT cycle .The glutamine synthetase (GS) fixes ammonium on a glutamate molecule to form glutamine. This glutamine reacts subsequently

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with 2-oxoglutarate to form two molecules of glutamate, this step being catalysed by the glutamine 2-oxoglutarate amino transferase (or glutamate synthase, GOGAT). Two classes of nuclear genes code for GS: the GLN2 and GLN1 genes. GLN2, present as a single nuclear gene in all the species studied so far, codes for the chloroplastic GS2, thought to be involved in the primary assimilation of ammonium coming from nitrate reduction in both C3 and C4 plants and in the re-assimilation of ammonium produced from photorespiration in C3 plants. The magnitude of the ammonium flux through the photorespiration pathway in the leaves of C3 plants was indeed estimated to exceed that produced from nitrate reduction by five- to ten-fold .

Conversely, the GLN1 gene family codes for cytosolic GS1 isoforms, present in different organs such as roots or stems and thought to be involved in ammonium recycling during particular developmental steps such as leaf senescence and in glutamine synthesis for transport into the phloem sap. Two different forms of glutamate synthase are present in plants: Fd-GOGAT and NADH-GOGAT use ferredoxin and NADH as the electron donors, respectively. Fd-GOGAT is predominantly localized in leaf chloroplasts whereas NADH-GOGAT is primarily located in plastids of non-photosynthetic tissues, such as roots, etiolated leaf tissues and companion cells.

In addition to the GS/GOGAT cycle, three enzymes probably participate in ammonium assimilation. Cytosolic asparagine synthetase (AS) catalyses the ATP-dependent transfer of the amido group of glutamine to a molecule of aspartate to generate glutamate and asparagine.Carbamoylphosphate synthase (CPSase) forms carbamoylphosphate, a precursor of citrulline and arginine, within plastids using bicarbonate, ATP and ammonium or the amide group of glutamine.

9.5 Sulphur Metabolism

Sulphur is one of the six macronutrients required by plants and is found in the amino acids Cystein (Cys) and Methionine (Met) and in a variety of metabolites. Sulphur is only 3% to 5% as abundant as nitrogen; sulfur assimilation has been less well studied. As a part of the Cys molecule, the sulfur group, called a thiol, is strongly nucleophilic (electron-donating), making it ideally suited for biological redox processes. When oxidized, two Cys

molecules can form a covalent linkage called a disulfide bond, which is readily broken by reduction to form two thiol groups. Disulfide ↔ dithiol interchange is so versatile that nearly all aerobic forms of life, including plants, have evolved to use this reaction as the dominant form of redox control. Redox control regulates enzymes and protects against oxidative damage.

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9.5.1 Sulphate Uptake and Transport

Free Cys is not used for redox control. It is much too readily oxidized to cystine, the disulfide form, which is visible in the laboratory as a white precipitate that is formed within hours after preparing a solution of Cys. A variety of more stable thiol compounds are involved in redox regulation. The most abundant is glutathione, an enzymatically synthesized tripeptide in which Cys is linked via peptide bonds to the γ-carboxyl group of Glu and the α-amino group of Gly. In plants glutathione is thought to be between 3 and 10 mm, and it is present in the major cellular compartments. The reduced form of glutathione is often referred to as GSH, whereas the disulfide form is GSSG. The balance between forms is overwhelmingly maintained in favour of GSH by the enzyme glutathione reductase, using NADPH as an electron source. The result is that the plant cytoplasm, chloroplast stroma, and mitochondrial matrix are highly buffered in the reducing state. Many intracellular enzymes require reducing conditions for activity, just as they require a specific pH or other properties of their chemical environment. The reason is that Cys residues in proteins can also form disulfide bonds, resulting in a disruption of structure and a loss of activity. There are special cases in which specific disulfide bonds are required for formation of tertiary and quaternary structure in a protein, but this is less common, especially for soluble intracellular proteins.

The transport of SO_4^{2-} occurs across several membrane systems as it enters and is distributed throughout the plant and within cells. Transport across the plasma membrane occurs with protons at a ratio of 1 SO_4^{2-}:3 H^+ (symport) and is driven by a proton gradient maintained by a proton ATPase. Transport across the tonoplast membrane is mediated by an unknown mechanism that is driven by the electrical gradient between the vacuole sap and cytoplasm. The phosphate/triose phosphate translocator of the inner chloroplast membrane or a proton/SO_4^{2-} symporter may mediate SO_4^{2-} transport into chloroplasts.

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The plasma membrane transporters of plants have been characterized. The sequences of cDNAs cloned from *Stylosanthes hamata*, Arabidopsis, soybean, barley, maize, resurrection grass, and Indian mustard showed that the plasma membrane transporters of plants are most closely related to fungal and animal proton/SO_4 2−cotransporters. Hydropathy analysis revealed that the plant transporters may span the membrane 12 times, a structural feature that is typical of many types of solute symporters.

In most of the species that have been analyzed, SO_4^{2-} transporters are encoded by a gene family. The situation in *S. hamata* is probably typical for most plants. In this species the individual transporters may have specialized functions, since they differ widely in affinity for SO_4^{2-}, and they show distinct spatial and regulated patterns of expression. High-affinity forms with K m for SO_4^{2-} of approximately 9 μm are expressed exclusively in roots, whereas the lower-affinity form with K m for SO_4^{2-} of approximately 100 μm is expressed principally in leaves but also in roots. The steady-state level of mRNA for the high-affinity form increases rapidly after sulphur starvation, whereas the lower-affinity form is unresponsive or responds more slowly to changes in external SO_4^{2-} supply. These results imply that the increase in SO_4^{2-} transport activity observed in roots of sulphur-starved plants is due to an increase in the expression of specific transporters. One of the earliest observations of SO_4^{2-} transport into roots was that the uptake rate varies in relation to the [SO_4^{2-}] of the bathing solution. The results with S. hamata suggest that this multiphasic behaviour may be due to the activities of separate transporters with different affinities for SO_4^{2-}.

Other factors that use the chemistry of disulfide ↔ dithiol interchange to mediate redox reactions include the proteins thioredoxin, glutaredoxin, and protein disulfide isomerase. These proteins are nearly ubiquitous and play fundamental roles in many different types of regulation. The dark reactions of CO_2 fixation must be strictly coordinated with the light reactions of photosynthesis. The coordination mechanism relies on the reductive activation of specific enzymes by thioredoxin, which is reduced by photo synthetically reduced Fd. New disulfide ↔ dithiol redox regulated processes are being

discovered each year, which attests to the prevalent roles that sulfur chemistry plays in biology.

9.5.2 Sulphate Assimilation

Sulphur is available to plants primarily in the form of anionic sulphate (SO_4^{2-}) present in soil. It is actively transported into roots and then distributed, mostly unmetabolized, throughout the plant. SO_4^{2-} is a major anionic component of vacuolar sap; therefore, it does not necessarily enter the assimilation stream. Gaseous sulphur dioxide (SO_2) is readily absorbed and assimilated by leaves, but it is significant as a nutrient source only in industrial areas with air pollution. Sulphur is assimilated in one of two oxidation states. SO_4^{2-} can be added to a hydroxyl group of an organic molecule. The reaction is referred to as sulfation and it is catalyzed by sulphotransferases. Some sulphur-containing phytoalexins such as camalexin may be important in combating plant pathogens. Although sulphur was long thought not to limit plant productivity, the recent restrictions on emissions of sulfurous air pollutants, the ingredients of acid rain, have resulted in sulfur deficiency in some agricultural areas of the world. Another example is that sulfur assimilation by plants has been implicated as a potential factor in moderating climate. Marine algae are prodigious producers of dimethylsulfoniopropionate, a sulphur-containing analog of betaine. Dimethylsulfoniopropionate degradation releases dimethylsulfide, which volatilizes from the ocean and seeds the formation of clouds in the atmosphere. The global scale of this process is such that algal growth may actually influence climate.

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In higher plants sulfation is a relatively minor fate for sulfur when compared with the reductive pathway. However, in marine algae, which produce large amounts of sulfated extracellular polysaccharides such as agar, sulfation accounts for a much greater proportion of the total assimilated sulphur.

Sulphur assimilation can be divided into three steps-

1. Activation
2. Reduction of Sulphides
3. Incorporation of Sulphide into Cystein

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1. Activation

SO_4^{2-} is an inert compound that must be activated before it can be metabolized. The low reactivity of SO_4^{2-} is a barrier to assimilation that is overcome by formation of a phosphate-SO_4^{2-} anhydride bond in the compound APS. The reaction is catalysed by ATP sulfurylase and is the sole entry point for metabolism of SO_4^{2-}. The equilibrium of the adenylation reaction favours the production of SO_4^{2-} and ATP, the reverse reaction. The K eq is 10−7 m in vitro, and the forward reaction can be measured only if enzymes that hydrolyse PPi and modify APS are included. Exactly how ATP sulfurylase operates in the forward direction in vivo has yet to be determined, since the conditions do not appear to be in equilibrium. The PPi concentration is approximately 0.3 mm in plant cells. There are two ATP sulfurylase isoforms in most plants: a major form localized in plastids and a minor form localized in the cytoplasm. Both enzymes have similar kinetic and structural properties.

Plant sulfo-transferases have been characterized that catalyze the sulfation of flavonol, desulfoglucosinolate, choline, and gallic acid glucoside. Sulfotransfer is the terminal step in the biosynthesis of these compounds. The function of sulfated flavonol and choline is unknown. Glucosinolates are the compounds responsible for the distinctive taste of mustards. Gallic acid glucoside, also known as turgorin or periodic leaf movement factor, is responsible for triggering nictinastic leaf movement in *Mimosa pudica*. Sulfation may regulate the process by activating the movement factor. The number of sulfated compounds in plants is not known. In contrast, sulfation plays a key role in the production of growth-regulating peptides in animals. However, recently, a sulfated regulator of cell proliferation, phytosulfokine-α, was identified from plants.

2. Reduction of Sulphide

SO_4^{2-} is reduced before incorporation into cys and the reduction pathways begin with APS. Eight electrons are required to reduce SO_4^{2-} to S^{2-}. The process occurs through the sequential action of two different enzymes. SO_4^{2-} reduction begins with APS in plants and eukaryotic algae. cDNAs that encode APS sulfotransferase have been cloned from a marine green alga and from several higher plants, most notably Arabidopsis. That the enzyme encoded by

the cloned cDNAs was named APS reductase rather than APS sulfotransferase has inadvertently confounded the subject. Although there were reasonable arguments for the new name, none of these were conclusive enough to warrant abridgment of the original name. Here we submit to historical precedent and refer to the enzyme as APS Sulf transferase.

NOTES

In next reduction step S-sulfoglutathione and SO_3^{2-} could be available in plastids. Plant SO_3^{2-} reductase catalyzes the reduction of SO_3^{2-} using electrons donated from reduced Fd. An Fd-dependent enzyme that reduces thiosulonate to thiosulfide has been measured in cell lysates, but it has not been purified or unambiguously demonstrated.

Another evidence indicating that APS sulfotransferase is a prime regulation point in SO_4^{2-} assimilation .The activity of this enzyme changes rapidly in a variety of plant species after sulfur starvation, exposure to reduced sulfur compounds, heavy-metal stress, or other stresses. Heavy metals induce the synthesis of phytochelatins, and high concentrations of metal ions significantly increase the demand for Cys. Recent studies indicate that one potential mechanism for regulating APS sulfotransferase activity may involve changes in the steady-state mRNA level. Sulfur starvation .induce the accumulation of APS sulfotransferase mRNA, but the response is limited to roots. By contrast, SO_3^{2-} reductase does not appear to be appreciably regulated at the mRNA level). The extent to which the regulation of mRNA abundance is responsible for changes in APS Sulf transferase activity has not yet been adequately explored.

3. Incorporation of reduced sulphur into Cysteine

The incorporation of S^{2-} into Cys is the last step in reductive SO_4^{2-} assimilation. The reaction is catalyzed by O-acetylserine (thiol) lyase from S^{2-} and OAS. The synthesis of OAS is catalyzed by Ser acetyltransferase .Ser acetyltransferase and OAS (thiol) lyase exists in an enzyme complex known as Cys synthase. The stability of the complex is affected by substrates (OAS disrupts it and S^{2-} stabilizes it), and it appears to form through specific protein-protein interactions. Yet, the free form of each enzyme has catalytic activity, and the complex is not required for channelling of OAS. Moreover, in chloroplasts the ratio of OAS (thiol) lyase to Ser acetyltransferase is 300:

therefore, only a fraction of the total OAS(thiol)lyase can be associated in the complex.

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The function of the complex is that association with OAS(thiol)lyase changes the kinetic behaviour of Ser acetyltransferase from the Michaelis-Menten type to positive cooperatively with respect to its substrates, Ser and acetyl-CoA .This suggests that OAS (thiol)lyase functions as a regulatory subunit that regulates Ser acetyltransferase in response to OAS and S^{2-}. Positive cooperativity is a form of allosteric regulation in which the velocity of a bisubstrate enzyme is highly sensitive to small changes in substrate concentration. One can think of the enzyme as having a hair-trigger control mechanism. The idea is appealing because Cys synthesis requires coordination of two converging pathways. If there is insufficient S^{2-} resulting from low activity of SO_4^{2-} reduction, the concentration of OAS will increase, causing dissolution of the Cys synthase complex. By contrast, overactivity of SO_4^{2-} reduction results in overabundance of S^{2-} and a shortage of OAS, a condition that would stabilize the complex.

9.6 Summary

Improving global plant productivity and product quality together with taking care of environmental quality and human wellbeing are the main challenges for the immediate. Such a goal depends on agricultural development and policy and can be achieved by providing the right nutrient source at the right rate, the right time and the right place. To improve sustainable agricultural production, it is also necessary to grow crops that can remove the nutrient applied to soil efficiently, and therefore require less fertilizer. Such global 'resource use efficiency' necessitates having a global view of plant physiology, plant uptake capacity, plant metabolism and plant response to restrictions, as well as a view of soil physical and chemical properties.

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway.

Sulphur is available to plants primarily in the form of anionic sulphate (SO_4^{2-}) present in soil. It is actively transported into roots and then distributed, mostly unmetabolized, throughout the plant. SO_4^{2-} is a major anionic component of vacuolar sap; therefore, it does not necessarily enter the assimilation stream. Gaseous sulphur dioxide (SO_2) is readily absorbed and assimilated by leaves, but it is significant as a nutrient source only in industrial areas with air pollution. Sulfur is assimilated in one of two oxidation states. SO_4^{2-} can be added to a hydroxyl group of an organic molecule. The reaction is referred to as sulfation and it is catalysed by Sulf transferases.

NOTES

9.7 Glossary

- **Biological nitrogen fixation :** Biological nitrogen fixation (BNF) is the process that changes inert N_2 to biologically useful NH_3.
- **Nitrification :** The term nitrification refers to the conversion of ammonium to nitrate
- **Chemoautotroph :** Chemoautotroph are the nitrifying bacteria, which are specialised to gain their energy by oxidising ammonium, while using CO_2 as their source of carbon to synthesise organic compounds.
- **Denitrification :** Denitrification refers to the process in which nitrate is converted to gaseous compounds (nitric oxide, nitrous oxide and N_2) by micro-organisms.
- **Ammonia assimilation :** Ammonia assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment.
- **Metabolism :** Metabolism (from Greek: "change") is the set of life-sustaining chemical transformations within the cells of living organisms.
- **Root nodules :** Root nodules occurs on the roots of plants (primarily Fabaceae) that associate with symbiotic nitrogen-fixing bacteria. Under nitrogen-limiting conditions, capable plants form a symbiotic relationship with a host-specific strain of bacteria known as rhizobia.

- **Nif- gene :** The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms
- **Nod factors :** Nodulation (Nod) factors are signalling molecules produced by bacteria known as rhizobia during the initiation of nodules on the root of legumes
- **Symbiosis :** Symbiotic relationships include those associations in which one organism lives on another (ectosymbiosis), or where one partner lives inside the other (endosymbiosis).

NOTES

9.8 Self -Learning Exercise

Section-A (Very Short Answer Type Questions)

1. Write the full form of GOGAT.
2. Name the artificial process which is involved in ammonia formation.
3. Name any two free living nitrogen fixing organisms ?
4. What is Leg-Hg?

Section-B (Short Answer Type Questions)

1. What do you mean by Nitrification?
2. Explain in short about nodule formation.
3. Briefly discuss the Haber process.
4. What is Nod factor?
5. Write the equation of nitrogen fixation.

Section-C (Long Answer Type Questions)

1. What is Biological Nitrogen fixation? Describe the mechanism of nitrogen fixation.
2. Enumerate the process involved in nitrate uptake and reduction.
3. Write an explanatory note on Sulphur metabolism.

Answer key of section –A

1. Glutamine 2 oxo glutarate amino transferase
2. Haber process
3. *Clostridium and* Azotobacter

4. Leg-Hg is produced in root nodules and its functions are to bind and regulate the levels of oxygen in the nodule.

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Unit - 10

Plant Growth Regulators and Elicitors

Structure of the Unit:

10.0 Objective

After going through this unit you will be able to understand:

- Auxins : physiological effects and mechanism of action
- Gibberllins : physiological effects and mechanism of action
- Cytokinins : physiological effects and mechanism of action

10.1 Introduction

Plant hormones (also known as phytohormones) are chemicals that regulate plant growth, which, in the UK, are termed 'plant growth substances'.Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and, moved to other locations, in other functional part of the plant.

Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones. Instead, each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death.

NOTES

Hormones are vital to plant growth, and, lacking them, plants would be mostly a mass of undifferentiated cells. So they are also known as growth factors or growth hormones. The term 'Phytohormone' was coined by Thimann in 1948.Phytohormones are found not only in higher plants, but in algae too, showing similar functions and in microorganisms, like fungi and bacteria, but, in this case, they play no hormonal or other immediate physiological role in the producing organism and can, thus, be regarded as secondary metabolites.

10.2 Auxins and their physiological effects

Auxins were the first plant hormones to be discovered. Both auxin and cytokinin differ from the other plant hormones and signaling agents in one important respect: They are required for viability. In 1926 Frits Went demonstrated the presence of a growth-promoting chemical in the tip of oat (*Avena sativa*) coleoptiles. it was eventually named auxin from the Greek *auxein*, meaning "to increase" or "to grow." Went's studies with agar blocks demonstrated unequivocally that the growth-promoting "influence" diffusing from the coleoptile tip was a chemical substance. The fact that it was produced at one location and transported in minute amounts to its site of action qualified it as an authentic plant hormone.

In the mid-1930s it was determined that auxin is indole-3-acetic acid (IAA).The principal auxin in plants is indole-3-acetic acid (IAA). IAA is produced mainly in the shoot apex bud and young leaves of plants. Others meristematic tissue, flowers, fruits and young seeds have also been shown to be sites of this hormone production. Although they are chemically diverse, a common featureof all active auxins is a molecular distance of about 0.5 nm

between a fractional positive charge on the aromatic ring and a negatively chargedcarboxyl group.

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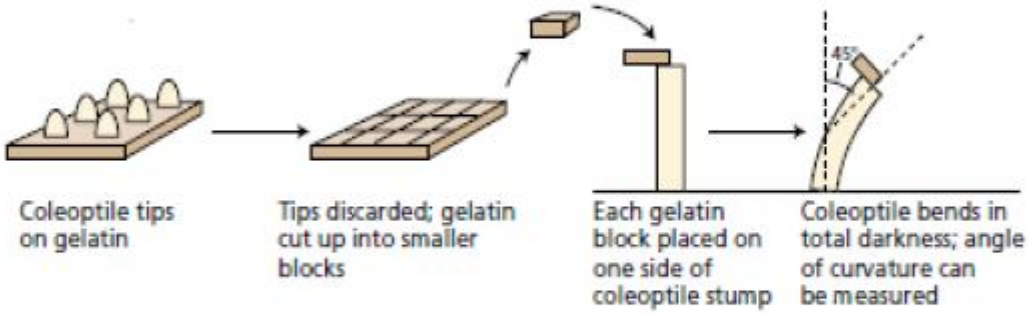


Fig. 10.1--Went Experiment showing that the active growth promoting substances can diffuse into gelatin block

Went used *Avena sativa* (oat) coleoptiles in a technique called the *Avena* coleoptile curvature test. The coleoptiles curved because the increase in auxin on one side stimulated cell elongation, and the decrease in auxin on the other side (due to the absence of the coleoptile tip) caused a decrease in the growth rate—a phenomenon called differential growth. Went found that he could estimate the amount of auxin in a sample by measuring the resulting coleoptile curvatureIAA biosynthesis is associated with rapidly dividing and rapidly growing tissues, especially in shoots. Although virtually all plant tissues appear to be capable of producing low levels of IAA, shoot apical meristems, young leaves, and developing fruits and seeds are the primary sites of IAA synthesis.The amino acid tryptophan is an intermediate in the synthesis of IAA. The IAA movement is strictly polar from the apex to the organ base, i.e. basipetal. This polarity in transport is a manifestation of cell polarity. it was discovered that IAA moves mainly from the apical to the basal end (*basipetally*) in excised oat coleoptile sections. This type of unidirectional transport is termed polar transport. Auxin is the only plant growth hormone known to be transported polarly. Because the shoot apex serves as the primary source of auxin for the entire plant, polar transport has long been believed to be the principal cause of an auxin gradient extending from the shoot tip to the root tip.Recently it has been recognized that a significant amount of auxin transport also occurs in the phloem, and that the phloem is probably the principal route by which auxin is transported *acropetally* (i.e., toward the tip) in the root. Thus, more than one pathway is responsible for the distribution of auxin in the plant.

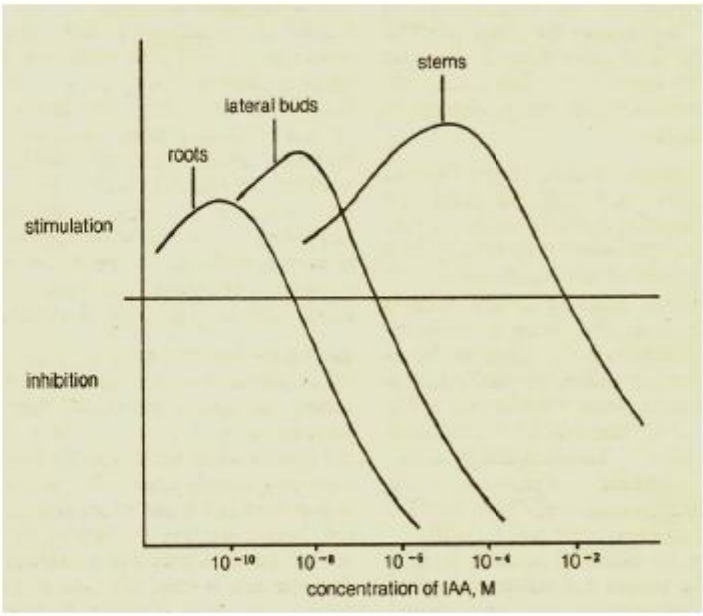
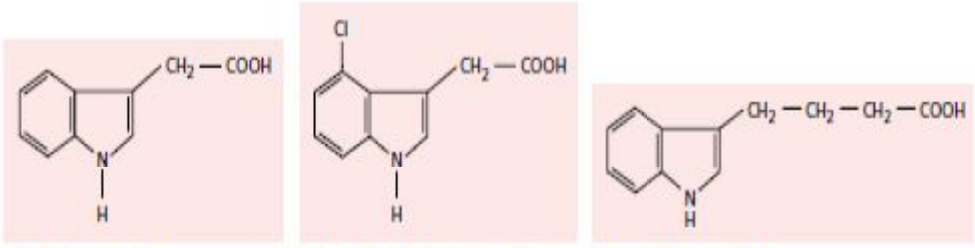


Fig. 10.2 Effects of Auxin concentration on the growth of different organs.

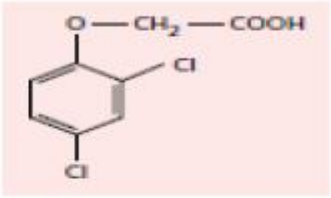


Indole-3-acetic acid (IAA)

4-Chloroindole-3-acetic acid (4-Cl-IAA)

Indole-3-butyric acid (IBA)

Fig 10.3 Structure of Natural Auxins



2,4-Dichlorophenoxyacetic acid (2,4-D)

Fig 10.4 Structure of Synthetic Auxin 2,4-D

Other natural occurring auxins are also based on the indole ring (e.g. indole acetonitrile acid and indole pyruvic acid). However the indole group is not essential for auxin activity as is shown by the auxin activity of certain synthetic compounds, e.g. naphthalenacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). The concentration of auxin is very important in determining the

nature of the growth response and the optimum auxin concentration differs for different organs.

NOTES

Multiple Pathways Exist for the Biosynthesis of IAA

IAA is structurally related to the amino acid tryptophan, and early studies on auxin biosynthesis focused on tryptophanas the probable precursor

1. The IPA pathway

The indole-3-pyruvic acid (IPA) pathway- is probably the most common of the tryptophan-dependent pathways. It involves a deamination reaction to form IPA, followed by a decarboxylation reaction to form indole-3-acetaldehyde (IAld). Indole-3-acetaldehyde is then oxidized to IAA by a specific dehydrogenase.

2.The TAM pathway

The tryptamine (TAM) pathway is the order of the deamination and decarboxylation reactions is reversed, and different enzymes are involved.

3.The IAN pathway

In the indole-3-acetonitrile (IAN) pathway tryptophan is first converted to indole-3-acetaldoxime and then to indole-3-acetonitrile.The enzyme that converts IAN to IAA is called *nitrilase*.

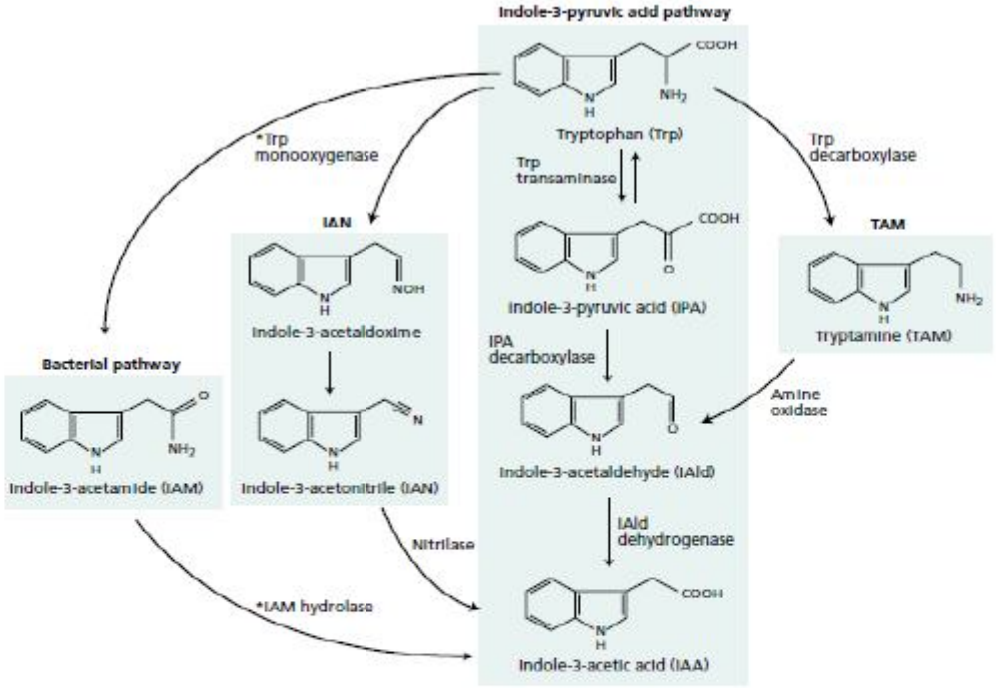


Fig 10.5 -Various steps IAA Biosynthesis

The highest concentrations of free auxin in the living plant are in the apical meristems of shoots and in young leaves because these are the primary sites of auxin synthesis.

