M.Sc. Previous Year

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PLANT PHYSIOLOGY AND METABOLISM

मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल

MADHYA PRADESH BHOJ (OPEN) UNIVERSITY - BHOPAL

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INTRODUCTION

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Plant physiology is a sub-discipline of botany specifically concerned with the functioning or physiology of plants which includes the life processes of plants both lower species as well as the higher green terrestrial plants. Principally, the plant physiology studies the different parts of plants and specific topics, such as the structure and function of leaves, stems and roots, water and sugar conductivity, and the reproductive organs of plants.

Fundamental processes of plants, such as photosynthesis, respiration, plant nutrition, plant hormone functions, tropisms, nastic movements, photoperiodism, photomorphogenesis, circadian rhythms, environmental stress physiology, seed germination, dormancy and stomata function, transpiration and plant water relations are studied by the plant physiologists. Characteristically, the plant physiology includes the study of plant response to environmental conditions and their variation, a field known as environmental physiology. Stress from water loss, changes in air chemistry, or crowding by other plants can lead to changes in the way a plant functions. These changes may be affected by genetic, chemical and physical factors.

In addition, the plant physiology deals with interactions between cells, tissues, and organs within a plant. Different cells and tissues are physically and chemically specialized to perform different functions. Roots and rhizoids function to anchor the plant and acquire minerals in the soil. Leaves catch light in order to manufacture nutrients. For both of these organs to remain living, minerals that the roots acquire must be transported to the leaves, and the nutrients manufactured in the leaves must be transported to the roots.

Biochemistry is both life science and a chemical science as it explores the chemistry of living organisms and the molecular basis for the changes occurring in living cells. Fundamentally, the term 'Biochemistry' refers to the study of the chemical substances and processes that occur in plants, animals, and microorganisms and also the changes they undergo during development process or life cycle. Biochemistry focuses on understanding how biological molecules give rise to the processes that occur within living cells and between cells, and also helps in understanding about the tissues, organs, and organism structure and functions of plants. Characteristically, the field of biochemistry studies the structures, functions and interactions of biological macromolecules, such as proteins, enzymes, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform the functions associated with plant life. The chemistry of the cell also depends on the reactions of smaller molecules and ions. These can be inorganic, for example water and metal ions, or organic, for example the amino acids, which are used to synthesize proteins. The mechanisms by which cells bind energy from their environment via chemical reactions are known as metabolism. The findings of biochemistry are applied primarily in medicine, nutrition, and agriculture.

This book, *Plant Physiology and Metabolism*, has been designed keeping in mind the Self-Instruction Mode (SIM) format and follows a simple pattern, wherein each Unit of the book begins with the Introduction followed by the Unit Objectives for the topics discussed. The content is then presented in a simple and easy-to-understand manner, and is interspersed with Check Your Progress questions to reinforce the student's understanding of the topic. A list of Questions and Exercises is also provided at the end of each Unit. The Summary, Key Terms and Further Reading further act as useful tools for students and are meant for effective recapitulation of the text.

UNIT 1 TRANSPIRATION AND ABSORPTION OF MINERALS IN PLANTS

Structure

1.0 INTRODUCTION

The process of water regulation in plants is modulated by various external and internal factors of the plant and its environment. Water status of the plant provides important insights to the health of the plant. The concept of water potential has been elaborated for the proper understanding of water movement mechanisms in plants. However, in order to analyse the physiological significance of water transport the mechanisms of transpiration needs attention. The fundamental understanding of transpiration in different types of plants provides us knowledge of its relevance in water utilization strategies in plants. Transpiration in nature possesses significance in the regulation of plant temperature and its water content. Water loss in the form of vapour contributes to the moisture content or relative humidity of the environment. The operation of water cycle by cloud formation and rainfall are partially controlled by the transpiration.

Water is an essential part of plant life which contributes up to more than 80% of fresh weight available to the plant tissues. In nature, the process of entry of water into plant body is regulated by several factors that are associated with soil composition, root structure and external micro-climate around the roots. In this context it is important to understand the basic physico-chemical properties of water molecules which render them suitable to be carried up to high elevations in huge Gymnospermous or Angiospermous plants. Although a number of theories have been formulated by various physiologists to explain the process of water transport in plants, in reality it is regulated by an assemblage of external and internal factors. Water molecules are electronegative and polar in nature due to the presence of one Oxygen atom bound to two Hydrogen atoms by the means of covalent bond. The electronegative nature of Oxygen results in attraction of electrons towards it. The angle between the two Hydrogen atoms joined to Oxygen forms an angle of 104.5°. This exhibits a difference in charge of δ^+ and δ^- ends at the Oxygen and Hydrogen atoms. However, the two polar charges being equal render water as a neutral molecule.

In this unit, you will study about the mechanism and types of transpiration, significance of transpiration in physiological homoeostasis of plants, absorption of minerals, water transport process in plants, and the water and mineral absorption from soil.

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1.1 UNIT OBJECTIVES

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

- Explain the mechanism and types of transpiration
- Discuss the significance of transpiration in physiological homoeostasis of plants
- Understand the absorption process of minerals
- Describe water transport process in plants
- Discuss the mechanism of water and mineral absorption from soil

1.2 TRANSPIRATION IN PLANTS

The process of **water regulation** in plants is modulated by various external and internal factors of the plant and its environment. Water status of the plant provides important insights to the health of the plant. The concept of water potential is very significant for the proper understanding of water movement mechanisms in plants. However, in order to analyse the physiological significance of water transport the mechanisms of transpiration needs attention. The fundamental understanding of transpiration in different types of plants provides us knowledge of its relevance in water utilization strategies in plants. Different plants are adapted to diverse habitats and they vary in their morpho-anatomical characters like stomatal position, stomatal frequency and water usage efficiency. Stomatal transpiration rate in plants is a function of its age and foliage development. Leaf formation and its expansion are associated with changes in surface area. The habit of the plant, its phyllotaxy and life period are the major factors which determines the light harvest efficiency. Shade loving and sun loving plants vary in their stomatal arrangement in both the surfaces of the leaves. **Transpiration index** of a plant and its stomatal frequency determines the rate of water loss for the various plants.

Transpiration in nature possesses significance in the regulation of plant temperature and its water content. Water loss in the form of vapour contributes to the moisture content or relative humidity of the environment. The operation of water cycle by cloud formation and rainfall are partially controlled by the rate of transpiration. The vegetation level of a region including tree and shrub percentages determine the rate of moisture formation by transpiration. Glycophytes and mesophytes possess normal transpiration rate and water use efficiency. However, xerophytes have adaptive measures in terms of stomatal count, transpiration rate and conservation of water content. Stomatal pores in leaves are formed by unique arrangement of the paired guard cells and their cell walls contain unequal thickness to provide rigidity. Various physiological regulators like Abscisic Acid, Potassium Ion, Malic Acid, CO_2 and Blue Light Modulate Stomatal Movement in plants. The process of stomatal movement is mostly regulated by osmotic changes in the guard cell. This process involves exchange of sap to and from the neighbouring subsidiary cells.

Water use efficiency of plants is an important factor which affects crop productivity and abiotic stress tolerance. Manipulation of transpiration rate can lead to protection of crops in the arid regions receiving low to average annual precipitation. Various strategies implying transgenic crop rearing through genetic engineering approach can produce plants

with increased water use efficiency. In this context it should be understood that stomatal count and its diurnal regulation plays an important role in the regulation of transpiration. Current understandings of the mechanism of stomatal movement have been gathered from various investigations across the last few decades of research. Initial theories proposed were formulated on the basis of Starch-Sugar inter-conversion evidences. Accumulation of photosynthates during the day time tends to increase the solute concentration of the guard cells. Thus the aperture of stomatal pore increases along with increase in the duration of light in the day time. In this process Potassium-Malate formed by combination of Malate and Potassium ion plays a pivotal role in regulating osmosis. Thus the rate of transpiration coincides with the increase in stomatal opening during the noon time. Physiologists prefer to mention transpiration as a necessary evil for the plant. The process of water loss in the form of vapours results in heat loss from the plant. However, excessive loss of water from foliar surface can result in dehydration and wilting in plants growing over dry soils.

Water stress management of plants is partially depended upon the foliar arrangement of a plant. The leaves depending upon their arrangement in the stem axis are categorized into dorsiventral and isobilateral types. The anatomical features in these type of leaves differ in terms of mesophyll differentiation and stomatal distribution. Thus, transpiration rate not only depends upon external climatic conditions but also on the morpho-anatomical features of the leaf. Dorsiventral leaves contain stomata either on both surfaces (amphistomatic) or on lower surface (hypostomatic) so as to regulate the rate of transpiration.

Transpiration in Plants

Transpiration is a vital physiological process in plants which regulate temperature, water status and water uptake from the soil. Transpiration is not a mere water loss process for plants but also bears significance in terms of water balance, crop productivity and moisture regulation of the environment. A very small amount of water is necessary to maintain cellular process and osmotic balance of the cell. The excess water exits in the form of water vapour from the foliar surfaces.Transpiration based upon the pathway of water loss has been broadly classified into three types, i.e., Stomatal Transpiration, Cuticular Transpiration and Lenticular Transpiration.

Stomatal Transpiration

Stomatal transpiration involves loss of water from the foliar surface of plants and through the stomatal aperture or pore. It has been evidenced that a major portion of the water content (90%) evaporates by the process of stomatal transpiration. In this pathway, the water molecules that are present in the mesophyll cells are due to higher pressure that is spread within the intercellular spaces and finally get evaporated through the stomatal slits. Water molecules transported through the xylem elements due to pressure dependent gradient provide hydrostatic pressure in the leaf tissue which results in the transpirational loss. This process of stomatal transpiration is a major pathway which operates involuntarily and depends upon internal water pressure and external environmental factors.

Cuticular Transpiration

Cuticular transpiration is a minor process which involves loss of water in vapour form exiting from the cuticle layer of the leaves. However, the amount of water lost through this process is very negligible in comparison with stomatal transpiration. The water molecules driven up by pressure flow accumulate in and around the mesophyll cells which move to the epidermal region and evaporate from the cuticular layer present in the outer surface of foliage. This process of transpiration does not account for more than 10% of water loss. However, the

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amount of cuticular transpiration also depends upon the anatomical feature of the leaf, i.e., Cuticle Thickening, Surface Area and Tissue Water Content.

Lenticular Transpiration

Lenticular transpiration involves loss of water from the lenticular openings present in the bark or stem and also in fruits. The formation of lenticels depends upon the age or maturity of the plant organ. This pathway of transpiration also involves very minor amount of water loss through evaporation.

The process of transpiration has similarities with evaporation in the fact that both involve loss of water in the form of vapour. However, the former is a biological process controlled by the osmotic pressure and suction pressure within the plant. Evaporation is a physical process of surface phenomenon which does not involve any biological component. However, transpiration is associated only with living cells. Thus water potential has a major role in determining the rate of transpiration from a plant surface. Transpiration rate is also associated with the changes in plant temperature. The comparison in the rate of transpiration with respect to evaporation from a unit surface area is termed as **transpiration index**. It indicates the differences in the rate of biological and physical process.

1.2.1 Mechanism of Stomatal Transpiration

The process of foliar transpiration occurs through the stomatal slits. This is a highly regulated process controlled by internal factors like osmotic potential and diffusion pressure of leaf mesophyll cells. The process of water uptake from soil and transport through xylem channels or conduits are inter-connected with the phenomenon of transpiration. The xylem elements draw water by the virtue of pressure gradient and capillary action. The water column reaches mesophyll cells which remain in vicinity to the xylem elements. The mesophyll cells absorb water dissolved with solutes which decrease the water potential of the cells. The intercellular spaces with lesser water content draw water from the mesophyll cells by the process of diffusion. Thus to understand the process of transpiration it is necessary to clarify the stages of advancement of water flow from the xylem to leaf tissue (Refer Figure 1.1).

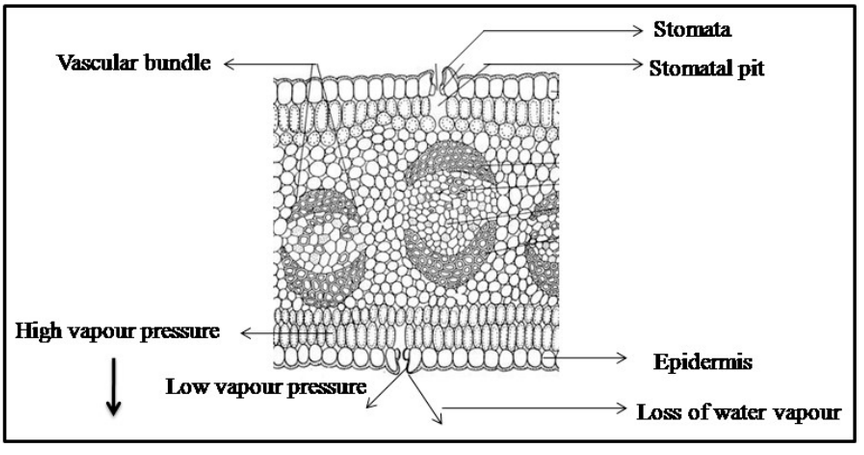

Fig 1.1 *Stomatal Transpiration*

The stages are as follows:

- Osmotic uptake of water from the xylem conduits into the mesophyll cells.
- Pressure driven diffusion.
- Diffusion mediated flow followed by evaporation.

Osmotic Uptake of Water from the Xylem Conduits into the Mesophyll Cells

The mesophyll cells accumulate photosynthates during the day time. Sucrose and Malic Acid are the main components which regulate osmotic pressure in the mesophyll cells as well in the guard cells. The difference in the water potential values result in the uptake of water from xylem elements to the mesophyll cells.

Pressure Driven Diffusion

Mesophyll cells absorb water from xylem and remain turgid in nature. The sub-epidermal region of leaves contain loosely arranged cells followed by the presence of spongy parenchyma tissue. These cells are loosely arranged and contain intercellular spaces. The water content in this region being low, it flows from the mesophyll cells by the process of pressure driven diffusion. The Diffusion Pressure Deficit (DPD) of the mesophyll cells is decreased due to absorption of water. This process further facilitates the water drive from mesophyll cells to the intercellular space beneath the stomatal chamber.

Diffusion Mediated Flow is followed by Evaporation

The external atmospheric vapour pressure around the stomatal pores is important to control the process of transpiration. Usually the water flowing into the intercellular spaces may form vapour. The water vapours accumulating gradually cause an increased vapour pressure within the leaf tissue (beneath the stomata). The external vapour pressure being lower than that of internal leaf space, water escapes in the form of vapour from the stomatal slits.

Thus, briefly, the process of transpiration is regulated by osmotic water uptake, followed by pressure driven diffusion. Soil water content is a primary external factor which may regulate sequential steps leading to transpiration. The external vapour pressure varies with the climatic conditions and wind speed. Thus the mechanism of transpiration is modulated by both external and internal factors.

Factors Affecting the Rate of Transpiration

The process of transpiration in various plant groups appear to be regulated by both external and internal factors.

External Factors

The external factors includes the following:

• Light Intensity

The intensity and irradiance of light perceived by the foliar surface affects the rate of transpiration. This process is primarily mediated by the regulation of stomatal aperture which in turn regulates the rate of water loss. Plants exhibiting Crassulacean Acid Metabolism (CAM) usually keep their stomata closed at day time.

• Moisture Content and Relative Humidity

The moisture content in the atmosphere is an important parameter which regulates the relative rate of transpiration. The water vapour pressure present in the environment around the leaves is one of the determinants of transpiration rate. The moisture content in atmosphere is denoted as relative humidity. The intercellular spaces in the leaf mesophyll cells mostly remain saturated with water vapour. Thus the process of water loss in the form of vapours is mostly due to pressure driven diffusion from high to low vapour pressure across the

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stomata. High humidity slows down the rate of transpiration due to lesser difference in vapour pressure between the external and internal surface of leaves. Wind velocity also controls the saturation rate of moisture in the environment thus affecting transpiration rate.

- **Temperature and Soil Water Availability**

Temperature usually controls various factors like stomatal movement, plant metabolism rate and water absorption by roots. Higher temperatures within the physiological range usually increase the rate of transpiration. However, temperatures more than 30°C may lead to stomatal closure and cease the rate of transpiration. Alternately, the rate of transpiration at higher temperature may exceed the rate of absorption thus causing dehydration to the plant.

Soil water content is important in respect of water absorption, root pressure generation and ascent of water column through xylem elements. The water pressure gradient formed in xylem causes to draw water towards the mesophyll cells. This process in turn transports water molecules in the intercellular spaces of leaf cell. Thus transpiration rate is dependent upon the rate of water absorption.

Internal Factors

Internal factors includes the following:

- **Root-Shoot Ratio**

The relative area of root and foliage in a plant regulates the relative rate of absorption and transpiration. Some plants have extensive root proliferation in the underground parts. The growth of primary and secondary roots mediates the rate of absorption. The shoot area (mostly foliage) determines the transpirational loss of water. In certain crops the foliage area is more developed in comparison with root area. This may result in higher transpiration rates.

- **Leaf Area and Stomatal Frequency**

The surface area of the leaf is important determinant of loss of water through transpiration. Stomatal frequency is defined as the number of stomata in unit area of leaf. The stomatal frequency varies in different plant species. Sun loving and shade loving plants possess different stomatal frequency depending upon their light requirement and heat tolerance ability. Young foliage with lesser surface area exhibit low rates of transpiration.

- **Leaf Structure**

The anatomical features in leaves regulate the rate of water vapour escaping from stomatal apertures. Certain leaves with xerophytic adaptation contain thick cuticle deposition to prevent transpiration. Isobilateral leaves possess no differentiation of palisade and spongy mesophyll cells. Thus less intercellular spaces are decreased due to absence of spongy mesophyll cells. Another important xerophytic feature is the presence of sunken stomata which reduces transpiration. Amphistomatic leaves (*Ixora* sp.) may undergo higher transpiration rate in comparison with Hypostomatic leaves (*Polyalthia* sp.).

1.2.2 Significance of Transpiration

The process of transpiration in plants involves a plethora of physiological effects which include regulation of water balance, ion uptake and temperature homoeostasis. Water absorption from soil and its subsequent transfer to aerial organs is regulated by osmotic process and tension force. Transpiration causes changes in the vapour pressure in the

intercellular spaces of mesophyll cells. The process of water escape in form of vapours causes to decrease the average temperature of the plant. Furthermore, transpiration accounts for more than 90% of the water loss from the plant. Around 5% of the water retained is involved in osmotic adjustment of the plant.

Water Transport Process

Water transport process is directly and partially regulated by the process of transpiration. The water column rising through the xylem elements is capable of tolerating the tension force created due to transpiration pull. The components of cohesion tension theory water transport mechanisms in plants. The process of transpiration pull results in the continuity of water column through the xylem conduits. The negative pressure formed in the xylem is compensated by the positive hydrostatic pressure from the roots. However, in absence of transpiration pull it is less likely that water column will cease to rise through the xylem elements. In Gymnospermous plants, foliage tissue bear less surface area to reduce transpiration. Similarly, the plants growing in arid region may undergo adaptive mechanisms to reduce transpiration. Thus it is important to understand that transpiration is not the sole regulator of water transport through the xylem elements.

Absorption and Translocation of Minerals

Absorption and translocation of minerals and salts are indirectly associated with transpiration pull. Earlier evidences opined that the process of mineral uptake was controlled by transpiration. However, ion uptake in roots is completely an independent process regulated by proton energised pumps, channels or carriers. However, transpiration only facilitates the movement of solutes (dissolved in sap) already loaded in the xylem column to appear in other parts of the aerial plant body. Thus creation of transpiration pull or negative pressure in the leaves is not directly associated with nutrient transport.

Maintenance of Cell Turgidity

Maintenance of cell turgidity is also regulated by the rate of transpiration. It is necessary to maintain the optimum hydration level of cells which is required for proper metabolic functioning of the plant. Transpiration causes to lose surplus water from the plant and maintains sufficient turgidity of the cells. However, the balance between water loss and absorption is crucial to maintain the water status of the plant. This is possible in the presence of adequate moisture/ water content in the soil. Dry soils or osmotically unsuitable soil content may lead to paucity in the water content of plant organs. In such a situation, high transpiration rates may appear detrimental to the plant. Thus stomatal regulation in response to soil water status is important to check transpiration. During normal conditions the moisture levels in soil optimum transpiration is likely to contribute to cell turgidity.

Temperature Homoeostasis

Temperature homoeostasis in plant organs appear to be an important outcome of foliar transpiration. Water molecules possess the characteristic property of high latent heat of vaporization which appears to be of 580 cal gm^{-1} at 30°C. Thus water evaporation from the foliar surface essentially absorbs the heat from plant and its surroundings. A major part of the irradiance (sunlight) absorbed by the leaf tissue is thus dissipated by transpiration. However, transpiration is not the sole method of heat dissipation in plants. Various arguments have been put forward by physiologists to explain the mechanisms of heat energy regulation in plants. Xerophytic or drought tolerant plants possess heat tolerance mechanisms inspite of

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minimum transpiration occurring through the foliages. Thus transpiration is likely to be supplemented by normal physical process of heat exchange between the plant and its surrounding atmosphere.

Transpiration Causes Plant Water Deficit

Transpiration has been commonly termed as a necessary evil because of the fact that a higher rate is likely to cause plant water deficit. Plants obtain benefits from transpiration although it causes possible effects of dehydration. Thus according to the statements from various physiological evidences it is necessary for the plant to maintain an optimum rate of transpiration. However, the requirement largely depends upon the physiological regulation and adaptation of the plant. Deciduous plants shed their leaves in the autumn season. This process naturally occurs due to leaf senescence which indirectly contributes to prevent transpiration. Evergreen plants are likely to imply regulation of transpiration through modulation of stomatal distribution and its movement.

Antitranspirants

Various compounds (natural and synthetic) are commonly applied in foliar surfaces of the plant to check the rates of transpiration. This method finds its application in water use management of agricultural crops. Usually water stress is a major issue which affects growth and productivity of crops. Antitranspirants usually block stomatal surface and prevent loss of water in form of vapour. Certain biological antitranpirants (biomolecules, hormones) act through metabolic regulation or cause stomatal closure in the leaves. **Abscisic Acid (ABA)** and **Salicylic Acid** are two biological antitranspirants used as foliar spray. Endogenous ABA in plant roots act as a messenger of water stress and thus brings about stomatal closure. The synthetic anti-transpirants commonly applied to plants are **Phenyl Mercuric Acetate (PMA)**, Fungicides or Herbicides. Moreover, colourless greasy substances or low viscosity oils can be applied on foliar surfaces to prevent transpiration. To imply an anti-transpirant it should ideally possess the properties like non-toxic nature, no permanent damage to stomatal apparatus and should not effectively hamper gaseous exchange and metabolic processes like photosynthesis and respiration.

Antitranspirants have been popularised in agriculture, floriculture and horticultural practices. Crops may be applied with antitranspirants to prevent water loss from the plant. This prevents wilting and dehydration. Fruit walls can be applied with antitranspirants to prevent water loss and wall cracks on the epicarp. This restores the quality and appearance of the fruits suitable for marketing. Transportation of seedlings/ornamental plants often involves application of antitranspirants on the foliar surface. This substitutes the frequent need for irrigation.

1.2.3 Structure and Functioning of Stomatal Apparatus in Plants

Stomatal apparatus is present in almost all groups of plants starting from Hepatics, Mosses, Pteridophytes, Gymnosperms and Angiosperms. However, there lie differences in the shape, arrangement and frequency of stomatal distribution in each of the plant groups. The stomatal apparatus or stoma (singular) refers to the two guard cells attached at two ends forming a central pore or aperture and subsidiary cells associated with the guard cells. The guard cells are main regulators of the stomatal aperture and exhibit osmotic movement coupled to diurnal fluctuations. The stomatal apparatus functions for transpiration and exchange of gaseous molecules between the plant and atmosphere (Refer Figure 1.2).

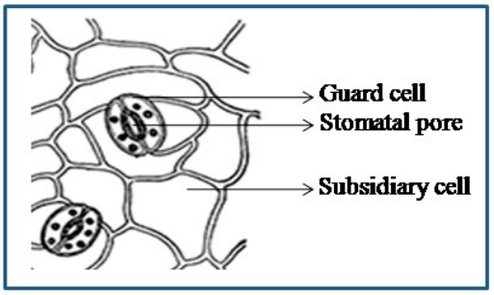

Fig 1.2 *Stomatal Apparatus in Plants*

The structure of stomata is unique in terms of its cytoskeletal arrangement and differential thickening of cell walls. In the poaceae family of monocots the guard cells are dumble shaped at the two ends and flat end walls meet each other to form the pore in between. Normally in most of the plant families guard cell is bean or kidney shaped with unequal thickness of cell wall. The inner walls forming the pore usually possess thick cell wall to attend rigidity. The outer lateral walls facing towards the subsidiary cells are thinner in nature. Moreover, there lie considerable differences in the pattern of cellulose micellation within each of the guard cells. Usually plant cells contain cellulose microfibrils arranged transverse to the axis of the cells. This feature allows the expansion of the cell along its length. However, in case of guard cells the arrangement is different and forms a pattern called as **radial micellation**. In this case, the cellulose microfibrils radiate from the pore to all sides of the cells. Guard cells by the virtue of such radial micellation can expand only transversely in an outward direction. The two guard cells are larger in comparison with other epidermal cells.

Thus expanding guard cells on their inner side touch the opposite walls only at the two ends. This allows the central pore to increase in its aperture. Thus, radial micellation and differential thickening of the cell walls contribute to radial expansion of guard cells thus allowing the stomatal pore to increase in aperture. The major physiological mechanism of guard cell functioning is regulated by osmotic adjustment of the guard cells. The regulation of stomatal movement leading to closing or opening of the aperture is crucial to control processes like transpiration, respiration and photosynthesis. In this context it is important to understand that the process of stomatal movement is regulated by both exogenous and endogenous factors like blue light, CO_2 concentration, ABA concentration, Potassium ion and malic acid. The primary control of guard cell expansion occurs through the formation of Potassium malate and associated ion exchange across the plasma membrane. This results in decreased Ψw of the guard cells. The guard cells now draw water from the adjacent subsidiary cells with higher Ψw maintained due to low accumulation of malate/ solutes. Thus endosmosis in the guard cells results in turgid form which causes them to distend/expand along the width.

This results in widening of the stomatal aperture. Conversely the breakdown of K-malate and subsequent ion exchange causes the Ψw of the guard cells to increase. Thus results in outward movement of water to the neighbouring subsidiary cells (exosmosis) thus leading to flaccidity of the guard cells. This causes closing of the stomatal aperture. The osmotic adjustment of the guard cells varies with diurnal fluctuation and in relation with other regulators.

1.2.4 Mechanism of Stomatal Movement in Plants

Various theories and hypothesis in the last few decades have been formulated to explain the mechanism of stomatal movements. Advancements in investigations along with the role of various biomolecules being deciphered, the refinement in the mechanism of stomatal movement has been successfully attained. The older hypothesis although not accepted now contain partial correlation with the physiological changes associated with the process.

Photosynthetic Theory

According to this theory proposed by Von Mohl (1856) the guard cells are capable to maintain osmolytes accumulating from the process of photosynthesis. However, this theory could not be accepted universally because it failed to explain the role of other components like CO_2 which affects stomatal movements independent of photosynthesis.

The Starch-Sugar Interconversion Hypothesis

This hypothesis was initially proposed by Lloyd in 1908 and later modified by Loftfield (1921), Sayre (1926) and Steward (1964). The main component of the theory is the inter conversion of starch into soluble sugars which increase the osmolyte accumulation in guard cells. The process of starch to sugar inter-conversion was attributed to the activity of a hydrolysing enzyme phosphorylase. Furthermore it was observed that during the day time the pH of the guard cell increased and it decreased at night. This change in pH was thought to be responsible for starch-sugar conversions. CO_2 infuses into the guard cells during the day time and gets utilized in the process of photosynthesis. However, the drop in pH during the night time results due to dissolution of CO_2 liberated by respiration. According to this hypothesis free glucose is osmotically active and involves ATP dependent reactions for its conversion to starch. Unfortunately the theory had various limitations - like it failed to explain the role of organic acids (malic acid), ions like K^+ Cl^-, H^+ blue light and endogenous regulators like ABA.

Modern Concept Theory

This concept explains the role of K^+ and malic acid in the regulation of osmotic potential in guard cells. Initially Imamura (1943) discussed the role of K^+ in regulating osmotic adjustments in the guard cell. In the day time starch is metabolized into sugars. According to Willmer (1973) PEP-carboxylase is responsible for formation of malate by carboxylation of PhosphoEonolPyruvate (PEP; formed as a product of photosynthesis). The Malate is the most common and abundant anion in the plant vacuoles. Malate formed is subsequently dissociated to $malate^{2-}$ or Mal^{2-} and H^+. The process of formation of malate results in acidification of the guard cell in the presence of protons. The protons are effluxed from the cell thus resulting in an increase in the negative charge of the cells. Changes in the membrane potential results in K^+ influx into the cells. The K^+ enters in vacuoles to form K^+-malate complex with $malate^{2-}$. This results in decreased Ψw of the guard cells and subsequent endomosis and stomatal opening. This event occurs during the day time in presence of $malate^-$ present in adequate amount. During the night phase opposite events occur resulting in exosmosis of guard cells and stomatal closure. The K^+ and Cl^- are transported out of the guard cells which cause dissociation of K^+-malate complex. Malic acid formed undergoes decarboxylation to CO_2 and pyruvate which results in sugar formation. This theory was acceptable in terms of its effectiveness in explaining the diurnal changes in stomatal movements (Refer Figure 1.3).

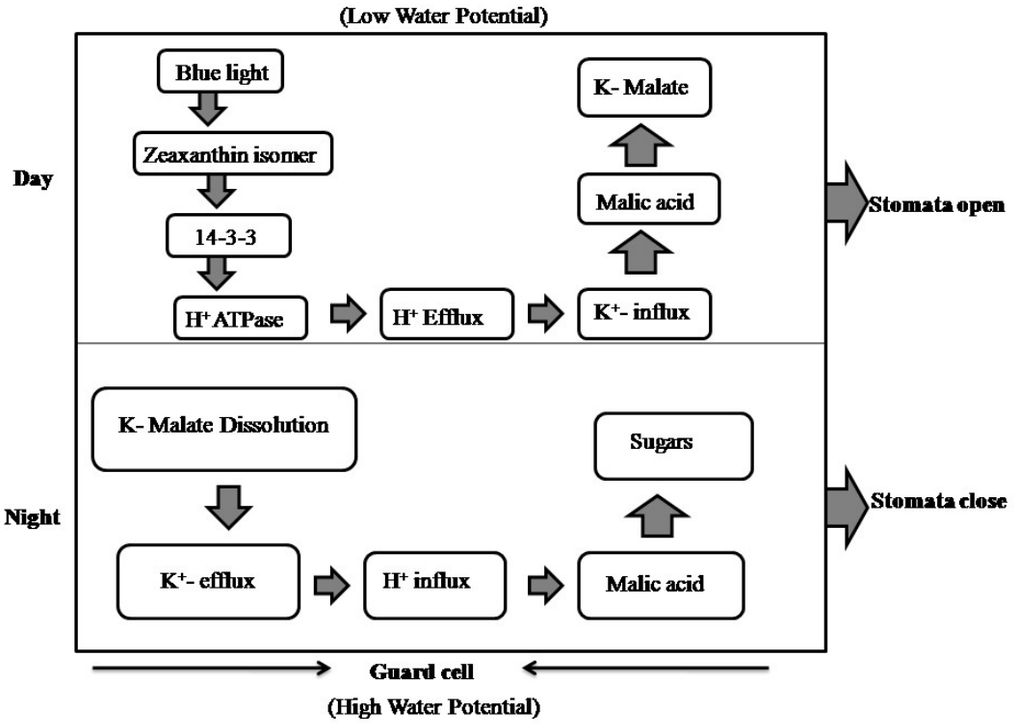

Fig 1.3 *Schematic Representation of the Regulation of Stomatal Movement*

Role of Biomolecules in Stomatal Movement

The following biomolecules hare significant role in the stomatal movement.

Potassium Ions (K^+)

Changes in membrane polarization associated with ion exchange is an important signaling event in guard cells. K^+ or Potassium ion plays an important role in maintaining the osmotic balance of the guard cells. Malic acid dissociation in the vacuole results in increased H^+ in the cells. H^+ efflux-ATPase functions to remove protons from the guard cell. This results in increase in negative charge in cytosol thus causing hyperpolarization. However, K^+ inward rectifying channels and passive voltage sensitive K^+ uptake mechanisms allow K^+ influx into the cells. The surge in K^+ in the cytosol and vacuole result in K-malate complex formation. Investigations have led to conclusions that K^+ influx occurs both by primary and secondary active processes.

Malic Acid

The presence of malate in cells appears from the photosynthetic pathway and is produced by the activity of PEP-carboxylase. Phospheonol pyruvate is metabolised to OxAloAcetate (OAA) catalyzed by PEP carboylase. OAA is further converted to malate catalyzed by malate dehydrogenase. Malic acid produced may remain stored in the vacuole of guard cells. However, dissociation of malic acid (day time) results in the formation of $malate^-$ and H^+. This reaction often results in the transient increase in proton concentration in the cytosol. Hyperpolarisation in the cells caused due to H^+ efflux is depolarized by inward movement of K^+. Malate forms complex with K^+ thus causing osmotic uptake of water in guard cells.

Carbon Dioxide (CO_2)

The effect of CO_2 on stomatal movement is largely regulated by its concentration. Low to moderate (physiological) concentrations of CO_2 induce opening of stomata. Such a process is accomplished by increased formation of malate from PEP via OAA formation. However,

extra physiological concentrations of CO_2 impose a feedback inhibition on the process of malate formation. Ambient CO_2 levels help in formation of ample amount of malate which causes acidification of the cytosol ($malate^{2-}$ and H^+). However, high concentration of CO_2 followed by excess malate formation inhibits the activity of PEP carboxylase. Thus CO_2 mediated control of guard cell movement operates through regulation of malate biosynthesis. It essentially controls the balance between malate formation and acidification of the cytosol in guard cells.

Abscisic Acid (ABA)

This biomolecule is an endogenous negative regulator of stomatal opening in plants. ABA is a hormone which transduces long distance signals associated with abiotic and biotic stress. Water stress-induced surge in ABA biosynthesis essentially occurs in the roots. It is followed by its long distance transport to leaves thus causing stomatal closure. ABA remains both in the apoplast and cytosol of guard cells. It causes subsequent acidification of the cytosol by increasing proton concentration. However, ABA inhibits the process of proton efflux by H^+ ATPase activity. Furthermore ABA activates the calcium influx channels thus leading to surge in cytosolic calcium oscillations. Increase in calcium and proton concentrations result in membrane depolarization which activates the K^+ outward channels. Thus dissociation of K-malate complex due to efflux of Potassium results in stomatal closure due to exosmosis. Alternately, ABA is also capable of acting through the Calcium dependent pathway to bring about stomatal closure. ABA induces a rise in the cytosolic calcium levels which in turn trigger rapid hyperpolarisation of membrane. Thus ABA induced Ca^{2+}-dependent pathway may also involve Ca^+-calmodulin (CaM) complex and CaM dependent Protein kinase activity.

Blue Light

The action of blue light on stomatal movement is reversible and is primarily mediated through carotenoid biosynthesis pathway. Blue light induces a metabolic surge in malate production in the guard cells. The photoreceptor for blue light triggers biosynthesis of zeaxanthin from antheraxanthin (a metabolic intermediate produced from violaxanthin). The activation of zeaxanthin by blue light photon results in the conformational change (isomerization) in its apoprotein. The conformational change in the structure of zeaxanthin results in activation of 14-3-3 moeity Protein which in turn activates H^+ ATPase in the plasma membrane of guard cells. This is followed by simultaneous activation of Ca^{2+} ATPase in the chloroplast membrane which transports calcium ions into the chloroplast. Thus blue light signaling is transduced by zeaxanthin to 14-3-3 Protein and mediated by secondary messenger of Ca^{2+}. These events are followed by the formation of K-malate complex as a tertiary response of blue light. In the day time sucrose accumulation can also influence stomatal opening. Physiologists have reported that sucrose remain as an active solute in the guard cells.

Some succulent plants undergo **Crassulacean Acid Metabolism (CAM)** and exhibit adaptive mechanisms to reduce transpiration and conserve water in tissues. Stomatal movement in CAM plants undergo a different regulation mediated by diurnal control of malic acid formation. Carbohydrates formed by photosynthetic pathway undergo incomplete oxidation to form malic acid during the night time. This malic acid produced is stored at the vacuoles. However, accumulation of malic acid results in the formation of K-malate complex. This leads to stomatal opening at night. During the day time the organic acids (oxaloacetate, malate) accumulated are oxidized to liberate carbon dioxide. Thus a decrease in organic acid levels results in dissociation of K-malate complex and subsequent stomatal closure.

'Check Your Progress'

1. What is water stress management in plants?
2. Define the term cuticular transpiration.
3. What does lenticular transpiration involves?
4. List the stages of the process of transpiration.
5. How does light intensity affects the rate of transpiration?
6. Define the term transpiration index.

1.3 ABSORPTION OF MINERAL

Water is an essential part of plant life which contributes up to more than 80% of fresh weight available to the plant tissues. In nature, the process of entry of water into plant body is regulated by several factors associated with soil composition, root structure and external micro-climate around the roots. In this context it is important to understand the basic physico-chemical properties of water molecules which render them suitable to be carried up to high elevations in huge gymnospermous or angiospermous plants. Although a number of theories have been formulated by various physiologists to explain the process of water transport in plants, in reality it is regulated by an assemblage of external and internal factors.

Plants are capable of absorbing water through the fine capillary networks present in the xylem tracheids and vessels. In this regard it should be understood that the vessels are more efficient conducting elements than tracheids. The vessels originate from a group of procambium cells which convert into hollow conducting elements by the process of programmed cell death. The continuity of water column through the xylem strands is maintained by the process of transpiration pull attained by the leaf mesophyll tissues. Soil composition of inorganic salts/metal ions regulates the osmotic phenomenon of water transport into the roots. This is further followed by cell to cell osmosis which drives water movement across the root tissues. Endodermis acts as a partial barrier to the apoplastic pathway of water transport across root tissues to xylem. Longitudinal deposition of lamellar suberin across cell walls of endodermal cells form structures called **casparian strips**.

The pressure of water column formed through xylem vessels is measurable in the units of Pascal (Pa)/Bar or atmosphere ($1Atm = 1.013 \times 10^5$ Pa). Plants growing in dry arid soils maintain lower osmotic potential of root tissues. However, desiccation or physiological dryness in the soil may cause difficulty in maintaining the pressure of upward directed water column. To understand the process of water transport in plants one needs to clarify the concepts of osmosis, diffusion and imbibition. The potential free energy of water provides it the driving force of movement across biological membranes. The free energy is regulated by the concentration of solute, membrane permeability and temperature. Temperature is an important determinant of membrane fluidity and causes subsequent changes in the kinetic energy of the biomolecules. Addition of solutes in pure water leads to decrease in its free energy, thus causing to decrease its driving force. Soil composition largely affects the degree of water availability to the plant roots. Briefly, the direction of flow of water from soil to root is crucial to maintain osmotic pressure and turgidity in the cells. Plant cells contain an outer elastic plasma membrane which is semi-fluidic in nature. The lipid composition of the membrane regulates its dynamic nature. Cell wall exerts counter pressure to the cell in respect to the osmotic pressure generated due to endosmosis. The net difference in the opposite directed

forces generates the suction pressure required by the cells to absorb water. Thus, in order to appreciate the enigma of water transport force generated in tall plants it is necessary to understand the bio-physical phenomenon of osmosis at the cellular level and capillary action, surface tension and other features at the macro level of the plant. The subsequent sections of the unit will explain the various physical and chemical properties of water suitable as a mobile solvent in plant tissues.

The Physico-Chemical Properties of Water

Following are the significant physico-Chemical properties of water:

• Polarity of Water

Water molecules are electronegative and polar in nature due to the presence of one Oxygen atom bound to two Hydrogen atoms by the means of covalent bond. The electronegative nature of Oxygen results in attraction of electrons towards it. The angle between the two Hydrogen atoms joined to Oxygen forms an angle of 104.5°. This exhibits a difference in charge of δ^+ and δ^- ends at the Oxygen and Hydrogen atoms. However, the two polar charges being equal render water as a neutral molecule (Refer Figure 1.4(A)).

• Formation of Hydrogen Bond and Capacity as a Good Solvent

The electronegative Oxygen atom of one water molecule associates with Hydrogen atom of the adjacent water molecule. These results in a chain formation of water molecules randomly aggregated together by Hydrogen bonds. The unique property of water as a universal solvent is also regulated by the partial electropositive and electronegative charges present at the two ends of the molecule (O and H atom). Oxygen atom of the water molecules can thus associate with electropositive atoms while the H atom can form Hydrogen bond with electronegative atoms like F, N, etc. most polar compounds present in the cells contain –OH or $–NH_2$ functional groups thus allowing them to get solubilised in the aqueous phase of water. Another unique property of water is the formation of hydration shell around charged macromolecules. This association results in the formation of suitable aqueous phase maintained for most of the cytoplasmic biomolecules suspended in the protoplasm.

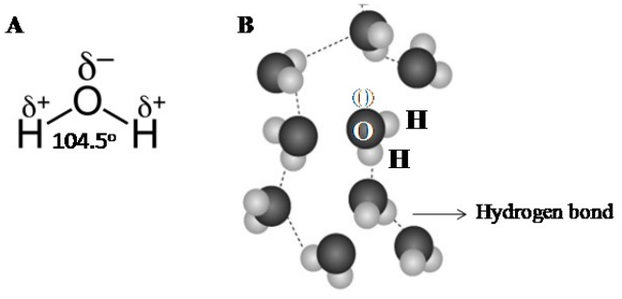

Fig. 1.4*(A) Structure of Water Molecule, (B) Water Molecules Possess Intermolecular Hydrogen Bonds (Cohesive Force)*

• Cohesive and Adhesive Properties of Water

The **cohesive property** of water is attained by the virtue of its Hydrogen bonding among the water molecules (Refer Figure 1.4(B)). This further result in the formation of a surface layer at the air-water interface. The force required to expand the surface is thus defined as **surface tension**. Water molecules can restrain high surface tension among different areas of entry into the cells. Surface tension phenomenon of water is also responsible for maintaining the continuity of water uptake and transport across the xylem elements. The **adhesive properties** of water are obtained due to its adhesion capacity with polar molecules.