Physiological Effects of Auxin

1. Cell Elongation

NOTES

Auxin was discovered as the hormone involved in the bending of coleoptiles toward light. The coleoptile bends because of the unequal rates of cell elongation on its shaded versus its illuminated side .Auxins Promote Growth in Stems and Coleoptiles,While Inhibiting Growth in Roots.As we have seen, auxin is synthesized in the shoot apex and transported basipetally to the tissues below. The steady supply of auxin arriving at the subapical region of the stem or coleoptile is required for the continued elongation of these cells. Because the level of endogenous auxin in the elongation region of a normal healthy plant is nearly optimal for growth, spraying the plant with exogenous auxin causes only a modest and short-lived stimulation in growth.

2. Root Elongation

It is particularly sensitive to auxin. IAA will promote the growth of excised root sections and intact roots, but only at very low concentrations (10^{-8} M or less). Higher concentrations of auxin, in the range that normally stimulates elongation of shoots (10^{-5} to 10^{-6} M), caused a significant inhibition of root growth. The inhibition of growth at higher auxin concentrations may be due to the auxin-promoted synthesis of ethylene, which inhibits cell elongation. Such inhibitory effects have been exploited in the production of herbicides based on 2,4-D or 2,4,5-T.

3. Increases Cell Extension

Auxin-Induced Proton Extrusion Acidifies the Cell Wall and Increases Cell Extension.

4. Phototropism

It is expressed in all shoots and some roots; it ensures that leaves will receive optimal sunlight for photosynthesis. Phototropism Is Mediated by the Lateral Redistribution of Auxin.

5. Gravitropism

It is growth in response to gravity enables roots to grow downward into the soil and shoots to grow upward away from the soil, which is especially critical

during the early stages of germination. Gravitropism Also Involves Lateral Redistribution of Auxin Statoliths Serve as Gravity Sensors in Shoots and Roots.

6. Auxin Regulates Apical Dominance

NOTES

In higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds—a phenomenon calledapical dominance. Removal of the shoot apex (decapitation) usually results in the growth of one or more of the lateralbuds. Not long after the discovery of auxin, it was found that IAA could substitute for the apical bud in maintaining the inhibition of lateral buds of bean (*Phaseolus vulgaris*) plants.

7. Auxin Promotes the Formation of Lateral and Adventitious Roots

8. Regulates Floral Bud Development

9 .Promotes Fruit Development

Much evidence suggests that auxin is involved in the regulation of fruit development. Auxin also promotes adventious root formation on stems . Certain synthetic auxins (e.g. NAA and indole butyric acid or IBA) are widely used as rooting compounds. Abscission has been correlated with low levels of auxin in the organ concerned and auxins have thus been used to prevent premature fruit drop. Other phenomena in which auxins have been implicated include apical dominance, phototropism, and epinasty.

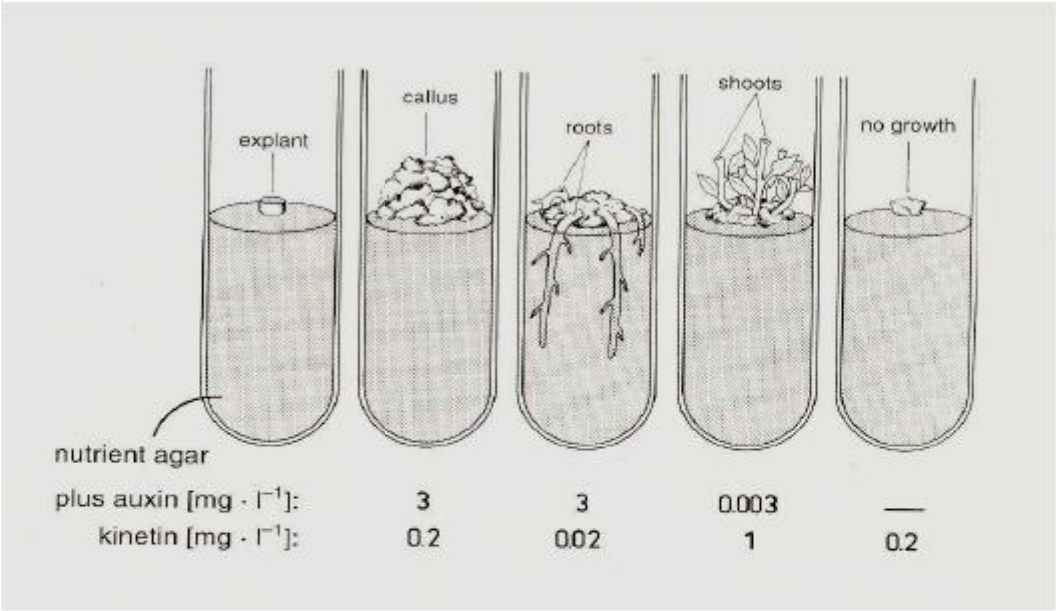


Fig 10.6 - Auxin (IAA) and Cytokinin (kinetin) as factors limiting mitotic

The four naturally occurring (endogenous) auxins are IAA, 4-chloroindole-3-acetic acid, phenylacetic acid and indole-3-butyric acid; only these four were found to be synthesized by plants. The other three endogenous auxins seems to have rather marginal importance for intact plants in natural environments. Alongside endogenous auxins, scientists and manufacturers have developed many synthetic compounds with auxinic activity.

NOTES

Synthetic auxin analogs include 1-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and many others.

10.3 Mechanism of action of Auxins

Auxin participates in phototropism, geotropism, hydrotropism and other developmental changes. The uneven distribution of auxin, due to environmental cues, such as unidirectional light or gravity force, results in uneven plant tissue growth, and generally, auxin governs the form and shape of plant body, direction and strength of growth of all organs, and their mutual interaction. Auxin stimulates cell elongation by stimulating wall-loosening factors, such as elastins, to loosen cell walls. The effect is stronger if gibberellins are also present. Auxin also stimulates cell division if cytokinins are present. When auxin and cytokinin are applied to callus, rooting can be generated if the auxin concentration is higher than cytokinin concentration. Xylem tissues can be generated when the auxin concentration is equal to the cytokinins.Auxin also induces sugar and mineral accumulation at the site of application.

Wound response

Auxin induces the formation and organization of phloem and xylem. When the plant is wounded, the auxin may induce the cell differentiation and regeneration of the vascular tissues.

Root growth and development

Auxins promote root initiation. Auxin induces both growth of pre-existing roots and adventitious root formation, i.e., branching of the roots. As more native auxin is transported down the stem to the roots, the overall development of the roots is stimulated. If the source of auxin is removed, for example the tips of stems are trimmed, the roots are less stimulated accordingly, and growth of stem is supported instead.In horticulture, auxins, especially NAA and IBA, are

commonly applied to stimulate root initiation when rooting cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation. Removal of the root tip can lead to inhibition of secondary root formation.

NOTES

Apical dominance

Auxin induces shoot apical dominance; the axillary buds are inhibited by auxin, as a high concentration of auxin directly stimulates ethylene synthesis in lateral buds, causing inhibition of their growth and potentiation of apical dominance. When the apex of the plant is removed, the inhibitory effect is removed and the growth of lateral buds is enhanced. Auxin is sent to the part of the plant facing light, and this promotes growth towards that direction.

Fruit growth and development

Auxin is required for fruit growth and development and delays fruit senescence. When seeds are removed from strawberries, fruit growth is stopped; exogenous auxin stimulates the growth in fruits with seeds removed. For fruit with unfertilized seeds, exogenous auxin results in parthenocarpy ("virgin-fruit" growth).Fruits form abnormal morphologies when auxin transport is disturbed. In *Arabidopsis* fruits, auxin controls the release of seeds from the fruit (pod). The valve margins are a specialised tissue in pods that regulates when pod will open (dehiscence). Auxin must be removed from the valve margin cells to allow the valve margins to form. This process requires modification of the auxin transporters (PIN proteins).

Flowering

Auxin plays also a minor role in the initiation of flowering and development of reproductive organs. In low concentrations, it can delay the senescence of flowers.

10.4 Gibberellins and their physiological effects

Gibberellins, or GAs, include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. In the 1930s Japanese scientists succeeded in obtaining impure crystals

of two fungal growth-active compounds, which they termed *gibberellin A* and *B*, but because of communication barriers and World War II, the information did not reach the West. Not until the mid-1950s did two groups—one at the Imperial Chemical Industries (ICI) research station at Welyn in Britain, the other at the U.S. Department of Agriculture (USDA) in Peoria, Illinois—succeed in elucidating the structure of the material that they had purified from fungal culture filtrates, which they named *gibberellic acid*: Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue.

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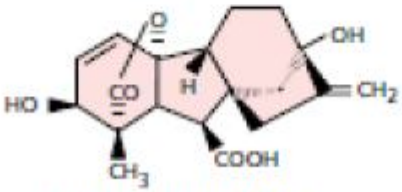
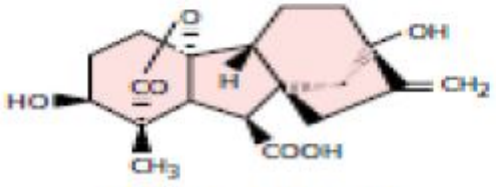


Gibberellic acid (GA_3)



Gibberellin A_1 (GA_1)

Absorption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling.

Gibberellins are synthesized via the Terpenoid Pathway in Three Stages

Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. Terpenoids are compounds made up of five-carbon (isoprene) building blocks: joined head to tail.

Fig. 10.7- Leaf sheath elongation in rice seedlings by Gibberellin

NOTES

Stage 1: Production of terpenoid precursors and ent-kaurene in plastids. The basic biological isoprene unit is isopentenyl diphosphate (IPP).2 IPP used in gibberellins biosynthesis in green tissues is synthesized in plastids from glyceraldehyde-3-phosphate and pyruvate

Stage 2: Oxidation reactions on the ER form GA12 and GA53. In the second stage of gibberellin biosynthesis, a methyl group on *ent*-kaurene is oxidized to a carboxylic acid, followed by contraction of the B ring from a six- to a five-carbon ring to give GA12-aldehyde. GA12-aldehyde is then oxidized to **GA12**, the first gibberellin in the pathway in all plants and thus the precursor of all the other gibberellins

Stage 3: Formation in the cytosol of all other gibberellinsfrom GA12 or GA53.

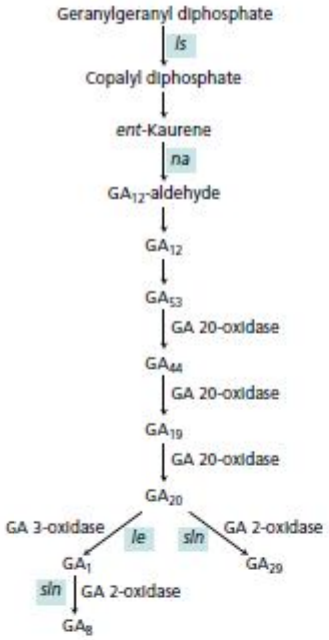


Fig 10.8 - Important steps in GA biosynthesis

GAs produce bolting of rosette-forming plants, increasing internodal length. Gibberellins Stimulate Cell Elongationand Cell Division For example, internodes of tall peas have more cells than those of dwarf peas, and the cells are longer. Mitosis increases markedly in the subapical region of the meristem of rosette long-day plants after treatment with gibberellinThey promote

flowering, cellular division, and in seeds growth after germination. In submergence-induced plants, gibberellin activates the cell division cycle first at the transition from G1 to S phase, leading to an increase in mitotic activity. To do this, gibberellins induces the expression of the genes for several cyclin-dependent protein kinases (CDKs), which are involved in regulation of the cell cycle Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA. Gibberellic Acid Enhances the Transcription of Amylase mRNA.

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10.5 Mechanism of action of Gibberllins

The gibberellins are metabolic products of the fungus Gibberella fujikuroi (conidial state Fusarium moniliforme). Three gibberellins are known: gibberellic acid ($C_{19}H_{22}O_6$), gibberellin A_1 ($C_{19}H_{24}O_6$) and gibberellin A_2 ($C_{19}H_{26}O_6$). A structure for gibberellic acid has been proposed. Gibberellin A_1 is a dihydro derivative of gibberellic acid. The structure of gibberellin A_2 has not yet been established.The most characteristic effects of GA on shoot growth are increased inter-node extension, increased leaf-growth and enhanced apical dominance.Under some circumstances, with some plant species, treatment with GA does not stimulate growth of intact roots, though some root sections do respond by increased growth. High concentrations of GA are only slightly inhibitory, results in increased dry weight. This is mainly due to increased carbon fixation and is believed to be a secondary effect of increased leaf growth.Not all plants respond to GA by increased shoot growth and the effect on some species is greater than that on others. In species in which dwarf mutants are known, the dwarf may frequently be induced by GA to grow in a form in-distinguishable from that of the tall phenotype, genetically tall plants themselves being unaffected. Many forms of dormancy are broken by GA. These include seed dormancy, dormancy of potato tubers and dormancy of shoot internodes and buds.GA will induce flowering of long-short-day plants kept permanently in short-day photoperiods. It will induce stem growth and, in long-day photoperiods but possibly not in short days, flowering in cold-requiring biennial long-day plants.It inhibits flowering of short-day plants in inductive short-day photoperiods. I t will induce stem growth and, in long-day photoperiods but possibly not in short days, flowering in cold-requiring

biennial long-day plants. It inhibits flowering of short-day plants in inductive short-day photoperiods.

10.6 Cytokinins and their physiological effects

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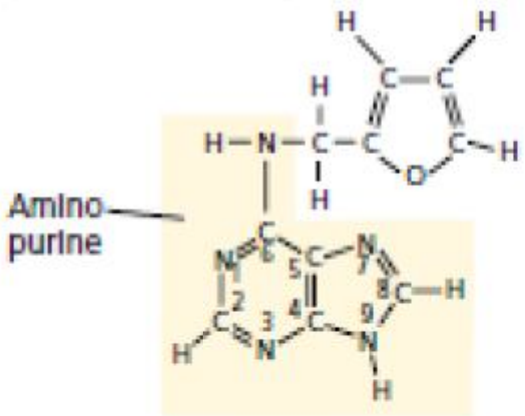
Cytokinins (CK) are a class of plant growth substances (phytohormones) that promote cell division, or cytokinesis, in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect apical dominance, axillary bud growth, and leaf senescence. F. Skoog discovered their effects using coconut milk in the 1940s at the University of Wisconsin–Madison.There are two types of cytokinins: adenine-type cytokinins represented by kinetin, zeatin, and 6-benzylaminopurine, and phenylurea-type cytokinins like diphenylurea and thidiazuron (TDZ). Most adenine-type cytokinins are synthesized in roots. Cambium and other actively dividing tissues also synthesize cytokinins. No phenylurea cytokinins have been found in plants. Cytokinins participate in local and long-distance signalling, with the same transport mechanism as purines and nucleosides.cytokinins are compounds that stimulate cell division or cytokinesis, although they may also do other things.



Kinetin

Proper regulation of cell division also requires auxin, which is needed to cause DNA synthesis before a cell can divide. White's nutrient medium, supplemented with an auxin and 10 to 20% coconut milk, will support the continued cell division of mature, differentiated cells from a wide variety of tissues and species, leading to the formation of callus tissue . This finding indicated that coconut milk contains a substance or substances that stimulate

mature cells to enter and remain in the cell division cycle.Eventually coconut milk was shown to contain the cytokinin *zeatin*, but this finding was not obtained until several years after the discovery of the cytokinins . The first cytokinin to be discovered was the synthetic analog kinetin.

Discovered of Kinetin

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In the 1940s and 1950s, Folke Skoog and coworkers at the University of Wisconsin tested many substances for their ability to initiate and sustain the proliferation of cultured tobacco pith tissue. They had observed that the nucleic acid base adenine had a slight promotive effect, so they tested the possibility that nucleic acids would stimulate division in this tissue. Surprisingly, autoclaved herring sperm DNA had a powerful cell division–promoting effect.

After much work, a small molecule was identified from the autoclaved DNA and named **kinetin**. It was shown to be an adenine (or aminopurine) derivative, 6-furfurylaminopurine after the discovery of kinetin, extracts of the immature endosperm of corn (*Zea mays*) were found to contain a substance that has the same biological effect as kinetin. Letham (1973) isolated the molecule responsible for this activity and identified it as *trans*-6-(4-hydroxy-3-methylbut-2-enylamino) purine, which he called **zeatin. Z**eatin is the most abundant naturallyoccurring free cytokinin, but *dihydrozeatin* (*DZ*) and *isopentenyl adenine* (*iP*) also are commonly found in higher plants and bacteria. The side chains of naturally occurring cytokinins are chemically related to rubber, carotenoid pigments, the plant hormones gibberellin and abscisic acid, and some of the plant defense compounds known as phytoalexinsIsoprene is similar in structure to the side chains of zeatin and iP.These cytokinin side chains are synthesized from an isoprene derivative. Large molecules of rubber and the carotenoids are constructed by the polymerization of many isoprene units; cytokinins contain just one of these units. The precursor(s) for the formation of these isoprene structures are mevalonic acid or pyruvate plus 3-phosphoglycerate. These precursors are converted to the biological isoprene unit dimethylallyl diphosphate (DMAPP). Root apical meristems are major sites of synthesis of the free cytokinins in whole plants. The cytokinins synthesized in roots appear to move through the xylem into the shoot, along with the water and minerals taken up by the roots.

Cytokinin responses include:-1.cell division (cytokinesis) 2.organ development (shoot formation)3.Delayed senescence and promotion of chloroplast development 4.Affect nutrient sink strength of organs 5.Promotion of lateral

bud growth 6.Promotion of cotyledon expansion (only in certain species) 7.Inhibition of auxin-induced elongation

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Cytokinins have found few uses in agriculture. They are used in plant tissue culture and they have been used to delay senescence. Possible future applications will depend on clever bio-engineering. For example, since cytokinins are important in regulating source-sink relationships, controlled production of cytokinins in fruit could potentially lead to increased nutrient mobilization into the fruit, thus, more nutritionally valuable food.

10.7 Mechanism of action of Cytokinins

The ratio of auxin to cytokinin plays an important role in the effect of cytokinin on plant growth. Cytokinin alone has no effect on parenchyma cells. When cultured with auxin but no cytokinin, they grow large but do not divide. When cytokinin is added, the cells expand and differentiate. When cytokinin and auxin are present in equal levels, the parenchyma cells form an undifferentiated callus. More cytokinin induces growth of shoot buds, while more auxin induces root formation. Cytokinins are involved in many plant processes, including cell division and shoot and root morphogenesis. They are known to regulate axillary bud growth and apical dominance. The "direct inhibition hypothesis" posits that these effects result from the cytokinin to auxin ratio. This theory states that auxin from apical buds travels down shoots to inhibit axillary bud growth. This promotes shoot growth, and restricts lateral branching. Cytokinin moves from the roots into the shoots, eventually signaling lateral bud growth. Simple experiments support this theory. When the apical bud is removed, the axillary buds are uninhibited, lateral growth increases, and plants become bushier. Applying auxin to the cut stem again inhibits lateral dominance.

Cytokinins modify apical dominance and promote lateral bud growth. Cytokinins delay leaf senescence and promote movement of nutrients. They influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as *cytokinin-induced nutrient mobilization*. They also Promote Chloroplast Development and Cell Expansion in Leaves and Cotyledons.The promotion of cell enlargement by cytokinins is most clearly demonstrated in the cotyledons of dicots with leafy cotyledons, such as mustard, cucumber, and sunflower. The cotyledons of these species expand as a result of cell enlargement during seedling growth. Cytokinin treatment promotes additional cell expansion, with no increase in the dry weight of the treated cotyledons. The first clue to the nature of the cytokinin receptor came

from the discovery of the *CKI1* gene. *CKI1* was identified in a screen for genes that, when overexpressed, conferred cytokinin-independent growth on *Arabidopsis* cells in culture.As discussed already, plant cells generally require cytokinin in order to divide in culture. However, a cell line that overexpresses *CKI1* is capable of growing in culture in the absence of added cytokinin.*CKI1* encodes a protein similar in sequence to bacterial two-component sensor histidine kinases, which are ubiquitous receptors in prokaryotes. Bacterial two-component regulatory systems mediate a range of responses to environmental stimuli, such as osmoregulation and chemotaxis. Typically these systems are composed of two functional elements: a *sensor histidine kinase*, to which a signal binds, and a downstream *response regulator*, whose activity is regulated via phosphorylation by the sensor histidine kinase. The sensor histidine kinase is usually a membrane-bound protein that containstwo distinct domains, called the input and histidine kinase, or "transmitter," domains. Detection of a signal by the input domain alters the activity of the histidine kinase domain. Active sensor kinases are dimers that transphosphorylate a conserved histidine residue. This phosphate is then transferred to a conserved aspartate residue in the receiver domain of a cognate response regulator and this phosphorylation alters the activity of the kinases. Most response regulators also contain *output* domains that act as transcription factors. The phenotype resulting from *CKI1* overexpression, combined with its similarity to bacterial receptors, suggested that the CKI1 and/or similar histidine kinases are cytokinin receptors. Support for this model came from identification of the *CRE1* gene Like *CKI1*, *CRE1* encodes a protein similar to bacterial histidine kinases.While cytokinin action in vascular plants is described as pleiotropic, this class of plant hormones specifically induces the transition from apical growth to growth via a three-faced apical cell in moss protonema. This bud induction can be pinpointed to differentiation of a specific single cell, and thus is a very specific effect of cytokinin.

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Cytokinins have been shown to slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues. A study that regulated leaf senescence in tobacco leaves found that wild-type leaves yellowed while transgenic leaves remained mostly green. It was hypothesized that cytokinin may affect enzymes that regulate protein synthesis and degradation.

10.8 Summary

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Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and, moved to other locations, in other functional part of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones. Instead, each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth, and, lacking them, plants would be mostly a mass of undifferentiated cells. So they are also known as growth factors or growth hormones. The term 'Phytohormone' was coined by Thimann in 1948. Phytohormones are found not only in higher plants, but in algae too, showing similar functions, and in microorganisms, like fungi and bacteria, but, in this case, they play no hormonal or other immediate physiological role in the producing organism and can, thus, be regarded as secondary metabolites.

10.9 Glossary

- **Auxins :** compounds that positively influence cell enlargement, bud formation and root initiation.
- **Cytokines :** they control the growth of stems, roots, and fruits, and convert stems into flowers.
- **Gibberellins :** include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells.

10.10 Self-Learning Exercise

Section – A (Very Short Answer Type Questions)

1. Went done experiment on which plant?

2. What is weed killer?
3. Who discovered Gibberelline?
4. Zeatine obtain from which plant?

Section – B (Short Answer Type Questions)

1. Write about Went Curvature method
2. Write a note on GA biosynthesis.
3. Write a Apical dominance.

Section – C (Long Answer Type Questions)

1. Define Auxins and its physiological effects
2. Explain mode of action of gibberlins
3. What are cytokinins explain their physiological effects

(Answer Key Section – A

1. oat (*Avena sativa*) 2. 2,4,-D 3. Kurosawa 4 .Zea mays

10.11 References

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Unit -11

Plant Growth Regulators

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Structure of the Unit

11.0 Objective

After going through this unit you will be able to understand:-

- Ethylene, Abscisic acid
- Brassinosteroides
- Polyamines
- Jasmonic-acid and salicylicacid
- Hormone receptors,plant rhythms and biologicalclock

11.1 Introduction

Plant hormones (also known as phytohormones) are chemicals that regulate plant growth, which, in the UK, are termed 'plant growth substances'.Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted

cells locally and, moved to other locations, in other functional part of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones. Instead, each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth, and, lacking them, plants would be mostly a mass of undifferentiated cells. So they are also known as growth factors or growth hormones. The term 'Phytohormone' was coined by Thimann in 1948.Phytohormones are found not only in higher plants, but in algae too, showing similar functions and in micro-organisms, like fungi and bacteria, but, in this case, they play no hormonal or other immediate physiological role in the producing organism and can, thus, be regarded as secondary metabolites.

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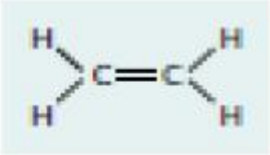
11.2 Ethylene

It is a gas that forms through the break-down of methionine, which is in all cells.



Ethylene

Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while

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underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stem's natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein Ethylene Insensitive2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones

11.3 Abscisic acid

Abscisic acid (also called ABA) is one of the most important plant growth regulators. It was discovered and researched under two different names before its chemical properties were fully known, it was called *dormin* and *abscicin II*. Once it was determined that the two compounds are the same, it was named abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly abscissed or freshly fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplasts, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, and seed and bud dormancy. It mediates changes within the apical meristem, causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly abscissed leaves, it was thought to play a role in the processes of natural leaf drop, but further research has disproven this. In plant species from temperate parts of the world, it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the

fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy.

In plants under water stress, ABA plays a role in closing the stomata. Soon after plants are water-stressed and the roots are deficient in water, a signal moves up to the leaves, causing the formation of ABA precursors there, which then move to the roots. The roots then release ABA, which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cells, which then lose turgidity, closing the stomata. ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone; its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones. Plants start life as a seed with high ABA levels. Just before the seed germinates, ABA levels decrease; during germination and early growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves, ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.

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11.4 Brassinosteroides

Brassinosteroids - are a class of polyhydroxysteroids, a group of plant growth regulators. Brassinosteroids have been recognized as a sixth class of plant hormones, which stimulate cell elongation and division, gravitropism, resistance to stress, and xylem differentiation. They inhibit root growth and leaf abscission. Brassinolide was the first identified brassinosteroid and was isolated from extracts of rapeseed (*Brassica napus*) pollen in 1979.

11.5 Polyamines

These are strongly basic molecules with low molecular weight that have been found in all organisms studied thus far. They are essential for plant growth and development and affect the process of mitosis and meiosis.

11.6 Jasmonic acid and Salicylic acid

These are produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are

believed to also have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth. Salicylic acid — activates genes in some plants that produce chemicals that aid in the defense against pathogenic invaders.

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11.7 Hormone receptors, Plant Rhythms and Biological-clock

Hormone receptors

The hormonal control of the growth and development of multicellular organisms has intrigued generations of biologists. In plants, virtually every aspect of the plant's life is regulated by a handful of small organic molecules collectively referred to as the phytohormones. Like animal hormones, the phytohormones act at very low concentrations and exert developmental control by regulating cell division, expansion, differentiation, and death. These effects can be highly complex, with a single cell responding to multiple hormones and a single hormone having differential effects on distinct tissues. Unlike the highly localized synthesis and transport via the circulatory or lymphatic systems characteristic of animal hormones, generally speaking, plant hormones are produced to various extents throughout the plant, and may elicit responses in the same cells or tissues where synthesis occurs. That said, phytohormones can be transported through the plant vasculature system or, at least in the case of auxin, through a complex cell-to-cell transport system that delivers auxin to its target cells in a highly regulated fashion .The origins of the concept of plant hormones can be traced to the late 19th century, when the German botanist Julian von Sachs proposed the existence of mobile endogenous compounds that act as specific "organ-forming substances." This coincided with a study by Charles and Francis Darwin on phototropism—the bending of plants toward light. The Darwins demonstrated that when grass seedlings were exposed to a lateral light source, a transported signal originating from the plant apex promoted differential cell elongation in the lower parts of the seedling that resulted in it bending toward the light source. This signal was subsequently shown to be indole-3-acetic acid (IAA or auxin), the first known plant hormone. Once identified, the effects of each hormone were initially elucidated largely by observing the physiological responses elicited by exogenous applications. However, the identification of hormone biosynthesis and response mutants, particularly in the model plant *Arabidopsis thaliana*, has verified many of these roles, as well as established new functions for each hormone.

Auxin: F-box proteins as receptors

IAA (auxin) regulates an amazingly diverse array of processes in plant growth and development ranging from embryo patterning to growth responses to tropic stimuli. Auxin response involves a large-scale reprogramming of gene expression affecting hundreds of auxin-regulated genes. The *Arabidopsis TIR1* gene was identified more than a decade ago as a positive regulator of auxin response. TIR1 is one of several hundred F-box proteins encoded in the *Arabidopsis* genome. F-box proteins function as substrate recognition modules for multisubunit Skp1–Cullin1–F-box protein (SCF) ubiquitin-ligases. Since mutations in *TIR1*, as well as in the genes encoding the core SCF subunits, confer diminished auxin sensitivity, it seemed likely that the SCF^{TIR1} complex might target negative regulators of auxin signaling for ubiquitin-mediated proteolysis. Such repressors became apparent through the combined approaches of forward and molecular genetics.*Aux/IAA* genes were initially identified as rapid transcriptional targets of auxin action. The short-lived proteins encoded by these genes were subsequently found to interact with members of the AUXIN RESPONSE FACTOR (ARF) family of transcription factors that control the expression of many auxin-regulated genes, and cotransfection assays demonstrated that Aux/IAA proteins negatively regulate ARF transcriptional activity. Meanwhile, several laboratories identified dominant gain-of-function mutations in *Aux/IAA* genes in screens for mutants with reduced auxin response. All of the dominant lesions mapped to a highly conserved motif (domain II) that confers instability to Aux/IAA proteins. These findings raised the possibility that Aux/IAA proteins might be substrates of the SCF^{TIR1} complex. Indeed, Aux/IAA proteins interact with TIR1 and exhibit increased stability in mutants with impaired SCF^{TIR1} function. In contrast, derivatives of Aux/IAA proteins containing mutations in domain II cannot interact with TIR1. Consequently, they exhibit increased stability, thus expla Gibberellins (GA): variation of the ubiquitin-ligase-based receptor theme

GAs are tetracyclic, diterpenoid plant hormones that regulate many aspects of plant development including stem elongation, seed germination, and the induction of flowering. Like the auxin and jasmonate pathways, genetic screens in both rice and *Arabidopsis* for GA-insensitive mutants identified mutations in genes encoding F-box proteins. The rice GID2 and *Arabidopsis* SLY1 F-box proteins are closely related and have been shown to assemble into SCF ubiquitin-ligase complexes. In response to GA, $SCF^{SLY1/GID2}$ targets members of

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a family of proteins that repress GA responses, known as DELLA proteins, for ubiquitin-mediated proteolysis. DELLA proteins have long been thought to function as transcriptional regulators. Only recently, however, has direct evidence in support of this possibility been obtained. Two recent reports demonstrated that DELLAs interact with the DNA-binding domain of PIF3 and PIF4, two basic helix–loop–helix transcription factors that regulate cell expansion in response to light and GA. DELLA-binding sequesters these transcription factors from their target promoters, preventing transcriptional activation . Meanwhile, a third study used expression profiling to identify putative direct DELLA target genes. All of the identified genes are GA-repressed and DELLA-induced, with several of them encoding GA biosynthesis enzymes, indicating a form of feedback regulation, while others encode potential negative regulators of GA signaling. Chromatin immunoprecipitation experiments detected DELLA association with the promoters of several of these genes, indicating that DELLAs can also regulate GA responses either by directly binding DNA or through other DNA-bound transcription factors. While the GA-dependent $SCF^{SLY1/GID2}$-mediated ubiquitination of the DELLA repressors is very reminiscent of the auxin and jasmonate pathways discussed above, in this case, the F-box proteins are not hormone receptors containing the dominant, auxin-unresponsive nature of these mutants.

Plant rhythms and Biological-clock

Many plants have a biological clock containing details of their own structure and other life forms that assist them with pollination and that bear a literal resemblance to a computer. The existence of biological clocks points to a single reality, the fact of Creation.The ability to measure time is an ability that one does not usually expect to see in other living things other than man. It may be thought that this is limited to man, but both plants and animals possess a time-measuring mechanism, or "biological clock."In the 1920s, when two scientists in Germany, Erwin Buenning and Kurt Stem, were studying the movement of bean plant leaves, they saw that the plants were moving their leaves towards the sun throughout the day, and that at night they were gathering their leaves vertically upwards and assuming a sleeping position.Some 200 years before these two scientists published their findings, the French astronomer Jacques d'Ortuous de Marian had also observed that plants possessed such a regular sleep rhythm. Experiments in a dark environment where temperature and

moisture were controlled showed that this situation did not change, and that plants possessed systems inside themselves which measure time. A circadian rhythm is any biological process that displays an endogenous, entrainable oscillation of about 24 hours. These 24-hour rhythms are driven by a circadian clock, and they have been widely observed in plants, animals, fungi, and cyanobacteria. The term *circadian* comes from the Latin *circa*, meaning "around" (or "approximately"), and *diem* or *dies*, meaning "day". The formal study of biological temporal rhythms, such as daily, tidal, weekly, seasonal, and annual rhythms, is called chronobiology. Although circadian rhythms are endogenous ("built-in", self-sustained), they are adjusted (entrained) to the local environment by external cues called zeitgebers, commonly the most important of which is daylight.

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Circadian Rhythms Exhibit Characteristic Features. Circadian rhythms arise from cyclic phenomena that are defined by three parameters:

1. **Period**, the time between comparable points in the repeating cycle. Typically the period is measured as the time between consecutive maxima (peaks) or minima (troughs).
2. **Phase**2, any point in the cycle that is recognizable by its relationship to the rest of the cycle. The most obvious phase points are the peak and trough positions.
3. **Amplitude**, usually considered to be the distance between peak and trough. The amplitude of a biological rhythm can often vary while the period remains unchanged.

Plant circadian rhythms tell the plant what season it is and when to flower for the best chance of attracting pollinators. Behaviors showing rhythms include leaf movement, growth, germination, stomatal/gas exchange, enzyme activity, photosynthetic activity, and fragrance emission, among others. Circadian rhythms occur as a plant entrains to synchronize with the light cycle of its surrounding environment. These rhythms are endogenously generated and self-sustaining and are relatively constant over a range of ambient temperatures. Important features include two interacting transcription-translation feedback loops: proteins containing PAS domains, which facilitate protein-protein interactions; and several photoreceptors that fine-tune the clock to different light conditions. Anticipation of changes in the environment allows appropriate changes in a plant's physiological state, conferring an adaptive advantage. A better understanding of plant circadian rhythms has applications in agriculture,

such as helping farmers stagger crop harvests to extend crop availability and securing against massive losses due to weather.

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Light is the signal by which plants synchronize their internal clocks to their environment and is sensed by a wide variety of photoreceptors. Red and blue light are absorbed through several phytochromes and cryptochromes. One phytochrome, phyA, is the main phytochrome in seedlings grown in the dark but rapidly degrades in light to produce Cry1. Phytochromes B–E is more stable with phyB, the main phytochrome in seedlings grown in the light. The cryptochrome (cry) gene is also a light-sensitive component of the circadian clock and is thought to be involved both as a photoreceptor and as part of the clock's endogenous pacemaker mechanism. Cryptochromes 1–2 (involved in blue–UVA) help to maintain the period length in the clock through a whole range of light conditions.

The central oscillator generates a self-sustaining rhythm and is driven by two interacting feedback loops that are active at different times of day. The morning loop consists of CCA1 (Circadian and Clock-Associated 1) and LHY (Late Elongated Hypocotyl), which encode closely related MYB transcription factors that regulate circadian rhythms in *Arabidopsis*, as well as PRR 7 and 9 (Pseudo-Response Regulators.) The evening loop consists of GI (Gigantea) and ELF4, both involved in regulation of flowering time genes. When CCA1 and LHY are overexpressed (under constant light or dark conditions), plant becomes arrhythmic, and mRNA signals reduce, contributing to a negative feedback loop. Gene expression of CCA1 and LHY oscillates and peaks in the early morning, whereas TOC1 gene expression oscillates and peaks in the early evening. While it was previously hypothesised that these three genes model a negative feedback loop in which over-expressed CCA1 and LHY repress TOC1 and over-expressed TOC1 is a positive regulator of CCA1 and LHY, it was shown in 2012 by Andrew Millar and others that TOC1 in fact serves as a repressor not only of CCA1, LHY, and PRR7 and 9 in the morning loop but also of GI and ELF4 in the evening loop.

11.8 Summary

Plant growth and development require the integration of a variety of environmental and endogenous signals that, together with the intrinsic genetic program, determine plant form. Central to this process are several growth regulators known as plant hormones or phytohormones. Despite decades of study, only recently have receptors for several of these hormones been

identified, revealing novel mechanisms for perceiving chemical signals and providing plant biologists with a much clearer picture of hormonal control of growth and developmentDespite the bewildering ability of higher plants to change their development with respect to the environment, there appear to be only a few hormones that function to organize growth and development. With the recent identification of three plant hormone receptors, the molecular identities of all the major plant receptors are now known. Some plant hormones such as cytokinins, ethylene, and brassinosteroids (BR) use well-characterized signaling modules such as those involving receptor kinases, but in the case of the ethylene and BR receptors, there appear to be additional functions aside from the hormone they perceive. Auxin and gibberellin perception require unique mechanisms where the receptors are components involved in ubiquitination-dependent proteolysis. With plant hormone receptors in hand, comparisons can now be made between plants and other kingdoms as to how hormones control growth and development.

11.9 Glossary

- **Plant hormones :** also known as phytohormones, are chemicals that regulate plant growth
- **Jasmonic acid** : have hormone properties, help regulating plant growth and development and they seem to participate in leaf senescence and in the defence mechanism against fungiCytokinins, they control the growth of stems, roots, and fruits, and convert stems into flowers.
- **Brassinosteroids :** Potent plant growth regulators of steroidal nature

11.10 Self -Learning Exercise

Section – A (Very Short Answer Type Questions)

1. Which is gaseous hormone ?
2. Which is growth retarding hormone?

Section – B (Short Answer Type Questions)

1 Write about Ethylene synthesis

2 Write a note on ABA mode of action.

3 Write a note on plant rhythm

Section – C (Long Answer Type Questions)

1. Define ABA and its physiological effects

2. Explain mode of action of Ethylene

3 Write short notes on brassinosteroids, jasmonic acid salicylicacid

4 Explain hormonal receptors in plants

Answer Key Section – A

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1. Ethylene 2. ABA

11.11 References

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Unit - 12

The Flowering Process

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Structure of the Unit

12.0 Objective

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After going through this unit you will be able to understand:-

- Photoperiodism and its significance
- LDP, SDP and Day neutral plants
- Endogenous clock and its regulation
- Floral induction and development
- Florigen concept
- Genetic and molecular analysis,
- Vernalization and devernalization

12.1 Introduction

Plants begin with a period of vegetative growth and then reproductive phase. The extent of vegetative growth is endowed with its genetic potentiality. They may grow into herbs or shrubs and some may develop into trees or climbers. After going through a period of vegetative growth, every plant responds to environmental clues and start producing floral structures, which may be in the form of characteristic single flowers or inflorescences.

Flowering time varies in large number of plant species (higher plants), after a period of vegetative growth, start flowering irrespective of the season. But some plants flower only in a particular season of the year. Based on the duration required for the plants to produce flowers, they have been classified into annuals, biennials and perennials. All plants have to acquire ripeness to flowering. Annuals complete their vegetative growth and flowering in one season and then they die. Similarly, Biennials produce vegetative growth in one season but they flower in the next season. However, perennials remain for many years and flower seasonally.

In fact, some trees do not flower till they reach a certain age. For example, coconut and Areca nut plants start producing flowers only when they reach an age of 6-8 years. Besides, bamboo plants, they grow for a number of years, and flower only once in their life span. As soon as they flower, produce seeds and

plants die (monocarpic plants). Interestingly there are many plants which flower throughout the year, ex., *Catharathus roseus.*

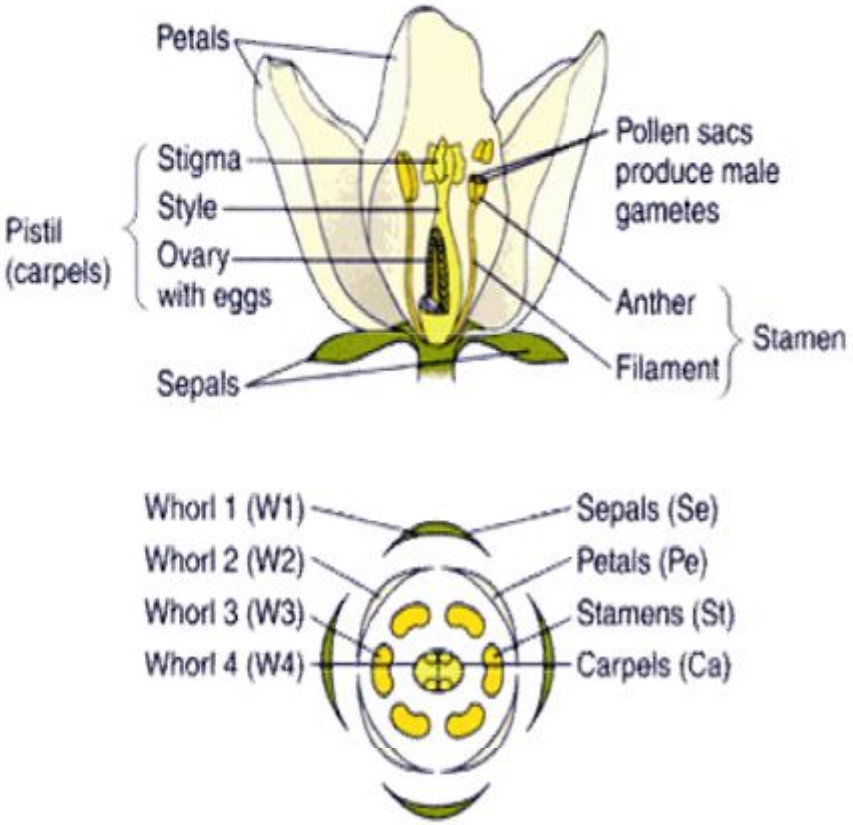

Fig. 12.1 Parts of a flower from outer to the central region

12.2 Photoperiodism

The reproductive cycles of many plants are regulated by the length of the light and dark period, called the photoperiod. In angiosperms, flowers are organs for sexual reproduction. This occurs even though they may have started growing at different times. Their flowering is a response to the changing length of day and night as the season progresses. The phenomenon is called photoperiodism which promote cross pollination. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of light and dark period or it is a physiological reaction of organisms to the length of day or night.

Photoperiod (Photo: light and Period: a specific length of time) is the relative length of daylight and night. The timing of flowering in plants is determined by

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the relative length of daylight and night (photoperiod).The seasons are controlled by the length of daylight. Between December and June, in the northern hemisphere, the amount of daylight increases daily. So, increased daylight indicates spring and summer are on the way. Between June and December, the opposite occurs. Photoperiodism affects flowering the shoot by inducing it to produce floral buds instead of leaves and lateral buds.

12.2.1 History

W. W. Garner and H. A. Allard (1920) discovered on photoperiodism and suggested the length of daylight that was critical but it was later discovered that the length of the night was the controlling factor. Photoperiodic flowering plants are classified as long-day plants or short-day plants even though night is also critical factor daylight being the controlling factor. Each plant has a different length critical photoperiod, or critical night length. It is the concurrence of the active forms of phytochrome or cryptochrome, created by light during the daytime, with the rhythms of the circadian clock that allows plants to measure the length of the night. Besides, photoperiodism in plants includes the growth of stems or roots during certain seasons and the loss of leaves. Artificial lighting can be used to induce extra-long days

12.2.2 Classification of Photoperiodism

Based on flowering response and development, plants are classified as Long-day plants (LDPs) Short-day plants (SDPs), Intermediate-day plants, or Day-neutral plants.

Long-day plants

Long-day plants (LDP)flower when the days are relatively long (generally fifteen hours or greater) and night length falls below their critical photoperiod .These plants typically flower in the northern hemisphere during late spring or early summer as days are getting longer. In the northern hemisphere, the longest day of the year (summer) is on or about 21 June. After that date, days grow shorter (i.e. nights grow longer) until 21 December (winter). This situation is reversed in the southern hemisphere (i.e., longest day is 21 December and shortest day is 21 June).

Examples:

- Arabidopsis , sugar beet, radish, spanich and lettuces.

Some long-day obligate plants are:

Carnation (*Dianthus*),Henbane (*Hyoscyamus*),Oat (*Avena*)

- Some long-day facultative plants are:
- Pea (*Pisum sativum*),Barley (*Hordeum vulgare*),Lettuce (*Lactuca sativa*)

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Short-day plants

Short-day plants flower when the days are relatively short (generally nine hours or less) and night lengths exceed their critical photoperiod. They cannot flower under short nights or if a pulse of artificial light is shone on the plant for several minutes during the night; they require a continuous period of darkness before floral development can begin. In some SDP species flowering is qualitative, meaning that short days are absolutely required, while in other SDP species flowering is quantitative, which means flowering is accelerated under short days, but short days are not an absolute requirement. Some examples of SDPs include

Examples: chrysanthemums (bloom in the fall), rice (*Oryza sativa*), poinsettias, morning glory (*Pharbitis nil*) ,the cocklebur (*Xanthium*)

Some short-day facultative plants are:

- Hemp (*Cannabis*),Cotton (*Gossypium*),Rice

Day-neutral plants

In day-neutral plants, flowering is not regulated by day lengths or day-neutral plants flower regardless of the day length. Besides, length of the day and light duration may also affected by temperature and development. A plant may respond to a certain day length at one temperature but exhibit a different response at another temperature. For example, poinsettia and morning glory are absolute SDPs at high temperature; however, they are absolute LDPs at low temperature and day-neutral at intermediate temperatures.
At the same time, cucumbers, roses, and tomatoes, do not initiate flowering based on photoperiodism. Instead, they may initiate flowering after attaining a certain overall developmental stage or age, or in response to alternative

environmental stimuli, such as vernalisation (a period of low temperature). Examples: Rice, Dandelions, Tomatoes, etc.

12.2.3 Regulation of Photoperiodism

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Flowering is regulated by chemicals produced in the plant, and a variety of plant hormones, including auxins, ethylene, gibberellins, cytokinins, and abscisic acid, also influence flowering in different species. The critical aspect of photoperiodism, however, is the measurement of seasonal time by detecting the lengths of day and night.

Phytochrome is responsible for measuring the dark period. Phytochrome, found in the leaves of plants, exists in two forms, P_r and P_{fr}. P_r absorbs red light during the day and is converted to P_{fr}. P_{fr} absorbs far-red light during the night and is converted to P_r. It is evident that P_{fr} inhibits flowering, and the length of the dark period has to be sufficient for the P_{fr} to fall below some critical level. When the P_{fr} falls below this level, chemical messages are sent to the floral regions, and flowering is initiated. While phytochrome definitely has been shown to trigger the flowering response, it is not the only chemical involved. It has been shown that a blue light photoreceptor may also play a role in photoperiodism. In addition, phytochrome is not translocated in the plant. It remains in the leaves. Hence, other chemicals which have not been positively identified are responsible for signalling the photoperiodic response.

Red light, of wavelength 660 nm, is the most effective in interrupting night length. Experimental results have confirmed this fact: Short-day (long-night) plants experiencing a long night will *not* flower if exposed briefly to 660 nm light sometime during the night. Long-day (short-night) plants exposed briefly to a 660 nm light will flower even if the total night length exceeds the critical number of hours. Shortening of night length by red light (R) can be annulled by a flash of far-red light (FR) of 730 nm. When this occurs, the plant perceives no interruption in night length. No matter how many times red light is flashed, as long as it is followed by far-red light the effects of red light are negated. This works in both short-day and long-day plants.

12.2.4 Significance of Photoperiodism

Photoperiodism is responsible for the distribution of many plants worldwide. Red light having a wavelength of 660 nm was found to be the most effective

for interrupting the dark period, and this effect can be reversed by a subsequent exposure to far-red light (730 nm). These observations led to the discovery of phytochrome, the pigment responsible for absorbing those wavelengths and apparently the light sensor in photoperiodism. Thus, photoperiodism results from an interaction between phytochrome and the plant's biological clock, which measures the time between successive dawns (rich in red light) and successive dusks (rich in far-red light). Under the appropriate conditions, these interactions are thought to activate the genes for flowering.

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The induction of flowering is the most studied aspect of crop growth relative to photoperiodism. It is perhaps the most important response of crops to photoperiod. This is so in most crops in which the economic product is the fruit or seed. But in sugarcane, tobacco, and forage crops, it is desirable if reproductive development is delayed or prevented to favour vegetative development. Light inhibits stem growth but promotes the expansion of leaves. In lettuce and radish, short days promote higher top: root ratio. This is desirable in lettuce because it is the top that is harvested but not in radish in which the economic organ is the tap root. At the same time, Poinsettia (*Euphorbia pulcherrima*) naturally produces colourful flowers in December where day length period is short. In chrysanthemum, short day length promotes flowering while long day length favours vegetative growth. Chrysanthemums have different critical photoperiods for floral initiation and for flower development. Further, the critical photoperiod can vary with cultivar and temperature. In some varieties of potato, tuber formation is induced by short photoperiod but in others, it occurs only during long days with low temperature. A similar response is exhibited by different varieties of onion in terms of bulb formation. For example, rag weed (a SDP) is not found in northern Maine because the plant flowers only when the day length is shorter than 14.5 hours. In northern Maine, days do not shorten to this length until August. This is so late in the growing season that the first frost arrives before the resulting seeds are mature enough to resist the low temperatures, and so the species cannot survive there. Besides, spinach (a LDP) is not found in the tropics because there the days are never long enough to stimulate the flowering process.

12.3 Endogenous clocks and its Regulations

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The earth rotates on its axis every 24 h, with the result that any position on the earth's surface alternately faces toward or away from the sun-day and night. That the metabolism, physiology, and behaviour of most organisms changes profoundly between day and night is obvious to even the most casual observer. These biological oscillations are apparent as diurnal rhythms. It is less obvious that most organisms have the innate ability to measure time. Indeed, most organisms do not simply respond to sunrise but, rather, anticipate the dawn and adjust their biology accordingly. When deprived of exogenous time cues, many of these diurnal rhythms persist, indicating their generation by an endogenous biological circadian clock. An internal mechanism in organisms that controls the periodicity of various functions or activities, such as metabolic changes, sleeps cycles, or photosynthesis. Photosensitive proteins and circadian rhythms are believed to have originated in the earliest cells, with the purpose of protecting the replicating DNA from high ultraviolet radiation during the daytime.

A circadian rhythm is any biological process that displays an endogenous, entrainable oscillation of about 24 hours. These rhythms are driven by a circadian clock, and rhythms have been widely observed in plants, animals, fungi and cyanobacteria. The term circadian comes from the Latin circa, meaning "around" (or "approximately"), and Diem or dies, meaning "day". The formal study of biological temporal rhythms, such as daily, tidal, weekly, seasonal, and annual rhythms, is called chronobiology. Although circadian rhythms are endogenous ("built-in", self-sustained), they are adjusted (entrained) to the local environment by external cues called zeitgebers, commonly the most important of which is daylight. Clocks can keep track of temporal cycles of a variety of lengths; another biologically significant clock periodicity is the CIRCANNUAL (approximately a year) rhythm. Lunar and semilunar rhythms are important in the behaviour of shoreline fish species such as grunions, whose spawning activity must be synchronized with the monthly cycle of the tides.

Circadian rhythms allow organisms to anticipate and prepare for precise and regular environmental changes; they have great value in relation to the outside

world. The rhythmicity appears to be as important in regulating and coordinating internal metabolic processes, as in coordinating with the environment. The simplest known circadian clock is that of the prokaryotic cyanobacteria. Recent research has demonstrated that the circadian clock of Synechococcus elongatus can be reconstituted in vitro with just the three proteins (KaiA, KaiB, KaiC) of their central oscillator. This clock has been shown to sustain a 22-hour rhythm over several days upon the addition of ATP.

12.3.1 History

The term *circadian* was coined by Franz Halberg in the 1950s.The earliest recorded account of a circadian process dates from the 4th century B.C.E., when Androsthenes, a ship captain serving under Alexander the Great, described diurnal leaf movements of the tamarind tree. The observation of a circadian or diurnal process in humans is mentioned in Chinese medical.

The first recorded observation of an endogenous circadian oscillation was by the French scientist Jean-Jacques d'Ortous de Mairan in 1729. He noted that 24-hour patterns in the movement of the leaves of the plant Mimosa pudica continued even when the plants were kept in constant darkness, in the first experiment to attempt to distinguish an endogenous clock from responses to daily stimuli.

The scientific literature on circadian rhythms began in 1729 by de Mairan when the French astronomer de Mairan reported that the daily leaf movements of the sensitive heliotrope plant (*Mimosa pudica*) persisted in constant darkness, demonstrating their endogenous origin .Presciently, de Mairan suggested that these rhythms were related to the sleep rhythms of bedridden humans. It took 30 years before de Mairan's observations were independently repeated These studies excluded temperature variation as a possible zeitgeber driving the leaf movement rhythms.

In 1896, Patrick and Gilbert observed that during a prolonged period of sleep deprivation, sleepiness increases and decreases with a period of approximately 24 hours. In 1918, J.S. Szymanski showed that animals are capable of maintaining 24-hour activity patterns in the absence of external cues such as light and changes in temperature in the early 20th century, circadian rhythms were noticed in the rhythmic feeding times of bees. Extensive experiments

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were done by Auguste Forel, Ingeborg Beling, and Oskar Wahl to see whether this rhythm was due to an endogenous clock. Ron Konopka and Seymour Benzer isolated the first clock mutant in Drosophila in the early 1970s and mapped the "period" gene, the first discovered genetic component of a circadian clock. Joseph Takahashi discovered the first mammalian 'clock gene' (CLOCK) using mice in 1994.

12.3.2 Characteristics

A biological rhythm must meet these three general characteristics:

1. The rhythm has an endogenous free-running period that lasts approximately 24 hours. The rhythm persists in constant conditions, (i.e., constant darkness) with a period of about 24 hours. And called the free-running period and is denoted by the Greek letter **T** (tau).
2. The rhythms are entrainable. The rhythm can be reset by exposure to external stimuli (such as light and heat), a process called entrainment. The external stimulus used to entrain a rhythm is called the Zeitgeber, or "Time giver". Travel across time zones illustrates the ability of the human biological clock to adjust to the local time; a person will usually experience jet lag before entrainment of his/her circadian clock has brought it into sync with local time.
3. The rhythms exhibit temperature compensation. In other words, they maintain circadian periodicity over a range of physiological temperatures.

12.3.3 Origin of Circadian Rhythms

Photosensitive proteins and circadian rhythms are believed to have originated in the earliest cells, with the purpose of protecting the replicating of DNA from high ultraviolet radiation during the daytime. As a result, replication was relegated to the dark. The fungus Neurospora, which exists today, retains this clock-regulated mechanism. Circadian rhythms allow organisms to anticipate and prepare for precise and regular environmental changes; they have great value in relation to the outside world. The rhythmicity appears to be as important in regulating and coordinating internal metabolic processes, as in coordinating with the environment. This is suggested by the maintenance (heritability) of circadian rhythms in fruit flies after several hundred

generations in constant laboratory conditions, as well as in creatures in constant darkness in the wild, and by the experimental elimination of behavioural but not physiological circadian rhythms in quail.

The simplest known circadian clock is that of the prokaryotic cyanobacteria. Recent research has demonstrated that the circadian clock of Synechococcus elongatus can be reconstituted in vitro with just the three proteins of their central oscillator. This clock has been shown to sustain a 22-hour rhythm over several days upon the addition of ATP. Previous explanations of the prokaryotic circadian timekeeper were dependent upon a DNA transcription/translation feedback mechanism.

It is now known that the molecular circadian clock can function within a single cell; i.e., it is cell-autonomous. At the same time, different cells may communicate with each other resulting in a synchronized output of electrical signalling. These may interface with endocrine glands of the brain to result in periodic release of hormones. The receptors for these hormones may be located far across the body and synchronize the peripheral clocks of various organs. Thus, the information of the time of the day as relayed by the eyes travels to the clock in the brain, and, through that, clocks in the rest of the body may be synchronized. This is how the timing of, for example, sleep/wake, body temperature, thirst, and appetite are co-ordinately controlled by the biological clock.

12.4 Flower Induction and Development

Plants growing in different regions of the globe are exposed to different climatic conditions and different day lengths. In fact they are adapted to such environs in such a way, they exhibit alternate vegetative and flowering cycles. Thus plants with their inherent genetic potentiality interact with environmental conditions; accordingly, they respond and behave.