Collectively the properties of cohesion, adhesion and surface tension provide capillary action to the water molecules. Capillary action is the property of water to rise to heights in tall trees. This is possible due to the unique tensile strength or squeezing property of water to enter vessel elements of 5 μm diameter. Finer diameter of xylem vessels allows water molecules to rise to higher heights.

- **Water Possess High Tensile Strength**

There is considerate amount of pressure which a water column can restrain before breaking its chain like aggregation. This property is known as **tensile strength** which results in the continuity of water column along the heights of xylem elements in plants. The subsequent positive or negative hydrostatic pressure developed due to compression or tension of water molecules results in the dynamic mobile property of water.

Water Possesses High Specific Heat Capacity and High Latent Heat of Vaporisation

The unique properties of water are exhibited by the virtue of its polar nature supported by Hydrogen bonding between the aggregates of molecules. Specific heat capacity is defined as the amount of heat required to raise the temperature of a substance by specific amount. The presence of extensive Hydrogen bonding renders water to capture a very high amount of specific heat. This in turn elevates the kinetic energy of the molecules. Interestingly, this high specific heat capacity is responsible to sustain marine life in frozen temperature. Talking about plants, the heat capacity of water regulates ambient temperatures within the biological system with reference to variable climatic conditions. Similarly the latent heat of vaporization is the amount of heat required for the substance to be converted from liquid to vapour/ gaseous state. In case of water, the Hydrogen bonds require higher amount of heat energy to be absorbed in order to convert it to gaseous or vapour states. At 25°C the heat of vaporisation is 44 KJ mol^{-1}. Thus the high latent heat of vaporisation results in the cooling effect of plant brought about by the process of foliar transpiration.

Cavitation/Embolism: This refer to the phenomena of xylem conduits being blocked due to air cavity. The process where air cavity forms due to increased vapour pressure of conducting water is known as **Cavitation**. **Embolism** is termed as the effect of air trapped in vessels due to bubble formation. Although the tracheids are not placed end to end, there are bordered pits and interconnectivities to maintain uninterrupted water flow. Plant physiologists, however, prefer to use cavitation and embolism as synonymous terms.

Diffusion

The effect of thermal agitation or kinetic energy allows water to move from its region of higher to the region of lower concentration. Such a process of water diffusion bears very important role in the process of water uptake by plants. The process of diffusion occurs without the help of any biological membrane. Although it is largely a physical process, the control of diffusion of water in plants is largely regulated by the membrane composition, soil water levels and upward transpiration pull mediated by leaves. According to the German scientist Adolf Fick, the rate of diffusion of molecules is directly proportional to the concentration gradient attained by it. However, in this context the distance travelled by the molecules is an important determinant of the rate of diffusion. Since diffusion is a physical process and it is not supplemented by additional source of energy, its rate decreases over increasing distances. For water molecules the process of diffusion necessarily occurs through shorter distances across the membranes of root cells. There are various other contexts of diffusion phenomena occurring in plants.

Thermodynamically the process of water diffusion in plants is regulated by its available chemical free energy (Refer Figure 1.5). This is, however, maximum for pure water devoid of any dissolved solutes. The process of solute dissolution in water decreases the rate of diffusion. The pressure developed due to movement of molecules from its higher to lower concentration along a gradient is called **diffusion pressure**. The difference/decrease in the diffusion pressure of a solvent in comparison to pure water resulting due to dissolution of solutes in pure water is known as **diffusion pressure deficit**.

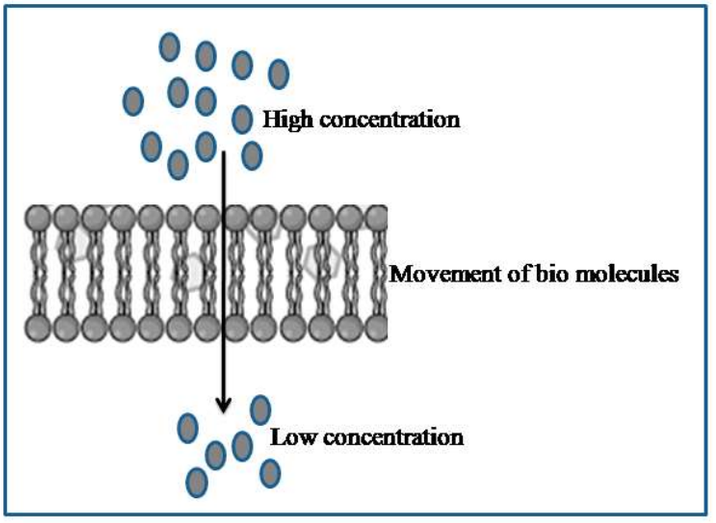

Fig. 1.5 *Mechanism of Diffusion Across Plasma Membrane*

Relevance of Diffusion in Plants

Water uptake in plants is associated with free diffusion across membranes. This process, however, is associated with the bulk flow of water from soil to root tissues. The process of free entry of water molecules across the membrane is thus associated with the presence of aquaporins (water channels) present abundantly in the root tissues. The details of aquaporins shall be described in the following section.

The process of gaseous diffusion across the epidermal layers of leaves involve exchange of various constituents like (CO_2, O_2, NO-, etc). The spongy parenchyma renders free intercellular spaces which regulate the vapour pressure in relation to the external microenvironment around the leaves.

Aquaporins

Investigations have revealed the presence of Integral Membrane Proteins which act as water channels for inward diffusion of water into root tissues. These channels allow unidirectional movement of water into the cells. Free diffusion across Aquaporins has been observed to be accomplished at a higher rate in comparison with that through membranes. Recent findings have revealed unique regulation of aquaporins in relation with the water levels present in the soil. Plants adapted to water stress or varied soil conditions have been reported to exhibit increased expression of aquaporins in the root cell membranes. Rice and other model crop systems have been implied to study the functioning of the aquaporins in relation to various external stimuli. The discovery of 'Aquaporin' has been acknowledged by Peter Agre in the year 2003. Various other organisms, such as Fungi, Bacteria and Animals have been reported to possess Aquaporin like Proteins associated with their cell membranes. The transmembrane component of the Protein contains membrane spanning alpha helices in

the cytopalsmic facet associated with a hydrophobic domain in the membrane. The hydrophobic region of the channel Protein is characterized by the presence of Asparagine-Proline-Alanine (NPA Motif) sequences which regulates the pathway of water transport across the channel. The specific Amino Acids in this NPA motif maintain the conformational form of the water path which is narrow in the middle and widened at the two ends.

Osmosis

Plant cells absorb sap from the adjoining cellular or intercellular areas or from the external environment in case of root tissues. Osmosis is largely a biological process governed by the concentration of solutes and membrane permeability across both sides of the membrane. In this process, water (solvent) moves from its region of higher concentration (low solute concentration) to the region of its lower concentration (higher solute concentration). Osmosis as a biological process appears to be the major determinant of water uptake and transport through plants. The movement of water molecules is in fact regulated by the differences in their free energy which provides them the gradient of flow across membranes. The movement of water during osmosis also facilitates the exchange of uncharged small solutes across the semi-permeable membrane. The large sized charged molecules are however unable to pass freely through the plasma membranes. However, explaining the phenomena of osmosis only in the light of concentration gradient of cell sap remains incomplete until one appreciates the component of pressure gradient established between the two regions. The cells are surrounded by a semi-permeable membrane and a cell wall. The enclosed system of intracellular region therefore encounters a particular magnitude of net pressure expressed as pressure potential. This along with the concentration gradient act to determine the direction of osmosis (Refer Figure 1.6).

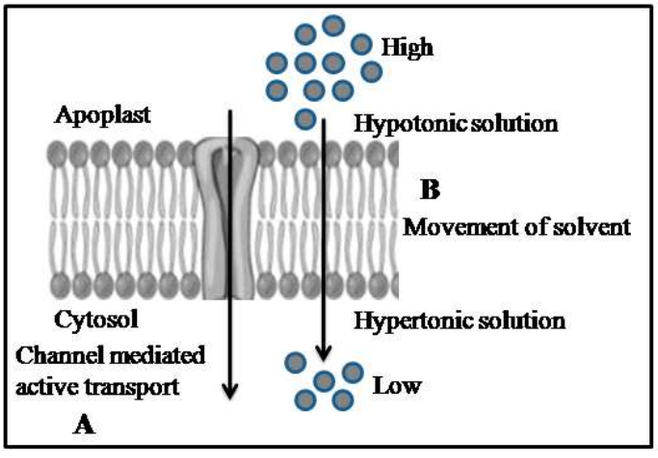

Fig. 1.6 *Mechanism of Water Transport in Plants (A) Active Transport; (B) Osmosis*

Plants absorb water available in the soil in form of a phenomenon known as endosmosis. The inward movement of solvent within a cell is regulated by higher solute levels within the cells. The concentration gradient for solutes between soil particles and the root cells needs to be maintained in such a manner that water with higher concentration within the soil enters into the root tissues. Thus, plant roots preferably maintain solute levels necessary to provide osmotic potential. In response to external low water content in the soils plant roots are capable of synthesizing **drought-induced metabolites** known as **compatible solutes**. These compounds are usually sucrose, proline or glycine-betaine like compounds which remain stable in the aqueous phase of the cytoplasm. The accumulation of compatible solute is important for a plant in order to absorb water from soil (endosmosis). Reverse flow of cell

sap out into the soil (exosmosis) or intercellular spaces results in cell flaccidity. This situation, if continues for a longer duration will result in shrinkage of root cell protoplasm and vacuole, a phenomenon known as **plasmolysis**. The initial stage of retraction of protoplasm from the cell membrane resulting due to exosmosis is termed as **incipient plasmolysis**. Plants experiencing mild to severe water stress have been reported to undergo higher accumulation of Abscisic acid and compatible solutes. In this context ABA being a stress hormone acts as a messenger for long distance signaling of water status from the soil, through the roots and up into the aerial organs of the plant. Essentially ABA induces closure of stomata thus preventing transpiration and water loss from the plants.

Osmosis in plants is simultaneously regulated by various components like gravity, pressure, temperature, membrane permeability, viscosity and concentration gradient. Root hairs are the active absorptive zone of plants which draw water from between the finer soil micelles. This **capillary water** present among the soil particles is the majorly available form of water to the plants. **Gravitational water** obtained from rain and other sources usually percolates down into the aquifers and underground water reservoirs. **Hygroscopic water** is tightly associated with the soil particles which is hard to be removed during absorption by roots. Thus water availability in soils largely depends upon its texture (sandy, loamy, clayey, laterite). This factor determines the Oxygen exchange capacity and water holding capability of soil particles. Root tissues are actively growing organs and therefore require good amount of Oxygen/aeration in order to carry on its metabolic processes. In soils affected by high salinity (natural salts like $CaCl_2$, $MgCl_2$, KCl and NaCl) the water concentration decreases and renders it unsuitable for agriculture. Extra-physiological concentrations of solutes accumulating in the rhizosphere render plants incapable of accumulating compatible metabolites in that range of concentration. The threshold of tolerance here largely depends upon the age and type of plants. Usually **glycophytes** and **mesophytes** are unable to restrain high salinity in comparison with halophytes.

The biological phenomenon of osmosis brings about various functional relevance in plants. The detailed analysis of the beneficial role of osmosis shall be dealt in the following section of the unit.

The main difference between diffusion and osmosis lies in the absence and presence of a semi-permeable membrane in the later case. Although diffusion is largely a physical process, physiologists prefer to associate it with biological systems. The term **Osmotic Pressure (OP)** is defined as the hydrostatic pressure developed by the virtue of solute concentration and sap accumulation within the cell. The outward pressure exerted by the cytoplasmic content towards the wall (membrane) is termed as **Turgor Pressure (TP)**. **Wall Pressure (WP)** is a counter inward pressure exerted by the cell wall. The **Suction Pressure** (**SP**) developed in any osmotically active cell is the net difference between the **Osmotic Pressure (OP)** and **Turgor Pressure (TP)**.

$$SP = OP - TP$$

For a fully turgid cell which has undergone endosmosis, the OP value is equal to the TP. Thus the cell has no net suction pressure.

$$OP = TP \text{ (Fully Turgid Cell)}$$

$$SP = \text{Zero}$$

This implies that the rate of osmotic movement of solvent continues until equilibrium is attained across the cell membranes in terms of osmotic concentration of cell sap.

Functional Relevance of Osmosis in Plants

- Primary plant growth and tissue turgidity is regulated by the process of osmosis in plants.
- Water absorption and cell to cell movement in roots is primarily driven by the process of osmotic phenomenon.
- Cell wall extension, organ growth, leaf expansion and root hair proliferation are commonly regulated by the osmotic drive of water and subsequent turgidity of cells.
- Stomatal regulation accomplished by the movement of guard cells is primarily an osmotic phenomenon modulated by various environmental signals or cues like blue light, ABA, CO_2 and K^+.
- Osmotic process largely regulates the overall health of the plant in respect to pathogen attack, cellular homoeostasis and biotic defence.
- Osmosis is capable of maintaining the hydrostatic pressure attained by the leaves and root tissues. It maintains the continuity of water column passing through xylem elements. The transpiration pull resulting from negative pressure gradient of water associates with osmotic uptake of water in the water driving process.
- Mechano-sensitive or photosensitive ion channels are associated with ion flux and subsequent osmotic movement in various plant organs, such as pulvinus, peduncle or leaf petiole. This process is associated with the trophic movements in various plant organs. The diurnal changes in the flaccidity or turgidity of the motor cells liberate movement of plant organs away from or towards the stimulus.
- Turgidity of young seedlings help them to expand their cotyledon and allow elongation of hypocotyl/epicotyl.

Water Potential

Understanding the fundamental process of water uptake and transport in plants is associated with the concept of water potential and its components in the biological system. Every living or non-living component in order to function by displacement possess certain degree of energy know as free energy. The potential energy stored among the water molecules provide a gradient to flow from its higher to lower concentrations. This free energy entrapped among the randomly arranged aggregates of water molecule is termed as **water potential**. The process of water uptake by plants driven by osmosis is largely accomplished due to the differences in the values of water potential. Biologically speaking, osmosis in plants involves movement of solvent component of the cell sap. The maximum possible value of water potential is observed for pure water (devoid of solutes) and considered to be zero. The water potential value for solvent (due to dissolved solutes) therefore decreases in its value and is mathematically expressed as a negative value less than zero. Thus, the differences in the water potential values are attained by the concentration gradient across cell membranes. This appears to be a driving force for osmotic movement of water. Water potential values indicate the general water status of the plant associated with soil moisture levels. **Water Potential** values are indicated by **Ψw (psi)**. The values lower than zero for any solvent is indicated by a negative sign **(–Ψw)**.

There are multiple components regulating water potential values in the cell. The effect of solute addition lowers the water potential values of a cell. The decrease in the solvent concentration attained due to the addition of solute is known as **Solute Potential (Ψs)**. The process of water uptake by cells results in the development of hydrostatic pressure. This component also regulates water potential values and is commonly known as **Pressure**

Potential (Ψp). Water movement against the gravitational force and up to the elevations vertically is also partially controlled by a factor known as **Gravity Potential (Ψg)**. The measure of solute potential of the cell is associated with the decrease in the free energy of water molecules induced by addition of solutes. This effect in the decrease in free energy is a result of the increasing entropy (Solute + Solvent) of the solution. Solute potential is also referred to as osmotic potential of the cell. The difference in the values of pressure potential across root to shoot and other aerial organs appears as the driving force for water transport through osmosis. However, when we consider the components of water potential in relation to cell to cell osmosis, the gravity component is often omitted because it exhibits negligible effects in comparison with osmotic and pressure potentials. To summarize, the value of water potential can be calculated as an additive effects of all the three components

$$\Psi w = \Psi s + \Psi p + \Psi g$$

For open systems devoid of membrane enclosures, the pressure component becomes almost similar to that of atmospheric pressure or sometimes negligible. During osmosis the attainment of concentration equilibrium on both sides of the membrane results in negligible value of Ψp almost equal to zero. At this phase both the solutions at opposite sides of the membrane are said to be in **isotonic** condition. Thus, at this stage the solute potential of the solution is similar to that of the water potential. The necessary method to calculate osmotic potential (solute potential) of a solution having non-dissociating solutes (for example Sucrose) is by applying **Van't Hoff equation**.

This equation involves the following four main components:

C - Concentration of the Solution (Molarity)

R - Universal Gas Constant (8.32 J mol.$^{-1}$ K^{-1})

T - Temperature of the Solution in Kelvin Scale

i - Dissociation Constant, usually a Value of 1 for Non-Dissociating Solutes

The application of negative value indicates the decrease in the water potential value caused due to addition of solutes with that of Pure Water (Zero). A Negative Hydrostatic Pressure/Pressure Potential (Ψp) can be evident in xylem columns or apoplastic spaces with less amount of water content.

$$\Psi s = - CiRT$$

For dissociating solutes the value of 'i' should be more than one and equal to the number of particles formed by dissociation in water. However, the limitation of this equation is that it works perfect for lower concentration of solutions with non-dissociable solutes. The equation finds routine practical application in laboratory experiments for determining isotonic concentration/solute potential of various plant tissues.

Significance of Water Potential

The measurement of water status of a plant in various environmental situations can be performed by calculating the water potential value of plant organs. Cell growth and turgidity, tissue proliferation and photosynthetic productivity are the major parameters regulated by water potential levels. The water status of soil regulates the water potential values in different plant organs. The process of water transport from roots to the aerial parts occur both by cell to cell osmosis followed by capillary pull through the xylem elements. Physiologically dry soils containing higher salt concentrations are not suitable for agricultural practice. Plants growing in such soils usually suffer from wilting and dehydration. The reason for such changes involves soil water potential values to remain much lower than that of root cells. Plants usually contain rigid cell walls surrounding the plasma membrane.

Thus increase in cell volume results in the formation of hydrostatic pressure within the cell. In the process of water movement from its higher to lower water potential there is usually no involvement of metabolic pumps. However, in case of phloem translocation the water movement coupled to transport of solutes may occur against the water potential gradient, thus, implying an energy driven process. Sucrose uploading occurs from the mesophyll tissue (source) with lower water potential and moves to sieve elements or root tissues (sink) with higher water potential. This process involves sucrose loading using metabolic pumps associated with flow of water in a opposite direction against its water potential gradient.

Chemical Potential

The term chemical potential refers to the state of free energy stored within water molecules which makes them capable of movement along a gradient. The aggregates of water molecules are not static in the liquid state but always in random movement around each other. This process results in continuous thermal agitation of the molecules which provide them the free energy. Thus chemical potential is an indication of the capacity of water to flow from its higher to lower concentration. In biological systems the chemical potential of water has been referred to as water potential by most physiologists. The unit for chemical potential is Joule in unit mole of substance ($J\ mol^{-1}$).

Imbibition

Plants often imply physical process of water transport which does not involve the presence of any differentially permeable membrane within it. Imbibition is such a process of adsorption which involves adherence of water molecules to the hydrophilic colloids of cell wall matrix and intercellular spaces. This process of water uptake is not an energy driven active process, but a passive physical process. Cell wall materials are usually composed of cellulose microfibrils (structural polysaccharides) interspersed within the pectin, hemicelluloses matrix (matrix polysaccharide). Thus water molecules due to their polar nature can easily intercalate between the cell wall layers due to their affinity with polar moieties of cell wall polysaccharides. Imbition results in the increase in volume of the plant material resulting due to intercalation of water.

The initial process of water absorption by root hairs starts with imbibitions of water to the cell wall layers, which is followed by other process of water uptake. The process of seed germination initiates through imbibitions. Thus, imbibitions results in formation of sufficient pressure within the layers of imbition. Earlier this was referred to as imbibition pressure which now physiologists prefer to call as Matrix Potential (Ψm). It is the force by which consumer molecules adhere to the surface of hydrophilic colloidal medium formed of polysaccharides. Thus matrix potential is usually more negative than the water potential. For any confined system, the water potential is equal to the summation of matrix potential and any other pressure in form of turgur pressure or pressure potential applied to the imbibant.

$$\Psi w = \Psi m + \Psi p$$

For any system where the imbibant is not confined the water potential equals to the matrix potential because no pressure or turgur force is associated with it.

$$\Psi w = \Psi m$$

1.3.1 Water Transport Process in Plants

Plants are capable of water uptake and transport to elevations as high as more than 10 ft from the ground. The Red Wood Tree of California (*Sequoiadendron* sp.) is one of the largest representative tree in the plant kingdom. Thus, mechanisms of water transport in

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plants have been a matter of brain storming physiological enigma to various plant physiologists since the last century. The process of water transport through root tissues is mostly facilitated by osmosis. Various mechanisms of water uptake may involve imbibition, diffusion, osmosis and active transport.

Water molecules are usually polar compounds which easily bind to each other by cohesive force of Hydrogen bonding. Thus water molecules are capable of forming long columns of network in their transport process. The composition of soil regulates the process of water potential gradient which remains as the main driving force of osmotic uptake across the cells. The process of xylem conduction is a capillary mediated pressure driven process. The negative hydrostatic pressure formed in root cells due to the uptake of water into xylem elements is termed as **Root Pressure**. This results in formation of a pressure gradient which involves bulk flow of water from the soil into the root tissues.

The bulk flow of water through the root tissues usually follows apoplastic, symplastic and transmembrane pathway. The process of symplastic transport is facilitated by desmotubule connections present between the cytoplasm of the cells.

Water conduction through xylem also regulates the process of phloem loading and unloading across the source and sink tissues of plants. The process of water movement associated with phloem translocation, however, occurs against the gradient. In such a condition, the xylem conduits involve bulk water flow to upper parts of the plant. In the mesophyll cells, water escapes in the form of vapour by exiting through stomatal guard cells at the foliar surface. This results in formation of a negative pressure in the leaves which in turn further draw water from the soil.

Thus, to appreciate the process of water transport, one needs to understand every phenomenon of water movement processes. Water uptake and transport therefore imply both physical and biological processes acting simultaneously or in association with each other.

Water availability for plants growing in various agricultural soils primarily depends upon the texture of soil and relative surface area of the soil particles. Sandy soil particles range from 500 μm to 1 mm in size while clayey particles are around 2 μm in diameter. The humus materials start accumulating in the O-horizon of the soil and mostly associate with the clayey particles. This humus-rich clayey soil contains larger surface area and subsequently higher water retention capacity among themselves. Moreover, soils suitable for agricultural practice must possess good drainage properties along with sufficient aeration. Sandy soils drain out too easily thus rendering dryness to the rhizosphere. Tightly packed smaller clay particles possess fewer channels among themselves which are too narrow to allow water escape.

Thus,in this type of soils the moisture is retained upto a greater extent. The moisture holding capacity of a soil is commonly termed as **field capacity**. It usually indicates the percentage of moisture held in a particular time span. To understand the primary process of water absorption by plant roots it is important to know the process of gradient mediated water flow in the soil. The process of water flow in the soil is largely a bulk flow of water facilitated by a negative hydrostatic pressure. This negative pressure is created by evaporation of surface water present in the soil-air interface. This allows further flow of water from the adjoining areas of the soil particles. Plants can uptake water mostly by the process of osmosis. The water potential of soil water is usually higher than that of root cell sap. The osmotic potential of soil is often negligible compare to that of roots. The capacity of water molecules to pass through the soil particles is known as soil hydraulic conductivity. Sandy soils contain loosely arranged particles and therefore possess higher hydraulic conductivity.

In the rhizosphere there is a unique association of water associated soil particles forming a film around the absorptive region of the roots. The primary and secondary lateral proliferation of roots results in the increase in surface area. The apical part of the root contains active absorptive zone comprised of the root hairs. These root hairs are fine epidermal extensions of the root tissue which contain fine plasma membrane and are metabolically active cells. The water entry into roots mostly occurs in the absorptive zone of the roots. The differentiated mature region of roots contain well developed exodermis or hypodermis region which undergoes lignification or suberization upon maturity. This layer of the root therefore becomes less permeable to water and nutrients. There are several external factors affecting water acquisition by plant roots. Nevertheless, plant height and foliage growth are important factors affecting water transport. Various factors operate in coordination to accomplish long distance transport of water from the roots to the aerial parts of the plant.

The anatomical features of the xylem components play a major role in water conduction in plants. The process of cell to cell osmotic uptake of water usually faces various resistances due to membrane and cell sap pressure. However, water transport through xylem elements is comparatively simpler and faces lesser resistance. However, blockages in the xylem conduits may result in severe water stress to foliar organs if persists for longer time. Plants, however, possess ways to compensate such loses. Another important regulator of water column in xylem is the transpiration pull and pressure gradient caused due to negative vapour pressure in the foliar surfaces of leaves. This factor is, however, subject to environmental or climatic variations. The following sections will thoroughly discuss each of the components associated with water absorption and transport in plants.

1.3.2 Water and Mineral Absorption from Soil

Water absorption by roots usually occurs through a combination of both physical and biological process. The soil particles are usually a mixture of sandy, clayey, silt and other organic matter. The roots uptake water through the absorptive zone of the root apex. The cell walls in the absorptive zone contain abundance of hydrophilic cell wall polysaccharides like pectin and cellulose. Root cells contain considerable volume of vacoular sap filled with various metabolites. This decreases the water potential of the sap thus allowing osmotic uptake of water from the soil. The absorptive zone of roots contains proliferated root hairs which serve as primary structures for water absorption. The root hair cells are metabolically active and thus allow exchange of water across their plasma membrane. Root hair cells are extensions of root epidermis and devoid of secondary deposition in their cell walls. The growing region of the roots above the quiescent centre is the main proliferation zone for formation of root hairs. Auxin gradient formed by basipetal transport through PIN efflux proteins are necessary for root hair formation. The differentiating zone of roots contains procambium cells being transformed into xylem and phloem elements. Beyond this differentiating zone the differentiated zone of roots contain thick exodermis which may contain deposition of lignin or suberin. This results in decrease in water absorption at the mature region of the root. The old and dead root hairs of the absorptive zone is replaced by new root hairs forming in the young apical region derived from root apical meristem

The root hairs form intimate contact with the water holding soil particles in the rhizosphere. The soil-air interface contains water particles which form small meniscus due to the surface tension properties of the water molecules. Flow of soil water across minute particles usually occurs by bulk flow process driven by negative hydrostatic pressure. The soil micelles are mostly negatively charged and they remain associated with cations and also

with water molecules forming Hydrogen bonds. By the virtue of adhesive forces water remains associated with the finer soil particles. The evaporation of water from exposed surface of the soil leads to further flow of water from adjoining areas of the soil. The osmotic potential of the soil particles is much lesser than that of the root cells. The hydrostatic pressure (pressure potential) being negative, the water potential value of soil water remains higher than that of root cell sap. The form of soil water available to the plant roots is termed as capillary water. This **capillary water** present among the soil particles is the majorly available form of water to the plants. **Gravitational water** obtained from rain and other sources usually percolates down into the aquifers and underground water reservoirs. **Hygroscopic water** is tightly associated with the soil particles which is hard to be removed during absorption by roots. Usually clayey soils rich in humus content bears higher water retention capacity (field capacity), ie., upto 40% moisture is retained even after 4 days of water saturation.

Mechanism of Water Absorption by Roots

The root hairs remain in contact with the soil micelles and therefore increase the surface area for absorption of water. Root hairs have been reported to constitute more than 60% of the surface area exposed in the apical region of the roots. The process of water absorption involves both active and passive absorption of water from the soil particles.

Detailed discussion on the pathway of water transport shall be dealt in the following sections of the unit.

1. Active Absorption

Active absorption of water involves expenditure of metabolic energy released through the process of respiration. Thus the process of respiration and sufficient aeration is very important for the roots in order to carry out water conduction into upper aerial parts of the plant. It has been observed that cyanide treatment (respiration blocker) results in inhibition of water absorption and transport through the roots.

Active Osmotic Uptake

Active osmotic uptake of water from the soil initiates after primary imbibition of water from the soil particles. The process of water uptake through osmosis occurs due to higher suction pressure developed in the root cells. The water potential of soil water being higher than that in root cell sap, water enters by the process of endosmosis. Further transport of water in the root tissue occurs by a process of cell to cell osmosis. Osmotic potential and turgor pressure are the important determinants which control the direction of flow of water. Root epidermis, cortex, hypodermis and vascular bundles possess differences in the water potential values. This difference is the main driving force for osmotic movement of water across the roots.

Active Non-Osmotic

Active non-osmotic absorption of water through roots can likely occur against the water potential gradient. The water potential value of soil may decrease in comparison with that of the root cell sap. This occurs usually in sodic or saline soils containing higher amount of Na, K or Mg salts. In such a situation, water transport against the water potential gradient occurs at the expense of metabolic energy produced by pathways like respiration. The process of active non-osmotic transport involves production of ATP molecules during the electron transport pathway of respiration. Investigations have revealed that cyanide or other metabolic inhibitors which block the pathway of respiration has also inhibited the process of active water absorption. Furthermore, auxins have also

been reported to increase the metabolic activity of the roots. The basipetal auxin gradient in the root apex is maintained by the activity of various PIN family auxin efflux proteins present in the plasma membrane.

2. Passive Transport

Passive transport of water from soil to the roots and up to the aerial organs is mostly associated with the negative pressure gradient formed in the leaves. This effect results from the phenomenon of transpiration which causes a loss in the water from foliar surface. Water molecules are capable of restraining tension force in the xylem conduits. Furthermore, they possess cohesive forces persisting due to extensive Hydrogen bonds among the molecules. This results in pull of water from the soil into the root cells. Aquaporin channels are also likely to be involved in the process of passive diffusion of water across the root membranes. The transpiration pull created in the leaves transmit the tension force created down to the xylem column. This causes obvious rise in the water column. Thus transpiration induced pull of water from soil into the plant does not involve any metabolic energy expended by the root tissues.

External Factors Affecting Water Absorption in Roots

Following are the factors that affecting water absorption in roots:

- **Soil Water Availability**

Soil water availability is the main factor which influences the rate of water transport across the roots. Field capacity of the soil depends upon the texture, particle size, humus accumulation and precipitation. Irrigated lands also differ in their moisture content depending upon the percentage of sand, silt and clay in the soil. The capillary form of water is the most easily available form to the plant roots. However, excessive water in the soils without proper drainage may lead to water logged anaerobic condition for the roots. This situation results in poor metabolic activity in the roots. Prolonged exposure to flooded soils causes partial or complete inhibition of water transport. This causes wilting of the plants.

- **Osmotic Potential**

Osmotic potential of the soil solution is an important determinant of osmotic water uptake through roots. Increased sodicity (NaCl, $NaSO_4$) or salinity (NaCl, KCl, $MgCl_2$) decreases the osmotic potential of the soil. This results in inhibition of water uptake due to osmosis. Although plant roots may imply non-osmotic uptake of water from the soil, alkaline or salt-stressed soils greatly reduce the probability of water absorption.

- **Soil-Air and Temperature**

Soil-air and temperature are edaphic factors which influence water permeability across membranes. Roots are metabolically active tissues which require an ambient supply of Oxygen from the soil-air interface. Physiologically dry soils containing high salt concentrations or water logged anaerobic condition results in Oxygen deficiency. Some plants contain adaptational mechanisms to develop aerenchyma formation in response to water logged anoxic conditions. This process is also regulated by ethylene formation in response to Oxygen deficiency. Increase in carbon dioxide concentration results in retardation of metabolic rate in the roots. Furthermore, temperature variations also influence water viscosity and membrane permeability across the root cells. Ambient temperature is essential to maintain optimum metabolic activity of the roots.

Various theories and evidences have been proposed to compare the importance of active and passive transport of water across the plant roots. It has been evidenced that gymnospermous plants growing in arid regions often exhibit poor magnitude of root pressure. Thus root pressure may not be sufficient to draw water upto high elevations. Actively transpiring plants have been reported to be able to absorb water even from dry or concentrated soil solutions. Physiologists consider the process of active water uptake in roots to be resulting from salt accumulation in the xylem elements. However, removal of salts from the xylem stream may result in non-osmotic uptake of water into the roots.

Soil mediated regulation of plant wilting largely depends upon the plant species growing in the soil. The amount of moisture present in the soil after the plant has permanently wilted is termed as **Permanent Wilting Percentage** or **Wilting Coefficient**. The value of wilting coefficient can be determined by observing a fully watered potted plant (soil covered with polythene) to wilt gradually after water uptake from the soil. The situation when the wilted plant does not recover even in presence of moisture is considered to be of permanent wilting. However, the moisture content which causes permanent wilting might depend upon the type of plant species. Roots of different plants are expected to show variation in the osmotic potential of their sap content. Thus the wilting coefficient value is not fixed for any soil content and largely depends upon the health and water status of the plant.

Pathway of Water Transport through Roots

The process of water transport across the roots occurs simultaneously by multipler routes of apoplastic, symplastic and transmembrane pathway. The apoplast refers to the free space in between cell wall and plasmamembrane or intercellular spaces which remain interconnected. The symplastic connection is maintained by the desmotubule/ plasmodesmatal connection between the cytoplasm of adjacent cells. However, apart from these two paths water may also enter the cell by crossing the plasma membrane which is referred to as the transmembrane pathway.

In the apoplastic pathway water molecules usually travel along the cell wall and in between the free spaces of the cells. In the symplastic pathway it travels thorug cytoplasm. In the transmembrane pathway it crosses the plasma membrane twice while entering as well as exiting from the cell. In this context it is worth mentioning that in the epidermis and cortex water follows all the three mentioned routes. However, during its passage through endodermis the apoplastic pathway is mostly blocked due to intercellular barrier of casparian strips. Casparian strips are thickenings composed of lamellar suberin depositedin the radial walls of the endodermis. Certain cells in the endodermis allow movement of water and are known as **Passage Cells**. The presence of casparian strips formed of suberin results in prevention of water and solutes through the apoplastic pathway. This restricts the entry of toxic heavy metals or other ions to enter into the xylem stream. Synplastic pathway or transmembrane pathway offers certain degree of selectivity in comparison with the free movement through apoplast. The process of suberiztaion in the endodermis starts at the differentiated region beyond the root apex. The part of the root in this region also exhibits differentiation of protoxylem elements formed from the procambium cells. Thus suberization in endodermis occurs simultaneously with the differentiation of protoxylem elements. This phenomenon results in symplastic pathway of water across the endodermis in comparison with apoplastic pathway present both in cortex and stele. The mature suberized region of the root may also uptake water through the breakages found in the cortical regions (Refer Figure 1.7).

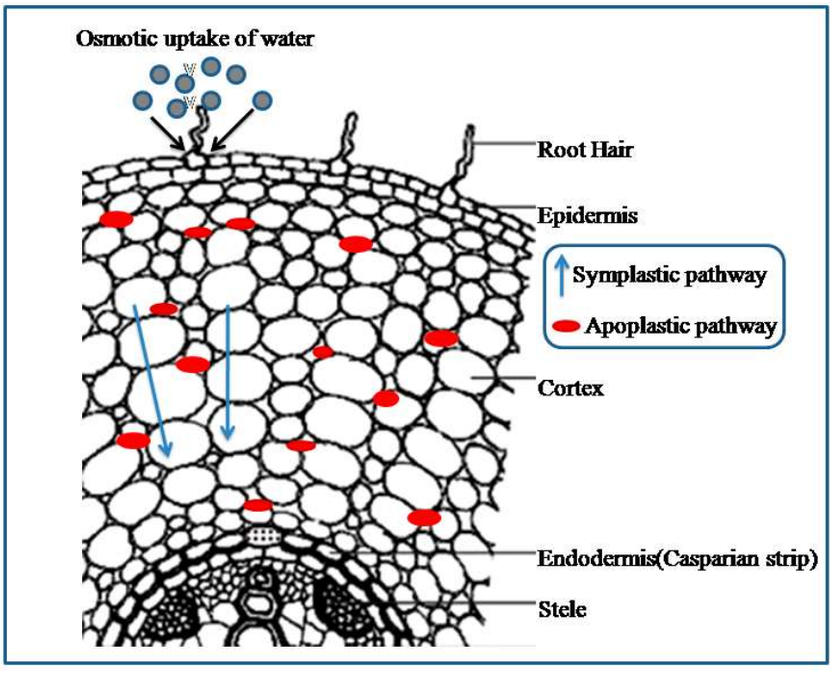

***Fig 1.7** Pathways of Water Uptake through Roots*

Solute Accumulation and Root Pressure Development in the Xylem Contributes to Water Uptake

The xylem sap in plants usually contains mixture of solutes dissolved in water. The bulk flow of water across soil particles is followed by osmotic uptake by root hairs. The accumulation of solutes in xylem decreases the Ψw of xylem sap. This results in further accumulation and uptake of water in the xylem. The cortical cells of root tissue containing lesser amount of solutes possess higher Ψw. Thus a positive hydrostatic pressure for flow of water develops in the root tissues. This is termed as the **Root Pressure**. The development of this hydrostatic pressure promotes water uptake into the xylem conduits. Solute accumulation in the xylem sap depends upon the solute composition present in the soil. Similarly, high transpiration rate may decrease root pressure and cause tension pull of water column within the xylem elements. Thus in order to accumulate root pressure the soil Ψw has to be sufficiently higher than that of root cell sap. Positive hydrostatic pressure develops in the aerial organs mostly during phases when the plant does not transpire. Such a case can be observed to be manifested in the form of **Guttation** (Refer Figure 1.8). It involves loss of water from the marginal areas of leaves in the form of water droplets. This phenomenon usually occurs early in the morning when the root pressure contributes to high hydrostatic pressure in the aerial organs. During this phase of the day the plant does not start to lose water by transpiration. The root pressure drives water up through the xylem.

Foliar tissues transport water at the marginal region which can escape through a loosely arranged tissue known as **epithem**. The opening through which the water escapes in the form of droplet is known as **hydathode**. Thus, evidences have unveiled the mechanisms of water transport through xylem. In this context it has been evident that positive xylem pressure is associated with high soil Ψw, solute accumulation in the xylem sap accompanied by lower rates of transpiration. However, the phenomenon of xylem mediated upliftment of water cannot be generalised because transpiration rate is variable and depends upon the duration of day, light intensity and climatic conditions. Moreover, the water status in the soil

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(available form) is also accounted to be a variable factor depending upon precipitation and amount of dissolved solutes. Thus solute accumulation and generation of root pressure are associated factors which partially contribute to ascent of water through the xylem elements. The root pressure can be experimentally visualized by cutting a well watered stem at a particular height. Considerable amount of root pressure may result in oozing out of sap at the cut end.

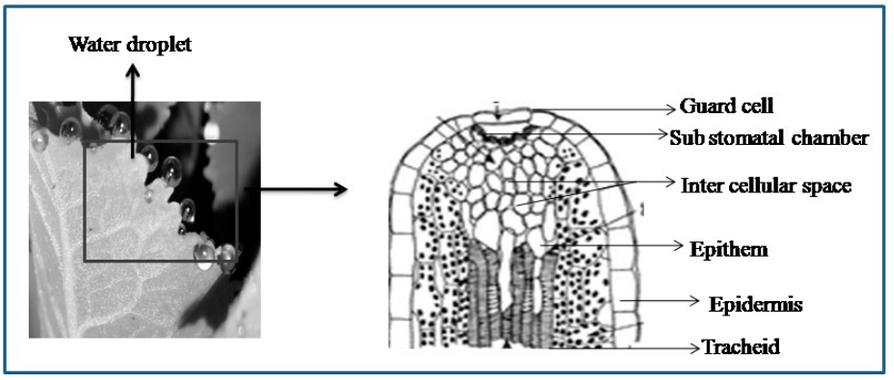

Fig. 1.8 *Process of Guttation*

Structural Features of Xylem Elements Facilitate Water Transport

Xylem elements in plants constitute a unique example of complex tissue comprised of parenchymatous and sclerenchymatous cells functioning in association with each other. The main function of water conduction in the xylem is performed by tracheids and vessels (trachea). These elements by the virtue of secondary wall thickenings also provide rigidity to the plant organs. The soft parenchymatous cells of xylem (ray and xylem parenchyma) contribute to lateral transfer of xylem sap and solutes/minerals in the stem. Almost 99% of water reaching the aerial organs has been evidenced to be transported by xylem. The two major components of xylem, i.e., tracheids and vessels possess anatomical features which facilitate water transport process across them. Tracheids are narrow elements with chisel shaped ends placed obliquely end to end. The ends of each tracheid contain simple or complex perforation plates. The secondary wall thickenings of the tracheids contain various ornamentations appearing in different forms, i.e., annular, spiral, scalariform or reticulate. The bordered pits are important part of the tracehids which facilitate water transport across them. These are regions which lack secondary cell wall. The primary wall areas degenerate by the process of programmed cell death. The bordered pits of two adjacent tracheids remain associated side by side often separated by a pit membrane. In some conifers a valve like thickening is present in the central region of the pit which is termed as **torus**. It acts as a valve to prevent invasion of gas/air bubble into adjacent tracheid elements. This is an important phenomenon which prevents air blockage to spread into neighbouring xylem elements.

Vessels are usually shorter and broader in shape compare to the tracheids. Unlike tracheids their end walls are free at the two ends. They possess pits which help in exit and entry of xylem sap. Vessels also exhibit similar kinds of secondary wall thickenings and pitted structures along its walls. However, due to their structural wideness they appear to serve as better conducting elements than tracheids. The diameter of vessels is wider while tracheids are narrow in width. Moreover the tracheids are placed end to end in oblique series which may offer certain amount of resistence to the water flow. Anatomists have hypothesised the origin of vessels to have resulted from differentiation of tracheids. Tracheids are universally present in all groups of vascular plants. However, vessels have been reported in certain Gymnosperms (Gnetales and Ephedrales) and mostly in Angiosperms. Some

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primitive families of Angiosperms (Winteraaceae and Trochodendraceae) have been observed to lack vessel members. During ascent of sap both the vessels and tracheid elements are capable of exchanging the water stream across the pits (Refer Figure 1.9).