Floral induction is the most important process in plant development since it leads to the formation of the reproductive structures. This induction occurs in response to environmental signals and to endogenous genetic pathways. To ensure reproductive success and species perpetuation, flowering must occur at an appropriate time of the year. In Arabidopsis, the florigen FT is the main

promoter of flowering under long days (as in spring), while in non-inductive short-day (as in winter) conditions flowering largely depends on the accumulation of gibberellins (GAs, particularly the bioactive GA4).Besides, Tempranillo (TEM) genes seem to play a key role as flowering inhibitors that prevent blooming at early stages after germination.

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12.4.1 Discovery of flowering response

G.Gassner and W.W. Garner observed that winter variety of petkus rye plants called *Secale cereal*, responded favorably to cold treatments. At the same time, Garner and Allard demonstrated how plants produce flower in response to different lengths of the day and night in a 24 hours day cycle. The above two phenomenon are popularly called as Vernalization and Photoperiodism respectively. The above studies have lead to the discovery of how plants rhythmically respond and behave to day and night duration or to temperature fluctuation in different seasons of the year and they also observed rhythmical behavior of the plants which is referred to as 'biological rhythm'. And the operational time measuring system found within the plant structures is called 'Biological Clock'.

12.4.2 Florigen Concept

Florigen specific flower-inducing substances were first postulated by Julius Sachs (1865), and have evidence from the discovery of photoperiodism. The seminal finding was that in photo periodically sensitive plants the day length is perceived by the leaves, whereas flower formation takes place in the shoot apical meristem. This finding demonstrates that a long-distance signal, called the floral stimulus or florigen moves from an induced leaf to the shoot apex. The floral stimulus can be transmitted from a flowering partner (donor) via a graft union to a non-flowering partner (receptor).

With the isolation of auxin as the first-identified plant growth hormone (1930) and the discoveries of cytokinins and gibberellins in the 1950s, attempts were made to extract florigin. In the early approaches, it was assumed that like the classical plant hormones, florigen would be a small organic molecule. Extracts prepared from flowering material were tested for flower-promoting activity in vegetative plants.

12.4.3 Molecular-Genetic analysis

The molecular and genetic mechanisms about floral induction, floral patterning, and floral organ have derived primarily from work in three dicot species: *Antirrhinum majus*, *Arabidopsis thaliana*, and *Petunia hybrida*. Although Antirrhinum and petunia have contributed fundamental breakthroughs to our understanding of flower development, it is from Arabidopsis that the most detailed and comprehensive picture of the molecular mechanisms underlying flower development has been obtained.

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Arabidopsis as a model plant for molecular-genetic studies, genetic and molecular analyses became popular approaches in studies on flowering. Through mutagenesis, many mutants were isolated in the quantitative long-day plant (LDP) *Arabidopsis thaliana*. Of interest here are those mutants that exhibit changes in flowering time in comparison with wild-type (WT) plants. Mutants flowering later than WT plants represent a loss-of-function that must involve positive regulators of flowering. Conversely, early-flowering mutants have lost an inhibitor of flowering. These molecular-genetic studies have led to identification of four pathways that regulate flowering in *Arabidopsis*: the photoperiod, vernalization, autonomous, and GA pathways

The genes called *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) are principal to long day induced flowering in *Arabidopsis*. *CO* encodes a nuclear zinc-finger protein, which in response to LD induces transcription of *FT* in the phloem of leaves. Does it happens in phloem or in leaf tissues, but where in the cells of leafs; is it mesophyll cells, bundle sheath cells or in cells in contact with phloem progenitors. Visualize phloem food conducting vessels are like human lymphocyte system. Neither *CO* nor *FT* is expressed in the shoot apex. Expression of *CO* from a meristem-specific promoter does not enhance flowering, but early flowering is induced in short days (SD) when *FT* is over expressed in the shoot apex. Expression of *CO* from a phloem-specific promoter is sufficient to generate a phloem-mobile stimulus that induces flowering, grafting experiments between *Arabidopsis* donor plants over expressing *CO* and *co* mutant shoots as receptor Because FT must act in the shoot apex in order to elicit flowering, this result gives a strong indication that

FT or its product is the signal that moves from an induced leaf to the shoot apex and induces flowering.

FT acts in the shoot apex by forming a complex with the bZIP transcription factor FD. The essential role of FD in flowering is demonstrated by the finding that *fd* mutants flower late and that *FT* over expression is partially suppressed by *fd* .

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12.4.4 Floral Architecture

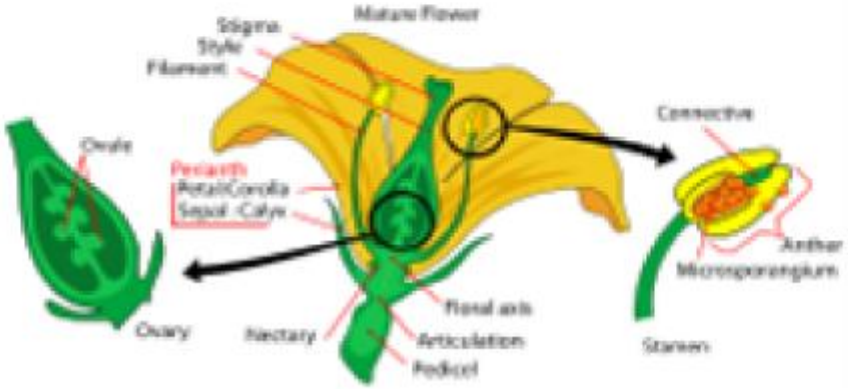

Fig. 12.2 --Flower Architecture

A flower's anatomy, as defined by the presence of organs (sepals, petals, stamens and carpels) and facilitate sexual reproduction in flowering plants. The flower arises from the activity of three classes of genes, which regulate floral development: genes which regulate the identity of the meristem, the identity of the flower organ and finally cadastral genes. They are-

- Meristem identity genes. Code for the transcription factors required to initiate the induction of the identity genes. They are positive regulators of organ identity during floral development.
- Organ identity genes. Directly control organ identity and also code for transcription factors that control the expression of other genes, whose products are implicated in the formation or function of the distinct organs of the flower.
- Cadastral genes. Act as spatial regulators for the organ identity genes by defining boundaries for their expression. In this way they control the extent to which genes interact thereby regulating whether they act in the same place at the same time.

The methodology for studying flower development involves two steps. Firstly, the identification of the exact genes required for determining the identity of the floral meristem. Secondly, genetic analysis is carried out on the aberrant phenotypes for the relative characteristics of the flowers, which allows the characterization of the homeotic genes implicated in the process.

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Principles of Flower Development

Flower development is the process by which angiosperms produce a pattern of gene expression in meristem that leads to the appearance of an organ oriented towards sexual reproduction, a flower. There are three physiological developments that must occur in order for this to take place: firstly, the plant must pass from sexual immaturity into a sexually mature state (i.e. a transition towards flowering); secondly, the transformation of the apical meristem function from a vegetative meristem into a floral meristem or inflorescence; and finally the growth of the flower's individual organs.

The first unifying principle in the flower development field is the ABC model. This model, initially proposed in the early 1990s based on genetic experiments in Antirrhinum and Arabidopsis, is striking in its simplicity and is applicable to a wide range of angiosperm species, both dicots and monocots, including economically important grass species such as rice and maize .The Arabidopsis flower, like most angiosperm flowers, consists of four organ types that are arranged in a series of concentric rings or whorls. From outside to inside, the flower consists of sepals in whorl 1, petals in whorl 2, stamens in whorl 3, and carpels in whorl 4. The ABC model postulates that three activities, A, B, and C, specify floral organ identity in a combinatorial manner. Specifically, A alone specifies sepals, A+B specifies petals, B+C specifies stamens, and C alone specifies carpels. A second major aspect is that A and C classes are mutually repressive. In the absence of A, C activity is present throughout the flower. Likewise, in the absence of C, A activity is present throughout the flower. Throughout the 1990s, the ABC genes were cloned from a wide range of species, and numerous molecular studies were performed. These molecular experiments largely support the major tenets of the ABC model.

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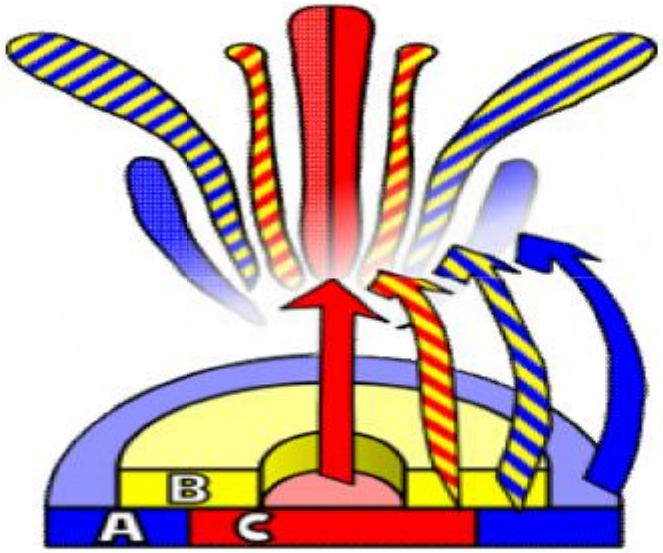

Fig. 12.3 : ABC model of flower development

The second major unifying principle involves the central role of the LEAFY (LFY) gene. LFY ortho logs are present in a wide range of flowering and nonflowering plant species .In many developmental contexts, LFY is necessary and sufficient to specify a meristem as floral. In addition, LFY serves two key roles in specifying flowers. First, LFY is a key integrator of the outputs of floral inductive pathways .Second, LFY is a key activator of the floral organ identity ABC genes.

Flower development can be divided into four steps that occur in a temporal sequence. First, in response to both environmental and endogenous signals, the plant switches from vegetative growth to reproductive growth; this process is controlled by a large group of flowering time genes. Second, signals from the various flowering time pathways are integrated and lead to the activation of a small group of meristem identity genes that specify floral identity. Third, the meristem identity genes activate the floral organ identity genes in discrete regions of the flower. Fourth, the floral organ identity genes activate downstream "organ building" genes that specify the various cell types and tissues that constitute the four floral organs.

Floral transition

The transition from the vegetative phase to a reproductive phase involves a dramatic change in the plant's vital cycle, perhaps the most important one, as the process must be carried out correctly in order to guarantee that the plant

produces descendents. This transition is characterised by the induction and development of the meristem of the inflorescence, which will produce a collection of flowers or one flower, where only one is produced. This morphogenetic change contains both endogenous and exogenous elements: For example, in order for the change to be initiated the plant must have a certain number of leaves and contain a certain level of total biomass. Certain environmental conditions are also required such as a characteristic photoperiod. Besides, plant hormones also play an important role in the process, with the gibberellins having a particularly important role.

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Formation of the floral meristem or the inflorescence

The meristem can be defined as the tissue or group of plant tissues that contain undifferentiated stem cells, which are capable of producing any type of cell tissue. Their maintenance and development, both in the vegetative meristem or the meristem of the inflorescence is controlled by genetic cell fate determination mechanisms. This means that a number of genes will directly regulate, for example, the maintenance of the stem cell's characteristics (gene WUSCHEL or WUS), and others will act via negative feedback mechanisms in order to inhibit a characteristic (gene CLAVATA or *CLV*).

12.5 Vernalization

Light has a profound influence on plants and it performs many important biological processes like photosynthesis, phototropism, photorespiration, photoperiodism, etc. Influence of light on plants in flowering is very fascinating, but not all the plants respond and flower to photoperiodic treatments. In addition, the temperature also has significant effects on plant growth, dormancy and flowering. Most of the metabolic processes are regulated by the temperature that is prevailing in the environment. But the effect of temperature in inducing the development of reproductive organs is fascinating.

Plants have different cycles of vegetative growth, flowering and fruiting. However, some biennials which produce vegetative structures in one season and induce flowering in the flowering season only after they are exposed to prolonged winter or cold treatment. Interestingly, such cold requiring plants

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also need proper photoperiodic treatment for flowering. Such biennial plants can be made to flower in the same season by subjecting them to cold treatment. Hence the process of acquiring the ability or capacity to accelerate the process of flowering, in response to cold treatment is referred to as Vernalization.

Vernalization (from Latin: vernus, of the spring) is the acquisition of a plant's ability to flower or germinate in the spring by exposure to the prolonged cold of winter.

Among many plants, Petkus rye (short day plants) was the first to be used for experimentation. The other examples are *Hyoscyamus niger* (long day plant) *Triticum aestivatum* (CV winter wheat), *Lunaria bienensis, Arabidopsis thalliana, Lolium perennial, Beta Vulgaris, Brassica oleracea,* etc. The winter rye called *Secale cereale* or Petkus rye is a biennial plant which requires cold treatment for successful cultivation as one season crop. The grains of these plants are known for their hardiness and quality for the purpose of milling and baking. Similarly,Many temperate plants have a vernalization requirement and must experience a period of low winter temperature to initiate or accelerate the flowering process, or, as the case with many fruit tree species, to actually break dormancy, prior to flowering. This ensures that reproductive development and seed production occurs at the optimum environmentally favorable time, normally following the passing of winter. Vernalization activates a plant hormone called florigen present in the leaves which induces flowering at the end of the chilling treatment. Some plant species do not flower without vernalization. Many biennial species have a vernalization period, which can vary in period and temperature. Typical vernalization temperatures are between 5 and 10 degrees Celsius (40 and 50 degrees Fahrenheit).

12.5.1 History

Vernalization is the process by which prolonged exposure to cold temperatures promotes flowering. Over the past century, this process has been studied extensively at the physiological level. Recent studies have provided some insight into the molecular basis of vernalization.

The word "vernalization" is coined by Trofim Lysenko to describe a chilling process he used to make the seeds of winter cereals behave like spring cereals.

Lysenko's (1928) studied vernalization and plant physiology drew wide attention due to its practical consequences for Russian agriculture. Severe cold and lack of winter snow had destroyed many early winter wheat seedlings. By treating wheat seeds with moisture as well as cold, Lysenko induced them to bear a crop when planted in spring. Later however, Lysenko inaccurately asserted that the vernalized state could be inherited - i.e., that the offspring of a vernalized plant would behave as if they themselves had also been vernalized and would not require vernalization in order to flower quickly.

12.5.2 Chemistry of floral induction in vernalized plants

The plants with their specific genetic makeup respond to different treatments like cold or photoperiods and produce flowers. Most of the cold requiring plants also require proper photoperiodic treatment. Gibberellins are known to overcome both cold treatment and photoperiodic treatment in long day plants, but it has no effect on short day plants. Synthesis of some unknown substance called vernalin during the period vernalization has been clearly demonstrated by grafting experiments. Furthermore for proper vernalization, plants require sufficient amount of water, oxygen and some vegetation growth. Though all the above said factors are provided to the plant, flower inducing substance won't be synthesized until and unless it is treated with proper cold condition at the stage of its development. It is during the cold treatment, the synthesis of the said flowering inducing factor is believed to be accelerated.

Arabidopsis thaliana rosette before vernalization shows no floral spike. Arabidopsis thaliana, also known as "thale cress," is a much-studied model species. In 2000, the entire genome of its five chromosomes was completely sequenced. Some variants, called "winter annuals", require vernalization to flower; others ("summer annuals") do not. The genes that underlie this difference in plant physiology have been intensively studied. The reproductive phase change of *A. thalliana* occurs by a sequence of two related events: first, the bolting transition (flower stalk elongates), then the floral transition (first flower appears). Bolting is a robust predictor of flower formation, and hence a good indicator for vernalization research. In Arabidopsis winter annuals, the apical meristem is the part of the plant that needs to be chilled. Vernalization of the meristem appears to confer competence to respond to floral inductive

signals on the meristem. A vernalized meristem retains competence for as long as 300 days in the absence of an inductive signal.

12.5.3 Various and Flowering

Age and Site of Vernalization

NOTES

Vernalization through cold treatment is very effective at the seed stage or seedling stage. In some cereals, even the embroyes can be successfully verbalized. However, in many cold recurring species, vernalization is not effective until and unless the plant possess at least few leaves. The requirement of few leaves for effective vernalization is called 'Ripeness to Flowering'. This suggests that plants need certain degree of photosynthetic obtain to respond for cold treatment.

Temperature Effect

For the normal growth and development, every plant requires on optimum temperature. But for vernalization the optimum temperature required is 3 ^{0}C to 17 ^{0}C, which varies depending upon the species involved. Even the duration of treatment varies from species to species. Individual requirements have to be determined independently by experimentation. In petkus rye the most effective range of temperature is 3 ^{0}C to 7 ^{0}C, whereas in Hyoscyamus niger 3-17 degree is optimal. However the efficiency of cold treatment in bringing about vernalization is determined by the number of days shortened between germination and flowering stage.

Effect of water and oxygen

Along with the cold treatment plants also require water and oxygen for effective vernalization. The seeds or embryos should possess at least 40-50% water in their cells, without which cold treatment has no effect. Similarly oxygen is very essential; probably it is required for biological oxidation.

Vernalin

During vernalization the meristematic cells found either in stem apex or leaves are stimulated to produce some substance. The presence of such substance has been demonstrated by grafting a vernalized plant to another non vernalized plant at normal temperatures. The plant that receives the graft, after sometime, starts producing flowers, which suggests some substance found in the

vernalized plants is transplanted to non vernalized plant and that is responsible for the induction of flowering in the latter plant. In some cases, if the cut shoot tip of the vernalized plant is placed above the decapitated stem of the non vernalized plant, flowering is induced in the receiver plant The above experiments clearly demonstrate that some substance is synthesized and such substance is now called 'Vernalin" and it is capable of diffusion.

NOTES

Devernalization

When vernalized seedlings or seeds are subjected to higher temperature like 35-40 ^{0}C the plants that develop from such treatment fail to flowers. Such a nullifying effect by higher temperatures is called Devernalization. Nevertheless, if the vernalized plants are maintained at sufficiently low temperatures for a long period of time, which has to be determined for every species, devernalization is not possible. This may be due to the putative vernalin have already acted upon the genetic material and committed it is flower formation. However, devernalized plants can be revernalized by subjecting the same seedling or seed again for another period of cold treatment by repetition of vernalization and devernalization cycles. Prolonged vernalization the effect decreases and seedlings lose their viability and potentiality to produce flowers.

Gibberellin as substitute for vernalization

Many plants which require cold treatment also require proper photoperiodic treatment for the induction of flowers, without which vernalization does not have any effect. If such plants are treated with gibberellins, they produce flowers without subjecting the plants to cold and photoperiodic treatments. For example, Henbane is a rosette leaved long day plant which requires cold treatment for flowering. If such untreated plants are sprayed with GAs, the plants produce flowers. It means gibberellins not only substitute vernalization but also photoperiodic treatment. But same gibberellins have no effect on other long day and cold requiring plants species called petkus rye. In some cases for the proper response to GA treatment, the plant should possess a cluster of leaves in rosette form as a precondition. The effect of GA on plants like Henbane has been attributed to its effect on the elongation of internodes at

which time GA also promotes and probably elaborates the factors required for the induction flowers.

12.6 Summary

NOTES

Timing of flowering is key to the reproductive success of many plants. In temperate climates, flowering is often coordinated with seasonal environmental cues such as temperature and photoperiod. Vernalization, the process by which a prolonged exposure to the cold of winter results in competence to flower during the following spring, is an example of the influence of temperature on the timing of flowering. In different groups of plants, there are distinct genes involved in vernalization, indicating that vernalization systems evolved independently in different plant groups.

Circadian rhythms occur as a biological rhythm with light, are endogenously generated and self-sustaining, and are relatively constant over a range of ambient temperatures. Circadian rhythms feature a transcriptional feedback loop, a presence of PAS proteins, and several photoreceptors that fine-tune the clock to different light conditions. Anticipation of changes in the environment changes the physiological state that provides plants with an adaptive advantage.

12.7 Glossary

- **Photoperiodism** : Photoperiodism is defined as the developmental responses of plants to the relative lengths of light and dark period or it is a physiological reaction of organisms to the length of day or night.
- **Day neutral plants** : In day-neutral plants, flowering is not regulated by day lengths or day-neutral plants flower regardless of the day length
- **Phytochrome** : Light-sensitive proteins called phytochromes are partially responsible for the timing of flowering
- **Circadian rhythms** : Circadian rhythms are patterns of physiological change that follow a 24-hour cycle, day after day.
- **Circaannual** : It is a biologically significant clock periodicity of approximately a year.

- **Florigen** : Florigen a specific flower-inducing substances which enhance flowering.
- **Vernalization** : It is the acquisition of a plant's ability to flower in the spring by exposure to the prolonged cold of winter, or by an artificial equivalent.
- **Devernalization** : when vernalized seedlings or seeds are subjected to higher temperature like 35-40 0C the plants that develop from such treatment fail to flowers. Such a nullifying effect by higher temperatures is called Devernalization.

NOTES

12.8 Self-Learning Exercise

Section-A (Very Short Answer Type Questions)

1 What is the function of phytochrome?
2 Write the name of two long day plants.
3 Define devernalization.
4 Give one example of model plant for molecular and genetic studies.

Section-B (Short Answer Type Questions))

1 Differentiate phytochrome and cryptochrome.
2 What are the main roles of vernalin in plants?
3 Briefly explain circadian rhythum.
4 What is critical photoperiod?

Section-C (Long Answer Type Questions))

1. What is florigen? Describe the genetic and molecular aspect of floral induction.
2. Define photoperiod and photoperiodism. Describe the classification and significance of photoperiodism.
3. Enumerate the characteristics and regulations of endogenous clocks in plants.

Answer key of section –A

1. Phytochrome is responsible for measuring the dark period, exists in two forms, Pr and Pfr. Pr which absorbs red light during the day and triggers the flowering response.

2. Spanish and Radish
3. When vernalized seedlings or seeds are subjected to higher temperature like 35- 40 0C the plants that develop from such treatment fail to flowers. Such a nullifying effect by higher temperatures is called Devernalization.
4. Arabidopsis

NOTES

12.9 Reference

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Unit - 13

Sensory Photobiology

NOTES

Structure of the Unit

13.0 Objective

After going through this unit you will be able to understand:-

- Phytochromes and Cryptochromes- History of Discovery and structure
- Photochemical and Biochemical properties of Phytochromes and Cryptochromes

- Photophysiology
- Cellular localization
- Photomorphogenesis
- Molecular mechanism of receptors

NOTES

13.1 Introduction

Light serves as a source of energy as well as information for plants. Plants are capable of sensing their physical, chemical and biological environment. They perceive signals containing information. This information is used by plants to adjust growth and morphology, and to trigger development. Through the process of photosynthesis plants capture solar energy. This energy is absorbed by chlorophylls and accessory pigments. Through other pigments that are in very small quantities in the plant, small amounts of energy are absorbed. This energy is not important as such but rather as a source of information about the environment.

Photobiology is the study of the interactions of light (non-ionizing radiation) and living organisms. This includes the study of photosynthesis, photo morphogenesis, circadian rhythms and ultra violet radiation effects. Moreover, it is a division of biology that deals with processes occurring in organisms upon exposure to visible, ultraviolet, and near-infrared radiation. Thus, Photobiology serves as the basis for increasing the efficiency of the photosynthesis of agricultural plants, for raising plants by artificial means, for accelerate the growth of farm animals, and for using radiation in medical practice and in the control of environmental pollution.

13.2 Phytochrome

Phytochrome is a photoreceptor or a pigment to detect light in plants. It is sensitive to light in the red and far-red region of the visible spectrum. Moreover, It is a chromoprotein present in trace amounts in all higher plants Many flowering plants use it to regulate the time of flowering based on the length of day and night (photoperiodism) and to set circadian rhythms. It also regulates other responses including the germination of seeds (photoblasty),

elongation of seedlings, the size, shape and number of leaves, the synthesis of chlorophyll, and the straightening of the epicotyl or hypocotyl hook of dicot seedlings. It is found in the leaves of most plants.

It involved in regulating light-dependent processes, such as leaf development and flowering. It consists of a phycobilin-related chromophore covalently linked to a polypeptide of ~124 kDa. Phytochrome exists in two forms, PR (666 nm) and PFR (730 nm), which are indefinitely interconvertible on absorption of light in the red and far-red regions of the spectrum, respectively. The spectral changes are the result of cis-trans isomerization in the phycobilin. The active form, PFR, plays a central role in light-promoted modulation of gene expression, which is of importance in plant growth and development in every phase of the life cycle; conversion of PFR to PR cancels these responses. The phytochromes are homodimeric proteins, with a linear tetrapyrrole chromophore covalently linked to a polypeptide.

NOTES

Other plant photoreceptors include cryptochromes and phototropins, which are sensitive to light in the blue and ultra-violet regions of the spectrum.

13.2.1 Discovery

In the year 1918 Garner and Allard discovered that flowering was controlled by photoperiod in Maryland Mammoth Tobacco, a spontaneous giant strain of tobacco that wouldn't flower in the field. In 1930 Site of induction is the leaf, Florigen /anti-florigen hypothesis developed. Circadian rhythm also identified as part of photoperiod detection. Action spectra for lettuce seed gemination and corn mesocotyl and coleoptile growth. Red light is most active, far-red light least effective.

The phytochrome pigment was discovered by Sterling Hendricks and Harry Borthwick at the USDA-ARS Beltsville Agricultural Research Center in Maryland during a period from the late 1940s to the early 1960s. They discovered that red light was very effective for promoting germination or triggering flowering responses. The red light responses were reversible by far-red light, indicating the presence of a photo reversible pigment.

The phytochrome pigment was identified using a spectrophotometer in 1959 by biophysicist Warren Butler and biochemist Harold Siegelman. Butler was also responsible for the name, phytochrome. In 1983 Peter Quail and Clark Lagarias

reported the chemical purification of the intact phytochrome molecule, and in 1985 the first phytochrome gene sequence was published by Howard Hershey and Peter Quail. By 1989, molecular genetics and work with monoclonal antibodies that more than one type of phytochrome existed.

NOTES

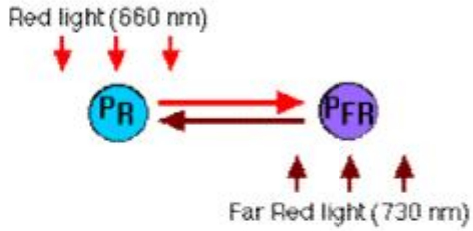
In 1996 a gene in the newly sequenced genome of the cyanobacterium Synechocystis was noticed to have a weak similarity to those of plant phytochromes, the first evidence of phytochromes outside the plant kingdom. Jon Hughes in Berlin and Clark Lagarias at UC Davis subsequently showed that this gene indeed encoded a bona fide phytochrome (named Cph1) in the sense that it is a red/far-red reversible chromoprotein. In 1990 five phy genes are identified in Arabidopsis. Multiple phy genes identified in many species. Arabidopsis mutants show different functions for different phys. In 2000, Phytochrome research finds itself intertwined with research on blue light receptors, protein turnover control, plant hormone biosynthesis, and many other developmental and signal transduction processes. Moreover, in 2005, the Vierstra and Forest, University of Wisconsin published a three-dimensional structure of the photosensory domain of Deinococcus phytochrome. This breakthrough paper revealed that the protein chain forms a knot - a highly unusual structure for a protein.

13.2.2 Structure

Phytochrome is found in various plants including all higher plants; very similar molecules have been found in several bacteria. A fragment of a bacterial phytochrome has three-dimensional protein structure. Phytochrome is a homodimer: two identical protein molecules each conjugated to a light-absorbing molecule (compare rhodopsin).

Plants have 5 phytochromes: PhyA, PhyB, as well as C, D, and E.

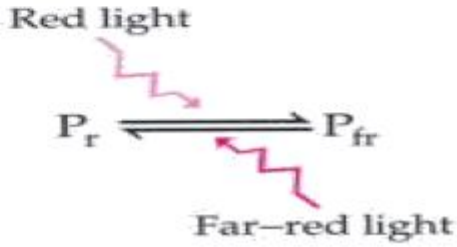
There is some redundancy in function of the different phytochromes, but there also seem to be functions that are unique to one or another. The phytochromes also differ in their absorption spectrum; that is, which wavelengths (e.g., red vs. far-red) they absorb best.



NOTES

Phytochromes exist in two interconvertible forms, PR because it absorbs red (R; 660 nm) light; PFR because it absorbs far red (FR; 730 nm) light.

These are the relationships: Absorption of red light by PR converts it into PFR. Absorption of far red light by PFR converts it into PR. In the dark, PFR spontaneously converts back to PR.



Chemically, phytochrome consists of a chromophore, a single bilin molecule consisting of an open chain of four pyrrole rings, bonded to the protein moiety. It is the chromophore that absorbs light, and as a result changes the conformation of bilin and subsequently that of the attached protein, changing it from one state or isoform to the other.

The phytochrome chromophore is usually phytochromobilin, and is closely related to phycocyanobilin and to the bile pigment bilirubin.The term "bili" in all these names refers to bile. Bilins are derived from the closed tetrapyrrole ring of haem by an oxidative reaction catalysed by haem oxygenase to yield their characteristic open chain. Chlorophyll too is derived from haem (Heme). In contrast to bilins, haem and chlorophyll carry a metal atom in the center of the ring, iron or magnesium, respectively.

The Pfr state passes on a signal to other biological systems in the cell, such as the mechanisms responsible for gene expression. This mechanism is almost certainly a biochemical process. It is known that although phytochromes are synthesized in the cytosol and the Pr form is localized there, the Pfr form, when generated by light illumination, is translocated to the cell nucleus. It has been proposed that phytochrome, in the Pfr form, may act as a kinase, and it has

been demonstrated that phytochrome in the Pfr form can interact directly with transcription factors.

13.2.3 Photochemical and Biochemical Properties

NOTES

Plants regulate important developmental processes such as seed germination, growth direction, growth rate, chloroplast development, pigmentation, flowering, and senescence, collectively termed photomorphogenesis. To perceive light signals, plants use several receptor systems that convert light absorbed by specific pigments into chemical or electrical signals to which the plants respond. This signal conversion is called photosensory transduction. Pigments used include cryptochrome, a blue light-absorbing pigment; an ultraviolet light-absorbing pigment; and phytochrome, a red/far-red light-absorbing pigment.

The phytochrome family of photoreceptors has an unusual feature that was a significant advantage in the study of the phytochrome regulation of photomorphogenesis, and the purification and characterization of the photoreceptor itself. The chromophore for phytochrome is photochromic, i.e., it undergoes a change in conformation that makes a stable change in its light absorption properties. When the chromophore is in one state, the phytochrome molecule is inactive, and when it is in the other state the phytochrome molecule initiates what are to this point poorly understood signalling processes. Phytochromes are synthesized in the inactive form, for which the absorption maximum is 660 nm. The red absorbing form, Pr, on absorption of a photon, converts to a form with an absorption maximum of 730 nm, Pfr (far red). The Pfr from is the active form, but it can be converted back to Pr (and inactivated) by the absorption of a far-red photon.

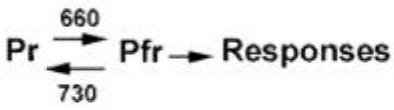


Fig. 13.1 Interconversion of the Pr and Pfr forms of Phytochrome

The photo reversibility of the activation of phytochrome has been a valuable tool in testing for phytochrome regulation of specific aspects of photomorphogenesis. The different absorption properties of the two molecules also made it possible to purify the protein. Phytochromes turned out to be large

multi-domain proteins, and the functions of these domains, and how the protein interacts with signaling partners have yet to be anywhere near fully explored.

Phytochrome consists of a compound that absorbs visible light (chromophore) bound to a protein. The chromophore is an open-chain tetrapyrrole closely related to the photosynthetic pigments found in the cyanobacteria and similar in structure to the circular tetrapyrroles of chlorophyll and hemoglobin. Phytochrome is one of the most intensely colored pigments found in nature, enabling phytochrome in seeds to sense even the dim light present well beneath the surface of the soil and allowing leaves to perceive moonlight

NOTES

Phytochrome can exist in two stable photointerconvertible forms, Pr or Pfr, with only Pfr being biologically active. Absorption of red light (near 666 nanometers) by inactive Pr converts it to active Pfr, while absorption of far-red light (near 730 nm) by active Pfr converts phytochrome back to inactive Pr. Plants frequently respond quantitatively to light by detecting the amount of Pfr produced. As a result, the amount of Pfr must be strictly regulated nonphotochemically by precisely controlling both the synthesis and degradation of the pigment.

Phytochrome has a variety of functions in plants. Initially, production of Pfr is required for many seeds to begin germination. This requirement prevents germination of seeds that are buried too deep in the soil to successfully reach the surface. In etiolated (dark-grown) seedlings, phytochrome can measure an increase in light intensity and duration through the increased formation of Pfr. Light direction also can be deduced from the asymmetry of Pfr levels from one side of the plant to the other. Different phytochrome responses vary in their sensitivity to Pfr; some require very low levels of Pfr (less than 1% of total phytochrome) to elicit a maximal response, while others require almost all of the pigment to be converted to Pfr. Thus, as the seedling grows toward the soil surface, a cascade of photomorphogenic responses is induced, with the more sensitive responses occurring first. This chain of events produces a plant that is mature and photosynthetically competent by the time it finally reaches the surface. Production of Pfr also makes the plant aware of gravity, inducing shoots to grow up and roots to grow down into the soil.

NOTES

In light-grown plants, phytochrome allows for the perception of daylight intensity, day length, and spectral quality. Intensity is detected through a measurement of phytochrome shuttling between Pr and Pfr; the more intense the light, the more interconversion. This signal initiates changes in chloroplast morphology to allow shaded leaves to capture light more efficiently. If the light is too intense, phytochrome will also elicit the production of pigments to protect plants from photo damage.

Temperate plants use day length to tailor their development, a process called photoperiodism. How the plant measures day length is unknown, but it involves phytochrome and actually measures the length of night. See also Photoperiodism.

Finally, phytochrome allows plants to detect the spectral quality of light, a form of colour vision, by measuring the ratio of Pr to Pfr. When a plant is grown under direct sun, the amounts of red and far-red light are approximately equal, and the ratio of Pr to Pfr in the plant is about 1:1. The plant should become shaded by another plant, the Pr/Pfr ratio changes dramatically to 5:1 or greater. This is because the shading plant's chlorophyll absorbs much of the red light needed to produce Pfr and absorbs almost none of the far-red light used to produce Pr. For a shade-intolerant plant, this change in Pr/Pfr ratio induces the plant to grow taller, allowing it to grow above the canopy.

It is not known how phytochrome elicits the diverse array of photomorphogenic responses, but the regulatory action must result from discrete changes in the molecule following photoconversion of Pr to Pfr. These changes must then start a chain of events in the photosensory transduction chain leading to the photomorphogenic response. Many photosensory transduction chains probably begin by responding to Pfr or the Pr/Pfr ratio and branch off toward discrete end points.

Seed Germination

The involvement of individual phytochromes in mediating Arabidopsis seed germination has been documented in many mutant studies. Three phytochromes, i.e. phyA, phyB and phyE are involved in the control of Arabidopsis seed germination. PhyA is responsible for the irreversible VLFR responses triggered by a wide variety of irradiations (ultraviolet, visible and FR

light), while phyB controls the R/FR photo reversible LFRs. Seed germination can be promoted by both VLFRs and LFRs. In addition, phyA promotes germination in continuous FR light in the HIR mode .However, phyE was also found to play a role in controlling seed germination in continuous FR light. This could be either because phyE is directly involved in the photoperception of FR light for this response, or because phyA requires phyE to mediate seed germination.

Seedling De-etiolation

Dark-grown seedlings undergo skotomorphogenesis (etiolation) and are characterized by long hypocotyls, closed cotyledons and apical hooks, and development of the proplastids into etioplasts. Light-grown seedlings undergo photomorphogenesis (de-etiolation) and are characterized by short hypocotyls, open and expanded cotyledons, and development of the proplastids into green mature chloroplasts. Phytochromes perform a variety of overlapping functions in regulating seedling de-etiolation.

Shade Avoidance

Plant development is regulated not only by the difference between light and darkness, but also by light quality, in particular the change of light quality due to shading by other plants. Light passed through or reflected from living vegetation is depleted in R and B wavebands, which are absorbed by chlorophyll and carotenoid pigments used for photosynthesis, leading to a reduction in the ratio of R to FR wavelengths (R:FR). This allows plants to initiate a suite of developmental responses called shade avoidance syndrome (SAS), which elevates leaves towards unfiltered daylight and enables plants to overtop competitors. These responses include elongation of stems and petioles, accelerated flowering time, and increased apical dominance. The ability of plants to monitor their light environments and change their architecture provides them with a competitive strategy to survive and complete their life cycle in dense stands.

13.3 Cryptochromes

Cryptochromes are a class of flavoproteins that are sensitive to blue light. They are found in plants and animals. Cryptochromes are involved in the circadian

rhythms of plants and animals, and in the sensing of magnetic fields in a number of species. The name Cryptochrome was proposed as a pun combining the cryptic nature of the photoreceptor, and the cryptogamic organisms on which many blue light studies were carried out.

NOTES

13.3.1 Discovery

Charles Darwin first documented plant responses to blue light in the 1800s; it was not until the 1980s that research began to identify the pigment responsible. In 1980, researchers discovered that the HY4 gene of the plant *Arabidopsis thaliana* was necessary for the plant's blue light sensitivity, and, when the gene was sequenced in 1993, it showed high sequence homology with photolyase, a DNA repair protein activated by blue light. By 1995, it became clear that the products of the HY4 gene and its two human homologs did not exhibit photolyase activity and were instead a new class of blue light photoreceptor hypothesized to be circadian photopigments. In 1996 and 1998, Cry homologs were identified in Drosophila and mice, respectively. In 1993 that Ahmad and Cashmore first reported the discovery of cryptochrome 1 (cry1) in Arabidopsis. It turned out to be a protein with considerable amino acid sequence similarity to prokaryotic DNA photolyases.

Recently plant cryptochromes were discovered by Ahmad and Cashmore, 1993; Hoffman et al., 1996, specifically mediate responses to blue light, showing a strong absorption peak in the blue region of the spectrum. However, because they also show a slight peak of absorption in the green region, cryptochromes have also been implicated in responses to green light.

13.3.2 Structure

The structure of cryptochrome involves photolyase, with a single molecule of FAD noncovalently bound to the protein. These proteins have variable lengths and surfaces on the C-terminal end, due to the changes in genome and appearance that result from the lack of DNA repair enzymes. The Ramachandran plot shows that the secondary structure of the CRY1 protein is primarily a right-handed alpha helix with little to no steric overlap. The structure of CRY1 is almost entirely made up of alpha helices, with several loops and few beta sheets. The molecule is arranged as an orthogonal bundle. Cryptochromes (CRY1, CRY2) are evolutionarily old and highly conserved

proteins that belong to the flavoproteins superfamily that exists in all kingdoms of life. All members of this superfamily have the characteristics of an N-terminal photolyase homology (PHR) domain. The PHR domain can bind to the flavin adenine dinucleotide (FAD) cofactor and a light-harvesting chromophore. Cryptochromes are derived from and closely related to photolyases, which are bacterial enzymes that are activated by light and involved in the repair of UV-induced DNA damage. In eukaryotes, cryptochromes no longer retain this original enzymatic activity.

13.3.3 Photochemical and Biochemical Properties Phototropism

In plants, cryptochromes mediate phototropism, or directional growth toward a light source, in response to blue light. This response is now known to have its own set of photoreceptors, the phototropins. Unlike phytochromes and phototropins, cryptochromes are not kinases. Their flavin chromophore is reduced by light and transported into the cell nucleus, where it affects the turgor pressure and causes subsequent stem elongation. To be specific, Cry2 is responsible for blue-light-mediated cotyledon and leaf expansion. Cry2 over expression in transgenic plants increases blue-light-stimulated cotyledon expansion, which results in many broad leaves and no flowers rather than a few primary leaves with a flower. A double loss-of-function mutation in Arabidopsis thaliana Early Flowering 3 (elf3) and Cry2 genes delays flowering under continuous light and was shown to accelerate it during long and short days, which suggests that Arabidopsis CRY2 may play a role in accelerating flowering time during continuous light.

Light capture

Cryptochromes are known to possess two chromophores: pterin (in the form of 5,10-methenyltetrahydrofolic acid (MTHF)) and flavin (in the form of FAD). Both may absorb a photon, and in Arabidopsis, pterin appears to absorb at a wavelength of 380 nm and flavin at 450 nm. Past studies have supported a model by which energy captured by pterin is transferred to flavin. Under this model of photo transduction, FAD would then be reduced to FADH, which probably mediates the phosphorylation of a certain domain in cryptochrome and triggered a signal transduction chain, possibly affecting gene regulation in the cell nucleus.

Circadian rhythms

Cryptochromes play a pivotal role in the generation and maintenance of circadian rhythms in plants and animals. In Drosophila, cryptochrome (dCRY) acts as a blue-light photoreceptor that directly modulates light input into the circadian clock, while in mammals, cryptochromes (CRY1 and CRY2) act as transcription repressors within the circadian clockwork. Some insects, including the monarch butterfly, have both a mammal-like and a Drosophila-like version of cryptochrome, providing evidence for an ancestral clock mechanism involving both light-sensing and transcriptional-repression roles for cryptochrome.

13.4 Photophysiology of Light induced Response

Photophysiology is the ability of plants to sense the environment and adjust their morphology, physiology and phenotype accordingly. Plants perceive and react with several stimuli includes- chemicals, gravity, light, moisture, infections, temperature, oxygen and carbon dioxide concentrations, parasite infestation, physical disruption, sound, and touch. Plants have a variety of means to detect such stimuli and a variety of reaction responses or behaviours.

Many plant-organs contain photo-sensitive compounds (phototropins, cryptochromes and phytochromes) each reacting very specifically to certain wavelengths of light. These light-sensors tell the plant if it's day or night, how long the day is, how much light is available and from where the light comes. Shoots grow towards light and roots usually grow away from light. These responses are called phototropism and skototropism respectively. They are brought about by light sensitive pigments like phototropins and phytochromes and the plant hormone auxin. Many plants exhibit certain phenomena at specific times of the day, for example certain flowers open only in the mornings. Plants keep track of the time of the day with a molecular clock]. This internal clock is set to the solar clock every day using sunlight. The internal clock coupled with the ability to perceive light also allows plants to measure the time of the day and so find the season of the year. This is how many plants know when to flower. The seeds of many plants sprout only after they are exposed to light. This response is carried out by phytochrome signalling. Plants are also able to sense the quality of light and respond appropriately, for

example in low light conditions plants produce more photosynthetic pigments whereas when the light is very bright and/or if the levels of harmful UV increase, plants produce more of their protective pigments that act as sunscreens

Phytochrome

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Phytochrome is a protein containing a covalently attached chromophore. Phytochrome exists in 2 interconvert able conformations with different absorption spectra. Pfr absorbs far red and is generally the biologically active conformation. Pr absorbs red. Absorption of red light converts Pr to Pfr while absorption of far red converts Pfr to Pr. Phytochrome responses is classically defined by their red/far red reversibility. For example, lettuce seeds require light to germinate. Red light induces germination but if followed by a pulse of far red light, germination is inhibited. It also contains a domain resembling a protein kinase and has been shown to autophosphorylate; however the functional significance of this in light signal transduction is unknown.

Phytochrome can measure light quality because if light contains more red than far red light, most phytochrome will be in the Pfr form. Phytochrome mediates a variety of photomorphogenic phenomena including leaf expansion and inhibition of stem elongation. One classic example is in the shade avoidance response of shade intolerant plants. Foliage readily absorbs red light and so in the shade of another plant there is higher amounts of far red light which will drive phytochrome to the Pr form. Pr does not inhibit stem elongation which allows shaded plants to elongate and grow to reach the sunlight.

Cryptochrome

Blue, green and UVA light are all perceived by a receptor called cryptochrome. It is a flavin protein with 2 chromophores attached, one for green, one for blue. There are 2 cryptochrome genes in Arabidopsis, CRY1 and 2. They have distinct but overlapping functions. Hy4/cry1 is a nonphotomorphogenic mutant defective for the blue light receptor. CRY proteins appear constitutively nuclear, although there are indications that there may be some CRY functions in the cytoplasm too.

Cryptochrome action requires the presence of phytochrome because some phytochrome mutants are non-photomorphogenic in blue or green light.

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However the cryptochrome mutant is photomorphogenic in red light (with far red reversibility) indicating that phytochrome action does not require cryptochrome. Evidence suggests that phytochrome and cryptochrome physically interact. CRY protein can be phosphorylated in vitro by the protein kinase activity of PHY-A. Furthermore, PHYB and CRY2 interact in plant extracts and exhibit FRET in plant cells. CRY1 and 2 also appear to directly interact with COP1, a factor involved in the negative regulation of photomorphogenesis in the dark.

Phototropin

Phototropin is a blue light receptor. Arabidopsis contains 2 phototropins. These are involved in phototropism, but not photomorphogensis (hypocotyl elongation).

Phototropism is the directional growth in response to directional light. Shoots are positively phototropic (grow toward light) while roots often show negative phototropism (grow away from light). Directional light causes a redistribution of auxin in shoot tissues such that the side away from the light accumulates higher levels and grows faster, causing bending toward the light. Phototropism is controlled mainly by blue/UVA light. However the receptor is different from cryptochrome because the cry mutants are still phototropic. A mutant identified as nonphototropic hypocotyl1 (nph1), acts at the level of light perception in the phototropic response but still retains normal photomorphogenetic responses. This gene was recently renamed Phototropin1 (PHOT1). Arabidopsis contains two phototropin genes, PHOT1 and PHOT2. PHOT protein is another flavoprotein containing 2 flavin mononucleotide chromophores. The protein also contains a protein kinase domain and blue light induces PHOT kinase activity.

13.5 Cellular Localization

Plant calculation and response claims to study the role of signalling, communication and behaviour to integrate data obtained at the genetic, molecular, biochemical and cellular levels, with the physiology, development and behaviour of individual organisms, plant ecosystems and evolution. A localization process that takes place at the cellular level; as a result of a cellular

localization process, a substance or cellular entity, such as a protein complex or organelle, is transported to, and/or maintained in, a specific location within or in the membrane of a cell.

Bacteria have subcellular localizations that can be separated when the cell is fractionated. The most common localizations referred to include the cytoplasm, the cytoplasmic membrane also referred to as the inner membrane in Gram-negative bacteria, the cell wall which is usually thicker in Gram-positive bacteria and the extracellular environment. Most Gram-negative bacteria also contain an outer membrane and peri plasmic space. Unlike eukaryotes, most bacteria contain no membrane-bound organelles; however there are some exceptions (i.e. magnetosomes). The cells of eukaryotic organisms are elaborately subdivided into functionally distinct membrane bound compartments. Some major constituents of eukaryotic cells are: extracellular space, cytoplasm, nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum (ER), peroxisome, vacuoles, cytoskeleton, nucleoplasm, nucleolus, nuclear matrix and ribosomes.

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In eukaryotes, numerous complex sub-cellular structures exist. The majority of these are delineated by membranes. Many proteins are trafficked to these in order to be able to carry out their correct physiological function. Assigning the sub-cellular location of a protein is of paramount importance to biologists in the elucidation of its role and in the refinement of knowledge of cellular processes by tracing certain activities to specific organelles. Membrane proteins are a key set of proteins as these form part of the boundary of the organelles and represent many important functions such as transporters, receptors, and trafficking. They are, however, some of the most challenging proteins to work with due to poor solubility, a wide concentration range within the cell and inaccessibility to many of the tools employed in proteomics studies.

Phytochrome has been localized within the cell plasma, the nucleus and the plastids by indirect immunofluorescence. Not all cells contain the same amount. In the epidermis, for example, occurs phytochrome nearly exclusively within the guard cells.

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Phytochrome has a part in the induction of chloroplast rotation within the thread-like green alga Mougeotia. It is distinguished between the weak light and strong light position of the laminiform chloroplast (epistrophe, oblique position). The chloroplast movement is an intracellular movement that varies from cell to cell, since each cell has its own light perception, which is not shared with other cells. The phytochrome system with its adaptation to long-waved light has no advantages for the alga. Algae and other organisms seem to have four physically different concepts for the light perception during chloroplast movements.

13.6 Photo morphogenesis and Molecular Mechanism of Receptors

Plants can sense light direction, quality (wavelength), intensity and periodicity. Light induces phototropism, photomorphogenesis, chloroplast differentiation and various other responses such as flowering and germination.

Light quality is mainly sensed by the presence of different light receptors specific for different wavelengths. The red/far red photoreceptors are called phytochrome. There are at least 2 classes of blue light receptors; cryptochrome recognizes blue, green and UV-A light, while phototropin perceives blue light. Plants exhibit different growth habits in dark and light. In the dark they have elongated stems, undifferentiated chloroplasts and unexpanded leaves. This is called skotomorphogenesis. Photomorphogenesis (light grown) involves the inhibition of stem elongation, the differentiation of chloroplasts and accumulation of chlorophyll, and the expansion of leaves. Thus the same stimulus causes opposite effects on cell elongation in leaves and stems. Photomorphogenesis can be induced by red, far red and blue light. Light perception and signaling has come from genetic analyses of photomorphogenesis. Essentially two types of mutant screens have been performed:

1. Screens for mutants grown in dark even in the light i.e. Insensitive or unresponsive to light. These are often designated as hy mutants for hypocotyl elongated a dark grown character. These mutants have identified

the known light receptors and a couple other genes that function as positive regulators of the light responses.

2. Screens for mutants that look light grown even in the dark. These are designated as cop for constitutive photomorphogenic or det for de-etiolated .These recessive mutants are epistatic to hy mutants indicating that they function as negative regulators of signal transduction steps downstream of the receptors. Besides, loss of function mutations allows photomorphogenic development in the absence of the inducing signal (light), the normal function of the DET and COP genes is to repress photomorphogenesis in the dark.

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The mode of action of the photoreceptors remains unclear, but several recent pieces of evidence have begun to shed light on it. Phytochrome and cryptochrome molecules linked to fluorescent protein tags have been used to study the subcellular localization of these molecules in living cells (Both the phytochromes and the cryptochromes are localized in the cell nucleus; the phytochromes display a light-dependent nuclear localization whereas cryptochromes are constitutively nuclear . In addition, several molecules interacting with phyA and phyB have recently been identified by yeast two-hybrid screening. Phytochrome-interacting factor (PIF3; a basic helix-loop-helix transcription factor) phytochrome kinase substrate and nucleoside diphosphate kinase have all been identified as binding to phytochrome.

Interestingly, both cry1 and cry2 interact with phyA in vitro. Furthermore, in vivo evidence suggests that cry1 is phosphorylated in response to red light. The significance of this interaction has proved elusive, although missense mutations in cry1 have a slight, dominant negative effect on phyA signaling. Nonetheless, no decrease in phytochrome signaling has been demonstrated in a cry1 null mutant.

13.6.1 Phytochrome and Photomorphogenesis

The sensitivity of phytochromes to red and far-red light makes this class of photoreceptor ideally suited for the sensing of shade in the terrestrial environment. Chlorophyllous leaves absorb most of the visible light spectrum, but are transparent in the far-red. Far-red light enriched shade light shifts the equilibrium between the Pr and Pfr forms significantly towards Pr. The

inactivation leads to the inhibition of seed germination in plants that normally thrive only in high light. When mature plants respond to shade, the loss of the Pfr signal, which inhibits stem elongation and stimulates leaf development, leads to increased height and limited allocation of resources to leaves until the shade condition has been overcome.

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The interconvert ability of the two forms of phytochrome is an important feature for sensing light intensity as well. In any natural light environment there will be photons of wavelengths that can be absorbed by each of the two forms of phytochrome. This results in individual molecules of phytochrome cycling between the two forms. It has been found that the cycling rate can be influenced by light intensity, and that cycling rate is converted to a signal regulating developmental adaptations to different light intensities.

13.6.2 Cryptochromes and Photomorphogenesis

Cryptochromes is a Blue Light Receptors. Cryptochromes share sequence similarity to the DNA repair enzyme photolyase but have no DNA repair activity. Cryptochromes and DNA photolyases share similarities not only in amino acid sequences but also in chromophore composition and in the light-dependent nature of their respective biochemical activities. Cryptochromes appear to be evolutionarily derived from gene duplication events of ancestral photolyase genes, because many organisms, including Arabidopsis and Drosophila, are known to have both cryptochromes and photolyases, functioning as photoreceptors and DNA repair enzymes, respectively.

Most plant cryptochromes are 70- to 80-kD proteins with two recognizable domains, an amino terminal PHR (for photolyase-related) domain that shares sequence homology with photolyases and a carboxyl terminal domain that has no strong sequence similarity to known protein .Photolyase contains two chromophores, a light-harvesting chromophore, which is either a folate (methenyltetrahydrofolate) or a deazaflavin, and a catalytic chromophore that is flavin adenine dinucleotide (FAD). Almost all residues known to be important for chromophore binding in photolyase are conserved in cryptochromes, whereas residues of photolyases that are critical for the binding of DNA lesions and the catalysis of DNA repair are not equally conserved in cryptochromes.

None of the blue light regulated responses exhibit the photo reversibility seen in the phytochrome system. The cryptochromes were the first blue light sensors characterized, and their discovery was based on the screening of mutant Arabidopsis plants for defects in photomorphogenesis in response to blue light. When the DNA sequence for cryptochrome 1 was obtained, it was found that there was significant homology to another class of light absorbing protein, the DNA photolyases involved in DNA repair. Both cryptochromes and photolyases have the same pair of chromophores, a pterin/deazaflavin and flavine adenine dinucleotide, but the chemistry that occurs after light absorption is quite different. The absorption spectrum of cryptochrome corresponds to the action spectrum of one class of blue light mediated photo morphogenic responses.

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13.6.3 Phototropins and Photomorphogenesis

It is also a Blue Light Photoreceptors. Like the cryptochromes, the phototropins were discovered by screening for non-responsive mutants, this time for differential growth towards a unidirectional light source. One of the genes that, when mutated, conferred a non-phototropic phenotype has been identified as the light sensor phototropin. The protein product of this gene has two flavin mononucleotide chromophores. The absorption spectrum for phototropin is consistent with the action spectrum for phototropism, and the molecule has been shown to be differentially activated in parallel with the light gradient created across a stem irradiated from one side. Phototropin 1 was identified as the sensor for phototropism, a second phototropin was found, and it appears to regulate phototropic responses to high light intensities and chloroplast movements. Phototropin 2 mediates both the accumulation of chloroplasts to optimize light absorption under low light intensities, and movements to minimize light interception under high light intensities. Like the phytochrome and cryptochrome systems, it turned out that there is redundancy between the two phototropins, and phototropin 1 will replace phototropin 2 in the chloroplast accumulation response, but not the chloroplast avoidance response.

13.7 Summary

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Light plays a major signalling role in plant development. A plant's ability to maximize its photosynthetic productivity depends on its capacity to sense, evaluate, and respond to light quality, quantity, and direction. Similarly, the timing of developmental phenomena, such as flowering or entrance into dormancy, depends on a system of measuring and responding to changes in day length.

A red, far-red-reversible chromoprotein, phytochrome, was the first photoreceptor identified. It is now known that multiple phytochromes exist and sometimes act independently of one another, sometimes redundantly, sometimes antagonistically, sometimes at the same time in development, and sometimes at different times. The first blue-light receptors to be identified were the two cryptochromes, chromoproteins that mediate several responses. More recently, another blue-light-absorbing chromoprotein, phototropin, has been identified as a photoreceptor mediating phototropism. A chimeric photoreceptor, phytochrome 3 (phy3), has been identified that contains both phytochrome and phototropin sequence motifs. For each of these photoreceptors, gene sequences are known, and plant biologists are working toward a greater understanding of their roles in plant development.