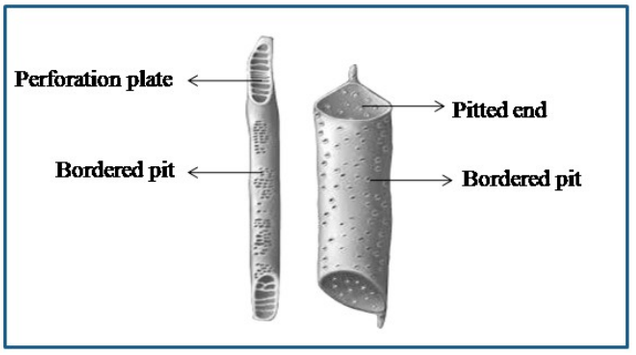

Fig. 1.9 *Structure of Tracheid and Vessel, Tracheids (Left) are Narrow with Perforation Plates at the Ends*

Water Transport through Xylem

The process of water transport across the xylem conduits is comparatively simpler and less resistible in comparison with that across extrastelar cells. Since tracheids and vessels function as hollow dead conducting strands, there are lesser reasons for water molecules to face obstruction. The sole factor associated with xylem mediated water transport is the driving force or root hydraulic conductivity which sets the magnitude of upward directed hydrostatic pressure. Evidences have revealed the speed of xylem sap ascent to be around 4-5 mm s^{-1}. However, water column through xylem may face obstructions due to the presence of perforation plates in the tracheids. Moreover the secondary wall thickenings across the tracheids and vessels are not always uniform. Hence the speed of ascent of sap is variable and depends upon the nature of resistance obtained. Furthermore, the root pressure or driving force for water uptake also influences the rate of sap translocation.

Nevertheless water transport through xylem elements is far more spontaneous than through living cells. Xylem tension pull can be experimentally demonstrated by puncturing the stem surface with a ink droplet. The pull created in the xylem elements will pull the drop inside the stem which can be visualized in the form of streaks. The secondary thickenings present in the tracheids and vessels facilitate to restrain from collapsing of the elements. Water conduction is associated with sufficient tension force or pressure drive which the xylem elements can easily withstand. Physiologist have analysed the magnitude of driving force or pressure required to drive the water column even upto a height of more than 90 meters. The tallest Red Wood Gymnosperms (*Sequoia* sp.) can grow upto a height of nearly 100 meters. The pressure or water force should be sufficient to overcome the frictional force produced during water uptake from soil and through the xylem elements. The pressure requirement has been stated to be near about 2 MPa in magnitude. Additionally a gravitational force operates for higher water columns sustaining in the xylem elements. Thus, considering such a margin physiologists state that 3 MPa pressure gradient between the top and base of the stem shall be sufficient to overcome both the components of frictional and gravitational forces. Briefly, water transport through xylem is largely a case of pressure driven uptake facilitated by root pressure, solute accumulation in the sap and also transpiration pull. The continuity of water column and the tension force that they withstand is by the virtue of their physical properties.

Plants are Capable of Overcoming Xylem Cavitation and Blockages

Roots can generate positive hydrostatic pressure which pulls the water column up through the xylem conduits. However, plants face certain degree of physical obstructions created in the xylem elements. Water column rising through xylem elements face tension force created by upward transpiration pull or pressure gradient. In this context it is likely that air molecules can pass through the minute pores present in the cell wall of xylem elements. Moreover evidence has suggested that xylem elements may form bubbles due to lower solubility of gases dissolved in water. This is due to the fact that high tension force causes the dissolved gases to form undissolved bubbles in the water column. This may also occur due to freezing of water column in cold temperatures. It is likely that the gas bubbles once formed in the xylem column will expand in length and volume. This phenomenon of xylem elements being blocked by gas or air bubble is termed as **cavitation** or **embolism**. This situation further results in complete or partial blockage in the transport of water column. The pit membranes of adjacent or neighbouring trcaheids and vessels are helpful in this regard. The water column can divert its pathway without further cavitation in the neighbouring xylem elements. However, prolonged and repetitive cavitation occurring in the xylem elements can result in more lethal consequences to the plant. Water stress and dehydration in the foliar tissues may thus lead to permanent wilting.

Plants are however capable of overcoming the physical resistance caused by cavitation. The tracheidal walls are interconnected by pit membranes. These connections prevent the transmission of air bubbles to the neighbouring tracheids. Water column can easily divert its pathway to the neighbouring conduits of xylem. Physiologists have also reported the possibilities of gas bubbles getting dissolved with time ad diurnal fluctuations. Situations when the plant does not transpire much of the tissue water, the xylem Ψp increases in magnitude. This results in dissolving of the air bubbles within the transportable sap. Furthermore, plants with normal and seasonal secondary growth develop new xylem conduits which substitute the older and blocked xylem strands.

The Cohesion-Tension Theory and its Critical Evaluation

The root pressure formed during water uptake in the xylem remains near to 0.1 MPa. Such lower magnitudes of hydrostatic pressure may not be sufficient to drive water to heights in more than 5 meters tall trees. The cohesion tension theory was initially proposed by **John Joly** and **Henry Horatio Dixon in the year 1894.** It explains the phenomenon of water transport across xylem elements. The theory is based upon two components operating during water transport through vessels and tracheids. The uprising water column is pulled by the creation of tension force caused due to negative hydrostatic pressure. Foliar transpiration from stomatal slits cause to create a negative Ψp in the leaves. Moreover the mesophyll cells contain sucrose and other osmolytes which decrease the Ψw. This further leads to the movement of water molecules actively from the xylem elements to upper foliar tissues of the plant. The inter-molecular forces of water aggregates persisting due to Hydrogen bonding allows the water molecules to tolerate the tension force created due to negative hydrostatic force. Thus, transpiration pull mediates the tension force created which is in turn resisted by the cohesive force present among the water molecules. These two components of cohesion and tension forces are jointly attributed to the process of water uptake through xylem (Refer Figure 1.10).

Various experimental evidences have been obtained to validate the effectiveness of the theory. Arguments have been put forward as to whether the water molecules are capable of enduring the amount of tension force created by transpiration pull. However, modification and repetition of refined experiments using pressure probe method have revealed that water

molecules can withstand the tension pull created by foliar tissues. Furthermore it was evidenced that both transpiring and non-transpiring leaves involved in experiments were capable of creating tension force in xylem elements. Physiologists have therefore acknowledged the theory to be sound and effective for explaining water uptake through xylem elements.

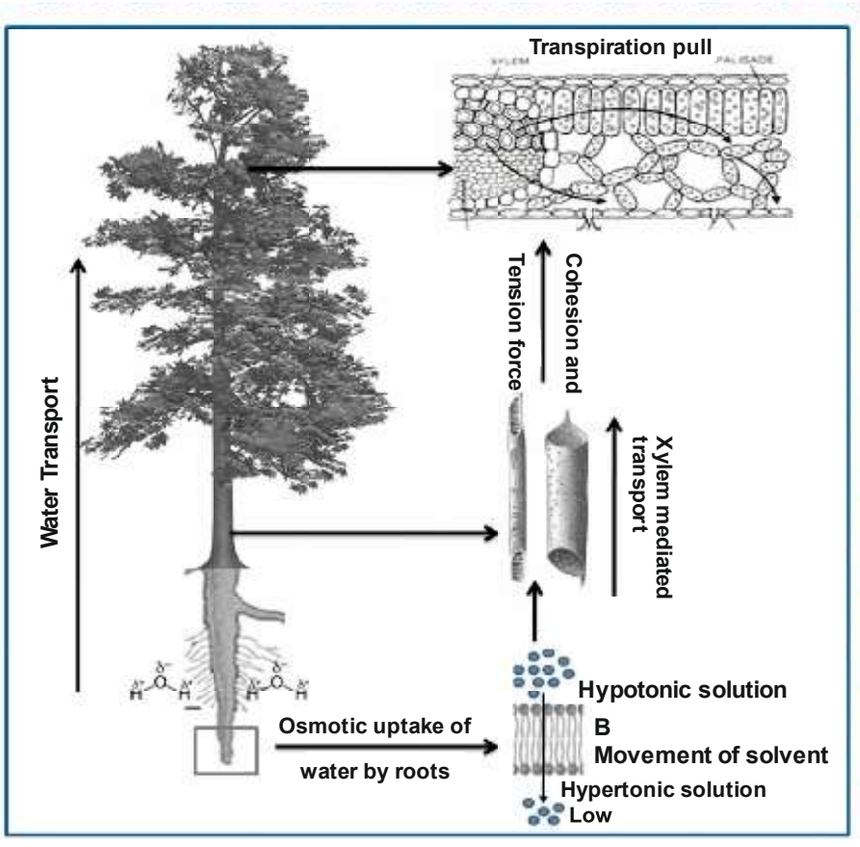

Fig. 1.10 *Components of Cohesion-Tension Theory*

The Soil-Plant Atmosphere Continuum Concept

The process of water transport from soil to the atmosphere via the plant is studied and analysed using the soil-plant-atmosphere continuum concept. The process of water uptake from the soil to plant body occurs along a gradient of pressure difference. The soil solution usually contains higher Ψw which allows the osmotic uptake of water from soil to plant root. The osmotic uptake of water and its transport across root tissues is followed by pressure driven upload into the xylem conduits. The aerial organs of plant usually possess lower values of Ψw which drives the water up into the foliar tissues. The mesophyll cells possess air space in small interstices among the cells which allow escape of water in the form of vapour from the stomatal slits. The process of transpiration is also governed by the relative vapour pressure around the leaves. Thus the connectivity of water uptake, transport and its loss by transpiration operates from the soil, through the plant and into the atmosphere. Therefore the continuum concept explains the interrelation between the processes occurring as a flow towards pressure gradient. External atmospheric vapour/relative humidity controls the rate of transpiration and subsequent tension force causing to draw the water column across the xylem elements.

Na^+/K^+ ATPase Pump

This electrogenic P-type membrane pump carries out the coupled transport of three Na^+ ions outside and two K^+ ions inside the cell. It is a transmembrane heterodimeric Protein and

comprises of **α** and **β** subunits, forming intracellular and extracellular domains and five pair of transmembrane helices. The α and β subunits have four isoforms each, expressed in tissue and cell specific manner, and they vary in their sensitivity towards Sodium and the steroid glycoside ouabain (inhibitor of Na^+ /K^+ ATPase in animals). The pump carries out a coupled ion transport accomplished by the hydrolysis of ATP and a subsequent phosphorylation event. It requires Mg^{2+}-ATP as its major substrate and has affinity towards specific ligands. The α subunit is 110 kDa in size and comprises of about 1000 Amino Acid residues. It has four isoforms (α_1, α_2, α_3 and α_4) which differ in their functional properties. The α_1 subunit is a housekeeping isoform expressed in most of the tissues. The saline environment triggers the quantitative expression of specific isoforms depending upon their Sodium binding affinity. The α subunit consists of five pairs of transmembrane helices forming the cation transport path and three cytoplasmic domains, which have the binding sites for Mg^{2+}-ATP and Na^+. These cytoplasmic domains are specified as nucleotide binding sites, phosphorylation site and a actuator site. These transmembrane helices move or rotate along to transport ions across the membrane. The α subunit is an active component of the pump carrying out phosphorylation and ion transport. The β subunit is 55 kDa in size composed of 370 Amino Acid residues, of which 30 Amino Acids form a cytosolic loop and 300 Amino Acids are folded to form the extracellular domain. The β subunit has three consecutive glycosylation sites on its extracellular folds. This subunit does not contain any active site for the substrate. It, however, interacts with the α subunit to restore the native structure of the enzyme. There are three S-S bonds present in the extracellular folds of the subunit necessary to provide the K^+ occlusion state of the enzyme.

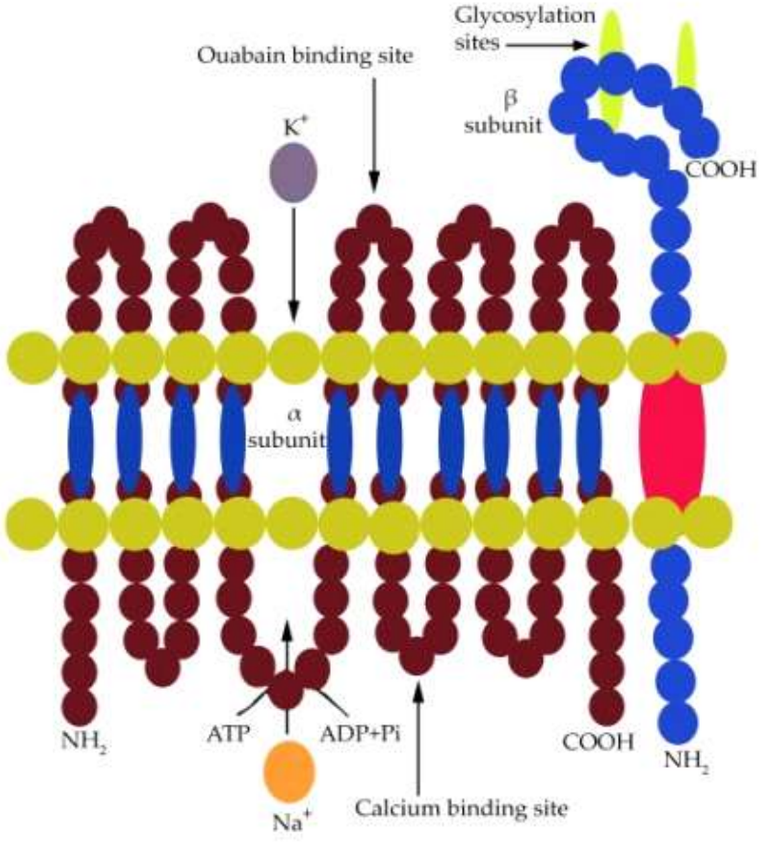

Fig. 1.11 *Structure of the Na-K ATPase*

The pump operates through a cycle of phosphorylation-dephosphorylation events accompanied with Na^+ and K^+ binding and hydrolysis of ATP. Sodium binding at the cytosolic loop of the α subunit initiates the Sodium-dependent phosphorylation of the pump, accomplished by hydrolysis of previously bound ATP. This results in the formation of an acyl-phosphate complex between the Pi of ATP released and a specific Asp residue or As portable residue, an α-Amino-Acid residue. This phosphorylated state of the pump, commonly stated as $[E_1P]$, now excludes three Na^+ ions out of the cell. Two Potassium molecules then bind to the extracellular fold of the enzyme and the enzyme attains $[E_2P]$ state. This is followed by occlusion of K^+ into the cell and subsequent dephosphorylation of the pump. Ouabain is a specific glycoside which can bind to the phosphorylated α subunit of the enzyme in the $[E_2P]$ state after it has excluded three Na^+ ions (Refer Figure 1.11).

Inhibition of the ATPase by Calcium

Calcium is a regulator of the Sodium pump. It inhibits the pump at the physiological concentration range of 0.08-5 μM. In excitable cells, depolarisation causes an increase in $[Ca^{2+}]_{cyt}$, which crosses the threshold level and inhibits specific isoforms of the pump in a concentration and affinity-dependent manner. Calcium at higher concentrations can, however, inhibit the pump in general, irrespective of the isomer. The mechanism of inhibition due to calcium can be explained as follows:

- The α subunit of the enzyme is associated with calcium inhibition, with α_2 isomer having greater affinity for calcium at physiological levels of 0.08-5 μM or even at higher concentration of 10 mM.
- Ca^{2+} in the concentration range of 10^{-5} M competes with magnesium for ATP and lowers the concentration of Mg^{2+}-ATP, which is a rate limiting substrate for the enzyme.
- Calmodulin and Calnaktin are the two Calcium-Binding Proteins which mediate the process of Calcium inhibition of this enzyme.
- Calmodulin and Calnaktin lower the threshold level of Calcium-Mediated inhibition from 100 μM to 2-5 μM of Ca^{2+}.
- These Proteins are unable to inhibit the pump in the absence of Ca^{2+}. Calcium-dependent inhibition of the pump is initiated by Calmodulin and Calnaktin and they interact with at least one or more Proteins in the membrane or cytosol to bring about inhibition of the pump.
- The process of Ca^{2+} inhibition associated with the α subunit of the enzyme is non-competitive and it does not compete with the binding site for Na^+.

'Check Your Progress'

7. Define the term DPD.
8. What is permanent wilting percentage?
9. What is osmotic pressure?
10. How are solute potential, suction pressure and surface tension different from each other?

NOTES

1.4 SUMMARY

- Different plants are adapted to diverse habitats and they vary in their morpho-anatomical characters, such as stomatal position, stomatal frequency and water usage efficiency.
- Transpiration index of a plant and its stomatal frequency determines the rate of water loss for the plant.
- Glycophytes and mesophytes possess normal transpiration rate and water use efficiency. However, xerophytes have adaptive measures in terms of stomatal count, transpiration rate and conservation of water content.
- Physiologists prefer to mention transpiration as a necessary evil for the plant.
- Dorsiventral leaves contain stomata either on both surfaces (amphistomatic) or on lower surface (hypostomatic) so as to regulate the rate of transpiration.
- The comparison in the rate of transpiration with respect to evaporation from a unit surface area is termed as transpiration index. It indicates the differences in the rate of biological and physical process.
- The process of transpiration is regulated by osmotic water uptake, followed by pressure driven diffusion.
- Transpiration causes changes in the vapour pressure in the intercellular spaces of mesophyll cells.
- Dry soils or osmotically unsuitable soil content may lead to paucity in the water content of plant organs. In such a situation high transpiration rates may appear detrimental to the plant.
- Abscisic Acid (ABA) and Salicylic Acid are two biological antitranspirants used as foliar spray. The synthetic antitranspirants commonly applied to plants are Phenyl Mercuric Acetate (PMA), Fungicides or Herbicides.
- The structure of stomata is unique in terms of its cytoskeletal arrangement and differential thickening of cell walls. In case of guard cells the arrangement is different and forms a pattern called as radial micellation.
- The major physiological mechanism of guard cell functioning is regulated by osmotic adjustment of the guard cells.
- Some succulent plants undergo Crassulacean Acid Metabolism (CAM) and exhibit adaptive mechanisms to reduce transpiration and conserve water in tissues.
- The process of water flow in the soil is largely a bulk flow of water facilitated by a negative hydrostatic pressure.
- The anatomical features of the xylem components play a major role in water conduction in plant.
- The roots uptake water through the absorptive zone of the root apex.
- The root hair cells are metabolically active and thus allow exchange of water across their plasma membrane.
- The root hairs form intimate contact with the water holding soil particles in the rhizosphere.
- This capillary water present among the soil particles is the majorly available form of water to the plants.

- Root hairs have been reported to constitute more than 60% of the surface area exposed in the apical region of the roots.
- Active osmotic uptake of water from the soil initiates after primary imbibition of water from the soil particles.
- Passive transport of water from soil to the roots and up to the aerial organs is mostly associated with the negative pressure gradient formed in the leaves.
- Physiologically dry soils containing high salt concentrations or water logged anaerobic condition results in Oxygen deficiency.
- The amount of moisture present in the soil after the plant has permanently wilted is termed as permanent wilting percentage or wilting coefficient.
- The amount of moisture present in the soil after the plant has permanently wilted is termed as permanent wilting percentage or wilting coefficient.
- Water conduction is associated with sufficient tension force or pressure drive which the xylem elements can easily withstand.
- Roots can generate positive hydrostatic pressure which pulls the water column up through the xylem conduits.
- The uprising water column is pulled by the creation of tension force caused due to negative hydrostatic pressure.
- The process of transpiration is also governed by the relative vapour pressure.
- Plants are capable of absorbing water through the fine capillary networks present in the xylem tracheids and vessels.
- The vessels originate from a group of procambium cells which convert into hollow conducting elements by the process of programmed cell death.
- The continuity of water column through the xylem strands is maintained by the process of transpiration pull attained by the leaf mesophyll tissues.
- Plant cells contain an outer elastic plasma membrane which is semi-fluidic in nature.
- The unique property of water as a universal solvent is also regulated by the partial electropositive and electronegative charges present at the two ends of the molecule (O and H atoms).
- Water molecules are usually polar compounds which easily bind to each other by cohesive force of Hydrogen bonding.
- Thermodynamically the process of water diffusion in plants is regulated by its available chemical free energy.
- Osmosis as a biological process appears to be the major determinant of water uptake and transport through plants.
- Plants absorb water available in the soil in form of a phenomenon known as endosmosis.
- Extra-physiological concentrations of solutes accumulating in the rhizosphere render plants incapable of accumulating compatible metabolites in that range of concentration.
- Osmosis is capable of maintaining the hydrostatic pressure attained by the leaves and root tissues.
- The necessary method to calculate osmotic potential (solute potential) of a solution having non-dissociating solutes (for example, Sucrose) is by applying van't Hoff equation.

NOTES

1.5 KEY TERMS

- **Amphistomatic leaf**: Leaves which contain stomata on both dorsal and ventral surfaces.
- **Hypostomatic leaf:** Leaves having stomata on the ventral surface.
- **Epistomatic leaf**: Leaves having stomata on the dorsal surface.
- **Stomatal frequency**: It is defined as the number of stomata in unit area of leaf.
- **Active absorption:** Process of absorption which involves expenditure of metabolic energy released through the process of respiration.
- **Capillary water:** The water molecule present in minute interstices of soil particles**.** The form of soil water available to the plant roots.
- **Epithem:** Foliar tissues transport water at the marginal region which can escape through a loosely arranged tissue called epithem.
- **Root pressure:** Positive hydrostatic pressure for flow of water which develops in the root tissues.
- **Torus:** In some conifers a valve like thickening is present in the central region of the pit membrane of tracheids.
- **Cavitation/embolism:** The phenomena of xylem conduits being blocked due to air cavity.
- **Chemical potential:** The term chemical potential refers to the state of free energy stored within water molecules which makes them capable of movement along a gradient.
- **Compatible solutes:** In response to external low water content in the soils plant roots are capable of synthesizing drought-induced metabolites.
- **Diffusion pressure:** The pressure developed due to movement of molecules from its higher to lower concentration along a gradient.
- **Diffusion:** The effect of thermal agitation or kinetic energy allows molecules/water to move from its region of higher to the region of lower concentration.
- **Gravitational water:** Obtained from rain and other sources usually percolates down into the aquifers and underground water reservoirs.
- **Hygroscopic water:** Tightly associated with the soil particles which is hard to be removed during absorption by roots.
- **Imbibition:** A process of adsorption which involves adherence of water molecules to the hydrophilic colloids of cell wall matrix and intercellular spaces.
- **Incipient plasmolysis:** The initial stage of retraction of protoplasm from the cell membrane resulting due to exosmosis.
- **Osmosis**: In this process, water (solvent) moves from its region of higher concentration (low solute concentration) to the region of its lower concentration (higher solute concentration).
- **Turgor pressure:** The outward pressure exerted by the cytoplasmic content towards the wall (membrane).
- **Water potential**: The potential energy stored among the water molecules provide a gradient to flow from its higher to lower concentrations.

NOTES

1.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Water stress management of plants is partially depended upon the foliar arrangement of a plant. The leaves depending upon their arrangement in the stem axis are categorized into dorsiventral and isobilateral types. The anatomical features in these type of leaves differ in terms of mesophyll differentiation and stomatal distribution. Thus, transpiration rate not only depends upon external climatic conditions but also on the morpho-anatomical feature of the leaf. Dorsiventral leaves contain stomata either on both surfaces (amphistomatic) or on lower surface (hypostomatic) so as to regulate the rate of transpiration.
2. Cuticular transpiration is a minor process which involves loss of water in vapour form exiting from the cuticle layer of the leaves. However, the amount of water lost through this process is very negligible in comparison with stomatal transpiration. The water molecules driven up by pressure flow accumulate in and around the mesophyll cells which move to the epidermal region and evaporate from the cuticular layer present in the outer surface of foliage. This process of transpiration does not account for more than 10% of water loss. However, the amount of cuticular transpiration also depends upon the anatomical feature of the leaf, i.e., cuticle thickening, surface area and tissue water content.
3. Lenticular transpiration involves loss of water from the lenticular openings present in the bark or stem and also in fruits. The formation of lenticels depends upon the age or maturity of the plant organ. This pathway of transpiration also involves very minor amount of water loss through evaporation.
4. The process of transpiration includes the following stages of advancement of water flow from the xylem to leaf tissue:
   - Osmotic uptake of water from the xylem conduits into the mesophyll cells.
   - Pressure driven diffusion.
   - Diffusion mediated flow followed by evaporation.
5. The intensity and irradiance of light perceived by the foliar surface affects the rate of transpiration. This process is primarily mediated by the regulation of stomatal aperture which in turn regulates the rate of water loss. Plants exhibiting Crassulacean Acid Metabolism (CAM) usually keep their stomata closed at day time.
6. Transpiration index is the comparison in the rate of transpiration with respect to evaporation from a unit surface area. It indicates the differences in the rate of biological and physical process.
7. Diffusion Pressure Deficit (DPD) is he difference/decrease in the diffusion pressure of a solvent in comparison to pure water resulting due to dissolution of solutes in pure water.
8. Permanent wilting percentage/wilting coefficient is the amount of moisture present in the soil after the plant has permanently wilted.
9. Osmotic pressure can be defined as the hydrostatic pressure developed by the virtue of solute concentration and sap accumulation within the cell.

10. Solute potential is the decrease in the solvent concentration attained due to the addition of solute, while suction pressure is the net difference between the osmotic pressure and turgor pressure and surface tension is the formation of a surface layer at the air-water interface. The force required to expand or contract the surface is termed as surface tension.

1.7 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Define stomatal frequency and transpiration index.
2. Name one natural and one artificial antitranspirant. Explain its applications.
3. How are CAM plants unique in their stomatal regulation? Mention its significance.
4. For a cell kept in an isotonic solution (0.2 mm) of Sucrose at a temperature of 25°C, find the value of its water potential.
5. Define cohesion-tension theory.
6. Explain the following terms:
   (i) Capillary Water
   (ii) Embolism
   (iii) Field Capacity
7. Define wilting coefficient.
8. Explain the mechanism of water transport in plants.
9. What value of suction pressure do you expect for a fully turgid cell? Provide reasons to support your answer.

Long-Answer Questions

1. Explain the phrase 'Transpiration is a Necessary Evil'.
2. Explain the effects of external CO_2 and blue light on regulation of stomatal movement.
3. Explain the mechanism of transpiration. Discuss various factors affecting transpiration.
4. Describe the structure of stomatal apparatus with labelled diagram. Schematically explain various endogenous and exogenous regulators affecting stomatal movement.
5. Differentiate between transpiration and guttation. Explain the types of transpiration.
6. What is water potential? Explain its significance in plant-water relations.
7. Explain the components affecting water potential of a cell. Differentiate between water potential and matrix potential.
8. Explain the significance of diffusion and osmosis in plants. What do you mean by diffusion pressure deficit and solute potential?
9. Define turgor pressure. Explain its significance in water uptake by roots. How is suction pressure related to turgor pressure of a cell?
10. Define root pressure. Describe briefly the pathways of water uptake through roots.

1.8 FURTHER READING

Hopkins, William G. and Norman P.A. Huner. 2013. *Introduction to Plant Physiology*, 4th Edition. Noida: Wiley India Pvt. Ltd.

Verma, S K and Mohit Verma. 1995. *A Textbook of Plant Physiology, Biochemistry and Biotechnology*. New Delhi: S Chand & Company Ltd.

Blackstock, James C. 2014. *Guide to Biochemistry*. Oxford: Butterworth-Heinemann.

Park, K. H. 2008. *Carbohydrate-Active Enzymes: Structure, Function and Applications*. Amsterdam: Elsevier.

Fromm, Herbert J. and Mark Hargrove. 2012. *Essentials of Biochemistry*. Berlin: Springer.

Fearon, William Robert. 2014. *An Introduction to Biochemistry*. Amsterdam: Elsevier.

NOTES

UNIT 2 PHYSIOLOGY: GROWTH HORMONES, SEED GERMINATION AND PHOTOPERIODISM

Structure

2.0 INTRODUCTION

The idea of chemical messengers involved in regulating plant growth, morphogenesis and various other physiological aspects originated as early in the eighteenth century. Animal systems produce various hormones which are produced and transported to long distance sites within the body. Plants produce hormones which act as the major signaling molecules or inducers of various responses.Moreover, the hormone is expected to exhibit dose-dependent or receptor-mediated response in various plant tissues. So far various hormones have been identified and their receptors have been characterized in plants.

Seed germination is a coordinated event of embryo emergence from the seed coat and formation of young seedling. The seed harbours the young embryonic plant which matures prior to emergence from the seeds. The process of seed germination initiates with the emergence of radical (root primordial) from the seed. In physiological sense, the germination process starts with seed hydration followed by increased respiration coupled to growth and emergence of seedling. Certain seeds after attaining maturity can germinate in the presence of moisture, Oxygen and other favourable conditions.

Autumn and spring seasons bring the appearance of colourful blooms of a variety of flowers, attracting tourists and botanists all over the world. Mechanisms of flowering and seed dispersal indicate the evolutionary success of Angiosperms. Flowering occurs in several seasons depending upon the geographical locations (Alpine, Temperate and Tropical vegetation). Some plants have perennial or annual life cycle, while some are biennial in their life cycle.

In this unit, you will study about growth hormones, role of phytohormones in plants, biosynthesis and signaling of hormones in various physiological processes, seed germination, seed dormancy, seed viability and photoperiodism.

2.1 UNIT OBJECTIVES

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

- Understand the role of phytohormones in plants
- Explain the biosynthesis and signaling of hormones in various physiological processes
- Identify the concept of seed development
- Explain the physiology of seed germination
- Understand the process of induction of flowering
- Understand the genetic regulation of formation of floral meristem
- Appreciate how plants respond to photoperiodic signals

2.2 GROWTH HORMONES

The idea of chemical messengers involved in regulating plant growth, morphogenesis and various other physiological aspects originated as early in the eighteenth century. According to German botanist Julius von Sachs, the chemical messengers regulate plant development. Furthermore, he also proposed that the gravity sensing mechanisms may also be associated with the polarity-induced growth in plants. Animal systems produce various hormones which are produced and transported to long distance sites within the body. Plants produce hormones which act as the major signaling molecules or inducers of various responses. According to the modern concept an effective phytohormone should ideally possess a specific receptor. Moreover, the hormone is expected to exhibit dose-dependent and receptor-mediated response in various plant tissues. So far various hormones have been identified and their receptors have been characterized in plants.

However, new morphogens like brassinosteroids have been recently reported to exhibit properties of phytohormone. New investigations in the field of plant signaling and development have provided clues on upcoming biomolecules (Serotonin, Melatonin, Hydrogen Sulphide, Nitric Oxide) which may be categorized as phytohormones.

Auxin was the first phytohormone to be discovered in plants. The action of auxin was established in terms of cell elongation and cell wall loosening. Investigations with various mutants have elucidated the complex regulation of Auxin and Cytokinin in regulating various plant processes. The phytohormones exhibit temporal and spatial regulations in terms of their biosynthesis, action and metabolism. Early experiments by Went have revealed the role of auxin in regulating shoot apex curvature. This was followed by extensive work on various physiological and molecular aspects related to auxin functioning in plants. The discovery of Gibberellins in 1950s appeared from observations of Backanae Disease in Rice. The discovery of other phytohormones like Cytokinin, Abscisic Acid (ABA) and Ethylene were related to

tissue culture experiments, characterization of molecules and observation of triple response respectively. ABA and Ethylene have been observed to modulate the process of stress signaling, fruit ripening and regulation of stomatal movements. The current unit shall focus on the extensive account of phytohormone discovery, biosynthesis and their molecular mode of action in plants. The synthetic chemicals implied as artificial hormones possess immense importance in plant breeding, horticulture and other biotechnological manipulations.

2.2.1 Auxins: Discovery and Chemical Nature

In the early period of nineteenth century, Charles Darwin and his son Francis investigated the phenomenon of phototropic curvature manifested as an effect of Asymmetric Auxin distribution in the shoot apex. This observation led to the conclusion that the growth region of coleoptiles was localized in the subapical region which responded to the direction of blue light. The work by Darwins was published in 1881 in the book '*The Power of Movement in Plants*'. Later investigations by Frits Went in 1926 revealed that a morphogen or chemical is present in the *Avena coleoptiles*. Went was successful in performing the experiments with Agar blocks and cut ends of Oat Colepotiles. The apical part of coleoptiles were cut and dipped in the agar medium and kept for a considerable amount of time. The agar blocks used for dipping the coleoptiles are placed into cut stumps of coleoptiles. The intensity of curvature produced by application of agar blocks was proportional to the amount of chemical accumulated in agar block and the time period of its application. This experiment for bioassay of auxin is termed as the Avena Curvature Test. However, characterization of the biomolecule from the oat coleoptiles was difficult due to its sparse quantity.

The word 'Auxin' has been derived form greek word '*Auxein*' which means to 'Increase or Grow'. The application of agar block in the Avena Curvature Experiment was applied asymmetrically which was responsible for their growth curvature. Later investigations by Kogl (1934) reported the characterization of biomolecules from human urine and *Rhizopus* culture which was called as Auxin.

The biomolecules characterized thus appeared to be indole acetic acid in its chemical nature. Later on various derivates of IAA (Indole -3-Acetaldehyde, Indole-3- Pyruvic Acid, Indole-Acetontrile) have been indentified in plant tissues. The central ring of auxin is comprised of an indole ring supplemented by various functional groups (Refer Figure 2.1).

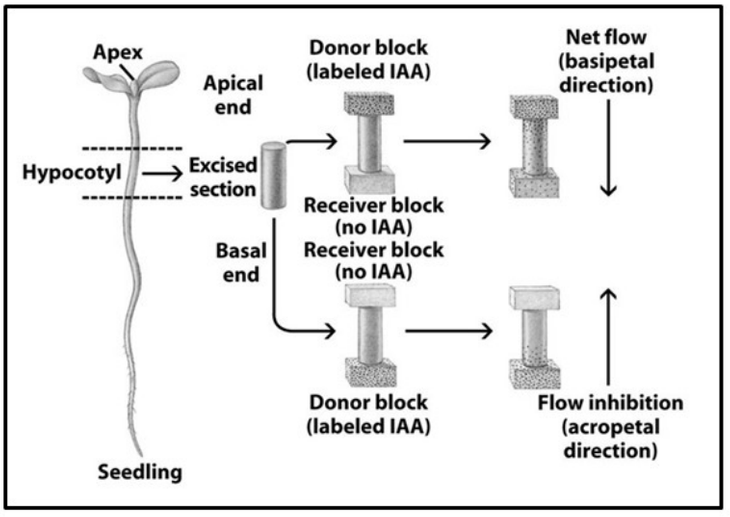

Fig 2.1 *Bioassay of Auxin*

Biosynthesis of Auxin

Auxin is an important plant hormone synthesized in most of the meristematic tissues especially in the shoot apices. However, fruits, seeds and young leaves are also potent sites for IAA production. IAA is chemically much similar to the Aromatic Amino Acid Tryptophan. The biosynthetic pathway of auxin has been reported to be accomplished through multiple pathways. Figure 2.2 illustrates the pathways of Auxin Biosynthesis

• IPA Pathway

The Indole-3-Pyruvic Acid (IPA) pathway is one of the common paths of auxin biosynthesis. IPA is formed by the catalytic activity of tryptophan transaminase. The biochemical reactions involved in this pathway are deamination reaction to form IPA followed by decarboxylation to form Indole-3-Acetaldehyde (IAld). IAld is metabolized to form IAA catalyzed by auxin dehydrogenase enzyme.

• TAM Pathway

The tryptamine pathway is almost similar to the IPA pathway but exhibits a reverse order of deamination and decarboxylation reaction catalyzed by different enzymes. Tyrptamine is formed by the enzyme tryptophan decarboxylase which then gets metabolized to IAld catalysed by Amine Oxidase.

The following step of IAA formation is similar to that of the IPA pathway.

• IAN Pathway

The Indole-3-AcetoNitrile (IAN) pathway involves conversion of tryptophan into Indole-3-Acetaldoxime catalyzed by Tryptohan Monooxygenase. Indole-3-Acetaldoxime is converted to Indole-3-Acetonitrile and then IAA.

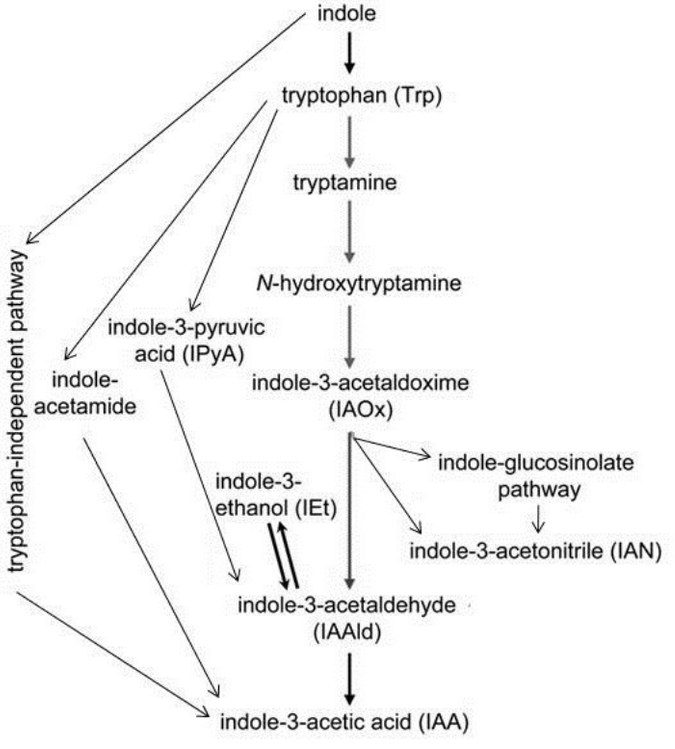

Fig. 2.2 *Pathways of Auxin Biosynthesis*

All the pathways are tryptophan dependent and multiple pathways may exist in same plant tissues. The IAN pathway is prevalent in three families of Brassicaceae, Musaceae and Poaceae. Certain reports also suggest the formation of auxin through Tryptophan-independent pathways. One of the important reports on tryptophan-independent pathway has been revealed from orange pericarp (*orp*) mutant of maize. IAA is likely to be synthesized from Indole and Indole-3-Glycerol Phosphate. IAN and IPA have been reported to be the possible intermediates of the tryptophan independent pathway. However, the immediate precursor of IAA is yet to be deciphered. The prevalence of Tryptophan-dependent and Tryptophan-independent pathways depend upon the developmental stage of the plant tissues.

Distribution and Transport of Auxin in Plants

Auxin is widely distributed in the plant tissues and its relative concentration varies in plant organs. A majority of auxin forms present in the tissue remains in the bound or conjugated form in the cells. Investigations have revealed the conjugate forms of auxin to be associated with low and high molecular weight molecules like myo-inositol, glucose or glycoprotein residues. *Zea mays* have been reported to exhibit glucose-auxin conjugation on various tissues. The intensity of the conjugated form depends upon the activity of the conjugating enzyme. The apical meristems and young leaves are the primary sites of auxin synthesis. Thus metabolism of conjugated auxin is a major factor which affects the availability of free auxin in the cells.

Environmental stimulus like light, gravity has been reported to regulate the hydrolysis of conjugate auxins to form free auxins. However, conjugate auxins are important in the storage and protective functions which prevent oxidation of other biomolecules. IAA degradation by oxidation may be accomplished by multiple pathways. Oxindole-acetic Acid has been reported to be one of the end products of auxin oxidation. In certain plant tissues peroxidative enzymes may be involved in the pathway of IAA oxidation. IAA oxidation has been reported to be regulated by enzymatic activity and light intensity. The distribution of IAA is mostly associated with the pH of the cytosol and localized in the cytosol and chloroplast. The IAA^- form is impermeable through the membrane while the IAAH form can freely cross the plasma and chloroplast membrane. Auxin is more likely to accumulate in the alkaline compartments of the cells. One third of the auxin amount is localized in the chloroplast while the rest is present in the cytosol. The conjugate form of auxin present in the chloroplast is in equilibrium with the forms present in the cytosol. Monocotyledonous seedlings have been reported to possess high auxin concentration in the coleoptiles while roots possess lower amounts.

The pattern of auxin transport is usually a polar gradient exhibiting acropetal or basipetal pattern of behaviour. In stem the auxin transport is basipetal in nature. The shoot-root polarity of auxin regulates a variety of developmental processes like stem elongation, leaf senescence, would healing and regulation of abscission. Physiological investigations have also revealed that a considerable amount of auxin is transported across the phloem. The polar transport of auxin is guided by a energy dependent and gravity regulated process. PIN Efflux Proteins present in the plasma membrane form a polar gradient mediated by the activity of cytoskeleton elements. The average velocity of polar auxin transport has been recorded to range from 5-20 cm. h^{-1}. The vascular parenchyma cells are associated with the polar transport of

auxin. The mechanism of polar auxin transport has been reported to be accomplished by two mechanism:

- Passive Diffusion of IAAH
- H^+-IAA^- Antiport Mechanisms

The undissociated form of auxin, i.e., IAAH is lipophilic due to its protonated nature. AUX 1 (permease type of carrier) are the important auxin uptake carriers responsible for Polar Suxin Transport or PAT. NPA (1- NaphthylPhth-alamic Acid) and TIBA (2,3,5-TriiodoBenzoic Acid) are the two commonly implied polar auxintransport inhibitors. These molecules prevent the process of auxin efflux in plants. Flavonoids have also been identified as auxin transport inhibitors. The PIN Efflux Proteins are rapidly recycled in the cytoplasm by the help of vesicles. The transport of Auxin across the phloem tissue is non-polar in nature.

Physiological Effects of Auxin

Following are the physiological effects of Auxin:

• Cell Elongation

Elongation of cells in the shoot and root region are the primary effects of auxin. The phototropic curvature of shoot tips mediated by blue light receptor (cryptochrome) causes asymteric distribution of auxin in the shaded side of the shoot. This results in formation of a curvature. The higher concentration of auxin at the subapical region of the seedlings regulate plant elongation in both dicotyledonous and monocotyledonous plants. The phenomenon of auxin-induced growth can be initiated within a lag time of 10-15 min. This rapid response is accomplished by acidification of cell wall. The dissociation of proton conjugated auxin results in the formation of IAA^- and H^+ in the cytosol. The excess protons loberated are extruded from the plasmamembrane.

This results in increased apoplastic pH. This event has been commonly explained by Acid hypothesis of cell wall growth and extension. The cell wall in the presence of lower pH undergoes extension or loosening followed by new cellulose deposition. Extensins and Xyloglucan Endo Transglycosylase (XET) are important enzymes regulating cell wall extension and growth. Auxin mediated gravity sensing in plants is regulated by asymteric distribution of statoliths (gravity sensing starch containing cells).

• Apical Dominance

The phenomenon by which the growth of terminal or apical bud in vascular plants dominates or reduces the growth of lateral buds is termed as apical dominance. The reason behind such an observation is due to higher concentration of auxin in the subapical region of the shoot apex. Decapitation of the shoot apex results in proliferation of the axillary or lateral buds. However, the phenomenon of apical dominance due to auxin has been reported to be reversed by cytokinin or ABA application.

• Root Initiation

The differential concentration of auxin present in the stem and root regulate the rate of shoot and root elongation. The higher concentration of auxin in roots inhibits the process of elongation but induces more number of lateral root branching. The application of synthetic auxin in the cut shoots promote rooting in horticultural and gardening practices.

- **Regulation of Abscission**

The formation of abscission layer of leaves and fruits is prevented by the activity of auxin. The abscission layer formed during ripening fruits is associated with the crosstalk mechanism involved between Auxin and Ethylene.

- **Parthenocarpy**

The process of parthenocarpic fruit formation is modulated by higher concentration of auxin in the ovaries. In this case the concentration of auxin is already higher in the pre-fertilization phase. Usually fruits develop due to increased auxin concentration at the post fertilization stages. However, in parthenocarpic fruits it occurs prior to the process of fertilization.

- **Respiration**

Physiological investigations have revealed a correlation between the growing organs exhibiting higher rate of respiration. Thus, the process of respiration is likely increased by the activity of Auxin. This has been attributed to increased source of ATP.

- **Callus Formation**

The role of auxin has been established in the process callus formation. The abscission layer formed in various plant organs or certain injured tissues undergo callus formation induced by the activity of auxin. The control of callus formation in tissue culture is also regulated by the relative rate of auxin concentration.

- **Vascular Differentiation**

The activity of auxin is essential in the formation of xylem and phloem tissue from the procambium cells. Furthermore, the activity of cambium and its seasonal growth is regulated by auxin activity.

The genetic control of auxin activity has been reported to be regulated by Auxin Response Factors (ARFs) which act as transcriptional activators of auxin response elements in the DNA. The AUX/IAA gene family Proteins in turn act as inhibitors of ARF.

2.2.2 Gibberellins: Discovery and Chemical Structure

The discovery of Gibberellins dates back to 1920s when a Japanese scientist Kurosawa investigated the physiological effects of Backanae Disease in Rice caused by the Fungi *Gibberella fujikuroi*. The rice seedlings affected by the disease exhibited slender and taller length. Later in 1935 Yabuta isolated the active substance and characterised it as Gibberellins. Yabuta and Sumiki (1938) reported the isolation of Gibberellins in two forms Gibberellin A and Gibberellin B. In 1950s the characterization and isolation of this hormone occurred in a commercial scale. The presence of Gibberellins was confirmed both in fungal and plant extracts. The various forms of Gibberellins are tetracyclic diterpene acids formed of Ent-Gibberellane skeleton with 19 or 20 C compounds. The various forms of Gibberellins vary in their structure in terms of oxidation state of carbon atom and position of hydroxyl group. Interestingly, the C19 GA molecules with 3-β-hydroxylation have been reported to be more active than C20 GA molecules.

Metabolic Regulation of Gibberellin Biosynthesis

The gibberellins are chemically terpenoids in nature formed of condensation of 5C precursor molecule known as Isopentenyl Pyrophosphate. This molecule undergoes series of conversions to form Ent-Kaurene by isomerisation and condensation to form Gibberellins. The process of Ent-Kaurene formation occurs in the Plastids which then migrates to endoplasmic reticulum and forms various forms of gibberellins. The formation of Gibberellin Acid (GA); GA_{19} or GA_{20} is followed by its oxidation and 3-β- hydroxylation to get converted into active forms.

Thus the process of GA formation is accomplished in three stages:

- Formation of terpenoid pathway and Ent-Kaurene in Plastids.
- Oxidation to form GA forms in the ER.
- Formation of other forms of GAs in the Cytosol (GA_{12} or GA_{53}).

Thus multiple components of the cells are involved in the formation of GA in plants. The main sites of GA synthesis are usually among developing fruits and seeds, young leaves, buds, and apical parts of shoots and roots. The enzymes and genes associated with GA biosynthesis pathway have been characterised. The GA-oxidase enzymes have been reported to be regulated by various environmental factors in plants. The various forms of the enzyme are regulated by 2-oxoglutarate and Fe^{2+} as cofactors. Active gibberellins are present in the free form in the cells. However, various forms of gibberellins form covalent conjugates with sugar molecules, especially Glucose.

This form of GA has been found to be prevalent in some seeds. The glycosylayated or glucosidic forms of GA are inactive in nature and remain in stored form in plant tissues. The GA_1 form of the hormone is essentially involved in the process of shoot elongation. The leaky mutants of pea plants have been investigated for the role of GA_1 in stem elongation. The gibberellins biosynthetic pathway is regulated by a mechanism of feed-back inhibition. The threshold of GA levels maintained in the cells is due to activation and inactivation of the transcription factors associated with GA synthesizing genes. Lower levels of GA in the cell triggers the process of GA biosynthesis (Refer Figure 2.3).

Moreover, environmental factors like photoperiods, temperature and sucrose level play important role in regulating the rate of GA biosynthesis. The light-dependent synthesis of GA is the primary mechanism which regulates flowering in long and short day plants. The transition of vegetative to floral meristem (floral evocation) is regulated by GA biosynthesis. Similarly, GA mediated responses can be mimicked by cold temperature (vernalization) which promotes GA biosynthesis.

The transport of GA in plants is non-polar in nature. The long distance transport of GA in the form of glycoside conjugates is mostly accomplished by phloem tissues. However, xylem mediated lateral transport of GA has also been reported. The monocot seeds exhibit movement of GA from scutellum to the aleurone layer of endosperm. This results in the triggering of α-amylase activity and subsequent mobilization of starch into soluble sugars. Plant tissues exhibit unique mechanisms for regulating the active levels of GA in cells. Formation of 2-β hydroxyl group in GA reduces its biological activity. Various other complex forms of sugar or thiol conjugates convert active GA into inactive forms.

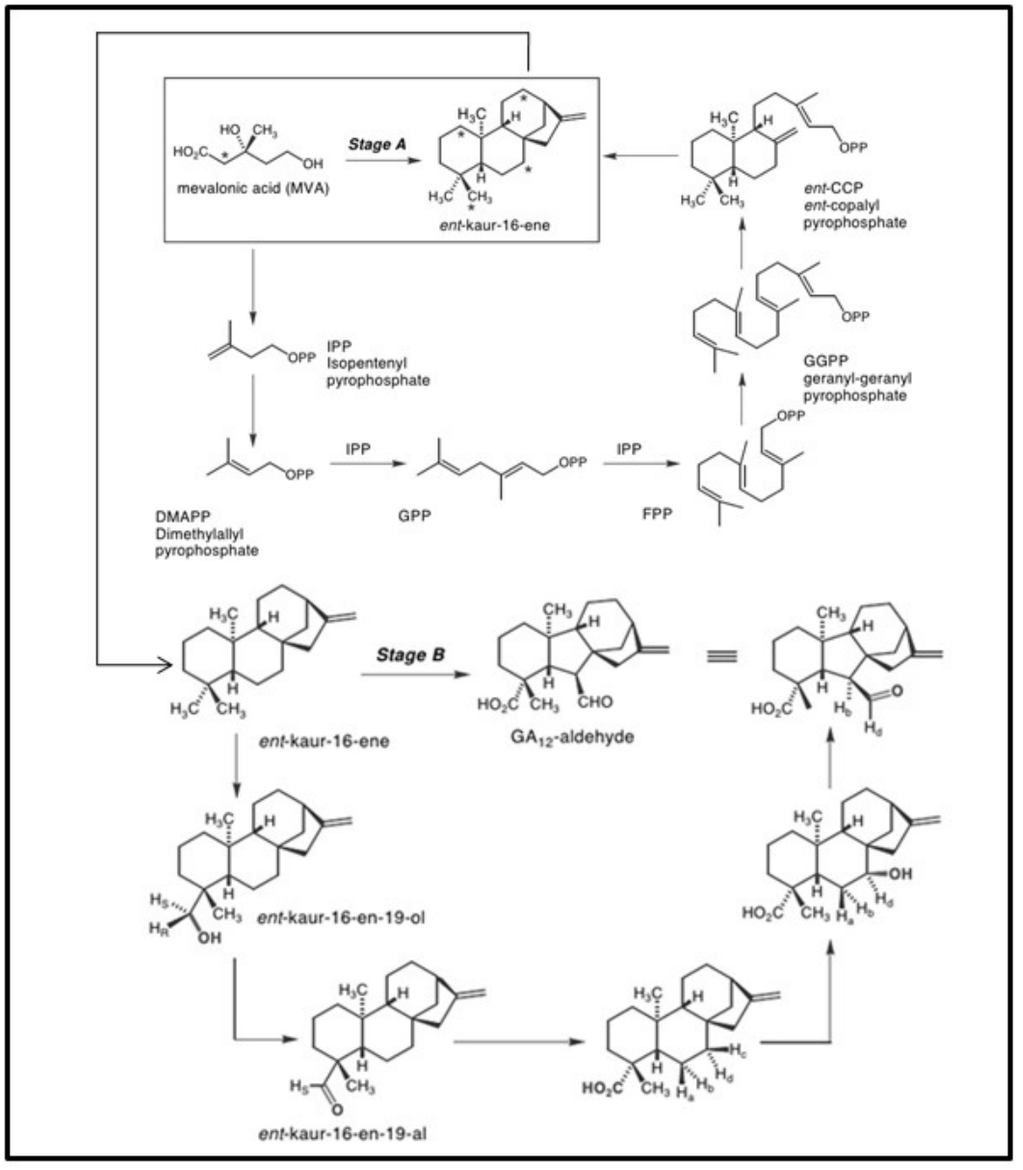

Fig. 2.3 *Pathway of GA Biosynthesis*

Physiological Effects of Gibberellins

Following are the physiological effects of Gibberellins:

• Seed Germination and Root Growth

Photoblastic seeds usually imply the GA-dependent pathway of germination in presence of light, for example, Lettuce and Tobacco. The dark dependent inhibition of photobalstic seeds can therefore be reversed by the effect of GA applications. GA usually tends to show very negligible effects on root growth. In certain cases it has been reported to be inhibitory for inducing root growth in stem cuttings.

• Bud Dormancy

Cold temperature mediated natural bud dormancy has been reported to be released by GA application. GA has also been reported to induce faster generation of sprouts from freshly harvested Potato Tubers. Thus GA helps in overcoming bud dormancy.

• **Intermodal Elongation**

GA induces elongation of intermodal regions of plants which may even appear effective for genetic dwarf varieties. Various dwarf varieties have been reported to be deficient in GA biosynthesis or possess higher concentrations of natural GA inhibitor. Such conditions can be overcome by exogenous GA application. Interesting observations have been obtained for stem elongation phenomenon exhibited by partially water submerged rice plants. Anaerobic conditions in the root trigger ethylene biosynthesis which markedly reduces ABA levels (GA antagonists) and induces GA formation.

• **Flowering Response**

The phenomenon of photoperiodism exhibited by long and short day flowering plants is controlled by the light-dependent endogenous regulation of GA biosynthesis. Various species of long day plants can be triggered to flower by GA application even at short day intervals. The phenomenon of bolting or vernalization (elongation of shoot meristem to form floral meristem) is induced by the application of GA or by cold temperature induced endogenous GA synthesis.

• **Parthenocarpy**

Formation of parthenocarpic fruits and pollen germination is stimulated by the activity of GA. Some fruits where auxin appears to be ineffective have been induced for parthenocarpic development by GA application. This technique finds application in horticultural practices of parthenocarpic grape and tomato development.

• **Activation of α-Amylase and Seed Germination**

Starch containing cereal seeds involve activation of α-Amylase Enzyme in the aleurone layers. The transport of GA from the scutellum to the aleurone layer triggers the transcription of GAMYB (A transcription factor) elements necessary for expression of α-Amylase Proteins.

2.2.3 Cytokinins: Discovery and Chemical Nature

The discovery of Cytokinin relates to the search of plant based factors regulating cell division and growth of plant organs. The physiological role of Cytokinin has partial similarity to Auxin and Gibberellins. The first report of Cytokinin in plant sources dates back to 1950 when Miller coincidentally reported a cell division promoting factor in degraded old DNA material. The Purine compound found in the DNA samples was reported to be 6-Furfurylaminopurine which was termed as Cytokinin or Kinetin. As the name suggests the hormone has potential role in cell division and cytokinesis. Zeatin in corn grains and coconut endosperm extract are two of the potential sources of cytokinin. Zeatin has been stated as the most abundant and active form of cytokinin which is chemically known as Trans-6-(4-Hydroxy-3-Methylbut-2-Enylamino) Purine. The molecular structure of Zeatin is similar to that of Kinetin in being the Adenine and Amino Purine derivatives.

In higher plants, Zeatin exists both in the cis and trans configuration which are interconvertible by the enzyme zeatin isomerase. The trans form of zeatin has been reported to be more active in plant tissues, although the cis form is also involved in certain physiological processes. Glucosyl transferase activity specific to the cis zeatin form has been detected in plant tissues. The purine forms of cytokinin isolated from various plant and bacterial species differ in the presence of their functional group associated with the Nitrogen or Carbon atom at various positions.

Moreover cytokinins may exsist in plants in the glycosidic forms. The presence of cytokinin is universal to tRNA molecules present in most of the organisms. Certain plant tRNA contain trans zeatin as a form of the modified base. Bacterial and fungal species associated with plant have been reported to possess various forms of cytokinin. These forms incude trans-zeatin, cis-zeatin and ribosides involved in plant-microbe ineractions. Crown gall forming bacteria like *Agrobacterium tumefaciens* and *Corynebacterium fascians* secrete cytokinin forms which stimulate the formation of Crown Gall or Witche's Broom like abnormalities in plants. The structure of cytokinin contains an isoprene unit. The precursors of isoprene units are derived from the mevalonic Acid/pyruvate pathway. The initial step of cytokinin synthesis involves transfer of isopentenyl group from dimethylallyl diphosphate to the adenosine ring. Physiologists have reported the possibility of tRNA acting as the sources of free cytokinin.

Root apical meristems are one of the major sites for cytokinin formation in plants. The long distance transport of cytokinin from the roots to the shoots mostly occurs through the xylem stream along with water and inorganic minerals. Environmental factors like water stress, nutrient deficiency and osmotic imbalance may result in decreased levels of cytokinin in the shoot.

However, supplementation with exogenous nitrate source has been reported to increase the levels of cytokinin. The transportable form of cytokinin present in the xylem stream is mostly in the form of zeatin riboside. Cytokinin transported to leaves and seeds are converted to glucosidic forms. The glucosidic forms of cytokinin are mostly inactive due to their chemical nature and compartmentalization in the cells. The regulation of cytokinin levels in the cell is associated with its inter-conversion into nucleotide and other purine forms. Cytochrome oxidase has been reported to regulate the levels of cytokinin in tissues. The irreversible reaction catalyzed by the enzyme inactivates the forms of cytokinin. Thus the activity of the enzyme is mostly induced in higher levels of cytokinin in the tissue. The gene encoding cytochrome oxidase has been indentified from Maize and Arabidopsis. The regulation of cytokinin activity has also been reported to be controlled by conjugation reactions. The Nitrogen present in the 3rd, 7th and 9th position of the Purine ring is likely to be conjugated to glucose. The hydroxyl group present in the side chain of cytokinin is also subject to conjugation with glucose units.

Physiological Effects of Cytokinin

Following are the physiological effects of cytokinin:

• Cytokinin Regulates Growth by Controlling Cell Cycle Components

The process of shoot and root growth has been reported to be controlled by the activity of cytokinin. Overexpression of cytokinin oxidase in plant tissues has been reported to exhibit decreased growth and elongation in the transgenics. The control of cell cycle exerted by cytokinin mostly operates through the regulation of cyclin-dependent kinase Proteins. Higher levels of cytokinin have been observed at the completion of synthetic, mitotic and G_1 phase of cell cycle.

• Auxin-Cytokinin Ratio Determines Growth in Tissue Culture

Usually higher levels of cytokinin appear to be inhibitory to root growth while it promotes shoot development. Plant tissue culture practices therefore imply a variable amount of auxin and cytokinin which regulates callogenesis, shooting and rooting for various explants.

• **Regulation of Apical Dominance**

Cytokinin modulates the effect of apical dominance by promoting the growth of axillary or lateral buds. The extent of apical dominance is usually regulated by the concentration of auxin in the subapical regions of the shoot. However, application of cytokinin has resulted in profuse growth of lateral buds which can complete with the growth of apical shoot. Cytokinin overproducing mutants have been reported to exhibit increased growth of lateral buds. Various plants like *Begonia, Tortella* and *Bryophyllum* respond to the application of cytokinin manifested by early proliferation of buds. *Funaria hygrometrica* has been reported to undergo higher rate of bud formation in the protonema manifested as an effect of cytokinin and light intensity.

• **Regulation of Leaf Senescence and Chloroplast Biogenesis**

The process of programmed death or senescence in detached plant leaves can be partially inhibited by the application of cytokinin. Usually senescence of plant leaves involve a rapid loss of RNA, Proteins and other nutrient reserve. Experiments by Richmond and Lang (1957) confirmed the role of cytokinin in delaying senescence in isolated leaf discs of Xanthium. This anti-senescence effect of cytokinin has been termed as the Richmond Lang effect.

Interestingly, for foliated plants application of cytokinin results in remobilization of nutrients to the hormone treated organs. This produces a gradient of source-sink relation across the plant. Thus, nutrient reallocation may appear as one of the important determinants of cytokinin mediated delay in senescence.

Moreover, cytokinin promotes chloroplast development in de-etiolated seedlings. Exposure to cytokinin treatment improves expansion of Grana and Protein development in the chloroplast.

2.2.4 Ethylene: Discovery, Biosynthesis and Chemical Nature

The discovery of Ethylene as a gaseous hormone resulted from observations at the Botanical Institute at Saint Petersbutg, Russia. The Russian graduate student Dimtri Neljubov (1901) observed the growth of etiolated seedlings manifested by reduction in stem elongation, increased lateral growth and nonexpasion of cotyledon (hook formation). This phenomenon was termed as the Triple Response. Dimtri identified the effect to be resulting from ethylene present in the coal gas emission of laboratory air. The plants were found to regain normal growth upon exposure to normal environmental conditions. In 1910, H.H.Cousin published the reports of ethylene to have been identified as a natural gas. Advancements in the gas chromatographic technique led to conclusions that ethylene is a phytohormone exhibiting significant effects on physiological processes.

Ethylene has been reported to be produced by various plant organs. The rate of ethylene production depends upon the age and nature of plant organs. Meristematic and nodal region of plants are the active sites of ethylene biosynthesis. Various physiological conditions associated with leaf abscission, flower senescence, fruit ripening and abiotic stress triggers ethylene biosynthesis in plants. Ethylene is biosynthesized from the precursor methionine which is metabolized to 1-Amino- Cyclopropane -1-Carboxylic (ACC) Acid catalyzed by the enzyme ACC synthase.

Methionine is the primary precursor in the pathway which gets converted to S-Adenosylmethionine catalyzed by the enzyme Adenosine Methionine Synthase. ACC formed from Adenosyl Methionine is converted to Ethylene catalyzed by ACC Oxidase. Ethylene is a simple olefinic gaseous hormone lighter than air (M.W. 28). Ethylene is oxidized to Ethylene

Oxide which liberates CO_2 in the cells. Ethylene is a freely diffusible biomolecule passing through the membranes and intercellular spaces. The surge in ethylene formation associated with fruit ripening can be measured by its absorption with Potassium Permanganate ($KMnO_4$). Atmospheric composition of gases include significant amount of ethylene obtained from plants and microorganisms.

The senescing and ripened sues produce higher amount of ethylene which may be more than 1 ng. gm^{-1}. FW^{-1}. Injured tissues or abscission results in higher emission of ethylene within a time span of 30 min. Certain strains of bacteria including *Escherichia coli* produce higher amount of Ethtylene from the precursor Methionine. Most of the plant groups are capable of producing ethylene in various tissues and organs.

Ethylene biosynthesis is regulated by the Yang Cycle where the CH_3-S group of methionine is recycled. This provides the sulphur pool necessary to maintain ethylene formation from the primary precursor S-Adenosyl-L-Methionine. Physiological investigations have proved that conversion of ACC into ethylene does not occur in anaerobic conditions. Formation of ethylene increases in the presence of aerobic conditions and during exogenous ACC supplementation. This observation implies that ACC biosynthesis appears to be the rate limiting step in the pathway of ethylene biosynthesis. Characterization of ACC synthase has been performed from various plant tissues. ACC synthase has been reported to be encoded by a multi-gene family. The enzyme is cytosolic in location and constitutes a very low percentage of the total Protein constituent. Abiotic factors like temperature, flooding or drought stress has been reported to alter the activity of ACC synthase. Endogenous levels of auxin have also been observed to modulate the activity of ACC synthase. Tomato tissues have been observed to express nine genes of ACC synthase regulated during wounding, fruit ripening or wound response. ACC oxidase which catalyzes the conversion of ACC to ethylene has also been stated to be a rate limiting step of ethylene biosynthesis. The enzyme has been found to be a Fe^{2+}-ascorbate family Protein. Radioisotope tracing technique has been implied to reveal the catabolic products of ethylene. This methodology has revealed the presence of ethylene oxide, CO_2 and ethylene glycol to be catabolic intermediates of ethylene. However, catabolism of ethylene does not seem to play an important role in regulating ethylene levels in tissues.

Alternately ACC formed in the penultimate step of ethylene biosynthesis can also form a conjugate to produce N-Malonyl-ACC which accumulates in the tissue. Stress-induced ethylene production has been associated with increased transcription of ACC synthase mRNA. Auxins both at endogenous and expogenous levels can trigger the activity of ACC synthase thus causing a surge in ethylene levels. Thus, various physiological responses earlier though to be mediated by auxin has been deciphered to be resulting from ethylene. Molecular data from Arabidopsis revealed the role of cytokinin in regulating ethylene levels by operating through ACC synthase activity. **Aminoethoxy-VinylGlycine** (**AVG**) and **AminoOxyacetic Acid** (**AOA**) are two important ethylene biosynthesis inhibitors which block the formation of ACC from S-Adenosyl Methionine.

Physiological Effects of Ethylene

The effects of ethylene were initially discovered in the context of seed germination and fruit ripening. However, this phytohormone has been reported to exhibit a plethora of effects in relation with aerenchyma formation, cell growth and expansion, senescence and stress signaling. Nevertheless, ethylene represents the major class of phytohormones in all plant groups.

• Fruit Ripening

Ethylene induces an onset of physiological changes associated with fruit ripening. The physiological action of ethylene is manifested by the increase in respiratory CO_2 emission, cell wall loosening, starch hydrolysis, sugar accumulation and degradation of organic acids. The relative proportion of sugars and organic Acid content determines the taste of the fruits. Briefly, fruits which respond to ethylene induced ripening process mediated by a surge in respiratory CO_2 are termed as climacteric fruits (for example, banana, tomato, etc.). Fruits which do not respond to ethylene-induced ripening are termed as non-climacteric fruits. Climacteric fruits exhibit an increased ethylene biosynthesis associated with the ripening stages. Veraison stages of grapes have been reported to be induced by exogenous ethylene which promotes ripening. The convincing proof of ethylene-induced fruit ripening has been reported by investigations on the never ripe mutant of tomato. This ethylene receptor mutant was incapable of ethylene binding thus leading to inhibition of ripening.

• Lateral Cell Expansion

The effect of ethylene manifested by triple response is associated with lateral expansion of cell wall. This effect of ethylene is mediated by reorientation of cortical cytoskeleton elements across the longitudinal walls. Thus ethylene induces new deposition of cellulose microfibrils across the longitudinal walls of the plant tissues.

This results in swelling and lateral expansion. The process of microtubular reorientation was observed in Pea cells where microtubule elements were tagged by yellow fluorescent Proteins. The observations obtained revealed new alignment of cytoskeletal elements on the longitudinal wall of cells in addition to the pre-existing components.

• Apical Hook Formation in Cotyledon

Ethylene induced unequal growth in the outer and inner surface of the etiolated cotyledons facilitate the soil-emergence of etiolated seedlings. The increased growth of the cotyledon at its outer surface results in such hook formation. The growth reverses to normal condition at the post-germination stages.

• Regulation of Seed and Bud Dormancy

Certain seeds of cereals and pulses respond to ethylene by breaking of dormancy. *Arachis hypogaea* has been reported to show correlations between endogenous ethylene production and seed germination. Furthermore, Potato Tubers treated with Ethylene exhibit faster germination of Sprouts.

• Regulation of Stem Elongation in Water Logged Plants

Various submerged wet land plants like *Nymphoides*, *Ranunculus* and *Oryza* exhibit ethylene-induced stem elongation. Water logged cereals contain abundant aerenchyma formed in the submerged parts. This provides sufficient Oxygen levels necessary for the process of ethylene formation from ACC. Moreover, Ethylene induces increased GA formation resulting in intermodal elongation of stems.

• Root Hair Formation

Ethylene has been observed to promote root hair and root emergence in cut stems of different plant species. Investigations with ethylene-inhibitors have also been performed to test the malleability of the process.

- **Regulation of Senescence and Abscission**

The antisenescence effects of cytokinin can be essentially reversed by exogenous ethylene. Leaf discs exhibit degradation of chloroplast and subsequent colour fading manifested by the effect of ethylene. The shedding of plant organs (leaf, branch and fruit) involves the formation of abscission layer. The physiological and biochemical regulation of abscission layer is mediated by the ratio of endogenous auxin and ethylene levels. Pre-shedding stage of abscission involves a decrease in auxin levels associated by increased sensitivity to ethylene. Ethylene formation triggers the activity of cell wall degrading enzymes which promote abscission in plant organs.

Interestingly the role of ethylene in various commercial applications including fruit ripening has provided its importance in floriculture and horticulture. Ethylene biosynthesis inhibitors can be implied with other anti-ripening compounds for post-harvest storage of seasonal fruits.

2.2.5 Abscisic Acid (ABA): Discovery and Chemical Nature

The preliminary investigations related to the control of seed and bud dormancy revealed the presence of inhibitory compounds detected by paper chromatography. This substance (termed as Dormin) was isolated from Sycamore trees during their autumn leaf fall at the onset of dormancy. Later on Dormin was reported to be chemically similar to Abscisin II responsible for shedding of Cotton Fruits. Thus, the biomolecule was designated to be phytohomone called abscisic acid. The effect of ABA-induced abscission is mediated by a surge in ethylene production in tissues. Seed maturation and its dormancy are largely regulated by temporal variations in the level of ABA. ABA has been reported to be present in most plant groups except liverworts. Interestingly, biosynthesis of ABA in plant tissues has been reported to be associated with cells containing chloroplasts or amyloplasts. Most of the plant tissues have been associated with the process of ABA biosynthesis.

ABA (15 C) shows partial chemical similarities to carotenoid molecules with carboxyl group at the second carbon which forms its cis-trans isomer. The biologically active form of ABA exists in the cis form within tissues. Presence of asymmetric carbon atom at 1' results in the formation of S and R enantiomers of ABA. The S enantiomer promotes rapid effects like stomatal closure in leaves. An equal mixture of both the forms induces the long term physiological effects in plants. ABA is commonly synthesized from a carotenoid intermediate which initiates from **Isopentyl Diphosphate (IPP).** IPP is metabolized to form violaxanthin catalyzed by zeaxanthin oxidase. This is followed by the formation of Neoxanthin and Xanthoxal where the later is formed by the enzyme **9(Nine)-*Cis*-Epoxycarotenoid Dioxygenase (NCED).** NCED activity is increased by water stress in the tissue and thus appears to be the rate limiting step of ABA synthesis. Xanthoxal is finally converted to ABA-aldehyde and ABA. The last step is catalyzed by a group of aldehyde oxidases. NCED is localized in the plastids of plant cells. Plant tissues exhibit transient fluctuations in the levels of ABA in different organs.

Developing seeds accumulate high amount of ABA which then decreases along with seed maturation. Similarly, water stress in the leaves exhibit 50 fold increase in ABA content which causes stomatal closure. Apart from its biosynthesis ABA is also regulated by its catabolism, conjugation or derivatization. ABA is easily transportable to long distance organs within the plants. Oxidation of free ABA results in the formation of phaseic acid. The glucose-ABA ester may be maintained in the tissues undergoing serial levels of drought or water stress effects. ABA transport across the cells is mostly regulated by the anion trap concept. The dissociated alkaline form of ABA^- causes alkalinisation of the apoplastic space.

Increase in the pH of xylem sap due to ABA transport results in the closure of stomata during drought stress.

Figure 2.4 illustrates the chemical structures of major phytohormones.

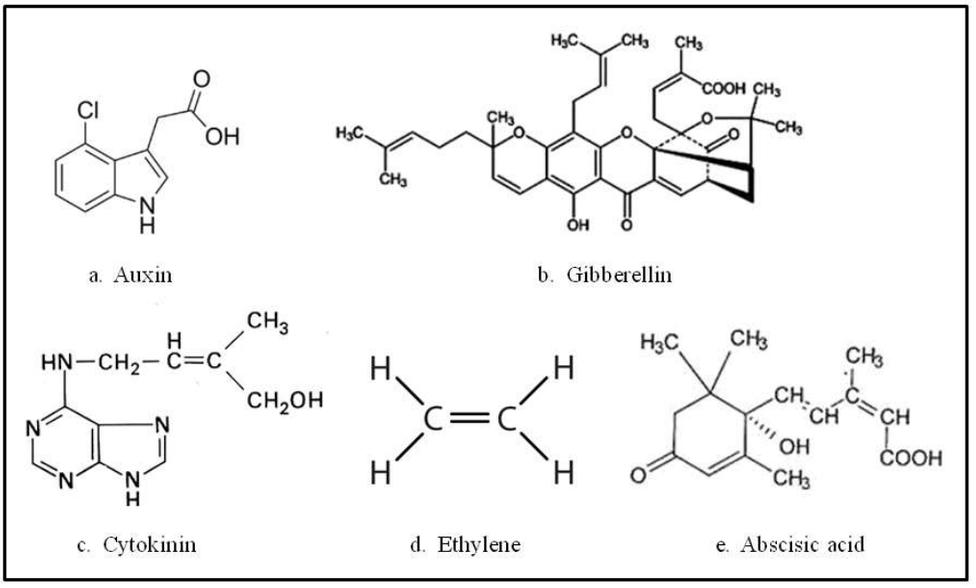

Fig 2.4 *Chemical Structure of Major Phytohormones*

Physiological Effects of ABA

Following are the physiological effects of ABA:

• ABA Levels Increase during Embryogenesis

Increase in the ABA levels during the later stages of seed filing phase is important to induce dormancy of seeds. It has been observed that at the initial phase of seed development the ABA levels remain at a lower concentration. However, followed by seed filing and endosperm development the seeds enter in a stage of dehydration. ABA levels have been observed to surge at this stage which, however, decreases at later stages of embryo maturity. Investigations with ABA deficient mutants of Arabidopsis has revealed that both the zygotic and maternal genotype regulate the biosynthesis and accumulation of ABA in the seeds. Higher levels of ABA accumulation in the seeds induce the synthesis of **Late Embryogenesis Abundant (LEA) Proteins** which facilitate in the process of desiccation tolerance. Furthermore, ABA regulates the accumulation of various storage Proteins in the seed. The accumulation of ABA is important for attaining dormancy of seeds. The embryo is maintained at a dormant stage during which the growth of embryo is restrained till future. Thus, seed dormancy is a temporary delay in the process of embryo emergence during unfavourable conditions. The ratio of ABA and GA is important to maintain the levels of dormancy in the seeds.

• ABA Inhibits Precocious Germination and Vivipary

The process of pre-harvest germination of seeds is prevented by the higher levels of ABA present in the mid and later stages of seed development. ABA deficient Viviparous Mutants (VP) in Maize exhibit precocious germination in the absence of ABA levels in seeds. ABA accumulation also occurs in the shoot buds which result in acquisition of dormancy during extreme cold temperatures. ABA inhibits GA-induced expression of α-amylase by inhibiting GA responsive MYB elements in seeds.

• ABA Induces Stomatal Closure in Response to Water Stress

ABA has been characterized as a stress hormone in response to various abiotic conditions like temperature, water and salt stress. Drought conditions induce increase in ABA concentration up to more than 50 fold in leaf tissues. ABA induced stomatal closure involves altered membrane potential of guard cells which result in decreased osmoticum and subsequent dissociation of K-malate. During stress conditions ABA is likely to be synthesized in roots and transported through shoots to the aerial parts of the plant. This long-distance signaling of ABA induced stomatal closure results in prevention of permanent wilting.

• ABA Regulates Root and Shoot Growth during Lower Water Potential

Water status in soil results in the regulation ABA-induced shoot and root growth in plants. During low water potential ABA promotes root growth faster than shots. The situation reverses during conditions of higher water potential in soil. Comparative investigations of wild type and ABA deficient mutants have revealed the differences in ABA-induced responses during variable water potential in soil. Thus, during dehydrating conditions ABA exerts a positive effect on root growth possibly by repressing ethylene biosynthesis. During similar conditions, ABA exerts slight inhibitory effect on shoot growth. Thus, soil dehydration results in increased root, i.e., shoot ratio in plants resulting from the action of ABA.

'Check Your Progress'

1. What is IPA pathway?
2. What happens in TAM pathway?
3. How is IAN pathway carried out?
4. What is triple response?

2.3 SEED GERMINATION PHYSIOLOGY

Seed germination is a coordinated event of embryo emergence from the seed coat and formation of young seedling. The seed harbours the young embryonic plant which matures prior to emergence from the seeds. The process of seed germination initiates with the emergence of radical (root primordial) from the seed. The hypocotyl elongation is followed by emergence of plumule which finally results in the formation of young cotyledonary leaf. In physiological sense, the germination process starts with seed hydration followed by increased respiration coupled to growth and emergence of seedling (Refer Figure 2.5).

Fig. 2.5 *Seed Germination*

Certain seeds after attaining maturity can germinate in the presence of moisture, Oxygen and other favourable conditions. Such seeds which are ready to germinate in presence of suitable inducers remain in their quiescent stage prior to maturity. However, certain seeds are unable to germinate due to presence of certain physiological constrains or anatomical barriers. Such a process results in the dormancy of the seeds. The viability of seeds depend upon its desiccation tolerance levels, ABA : GA ratio and antioxidant machinery. Recalcitrant seeds usually loose viability after certain span of their maturity and therefore are unable to germinate. This type of seed is unable to tolerate desiccation and dehydrating stages and cannot be stored for longer durations. Orthodox seeds are capable of germinating even after prolonged period of desiccation and storage.

Usually it has been observed that the orthodox seeds possess higher activity of antioxidant enzymes necessary to quench ROS (Reactive Oxygen Species) levels. The process of seed development occurs briefly in three phases. The first two phases involve cell division and expansion. The last phase involves reserve matter accumulation accompanied by a decrease in the moisture content. Briefly, the maturation stage involves loss of moisture associated with increased desiccation tolerance. In recalcitrant seeds, the post maturation phase of desiccation tolerance may not exist prior to germination. The pre-germination stage of quiescent seeds usually involves hard and dry seed coat barriers.

However, imbibition of water results in hydration and expansion of cells in the seed coat, aleurone layer and endosperm. This is followed by a surge in metabolism and remobilization of dissolved sugars into the growing embryo. Thus, the present unit shall focus on the physiological events associated with seed germination and seedling growth.

2.3.1 Stages of Seed Development

The process of seed development and maturation is an important process in the life cycle of plants. The stages of seed development and its maturation are largely under the hormonal control exerted by the effect of ABA and GA. The development of embryo and determination of its polarity is regulated by the localization of auxin concentration within the embryo. The process of cell division in the endosperm is influenced by the activity of cytokinin. In plants like legumes cytokinin regulates the process of cotyledon expansion and depletion of endosperm. At the advanced stage of seed development ABA plays a pivotal role in the process of seed maturation and desiccation tolerance. ABA promotes the process of accumulation of seed storage protein, induces desiccation tolerance and prevents precocious germination (vivipary). Briefly, the stages of seed development involve expansion of tissues by cell division, seed filling and desiccation tolerance.

Stage I: The first stage of seed development associated with the post-fertilization phase mainly involves histodifferentiation of various layers within the seed. The seed coat develops to form a hard impermeable barrier for moisture and Oxygen. This boundary is, however, prominent at the later stages of seed development. The advancement of seed development is usually analyzed by counting the Days After Anthesis (DAA) or Days After Pollination (DAP).

In the early stage of seed development the embryo polarity is determined by spatial distribution of auxin gradient. The concentration of cytokinin and gibberellin help in cell proliferation and endosperm development. In the histodifferentiation phase of seed development there is a remarkable increase in the moisture content. Monocot seeds possess an additional structure of scutellum. The shoot apex and root apex are covered by sheath like structures called coleoptiles and coleorhizae. The pattern of endosperm development depends upon its type and variants in different species.

Stage II: In the second phase of seed development the process of cytodifferentiation continues which is followed by an increase in the size of the seed. Physiologically speaking the process of seed maturation is characterized by an increase in the dry mass accumulation followed by decrease in the moisture content. In the second phase of seed development reserve material accumulation is the key process affecting dry matter accumulation. In the post-pollination phase the seed appears to be an important sink associated with gathering of assimilates.

The assimilates are usually photosynthetic products or remobilized sugar molecules which are essentially transported through apoplastic loading. The external weather conditions and maintenance of transpiration stream are essential in the process of sugar transport to the seeds. Starch is the main form of storage carbohydrate in seeds. This is synthesized primarily from sucrose.

However, sucrose transported into the seeds may also be metabolized into oils and lipids. The amount of Protein content accumulating in the seeds depends upon the rate of Nitrogen accumulation in the seeds prior to the second stage of development. Cereal seeds contain Protein content of around 12-15% of their dry weight which may range from 20-40% for Legume seeds. Canola, Linseed, Castor, Sunflower are some of the oil seeds which store a majority of Lipids.

Stage III: The final stage of seed development is briefly stated as the maturation stage. The dry weight accumulation slowly ceases in this stage which is followed by a decrease in the moisture content. During this phase the GA levels decrease and accompany a surge in the ABA levels. The decrease in moisture content occurs after complete accumulation of reserve material. Thus seeds approaching maturation and quiescence are associated with desiccation tolerance, synthesis of **Late Embryogenesis Abundant (LEA) Proteins** and up regulation of antioxidant system necessary for redox homoeostasis. Seeds produced within dry fruits possess lesser moisture content. Usually orthodox seeds contain 25-35% moisture at its maturation stage which may increase to 60% in the case recalcitrant seeds.

2.3.2 Seed Dormancy

The physiological inability of the viable seeds to germinate even in the presence of suitable conditions is referred to as dormancy. The stage of dormancy may persist for certain duration and usually occurs due to anatomical and biochemical reasons. Certain seeds are capable of germination immediately after their harvest. However, dormant seeds usually undergo a stage of metabolic suspension and growth retardation. The vigor of seeds is usually determined at the maturation phase of seed development.

During this stage the rate of photosynthate assimilation determines the rate of seed vigor. This factor has been partially known to affect the levels of seed dormancy. Certain seeds capable of germinating after harvest may appear to remain dormant due to certain constrains in the suitable environmental conditions. This is referred to as **secondary dormancy**. This can, however, be overcome by chemical or temperature treatments. Thus, seeds released from plants in a dormant state are referred to as **primary dormancy**. Further investigations are necessary to decipher the reasons of secondary dormancy. Temperature, moisture levels and CO_2/O_2 ratio are the main factors affecting secondary dormancy. Primary dormancy may be due to barriers in the seed coat permeability or growth and metabolic suspension of the embryo. In the case of coat-imposed dormancy, the seed coat and surrounding tissue of endosperm or aleurone layer impose a barrier to the process of water imbibition and gaseous diffusion. This prevents the resumption of metabolism within the seed. In embryo-induced dormancy the growth and metabolism of embryo ceases due to desiccation tolerance.

In certain cases the cotyledon may contribute towards imposing embryo dormancy. The levels of ABA and GA are important determinants of the levels of seed dormancy. ABA levels increase during the seed maturation stage of its development which imposes the process of dormancy. ABA induces an inhibition of GA-mediated amylase activation. This prevents the process of germination and mobilization of food reserve.

Factors Affecting Seed Dormancy

Primary seed dormancy is usually imposed either due to hardiness of seed coat or metabolic suspension of the embryo. Thus, various factors in association may result in the imposition of seed dormancy are discussed below.

• Seed Coat Barrier

The seeds of angiosperms belonging to different families like *Leguminosae*, *Malvaceae* and *Chenopodiaceae* often possess very hard seed coat. This tissue barrier results in poor permeability of water and causes Oxygen paucity. In natural process, the outer covering of seed coat is subject to microbial decay in soils which facilitates germination. Artificial process of scarification, pressure application and temperature treatment may release seed coat-imposed dormancy.

• Embryo-Induced Dormancy

In certain seeds the embryos are observed to exhibit dormancy during the desiccation stage. This result in physiological dormancy of the seeds attained due to higher levels of ABA. Thus, dormancy imposed by embryo does not allow the seeds to germinate immediately after harvest. A certain amount of resting period is important to follow germination. This time span is necessary for completing the growth of embryo.

• Presence of Germination Inhibitors

Certain secondary metabolites like coumarins, ferulic Acid, prascorbic Acid and abscisic Acid are present in various tissues within the seed. These metabolites prevalent in the seed coat, endosperm, embryo or juicy pulp surrounding the seeds appear as natural inhibitors of germination.

• Temperature and Light Requirement

Certain seeds obtained from seasonal fruits require low temperature for germination. Thus seeds harvested in the autumn may remain dormant till the onset of spring when they are suitable to germinate, for example, Apple, Wild Rose and Peach.

Similarly in many species the process of germination is affected due to light sensitivity. These seeds which exhibit light-dependent dormancy are termed as **photoblastic** seeds. The seeds which require light to induce germination are known as **positively photoblastic** seeds (such as, Tobacco, Tomato and Lettuce), while the ones which are inhibited by light are **negatively photoblastic** seeds (such as, Black Cumin, *Silene* sp.).

Thus, dormancy of seeds can be overcome by a number of methods which involve scarification of seed coat, temperature alteration, light requirement and chemical treatment. In positively photoblastic seeds, application or red light might induce the process of germination. The intensity of the light received determines the amount of P_r form of phytochrome which is converted to P_{fr} form.

However, in certain species of Pepper and Lettuce the far red light has been repoted to promote germination. Hormones, such as, GA, Cytokinin and Ethylene are known to

promote germination. KNO_3 and Thiourea are potent chemicals which can help in overcoming dormancy.