13.8 Glossary

- **Photobiology :** the study of the interactions of light (non-ionizing radiation) and living organisms.
- **Photophysiology :** the ability of plants to sense the environment and adjust their morphology, physiology and phenotype accordingly
- **Photomorphogenesis :** Effects of light on plant development and cellular metabolism
- **Phytochromes :** A pigment that controls most photomorphogenic responses in higher plants
- **Pr :** red light-absorbing form
- **Pfr :** far red light-absorbing form

- **Cryptochromes** : Cryptochromes are a class of flavoproteins that are sensitive to blue light
- **Photo stationary state :** absorption spectra of Pr and Pfr forms overlap, resulting in a equilibrium between the two forms.
- **Phototropins** : It is a Blue Light Photoreceptors

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13.9 Self Learning exercise

Section-A (Very Short Answer Type Questions)

1. Where are phytochromes produced?
2. Name two morphogenetic processes which are under the control of phytochrome?
3. Name one blue light photoreceptors.
4. Define photomorphogenesis.

Section-B (Short Answer Type Questions)

1. Briefly describe Phytochrome.
2. What is the difference between florigen and phytochrome?
3. What are the two forms of phytochrome and how are they switched?

Section-C (Long Answer Type Questions)

1. What do you mean by Photomorhogenesis? Describe the molecular mechanism of receptors.
2. What are photoreceptors? Briefly describe the discovery, structure, photochemical and biochemical properties of phytochrome.
3. Write an explanatory note on Photophysiology and cellular localization.

Answer key of Section –A

1. Leaves 2. Germination and flowering
3 Phototropin 4 Light driven plant growth and development.

13.10 References

NOTES

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Unit - 14

Secondary Metabolites-Alkaloids and Steroids

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Structure of the Unit

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14.0 Objective

After going through this unit you will be able to understand:-

- About the Secondary metabolites
- Different Types of Secondary metabolites
- About the alkaloids and their History, Distribution and nomenclature
- Alkaloids Classification , Chemical properties and biological significance
- Steroids- Definition, History nomenclature, biosynthesis. and biological role

14.1 Introduction

Plants are used for variety of purposes including perfumes, cosmetics, pharmaceuticals, agrochemicals, food additives etc. Metabolites are the intermediates and products of metabolism and are restricted to small molecules. They have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity defense, and interactions with other organisms (e.g. pigments, odorants, and pheromones). Plants synthesize a vast range of organic compounds that are classified as primary and secondary metabolites. Primary metabolite have essential roles associated with photosynthesis, respiration and is directly involved in normal "growth", development, and reproduction. Whereas, secondary metabolite is not directly involved in those processes, but usually has an important ecological function. The metabolite forms a large network of metabolic reactions, where outputs from one enzymatic chemical reaction are inputs to other chemical reactions.

14.2 Plant Secondary Metabolism

Secondary metabolites are compounds which are biosynthetically derived from primary metabolites and present in plant kingdom. Primary metabolites include energy rich fuel molecules, such as sucrose and starch, structural components such as cellulose, informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments, such as chlorophyll. In addition to having fundamental roles in plant growth and development, some primary metabolites are precursors (starting materials) for the synthesis of

secondary metabolites. Secondary metabolites largely fall into three classes of compounds: alkaloids, terpenoids, and phenolic. However, these classes of compounds also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom. Plant secondary metabolites aid in the growth and development of plants but are not required for the plant to survive. They facilitate the primary metabolism in plants. Primary metabolism consists of chemical reactions that allow the plant to live. Whereas, secondary metabolism plays a pinnacle role in keeping all the of plants systems working properly. The main role of secondary metabolites in plants is defence mechanisms. They are used to fight off herbivores, pests, and pathogens. Besides, secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defence systems.

Role of Secondary metabolism in plants-

1. In Defense - herbivores, microbes, competing plants.
2. Attraction- pollinating insects, seed dispersing animals, root nodule bacteria.
3. In UV protection.

On the basis of biosynthetic origins, plant secondary metabolites can be divided into three major groups:

1. Flavonoids and allied phenolic and polyphenolic compounds,
2. Terpenoids and
3. Nitrogen-containing alkaloids and sulphur-containing compounds.

14.3 Alkaloids

14.3.1 Introduction

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms and found in 20% species of flowering plants. This group also includes some related compounds with neutral and even weakly acidic properties. In addition to nitrogen, they have carbon, hydrogen, oxygen, sulphur and traces of other elements such as chlorine, bromine, and

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phosphorus. They are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products. Although alkaloids are toxic to other organisms, however they have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples., cocaine (local anesthetic and stimulant), psilocin (psychedelic) caffine, nicotine (stimulant,), morphine (the analgesic) ,berberine (antibacterial), vincristine (the anticancer compound), reserpine (the antihypertension agent), galantamine (the cholinomimeric), atropine (the spasmolysis agent), vincamine (the vasodilator), quinidine (the antiarrhythmia compound), ephedrine(the antiasthma) and quinine is the therapeutic, and the antimalarial drug .

14.3.2 Nomenclature

The nomenclature of alkaloids is not very exact but those with heterocyclic rings are called true alkaloids and those without such rings are protoalkaloids .They are derived from amino acids and other multicarbon units e.g. acetate. Alkaloids which are not derived from amino acids are called as pseudo alkaloids. The term "alkaloids" (German: Alkaloide) was introduced in 1819 by the German Scientist Carl Friedrich Wilhelm Meißner, and is derived from late Latin root Latin: alkali (which, in turn, comes from the Arabic al-qalwī – "ashes of plants" .

There is no unique crieteria of alkaloids nomenclature. Many individual names are formed by adding the suffix "ine" to the species or genus name. For example, atropine is isolated from the plant *Atropa belladonna*, strychnine is procured from the seed of Strychnine tree (*Strychnos nux-vomica* L.). When many alkaloids are extracted from single plant then their names often contain suffixes "idine", "anine", "aline", "inine", etc. There are also almost 86 alkaloids containing the root "vin" (extracted from the Vinca plant) like vincristin ,vinblastin etc. Some criteria of alkaloid nomenclature are:

1. According to Plant source- papaverine.
2. According to Physiological action- morphine,narcotine.
3. According to Name of scientist who discovered- palatrine is by P.J.Pelletior.
4. Sometimes Greek words are also used in nomenclature.

14.3.3 History

Alkaloids are mostly present in plants and are mainly found in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species. Many plant families are particularly rich in alkaloids; all plants of the poppy family (Papaveraceae) are thought to contain them, for example. The Ranunculaceae (buttercups), Apocynaceae (*Cataranthus roseus*), Solanaceae (nightshades), and Amaryllidaceae (amaryllis) are other prominent alkaloid-containing families. A few alkaloids have been found in animal species, such as the New World beaver (*Castor canadensis*) and poison-dart frogs (Phyllobates). Ergot and a few other fungi also produce them.

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A significant contribution to the chemistry of alkaloids in the early years of its development was made by the French researchers Pierre Joseph Pelletier and Joseph Bienaimé Caventou, who discovered quinine (1820) and strychnine (1818). At the same time, other alkaloids were discovered around that time, including xanthine (1817), atropine (1819), caffeine (1820), coniine (1827), nicotine (1828), colchicine (1833), sparteine (1851), and cocaine (1860).

The first complete synthesis of an alkaloid was achieved in 1886 by the German chemist Albert Ladenburg. He produced coniine by reacting 2-methylpyridine with acetaldehyde and reducing the resulting 2-propenyl pyridine with sodium. The development of the chemistry of alkaloids was accelerated by the emergence of spectroscopic and chromatographic methods in the 20th century, recently more than 13,000 alkaloids had been identified, approximately.

14.3.4 Classification of Alkaloid

Alkaloid, are naturally occurring organic nitrogen-containing bases. Alkaloids are characterized by a great structural diversity and there is no unique classification of alkaloids.. Alkaloids are often classified on the basis of their chemical structure. For example, those alkaloids that contain a ring system called indole are known as indole alkaloids. On this basis of chemical structure, the principal classes of alkaloids are the pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinoline's, indoles, quinolines, and the terpenoids and steroids. Alternatively, alkaloids can be classified according to the biological

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system in which they occur. For example, the opium alkaloids occur in the opium poppy (*Papaver somniferum*). Hegnauer (1963) conveniently classified alkaloids into six important groups, corresponding to the six amino-acids legitimately considered as the starting points for their biosynthesis, such as: anthranilic acid, histidine, lysine, ornithine phenylalanine and tryptophan. Price* (1963) further took a leading clue from the earlier observation and considered in details the alkaloids present in one of the families, (Rutaceae) and logically placed them in the following nine chemical-structural categories, namely: acridines, amides, amines, benzylisoquinolines, canthinones, imidazoles, indolquinazolines, furoquinolines, and quinazolines

Moreover, recent classifications are based on similarity of the carbon skeleton (e.g., indole-, isoquinoline-, and pyridine-like) or biogenetic precursor (ornithine, lysine, tyrosine, tryptophan, etc.).

Alkaloids are also divided into the following major groups:

1. "True alkaloids", which contain nitrogen in the heterocycle and originate from amino acids. Examples are atropine, nicotine, and morphine. This group also includes some alkaloids that besides nitrogen heterocyclic contain terpene (e.g.,evonine) or peptide fragments (e.g. ergotamine). This group also includes piperidine alkaloids coniine and coniceine although they do not originate from amino acids.
2. "Protoalkaloids", which contain nitrogen and also originate from amino acids. Examples include mescaline, adrenaline and ephedrine.
3. Polyamine alkaloids – derivatives of putrescine, spermidine, and spermine.
4. Peptide and cyclopeptide alkaloids.
5. Pseud alkaloids – alkaloid-like compounds that do not originate from amino acids. This group includes, terpene-like and steroid-like alkaloids, as well as purine-like alkaloids such as caffeine, theobromine, theacrine and theophylline.

Another classification of alkaloids is in the following four heads, namely:

(a) **Biosynthetic Classification**

In this particular instance the significance solely lies to the precursor from which the alkaloids are produced in the plant biosynthetically. Therefore, it is

quite convenient and also logical to group together all alkaloids having been derived from the same precursor but possessing different taxonomic distribution and pharmacological activities.

Examples (i) Indole alkaloids derived from tryptophan.
(ii) Piperidine alkaloids derived from lysine.

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(iii) Pyrrolidine alkaloids derived from ornithine.

(iv) Phenylethylamine alkaloids derived from tyrosine.
(v) Imidazole alkaloids derived from histidine.

(b) Chemical Classification

It is the most widely accepted and common mode of classification of alkaloids for which the main criterion is the presence of the basic heterocyclic nucleus (i.e., the chemical entity).

Examples

(i) Pyrrolidine alkaloids e.g., Hygrine;
(ii) Piperidine alkaloids e.g., Lobeline;
(iii) Pyrrolizidine alkaloids e.g., Senecionine;
(iv) Tropane alkaloids e.g., Atropine;
(v) Quinoline alkaloids e.g., Quinine;
(vi) Isoquinoline alkaloids e.g., Morphine;
(vii) Aporphine alkaloids e.g., Boldine;
(viii) Indole alkaloids e.g., Ergometrine;
(ix) Imidazole alkaloids e.g., Pilocarpine;
(x) Diazocin alkaloids e.g., Lupanine;
(xi) Purine alkaloids e.g., Caffeine;
(xii) Steroidal alkaloids e.g., Solanidine;
(xiii) Amino alkaloids e.g., Ephedrine;
(xiv) Diterpene alkaloids e.g., Aconitine.

(c) Pharmacological Classification

In this type, the alkaloids exhibit a broad range of very specific pharmacological characteristics. Perhaps this might also be used as a strong basis for the general classification of the wide-spectrum of alkaloids derived from the plant kingdom, such as: analgesics, cardio-vascular drugs, CNS-stimulants and depressants, dilation of pupil of eye, mydriatics,

anticholinergics, sympathomimetics, antimalarials, purgatives, and the like. However, such a classification is not quite common and broadly known.

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Examples

(i) Morphine as Narcotic analgesic;
(ii) Quinine as Antimalarial;
(iii) Strychnine as Reflex excitability;
(iv) Lobeline as Respiratory stimulant;
(v) Boldine as Choleretics and laxatives;
(vi) Aconitine as Neuralgia;
(vii) Pilocarpine as Antiglaucoma agent and miotic;
(viii) Ergonovine as Oxytocic;
(ix) Ephedrine as Bronchodilator;
(x) Narceine as Analgesic (narcotic) and antitussive.

(d) **Taxonomic Classification**

This classification is mainly deals with the 'Taxon' i.e., the taxonomic category. The most common taxa are the genus, subgenus, species, subspecies, and variety. Therefore, the taxonomic classification encompasses the plethora of alkaloids exclusively based on their respective distribution in a variety of Plant Families, sometimes also referred to as the 'Natural order'. A few typical examples of plant families and the various species associated with them are stated below, namely:

(i) Cannabinaceous Alkaloids: e.g., Cannabis sativa Linn., (Hemp, Marijuana).
(ii) Rubiaceous Alkaloids: e.g., Cinchona Sp. (Quinine); Mitragyna speciosa Korth (Katum, Kratum, Kutum); Pausinystalia johimbe (K. Schum) (Yohimbe).

14.3.5 Chemical Structure and Properties

Chemically, alkaloids are alkaline or basic in nature and extremely variable heterocyclic compounds. Its Ph value found in cytosol is 7.2 and in vacuole 5-6. Generally, an alkaloid contains at least one nitrogen atom in an amine-type structure—i.e., one derived from ammonia by replacing hydrogen atoms with hydrogen-carbon groups called hydrocarbons. This or another nitrogen atom can be active as a base in acid-base reactions. The name alkaloid ("alkali-like") was originally applied to the substances because, like the inorganic alkalis, they react with acids to form salts. Most alkaloids have one or more of their nitrogen

atoms as part of a ring of atoms, frequently called a cyclic system. Alkaloid names generally end in the suffix -ine, a reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste, and used in pharmacy. They are active in small concentration.

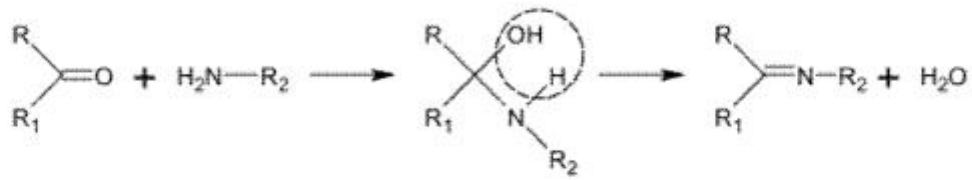
Many alkaloids contain oxygen in their molecular structure; those compounds are usually colorless crystals at ambient conditions. Oxygen-free alkaloids, such as nicotine or coniine, are typically volatile, colorless, oily liquids. Some alkaloids are coloured, like berberine (yellow) and sanguinarine (orange). Several alkaloids are weak bases, but some, such as theobromine and theophylline, are amphoteric. Many alkaloids dissolve poorly in water but readily dissolve in organic solvents, such as diethyl ether, chloroform or 1,2-dichloroethane. Caffeine cocaine, codeine and nicotine are water soluble, whereas others, including morphine and yohimbine are highly water soluble. Alkaloids and acids form salts of various strengths that are usually soluble in water and alcohol and poorly soluble in most organic solvents. Exceptions include scopolamine hydrobromide, which is soluble in organic solvents, and the water-soluble quinine sulphate. Some alkaloids have a bitter taste or are poisonous when ingested.

14.3.6 Biosynthesis

Biological precursors of most alkaloids are amino acids, such as ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, aspartic acid, and anthranilic acid. Nicotinic acid can be synthesized from tryptophan or aspartic acid. Although there are so many ways of alkaloid biosynthesis, however there are some typical reactions involved in the biosynthesis of of alkaloids, including synthesis of Schiff bases and Mannich reaction.

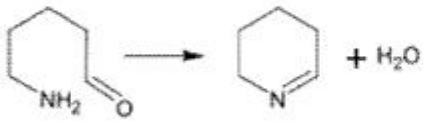
Synthesis of Schiff bases

Schiff bases can be obtained by reacting amines with ketones or aldehydes. These reactions are a common method of producing C=N bonds.



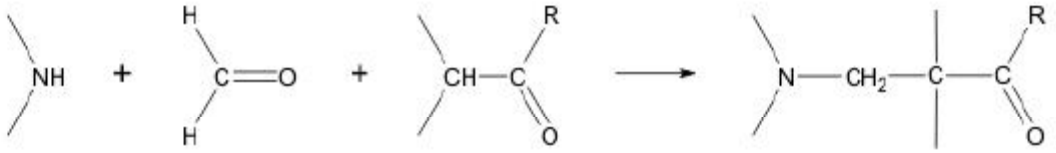
NOTES

In the biosynthesis of alkaloids, such reactions may take place within a molecule such as in the synthesis of piperidine:

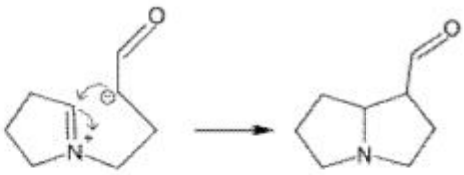


Mannich reaction

An integral component of the Mannich reaction, in addition to an amine and a carbonyl compound, is a carbanion, which plays the role of the nucleophile in the nucleophilic addition to the ion formed by the reaction of the amine and the carbonyl.



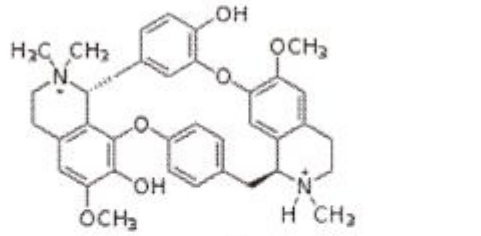
The Mannich reaction can proceed both intermolecularly and intramolecularly:



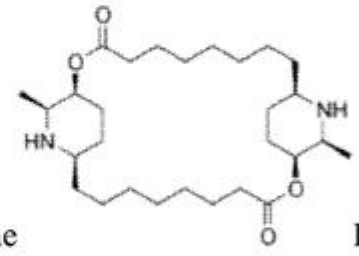
Dimer alkaloids

Moreover, above described monomeric alkaloids, there are also dimeric, and even trimeric and tetrameric alkaloids formed upon condensation of two, three, and four monomeric alkaloids. Dimeric alkaloids are usually formed from monomers of the same type through the following mechanisms:

- Mannich reaction, resulting in, e.g., voacamine
- Michael reaction (villalstonine)
- Condensation of aldehydes with amines (toxiferine)
- Oxidative addition of phenols (dauricine, tubocurarine)
- Lactonization (carpaine).



Toxiferine



Dauricine

14.3.7 The Biological role

Alkaloids are multifunctional in nature and have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. The function of alkaloids in plants is not yet understood. They are known to be simply waste products of plants' metabolic processes and serves specific biological functions. In some plants, the concentration of alkaloids increases prior to seed formation and then drops off when the seed is ripe. Alkaloids may also protect some plants from destruction by certain insect species. Although it was assumed that the alkaloids are the final products of nitrogen metabolism in plants, as urea in mammals however, later shown that alkaloid concentration varies over time.

(i) Biological significance-

1. Protect plants against fungi, insects and animals.
2. Excretory product of plants.
3. Some alkaloids are reservoir of N and help in protein synthesis.
4. Behaves as growth regulator hormones.
5. Helpful in neutrilize the harmful effect of acids in plants.
6. Also protect plants from plant diseases.
7. Colchicine is used in multiplication of chromosomes.
8. They inhibit enzyme activity and seed germination. Some remove bad effect of tannins.

(ii) Medicinal significance-

NOTES

The medicinal properties of alkaloids are very diverse. Morphine is a powerful narcotic used for the relief of pain, though its addictive properties limit its usefulness. Codeine, the methyl ether derivative of morphine found in the opium poppy, is an excellent analgesic that is relatively nonaddictive. Certain alkaloids act as cardiac or respiratory stimulants. Quinidine, which is obtained from plants of the genus Cinchona, is used to treat arrhythmias, or irregular rhythms of the heartbeat. Many alkaloids affect respiration, but in a complicated manner such that severe respiratory depression may follow stimulation. The drug lobeline (*Lobelia inflata*) is safer in this respect and is therefore clinically useful. Ergonovine (*Claviceps purpurea*) and ephedrine (Ephedra species) act as blood-vessel constrictors. Ergonovine is used to reduce uterine hemorrhage after childbirth, and ephedrine is used to relieve the discomfort of common colds, sinusitis, hay fever, and bronchial asthma.

Table – 1 : Medicinal role of Alkaloids

S.No.	Alkaloid	Role
1.	Ajmaline	antiarrhythmic
2.	Atropine, scopolamine, hyoscyamine	anticholinergic
3.	Caffeine	Stimulant, Adenosine receptor antagonist
4.	Codeine	cough medicine, analgesic
5.	Colchicine	remedy for gout
6.	Emetine	antiprotozoal agent
7.	Ergot alkaloids	sympathomimetic,vasodilator, antihypertensive
8.	Morphine	analgesic
9.	Nicotine	Stimulant, Nicotinic acetylcholine

		receptor agonist
10.	Physostigmine	inhibitor of acetylcholinesterase
11.	Quinidine	Antiarrhythmic
12.	Quinine	antipyretics, antimalarial
13.	Reserpine	antihypertensive
14.	Tubocurarine	Muscle relaxant
15.	Vinblastine, vincristine	antitumor
16.	Vincamine	vasodilating, antihypertensive

NOTES

Many alkaloids possess local anesthetic properties, though clinically they are seldom used for this purpose. Cocaine (*Erythroxylon coca*) is a very potent local anesthetic. Quinine (Cinchona species) is a powerful antimalarial agent that was formerly the drug of choice for treating that disease, though it has been largely replaced by less toxic and more effective synthetic drugs. The alkaloid tubocurarine is the active ingredient in the South American arrow poison, curare (*Chondrodendron tomentosum*), and is used as a muscle relaxant in surgery. Two alkaloids, vincristine and vinblastine (*Vinca rosea*), are widely used as chemotherapeutic agents in the treatment of many types of cancer.

Nicotine obtained from the tobacco plant (*Nicotiana tabacum*) is the principal alkaloid and chief addictive ingredient of the tobacco smoked in cigarettes, cigars, and pipes. Some alkaloids are illicit drugs and poisons. These include the hallucinogenic drugs mescaline (from *Anhalonium* species) and psilocybin (Psilocybe mexicana). Synthetic derivatives of the alkaloids morphine and lysergic acid (*C. purpurea*) produce heroin and LSD, respectively. The alkaloid coniine is the active component of the poison hemlock (*Conium maculatum*). Strychnine (*Strychnos* species) is another powerful pois.

In Opium - Latex contains the alkaloids morphine and related alkaloids such as codeine Alkaloids of Opium Poppy (Papaver)Maturing capsule. The piperidine alkaloid coniine (the first alkaloid to be synthesized)-Coniine is extremely

toxic, causing paralysis of motor nerve endings-"The death of Socrates"-the philosopher Socrates drank and extract of coniine-containing hemlock (339 B.C.) Sanguinarine-Antibacterial showing antiplaque activity, used in toothpastes and oral rinses.

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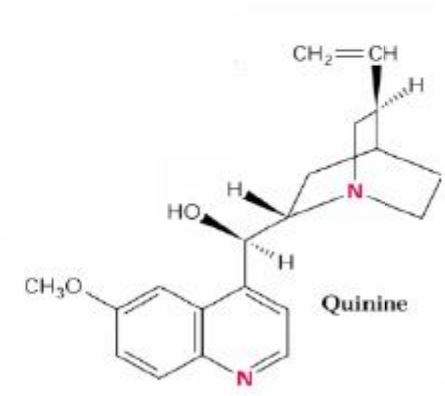
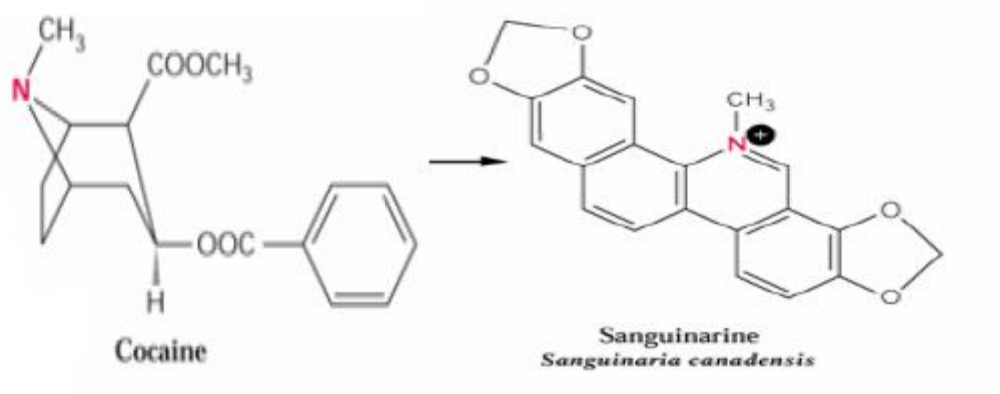
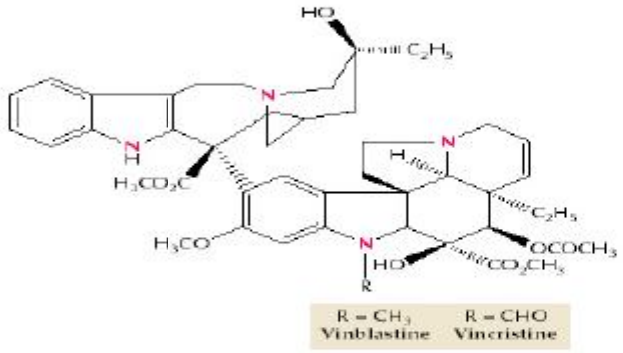




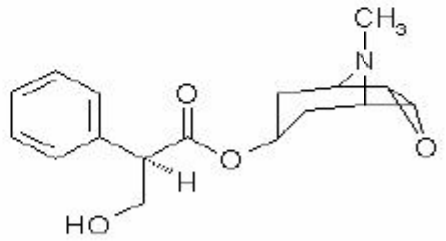
Madagascar periwinkle (*Catharanthus roseus*/ vinka) Terpenoid Indole Alkaloids



Vinblastine and Vincristine are the compounds commonly used for cancer therapy .Vinblastine is a component of chemotherapy for metastatic testicular cancer, Hodgkin's disease and other lymphomas Vincristine is the preferred treatment for acute leukaemia in children Both drugs expensive, Catharanthus the only source (low levels)

NOTES

Tropane Alkaloids- -Datura, a rich source of scopolamine and hyoscyamine used as a sedative Scopolamine can cause death in infants.



Purine Alkaloids

Caffeine the most important example is, coffee, tea, mate', cacao, camellia

Purine alkaloid biosynthesis starts with xanthosine, a nucleotide degradation production.

(iii) Use in agriculture

In addition to the development of a wide range of relatively low-toxic synthetic pesticides, some alkaloids, such as salts of nicotine and anabasine, were used as insecticides. Their use was limited by their high toxicity to humans. At the same time Azadirachtin isolated from neem tree is also used as biopesticides.

(iv) Use as psychoactive drugs

Alkaloids and their extracts, and later pure alkaloids, have been used as psychoactive substances. Cocaine, caffeine, and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as psilocybin, dimethyl tryptamine and ibogaine) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers. However there are alkaloids that do not have strong psychoactive effect themselves, but are precursors for semi-synthetic psychoactive drugs. Example, ephedrine and pseudoephedrine are used to produce methcathinone and methamphetamine. At the same time Thebaine is used in the synthesis of many painkillers such as Oxychodone.

NOTES

14.4 Steroid

14.4.1 Introduction

Steroids are the group of organic compounds belonging to the general class of biochemicals called lipids, which are easily soluble in organic solvents and slightly soluble in water. Additional members of the lipid class include fatty acids, phospholipids, and triacylglycerides. The unique structural characteristic of steroids is a four-fused ring system. Members of the steroid family are ubiquitous, occurring, for example, in plants, yeast, protozoa, and higher forms of life. Steroids exhibit a variety of biological functions, such as participation in cell membrane structure, regulation of physiological events. A member of a group of compounds, occurring in plants and animals, that are considered to be derivatives of a fused, reduced ring system, cyclopenta[α]-phenanthrene, which consists of three fused cyclohexane rings in a nonlinear or phenanthrene arrangement. Any of a large group of fat-soluble organic compounds containing a characteristic chemical ring system. The majority, including the sterols, bile acids, many hormones, and the D vitamins, have important physiological action. Naturally occurring steroids have an oxygen-containing group at carbon-3. Shorthand formulas for steroids indicate the presence of double bonds, as well as the structure and position of oxygen-containing or other organic groups.