- **Seed Viability**

The post-maturation phase of seed germination involves a certain time span for which the seed remains physiologically active or viable. The time span for which the seed remains viable depends upon the species. Thus, viability may vary from weeks to years and depend upon the plant species and environmental conditions prevailing during the storage. According to Ewart (1908), the seeds have been classifies into following three types based upon their viability:

- Microbiotic Seeds: These are seeds with life span of a few weeks to 5 years.
- Mesobiotic Seeds: These life span ranging from 3-15 years.
- Macrobiotic Seeds: These seeds have possess viability for a range of 15-100 years.

The orthodox seeds of a majority of crop plants usually remain viable for 1-4 years. Thus, they appear to be microbiotic in nature. Certain wild plants including *Cassia* sp. and certain legumes exhibit mesobiotic to macrobiotic nature. The seed of a species of Indian Lotus (*Nelumbo nucifera*) has been reported to be viable for more than 150 years.

2.3.3 Physiological Situation of Quiescent Seed

The seeds which have attained complete maturity prior to germination remains in a physiologically inactive state. During this stage the seed possess a hard impervious seed coat comprised of nonliving material. The seed coat contains various secondary metabolites necessary for protection from biotic stress. In certain seeds the seed coat is impermeable to Water and Oxygen. The structure of seed coat and endosperm impose a barrier for the embryo to emerge from the seed. Physiologically the quiescent seeds possess poor activity of hydrolytic enzymes. The rate of aerobic respiration is minimum for the quiescent seeds. Thus results in a very poor Oxygen uptake in the seeds. However, antioxidant enzymes like peroxidase, catalase, superoxide dismutase and glutathione reductase have been found to show ambient activity.

Significant amount of neurotransmitters like melatonin, catecholamines and serotonin have also been found to be present in the quiescent seeds. This process helps to maintain redox homoeostasis and free radical scavenging. Due to absence of water the seeds undergo a desiccation phase and do not possess sufficient turgour pressure in the cells. The cells do not possess sufficient respirable substrate. Reserve food material present in the endosperm remains in the insoluble state.

Physiological and Biochemical Events Associated with Seed Germination and Seedling Growth

The seeds germinate in the presence of suitable external conditions of temperature, moisture and Oxygen availability. The process of germination in a physiological sense is associated with a surge in aerobic respiration followed by radical emergence.

The process of germination is accomplished in three stages mainly, which are as follows:

- Imbibition and lag phase.
- Hydration of seeds and surge in metabolism.
- Radical emergence and seedling growth.

The process of seed germination thus resumes the metabolic state of the embryo and activates the hydrolytic enzymes necessary for remobilization of food reserve. The various physiological and biochemical events associated with seed germination are as follows:

Water Uptake and Hydration

The process of water uptake by the seed coat occurs by a physical process of imbibition. This involves absorption of water molecules by the hydrophilic colloidal matrix of the cell wall. Various hydrophilic polar substances like Proteins, -OH Molecules, Pectin and Hemicelluloses contribute to the process of water absorption. This results in improved hydration of the seeds and better permeability of seed coat to Water and Oxygen. Furthermore, the seed coat now appears to be less resistant to the outward growth of the embryo.

Surge in Aerobic Respiration

The process of hydration of seeds results in increased uptake of Oxygen in the seed tissues. This leads to increased rate of aerobic respiration with Oxygen uptake up to 100 $\mu l\ gm^{-1}\ hr^{-1}$. Sucrose has been observed to be the main form of aerobic respiration which is essentially supplied by the endosperm.

Activation and Mobilization of Reserve Material

The activity of gibberellic Acid results in the formation of soluble sugars produced by starch degradation. Activation of α-Amaylase and β-Amylase result in formation of soluble sugars from starch. The activity of maltase is also regulated by the activity of GA. In monocotyledonous seeds the DNA and RNA content decreases in the endosperm but increases in the embryonic axis. This is attributed to the increased transport of assimilates and other molecules in the young seedling. The aleurone layer has been reported to exhibit increased RNA content during germination. The embryonic axis exhibits increased rate of cell division and growth. In oil seeds, the germination is accompanied by mobilization of oil bodies. The oil body membrane Proteins like Caleosins, Steroleosins and Caleosins are slowly hydrolysed followed by Lipid breakdown. The activity of lipases results in the breakdown of fats to fatty acids. B-oxidation of fatty acids results in the formation of Acetyl-CoA. The process of imbibition and GA activity stimulate the function of lipase. The process of glyxoylate cycle allows conversion of Acetyl-CoA to Sucrose. This Sucrose formed is transported to the young embryo and seedling. The process of glyoxylate cycle is mainly operative until the seedling is capable of photosynthesis.

Proteins are metabolized from specialized structures called aleurone grains. The activity of peptidases increase during seed germination. The formation of peptide fragments and Amino Acids which act as a source of energy by oxidation. Moreover, the Amino Acids may also be used for synthesis of new Proteins. Various inorganic elements like Potassium, Calcium and Magnesium are stored in the seeds in the form of Phytin. These elements are released by the activity of phytase. The elements act as important cofactors for enzymes.

Emergence of Seedling

The process of seed germination occurs after the metabolic activation of the seeds. This event is visible through the process of radical emergence from the seed. This process is followed by plumule growth which results in the extension of hypocotyl. The imbibitional pressure supplemented by the activity of hydrolytic enzymes (cellulase and pectinase) help in penetrance of the seedling through the seed coat. The young seedling contains the epicotyls which bears young leaves. The cotyledonary leaves are slowly shed off and new leaves emerge. The radical grows into the soil to produce primary root and root hairs.

'Check Your Progress'

5. What is LAE?
6. Define dormancy.
7. What are the stages that are involved in the process of germination?

2.4 PHOTOPERIODISM: PHYSIOLOGY OF FLOWERING

Autumn and Spring seasons bring the appearance of colourful blooms of a variety of flowers, attracting tourists and botanists all over the world. Mechanisms of flowering and seed dispersal indicate the evolutionary success of Angiosperms. Flowering occurs in several seasons depending upon the geographical locations (Alpine, Temperate and Tropical Vegetation). Some plants have perennial or annual life cycle, while some are biennial in their life cycle. **Monocarpic plants** flower for a single time in their life cycle and die after seed set. Annual or perennial plants may change their life cycle often depending upon climate. Flowering proceeds with the attainment of reproductive phase in the life cycle of a plant. The internal and external factors regulating the event of flowering involve attainment of a certain vegetative age of the plant followed by transition from vegetative to reproductive phase and induction of flowering. Proper growth and nutrition of the juvenile plant is necessary to exhibit transition into the reproductive phase. Development of floral parts involves the formation of sepals, petals, stamens and pistils at specific places around the **thalamus**, which is the receptacle of flower. Floral induction starts with enhanced cell division activity at the vegetative shoot apical meristem, followed by **formation of buds** either **solitary** or in **inflorescence**. Inflorescence is the arrangement of flowers around a **floral axis (Rachis)** and provides specific taxonomical characters of several plant families.

Flowering is primarily regulated by photoperiodic cycles of day and night. Flowers blooming specifically in winter (for example, *Chrysanthemum* sp.) require a certain period of short days necessary to induce flowering. Some plants specifically bloom during the night time, for which a specific duration of light is necessary to induce flowering in dark (for example, *Cestrum nocturnum*). Blooming of flower involves the opening of buds followed by **anthesis** (**maturation of stamens**). The process of induction of flowering, however, starts as early as the development of floral meristem, even prior to bud formation. Nutrient and water availability is crucial for the vegetative growth of a juvenile plant. Plants in adverse or stress conditions attain early reproductive maturity and flowering as an adaptation to achieve reproductive success. Several genes, phytohormones and other biomolecules are involved in the process of floral induction and development. **Floral organogenesis** involves the activity of **homeotic genes** responsible for the development of floral organs. The following sections of the unit will elaborate the factors and events associated with photoperiodism and physiology of floral induction.

2.4.1 Classification of Plants based on Photoperiodism

The photoperiodic response in plants is regulated by the intensity and duration of light wavelengths. The photoreceptors namely phytochrome and cryptochrome are responsible for the perception of red and blue wavelength in the visible spectrum. Photoperiodism is the phenomenon of light-induced periodic response in plants manifested by various morphological and physiological changes. The magnitude and nature (reversibility) of the photoperiodic

response varies upon the mechanism of signaling pathway. Flowering in plants is largely a photoperiodic phenomenon regulated by the wavelength and intensity of light. Molecular studies in Arabidopsis have revealed the mechanism of photoperiodic response in plants. The key elements associated with flowering are the phytochrome and cryptochrome receptors encoded by the **PHY** and **CRY Genes**. Arabidopsis has been reported to possess five forms of phytochrome responsible for perception of red, far- red and blue light. Moreover, the cryptochromes are responsible for blue light sensing in plants.

However, the CRY Proteins may also be involved as signaling intermediates of phytochrome-induced responses. Plants possess the capacity of sensing the duration of day length and respond to the cyclic repletion of light and dark phase. The duration of day and light phase varies upon the latitudinal location of Earth. The equator region exhibits equal duration of light and dark phase, which changes along with increase in latitude. Higher latitudinal locations usually have longer nights and shorter day periods in winters. This situation reverses in the summer seasons. Interestingly, the plants native to a particular geographical location is capable of sensing the photoperiodic fluctuations associated with seasonal variations. This results in the origin of short day, long day and day neutral plants which flower upon sensing the night and day periods. The classification of plants according to their photoperiodic response has been obtained for the better understanding of flowering. Initially physiologists considered that flowering resulted from the sensing of day duration which provided higher accumulation of photosynthates.

However, in 1920s Garnard and Allard conducted experiments in the U.S. Department of Agricultural Laboratories which revealed that the duration of light sensed is an important determinant of flowering in plants. The flowering response regulated by photoperiodic duration can be broadly classified as follows:

Short-Day Plants (SDP)

Plants which flower in response to shorter day durations or are accelerated by shorter day lengths are termed as Qualitative or Quantitative Short Day Plants, respectively. These plants require 6-8 hours of day duration followed by a longer night phase of 14-16 hours. However, it is important to understand that the night phase is the critical period which if interrupted (660-665 mμ) may inhibit flowering. *Nicotiana tabacum*, *Glycine max* and *Xanthium pennsylvanicum* are some of the short day plants. The interruption of the night period by red light possibly occurs at the middle of critical dark period. However, the inhibitory effect of red light can be reversed by subsequent application of far red light. The extension of night period promote early flowering of short day plants.

Long-Day Plants (LDP)

Plants which require longer photoperiod duration of 14-16 hours are known as Long-Day-Plants (LDP) and also sometimes as Short Night Plants (SNP). Interruption of the dark period with a light flash or extension of the day duration can result in early flowering in LDP. *Hyoscyamus niger*, *Spinacea* sp. and *Beta vulgaris* are some of the examples of plants flowering in longer day durations. The plants which flower in long day or are accelerated by longer day durations are termed as Qualitative or Quantitative Long Day Plants, respectively. The major differentiation between LDP and SDP lies in the fact that flowering in LDP occurs at a day duration exceeding the critical day length, while flowering in SDP results due to day duration lesser than the critical day length. Moreover**,** for SDP to flower the night duration should be essentially more than the critical night length (Refer Figure 2.6).

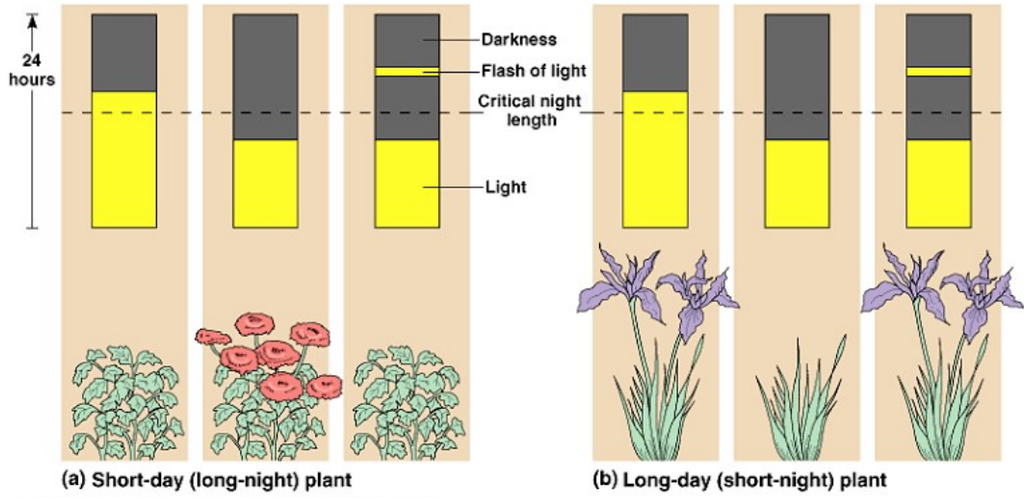

Fig. 2.6 *Photoperiodism for Short and Long Day Plants*

Long Short Day Plants

These plants require longer day durations at the initial phase of growth but flower during shorter day durations. Certain plants like *Bryophyllum*, *Cestrum nocturnum* and *Kalanchoe* sp. flower during the late summers and autumn season when the day durations decreases.

Short Long Day Plants

These plants require initial shorter day durations but flower in the presence of longer day duration of spring season. *Trifolium repens*, *Campanula medium, Triticum aestivum* and *Secale cereale* are some of the plants which flower in the presence of longer day durations of late winter and spring.

Day-Neutral Plants

These plants are insensitive to the duration of photoperiod and can flower in the presence of variable day lengths ranging from 5-24 hours. Certain plants native to the equatorial region is adapted to flower in constant duration of day, for example, *Phaseolus vulgaris*. Certain desert plants like *Abronia villosa* flower in the presence of ample amount of water.

2.4.2 The Night-Break Experiment of Flowering

In plants like *Xanthium* and *Pharbitis* a short exposure of light during the night period may result in the inhibition of flowering. This result due to the conversion of the cis-trans form of the flowering-inducing photopigment called phytochrome. The phytochorme and its functional details shall be discussed in the following section of the unit.

However, the two forms of the pigment designated as P_r and P_{fr} (absorbing red and far red light) are responsible for flowering in the short and long day plants, respectively. The P_r form induces flowering in the short day (long night) plants while the P_{fr} form induces flowering in long day (short night) plants. In *Xanthium* a short exposure of red light in the night period inhibits flowering. This result due to the conversion of P_r back to the P_{fr} form. This inhibitory effect of red light is usually effective at a very low exposure or brief flash.

However, subsequent exposure of far red light may result in flowering due to reconversion of P_{fr} into the P_r form. Thus, alternating exposure of red and farred light determines the fate of flowering in the night break experiment.

NOTES

R + FR ——————— Flowering

R + FR + R ——————— Not Flowering

R + FR + R + FR ——— Flowering

For long day plants, a longer exposure of light in the night period promotes flowering. However, in the night break experiment the duration and intensity of light exposure essentially modulates the flowering response. The exposure was found to be effective when applied at the middle of the duration of dark period. The findings of the night break experiment led to the inference that the inter-conversion of the P_r and P_{fr} form is a rapid and transient process regulated by even a short exposure of light and dark phases. This experiment was helpful in probing the process of artificial flowering in certain horticultural species like *Kalanchoe*, *Chrysanthemum* or *Euphorbia pulcherima*.

Further experiments in Soybean revealed that apart from the night duration a specific exposure of dawn at a particular time phase and in repeated cycles is responsible for flowering. This proved that the photoperiodic regulation of flowering was effective through a process of circadian oscillation.

2.4.3 Perception of Photoperiodic Stimulus

The concept of flowering modulated by photoperiodic response has been observed by foliation-defoliation experiments in Cocklebur plant (*Xanthium pennsylvanicum*). The plant responds to shorter day durations for flowering. Thus, longer day phases followed by shorter night periods inhibit flowering. The defoliated plant kept in shorter day duration, however, does not flower. Moreover, a single leaf kept in the plant is also sufficient to promote flowering in the shorter day periods. This implies that the perception of photoperiodic stimulus is essentially carried out by the foliage organs of the plant, i.e., leaf. The experiment involved separate exposure of the foliated branches to longer and shorter photoperiods. This process also interestingly led to flowering resulting due to exposure to short day duration. The defoliated branch was grafted to a plant which was exposed to a short day period. This also led to flowering in the defoliated branch. Thus, the results obtained from the experiment in Cocklebur implied that leaves are the primary site of perception of photoperiodic stimulus (Refer Figure 2.7).

Moreover, the floral stimulus is transmittable across various parts of the plants. The defoliated branch grafted to a foliated plant also flowered due to transmission of floral stimulus. Later investigations revealed that the floral stimulus appeared to be floral hormone or mRNA molecule which was transmitted from the leaves to the shoot apical meristem. The following sections of the unit will summarize the molecular mechanism of transmission of floral stimulus in plants. The experiment performed in cocklebur plant can be summarized as follows:

Defoliated Plant + Short Day Period → NO Flowering

Single Leaf in Plant + Short Day Period → Flowering

Defoliated Plant Grafted to a Foliated Plant + Short Day Period → Flowering

Foliated Plant + Long Day Duration → No Flowering

Separate Exposure of Two Foliated Branches to Long and Short Day Period → Flowering

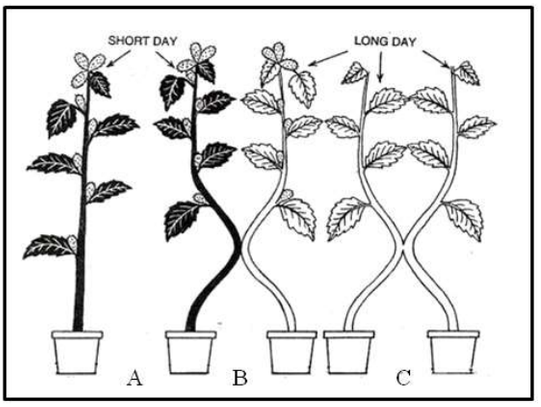

***Fig 2.7** Photoperiodic Stimulus is Received by Leaves*

Concept of Photoperiodic Induction

The process of flowering is controlled by the exposure to a number of photoperiodic inductions in a plant. The duration of photoperiod in a 24 hour cycle required by a plant is known as the inductive cycle. The time period required for flowering in a SDP or LDP can be modulated by the exposure to a number of inductive cycles. Continuous exposure to inductive cycles has been observed to be more effective than the discontinuous exposures for flowering in those plants. *Xanthium* is a short day plant which flowers after around 64 days from exposure to a single inductive cycle. However, increasing the number of inductive cycle up to 8 or 10 hour may result in early flowering. Thus, the process of floral evocation is attained by exposure to inductive cycles and is essentially a non-reversible process. The exposure of plants to non-inductive cycles later to inductive stimulus also results in flowering. The process of floral induction is operative through non- reversible changes at the molecular level. The transition of the vegetative meristem into the floral meristem results in flowering in the plants.

Phytochrome Functioning and Photoperiodism in Plants

The photoreceptor called phytochrome is a cytosolic soluble Protein of molecular weight around 250 kDa. The Protein-pigment complex is formed of two homodimers of 125 kDa each. The perception of red and far red light results in a cis-trans isomerisation of the first ring of the tetra-pyrrole chain. The apoprotein and chromophore part together function in the perception of light stimulus. Phytochrome has been detected in various classes of plant ranging from Algae, Bryophytes, Gymnosperms and Angiosperms. The chromophore part of the Pigment-Protein Complex is synthesized in the plastids while the apoprotein part is encoded by nuclear genome. The autocatalytic assembly to form the holoprotein form occurs in the cytosol. The two classes of phytochrome vary in their spatial location, molecular weight and spectral properties. The Type I phytochrome is localized in the etiolated seedlings, while Type II is present in the green plants and seeds. PHYA gene encodes the Type I phytochrome while PHYB, PHYC, PHYD and PHYE genes encode Type II phytochrome. The two inter-convertible forms of phytochrome are P_r and P_{fr} which induce flowering in short and long day plants, respectively. The P_r form appears to be physiologically inactive and is capable of absorbing light of wavelength in the range of 660- 665 nm. The P_{fr} form absorbs light of wavelength range 730-735 nm. The day period results in formation of P_{fr} form of the pigment which promotes flowering in LDP but inhibits the process in SDP. Exposure of the plants to subsequent night period results in reconversion of P_{fr} form back into the P_r form. This results in promotion of flowering in SDP. Thus the night break experiment applicable to SDP and LDP result in alteration of the P_r/P_{fr} ratio in the plant. The inter-conversion of P_r and P_{fr} form leaves certain residual levels of each of the forms unconverted. The photoperiodic response

controlled by phytochrome involves various physiological processes like seed germination, leaf elongation, plastid biogenesis and bud dormancy. The P_r form is bluish in colour while the P_{fr} form is olive-green in nature. Phytochrome constitutes around 0.2-1% of total extractable cytosolic Proteins in leaves and etiolated seedlings (Refer Figure 2.8).

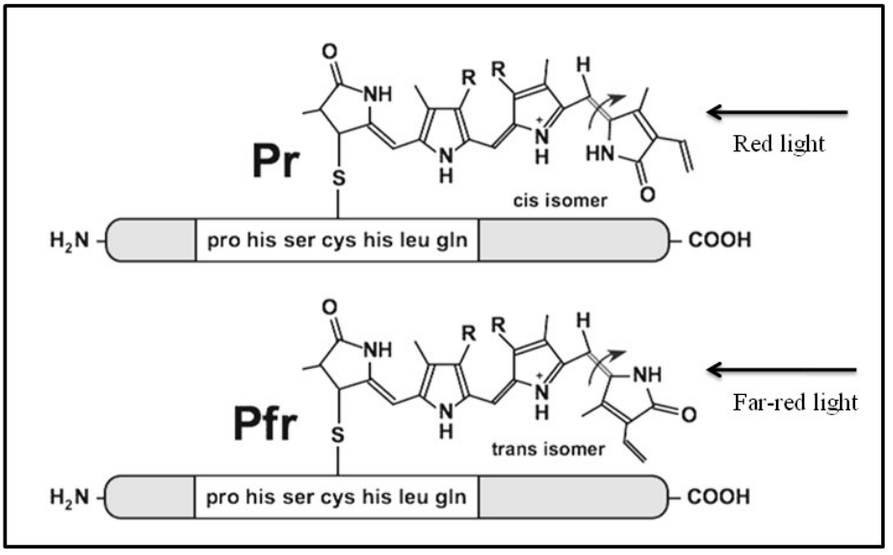

Fig 2.8 *Phytochrome Functioning in Red and Far Red Light*

2.4.4 Floral Evocation, Competence and Determination of Flowering

The process of floral evocation and formation of floral meristem involves the attainment of two specific stages. The apical bud becomes competent to turn into a flower upon receiving suitable conditions. The competent bud then proceeds to the next stage of development of flower which is termed as determination. Thus attainment of determination of flowering is the active state of flower development which is irreversible even if the bud is removed from the system (Refer Figure 2.9).

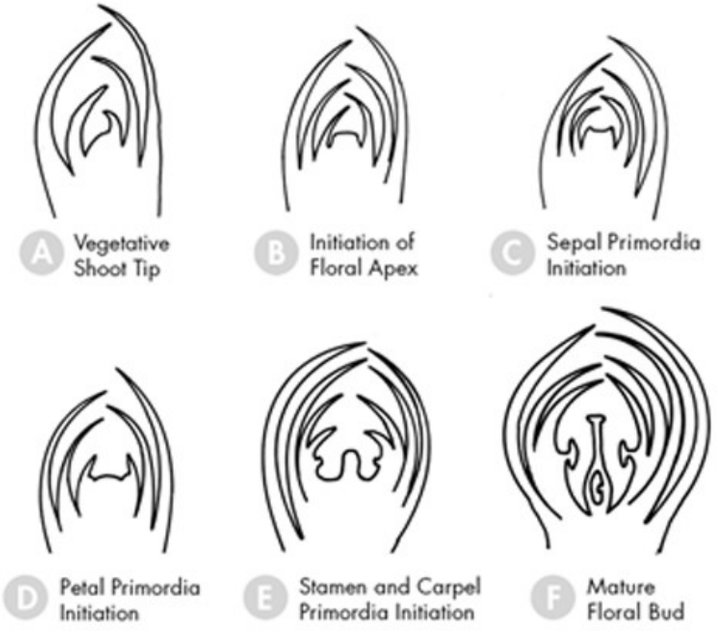

Fig. 2.9 *The Successive Stages of Transition from Vegetative Shoot Meristem to Floral Meristem and Formation of Bud*

Factors Associated with Induction of Flowering

Induction of flowering is a coordination of several external and internal events associated with the elicitation of floral stimulus. Climate, temperature, photoperiodic cycles, nutrient and water availability in the soil are some major external factors associated with flowering. The internal factors involved include the coordination of the homeotic genes and their cadastral activity regulating floral organogenesis. Hormones, like Gibberellic Acid (GA) and sugars also contribute to induction of flowering. Plants allocate most of their nutrients to the reproductive organs, which are the major sinks for solutes transported through the vasculature. Induction of flowering involves the allocation of higher levels of sucrose and transport of GA necessary for the elongation of the floral axis.

External Factors Associated with Flowering

Following are the factors that effects Flowering:

- **Climate**

Induction of flowering is partially regulated by climate. Naturally occurring plants flowering in a particular duration of the year are thus seasonal and depend upon climate. The hybrid varieties of such flower are, however, cultivated throughout the year. The seasonal flowers however show optimum growth during the suitable climate.

- **Nutrient and Water Status**

Plants growing in an ambient condition of nutrient rich soil are likely to have a healthy and fast growing adult vegetative phase, which leads to the induction of flowering with the advance of suitable conditions. Plants growing in adverse conditions of nutrient deficiency or which are subjected to biotic or abiotic stress, are likely to show early attainment of flowering and reproduction. This advancement in the reproductive maturity of the plant may often account for the reduction in the number of flowers and seed size. Nutrient deficiency in plants leads to less allocation of resources to the reproductive organs.

- **Temperature**

Temperature affects floral induction, for example, vernalization.

- **Circadian Rhythm**

The cyclic durations of day and night within a period of 24 hours regulate flowering in plants. The period of day light altering with night phase determines the time of flowering in different plant species. A shift in the oscillations of diurnal cycle may result in a delay or alteration in the induction of flowering.

- **Photoperiodism**

The response of light and dark periods is perceived by specific photoresponsive pigments in plant, namely phytochromes and cryptochromes. Photomorphogenic response involves induction of flowering as an effect of specific duration of light period to which a plant is subjected. Plants flowering in different latitudes adapt according to the duration of light and dark periods within a 24 hours cycle. Photoperiodic responses are similar to those of circadian rhythm. Circadian rhythm denotes the specific oscillations provided to the plant, while photoperiodism refers to the sensing of light duration necessary to induce flowering.

2.4.5 The Evolution of 'Florigen' Concept in the History of Physiology of Flowering

The idea of a molecule responsible for flowering was anticipated to be mobile and transportable within plant parts and preferably through the phloem cells. Apical and axillary meristems undergo floral induction and contain high amount of flower-inducible factors. In the year 1937, Mikhail Chailakhyan postulated the existence of a flower-inducing factor 'Florigen', which can be transported within one plant and preferably also through grafts, to induce flowering in a uninduced plant. Investigations on the effect of Tibberellin in inducing flowering were interpreted to be responsible for formation of Florigen and thus transportable to aerial parts of plants. Later workers investigated the effect of Auxin, Cytokinin and Carbohydrates to control flowering. Failure to isolate Florigen lead to the conclusion that this factor was comprised of more than one molecule or rather a ratio of different hormones. The concept of Florigen was established with the identification of Flowering Locus T (FT) in *Arabidopsis* sp. through genetic screening. Later, scientists reported Florigen molecule to be an FT mRNA molecule transported through plants, which was, however, later reported to be a Protein transported through phloem and accumulating in shoot apex (Refer Figure 2.10).

Table 2.1 illustrate the concept of evolution of the molecular regulations of floral inducation in plants.

Table 2.1 *Evolution of the Molecular Regulations of Floral Induction in Plants*

Author and Year	Event Reported	Conclusion
Mikhail Chailakhyan, 1937	Induction of flowering by grafting florally induced scion.	Presence of a transportable flowering-inducing factor Florigen.
Mikhail Chailakhyan and Anton Lang, 1975	Gibberellin-mediated floral induction in long day plants grown in non-inducing photoperiods.	Florigen was suggested to be comprised of two hormones-gibberellin and anthesin.
JD Zeevart, 1976	Regulation of flowering.	Florigen was as a ratio of control of the ratio of some hormones responsible for flowering.
Bernier, 1993	Effect of carbohydrates and hormones in inducing flowering.	Florigen seemed to refer to the regulation of more than one type of biomolecule regulating flowering.
Huang, 2007	Detection of mRNA of Arabidopsis FT gene.	Florigen was stated as mRNA from FT locus being transported to plant parts.
Corbesier, 2007	A transcriptional activation of FT locus in Arabidopsis and transport of FT protein from leaf to plant apex.	Florigen was confirmed to be a protein to be translated in leaves and transported through phloem to apical parts.
Notaguchi, 2008	Involvement of FT protein in inducing flowering by graft transmission.	Confirms florigen hypothesis, by stating that FT protein (florigen) is graft transmissible and capable to induce flowering in donor plant.

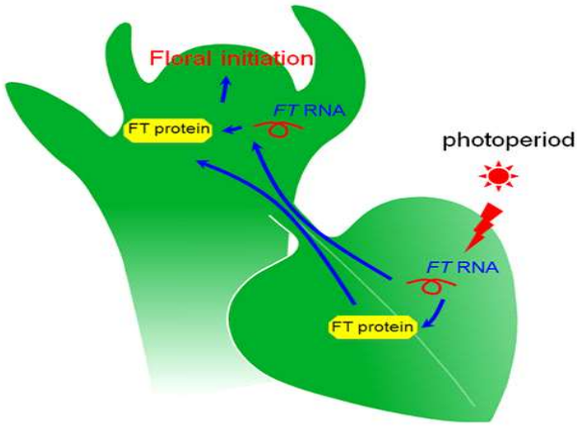

Fig.2.10 *The Initiation of Floral Induction begins with Transport of FT Protein from Leaf through Phloem to the Shoot Apical Meristem*

Three Classes of Genes Regulate Floral Induction

Present day research with several mutants (plants with non-functional gene/gene used to study the function of a gene observed by morphogenetic response) have revealed the involvement of three types of genes regulating the event of floral induction.

- **Floral Organ Identity Genes:** The Proteins formed by these type of genes regulate the expression of other genes involved in the process of flowering by floral organogenesis.
- **Cadastral Genes:** These genes control site specific expression or spatial regulation of floral organ identity genes, thus ensuring floral organogenesis at the specific region of meristem.
- **Meristem Identity Genes:** These genes activate the floral organ identity genes by acting as positive regulators.

Homoeotic Genes (MADS Box Gene)

Plants possess developmental genes belonging to a specific class of transcription factors called MADS Box genes. These homoeotic genes are responsible for organogenesis in plants. The process of floral organogenesis has been affirmed to be associated with specific set of homeotic genes responsible for the development of floral parts–sepals, petals, stamens and pistils.

Three Types of Homoeotic Genes Regulate Flowering in Plants (The ABC Model)

Various homeotic genes have been recognised to have role in the development of floral organs. Based on the type of function performed by these genes, they have been categorized into three classes as A, B and C type genes. The five different genes involved in flowering are namely APETALA1 (AP1), APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (PI), and AGAMOUS (AG).

Features of the ABC Model of Flowering

The popular ABC model was proposed in 1991, which highlights the effect of three different types of homeotic genes A, B and C, singly and in association, considered responsible for floral organogenesis. The Gene Types A and C are cadastral genes as they repress each other's activity and are spatially distributed to form the first and last whorl of flower, i.e., sepals and carpels, respectively. Gene Type B functions in association with Types A and C, leading to the formation of petals and stamens, respectively (Refer Figures 2.11 and 2.12).

Type A: AP1 and AP2 regulate organogenesis in the first and second whorls. Mutation of Type A activity leads to the formation of carpels in place of sepals in the first whorl, and of stamens in place of petals in the second whorl.

Type B: AP3 and PI regulate organogenesis in the second and third whorls. Mutation of Type B activity results in the formation of sepals in place of petals in the second whorl, and of carpels in place of stamens in the third whorl.

Type C: AG regulates organogenesis in the third and fourth whorls. Mutation of C results in the formation of petals in place of stamens in the third whorl and sepals in the fourth whorl.

Type A: AP2	Sepals: Type A
Type B: AP3/PI	Petals: Type A + Type B
Type C: AG	Anthers: Type B + Type C, Carpels: Type C

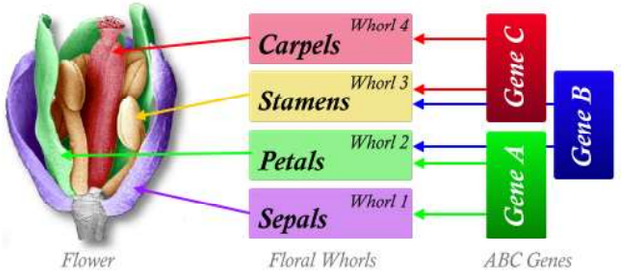

Fig. 2.11 *The ABC Model Depicts the Functional Role of the Three Types of Genes A, B and C in Floral Organogenesis*

The functions of these genes were deciphered through mutant analysis which alters the floral structure thereby identifying the floral organs produced by each gene. Plants with *AP*2 mutation are devoid of sepals and petals. Plants with *AP*3 or *pi* mutations develop sepals in place of petals in the second whorl, and carpels in place of stamens in the third whorl. Plants with homozygous genotype for the *AG* mutation lack both stamens and carpels.

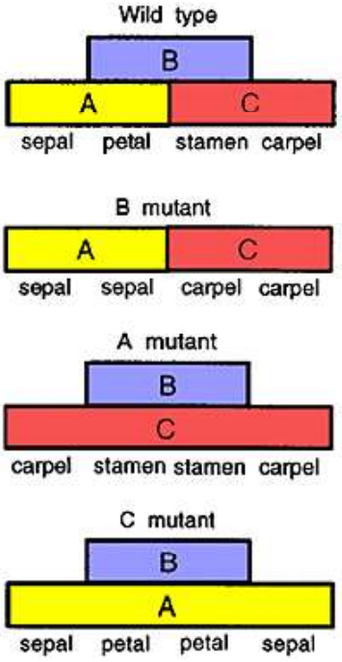

Fig. 2.12 *The Mutations in the A, B and C Types of Genes cause Alterations in the Pattern of Floral Organogenesis*

The Genetic Regulation of Transition from Vegetative to Reproductive Phase

The initiation of floral induction begins with the message of transition of vegetative to reproductive phase. As described earlier in this unit, the events of formation of floral meristem from shoot apical meristem are highly regulative in terms of environmental cues (Nutrition, Sucrose, GA, Photoperiod) which trigger the downregulation or upregulation of several genes necessary to transit from vegetative to reproductive phase. The CONSTANS (CO) (Refer Figure 2.13) gene has been investigated in Arabidopsis and has been affirmed to be the early responsive element of molecular crosstalk during floral induction. The CO locus responds to certain duration of photoperiod (day length) in Arabidopsis. CO Protein is a specific transcription factor, which after being produced triggers expression of FT Protein responsible for induction of flowering. The levels of CO Protein remain high in the dusk. Long day length triggers greater synthesis of CO Protein, which remains stable in light. The CO Protein activates AGAMOUS-LIKE 20 (AG) gene and LEAFY (LFY) gene, which are termed meristem identity gene, and are responsible for the expression of homeotic (A,B,C) genes. The FT Protein transported to shoot apex associates with FD Protein. The FD/FT complex up regulates the expression of two more genes APETALA 1 (AP1) and SOC 1 which in association with LFY trigger flowering. Expression of SOC Gene is also sensitized by high levels of GA and Sucrose.

The CONSTANS (CO) Protein is one of the early responsive elements of floral induction sensitized by longer day lengths. CO Protein in turn triggers FT Protein accumulation and its long distance transport to the shoot apex to induce flowering. FD/FT Protein complex in the shoot apex regulate several genes. The induction of flowering is regulated by the balance of P_r and P_{fr} forms of phytochrome in both high and low light intensities which separately induce CO and FT Protein formation in long and short day plants, respectively.

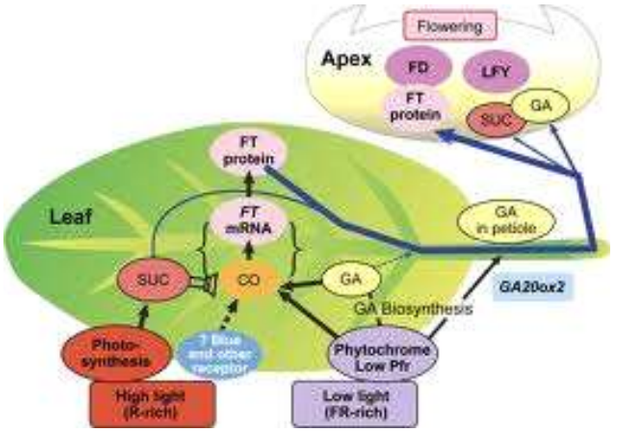

Fig. 2.13 *The CONSTANS (CO) Protein*

Phytochrome and Cryptochrome Regulate Floral Induction

The light sensing pigments, namely phytochrome and cryptochrome absorb light in the Red and Blue wavelengths, respectively. Certain duration of light and dark phases for both short and long day plants, is necessary for floral induction. Phytochrome contains two reversible form of pigments P_r and P_{fr} form inducible by Red and Far red Light. PHYA and PHYB are the two major red light receptors in plants which respond to flowering (Refer Figure 2.14).

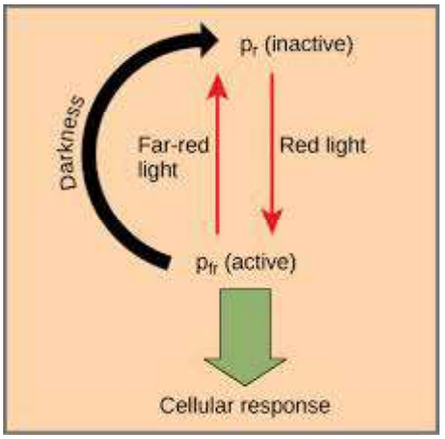

Fig. 2.14 *The Two Forms of Phytochrome*

The photomorphogenic response of flowering is regulated by the amount of P_r and P_{fr} forms of phytochrome prevalent in long day and short day plants. Both P_r and P_{fr} forms induce genes which initiate flowering by the activation of CO Protein. Thus, long and short day plants contain phytochrome-responsive genes which are regulated by a balance of P_r and P_{fr} forms.

The two major blue light receptors characterized in plants are CRY 1 and CRY 2 which induce flowering in response to specific exposure to photoperiods. The transcriptional regulation of CO and FT Proteins is mediated by CRY Proteins, thereby regulating floral induction. The event of flowering is temporally regulated by stabilization and ubiquitinylation of the CO Protein which activates the level of FT Protein in the shoot apex. CRY (Blue Light Receptor) mediated signal, on exposure to long day, negatively regulates COP1 Protein, thus rendering CO available to induce the expression of FT Protein necessary for flowering.

In the absence of active CRY signaling under short day periods, COP1 associates to CO Protein, leading to its ubiquitinylation, thus inhibiting flowering.

Molecular Mechanism of Vernalization (Low Temperature Induced Flowering)

Vernalization involves the attainment of competency of floral meristem formation induced by low temperature. Effect of natural low temperature or short day period can be mimicked by providing low temperature conditions to fasten attainment of reproductive phase in adult vegetative plants. Vernalization involves epigenetic changes associated with the repression of FLC Locus which represses flowering in shoot meristems. The epigenetic regulation of repression of FLC Locus involves DNA Methylation-Demethylation events associated with the signal of vernalization. These changes are, however, reversible in the next generation of the plant. Repression of FLC Locus transits the shoot apical meristem to floral meristem by further activating the homeotic genes. Vernalization involves exposure to low temperature (1-10°C) for a certain duration, followed by photoperiod necessary for flowering. Vernalization occurs in the shoot apex and the effect is reversible, i.e., devernalization is possible under opposite conditions of temperature and light. Flowers booming in the spring season undergo vernalization after exposure to specific duration of low temperature followed by some long days with the advent of summers.

Recent Advances Associated with the Molecular Mechanism of Floral Induction

- The Flowering Locus T (FT) Protein has been investigated in plant systems namely Arabidopsis, Cucurbita and Rice. FT Protein fulfils all the characteristic features hypothesised by Chailakhyan. The Protein regulates flowering induction in (Long Day Plant) Arabidopsis and rice (Short Day Plant). The initiation of floral induction begins with translation of FT Protein from FT mRNA in the leaves followed by its transport to the leaves. The Protein gets transported with photoassimilate to the shoot apex. The FT Protein in the apex acts as a transcriptional regulator for the expression of developmental genes necessary for transition of vegetative to reproductive phase. The movement of FT Protein by long distance transport from the phloem to the shoot apex has been reported to be associated with cytosolic trafficking (Cytoplasmic movement of Protein vesicles facilitated by Cytoskeleton elements) and intercellular transport by dilation of plasmodesmata.
- Recent reports in Arabidopsis suggest the systemic regulation of FT RNA by its long distance movement from stock plant shoot apex to grafted branch. Thus FT Protein and FT RNA both involve in the long distance signaling of floral induction.
- Arabidopsis cotyledons have been found to produce FT Protein in sufficient levels before the emergence of the first leaf. This suggests the role of cotyledons in floral induction in Arabidopsis.
- The differential flowering response in primary and secondary apical meristems of Tomato plants is regulated by Florigen interacting with two other Proteins SFT and SP. Florigen has also been suggested to be associated with leaf growth and maturation and abscission.
- Genes responsible for control of flowering through vernalization have been characterized in Arabidopsis and Cereals. VRN1 and FT genes have been characterised and observed to have positive regulation on floral induction stimulated by vernalization, while VRN2 has been stated to repress FT activity in the absence of vernalization, thus repressing flowering at the early vegetative state.

NOTES

A cross talk exists between external cues (Light, Temperature, Sucrose), Plant Hormones (GA) and Gene Expression to coordinate the initiation of flowering (Refer Figure 2.15).

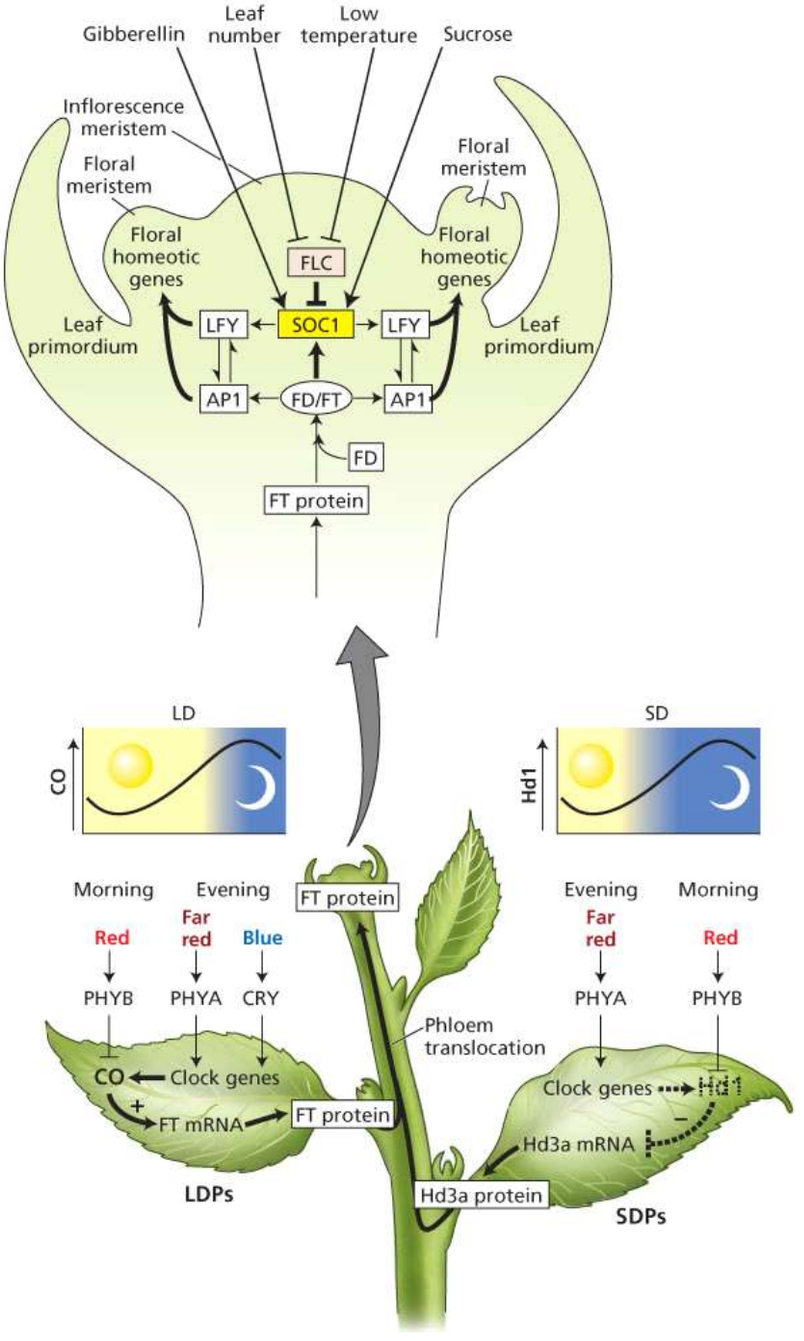

Fig. 2.15 *Scheme Showing Cross Talk between Several Factors Regulating Floral Induction*

'Check Your Progress'

8. What results in early flowering in long day plants?
9. Define evocation.
10. What are short day plants?

2.5 SUMMARY

- Plants produce hormones which act as the major signaling molecules or inducers of various responses. According to the modern concept an effective phytohormone should ideally possess a specific receptor.
- The phytohormones exhibit temporal and spatial regulations in terms of their biosynthesis, action and metabolism.
- The central ring of Auxin is comprised of an indole ring supplemented by various functional groups.
- IAA is chemically much similar to the Aromatic Amino Acid tryptophan. The Biosynthetic pathway of Auxin has been reported to be accomplished through multiple pathways. All the pathways are tryptophan dependent and multiple pathways may exist in same plant tissues.
- Elongation of cells in the shoot and root region are the primary effects of Auxin. The dissociation of proton conjugated auxin results in the formation of IAA^- and H^+ in the cytosol.
- The discovery of Gibberellins dates back to 1920s when a Japanese scientist Kurosawa investigated the physiological effects of Backanae Disease in Rice caused by the Fungi *Gibberella fujikuroi.*
- The formation of GA19 or GA20 is followed by its oxidation and 3-β-Hydroxylation to get converted into active forms.
- Photoblastic seeds usually imply the GA-dependent pathway of germination in presence of light, for example Lettuce and Tobacco.
- Formation of parthenocarpic fruits and pollen germination is stimulated by the activity of Gibberellic Acid (GA).
- The transport of Gibberellic Acid from the scutellum to the aleurone layer triggers the transcription of GAMYB (a transcription factor) elements necessary for expression of α-Amylase Proteins.
- In higher plants Zeatin exists both in the cis and trans configuration which are interconvertible by the enzyme Zeatin Isomerase.
- The process of shoot and root growth has been reported to be controlled by the activity of Cytokinin.
- Cytokinin modulates the effect of apical dominance by promoting the growth of axillary or lateral buds.
- Ethylene biosynthesis is regulated by the Yang Cycle where the CH_3-S group of methionine is recycled.
- Ethylene induces an onset of physiological changes associated with fruit ripening.
- An inhibitory substance (termed as Dormin) was isolated from Sycamore trees during their autumn leaf fall at the onset of dormancy. Later on Dormin was reported to be chemically similar to Abscisin II responsible for shedding of Cotton Fruits.
- ABA (15 C) shows partial chemical similarities to carotenoid molecules with carboxyl group at the second carbon which forms its cis-trans isomer.
- ABA inhibits GA-induced expression of α-amylase by inhibiting Gibberellic Acid responsive MYB elements in seeds.

- The seed harbours the young embryonic plant which matures prior to emergence from the seeds.
- Recalcitrant seeds usually loose viability after certain span of their maturity and therefore are unable to germinate.
- The first stage of seed development associated with the post-fertilization phase mainly involves histodifferentiation of various layers within the seed.
- The physiological inability of the viable seeds to germinate even in the presence of suitable conditions is referred to as dormancy.
- Dormancy of seeds can be overcome by a number of methods which involve scarification of seed coat, temperature alteration, light requirement and chemical treatment.
- The post-maturation phase of seed germination involves a certain time span for which the seed remains physiologically active or viable.
- The rate of aerobic respiration is minimum for the quiescent seeds.
- Mechanisms of flowering and seed dispersal indicate the evolutionary success of Angiosperms.
- The key elements associated with flowering are the phytochrome and cryptochrome receptors encoded by the Phy and Cry Genes.
- Garnard and Allard conducted experiments in the U.S. department of agricultural laboratories which revealed that the duration of light sensed is an important determinant of flowering in plants
- In plants like *Xanthium* and *Pharbitis* a short exposure of light during the night period may result in the inhibition of flowering.
- The P_r form induces flowering in the short day (long night) plants while the P_{fr} form induces flowering in long day (short night) plants.
- The process of flowering is controlled by the exposure to a number of photoperiodic inductions in a plant. The duration of photoperiod in a 24 hour cycle required by a plant is known as the inductive cycle.
- The photoreceptor called phytochrome is a cytosolic soluble Protein of molecular weight around 250 kDa. The Protein-Pigment Complex is formed of two homodimers of 125 kDa each.
- In the year 1937, Mikhail Chailakhyan postulated the existence of a flower- inducing factor-'Florigen', which can be transported within one plant and preferably also through grafts, to induce flowering in a uninduced plant.
- The events of formation of floral meristem from shoot apical meristem are highly regulative in terms of environmental cues (Nutrition, Sucrose, Gibberellic Acid, Photoperiod) which trigger the downregulation or upregulation of several genes necessary to transit from vegetative to reproductive phase.
- The two major blue light receptors characterized in plants are CRY 1 and CRY 2 which induce flowering in response to specific exposure to photoperiods.
- Vernalization involves the attainment of competency of floral meristem formation induced by low temperature.

- Effect of natural low temperature or short day period can be mimicked by providing low temperature conditions to fasten attainment of reproductive phase in adult vegetative plants.

2.6 KEY TERMS

- **Apical dominance:** The phenomenon of Auxin induced elongation of apical shoot and suppression of later branches.
- **Bolting:** The processs of stem apex elongation resulting in flowering.
- **Richmond-Lang effect:** The antisenescence effect of cytokinin resulting in suppression of chlorophyll degradation in leaf discs.
- **Orthodox seeds:** Seeds which can endure desiccation tolerance and remain viable after long term storage (depending upon the species, i.e., 1-4 years).
- **Seed dormancy:** The physiological inability of the viable seeds to germinate even in the presence of suitable conditions is referred to as dormancy.
- **Positively photoblastic seeds:** The seeds which require light to induce germination.
- **Negatively photoblastic seeds:** Phenomenon where seed germination is inhibited by light.
- **Competence:** The capability of flowering upon receiving suitable photoperiod.
- **Vernalization:** Cold temperature induced flowering is known vernalization.

2.7 ANSWERS TO 'CHECK YOUR PROGRESS'

1. IPA pathway is the Indole-3-Pyruvic Acid pathway is one of the common paths of Auxin Biosynthesis. IPA is formed by the catalytic activity of tryptophan transaminase. The biochemical reactions involved in this pathway are deamination reaction to form IPA followed by decarboxylation to form indole-3-Acetaldehyde (IAld). IAld is metabolized to form IAA catalyzed by Auxin Dehydrogenase Enzyme.
2. TAM pathway is the tryptamine pathway which is almost similar to the IPA pathway but exhibits a reverse order of deamination and decarboxylation reaction catalyzed by different enzymes. Tyrptamine is formed by the enzyme tryptophan decarboxylase which then gets metabolized to IAld catalysed by amine oxidase.
3. IAN pathway is the Indole-3-Acetonitrile pathway involves conversion of Tryptophan into Indole-3-Acetaldoxime catalyzed by Tryptohan Monooxygenase. Indole-3-Acetaldoxime is converted to Indole-3-Acetonitrile and then IAA.
4. Triple response is the morphological response of Ethylene in etiolated seedlings manifested by suppression of hypocotyl growth, increased lateral growth and formation of cotyledonary hook.
5. LEA, i.e., Late Embryogenesis Abundant Proteins is a ABA induced synthesis of desiccation tolerance Proteins produced during mature phase of seed development.
6. The physiological inability of the viable seeds to germinate even in the presence of suitable conditions is referred to as dormancy. The stage of dormancy may persist for certain duration and usually occurs due to anatomical and biochemical reasons.

7. The process of germination is accomplished in the following three stages:
   - Imbibition and lag phase
   - Hydration of seeds and surge in metabolism
   - Radical emergence and seedling growth
8. Interruption of the dark period with a light flash or extension of the day duration can result in early flowering in LDP, i.e. Long Day Plants.
9. Evocation is the irreversible conversion of vegetative to floral meristem regulated by molecular changes.
10. Plants which flower in response to shorter day durations or are accelerated by shorter day lengths are termed as qualitative or quantitative short day plants, respectively.

2.8 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Explain the biosynthetic pathway of Auxin and Gibberellins.
2. Name one synthetic Auxin and Cytokinin.
3. What do you mean by Richmond-Lang effect?
4. List the physiological roles of Auxin and Abscisic Acid in plants.
5. What do you mean by quiescent seeds?
6. Define positive and negative photobalstic seeds.
7. What are the physiological changes associated with seed germination?
8. Write a short note LEA Proteins.
9. What do you mean by floral evocation? Diagrammatically explain the steps involved in the development of floral meristem from shoot apical meristem.
10. What are MADS box genes?

Long-Answer Questions

1. Explain the role of gibberellin in seed germination and flowering.
2. Discuss the significance of triple response.
3. Elaborate a note on the role of Cytokinin in plants.
4. Write a note on seed dormancy and its types.
5. Explain the physiological reasons for dormancy of seeds.
6. Define the terms competence and determination of flowering. Briefly explain the role of phytochrome and cryptochrome in induction of flowering.
7. Define photoperiodism. Explain with appropriate examples the classification of plants based on photoperiodic response.
8. Elaborate a note on the significance of night break experiment of flowering.
9. Explain with reference to an experiment that leaves are the primary organ of photostimulus perception.

10. Explain the effect of photoperiod on CO Protein. Also discuss about the cross talk between several factors and gene expression involved in floral induction.
11. Explain the mechanism of ABC model of flowering and the developmental abnormalities likely to form on mutation in the Type C gene of the model.

2.9 FURTHER READING

Hopkins, William G. and Norman P.A. Huner. 2013. *Introduction to Plant Physiology*, 4th Edition. Noida: Wiley India Pvt. Ltd.

Verma, S K and Mohit Verma. 1995. *A Textbook of Plant Physiology, Biochemistry and Biotechnology*. New Delhi: S Chand & Company Ltd.

Blackstock, James C. 2014. *Guide to Biochemistry*. Oxford: Butterworth-Heinemann.

Park, K. H. 2008. *Carbohydrate-Active Enzymes: Structure, Function and Applications*. Amsterdam: Elsevier.

Fromm, Herbert J. and Mark Hargrove. 2012. *Essentials of Biochemistry*. Berlin: Springer.

Fearon, William Robert. 2014. *An Introduction to Biochemistry*. Amsterdam: Elsevier.

UNIT 3 ENZYMES AND BIOLOGICAL OXIDATION

NOTES

Structure

3.0 INTRODUCTION

Enzymes are diverse types are proteinaceous molecules which function as reaction catalyst of various biochemical reactions of metabolism. In certain cases, enzymes possess higher catalysis power in comparison with inorganic or organic chemical catalysts. The enzymes possess high specificity for the substrates which they metabolize to form products. Enzymes function in mild temperature and pH in aqueous environments. The enzymes essentially function in suitable micro-environments of particular cofactor, pH, and temperature. The specialized feature of the enzyme catalyzed biochemical reaction lies in the fact that the enzyme provides the confines of its active site. The chemical transformation of the substrate bound to the active site occurs by biochemical process.

Aerobic respiration is a catabolic process associated with organic compounds oxidized to store energy in the form of ATP molecules. Plants exhibit glucose oxidation pathway in the cells which serve to be the major component of aerobic respiration. However, various other sources like triose phosphate, lipids and Complex carbohydrates can also act as substrate for respiration. The biochemical reaction of respiration is almost reverse of the photosynthetic pathway where sucrose in oxidized to form CO_2 and water. The Oxygen molecules are reduced to water and they also act as the terminal electron acceptor in the respiratory pathway. The decrease in free energy associated with the process of respiration is coupled to ATP synthesis and energy storage within the cells.

In this unit, you will study about concept of enzyme activity and its structure, classification of enzyme, mechanism of enzyme action and inhibition, mechanism of glycolysis, importance of TCA cycle, oxidative phosphorylation, electron transport and pentose phosphate pathway.

NOTES

3.1 UNIT OBJECTIVES

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

- Understand the concept of enzyme activity and its structure
- Discuss the classification system of enzymes
- Explain the mechanism of enzyme action and inhibition
- Understand the mechanism of glycolysis
- Analyse the importance of TCA cycle
- Explain the mechanism of oxidative phosphorylation and electron transport
- Discuss the significance of pentose phosphate pathway

3.2 ENZYMES

Enzymes are diverse types are proteinaceous molecules which function as reaction catalyst of various biochemical reactions of metabolism. In certain cases, enzymes possess higher catalysis power in comparison with inorganic or organic chemical catalysts. The enzymes possess high specificity for the substrates which they metabolize to form products. Enzymes function in mild temperature and pH in aqueous environments. The enzymes essentially function in suitable micro-environments of particular cofactor, pH, and temperature. They sequentially catalyze the steps of biochemical reaction in a metabolic pathway. They convert various nutrient molecules into their derivative forms or function in transformation of chemical energy within the cell. The function of enzyme is also associated with the biosynthesis of macromolecules within the cell. These molecules act as storage compounds or function as structural components of the cell. The study of function of enzymes is necessary in the investigation of various diseases related to metabolism. The absence or hyperactivity of certain enzymes result in physiological disorder manifested as a diseases.

Moreover, various drugs interact with enzymes and accomplish their action in the cell. Enzymes possess immense practical applications in the food industry, food processing and agricultural aspects. Most of the enzymes are chemically Proteins in nature with exceptions for ribozymes which are self-catalytic RNA molecules. The discovery of enzyme unknowingly dates back to the ancient period when ancient Greeks popularized the process of alcoholic fermentation, cheese making and bread leavening. The fact that yeast mediates fermentation of sugary compounds was thought to be resulting from the activity of chemical compound.

However, in 1857 Louis Pasteur reported the importance of yeast as a living organism which undergoes fermentation as a part of its metabolic process. In 1878 Friedrich W. Kuhne coined the word 'Enzyme' which has been derived from the Greek word '*Enzumas*' meaning (the leaving of bready by yeasts). In 1887, Edward Buchner discovered the activity of Zymase necessary for the fermentation of Grape Juice. The extract produced in the experiment was a cell free enzyme extract. The initial idea that enzymes are Proteins in nature came from the investigations of James B. Summer in 1926. James isolated the Enzyme Urease in crystalline form from the Jack Bean Seeds (*Canavalia ensiformis)*.

Later on, various other reports on the isolation of pepsin, trypsin, chymotrypsin and chymotrypsinogen led to the conclusion that enzymes are proteinaceous in nature. The catalytic activity of the enzyme depends upon the nature of their native conformation. The primary,

secondary, tertiary and quaternary structure of the Protein is important in maintaining the catalytic activity of the enzyme. The quaternary structure formed for an enzyme results in the formation of an active site on its surface. Active sites are usually regions formed by stabilization of various chemical interactions among the Amino Acids. This region binds to particular substrates specific for the enzyme. The active-site mediated binding of substrate is supported by structural complimentary between the substrate and the active site. Enzymes may require the association of various chemical substances known as cofactors. Cofactors are usually inorganic non-protein substances like metal ions (Ca^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+} or Mn^{2+}) which stabilize the enzyme-substrate interaction and help in the catalytic activity of the enzyme (Refer Figure 3.1).

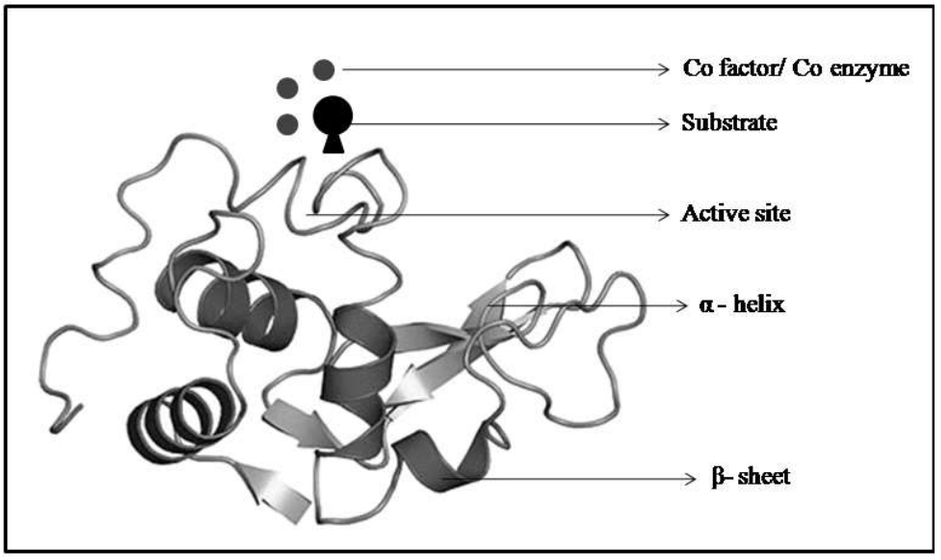

Fig 3.1 *Structure of an Enzyme: Folded in Tertiary Structure*

Coenzymes are, however, metaloorganic compounds which do not bind to the enzyme, but facilitate in its activity (Refer Figure 3.2). These biomolecules are mostly derived from various vitamins and organonutrients which function as carriers of various functional groups. Certain enzymes may require the association of both coenzymes and cofactors necessary for metabolizing a substrate. The chemical activators which are tightly bound to the enzymes by means of covalent bonds are known as prosthetic groups. The functional catalytic form of the enzyme in association with its cofactors or coenzymes is referred to as the **holoenzyme**. The proteinaceous part of the enzyme excluding the cofactor or coenzyme is known as the **apoenzyme**. The association of the cofactors are required to trigger the catalytic activity of the apoprotein which then constitutes the holoenzyme. The activities of certain enzymes result from their activity modulation by Phosphorylation, Glycosylation or Nitrosylation. The spatio-temporal regulation of enzymes is important to control any biochemical pathway. Plant enzymes exhibit specific compartmentalizations in respect to their distribution in the cells. The enzymes are non-uniformly distributed in the protoplasm and other organellar components. The site or location of the enzyme in the cell depicts the nature of its action. Most RNA synthesizing enzymes are localized in the nucleolus.

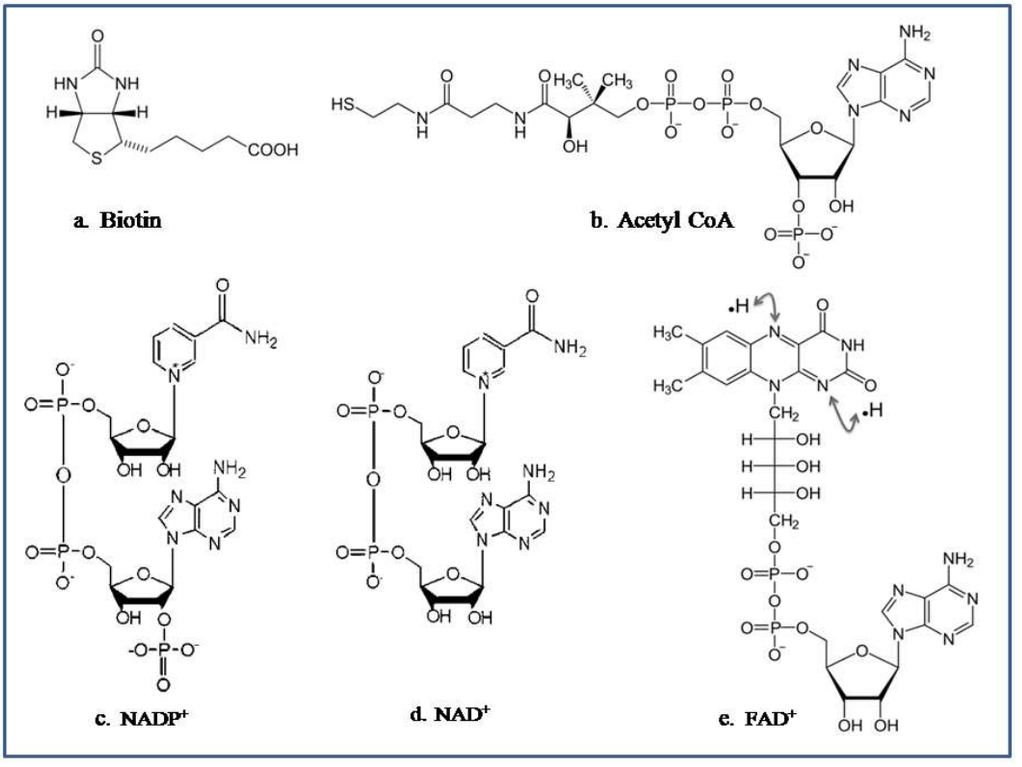

Fig 3.2 *Structure of Various Coenzymes*

The enzymes necessary for translation are mostly present in the cytoplasm. The respiratory enzymes remain both in the cytosol and mitochondria (intermembrane space and matrix). The photosynthetic enzymes are present in the chloroplast. Certain biochemical pathways like photorespiration involve three organelles (chloroplast, mitochondria and peroxysomes) to accomplish their enzymatic reactions. Various enzymes are present in the plasma membrane and function as sites of biochemical reactions. The enzymes have been classified into various classes on the basis of the nature of biochemical reaction catalyzed by them. The class and subclass of the enzyme is being denoted by the Enzyme Commission Number (E.C. Number). This classification is a four level classification which categorizes the enzymes into a class and respective subclasses.

The specialized feature of the enzyme catalyzed biochemical reaction lies in the fact that the enzyme provides the confines of its active site. The chemical transformation of the substrate bound to the active site occurs by biochemical process. The concept of formation of enzyme-substrate Complex was reported by Charles-Adolphe Wurtz in 1880. The biochemical reactions are driven by the availability of free energy. The difference between the ground state and transition state is known as the activation energy. Enzymes present as biocatalysts lower the activation energy of a reaction. This results in easier transformation of a substrate to its product. The activity of the enzyme increases the rate of the reaction without changing its equilibrium. The intermediate steps of the reaction are sequentially converted to the final state of product. Certain cases where the product is formed by serial reactions are regulated by the step having highest activation energy. The present unit shall elaborate the structure, chemical nature and classification of enzymes in plants. The mechanism of enzyme kinetics is important to understand the nature of substrate transformation and metabolism catalyzed by enzymes.

NOTES

3.2.1 Classification of Enzymes

The older system of classification of enzymes was based upon the name of substrate and the nature of reaction catalyzed by the enzyme. The broad group of classification for enzymes were primarily:

- Hydrolyzing Enzymes
- Desmolysing Enzymes

The hydrolysing enzymes function to split the molecules by addition of water molecules. The major Hydrolyzing Enzymes associated with the breakdown of sugars include Invertase, Maltase, Lactase, Cellulose or Inulase. These enzymes were collectively termed as the **Carbohydrates**. The other enzymes of esterases, i.e., Lipase and Phosphatase are involved in hydrolyzing the Ester group. The major proteolytic enzymes associated with hydrolytic reactions include Pepsin, Peptidase, Protease, etc. The Amidases were termed for the enzymes associated with hydrolysis of Amide group, for example, Ureases and Asparaginase. The other class of hydrolyzing enzymes include Phosphorylases.

Apart from the hydrolysing enzymes, the other category of enzyme was termed as the desmolysing type. The various types of desmolysing enzymes include Aldolase, Dehydrogenase, Transphosphorylases, Hydrases, Transaminase, Carboxylase, Peroxidase and Catalases. The category of desmolysing enzyme was broadly based upon the reactions which involved the breakage of Carbon chain or intra or inter molecular transfers of atoms. However, the older system of classification of enzymes was obscure in terms of clear indication of the type of enzyme. According to the older system of enzymes the names used often did not indicate the type of substrate or reaction they catalyze.

The new system of classification of enzyme involves systematic nomenclature of the enzymes and their categorization according to the reaction catalyzed by them. The Commission on Enzymes of the International Union of Biochemistry (1961) has recommended this new system of classification. According to this system of classification the enzymes are referred by four digit code numbers.

The features of the new system of classification are as follows:

- The enzymes have been categorized into six major classes.
- The major classes have been divided into various subclasses.
- The enzymes are represented by a specific code number consisting of four digits. The first digit represents the major class while the second and third digit represents the subclasses. The last digit denotes the specific name of substrate used and the nature of the reaction.

The major classes of the enzymes are as follows:

1. **Oxido-Reductases:** Enzymes which catalyze oxidation-reduction process.
2. **Transferases:** Enzymes which catalyze group transfer.
3. **Hydrolases:** Enzymes which catalyze isomerisation reactions.
4. **Ligases:** Enzymes catalyzing reactions in which two molecules are linked and coupled. to breakdown of pyrophosphate bond of ATP or similar triphosphates.

The subclasses of the enzymes are as follows:

1.1 Subclass: Oxidoreeductases acting on the CH.OH group of the donor.

2.1 Subclass: Transferases transferring one carbon group.

3.1 Subclass: Hydrolase acts on the ester links.

NOTES

4.1 Subclass: Lyase acting on the C-C bond.

5.1 Subclass: Isomerase acts as a epimerase and racemase.

6.1 Subclass: Ligase which forms C-O bonds.

1.1.1 Sub-subclass- Oxidoreductase acting on the CH.CH group of the donor with coenzyme NAD or NADP as acceptors.

2.1.1 Sub-subclass- Transferase which catalyze the transfer of one-carbon groups and a methyl transferase.

3.1.1 Sub-subclass- Hydrolase acting on carboxylic ester links.

4.1.1 Sub-subclass- Lyase (C-C lyase) carboxylase.

5.1.1 Sub-subclass- Isomerase functioning as a racemase and epimerase on Amino Acids and derivatives.

6.1.1 Sub-subclass- Ligases which form C-O bond.

Thus, enzymes like alcohol dehrdogenase or carboxyesterases are represented as 1.1.1.1 and 3.1.1.1, respectively. Pyruvate decarboxylases are represented as 4.1.1.1.

3.2.2 Structure of Enzyme

Enzymes are proteinaceous molecules which assort in the quaternary conformation to function as catalytic units. The functionality of the enzyme is thus maintained by its active site which binds to the substrate and forms a substrate enzyme Complex. Enzymatic Protein molecules thus consist of more than one polypeptide chain and are composed of 10 different types of Amino Acids. The sequence of Amino Acids is specific for each of the enzymes. Mutation in the triplet codon may result in alteration of the Amino Acid composition.

This results in misfolding of Proteins and enzymes which lead to termination in their catalytic activity. Various genetic diseases occur due to non-functionality, hyperactivity or absence of certain metabolic enzymes in the cell. The quaternary structure of the Protein is essential to be maintained by means of stable Sulphide bonds prevalent among the constituent subunits. Protein denaturants like mercaptoethanol, urea and DiThioeriThritol (DTT) may cause disruption of the bonds forming the quaternary structure of Proteins. This may result in loss of catalytic activity of the enzyme. The activity of the enzyme is exhibited at a particular pH and range of temperature. The ambient range of temperature necessary for any enzyme activity usually ranges from 25°C to 37°C.

However, in certain cases enzymes may function at temperatures beyond this range. All enzymes function as biocatalysts in nature. They differ from chemical catalysts in terms of their proteinaceous nature and being products inside the cells. Enzymes act on a specific substrate or a group of substrate to metabolize it into the product. Enzymes which are produced and function inside the cell are termed as endoenzymes. A majority of the enzymes responsible for growth and metabolism or various cellular processes are usually endoenzymes. The exo-enzymes are secreted by the heterotrophic organisms like Bacteria and Fungi which produce and secrete them at the extracellular region. Cellulase, Pectinase, and Ligninase are enzymes which breakdown Complex organic substances into simpler forms.

Mostly the exoenzymes are digestive or hydrolytic enzymes functioning in breakdown of insoluble compounds to soluble forms. The germinating seeds secrete hydrolytic enzymes which convert insoluble forms of metabolite into soluble sugars in the endosperm. The enzymes which are present in the organism irrespective of its metabolic state are termed as the constitutive enzyme. The inducible enzymes are the ones synthesized in the cell in response

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to external substrate. The substrate or other chemical agent capable of inducing the synthesis of the enzyme is known as an inducer. The process of inducible expression of enzyme by the action of external substrate is common in bacterial members. In higher plants nitrate reductase is an example of inducible enzyme which functions in presence of external source of nitrate. The activity of Gibberellic Acid stimulates the finction of α-Amylase in the endosperm which breaks down Starch into Sugar forms.

The parts of the enzyme necessary for its functioning are as follows:

- **Protein Part (Apoenzyme):** The Protein part of the enzyme functions in its quaternary or tertiary form of folded Protein. Certain enzymes are composed of subunits of **Proteins** exhibiting similar molecular weight. The Protein part of the enzyme is termed as the apoenzyme which associates with cofactor or prosthetic group to exhibit its catalytic activity.
- **Active Site:** The active site of the enzyme is the most important region necessary to maintain its catalytic activity. This region is specific to the enzyme which may bear more than one active sites together in the same enzyme. The active site is comprised of hydrophilic Amino Acids which orient in specific alignment in the Folded Protein. The active site binds to the specific substrate and catalyzes the reaction. The interaction between the substrate and enzyme is a Complex process. Hydrogen bonds, hydrophobic interactions and ionic bonds facilitate the process of substrate-enzyme Complex formation. The energy derived from the enzyme-substrate binding is termed as the binding energy. In another sense this binding energy is a form of free energy by which the enzyme lowers the activation energy of the reaction.

  Thus, the catalytic power of an enzyme largely depends upon the amount of free energy released in the form of weak bonds and ionic interactions between the substrate and enzyme. The weak interactions are optimised in the process of transition of the substrate into the product. According to Emil Fischer the structure of the enzyme at the active site is complementary to the specific substrate of the enzyme.
- **Prosthetic Group (Non-Protein Part of the Enzyme**): The non-protein part of the enzyme associated with the active site of the enzyme is necessary for the catalytic activity of the enzyme. The prosthetic groups usually consist of an organic compound or inorganic metal ion (Cu, Zn, Mo, etc). The organic compounds usually contain flavin nucleotides or heme containing iron groups. The prosthetic group is tightly bound to the enzyme by means of covalent linkage.
- **Cofactor:** The cofactors are not structural part of the enzyme but remain associated with it. The presence of cofactor is essential to accomplish the metabolic reactions catalyzed by the enzyme. The cofactors are not tightly associated with the enzyme are categorized into following two types based upon its structure:

  (i) **Specific Coenzymes:** The various specific coenzymes necessary for the catalytic activity of enzymes include Hydrogen carriers (Oxidation-Reduction Reactions) like Coenzyme I, Coenzyme II, Lipoic Acid, Glutathione or Flavin Adenine Dinucleotide. The other types of coenzymes include Coenzyme A as an Acyl group carrier, Pyridoxal Phosphate as Amino Group carrier and Biotin as a form of CO_2 carrier. Thiamine Pyrophos Phate (TPP) or Coenzymes F are some of the other coenzymes necessary for enzyme activity.

(ii) **General Activators:** Certain metal ions and inorganic molecules may act as general inducer or activator of enzymes.

3.2.3 Theories for Enzyme Action

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The activity of enzyme involves substrate-enzyme interaction and utilization of free energy which helps in the formation of enzyme-substrate Complex. The two main theories proposed for explaining the mode of action of enzyme are Fischer's Lock and Key Hypothesis and Koshland's Induced Fit Theory.

Lock and Key Hypothesis

The structural complementarity in the mode of interaction among the substrate and enzyme has been explained by the Lock and Key Hypothesis. This theory has been widely accepted which explains that the active site possess structural features facilitating the binding of substrate. The specificity of the enzyme-substrate is similar to the Lock-Key specificity. The enzyme catalyzes the formation of product from the transition of the substrate. The enzyme is released free to accept substrate for new reaction (Refer Figure 3.3).

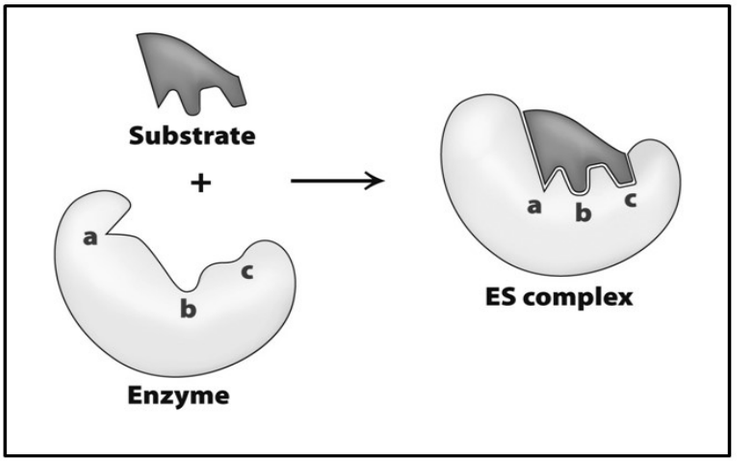

Fig. 3.3 *Lock and Key Hypothesis of Enzyme-Substrate Binding*

Induced Fit Theory

According to this theory the presence of substrate in the vicinity of the enzyme acts as an inducer of the enzyme. The presence of the substrate results in the formation of conformational change in the active site of the enzyme. This results in the formation of chemical interaction between the enzyme and substrate. The substrate is held in the active site by help of Hydrogen bonds. Furthermore, the electrophilic and nucleophilic interactions prevalent in the catalytic groups of the enzyme weaken the bonds formed between the substrate and the active site. Moreover, for non-substrate molecules the active site does not undergo suitable interactions with the molecule present. Thus, the enzymatic reaction does not take place (Refer Figure 3.4).

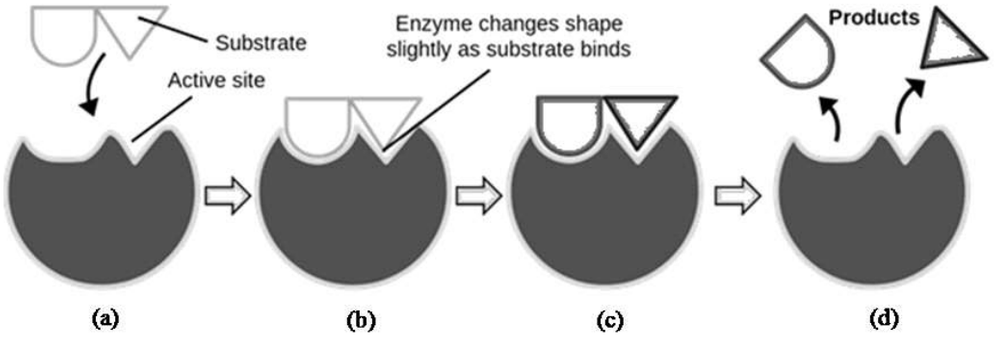

Fig. 3.4 *Induced Fit Theory of Enzyme Action (a-d) Substrate-Induced Changes in the Shape of Enzyme*

3.2.4 Mechanism of Enzyme Action

The enzyme utilizes the binding energy to form the transitory enzyme-substrate Complex. This binding energy is formed due to Hydrogen bond and various ionic interactions present between the enzyme and substrate. The binding energy is referred to as the free energy utilized by the enzyme to lower the activation energy of the reaction. Emil Fischer in 1894 proposed that the substrate fits into the active site of the enzyme due to its structural complementarities. This theory was found to possess limitations. Complete structural complementarities between the enzyme and substrate at the active site would, in general, impede the reaction and product formation. According to the modern theories of enzyme action proposed by Michael Polanyi (1921), Haldane (1930), and Linus Pauling (1946) the enzymatic reaction involves formation of a transition state stage established between the Enzyme and the Substrate. Thus, the substrate should essentially gather an increased free energy necessary for undergoing the transformation. Initially the enzyme-substrate Complex forms by weak interactions which are followed by stringer forces operative in the transition state.

Thus, the electrophilic interactions occurring at the transition state of the enzyme-substrate Complex is very essential to complete the reaction. The free energy released in this context essentially lowers the activation energy of the reaction. In this case, the summation of two components of energy, i.e., positive free energy and the negative binding energy lower the activation energy of the biochemical reaction. The weak interaction between the enzyme and substrate is the main driving force for the catalytic activity of the enzyme. The larger size of the enzyme helps in providing multiple sites of weak interactions between the active site and the substrate. It has been reported that enzymatic interactions can lower the activation energy by as low as 5.7 kJ/mol. The amount of energy available for formation of weak interaction varies forms 3-30 kJ/mol.

To understand the process of enzyme catalysis in the light of thermodynamics it is essential to know the reasons of higher activation energy in absence of the enzyme. In uncatalyzed reactions usually there in low entropy caused due to less freedom of the molecules. The solvation shell of the biomolecules is formed by Hydrogen bonded water molecules present in the aqueous environment. This results in increased activation energy of the reaction. Higher energy is required to cause structural distortion of the substrate or the alignment of the catalytic groups. This situation is overcome by the free energy and binding energy provided by enzyme-substrate interaction.

According to Arrhenius, low energy rich molecules require higher energy to complete chemical reactions. The stability of the molecules requires to be overcome to undergo transition and transformation into products. This energy required for a chemical reaction is called the

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activation energy. Higher temperature facilitates a chemical reaction occurring due to higher kinetic energy of the molecules. This is caused as a result of thermal agitation. In presence of the enzyme-substrate Complex weak interactions result in breaking the solvation shield of the molecule. Furthermore, the binding energy present helps to compensate any thermodynamic distortion caused by electron redistribution and substrate transformation. Thus, the binding energy is important in imparting enzyme-substrate specificity of a reaction (Refer Figure 3.5).

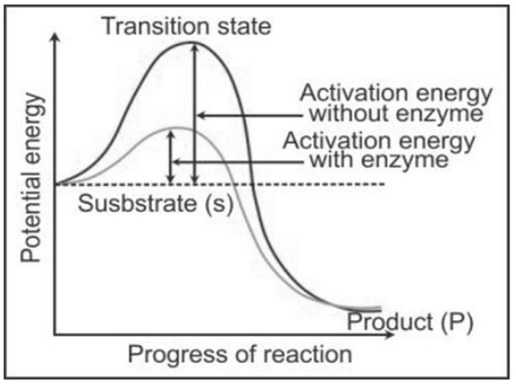

Fig. 3.5 *Lowering of Activation Energy by Enzyme Activity*

3.2.5 The Michaelis-Menten Constant (Mechanism of Enzyme Catalysis)

The mechanism of enzyme-induced catalysis of reaction can be explained by the concept of Michaelis-Menten Equation. The concept explains the regulation of the rate of enzymatic reaction which depends upon the concentration of substrate and available molecules of enzyme in a reaction medium. In situations of increasing substrate concentration and constant enzyme molecules, the reaction rate in relation to the increasing substrate concentration follows a hyperbolic curve. This results due to saturation in the rate of reaction after optimum substrate concentrations. Lower concentration of substrate exhibits a direct proportionality to the reaction rate. This results due to binding of the substrate to the enzyme molecules. However, enzyme molecules become limiting at a later stage which results in the appearance of steady state. At this stage the reaction rate does not increase with increasing the substrate concentrations. The maximum velocity attained by optimum concentration of substrate is denoted as the $V_{max.}$ The substrate concentration necessary for attaining half velocity ($1/2/V_{max}$) of the reaction is known as K_m value for the enzyme. This **K_m value** is known as the **Michaelis-Menten Constant**. This value of K_m is essential to understand the affinity of an enzyme to its substrate. Lower K_m value indicates higher affinity of the enzyme to the substrate.

This results in attaining $1/2V_{max}$ at a lower substrate concentration. During steady state conditions, the kinetic value of enzyme catalyzed reactions show relations between the following parameters:

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

V= Velocity or Rate of Reaction, V_{max} = Maximum Velocity Attained by the Enzyme, [S]= Substrate Concentration, K_m = Michaelis-Menten Constant.