14.4.2 Nomenclature

"Steroids are compounds possessing the skeleton of cyclo penta phenanthrene or a skeleton derived there from by one or more bond scissions or ring expansions or contractions. Methyl groups are normally present at C-10 and C-13. An alkyl side chain may also be present at C-17.Sterols are steroids carrying a hydroxyl group at C-3 and most of the skeleton of cholestane. Additional carbon atoms may be present in the side chain."

Nomenclature for steroid skeleton is on the right.

- By removing carbon 242, campesterol is obtained.
- By removing carbons 241 and 242, cholesterol is obtained.

- Removing a hydrogen from carbons 22 and 23 yields stigmasterol (stigmasta-5,22-dien-3β-ol).
- By hydrogenating the double bond between carbons 5 and 6, β-sitostanol is obtained.
- By hydrogenating the double bond between carbons 5 and 6 and removing carbon 241, campestanol is obtained.
- Removing carbon 242 and hydrogens from carbons 22 and 23, and inverting the stereochemistry at C-24 yields brassicasterol (ergosta-5,22-dien-3β-ol).
- Further removal of hydrogens from carbons 7 and 8 from brassicasterol yields ergosterol (ergosta-5,7,22-trien-3β-ol). Important: Ergosterol is not a plant sterol. Ergosterol is a component of fungal cell membranes, serving the same function in fungi that cholesterol serves in animal cells. Esterification of the hydroxyl group at carbon 3 with fatty/organic acids or carbohydrates results in plant sterol esters, i.e. oleates, ferulates and (acyl) glycosides.

NOTES

Gonane, is the simplest possible steroid and is composed of seventeen carbon atoms in carbon-carbon bonds that form four fused rings in a defined three-dimensional shape. The three cyclohexane rings (designated as rings A, B, and C in the gonane and cholesterol figures above right) form the skeleton of a perhydro- derivative of phenanthrene. The D-ring has a cyclopentane structure; hence, though it is uncommon, per IUPAC steroids can also be named as various hydro-derivatives of cyclopenta[a]phenanthrene. When the two methyl groups and 8-carbon side chain (at C17, as shown for cholesterol) are present, the steroid is said to have a cholestane framework.

14.4.3 History

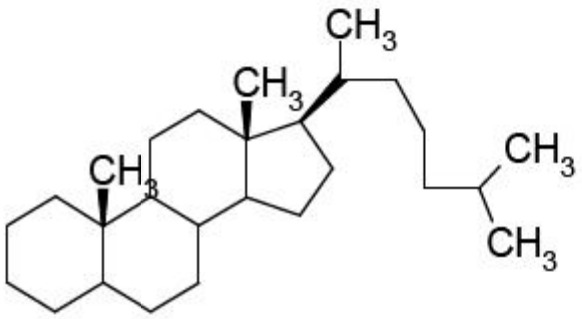
The term "steroids" was coined by Callow RK et al. (Proc Royal Soc London series A 1936, 157, 194) "for the group of compounds comprising the sterols, bile acids, heart poisons, saponins, and sex hormones". As natural steroids are derived from squalene by cyclization, unsaturation and substitution, they may be considered as modified triterpenes. Fatty acid esters of steroids are found mainly in the blood but their exact role is not known to date.

14.4.4 Classification of Steroids

Steroids are classified as:

NOTES

1. Anabolic Steroids- -Interact with androgen receptor; enhance muscle mass/athlete's performance; male sex hormones
2. Glucocorticoids= -regulate metabolism and immune function; anti-inflammatory activity
3. Mineralocorticoids= -maintain blood volume and renal excretion
4. Progestins- -Development of female sex organs and characteristics
5. Phytosteroids-
6. Ergosteroids- -Steroids of the fungi; vitamin D related



Steroid

The classification of steroids is based on chemical structure and on the nature of the physiological effect or function. There are eight groups of steroids.

The first group comprises the sterols, which contain a branched side chain R made up of 8–10 carbon atoms. Sterols are components of plant and animal lipids, and the most important sterol—cholesterol—participates in the biosynthesis of steroid hormones.

The second group—D vitamins—is made up of unsaturated isomers of sterols (with ring B open). These isomers act to regulate calcium metabolism and the formation of the skeleton in vertebrates.

The third group includes the bile alcohols and bile acids, which contain a hydroxyl or carboxylic group in the side chain (consisting of eight or five carbon atoms). These substances aid in the digestion of food in the intestines of vertebrates.

The fourth group is that of the aglycone (genins) of steroid saponins and steroid glycoalkaloids. Typical representatives of this group are diosgenin (II, where X = O) and solasodine (II, where X = NH). Both groups of aglycones are characteristic of plants of the Liliaceae, Scrophulariaceae, and Solanaceae families, and in the form of glycosides, they have surface-active and hemolytic properties.

NOTES

The fifth group comprises steroid alkaloids of other types. Among them are C27 alkaloids with modified steroid skeletons (jerveratrum, ceveratrum), which stimulate the contraction of striated muscle, C21 alkaloids, which possess bactericidal and amebicidal action, and modified C21 alkaloids from the glands of amphibians (samandarine, "toad poisons"), which are cardiotox-ic, as well as highly toxic to the central nervous system.

The sixth group is that of the cardiac genins, containing a side chain in the form of an unsaturated five-membered ring (C23 car-denolides) or six-membered lactone ring (C24 bufodienolides). These substances can strengthen the contraction of cardiac muscles by inhibiting the enzyme ATPase in the membrane of the heart cells. Cardenolides are found in many plants, while bufodienolides are found primarily in the venom from the cutaneous glands of toads.

The seventh group comprises the steroid sex hormones and the products of the hormones' conversions. These substances determine the development and operation of the sexual system in animals. They include progesterone and related C21 compounds, in which the side chain R contains two carbon atoms, as well as the male sex hormones—androgens—which contain 19 carbon atoms, and the female sex hormones—estrogens—which contain 18 carbon atoms. A hydroxyl or carbonylic group replaces the side chain in androgens and estrogens.

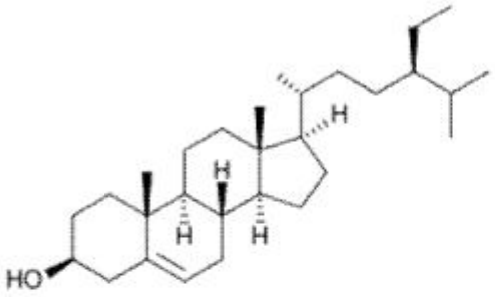
The last group includes the hormones of the adrenal cortex—corticosteroids (where R = COCH2OH)—which regulate the balance of electrolytes and the metabolism of carbohydrates in vertebrates. Certain triterpene antibiotics (fusidic acid, cephalosporin P]) and other triterpenes are similar to steroids.

Sterols are plant fats found in all plant-based foods. Sterols, including cholesterol, are in the same large classification family of steroids but they do

NOTES

not have the negative effects that are often associated with steroids. Sterolins are glucosides, which are molecular structures joined to the sterol. Sterolin is easily destroyed, and without it, the sterol does not have the same immune-enhancing benefits. In nature, plants never contain sterols only. The sterols are always associated with their glucoside sterolin. The original research on sterols and sterolins was based on an extract of the African Hypoxis plant or "African Potato". Its nomenclature derived due to the potato-like appearance of the Hypoxis plant. Due to the presence of other potentially harmful substances contained within the Hypoxis plant, other plants were investigated as sources for the sterols and sterolins used in supplementation.

Phytosterols, which encompass plant sterols and stanols, are steroid compounds similar to cholesterol which occur in plants and vary only in carbon side chains and/or presence or absence of a double bond. Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil, and soluble in alcohols. Phytosterol-enriched foods and dietary supplements have been marketed for decades. Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified. Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil, and soluble in alcohols.



β-Sitosterol

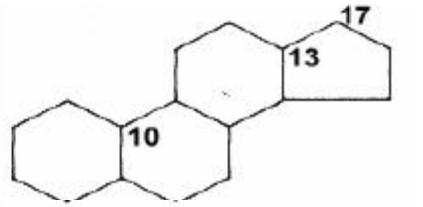
14.4.5 Chemical Structure and Properites

Steroids are the group of organic compounds belonging by virtue of their chemical nature to the isoprenoids. The various types of steroids are widely distributed throughout the biological world and are encountered in micro-organisms, plants, and animals. One of the main directions in the chemical

evolution of steroids has been that toward specialization as biological regulators —hormones and other substances. The core of steroids composed of 17 carbon atom bounded together and form one four fused ring (3 cyclohexane ring ABC and one cyclopentane ring D ring.In a formal sense, all steroids are derivatives of the hypothetical hydrocarbon sterane (I, where R = H); biogenetically, the steroids derive from squalene, which is converted into the immediate polycyclic steroid precursors lanosterol (in animals) or cy-cloartenol (in plants). These precursors contain 30 carbon atoms (C30). Nearly all steroids are crystalline substances that possess optical activity and dissolve more readily in organic solvents than in water.

NOTES

Steroids form an important group of compounds based on the fundamental saturated tetracyclic hydrocarbon : 1,2-cyclopentanoperhydrophenanthrene (sterane or gonane).



Sterane

This nucleus, partially or completely hydrogenated, is generally substituted by methyl groups at C10 and C13. A chemical group (ketone, hydroxyl...) or an alkyl side-chain may also be present at C17. Steroids may possess a nucleus derived from sterane by one or more C-C bond scissions or ring expansions or contractions.

14.4.6 Biosynthesis

In industry, chemical and microbiological methods have been introduced for the partial synthesis of steroid hormones from available raw materials (sterols, bile acids, saponins), and in the 1960's and 1970's methods have been introduced for complete chemical synthesis from the simplest starting materials. The synthesis of "artificial" steroid hormones with specialized physiological effects (contraceptive, anabolic), in particular, fluorine-containing and nitrogen-containing analogs, is acquiring increasing importance

NOTES

Biosynthesis of steroids is initially similar in both plants and animals, except that in plants lanosterol is replaced by the related compound cycloartenol, which contains a three-membered ring (C9, C10, C19) in lieu of the nuclear double bond of lanosterol. The side chains of the phytosterols, such as stigmasterol, and of the sterol ergosterol of yeasts and other fungi contain extra carbon atoms that are incorporated in reactions involving S-adenosylmethionine, which donates methyl groups in numerous biological processes. Although most plant tissues contain only traces of cholesterol, this sterol is the biogenetic precursor of such important plant steroids as the sapogenins, glycosides, and alkaloids. Because pregnane derivatives are intermediates in some of these transformations, plants and animals appear to have important features of steroid metabolism in common.

Steroid biosynthesis is an anabolic metabolic pathway that produces steroids from simple precursors. A unique biosynthetic pathway is followed in animals compared to many other organisms, making the pathway a common target for antibiotics and other anti-infective drugs. In addition, steroid metabolism in humans is the target of cholesterol-lowering drugs such as statins.

Mevalonate pathway

This pathway or HMG-CoA reductase pathway is starts with acetyl-CoA and ends with dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). DMAPP and IPP in turn donate isoprene units, which are assembled and modified to form terpenes and isoprenoids, which are a large class of lipids that include the carotenoids, and form the largest class of plant natural products. Here, the isoprene units are joined together to make squalene and then folded up and formed into a set of rings to make lanosterol.Lanosterol can then be converted into other steroids such as cholesterol and ergosterol.

14.4.7 Biological role

Steroid and their metabolites are used signalling molecules. The chemistry, biochemistry, and physiological activity of steroids are under intense study in light of the great importance of steroids in medicine, veterinary science, and animal husbandry. The most notable examples are the steroid hormones. Steroids along with phospholipids function as components of cell membranes.

Steroids such as cholesterol decrease membrane fluidity. Similar to lipids, steroids represent highly concentrated energy stores. However, steroids are not typically used as sources of energy. In mammals, they are normally metabolized and excreted.

In Seed Dormancy and Germination

NOTES

Sterols have been proposed to have a positive function on seed germination. Regulation of the seed germination rate is key for a high seedling establishment, resulting in weed control and efficient crop production, especially under suboptimal growth conditions.

In Plant Architecture and Biomass

Plant architecture is the three-dimensional organization of the pla Sterols also play a role in vascular tissue development nt. For the aerial part, this includes plant height, branching/tillering pattern, foliar arrangement and morphology, and reproductive organ structure. Plant architecture is a trait of major agronomic importance as it has a strong effect on harvest index and grain yield potential.

In Root Development

Roots are an important determinant of crop productivity given their role in water and nutrient uptake from the soil. They have both positive and negative effects on root growth, depending on the applied concentration: Root growth is stimulated by low concentrations and inhibited by high concentrations of exogenous steroids.

In Flowering Time

Floral induction is a complex developmental process that requires the integration of endogenous signals (including hormones) and environmental cues (especially temperature and photoperiod) to ensure that flowering occurs under appropriate environmental conditions .Plants that flower late tend to have high total seed production as a result of extended vegetative growth phase and source strength. However, delayed flowering in crops is generally undesirable.

In Male and Female Fertility

Seed production in flowering plants relies on the formation of male and female gametophytes in the reproductive organs and is regulated by various external and internal factors. Systematic phenotypic analysis of the male reproductive organs of these mutants has revealed defects in anther development and in pollen number, viability, and release efficiency.

NOTES

In Fiber Production

Another important role of steroids is in fiber production .In cotton plant (*Gossypium herbaceum),* fiber yield and quality are the most important traits. Cotton fibers consist almost exclusively of cellulose grown in a protective capsule (boll) around the seeds to aid their dispersal. Besides being a major constituent of cotton fiber, cellulose is a biopolymer that contributes to cell wall formation during cell elongation and expansion. A crucial role for cellulose synthesis has been established for phytosterols.

In Stress Tolerance

In addition to their impact on many plant growth and developmental processes, steroids play a unique role in biotic and abiotic stress tolerance, including disease resistance and tolerance against drought, salt, heat, cold, hypoxia, pesticides, and heavy metals.

14.5 Summary

Metabolites are organic compounds synthesized by organisms using enzyme-mediated chemical reactions called metabolic pathways. Primary metabolites have functions that are essential to growth and development and are therefore present in all plants. In contrast, secondary metabolites are variously distributed in the plant kingdom, and their functions are specific to the plants in which they are found. Moreover, secondary metabolites are often coloured, fragrant, or flavourful compounds and they typically mediate the interaction of plants with other organisms.

Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the

components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential. Mostly, secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used as medicines. It is only since the late twentieth century that secondary metabolites have been clearly recognized as having important functions in plants. Thus, Metabolites are the intermediates and products of metabolism. The term metabolite is usually restricted to small molecules.

NOTES

14.6. Glossary

- **Metabolites :** the intermediates and products of metabolism
- **Metabolism :** It is the set of life-sustaining chemical transformations within the cells of living organisms.
- **Alkaloids :** a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms
- **True alkaloids :**, which contain nitrogen in the heterocyclic and originate from amino acids.
- **Pseud alkaloids :** alkaloid-like compounds that do not originate from amino acids.
- **Sterols :** sterols of plants are called phytosterols .It include campesterol, sitosterol, and stigmasterol. Ergosterol is a sterol present in the cell membrane of fungi.
- **Zoosterols :** sterols of animals are called zoosterols. The most important zoosterol is cholesterol;
- **Analgesic :** or painkiller, is any member of the group of drugs used to achieve analgesia — relief from pain.

- **Biomass :** is the mass of living biological organisms in a given area or ecosystem at a given time.
- **Stimulants :** are drugs that make you feel more alert

NOTES

14.7 Self-Learning Exercise

Section-A (Very Short Answer Type Questions)

1. What are metabolites?
2. Name one purine and one tropane alkaloids.
3. Who coined the term steroids?
4. Steroids belongs to class _____ .

Section-B (Short Answer Type Questions)

1. Differentiate primary and secondary metabolites.
2. What are the main biological roles of alkaloids?
3. Briefly explain the main classes of steroids.
4. What are sterols?

Section-C (Long Answer Type Questions)

1. What are secondary metabolites? Briefly describe the biosynthetic pathways and role of secondary metabolites in plant growth and development.
2. What are Alkaloids? Describe the classification of alkaloids.
3. Write in detail the biological significance of Steroids.

Answer key of Section –A

1. Metabolites are the product of metabolism.
2. Caffeine and Scopolamine
3. R.K. Callow
4. Lipid

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NOTES

Unit - 15

Stress Physiology

Structure of the Unit

15.0 Objective

After going through this unit you will be able to understand:-

- Stress
- Different types of stress

- Mechanism of Abiotic and Biotic Stress
- HR and SAR, Drought, Temperature (Freezing and heat stress)
- Salinity, Metal stress and Oxidative Stress
- Effect of stress on plants

NOTES

15.1 Introduction

The term Stress means any change in any environmental condition that has an impact on the plant life, affecting its biochemical and physiological response to such changes that ultimately lead to damage or injury. Moreover it can be defined as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival. This includes a wide range of factors which can be divided into two main categories: abiotic or environmental stress factors, and biotic or biological stress factors. Normally, plants do not grow in optimum conditions during their life cycle, but suffer many adverse situations that cause different types of stress, and prevent them from reaching maximum development. Thus, the stressful situations may cause a series of physiological changes in the plant to maintain the plant's vital functions.

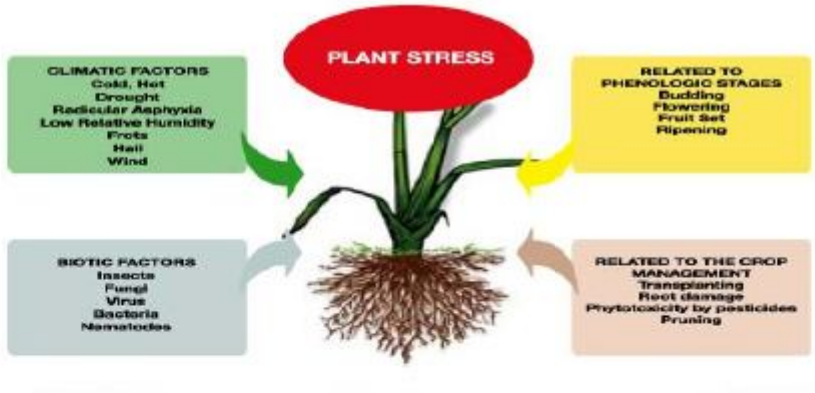

Fig. 15.1- The basic concepts of Plant Stress

Energy is required for all vital activities in organism as well as maintenance of complex order over time. The results in a constant flow of energy through all biological organisms, provides the dynamic driving force for the performance of important maintenance processes such as cellular biosyntheses and transport to maintain its characteristic structure and organization as well as the capacity

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to replicate and grow. The maintenance of steady-state results in a meta-stable condition called homeostasis. Any change in the surrounding environment may disrupt homeostasis. Environmental modulation of homeostasis is defined as biological stress. Thus, it follows that plant stress implies some adverse effect on the physiology of a plant induced upon a sudden transition from some optimal environmental condition where homeostasis is maintained to some suboptimal condition which disrupts this initial homeostatic state. Thus, plant stress is a relative term since the experimental design to assess the impact of a stress always involves the measurement of a physiological phenomenon in a plant species under a suboptimal, stress condition compared to the measurement of the same physiological phenomenon in the same plant species under optimal conditions. The importance of studying plant stresses in combination is the ultimate goal of creating stress-tolerant crops either transgenically or through conventional breeding has pervaded almost all aspects of plant science, and is pursued by both public and private sector researchers.

15.2 Plant Response to Abiotic and Biotic Stress

Plants produce several response to specific multiple stress conditions, Plants have efficient balance resource allocation between growth and defence against stress, as responding to stress can be costly and reduce fitness in terms of growth and yield. Plant stress can be divided into two primary categories. Abiotic stress is a physical (e.g., light, temperature) or chemical effect that the environment may impose on a plant. Biotic stress is a biological effect, (e.g., insects, disease) to which a plant may be exposed during its lifetime. Some plants may be injured by a stress, which means that they exhibit one or more metabolic dysfunctions. If the stress is moderate and short term, the injury may be temporary and the plant may recover when the stress is removed. If the stress is severe enough, it may prevent flowering, seed formation, and induce senescence that leads to plant death (Fig-1). Such plants are considered to be susceptible. Whereas, some plants escape the stress altogether, such as ephemeral, or short-lived, desert plants.

The occurrence of simultaneous biotic and abiotic stresses presents an added degree of complexity, as the responses to these are largely controlled by

different hormone signalling pathways that may interact and inhibit one another .The exposure of plants to a pest or pathogen increases the effects of an abiotic stress such as water, whereas long-term abiotic stress can weaken plant defences and cause enhanced pathogen susceptibility. Some plants could have genes added to them from other species of plants that have a resistance to a specific stress. Plants implanted with these genes would then become transgenic plants because they have the genes from another species of plant in them. Scientists first have to isolate the specific gene in a plant that is responsible for its resistance. The gene would then be taken out of the plant and put in to another plant. The plant that is injected with the new resistant gene would have a resistance to an abiotic stressor and be able to tolerate a wider range of conditions.

Stress perception and plant response occurs via signal transduction pathways that regulate expression of several classes of stress responsive genes. Products of these genes include those that are directly involved in plant protection and those that fulfil regulatory functions. The first group of the gene products includes chaperones, osmotins, anti-freeze proteins, mRNA binding proteins, enzymes involved in Osmolite biosynthesis, water channel proteins, sugar and proline transport proteins, detoxification enzymes and a variety of proteases, as well as a range of antimicrobial, insecticidal and other proteins and peptides. The proteins with regulatory function involve transcription factors and those that are engaged in signal transduction pathways, such as protein kinases and hormone biosynthetic enzymes. Gene function can be enhanced through reverse genetics approaches such as genetic engineering or novel alleles can be sought through germ plasm screening or mutagenesis. The new knowledge acquired through research of abiotic and biotic stress tolerance mechanisms in plants will help in the application of stress responsive determinants and in engineering plants with enhanced tolerance to stress.

15.3 Mechanism of Abiotic and Biotic stress

In Nature plants are exposed to different stress factors in combination. Being sessile, they have developed specific mechanisms that allow them to detect precise environmental changes and respond to complex stress conditions, minimizing damage while conserving valuable resources for growth and

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reproduction. Plants activate a specific and unique stress response when subjected to a combination of multiple stresses. This is particularly true for combinations of biotic and abiotic stresses, the signalling pathways of which can act antagonistically. While valuable, the results of studies investigating stress factors in isolation do not explain the effects of more than one stress on plants. The changing climatic conditions, combined with an increasing pressure on global food productivity due to population increase, result in a demand for stress-tolerant crop varieties that has never been greater. The mechanisms of plant responses to multiple simultaneous stresses are crucial in providing opportunities for the development of broad-spectrum stress-tolerant crops. Plants interact with not only climatic factors (such as irradiation, temperature, and drought) but also soil factors (such as salinity) and biotic factors (such as herbivores and pathogens). All these factors put the plant under interrelated stresses. Moreover, daily sudden changes in the temperature and the presence of heavy metals, toxins, and oxidants due to human activities could result in extra stresses on plants.

Basic stresses such as drought, salinity, temperature, and chemical pollutants are simultaneously acting on the plants causing cell injury and producing secondary stresses such as osmotic and oxidative ones. Plants could not change their sites to avoid such stresses, but have different ways and morphological adaptations to tolerate these stresses. Some of these are, the dominance of sporophyte that embraces the sensitive gametophyte, the presence of epidermis with stomata for gases exchange, the formation of dormant organs, and the presence of conducting tissues for long distant transport. Other ways of defence at the molecular level are very important for the survival and growth of plants. Plants show a series of molecular responses to these stresses.

Abiotic stress factors such as heat, cold, drought, salinity, and nutrient stress have a huge impact on world agriculture, and it has been suggested that they reduce average yields by >50% for most major crop plants . Further to this, plants must defend themselves from attack by a vast range of pests and pathogens, including fungi, bacteria, viruses, nematodes, and herbivorous insects. Each stress elicits a complex cellular and molecular response system implemented by the plant in order to prevent damage and ensure survival, but

often at the detriment of growth and yield. Heat stress as well as other stresses can trigger some mechanisms of defence such as the obvious gene expression that was not expressed under "normal" conditions. In fact, this response to stresses on the molecular level is found in all living things, especially the sudden changes in genotypic expression resulting in an increase in the synthesis of protein groups. These groups are called "heat-shock proteins" (Hsps), "Stress-induced proteins" or "Stress proteins" . Almost all kinds of stresses induce gene expression and synthesis of heat-shock proteins in cells that are subjected to stres.

15.4 Types of Stress

Plant Stresses are divided into two groups- Abiotic and Biotic Stresses. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way. Whereas a biotic stress would include such living disturbances as fungi or harmful insects, abiotic stress factors, or stressors, are naturally occurring, often intangible, factors such as intense sunlight or wind that may cause harm to the plants and animals in the area affected.

15.4.1 Abiotic Stress

Abiotic stresses such as drought, salinity and mineral toxicity have negatively impact growth, development, yield and seed quality of plants. Similarly, large losses of grain yields in plants occur as a result of pathogen attack, in particular during vulnerable stages of grain development and germination. Abiotic stress comes in many forms. The most basic stressors include: High winds ,Extreme temperatures, Drought, Flood Other natural disasters, such as tornadoes and wildfires. Lesser-known stressors generally occur on a smaller scale. They include: poor edaphic conditions like rock content and pH levels, high radiation, compaction, contamination, and other, highly specific conditions like rapid dehydration during seed germination.

Similarly, abiotic stress, is a natural part of ecosystem, and affects organisms in a variety of ways. Although these effects may be either beneficial or

detrimental, the location of the area is crucial in determining the extent of the impact that abiotic stress will have. The higher the latitude of the area affected, the greater the impact of abiotic stress will be on that area. Morever, abiotic stress has enabled species to grow, develop, and evolve, furthering natural selection as it picks out the weakest of a group of organisms. Both plants and animals have evolved mechanisms allowing them to survive extremes. Roots are plant's first line of defence against abiotic stress. If the soil holding the plant is healthy and biologically diverse, the plant will have a higher chance of surviving stressful conditions.

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15.4.2 Biotic Stress

Biotic stress is a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. The damage caused by these living and nonliving agents is very similar. For example, browning of leaves on an oak tree caused by drought stress may appear similar to leaf browning caused by oak wilt, a serious vascular disease, or the browning cause by anthracnose, a fairly minor leaf disease. Similarly, biotic stresses cause damage to plants via living organisms, including fungi, bacteria, insects, and weeds. Viruses, although they are not considered to be living organisms, also cause biotic stress to plants. Fungi cause more diseases in plants than any other biotic stress factor. It has been estimated over 8,000 fungal species are known to cause plant disease. On the other hand, only about 14 bacterial genera cause economically important diseases in plants. They can cause plant wilt, leaf spots, root rot, or seed damage. Insects can cause severe physical damage to plants, including to the leaves, stem, bark, and flowers. Insects can also act as a vector of viruses and bacteria from infected plants to healthy plants. The relationship between biotic stress and plant yield affects economic decisions as well as practical development. The impact of biotic injury on crop yield impacts population dynamics, plant-stressor coevolution, and ecosystem nutrient cycling. Biotic stress also impacts horticultural plant health and natural habitats ecology.

15.5 Hypersensitive response (HR) & Systemic acquired resistance (SAR)

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HR refers to hypersensitive response, is a mechanism used by plants to prevent the spread of infection by microbial pathogens. HR is characterized by the rapid death of cells in the local region surrounding an infection. It also serves to restrict the growth and spread of pathogens to other parts of the plant. Whereas, SAR stands for Systemic Acquired Resistance, a pathogen-induced plant defense mechanism against further pathogen attacks. SAR is analogous to the innate immune system found in animals. Similarly, HR is analogous to the innate immune system found in animals, and commonly precedes a slower systemic response, which ultimately leads to systemic acquired resistance (SAR).