The K_m values of enzymatic reaction may vary according to the pH, temperature and ionic strength of the medium. The amount of coenzyme present in the medium also influences the K_m value. The K_m values are usually calculated in terms of molarity. The K_m of enzymes

range from (1 mM -1 μM). The concept of Michaelis-Menten Constant is helpful in understanding the nature of enzyme-substrate reaction. The velocity of the reaction indicates the further requirement of enzyme or substrate molecule to speed up the reaction. Certain enzymes which can bind to two similar kinds of substrates (Hexose Sugars) will exhibit higher reaction rate for the substrate with lower K_m value. The measurement of K_m value provides indication about the endogenous concentration of the substrate participating in the biological reaction. Enzymes which catalyze reactions with higher availability or high concentration of substrate usually exhibit higher K_m value.

3.2.6 Isoenzymes

Enzymes which exist in multiple forms in various tissues and organs but catalyze the same biochemical reaction are referred to as isoenzymes or isozymes. These enzymes possess differences in their molecular structure and kinetic properties. The isoenzymes possess variable molecular weight which is attributed to the differences in their subunit arrangement and Amino Acid composition. Different genes of a single gene family usually encode for isoenzymes in tissues. Isoenzymes comprised of dissimilar subunits are called Heteropolymers. Isoenzymes may vary in their structural feature due to their encoding by alleles of the same gene. The minute differences in the structure of the isoenzymes are also attributed to the post-translational modifications like Glycosylation, N-Nitrosylation or Methylation. The activity of isoenzyme exhibit temporal and spatial regulation in plants and animal tissues. This results in different metabolic requirement of the tissues varying with its age and stage of development. The first enzyme reported to possess isoenzyme forms was lactate dehydrogenase. In plants peroxidase has been reported to possess as high as 18 isoenzyme forms in Maize. This enzyme is a major antioxidative enzyme with detoxification and ion homoeostasis function. The various isoenzymes of peroxidase protects different plant tissues associated with development, senescence or environmental stress. Aspartate Kinase in plants is associated with biosynthesis of Amino Acids like Lysine and Alanine from Aspartate. This enzyme exists in two isozyme forms.

Mechanisms of Enzyme Inhibition

Various metabolic inhibitors are associated with the inhibition of enzyme action partially or completely. The process may appear to be reversible or irreversible process which depends upoon the nature of the inhibitor. The efficiency of the inhibitor depends upon the concentration of the enzyme and substrate.

- **Competitive Inhibitor:** The inhibitors which possess structural similarity to the substrate may compete for the same active site of the enzyme thus preventing the binding of substrate to the enzyme. Prior occupancy of the active site with the application of competitive inhibitor prevents the catalysis of the substrate.

  However, this type of inhibiton is a reversible type, where removal of the inhibitor may restore the activity of the enzyme, for example malonic acid is a competitive inhibitor of the enzyme succininc dehydrogenase which catalyzes the conversion of succininc acid to fumaric acid.
- **Non-Competitive Inhibitor:** The chemical inhibitors which bind to the enzyme at regions different from active sites and distort the functionality of the enzyme. These types of inhibitors usually cause irreversible changes in the structure of the enzyme, for example heavy metals, acid, etc.
- **Allosteric Inhibition (Feedback Inhibition):** Metabolic pathways exhibit the phenomenon of allosteric inhibition. This process involves binding of an end product of the pathway to one of the initial step enzyme thus causing inhibition in its activity.

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The product which exerts feedback inhibition is completely different in structure in comparison with the substrate of the enzyme. The enzyme undergoing the process of inhibition is termed as **allosteric enzyme**. The allosteric site is different from the active site of the enzyme.

The binding of the product to the allosteric site results in the conformational change in the enzyme which renders the active site to be unsuitable to accept the substrate. The process of allosteric inhibition is reversible with the decrease in the concentration of the end product formed in the pathway. Threonine dehydratase in *E. coli* exhibits the property of allosteric inhibition by the end product of the pathway, i.e., L-Isoleucine.

'Check Your Progress'

1. What is the difference between binding energy and activation energy?
2. What is Michaelis-Menten Constant?
3. Define allosteric inhibition.

3.3 BIOLOGICAL OXIDATION

Aerobic respiration is a catabolic process associated with organic compounds oxidized to store energy in the form of ATP molecules. Plants exhibit glucose oxidation pathway in the cells which serve to be the major component of aerobic respiration. However, various other sources like triose phosphate, lipids and Complex carbohydrates can also act as substrate for respiration. The biochemical reaction of respiration is almost reverse of the photosynthetic pathway where sucrose in oxidized to form CO_2 and water. The Oxygen molecules are reduced to water and they also act as the terminal electron acceptor in the respiratory pathway. The decrease in free energy associated with the process of respiration is coupled to ATP synthesis and energy storage within the cells. The method of energy release in the process of respiration is through sequential steps or phases. This prevents excessive flow of energy through Protein and carrier components which may otherwise cause cellular damage. The four phases of Respiration are mainly Glycolysis, Citric Acid Cycle, Pentose Phosphate Pathway and the Electron Transport Chain in Mitochondria. Glycolysis is the preliminary phase of respiration which occurs in the cytosol and plastids. The process of glycolysis accomplishes by the formation of pyruvic acid, catalyzed by various enzymes. Pyruvate is formed by the oxidation of sugars like sucrose which is converted to intermediate metabolites like hexose phosphates and triose phosphates.

Apart from ATP release the process of glycolysis also liberates NADH which acts as the reducing power in the cell. Oxidation of glucose liberates around 686 Kilo Cal of energy. The pathway of citric acid cycle involves complete oxidation of pyruvic acid into CO_2. A significant amount of reducing power formed by 16NADH and $4FADH_2$ is formed as products of the citric acid cycle. The majority of enzymes associated with the process of citric acid cycle are localized in the inner matrix of mitochondria. The pentose phosphate pathway operates in the cytosol and plastid where glucose-6 Phosphate is oxidized to Ribulose-5-phosphate and forms CO_2 molecules. Reducing power formed in this pathway is in the form of NADPH. The product of glucose oxidation, i.e., ribulose 5-phosphate is further metabolized into three and seven carbon sugars. The electron transport chain is associated with the process of oxidative phosphorylation. This involves ATP synthesis coupled to the energy released during electron transfer. The majority of electron carriers/proteins are localized in the inner membrane of the mitochondria. NADH transfers the electron through various

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intermediate carriers and it finally reaches Oxygen molecules (terminal electron acceptor). Thus the redox reactions associated with electron transfer process coupled to ATP biosynthesis is termed as **oxidative phosphorylation**. Nicotinamide Adenine Dinucleotide (NAD) is an important cofactor or organic coenzyme associated with the process of enzymatic reactions. NAD accepts electrons and gets converted from its oxidised form to NADH (reduced form). This molecule possesses reduction potential of around –320 mV which provides it the property of a very good reductant. The phosphorylated form of this molecule, i.e., Nicotinamide Adenine Dinucleotide Phosphate (NADP) also functions as a reductant in photosynthesis and pentose phosphate pathway. The free energy released by oxidation of NADH is around 220 kJ mol^{-1} which helps in the process of ATP generation.

Substrate Level Phosphorylation is another mechanism of ATP synthesis which involves the transfer of Inorganic Phosphate (PI) molecules from the substrate to the ADP molecules. The process of reverse glycolysis and formation of glucose from pyruvic acid is termed as gluconeogenesis. This pathway is not much common in plants. However, metabolism in germinating oil seeds has been reported to be associated with the process of gluconeogenesis which involves the conversion of oils to sucrose. The progress of glycolysis in germinating seeds overlaps with gluconeogenesis and also sucrose formation from triose phosphate (photosynthetic pathway). At the termination point of glycolytic pathway there are alternative paths for the carboxylation of Phospheonol Pyruvate (PEP). The formation of oxaloacetate, malate and pyruvate is equilibrated in different cells. Plants with CAM or C_4 metabolism require a surge in the organic acid content. Thus PEP carboxylase, and pyruvate kinase activity vary in different types of metabolic pathways. Physiologists report the dominance of pyruvate over malate in normal C_3 plants undergoing glycolysis and TCA cycle. The metabolic intermediates of glycolysis are interconnected to starch/triose phosphate metabolism in chloroplast and sucrose catabolism in the cytosol. The pool of phospheonol pyruvate thus formed is important to regulate the activity of PEP carboxylase and pyruvate kinase activity in the respiring cells. The process of glycolysis produced ATP and NADH as the major reducing powers. This mechanism can also serve as the main energy source in non-respiring roots under anoxic conditions. The respiratory metabolism in the germinating oil seeds requires the conversion of fats into mobile form of carbon.

Thus carbon compounds are then transported from the endosperm to other aerial parts of the seedling. The process of conversion of lipids to sucrose initiates from triacyl glycerol which is later followed by oxidation of fatty acids to form Acetyl-CoA. This process is termed as the β-oxidation pathway. The process of such conversion of fatty acids occurs in specialized unit membrane bound organelles called glyoxysomes. The Acetyl-CoA formation in the β-oxidation pathway is followed by citrate and malate metabolism.

Various sources like triose phosphate, lipids and Complex carbohydrates can also act as substrate for respiration. The Oxygen molecules are reduced to water and they also act as the terminal electron acceptor in the respiratory pathway. The method of energy release in the process of respiration is through sequential steps or phases. This prevents excessive flow of energy through Protein and carrier components which may otherwise cause cellular damage. The electron transport chain is associated with the process of oxidative phosphorylation. This involves ATP synthesis coupled to the energy released during electron transfer. The majority of electron carriers/proteins are localized in the inner membrane of the mitochondria. NADH transfers the electron through various intermediate carriers and it finally reaches Oxygen molecules (terminal electron acceptor).

Thus the redox reactions associated with electron transfer process coupled to ATP biosynthesis is termed as **Oxidative Phosphorylation**. Nicotinamide Adenine Dinucleotide (NAD) is an important cofactor or organic coenzyme associated with the process of enzymatic

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reactions. NAD accepts electrons and gets converted from its oxidised form to NADH (reduced form). This molecule possesses reduction potential of around –320 mV which provides it the property of a very good reductant. The phosphorylated form of this molecule, i.e., Nicotinamide Adenine Dinucleotide Phosphate (NADP) also functions as a reductant in photosynthesis and pentose phosphate pathway. The free energy released by oxidation of NADH is around 220 kJ mol^{-1} which helps in the process of ATP generation. The process of energy release and ATP synthesis is associated with electron transport pathway across the carriers in the mitochondrial membrane. The ultimate step of aerobic respiration involves the oxidation of reduced coenzymes produced in glycolysis and Krebs cycle. The process of oxidation is accomplished by O_2 and operates through FAD, Ubiquinone (UQ), Cytochrome b, Cytochrome c, Cytochrome a, Cytochrome a_3. It is important to understand the distribution of the four complexes in the inner mitochondrial membrane. The oxidation of NADH and $FADH_2$ are oxidized on the side of the membrane. Ubiquinone is a mobile carrier which diffuses freely within the inner membrane. The Cytochrome – c Complex is loosely bound to the outer side of the inner mitochondrial membrane. Cytochrome b_{556} and Cytochrome b_{557} are present in the outer side of the inner membrane.

The major sites of phosphorylation associated with the process of electron transfer are:

- Electron transfer between NADH dehydrogenase to UQ via Fe-S present in Complex I.
- Transfer of electron to Cytochrome.
- Electron transfer from Cytochrome a to Cytochrome a_3 in Complex IV.

The oxidation of NADH molecules liberate 3ATP each and $FADH_2$ yields 2ATP each. The oxidation reaction associated with the coenzymes require $1/2O_2$ molecule and 2H atom. The Flavoproteins and Ubiquinone are Hydrogen carriers in the electron transport components while the cytochromes serve as single electron carriers. In the course of electron transfer, the FAD molecule and Fe molecule in different cytochrome complexes undergo successive oxidation and reduction to Fe^{2+} and Fe^{3+}. The energy released during the process of electron transfer is utilized in the process of phosphorylation of ADP molecules to form ATP. This process of phosphorylation associated with oxidation of coenzymes is known as **oxidative phosphorylation**. The phosphorylating Complex, i.e., ATPases are situated in the cristae of mitochondria. **2, 4-dinitrophenol** has been reported to be a commonly used uncoupler of oxidative phosphorylation and the electron transport chain. The transport of electrons from reduced NADH to FAD, Cytochrome b to Cytochrome c and reduced Cytochrome a to Cytochrome a_3 involve the formation of one ATP molecule in each step, i.e., 7.6 Kilo Cal energy. Physiologists have reported that the two NADH molecules generated in glycolysis are extra-mitochondrial. Thus they are likely to produce two molecules of ATP each during terminal oxidation. Thus 4 ATP molecules are liberated by the terminal oxidation of glycolysis –mediated NADH oxidation. The complete oxidation of glucose molecule liberates around 36 ATP in a Eukaryotic cell. The energy efficiency of respiration is around 40% where 273.6 Kilo Cal energy is obtained from 36 ATP molecules. The total energy retained within one glucose molecule is 686 Kilo Cal. Various theories have been proposed to explain the mechanism of ATP synthesis associated with electron transfer process of mitochondria. The chemisosmotic theory has been observed to be more appropriate in relation to proton-mediated ATP generation.

Pentose phosphate pathway is an alternative pathway for oxidation of sugar molecules. This pathway produces some of the metabolic intermediates of glycolysis and is termed as the **Hexose Monophosphate Shunt**. The biochemical reactions associated with this pathway mostly operates in the cytosol and plastids. Similar to glycolysis the metabolic intermediates

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associated with this pathway also contribute as precursors to various metabolic products. The regulation of glycolysis and pentose phosphate pathway in respiring tissue depends upon photosynthesis and metabolic activity. Furthermore, respiratory efficiency in plant tissues has often been observed to be insensitive to cyanide inhibition. This process of cyanide-insensitive respiratory pathway is mediated by a mitochondrial carrier alternate oxidase. The cyanide resistance pathway possesses implications in the thermogenic emission of pollination attractants associated with certain families of angiosperms. The excess heat energy produced in this pathway is thus effective in releasing volatile compounds from the inflorescences.

Furthermore, it has been observed that various abitoic factors like nutrient deficiency, temperature and osmotic stress are likely to disrupt the process of mitochondrial electron transport. In such a context, the alternative pathway of cyanide resistant respiration maintains the electron flow across mitochondria. Moreover, it prevents over-reduction of the ubiquinone Complex which may otherwise result in the generation of free radical species. Animal systems also possess an uncoupler Protein which functions in a manner similar to alternate oxidase present in plants. This Protein present in the inner membrane of mammalian mitochondria increases the proton permeability of the membrane.

3.3.1 Glycolysis: Embden-Meyerhof-Parnas (EMP) Pathway

The word glycolysis as the name suggests is involved with splitting of carbohydrate molecules (Glycos: Sugars; Lysis: to Break) to form hexose and triose phosphates. The pathway yields pyruvate which functions as a substrate for citric acid cycle. Moreover it produces energy molecules in the form of ATP and NADH. The pathway of glycolysis can proceed even in the absence of Oxygen. However, in anaerobic conditions like anoxia in roots (water logged) the process of glycolysis is followed by fermentation of pyruvate and subsequent recycling of NADH. The pathway of glycolysis is universal to both Prokaryotes and Eukaryotes. However, there lie differences in the process of glycolysis between plants and animal systems. The main respiratory substrate in animal cells is usually glucose. In plants sucrose is the main transportable form of sugar. Thus for Glycolysis in plants Sucrose has been mentioned as one of the important substrate other than Glucose. The process of Sucrose Degradation occurs in the cytosol where Sucrose Synthase Catalyzes the degradation of Sucrose-UDP Complex to Fructose and UDP-Glucose. This step is followed by the activity of UDP-Glucose phosphorylase which converts UDP-Glucose into UTP and Glucose-6-phosphate. In another pathway the activity of invertase present in the Cytosol, Apoplast, Cell Wall and Vacuole converts Sucrose into Glucose and Fructose. Later these sugars are phosphorylated by the activity of kinase enzyme. There are various sources of obtaining sugars as substrate for glycolysis. Plant cells exhibit glycolytic pathway alternately both in the plastids and cytosol. The chloroplast or amyloplasts are sources of starch synthesis and breakdown.

The starch metabolism pathway provides sources of hexose phosphates in glycolytic pathway. Photosynthesis provides triose phosphates for the oxidative pathway of respiration. The process of glycolysis produces pyruvic acid from the carbohydrates. At the initial stage of glycolysis hexose units are phosphorylated and split into two molecules of triose phosphate.

The number of ATP consumed for the initial phase of glycolysis depends upon the activity of sucrose synthase or inverstase. The reaction catalyzed by phosphofructokinase is the control point for glycolysis in plants and animals. The energy conserving phase of glycolysis initiates with the activity of glyceraldehyde-3-phosphate dehydrogenase which catalyzes the oxidation of aldehyde into carboxylic acid. Energy is conserved in the form of NAD^+ into NADH. In the subsequent stages of glycolysis 1, 3-biphosphoglycerate acts as a strong donor of phosphates. This is obtained by the high free energy of hydrolysis (-49.3 kJ mol^{-1}) of the phosphorylated carboxylic acid in 1, 3-biphosphoglycerate.

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Enzymatic Steps of Glycolysis

The entire phase of glycolysis is comprised of ten subsequent reactions of which the first five reactions constitute the preparatory phase. In this phase, two molecules of ATP are consumed and glucose is metabolized into two molecules of glyceraldehyde-3–phosphate. In the pay off phase of glycolysis, two molecules of glyceraldehyde-3-phosphate are converted into two molecules of pyruvic acid. This process is followed by the formation of 2 NADH and 4 ATP molecules as the source of reductants.

Prepatory Phase of Glycolysis

- Glucose in the presence of ATP is phosphorylated to glucose- 6-phosphate, catalyzed by the enzyme hexokinase.
- Glucose-6-phosphate is converted to fructose-6-phosphate. This step is a isomerisation reaction catalyzed by the enzyme phosphohexose isomerise.
- Fructose-6-phosphate reacts with another molecule of ATP to form fructose 1, 6-biphosphate catalyzed by phosphofructokinase.
- In the last step of preparatory phase, the fructose 1, 6-biphosphate is splitted by the activity of aldolase to form two triose molecules namely glyceraldehyde-3-phosphate and dihydroxy acetone phosphate. Dihydroxyacetone phosphate formed is isomerized to form glyceraldehyde-3-phosphate catalyzed by triosephosphate isomerase.

Pay-Off Phase of Glycolysis

- The pay-off phase initiates with the activity of glyceraldehyde-3-phosphate dehydrogenase activity which phosphorylates 2 molecules of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. The reaction involves reduction of cofactor NAD^+ into $NADH + H^+$ and consumes inorganic phosphate (Pi) from H_3PO_4 molecules.
- The two molecules of 1, 3 biphosphoglycerate formed react with 2 ADP molecules to form 2 molecules of ATP and two molecules of 3-phosphoglycerate. This reaction is catalyzed by the enzyme phosphoglycerate kinase and Mg^{2+}.
- The 3-phophoglycerate formed in the earlier step undergoes change in the position of phosphorylated Carbon which is catalyzed by the enzyme phosphoglyceromutase and presence of Mg^{2+}. The product thus formed is 2-phosphoglycerate.
- Two molecules of 2-phosphoglycerate undergo dehydration to form two molecules of phospheonol pyruvate.This step involves the elimination of two water molecules catalyzed by Enolase and Mg^{2+}.
- Phospheonol pyruvate thus formed may also function as the substrate for organic acid metabolism or may proceed to the ultimate step of glycolysis.
- The last step is catalyzed by the enzyme pyruvate kinase and K^+/ Mg^{2+} which catalyzes the conversion of phospheonol pyruvate to pyruvic acid (two molecules).

The carboxylic group of pyruvic acid has been observed to be derived from the 4th and 5th carbon atom of the glucose molecule. The process of glycolysis involves all intermediate metabolites between glucose and pyruvic acid to be involved in their phosphorylated forms. Except certain steps of glycolysis (1, 3, 7 and 10) the rest of the reaction are reversible in nature. In the process of oxidation of one molecule of hexose sugar there is a net gain of 2 ATP molecules. Although four ATP molecules are produced in process of glycolysis, two ATP molecules are consumed in the process. The process of ATP formation in glycolysis is termed as **substrate level phosphorylation**. In this process the phosphate moiety is directly transferred from the substrate molecule to ADP thus forming ATP.

The process of oxidative phosphorylation involves ATP formation coupled to the oxidation process in the terminal step of respiration. Additionally two molecules of NADH (reduced coenzyme). These extramitochondrial NADH molecules are oxidised in the electron transport chain to form two molecules of ATP each. Other than glucose starch, fructosan (fructose polymer) or sucrose can also act as the respiratory susbstrates. Starch can be degraded by the activity of starch phosphorylase/amylase to form glucose. Sucrose is acted upon by invertase to form one units of glucose and fructose. Fructofuranosidases catalyze the conversion of fuctosans into fructose. The hexoses formed are subsequently phosphorylated by kinases which then enter the process of glycolysis (Refer Figure 3.6).

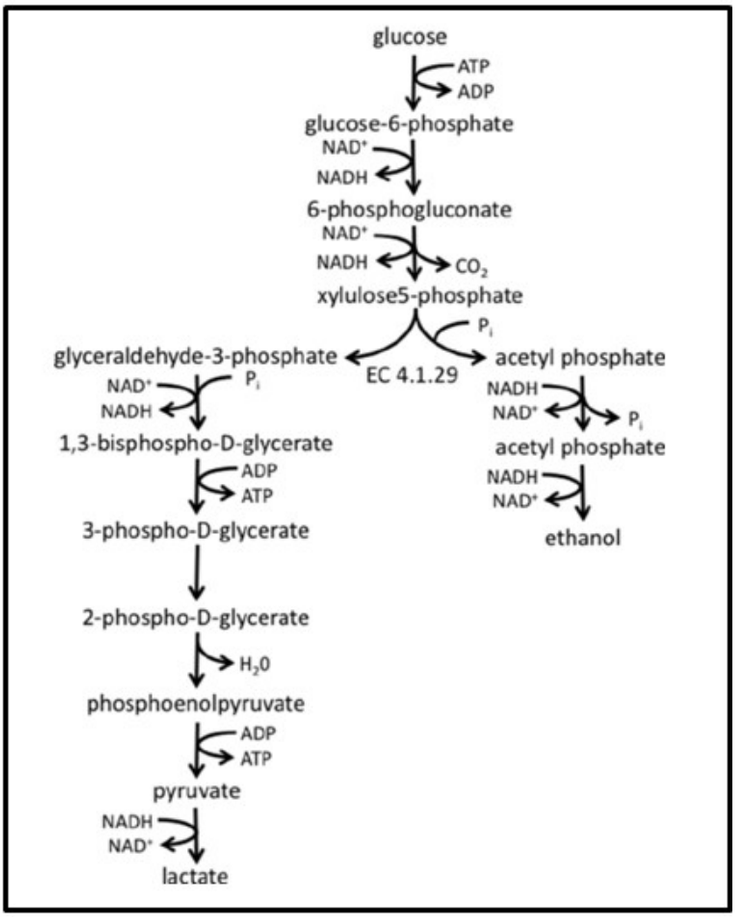

Fig.3.6 *Pathway of Glycolysis*

Regulation of Glycolysis

Glycolysis is regulated by the phosphorylation of fructose-6-phosphate and phospheonol pyruvate formation. Apart from ATP and AMP molecules the levels of phospheonol pyruvate regulate the activity of phosphofructokinase. PEP exerts an inhibitory effect on the activity of phosphofructokinase which is decreased in the presence of inorganic phosphate (Pi). This implies that the cytosolic ratio of PEP : Pi is an important determinant of the rate of glycolytic reactions. Phospheonol pyruvate is likely to get diverted towards the pathway of oxaloacetate and malate synthesis. As discussed earlier, the rate of organic acid formation depends upon the age of plant tissue, nature of organ and lastly is different for C_3 and C_4 plants. The activity of the enzymes PEP carboxylase and pyruvate kinase is modulated by feedback inhibition by the metabolic intermediates of the citric acid cycle. These also include the derivates of the products of TCA cycle like malate, glutamate and 2–oxoglutarate.

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Thus, briefly there are two major points of control operative in plant glycolysis pathway, i.e., the modulation of activity of pyruvate kinase and PEP carboxylase which regulate PEP levels and secondly PEP-mediated regulation of fructose 1, 6-biphosphate formation from fructose-6-phosphate. Physiologists report that the process of feed back control of glycolysis provides regulation of pyruvate formation independent of other metabolic processes. Thus the regulation of glycolytic products in plants is adjustable to the extent of their requirement for various biosynthetic precursors. The formation of pyruvate can be bypassed by the activity of PEP carboxylase to form malate. This biomolecules can then enter the TCA cycle. In certain plants like tobacco leaves a very low activity of pyrivate kinase has been detected to be operative during respiration. Moreover, the regulation of fructose 1, 6-biphosphate formation is also Complex. The variable levels of fructose 1, 6-biphosphate in the cytosol stimulates the activity of phosphofructokinase and inhibits fructose 1, 6-biphosphatase. Thus fructose, 1, 6-biphosphate is a main regulatory intermediate of glycolysis. Further understandings of the regulation of glycolysis shall be possible by the study of metabolic profiling of plant tissues subjected to various physiological conditions.

Anaerobic Respiration (Fermentation Pathway)

Anoxic conditions in plants result in the operation of anaerobic pathway of fermentation which liberates ethanol or lactic acid and subsequent formation of two molecules of ATP. The process of fermentation is necessary for the operation of glycolysis and recycling of NADH formed in glycolysis. Ethanolic fermentation involves the decarboxylation of pyruvic acid to acetaldehyde catalyzed by the enzyme pyruvate decarboxylase and cofactor thiamine pyrophosphate. Acetaldehyde is converted to ethylalcohol catalyzed by the enzyme alcohol dehydrogenase. This step is associated with the oxidation of $NADH + H^+$ to NAD^+. In the process of lactic acid fermentation pyruvate is converted to lactic acid catalyzed by the activity of lactate dehydrogenase. In this step similar mechanism of NAD^+ formation is operative to recycle the cofactor through glycolysis.

However, a low production of ATP (2 molecules) during fermentation is associated with energy loss in the form of heat. Thus the process of fermentation is less efficient in releasing optimum energy form the sugar molecules. The process of fermentation nearly possesses an efficiency of 4% in the form of energy turnover from sugars. In the aerobic process the pyruvate formed in glycolysis enters mitochondria to undergo TCA cycle. Thus during the anaerobic process of respiration the plant cell has to preferably undergo more frequent steps of glycolysis in order to generate more number of ATP molecules.

This phenomenon is termed as **Pasteur Effect** which was observed by microbiologist Louise Pasteur in Yeast cells. The cells shifted from aerobic to anaerobic process exhibited a surge in the glycolytic metabolites mediated by increased gene expression of glycolysis related enzymes.

3.3.2 Kreb's Cycle (Tricarboxylic Acid/Citric Acid Cycle)

During the initial periods of the nineteenth century experiments revealed the consumption of Oxygen in the process of aerobic respiration of cells. Later on in 1937 Hans A. Krebs reported the discovery of the citric acid cycle. The Kreb's cycle hence named is also termed as the tricarboyxlic acid cycle. As the name suggests, the initial stable products of the pathway are tricarboxylic compounds, i.e., citrate and isocitrate. In this pathway the pyruvate is broken down into CO_2 and H_2O. To understand the process of citric acid cycle it is important to know the structural features of mitochondria. The majority of the enzymes associated with TCA cycle are localized in the aqueous matrix of the mitochondria. The mitochondria are autonomous organelle present universally in all plant and animal cells.

The number of mitochondria present in the cell depends upon the metabolic activity of the tissue. Plant mitochondria are comprised of the outer membrane which surrounds the invaginated inner membrane. The invaginations in the inner membrane are known as cristae. These cristae increase the surface area of the inner membrane of mitochondria which comprise of more than 50% of the mitochondrial Proteins. The inter membrane space also harbours various enzymes and Proteins associated with metabolic process. The outer membrane of mitochondria is permeable to solutes having molecular weight less than 10,000 Da. These molecules usually comprise of ions and other metabolites (Refer Figure 3.7).

However, the inner membrane is usually impermeable to most of the biomolecules. The pyruvate molecules produced in the cytosol is transported to the matrix of mitochondria. The transport across the inner membrane is facilitated by specialized pyruvate transporters. The transport of pyruvate into the matrix is followed by its decarboxylation by oxidation reaction catalyzed by pyruvate dehydrogenase.

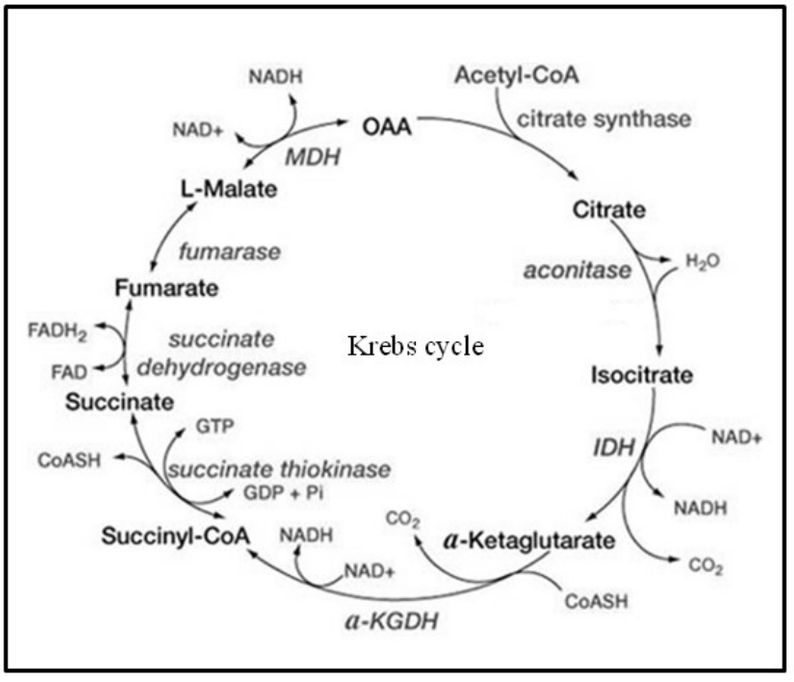

Fig. 3.7 *Pathway of Kreb's Cycle*

Enzymatic Steps of Citric Acid Cycle

In the presence of Oxygen the process of aerobic respiration proceeds through Kreb's cycle which involves oxidation of pyruvate and formation of tricarboxylic acid as the initial stable products. All enzymes of this pathway except succinate dehydrogenase are present in the aqueous matrix. The succinate dehydrogenase enzyme is localized in the inner mitochondrial membrane. Assay of succinate dehydrogenase is performed to assess viability of mitochondria in in-vitro extraction protocols. Thus this enzyme serves as the marker molecule for mitochondria.

Oxidative Decarboxylation of Pyruvic Acid

The initial step of TCA cycle involves conversion of pyruvate into Acetyl-CoA. This reaction is catalyzed by the enzyme Pyruvate Dehydrogenase Complex. Pyruvate

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dehydrogenase enzyme Complex is a large Protein assembly composed of following three different enzymes:

- Pyruvate Dehydrogenase
- Dihydrolipoyl Dehyrogenase
- Dihydrolipoyl Transacetylase

This enzyme Complex is involved in the oxidative-decarboxylation of pyruvic acid to form Acetyl-CoA. The three intermediate steps associated with the process include decarboxylation, oxidation and conjugation to CoA. The various coenzymes and prosthetic groups necessary for the reactions include Thiamine Pyrophosphate (TPP), NAD^+, FAD^+, CoA and Lipoate.

Oxidation of Acetyl Group in Acetyl-CoA

The next part of the TCA cycle is accomplished in eight steps of enzymatic reactions where Acetyl-CoA is converted to various organic acids.

- Acetyl-CoA combines with a 4-C organic acid oxaloacetate to form a tricarboxylic acid citrate. This reaction is catalyzed by the enzyme citrate synthase. Coenzyme A (CoA) is liberated in this reaction.
- Citrate is converted to isocitrate catalyzed by the enzyme aconitase. The process of conversion occurs in two steps namely dehydration of citrate to cis-aconitate and further rehydration to isocitrate.
- Followed by the formation of isocitrate the molecule is oxidatively decarboxylated to form α-Ketoglutarate. In this reaction NAD^+ is reduced to $NADH^+ + H^+$ and another molecule of CO_2 is released. The reaction is catalyzed by isocitrate dehydrogenase. This enzyme possesses different isozymes present in the cytosol and mitochondrial matrix. They imply both NAD^+ and $NADP^+$ as co factors. However, the respiratory pathway implies NAD^+- dependent isocitrate enzyme.
- α-Ketoglutarate reacts with coenzyme-A and NAD^+ to form Succinyl-CoA. The reaction is a oxidative decarboxylation process and is catalyzed by the enzyme α-Ketoglutarate dehydrogenase. Arsenite has been reported to inhibit this irreversible reaction.
- Succinyl-CoA is further metabolized to succinate catalyzed by Succinyl-CoA-synthetase. This step is important as it involves a substrate level phosphorylation step. In the presence of inorganic phosphate (H_3PO_4/Pi) the thioester bond of Succinyl-CoA is hydrolyzed. This reaction leads to release of energy which drive the synthesis of ATP from ADP+Pi. Coenzyme A (CoA) isliberated from Succinyl-CoA in this reaction.
- Succinate is further converted into fumarate catalyzed by the enzyme succinate dehydrogenase. The reaction also involves reduction of Flavine Adenine Dinucleotide (FAD) to $FADH_2$. Malonate has been observed to be an inhibitor of this reaction. Succinate dehydrogenase has been reported to be the only membrane bound enzyme associated with TCA cycle.
- Fumarate is hydrolyzed in presence of one molecule of water to form Malate. The reaction is catalyzed by the enzyme Fumarase.
- The last step of TCA cycle involves oxidation of Malate to form Oxaloacetate, catalyzed by the Enzyme Malate Dehydrogenase. NAD^+ is reduced to $NADH + H^+$ in this step.

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Significance of Kreb's Cycle

Kreb's cycle involves most of the reactions to be reversible except the reactions involved with Acetyl-CoA, Citrate and Succinyl-CoA formation. The release of CO_2 in this process occurs sequentially from the third, fouth, second, fifth and first Carbon atom of Glucose. The understanding of energy conservation process occurring through the steps of Kreb's cycle is associated with the formation of NADH and $FADH_2$. A considerable amount of free energy released during the formation of pyruvate or Acetyl-CoA. The NADH and $FADH_2$ produced in the process are oxidized by terminal oxidation occurring through the mitochondrial electron transfer pathway. Additionally the ATP formed in the process of Kreb's cycle is evident from the substrate level phosphorylation. The reactions of citric acid cycle are not identical in plant and animal systems. Plants produce ATP in the step catalyzed by Succinyl-CoA synthetase. This step produces GTP in animal systems. Moreover, plant systems possess NAD^+ malic enzyme evident in the matrix of mitochondria. This enzyme catalyzes the oxidative decarboxylation of malate to pyruvate. This enzyme is associated with the other pathways associated with PEP-derived metabolism apart from glycolysis.

Various anaplerotic reactions have been associated with the activity of PEP carboxylase which produces malate and other metabolic intermediates. CAM plants effectively produce malate through PEP metabolism. α-ketoglutarate produced in the TCA cycle is also a precursor for Glutamate Biosynthesis. Another important metabolic intermediate is Succinyl-CoA which produces porphyrins (precursor of Chlorpphyll, Phytochrome and Phycocyanin). In this sense TCA cycle acts as an amphibolic pathway where metabolic intermediates serve for both anabolic and catabolic functions. Table 3.1 shows the ATP balance sheet in respiration.

Table 3.1 *The ATP Balance Sheet in Respiration*

Reactions	Total ATP Production or Breakdown
Glycolysis	
Hexose Phosphorylation in Step 1 and 3	-2 ATP
Terminal Oxidation of 2 NADH in Step 6	+4 ATP
Substrate Level Phosphorylation in Step 7 and 10	+ 4 ATP
	Net Gain: 8 – 2 = 6ATP
TCA cycle	
Terminal Oxidation of 2NADH each in Step 1, 4 and 5	+ 18 ATP
Substrate Level Phosphorylation in Step 6	+ 2 ATP
Terminal Oxidation of 2 $FADH_2$ in Step 7	+ 4 ATP
Terminal Oxidation of 2 NADH in Step 9	+ 6 ATP
	Net Gain: 30 ATP
	Total ATP Produced: 6 + 30 = 36 ATP

Mitochondrial Electron Transport Pathway (Terminal Oxidation)

The process of energy release and ATP synthesis is associated with electron transport pathway across the carriers in the mitochondrial membrane. The ultimate step of aerobic respiration involves the oxidation of reduced coenzymes produced in glycolysis and Krebs Cycle. The process of oxidation is accomplished by O_2 and operates through FAD, Ubiquinone (UQ), Cytochrome b, Cytochrome c, Cytochrome a, Cytochrome a_3. The oxidation of NADH molecules liberate 3ATP each and $FADH_2$ yields 2ATP each. The oxidation reaction associated with the coenzymes require $1/2O_2$ molecule and 2H atom. The flavoProteins and ubiquinone are Hydrogen carriers in the electron transport components while the cytochromes serve as single electron carriers. In the course of electron transfer the FAD molecule and Fe molecule in different cytochrome complexes undergo successive oxidation and reduction to Fe^{2+} and Fe^{3+}. The energy released during the process of electron transfer is utilized in the process of phosphorylation of ADP molecules to form ATP. This process of phosphorylation

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associated with oxidation of coenzymes is termed as **Oxidative Phosphorylation**. The phosphorylating Complex, i.e., ATPases are situated in the cristae of mitochondria. **2,4-dinitrophenol** has been reported to be a commonly used uncoupler of oxidative phosphorylation and the electron transport chain. The transport of electrons from reduced NADH to FAD, Cytochrome b to Cytochrome c and reduced Cytochrome a to cytochrome a_3 involve the formation of one ATP molecule in each step, i.e., 7.6 kilo Cal energy. Physiologists have reported that the two NADH molecules generated in glycolysis are extra-mitochondrial. Thus they are likely to produce two molecules of ATP each during terminal oxidation.

Thus 4 ATP molecules are liberated by the terminal oxidation of glycolysis–mediated NADH oxidation. The complete oxidation of glucose molecule liberates around 36 ATP in a Eukaryotic cell. The energy efficiency of respiration is around 40% where 273.6 kilo Cal energy is obtained from 36 ATP molecules. The total energy retained within one glucose molecule is 686 kilo Cal.

Mechanism of Electron Transport

The electron transport Complex in mitochondria is comprised of four multi-protein complexes localized in the inner membrane. The membrane bound complexes are indicated as **Complexes I, II, III** and **IV**. The ubiquinone and Cytochrome-c complexes are loosely bound to the membrane and function as mobile carriers between the complexes. The process of electron transfer is as follows:

Complex I: This Complex is composed of NADH-dehydrogenase which is comprised of flavoprotein Flavin Mono Nucleotide (FMN) and associated with non-heme Iron-Sulphur (Fe-S) Proteins. This Complex involves electron transfer from mitochondrial NADH/NADPH to Ubiquinone (UQ). Plants possess additional external dehydrogenase Complex which can oxidize NADH + H^+ (Refer Figure 3.8).

Complex II: This Complex contains succinate dehydrogenase which contains Flavin Adenine Dinucleotide (FAD) as a prosthetic group. This Complex is associated with a Fe-S Non-Heme Iron Protein. The Complex receives electron from succinic acid and transfers it to Ubiquinone.

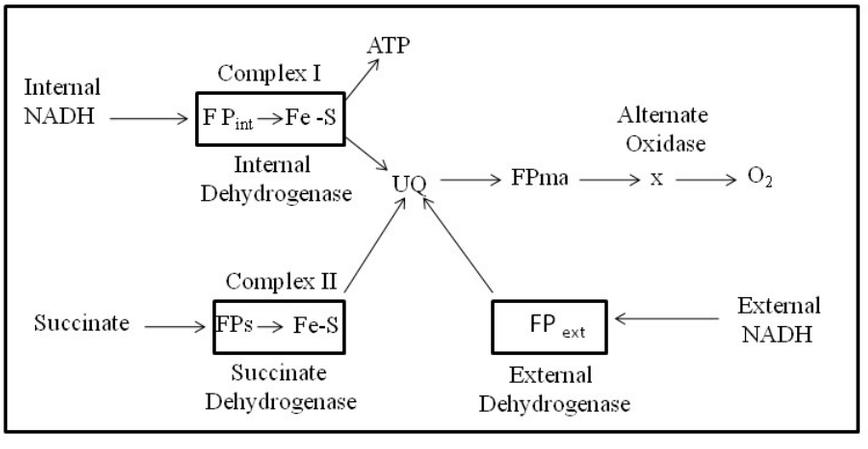

Fig. 3.8 *Electron Transport Chain in Mitochondria*

Complex III: The Complex consists of dihydroubiquinone (UQH_2), Cytochrome–C-oxidoreductase along with two forms of Cytochrome b (557 and 560), non Heme Fe-S Proteins and Cytochrome C_1. Plant mitochondria possess an additional flavoportein associated

with this Complex. This Complex receives electron from UQH_2 and transfers to Cytochrome C. In this process the protons are released from UQH_2.

Complex IV: The main component of this Complex is Cytochrome C, Oxygen, Oxidoreductase, Cytochrome a and Cytochrome a_3. The enzyme in this Complex contains two copper centres CuA and CuB. Electrons are transferred from Cytochrome- C to this Complex and further to $1/2O_2$. Followed by this step proton molecules are released to form water molecules.

It is important to understand the distribution of the four complexes in the inner mitochondrial membrane. The oxidation of NADH and $FADH_2$ are oxidized on the side of the membrane. ubiquinone is a mobile carrier which diffuses freely within the inner membrane. The Cytochrome c Complex is loosely bound to the outer side of the inner mitochondrial membrane. Cytochrome b_{556} and Cytochrome b_{557} are present in the outer side of the inner membrane. The major sites of phosphorylation associated with the process of electron transfer are:

- Electron transfer between NADH dehydrogenase to UQ via Fe-S present in Complex I.
- Transfer of electron to Cytochrome c.
- Electron transfer from Cytochrome a to Cytochrome a_3 in Complex IV.