The HR is triggered by the plant when it recognizes a pathogen. The identification of a pathogen typically occurs when a virulence gene product, secreted by a pathogen, bind to, or indirectly interact with the product of a plant resistance gene (R gene).R genes are highly polymorphic, and many plants produce several different types of R gene products, enabling them to recognize virulence products produced by many different pathogens. The activation of R genes triggers an ion flux, involving an efflux of hydroxide and potassium outside the cells, and an influx of calcium and hydrogen ions into the cell.After this the cells involved in the HR generate an oxidative burst by producing reactive oxygen species (ROS), superoxide anions, hydrogen peroxide, hydroxyl radicals and nitrous oxide. These compounds affect cellular membrane function, in part by inducing lipid per oxidation and by causing lipid damage. Several enzymes have been shown to be involved in generation of ROS. For example, copper amine oxidase, catalyses the oxidative deamination of polyamines, especially putrescine, and releases the ROS mediators hydrogen peroxide and ammonia. Other enzymes thought to play a role in ROS production include xanthine oxidase, NADPH oxidase, oxalate oxidase, peroxidases, and flavin containing amine oxidases. In some cases, the cells surrounding the lesion synthesize antimicrobial compounds, including phenolic, phytoalexins, and pathogenesis related (PR) proteins, including β-glucanases and chitinases. These compounds act by puncturing bacterial cell

walls; or by delaying maturation, disrupting metabolism, or preventing reproduction of the pathogen. The induction of cell death and the clearance of pathogens also require active protein synthesis, an intact actin cytoskeleton, and the presence of salicylic acid.

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15.6 Water Deficit and Drought Resistance

15.6.1 Water deficit

One of the most important abiotic stresses affecting plants is water stress. A plant requires a certain amount of water for its optimal survival; too much water (flooding stress) can cause plant cells to swell and burst; whereas drought stress (too little water) can cause the plant to dry up, a condition called desiccation. Either condition can be deadly to the plant.

Water scarcity is the lack of sufficient available water resources to meet the demands of water usage. Water scarcity involves water stress, water shortage or deficits, and water crisis. In water stress is it is the difficult to obtain sources of fresh water for use during a period of time and may result in further depletion and deterioration of available water resources. Water shortages may be caused by climate change, such as altered weather patterns including droughts or floods, increased pollution, and increased human demand and overuse of water. A water crisis is a situation where the available potable, unpolluted water within a region is less than that region's demand. Water scarcity is being driven by two converging phenomena: growing freshwater use and depletion of usable freshwater resources.

Water scarcity can be a result of two mechanisms: physical water scarcity and economic water scarcity, where physical water scarcity is a result of inadequate natural water resources to supply a region's demand, and economic water scarcity is a result of poor management of the sufficient available water resources.

Causes of water scarcity

There are several principal manifestations of the water crisis. Groundwater over drafting (excessive use) leading to diminished agricultural yields. Overuse and pollution of water resources harming biodiversity Regional conflicts over

scarce water resources sometimes resulting in warfare Waterborne diseases and the absence of sanitary domestic water are one of the leading causes of death worldwide. Water is the underlying tenuous balance of safe water supply, but controllable factors such as the management and distribution of the water supply itself contribute to further scarcity.

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Vegetation and wildlife are fundamentally dependent upon adequate freshwater resources. Marshes, bogs and riparian zones are more obviously dependent upon sustainable water supply, but forests and other upland ecosystems are equally at risk of significant productivity changes as water availability is diminished. In wetlands, considerable area has been simply taken from wildlife use to feed and house the expanding human population. But other areas have suffered reduced productivity from gradual diminishing of freshwater inflow, as upstream sources are diverted for human use.

Effects of water scarcity on environment

Water scarcity has many negative impacts on the environment, including lakes, rivers, wetlands, and other fresh water resources communities' water resources. The resulting water overuse that is related to water scarcity, often located in areas of irrigation agriculture, harms the environment in several ways including increased salinity, nutrient pollution, and the loss of floodplains and wetlands. These wetlands are important not only because they are the habitats of numerous inhabitants such as mammals, birds, fish, amphibians, and invertebrates, but they support the growing of rice and other food crops as well as provide water filtration and protection from storms and flooding.

15.6.2 Drought Resistance

Drought has profound effect on growth, yield and plant quality. It also enhances the effects of wind. When drought occurs the soil becomes very dry and light. The wind picks up this dry dirt and carries it away. This action severely degrades the soil and creates a poor condition for growing plants.

Drought tolerance refers to the degree to which a plant is adapted to arid or drought conditions. Desiccation tolerance is an extreme degree of drought tolerance. Plants naturally adapted to dry conditions are called xerophytes. Drought tolerant plants typically make use of either C4 carbon fixation or

Crassulacean acid metabolism (CAM) to fix carbon during photosynthesis. Both are improvements over the more common but more basal C3 pathway in that they are more energy efficient. CAM is particularly good for arid conditions because carbon dioxide can be taken up at night, allowing the stomata to stay closed during the heat of day and thus reducing water loss.

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Many adaptations for dry conditions are structural, including the following:

(i) Adaptations of the stomata to reduce water loss, such as reduced numbers or waxy surfaces.

(ii) Water storage in succulent above-ground parts or water-filled tubers.

(iii) Adaptations in the root system to increase water absorption.

(iv) Trichomes (small hairs) on the leaves to absorb atmospheric water.

(v) Importance in agriculture

Although arid conditions can lowers the yield of many crops. However, plant breeding programs for improved yield during drought conditions have great economic importance, and these programs may be broad in scope. Drought Resistance is the ability of a plant to maintain favourable water balance and turgidity even exposed to drought conditions there by avoiding stress and its consequences. Stress avoidance due to morphological anatomical characteristics which themselves are the consequences of the physiological processes induced by drought these xerophytic characteristics are quantitative and vary according to environmental conditions. A favourable water balance under drought conditions can be achieved by transpiration before as soon as stress is experienced.

Control measures of Drought resistance

A) Mechanism for conserving water:-

1. **Stomatal mechanism**: -Stomata of different species vary widely in their normal behaviour and range. In some species stomata remain open continuously or remain closed continuously. Many cereals open their stomata only during a short time in the early morning and remain closed during rest of the day. There is a difference in this respect between varieties of the same crop as shown by the example in two varieties of

oat one is more resistant to drought open its stomata more rapidly in the early morning when moisture stress is at its minimum and photosynthesis can precede with the least loss of water .However mechanism of conserving water based on the closure of stomata will inevitable load to reduce photosynthesis and may lead to drought induced starvation injury.

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2. **Increased / Photosynthetic efficiency** :- On possibility for overcoming limitations on photosynthesis, imposed bicoastal closure as means for increasing resistance to loss of water by transpiration there by transpiration there by accumulations of CO2 would be at higher rate for a given stomatal opening . A number of imperfect crop plants (maize, sugarcane sorghum prose, fox tail & finger millets) as well as certain forage species Bermuda grass (Cynodon dactyl on) Sudan grass Bahia grass (*Paspalum notatum*) Rhodes grass (chloris Guyana) and certain A triplex sp. fixed most of CO2 into the C4 of molic and aspartic acids so called C4 dicarboxylic acid (C4) pathway.

3. **Low rate of cuticular transpiration**: - The typical example is the cactus. Thick cuticle results in low rate of transpiration.

4. Decreasing transpiration by a deposit of lipids layers on the surface of the leaves on exposure to moderate drought e.g. soybean.

5. **Reduce leaf area**: - The principal means of reducing water loss of xenomorphic plants is their ability to reduce their transpiring surface. Apart from the common means of keeping the aerial parts small perhaps the simplest form of this reduction of the transpiring surface is the sealing or of leaves at the time of water stress a characteristic phenomenon exhibited by many grasses.

6. **Leaf surface**: - Various morphological characteristics of leaves he reduce the transpiration rate and may affect survival of plants drought conditions. Leaves with thick cuticle waxy surface and the presence of spines etc. are common and effective.

7. **Stomatal frequency and location**: - A smaller number of stomata retard the development of water deficits. In certain species, the stomata

are located in depression or cavity in the leaves which is feature can further reduce transpiration by limiting the impingement of currents.

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8. **Effect of awns**: - Awned varieties of wheat predominate in the drier at warmer regions and have been found to yield better than awnless one especially under drought conditions though there are exceptions. Awns have chloroplasts stomata and so as photosynthesized.

B) By improving water uptake

1. **Efficient root system**:-

The root systems of drought resistant plants are characterized by wide variety of apparent adaptations. These responded to such predominant soil conditions as the duration of soil dryness and the depth that is normally wet. Plants become adapted to dry conditions mainly by developing an extensive root system rather that structural modification of the roots .The conceres "extensive root system" includes additional growth of secondary hair roots.

2. **High root to top ratio (R/T)** :-

A high root to top ratio is very effective mean to adoption of plants to dry conditions of the growth rate of the roots considerably exceeds that of the shoots. The transpiring surface is there by reduced while root system of the individual plant obtains it's water from a large volume of soil has shown that an increased root top ratio may actually result in greater amount of total dry matter of plants grown under dry conditions as compared a similar ones grown with full moisture.

3. **Difference in osmotic potential of plants**: -

It has been calculated a difference in soil moisture content includes per manual wilting could supply a plant with enough water to keep it alive for few days. This could mean in certain cases the difference between survival and death.

4. **Conservation of water spenders to water stress**:-

Because of increased water absorption water spenders are characterized by very high rate transpiration. However as soon as the absorption rate

becomes insufficient to keep up with water loss the water spender generally develops some of the characteristics of the water savers.

15.7 Salinity Stress

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Salinity is one of the most important factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield. Salinization affects many irrigated areas mainly due to the use of brackish water. High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, geno toxicity. Together, these effects reduce plant growth, development and survival.

During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected .At the time with the exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. The osmotic effects of salinity stress is observed immediately after salt application and continue for the duration of exposure, resulting in inhibited cell expansion and cell division, as well as stomatal closure .During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth .In fact, excess sodium and more importantly chloride has the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes

Moreover, Soil type and environmental factors, such as vapour, pressure deficit, radiation and temperature may further alter salt tolerance .In fields, in fact, the salt levels fluctuate seasonally and spatially, and variation will occur due to the circumstances influencing each particular plant .In addition, the continuous use of same soil for growing vegetables results in an increase of salinization.

Salt stress affects plants adversely in two ways- 1.High solute content in rooting medium creates water stress by decrease osmotic potentials. 2. Direct toxic effect of high concentration of ions. Soil salinity is a major factor that

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limits the yield of agricultural crops, jeopardizing the capacity of agriculture to sustain the burgeoning human population increase. At low salt concentrations; yields are mildly affected or not affected at all. As the concentrations increase, the yields move towards zero, since most plants, glycophytes, including most crop plants, will not grow in high concentrations of salt and are severely inhibited .The reason is that they have evolved under conditions of low soil salinity and do not display salt tolerance. On the contrary halophytes can survive salinity in excess of amounts. Halophytes are known to have a capability of growth on salinized soils of coastal and arid regions due to specific mechanisms of salt tolerance developed during their phylogenetic adaptation. Depending on their salt-tolerating capacity, these plants can be either obligate and characterized by low morphological and taxonomical diversity with relative growth rates increasing up to 50% sea water or facultative and found in less saline habitats along the border between saline and non-saline upland and characterized by broader physiological diversity which enables them to cope with saline and non-saline conditions. Measurements of ion contents in plants under salt stress revealed that halophytes accumulate salts whereas glycophytes tend to exclude the salts .Together, these effects reduce plant growth, development and survival. Plants sensitive or tolerant to salinity differ in the rate at which salt reaches toxic levels in leaves.

15.8 Metal toxicity

Metal toxicity is the toxic effect of certain metals in certain forms and doses on life. Some metals are toxic when they form poisonous soluble compounds. Certain metals have no biological role, i.e. are not essential minerals, or are toxic when in a certain form. In the case of lead, any measurable amount may have negative health effects. Often heavy metals are thought as synonymous, but lighter metals may also be toxic in certain circumstances, such as beryllium, and not all heavy metals are particularly toxic, and some are essential, such as iron. The definition may also include trace elements when considered in abnormally high, toxic doses.

Sometimes toxic metals imitate the action of an essential element in the body, interfering with the metabolic process to cause illness. Many metals, particularly heavy metals are toxic, but some heavy metals are essential, and

some, such as bismuth, have a low toxicity. Most often the definition of toxic metals includes at least cadmium, lead, mercury and the radioactive metals.[citation needed] Metalloids (arsenic, polonium) may be included in the definition. Radioactive metals have both radiological toxicity and chemical toxicity. Metals in an oxidation state abnormal to the body may also become toxic: chromium(III) is an essential trace element, but chromium's a carcinogen.

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Toxicity is a function of solubility. Insoluble compounds as well as the metallic forms often exhibit negligible toxicity. The toxicity of any metal depends on its ligands. In some cases, organometallic forms, such as methyl mercury and tetraethyl lead, can be extremely toxic. In other cases, organometallic derivatives are less toxic such as the cobalt ocenium cation. Decontamination for toxic metals is different from organic toxins: because toxic metals are elements, they cannot be destroyed. Toxic metals may be made insoluble or collected, possibly by the aid of chelating agents. Alternatively, they can be diluted into a sufficiently large reservoir, such as the sea, because immediate toxicity is a function of concentration rather than amount. However, bioaccumulation has the potential to reverse this.

Similarly, Toxic metals can bio-accumulate in the body and in the food chain. Therefore, a common characteristic of toxic metals is the chronic nature of their toxicity. This is particularly notable with radioactive heavy metals such as radium, which imitates calcium to the point of being incorporated into human bone, although similar health implications are found in lead or mercury poisoning. The exceptions to this are barium and aluminium, which can be removed efficiently by the kidneys.

Some toxic metals are:

- Antimony (a metalloid)
- Arsenic (see arsenic poisoning) is a metalloid
- Barium
- Beryllium
- Cadmium - cadmium poisoning

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- Lead - lead poisoning
- Mercury - mercury poisoning
- Osmium
- Thallium
- Vanadium
- Radioactive metals:
  - Actinium
  - Thorium
  - Uranium
  - Radium
  - The transuraniums, such as plutonium, americium, etc.
  - Polonium
  - Radioactive isotopes of metallic elements not otherwise strongly toxic, e.g. cobalt-60 and strontium-90.

Aluminium has no known biological role and its classification into toxic metals is controversial. Significant toxic effects and accumulation to tissues have been observed in renally impaired patients. However, individuals with healthy kidneys can be exposed to large amounts of aluminium with no ill effects. Thus, aluminium is not considered dangerous to persons with normal elimination capacity. Vanadium poisoning is observed as an anti-corrosive component of automotive steel, fragments of which can be left in passengers during an automobile accident.

Besides some trace elements and Non-metals with toxicity are-Chromium as hexavalent Cr (VI), Nickel – nickel salts are carcinogenic, Copper – copper toxicity, Zinc - zinc toxicity, Iron – poisoning. Some heavy non-metals may be erroneously called "metals", because they have some metallic properties. Selenium – a non-metal; essential element, Tellurium.

15.9 Temperature Stress

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Plants are pokiotherms that is they assume the temperature on their environment. Any change in temperature affects plant greatly, so temp. Stress has important effect on agriculture. Temperature stress is a major factor due to the changing climate. The difficulty of climate change is further added considering its precisely projecting potential agricultural impacts .Temperature stress has devastating effects on plant growth and metabolism, as these processes have optimum temperature limits in every plant species.

High temperature (HT) stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development. The growth and development of plants involves a countless number of biochemical reactions, all of which are sensitive to some degree to temperature. Besides, Low temperature (LT) or cold stress is another major environmental factor that often affects plant growth and crop productivity and leads to substantial crop losses. Chilling stress results from temperatures cool enough to produce injury without forming ice crystals in plant tissues, whereas freezing stress results in ice formation within plant tissues. Temperature stress can also affect plant growth and development. A plant has an optimal temperature range at which is grows and performs best. If the temperature is too cold for the plant, it can lead to cold stress, also called chilling stress. Extreme forms of cold stress can lead to freezing stress. Cold temperatures can affect the amount and rate of uptake of water and nutrients, leading to cell desiccation and starvation. Under extremely cold conditions, the cell liquids can freeze outright; causing plant death .Hot weather can affect plants adversely, too. Intense heat can cause plant cell proteins to break down, a process called denaturation. Cell walls and membranes can also "melt" under extremely high temperatures, and the permeability of the membranes is affected.

15.9.1 Freezing Stress

On the basis of response to temperature plants are classify into following:

(i) Chilling sensitive plants: Seriously injured by temperature above 00C, below 150C

(ii) Chilling resistant Plants: Able to tolerate low temperature, seriously injured when ice start to form in tissues

(iii) Frost Resistant Plants: tolerate exposure to very low temperatures (-500C to -1000C) even when immersed in liquid N2.

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Chilling injury occurs at Low temperature but non-freezing temperatures. Chilling Effect is manifested by physiological and cytological changes Cytological changes may be reversible or irreversible depending upon time of exposure to low temperature.

Symptoms of chilling injury include following changes-

Cellular changes: Changes in membrane structure and composition decreased protoplasmic streaming, electrolyte leakage and plasmolysis. Altered metabolism: Increased or reduced respiration, depending on severity of stress, production of abnormal metabolites due to anaerobic condition.

Cytological Changes: Swelling of plastid membranes and mitochondrial membranes, Swelling of chloroplast thylakoids,Decrease in size and no. of starch grains, Grana disintegration and increase in size and no. of plastoglobules ,Mitochondria with reduced cristae and transparent matrix, Rough ER completely disappears ie., Ribosomes are lost from the membrane, Swelling of dictyosomes, Longer exposure to chilling-disintegration of dictyosomes

Besides, Plasmolysis: Plasmalemma - pressed against the tonoplast and deleted into the vacuole as sac like intrusions, Formation of crystalline deposits in root cells, epidermal, mesophyll and vascular cells of leaves -leading to tonoplast disruption, Tonoplast injury is irreversible, During hardening at low or above zero temp the lipid bodies accumulate in cytoplasm in close association with plasmalemma.

At the same time following features can also be noticed. Surface lesions on leaves and fruits, Abnormal curling, lobbing and crinkling of leaves Water soaking of tissues, Cracking, splitting and dieback of stems, Internal discolouration (vascular browning),Increased susceptibility to decay ,Failure to ripen normally, Loss of vigour (potato lose the ability to sprout if chilled),Reduced plant growth and death.

15.9.2 Heat Stress

Heat is another thing that plants can deal with if they have the proper pre-treatment. This means that if the temperature gradually warms up the plants are going to be better able to cope with the change. A sudden long temperature increase could cause damage to the plant because their cells and receptors haven't had enough time to prepare for a major temperature change.Heat stress can also have a detrimental effect on plant reproduction. Temperatures 10 degrees Celsius or more above normal growing temperatures can have a bad effect on several plant reproductive functions. Pollen meiosis, pollen germination, ovule development, ovule viability, development of the embryo, and seedling growth are all aspects of plant reproduction that are affected by heat.

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High temperature also affects the metabolism and structure of plants, especially cell membranes and many basic physiological processes such as photosynthesis, respiration, and water relations. On the molecular level, this effect of heat stress reflects the temperature dependence of Michaelis–Menton constant (Km) of every enzyme participating in the process). Plants must cope with heat stress for survival, so they developed different mechanisms including the maintenance of cell membrane stability, capturing the reactive oxygen species, synthesis of antioxidants, accumulation and osmoregulation of osmoticum, induction of some kinases that respond to stress, Ca-dependent kinase proteins, and enhancing the transcription and signal transfer of chaperones .The induction and synthesis of heat-shock proteins due to high temperature exposure are common phenomena in all living organisms from bacteria to human beings .It seems that the synthesis of these proteins is costly energy wise that is reflected on the yield of the organism.

Plant responses to high temperature include;-

(i) Seed germination

Seed germination and seedling vigour are important traits for obtaining a good plant sps. and subsequent high yields of a crop. Seed germination is highly dependent on temperature as temperature is one of the basic requisites of this process. However, the range of temperature in which seeds perform better

germination depends largely on crop species. Soil temperature is one of the major environmental factors that influence not only the proportion of germinated seeds, but also the rate of emergence and the subsequent establishment, even under optimum soil and irrigation conditions.

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(ii) Growth and morphology

The most important effect of heat stress on plants is the retardation of growth. As heat stress often occurs simultaneously with drought stress, the combination of drought and heat stress induce more detrimental effect on growth and productivity of crops than when each stress was applied individually . In higher plants, heat stress significantly alters cell division and cell elongation rates which affect the leaf size and weight. Exposure of plants to severe heat stress decreased the stem growth resulting in decreased plant height HT stress also cause reduced cell size, closure of stomata and curtailed water loss, increased stomatal density and trichomatous densities, and larger xylem vessels in both roots and shoots .

(iii) Physiological effects

Physiological processes of plants are largely affected by the alteration of surrounded environmental temperature. The ability of plants to cope with extreme temperature is a complex process and is determined by environmental factors and also by the genetic capability of the plant. The increase of temperature up to a certain level increases plant growth, photosynthesis, respiration and enzyme activity and after that these parameters tend to decline. Respiration rapidly increases with temperature and drops drastically after an extreme tolerable temperature. Photosynthesis is a comparatively less sensitive than respiration process but its declining pattern is as like as respiration. Temperature plays one of the most important roles in the rate and ability of a plant to photosynthesize effectively. In general, there is a positive correlation between change in temperature and photosynthesis. But when temperatures exceed the normal growing range (15°C to 45°C) of plants heat injury takes place and HT hurts the enzymes responsible for photosynthesis. Even in the absence of heat stress injury, photosynthesis would be expected to decline as temperature increases because photorespiration increases with temperature faster than does photosynthesis.

(iv) Water relations

Plant water status is considered as the most important variable under changing ambient temperatures .Plant water relation is more affected under the combined heat and drought stress, than the condition of heat and sufficient moisture level. High temperatures affect seedlings by increasing evaporative demand and tissue damage. High temperatures induced increased transpiration and water transportation is another necessary tool for plant survival under extreme temperatures.

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(v) Reproductive development

Reproductive development of plants is more sensitive to HT because plant fertility is considerably reduced as temperatures increase. In case of bean, and peach, elevated temperatures during flower development can markedly reduce the fruit set. The decrease in the fruit set has generally been attributed to low pollen viability and germinability at HT in crop species such as tomato and groundnut.

15.10 Oxidative Stress

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signalling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signalling. In Humans , oxidative stress is thought to be involved in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome Sickle Cell Disease, lichen planus, vitiligo, autism, and chronic fatigue syndrome. However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens.

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(i) Chemical and Biological effects

Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defences, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinone's) into more aggressive radical species that can cause extensive cellular damage.

Table – 1 Oxidative Stress: Oxidative Reduction rections

S.No.	Oxidants	Description
1	•O2-, superoxide anion	One-electron reduction state of O2, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe2+ from iron-sulfur proteins and ferritin. Undergoes dismutation to form H2O2 spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed •OH formation.
2	H2O2, hydrogen peroxide	Two-electron reduction state, formed by dismutation of •O2- or by direct reduction of O2. Lipid soluble and thus able to diffuse across membranes.
3	•OH, hydroxyl radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components

	ROOH, organic hydro peroxide	Formed by radical reactions with cellular components such as lipids and nucleobases.
4	RO•, alkoxy and ROO•, peroxy radicals	Oxygen centred organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.
5	HOCl, hypochlorous acid	Formed from H_2O_2 by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.
6	ONOO-, peroxynitrite	Formed in a rapid reaction between $•O_2$- and NO•. Lipid soluble and similar in reactivity to hypochlorous acid. Protonation forms peroxynitrous acid, which can undergo homolytic cleavage to form hydroxyl radical and nitrogen dioxide.

NOTES

(ii) **Oxidative stress and diseases**

Oxidative stress is responsible for neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease, Lou Gehrig's disease (aka MND or ALS) and Multiple sclerosis Reactive oxygen species, and reactive nitrogen species production, antioxidant defence indicates oxidative damage may be involved in the pathogenesis of these diseases, while cumulative oxidative stress with disrupted mitochondrial respiration and mitochondrial damage are related with Alzheimer's disease, Parkinson's disease, cancer, and other neurodegenerative diseases. Besides, oxidative stress is thought to be linked to certain cardiovascular disease, It also plays a role in the ischemic cascade due to oxygen reperfusion injury following hypoxia. This cascade includes both strokes and heart attacks. Oxidative stress has also been implicated in chronic fatigue syndrome. Moreover, oxidative stress also contributes to tissue injury following irradiation and hyperoxia, as well as in diabetes. In addition, oxidative stress is likely to be involved in age-related

development of cancer. The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and it may also suppress apoptosis and promote proliferation, invasiveness and metastasis. Infection by Helicobacter pylori which increases the production of reactive oxygen and nitrogen species in human stomach is also thought to be important in the development of gastric cancer.

NOTES

15 .11 Summary

Plants as sessile organisms are exposed to persistently changing stress factors. The primary stresses such as drought, salinity, cold and hot temperatures and chemicals are interconnected in their effects on plants. These factors cause damage to the plant cell and lead to secondary stresses such as osmotic and oxidative stresses. Abiotic stress is produced by natural environment factors such as extreme temperatures, wind, drought, and salinity.

Salinity is a significant problem affecting agriculture worldwide and is predicted to become a larger problem in the coming decades.

Heavy metals toxicity caused by increasing levels of pollution and use of chemicals in industry is a growing threat to our health and development of our children. High levels of toxic metals deposited in body tissues and subsequently in the brain, may cause significant developmental and neurological damage, including autistic symptoms, depression, increased irritability, anxiety, insomnia, hallucinations, memory loss, aggression and many other disorders.

Drought is an extended period when a region receives a deficiency in its water supply, whether atmospheric, surface or ground water. Drought tolerance refers to the degree to which a plant is adapted to arid or drought conditions. Desiccation tolerance is an extreme degree of drought tolerance. Plants naturally adapted to dry conditions are called xerophytes.

At the same time, the extreme temperatures influence the metabolism, viability, physiology, and yield of many plants. Plants exposed to extreme temperatures often show a common response in the form of oxidative stress. However, the extent of damage caused by extreme temperatures depends greatly on the

duration of the adverse temperature, the genotypes of the exposed plants, and their stage of growth.

15.12 Glossary

NOTES

- **Stress** : Stress is any change in any environmental factor that has an impact on the plant by affecting its biochemical and physiological response to such changes, and may on occasions lead to damage or injury.
- **HR** : HR refers to Hypersensitive Response, which is a mechanism used by plants to prevent the spread of infection by microbial pathogens
- **SAR** : SAR stands for Systemic Acquired Resistance, a pathogen-induced plant defence mechanism against further pathogen attack
- **Water scarcity** : Water scarcity is the lack of sufficient available water resources to meet the demands of water usage within a region
- **Metal toxicity** : Metal toxicity is the toxic effect of certain metals in certain forms and doses on life
- **Antioxidant** : An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals
- **Salinity** : Salinity is the concentration of dissolved salts found in water. It is measured as the total amount of dissolved salts in parts per thousand.
- **Drought** : Drought is an extended period when a region receives a deficiency in its water supply, whether atmospheric, surface or ground water. A drought can last for months or years, or may be declared after as few as 15 days

- **Free radicals** : Free radicals are molecules with unpaired electrons. In their quest to find another electron, they are very reactive and cause damage to surrounding molecules.

NOTES

15.13 Self-Learning Exercises

Section- A (Very Short Answer Type Questions)

1. Write the full form of HR and SAR.
2. What do you mean by Stress?
3. What is Oxidative stress?
4. Name of two toxic metals.

Section-B (Short Answer Type Questions)

1. Differentiate biotic and biotic stress.
2. What do you mean by Drought resistance?
3. What are free radicals?
4. Explain in short about temperature stress.
5. Write the name of two factors of Water crises.

Section-C (Long Answer Type Questions)

1. Discuss the mechanism of biotic and abiotic stress.
2. Explain the effect of Heat stress on plants..
3. Discuss the chemical and biological role of oxidative stress
4. What is salinity stress? Discuss the effect of salinity on plant growth.

Answer key of Section –A

1. HR refers to Hypersensitive response, Whereas, SAR stands for Systemic Acquired Resistance.
2. Stress is defined as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival.

3. Oxidative stress is defined as the relationship between reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.
4. Lead and Mercury.

NOTES

15.14 References

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