3.3.3 Mechanism of Oxidative Phosphorylation

The method of energy release in the process of respiration is through sequential steps or phases. This prevents excessive flow of energy through Protein and carrier components which may otherwise cause cellular damage. The electron transport chain is associated with the process of oxidative phosphorylation. This involves ATP synthesis coupled to the energy released during electron transfer. The majority of electron carriers/proteins are localized in the inner membrane of the mitochondria. NADH transfers the electron through various intermediate carriers and it finally reaches Oxygen molecules (terminal electron acceptor). Thus the redox reactions associated with electron transfer process coupled to ATP biosynthesis is termed as oxidative phosphorylation.

The electron transport Complex in mitochondria is comprised of four multi-protein complexes localized in the inner membrane. The membrane bound complexes are indicated as Complexes I, II, III and IV. The ubiquinone and Cytochrome c complexes are loosely bound to the membrane and function as mobile carriers between the complexes. The Complex I is composed of NADH-dehydrogenase which is comprised of flavoprotein Flavin Mono Nucleotide (FMN) and associated with non-heme iron-sulphur (Fe-S) Proteins. This Complex involves electron transfer from mitochondrial NADH/NADPH to ubiquinone (UQ). Plants possess additional external dehydrogenase Complex which can oxidize $NADH+H^+$. Complex II contains succinate dehydrogenase which contains (FAD) Flavin Adenine Dinucleotide as a prosthetic group. The Complex receives electron from succinic acid and transfers it to ubiquinone (Complex III). This Complex consists of dihydroubiquinone (UQH_2)-Cytochrome – C Oxidoreductase along with two forms of Cytochrome b (557 and 560), non-heme Fe-S Proteins and Cytochrome C_1.

The main component of Complex IV is Cytochrome C: Oxygen Oxidoreductase, Cytochrome a and Cytochrome a_3. The enzyme in this Complex contains two copper centres CuA and CuB. Electrons are transferred from Cytochrome- C to this Complex and further to $1/2O_2$. Followed by this step proton molecules are released to form water molecules.The sites of ATP synthesis are associated with intermediate stages of electron transport from reduced coenzymes to Oxygen. Electron transfer from reduced coenzyme (NADH) to Oxygen via

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Complexes I-IV is coupled with synthesis of ATP from ADP and Pi. This process includes mainly three sites, i.e., as follows:

- Transport of electrons from NADH dehydrogenase to UQ through the Fe-S Complex.
- From Complex III to Cytochrome c.
- From Cytochrome a to Cytochrome a_3.

Each molecule of NADH produces 3 ATP molecules while $FADH_2$ oxidation liberates 2 molecules of ATP. The difference in ATP count is accounted for the bypass of the first phosphorylation step for $FADH_2$ oxidation.

Mechanism of ATP Synthesis

The mechanism of ATP synthesis associated with mitochondrial electron transport has been supported by the chemiosmotic theory. Peter Mitchell in 1961 proposed the theory of proton mediated electrochemical gradient energising the process of ATP formation. This has also been attributed to the asymmetric distribution of electron carriers localized in the inner mitochondrial membrane. The event of ATP generation is accompanied by proton transport from the matrix to the inner membrane of mitochondria. The electron transport Complexes I, III and IV serve to function in proton pumping from the matrix towards the inter membrane space. The impermeability of the inner mitochondrial membrane towards proton transport results in formation of a proton mediated electrochemical gradient which is also mentioned as the proton motive force. The matrix region of the mitochondria has a higher alkalinity and is negatively charged. The inter-membrane space contains a higher concentration of proton which results in acidic pH and has higher positive charge. Thus, the proton electrochemical gradient results in the generation of free energy which drives the synthesis of ATP from ADP + Pi. It has been reported that for a pair of electrons being transferred from NADH to $1/2O_2$ 4 protons are pumped by Complex I, 4 protons by Complex III and protons by Complex IV.

The ATP synthesising F_0-F_1 particle is localized in the cristae of inner mitochondrial membrane. The ATPase is comprised of F_1 or head piece and a basal part called F_0 particle. The head piece contains the catalytic site for converting ADP + Pi into ATP. The F_1 particle is a cluster of small three different polypeptides called a, b and c which forms the channel Protein for proton transport. Thus, the passage of protons across this channel is associated with ATP formation accomplished by the event of phosphorylation. Physiologists suggest the involvement of a rotational movement of the F_0 particle associated with the movement of proton ions. The catalytic subunit of the F_1 particle undergoes conformational changes in its structure which results in the formation of ATP from ADP + Pi. The structural details have been confirmed by X-ray studies. For a single ATP molecule formed, $3H^+$ are transported across the membrane. The components of the chemiosmotic theory explain ATP synthase to be the main site of ATP synthesis. It also explains that the electron carrier complexes are mainly associated with free energy conservation and generation of proton motive force. Various physiological uncouplers like 2,4-dinitrophenol and *p*-TriFluoromethoxyCarbonyl cyanide Phenylhydrazone (FCCP) function as inhibitors of ATP synthesis. The mechanism of inhibition of ATP synthesis is accomplished by membrane leakage and disruption of proton mediated gradient across the mitochondrial membrane. Isolated mitochondrial extracts supplemented by ADP has been observed to show higher rates of electron flow. Apart from the synthesis of ATP, the proton gradient across the membrane also facilitates the movement of the products of citric acid cycle. Furthermore, the ADP/ATP transporter facilitates the

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exchange of ADP and ATP across the membranes. Inorganic phosphate (Pi) uptake is undertaken by active phosphate transporter which functions by the proton gradient. The pH difference across the membrane regulates the accumulation of Pi in the matrix of mitochondria. Inner membrane of the mitochondria possesses transporters for malate and succinate which are exchanged for Pi. The extra-mitochondrial NADH produced during glycolysis is impermeable through the inner membrane of mitochondria. The outer mitochondrial membrane is permeable to NADH.

Thus, plant mitochondria possess an external NADH dehydrogenase present in the outer surface of the inner membrane. This enzyme is composed of a flavoprotein and transfers electron to Ubiquinone. In plant and animal systems, the glycero-phosphate pathway bypasses the first phosphorylation site and thus two ATP molecules are produced due to the oxidation of NADH molecules.

Theories of Oxidative Phosphorylation

The mechanism of oxidative phosphorylation coupled with ATP synthesis has been explained by the following three theories.

The Chemical Coupling Theory: The theory has been proposed by Slater in 1953. This theory is based on substrate level phosphorylation of glyceraldehyde-3-phosphate dehydrogenase produced in glycolysis. This process is accompanied by the formation of ATP and 3-phosphoglyceric acid. This theory described the involvement of various high energy phosphorylation intermediates which are involved in the process of electron transfer. However, this theory did not find acceptance because the phosphorylating intermediates were not identified in the pathway of respiratory carriers.

The Conformational Coupling Theory: The theory was proposed by Boyer in 1964 which states that the free energy released during electron transport is associated with the conformational change in the structure of Protein carriers. According to Boyer the energy acting as a driving force for ATP synthesized is probably obtained by close association of carboxyl and sulphydryl group. This results in the formation of Acyl-S-Linkage as a high energy intermediate for ATP synthesis. Similar mechanisms of conformational coupling theory proposed by Green and Ji (1972) mentioned that the mechanical and electrical force produced by electron transport across the Protein carriers result in ATP generation. The process is accomplished by mechano-electric force induced conformational change in the ATPase component of mitochondria.

The Chemiosmotic Theory: This theory was proposed by Peter Mitchell in 1961 and remains as the most acceptable mechanism of mitochondrial and chloroplastidial phosphorylation. The theory explains the involvement of a membrane bound ATPase enzyme associated with ATP synthesis. The enzyme ATPase can reversibly catalyze the formation of ADP + Pi form ATP. According to Mitchell the activity of ATPase regulates the H^+ and OH concentration across the membrane. The reaction equilibrium catalyzed by ATPase has been reported to be tending towards ATP synthesis. The differential distribution of H^+ and OH across the inner membrane of mitochondria essentially provides the forward and reverse shift in equilibrium of the reaction. The theory proposed by Mitchell also anticipated the involvement of membrane transporters associated with transport of OH^- and H^+ in exchange of Cl^- and K^+. Thus according to this theory the transport of metabolites is facilitated by membrane potential gradient and ATP generation (Refer Figure 3.9).

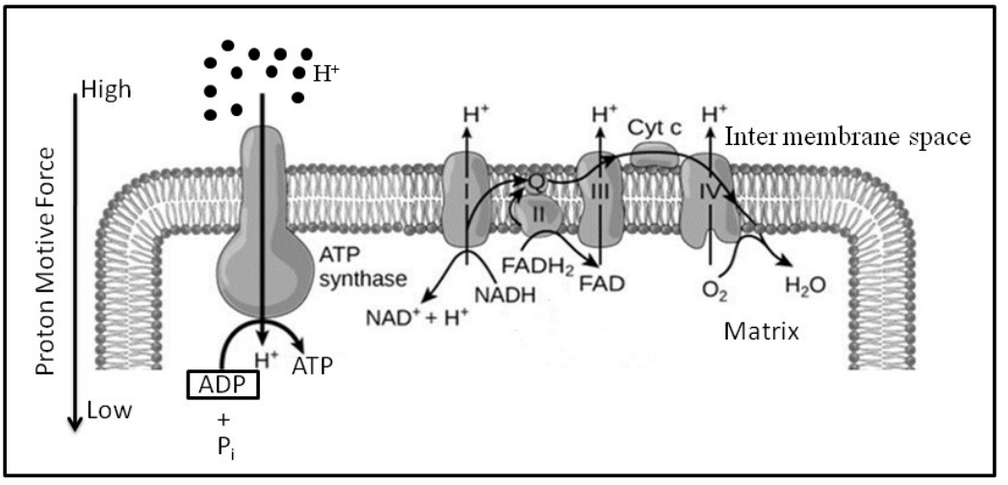

Fig. 3.9 *Chemiosmotic Mechanism of ATP Synthesis in Mitochondria*

3.3.4 Pentose Phosphate Pathway

This metabolic pathway is an alternative route associated with the oxidation of sugar molecules. The enzymes associated with the oxidative pentose phosphate pathway are mostly localized in the cytosol and plastids. Since this pathway involves direct oxidation of glucose-6- phosphate without entering glycolysis it is also termed as the direct oxidation pathway or hexose monophosphate shunt. The pathway initiates with the presence of 6 molecules of glucose-6-phosphate which proceed through various enzymatic steps:

- The six molecules of glucose-6-phosphate are metabolized into 6 phosphoglucono-lactone catalyzed by the enzyme glucoe-6-phosphate dehydrogenase. The reaction is catalyzed by coenzyme NADP.
- 6 phosphoglucono-lactone is hydrolyzed by lactonase to produce 6-phosphogluconic acid.
- 6-phosphogluconic acid undergoes oxidative decarboxylation catalyzed by the enzyme 6-phophogluconic acid dehydrogenase. The reaction produces 6 molecules of CO_2 and 6 molecules of ribulose-5-phosphate.
- 6 molecules of ribulose-5-P isomerize into 4 molecules of xylulose-5-phosphate and 2 molecules of Ribose-5-phosphate. The reaction is catalyzed by the activity of Ribulose-3-epimerase and pentose phosphate isomerise a respectively.
- 2 molecules of xylulose-5-phosphate and 2 molecules of ribose-5-phosphate combine in the presence of transketolase to form 2 molecules of sedoheptulose-7-phosphate and 2 molecules of 3-phosphoglyceraldehyde.
- 2 molecules of sedoheptulose-7-phosphate and 2 molecules of 3-phosphoraldehyde combine in the presence of transketolase to form 2 molecules of fructose-6-phosphate and 2 molecules of erythrose -4-phosphate.
- 2 molecules of erythrose-4-phosphate react with two molecules of xylulose-5-phosphate in the presence of transketolase to form 2 molecules of fructose-6-phosphate and 2 molecules of 3-phosphoglyceraldehyde.
- 1 molecule of 3-phosphoraldehyde undergoes isomerisation to form dihydroxyacetone phosphate catalyzed by phosphotriose isomerise.
- 1 molecule of 3-phosphoglyceraldehyde combines with dihydroxyacetone phosphate catalyzed by aldolase to form one molecules of fructose 1, 6-biphosphate. This molecule is further metabolized into fructose 6-phosphate catalyzed by phosphatase.

- The last step of the pathway involves isomerisation of fructose -6-phosphate (produced in earlier steps) into 5 molecules of glucose-6-phosphate. This step is catalyzed by the enzyme phosphohexose isomerase.

The Pentose phosphate pathway briefly undergoes oxidation of 6 molecules of glucose-6-P to produce 6 mols. of CO_2 and 12 molecules of reduced coenzymes $NADPH_2$. This is followed by regeneration of 5 molecules of glucose- 6- phosphate (Refer Figure 3.10).

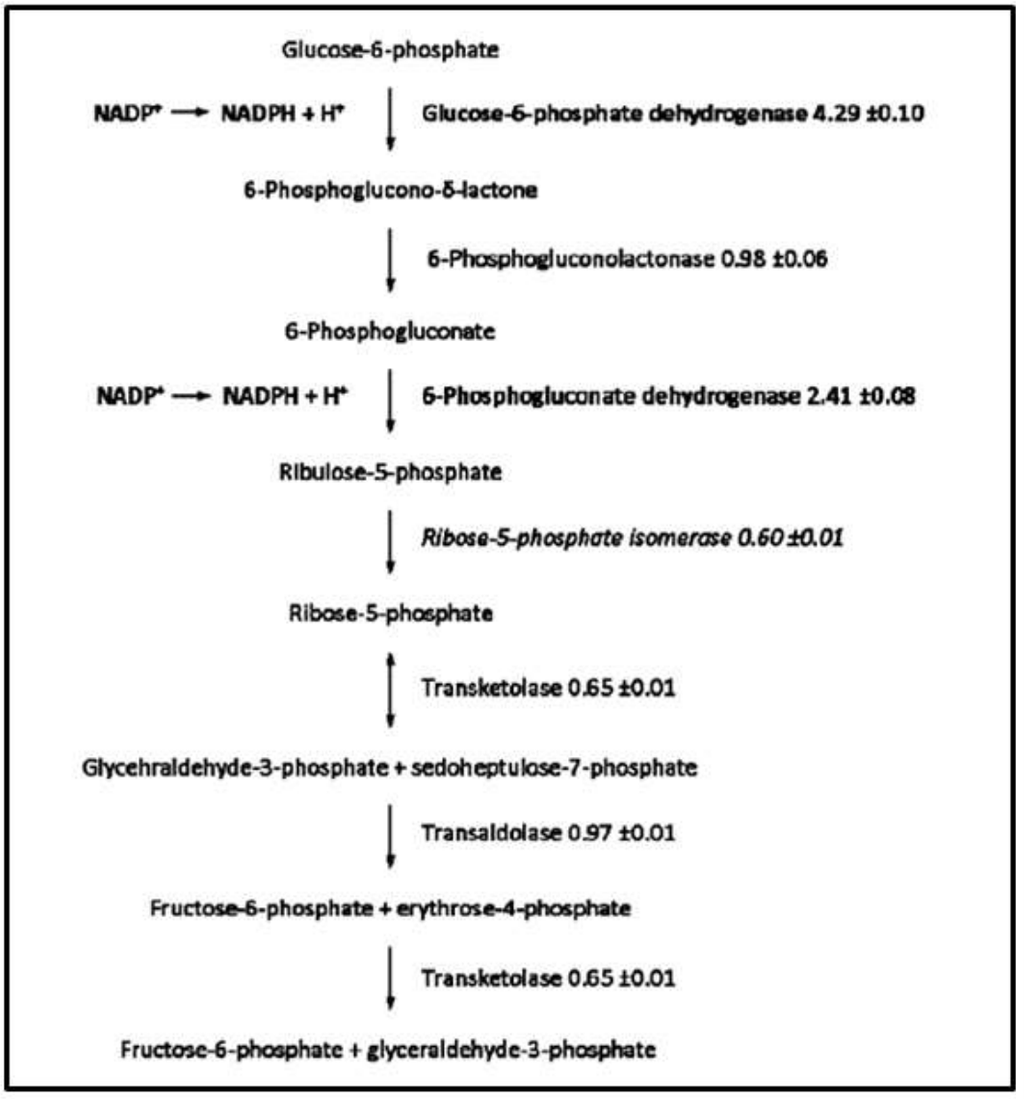

Fig. 3.10 *Pentose Phosphate Pathway*

Significance of Pentose Phosphate Pathway

The pentose phosphate pathway is an alternative pathway associated with oxidation of sugar molecules. This pathway involves oxidation of glucose- 6-phophate by passing the steps of glycolysis. Hence the pathway is commonly known as the hexose monophosphate shunt. A majority of the enzymes associated with this pathway are localized in the cytosol and plastids of respiring tissues. In certain cases the plastidial pathway dominates over the cytosolic pathway. Physiological investigations performed by radioactive tracer technique have revealed the dominance of glycolysis as the major catabolic pathway of respiration. More than 80% of the carbon flux in plants is due to operation of glycolysis. Physiologists have reported the

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regulation of pentose phosphate pathway modulated by the developmental stage of plant tissues. Transition of plant tissues from meristematic to differentiated forms have been reported to be associated with a surge in pentose phosphate pathway. The pathway has been importantly associated with the metabolic precursors of various biosynthetic pathways.

The NADPH produced in the pathway is associated with reducing steps of the pathway. The amyloplast and etioplasts present in dark growing tissue undergo pentose phosphate pathway. This pathway also supplies NADPH required for nitrogen assimilation and lipid metabolism. Ribose-5-phosphate produced in this pathway is utilized as a precursor of ribose and deoxyribose sugar associated with DNA and RNA synthesis. This pathway also produces a 4C metabolic inhibitor called erythrose- 4- phosphate combines with phospheonol pyruvate to produce precursors of aromatic Amino Acids, lignin and phenol biosynthesis. Moreover this pathway has been reported to be associated with replenishing the calvin cycle intermediates in non-green young leaves. In photosynthetic tissue, the pathway is importantly associated with CO_2 fixation through RUBP. It also regulates the metabolic rate of shikimate pathway. The pathway is partially regulated by the initial reaction of glucose-6-phosphate dehydrogenase. The enzyme activity is regulates by the ratio of NADPH : $NADP^+$.

Cyanide Resistant Respiratory Pathway

Plant mitochondria harbour a Complex set of electron carrier complexes which determine the energy conservation efficiency during various pathways of respiration. The process of oxidative phosphorylation results in the formation of ATP. However, plants also possess certain non-phosphorylating energy dissipating pathways. The operation of such pathways result in thermogenic responses associated with various physiological functions.

Thus the non-phosphorylating pathway possesses certain degree of usefulness to the plant. Physiological investigations in various plant systems have revealed certain degree of (10-100%) cyanide–resistance associated with the pathway of respiration. The enzyme associated with the process has been reported to be a cyanide-resistant oxidase component involved in the mitochondrial electron transport pathway. This enzyme has been termed as alternate oxidase. The alternate oxidase pathway involves reduction of Oxygen to water by the transfer of four electrons. SalicylHydroxamic Acid (SHAM) has been reported to be an uncoupling inhibitor of this pathway. The passage of electrons from alternate oxidase to ubiquinone Complex bypasses two sites of proton pumping at Complexes III and IV.

Thus in the absence of ATP formation at the electron transfer pathway a considerable energy is lost in the form of heat energy. However, the cyanide-resistant pathway is not always sufficient to generate sufficient heat energy. The alternative pathway of respiration associated with plant tissues is associated with oxidation of respiratory substrates overproduced in other pathways. Sometimes surplus of substrates are present after their utilization in growth, storage and ATP synthesis. Thus alternate pathway is mostly operative after saturation of the main electron transport pathway. In-vivo experiments have revealed the saturation of the main pathway in cell extracts induced with for higher rate of respiration. Thus activation of the alternate oxidase pathway occurs simultaneously to the saturation of cytochrome pathway. This ensures the mitochondrial process to maintain the proper count of ATP. Various unfavourable conditions associated with abiotic stress, viz., cold temperature, phosphate deficiency or osmotic stress may trigger the alternate oxidase pathway in plants which otherwise inhibits the main pathway. Moreover, the alternate oxidase pathway is associated with prevention of overreduction of ubiquinone Complex. This may otherwise lead to formation of free radicals in the mitochondria.

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Mechanism of Cyanide Resistant Pathway

Application of cyanide results in the blockage of mitochondrial electron transport pathway associated with aerobic respiration. Cyanide is a toxic molecule which inhibits the activity of cytochrome oxidase present in the mitochondrial membrane. Thus the process of respiration affected by application of cyanide is termed as cyanide sensitive respiration. Plant mitochondria possess an alternative pathway for completion of the electron transport pathway accomplished by the activity of alternate oxidase. Thus physiological investigations have revealed that cyanide application may trigger the alternate oxidase-mediated respiratory pathway also known as cyanide resistant pathway.

- The transfer of electrons from reduced coenzymes to ubiquinone Complex is similar to that of usual aerobic respiration.
- The movement of electrons from the ubiquinone Complex appears to be at the diversion point in this pathway. The electrons are transferred to a flavoprotein Complex known as Fpma. This Protein possesses a moderate value of redox potential.
- Electrons are transferred from the Fpma to the alternate oxidase Complex.
- The final step of reduction of O_2 molecules occurs by transfer of electrons from alternate oxidase to Oxygen molecules. However, reduction of Oxygen has often been reported to result in the formation of H_2O_2. Excess of Hydrogen peroxide formed may be detoxified by the activity of catalase.

The nature of alternate oxidase has been reported to be comprised of iron-containing Protein which is, however, different from heme or Fe-S containing Protein. An inhibitor of alternate oxidase is m-chloroben- zhydroxamic acid. The P/O ratio (number of ADP molecules phosphorylated to ATP for one O atom) for cyanide respiration is usually one. The first phosphorylation site of electrontransport chain is common for both cyanide-resistant and normal pathway of respiration.

Significance of Cyanide Resistant Pathway

The cyanide resistant pathway is an alternate mechanism to diisipate energy from the mitochondrial electron carrier complexes. This pathway mediated by alternate oxidase thus prevents over-reduction of the ubiquinone Complex. The regulation of this alternate pathway has been reported to be effective under saturating conditions of the normal pathway (surplus respiration). Thus planst possess this pathway as an adaptive mechanism associated with ATP synthesis, carbon precursor biosynthesis and energy transformation. In the cyanide resistant respiration a major part of the energy liberated by oxidation of respiratory substrate is lost in the form of heat. A substantial part of the energy is utilized for the formation of ATP. Thus the value of P/O ratio for 1 NADH molecule in the cyanide resistant respiration is 1. Moreover, the cyanide pathway helps in surplus ATP drainage which may otherwise inhibit the process of TCA cycle and electron flow across mitochondria. Thus the cyanide pathway operates to replenish oxidized NADH and helps in continuous operation of TCA cycle.

Cyanide resistant respiration has been reported to be associated with climacteric rise in CO_2 emission associated with ethylene-induced fruit ripening. The process of CO_2 burst is evident in the pre-ripening stage of climacteric fruits. The climacteric response induced by ethylene is later considered to be carried through the cyanide resistant pathway. This results in heat generation and emission of volatile aroma from fruits. Subsequent formation of H_2O_2 and superoxide molecules results in the oxidation and loosening of cell membrane associated with the ripening process.

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Physiologists have reported around 10-100% of cyanide resistant respiration to have been accomplished in different plant species. Plants of certain families like *Arundinaceae*, *Liliaceae*, or *Araceae* exhibit thermogenic emissions from their inflorescence. *Arum maculatum, Symplocarpus foetidus* and *Sauromatum guttatum* have been observed this exhibit this process of thermogenecity. The inflorescence in this thermogenic plants at the pre-pollination stage undergo higher rate of respiration accomplished through the alternate oxidase pathway. The upper part of the stalk of inflorescence called appendix tissue undergoes a thermogenic increase in temperature to as high as 25°C higher than normal. This results in liberation of volatile compounds from the inflorescences which are mostly Terpenes, Amines and Indoles. This molecules act as pollinator attractant for insects. In certain plants, salicylic acid has been identified as one of such attractants. Cyanide resistant respiration may also be operative during abiotic stress induced conditions of nutrient deficiency, temperature abnormalities or oxidative stress.

Inhibitors and Uncouplers of Mitochondrial Electron Transport Chain

The process of electron flow across various electron carrier complexes is dependent upon the process of ATP formation. Various physiological inhibitors are effective in uncoupling of the process of oxidative phosphorylation associated with the process of electron transport. A major type of inhibitors implied are lipid soluble hydrophobic compounds like 2,4-dinitrophenol or carbonylcyanide p-trifluoromethoxy-phenylhydrazone. Certain ionophores like valinomycin or gramicidin-A function as uncouplers of oxidative phophorylation in the presence of Na^+ or K^+. Furthermore, inhibitors also function in inhibiting the activity of ATPase localized in the inner membrane. Oligomycin has been reported to inhibit the ATPase activity. Moreover, the process of oxidative phosphorylation can also be inhibited by blocking the antiport exchange of ADP-ATP across the inner membrane (for example, Atractylic Acid). The flow of electrons across the phosphorylating sites or complexes is essential to accomplish the process of ATP formation. Amytal and Rotenone inhibit electron flow from Comlex I to Ubiquinone. Antimycin-A inhibit electron flow in Complex III, while azides and cyanides block electron flow from cytochrome to O_2 by inhibiting the enzyme cytochrome oxidase.

Respiratory Quotient

The Respiratory Quotient (RQ) is a parameter which indicates the nature of substrate used for respiration. In quantitative terms it is represented as the ratio of the volume of CO_2 released to the volume of O_2 consumed in the process of respiration. Thus the value of R.Q. depends upon the chemical nature of the substrate. Usually carbohydrates (hexoses) involved in the process of respiration exhibit RQ value of 1 where $6CO_2$ molecules are released for $6O_2$ molecules consumed (CO_2/O_2). However, fat molecules being poorer in Oxygen release less volume of CO_2 in comparison to the amount of O_2 consumed in respiration. Thus the CO_2/O_2 ratio being low the RQ value appears to be <1. For CAM plants undergoing partial oxidation of organic acid the CO_2 liberated being NIL, RQ value stands to be ZERO. Organic acids undergoing complete oxidation reveal that the O_2 molecules required are lesser than the number of CO_2 molecules released. Thus the RQ value exceeds 1 RQ for anaerobic respiration is Infinity.

'Check Your Progress'

4. Differentiate between oxidative phosphorylation and substrate level phosphorylation.
5. What are thermogenic plants?
6. Define uncouplers.
7. What is hexose monophosphate shunt?

3.4 SUMMARY

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- Enzymes are diverse types are proteinaceous molecules which function as reaction catalyst of various biochemical reactions of metabolism.
- The enzymes essentially function in suitable microenvironments of particular cofactor, pH, and temperature.
- The study of function of enzymes is necessary in the investigation of various diseases related to metabolism.
- Most of the enzymes are chemically Proteins in nature with exceptions for ribozymes which are self-catalytic RNA molecules.
- The quaternary structure formed for an enzyme results in the formation of an active site on its surface. Active sites are usually regions formed by stabilization of various chemical interactions among the Amino Acids.
- Enzymes may require the association of various chemical substances known as Cofactors.
- Cofactors are usually inorganic non-protein substances like metal ions (Ca^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+} or Mn^{2+}) which stabilize the enzyme-substrate interaction and help in the catalytic activity of the enzyme.
- The functional catalytic form of the enzyme in association with its cofactors or coenzymes is referred to as the holoenzyme.
- The association of the cofactors are required to trigger the catalytic activity of the apoprotein which then constitutes the holoprotein.
- The biochemical reactions are driven by the availability of free energy. The difference between the ground state and transition state is known as the activation energy.
- The active site is comprised of hydrophilic Amino Acids which orient in specific alignment in the Folded Protein.
- The prosthetic groups usually consist of an organic compound or inorganic metal ion (Cu, Zn, Mo, etc).
- The structural complementarity in the mode of interaction among the substrate and enzyme has been explained by the Lock and Key Hypothesis.
- The older system of classification of enzymes was based upon the name of substrate and the nature of reaction catalyzed by the enzyme. The Commission on Enzymes of the International Union of Biochemistry (1961) has recommended the new system of classification.
- The enzyme utilizes the binding energy to form the transitory enzyme-substrate Complex. Thus, the substrate should essentially gather an increased free energy necessary for undergoing the transformation.
- The weak interaction between the enzyme and substrate is the main driving force for the catalytic activity of the enzyme.
- The substrate concentration necessary for attaining half velocity ($1/2/V_{max}$) of the reaction is known as K_m value for the enzyme. This K_m value is known as the Michaelis-Menten Constant.

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- The activity of isoenzyme exhibit temporal and spatial regulation in plants and animal tissues.
- The biochemical reaction of respiration is almost reverse of the photosynthetic pathway where sucrose in oxidized to form CO_2 and water.
- The four phases of respiration are mainly glycolysis, citric acid cycle, pentose phosphate pathway and the electron transport chain in mitochondria.
- The process of glycolysis accomplishes by the formation of pyruvic acid, catalyzed by various enzymes.
- The redox reactions associated with electron transfer process coupled to ATP biosynthesis is termed as oxidative phosphorylation.
- Substrate level phosphorylation is another mechanism of ATP synthesis which involves the transfer of inorganic phosphate (Pi) molecules from the substrate to the ADP molecules.
- The progress of Glycolysis in germinating seeds overlaps with Gluconeogenesis and also Sucrose formation from Triose Phosphate (Photosynthetic Pathway).
- At the termination point of Glycolytic Pathway there are alternative paths for the Carboxylation of Phospheonol Pyruvate (PEP).
- The metabolic intermediates of Glycolysis are interconnected to Starch/Triose Phosphate metabolism in Chloroplast and Sucrose catabolism in the Cytosol.
- The respiratory metabolism in the germinating oil seeds requires the conversion of fats into mobile form of Carbon.
- The starch metabolism pathway provides sources of hexose phosphates in glycolytic pathway.
- Glycolysis is regulated by the phosphorylation of fructose-6-phosphate and phospheonol pyruvate formation.
- Cytosolic ratio of PEP : Pi is an important determinant of the rate of glycolytic reactions.
- Anoxic conditions in plants result in the operation of anaerobic pathway of fermentation which liberates ethanol or lactic acid and subsequent formation of two molecules of ATP.
- The process of fermentation nearly possesses an efficiency of 4% in the form of energy turnover from sugars.
- In the presence of Oxygen, the process of aerobic respiration proceeds through Kreb's cycle which involves oxidation of pyruvate and formation of tricarboxylic acid as the initial stable products.
- The process of energy release and ATP synthesis is associated with electron transport pathway across the carriers in the mitochondrial membrane.
- Physiological investigations performed by radioactive tracer technique have revealed the dominance of glycolysis as the major catabolic pathway of respiration.
- The electron transport chain is associated with the process of oxidative phosphorylation.
- The redox reactions associated with electron transfer process coupled to ATP biosynthesis is termed as oxidative phosphorylation.

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- The free energy released by oxidation of NADH is around 220 kJ mol^{-1} which helps in the process of ATP generation.
- The majority of electron carriers/proteins are localized in the inner membrane of the mitochondria.
- The process of oxidation is accomplished by O_2 and operates through FAD, Ubiquinone (UQ), Cytochrome b, Cytochrome c, Cytochrome a, Cytochrome a_3.
- The major sites of phosphorylation associated with the process of electron transfer are (a) Electron transfer between NADH dehydrogenase to UQ via Fe-S present in Complex I, (b) Transfer of electron to Cytochrome c, (c) Electron transfer from Cytochrome a to Cytochrome a_3 in Complex IV.
- The oxidation of NADH molecules liberate 3ATP each and $FADH_2$ yields 2ATP each.
- The energy released during the process of electron transfer is utilized in the process of phosphorylation of ADP molecules to form ATP.
- The mechanism of ATP synthesis associated with mitochondrial electron transport has been supported by the chemiosmotic theory.
- The impermeability of the inner mitochondrial membrane towards proton transport results in formation of a proton mediated electrochemical gradient which is also mentioned as the proton motive force.
- The ATP synthesising F_0-F_1 particle is localized in the cristae of inner mitochondrial membrane.
- 2,4-dinitrophenol has been reported to be a commonly used uncoupler of oxidative phosphorylation and the electron transport chain.
- Isolated mitochondrial extracts supplemented by ADP has been observed to show higher rates of electron flow.
- The energy efficiency of respiration is around 40% where 273.6 kilo Cal energy is obtained from 36 ATP molecules.
- Pentose phosphate pathway is an alternative pathway for oxidation of sugar molecules. The biochemical reactions associated with this pathway mostly operates in the cytosol and plastids.
- The amyloplast and etioplasts present in dark growing tissue undergo pentose phosphate pathway.
- The alternate oxidase pathway involves reduction of Oxygen to water by the transfer of four electrons.
- The cyanide resistance pathway possesses implications in the thermogenic emission of pollination attractants associated with certain families of angiosperms.

3.5 KEY TERMS

- **Coenzymes:** These are metaloorganic compounds which do not bind to the enzyme, but facilitate in its activity.

- **Cofactors:** These are usually inorganic non-protein substances like metal ions (Ca^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+} or Mn^{2+}) which stabilize the enzyme-substrate interaction and help in the catalytic activity of the enzyme.
- **Prosthetic group:** The non-protein part of the enzyme which binds to it firmly through covalent or ionic bonds.
- **Apoenzyme:** The proteinaceous part of the enzyme excluding the cofactor or coenzyme is known as the apoenzyme.
- **Holoenzyme:** The association of the cofactors are required to trigger the catalytic activity of the apoprotein which then constitutes the holoenzyme.
- **Active site:** The substrate binding site of the enzyme necessary for catalysis of reaction.
- **Isoenzymes:** Enzymes which exist in multiple structural forms in various tissues and organs but catalyze the same biochemical reaction are referred to as isoenzymes or isozymes.
- **Glyoxysomes**: Specialized unit membrane bound organelles where β-oxidation of fatty acids is accomplished.
- **Anoxia:** Anaerobic conditions associated with flooding in rhizosphere.
- **Pasteur effect:** A surge in the glycolytic metabolites mediated by increased gene expression of glycolysis related enzymes during anaerobic conditions.
- **Terminal oxidation**: Oxidation of coenzymes NADH, $FADH_2$ accomplished through electron transport across mitochondrial membrane and terminal acceptance by O_2.
- **Anaplerotic reaction**: The biochemical reactions which replenish metabolic intermediates of major pathways.
- **Cyanide resistant pathway:** Alternate oxidase mediated electron transport in mitochondria causes cyanide resistant respiration in plants.
- **Respiratory Quotient (RQ):** It is represented as the ratio of the volume of CO_2 released to the volume of O_2 consumed in the process of respiration.

3.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Binding energy is the free energy utilized by the enzyme to form a stable enzyme-substrate Complex in a transition state, while activation energy is the energy required to cross the stable state barrier of the substrate to participate in the reaction is termed as activation energy.
2. Michaelis-Menten Constant is the substrate concentration necessary for attaining half velocity ($1/2/V_{max}$) of the reaction is known as K_m value for the enzyme. This K_m value is known as the Michaelis-Menten Constant.
3. Allosteric inhibitionis the process that involves binding of an end product of the pathway to one of the initial step enzyme thus causing inhibition in its activity.
4. Oxidative phosphorylation is the redox reactions associated with electron transfer process coupled to ATP biosynthesis, while substrate level phosphorylation is the mechanism of ATP synthesis which involves the transfer of inorganic phosphate (Pi) molecules from the substrate to the ADP molecules.

5. Thermogenic plants are of certain families of Angiosperms exhibit alternate oxidase mediated respiration accompanied by heat formation. This helps the inflorescence appendix to liberate volatile pollinator attractants.
6. Uncouplers are physiological inhibitors associated with the uncoupling of ATP formation and electron transport. The inhibitors function through ATPase activity inhibition or by blocking the electron flow across the complexes.
7. Hexose monophosphate shunt is the pentose phosphate pathway involves direct oxidation of glucose-6-phosphate without entering glycolysis it is also termed as the hexose monophosphate shunt.

3.7 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Define allosteric inhibition. Differentiate between allosteric inhibition and non-competitive inhibition.
2. What are the factors which affect enzyme activity?
3. Explain the term isozyme.
4. Mention the various types of coenzymes used for enzymatic activity.
5. Define anabolism. Mention the anabolic pathways associated with aerobic respiration.
6. What are zymogens? Differentiate between apoenzyme and coenzyme.
7. How many ATP molecules do you expect to be produced from TCA cycle?
8. Define the terms oxidative phosphorylation and respiratory quotient.
9. Name two inhibitors of oxidative phosphorylation and electron transport pathway in mitochondria.
10. Differentiate between oxidative and substrate level phosphorylation. Give examples.
11. Define anaplerotic reactions. Give examples.

Long-Answer Questions

1. Explain the mechanism of enzyme action and the significance of Michaelis-Menten Constant.
2. Explain briefly the system of classification of enzymes with help of suitable examples.
3. Schematically represent the steps of EMP pathway. Mention the net gain of ATP in this process.
4. Explain the steps of TCA cycle associated with oxidative decarboxylation and substrate level phosphorylation.
5. Discuss the function of pyruvate dehydrogenase. Mention its components.
6. Define terminal oxidation. Mention the steps associated with ATP synthesis during mitochondrial electron transport.
7. Mention the total ATP count produced in Glycolysis TCA cycle and terminal oxidation of coenzymes.

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8. Explain the various theories proposed for the mechanisms of oxidative phosphorylation in mitochondria. Elaborate the features of chemiosmotic theory.
9. Schematically explain the various enzymatic steps of pentose phosphate pathway. Why is it called the hexose monophosphate shunt?
10. Explain the significance of pentose phosphate pathway.
11. 'Plants possess alternative respiratory pathway associated with heat dissipation'. Explain the statement with suitable examples.

3.8 FURTHER READING

Hopkins, William G. and Norman P.A. Huner. 2013. *Introduction to Plant Physiology*, 4th Edition. Noida: Wiley India Pvt. Ltd.

Verma, S K and Mohit Verma. 1995. *A Textbook of Plant Physiology, Biochemistry and Biotechnology*. New Delhi: S Chand & Company Ltd.

Blackstock, James C. 2014. *Guide to Biochemistry*. Oxford: Butterworth-Heinemann.

Park, K. H. 2008. *Carbohydrate-Active Enzymes: Structure, Function and Applications*. Amsterdam: Elsevier.

Fromm, Herbert J. and Mark Hargrove. 2012. *Essentials of Biochemistry*. Berlin: Springer.

Fearon, William Robert. 2014. *An Introduction to Biochemistry*. Amsterdam: Elsevier.

UNIT 4 PHOTOSYNTHESIS

NOTES

Structure

4.0 INTRODUCTION

Photosynthesis is a major photochemical process which harnesses light energy and converts it into biochemical forms to store and utilize within the cells. The most abundant Protein on Earth (RuBisCO) is interestingly involved in the Carbon fixation process in green autotrophic organisms like Algae, Bryophyte, Pteridophyte Gymnosperms and Angiosperms. Apart from Plants, Bacteria also employ the mechanism of Photosynthesis to store their energy.

Chloroplasts are semi-autonomous organelles within photosynthetic cells and are capable of transient movement in response to light. Various theories and hypothesis have been proposed to explain the origin and transformation of chloroplasts in Eukaryotic cells. Light energy necessary for photosynthesis is available in form of photons designated to form Quanta (pockets of energy).

A competitive relation exists between the carboxylation and oxygenation reaction of RuBisCO which acts upon the substrate Ribulose-1, 5 Bisphosphate (RuBP). Interestingly, RuBisCO (Ribulose-1, 5-Bi-phosphate Carboxylase Oxygenase) possess both carboxylation and oxygenation properties. The process of oxygenation of RuBP mediated by RuBisCO results in CO_2 loss from Calvin cycle. This results in a drop in the rate of Carbon fixation occurring during photosynthesis.

In this unit, you will study about photosynthesis, wavelength dependent photochemical reactions in chloroplast, ultrastructure or chloroplast, mechanism of light reaction, C_3 and C_4 cycles, CAM pathway and photorespiration.

4.1 UNIT OBJECTIVES

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

- Understand what photosynthesis is
- Explain the wavelength dependent photochemical reactions in chloroplast
- Describe the ultrastructure of chloroplast
- Explain the mechanism of light reaction
- Understand the C_3 and C_4 cycles

- Analyse CAM pathway
- Explain photorespiration

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4.2 PHOTOSYNTHESIS

Photosynthesis is a major photochemical process which harnesses light energy and converts it into biochemical forms to store and utilize within the cells. The most abundant Protein on Earth (RuBisCO) is interestingly involved in the Carbon fixation process in green autotrophic organisms like Algae, Bryophyte, Pteridophyte Gymnosperms and Angiosperms. Apart from plants, bacteria also employ the mechanism of photosynthesis to store their energy. However, pigment composition and the energy carriers are different in bacteria. Photosynthesis is an oxido-reductive process occurring in the specialized organelles called **chloroplasts**. Chloroplast are largely present in the mesophyll tissue of leaves, green young buds, young green fruit walls and also in stems with adaptive features. Chloroplasts are semi-autonomous organelles within photosynthetic cells and are capable of transient movement in response to light. Various theories and hypothesis have been proposed to explain the origin and transformation of chloroplasts in Eukaryotic cells. Light energy necessary for photosynthesis is available in form of photons designated to form Quanta (pockets of energy). The specific wavelengths of light energy are perceived by Specific Pigment-Protein complexes present in the chloroplast. The excitation of chlorophyll molecules energised by photon molecules result in emission of electrons being transmitted across various intermediate Electron Carrier Proteins.

Thus the energy harnessing and transfer process of photosynthesis is accomplished by **Fluorescence Resonance Energy Transfer (FRET)** method. Chloroplasts possess membrane bound stacked structures called thylakoids. The thylakoids are arranged in stacks called **grana**. The structure of thylakoid is important for the clear understanding of the process of electron transfer and ATP production associated with photochemical reactions. The chlorophyll molecules are the primary pigments associated with photosynthesis. They form cluster of molecules known as **Quantasome**. These quantasomes form energy harvesting units of chloroplasts. The chlorophyll - A molecules cluster in form of two photosystems known as PS-I ans PS-II. The core reaction centre of photosynthesis is thus formed of Chl A molecules capable of receiving photons of wavelength 680 nm (PS-II) and 720 (PS-I) nm, respectively.

The chloroplasts contain their circular DNA possessing similarities to Prokaryotic DNA. This organelle also contains its own ribosomes necessary for biosynthesis of chloroplastidial Proteins encoded by plastidial DNA. The sequence of plastidial DNA and InterTranscribed Spacers (ITS) are important taxonomic characters. Apart from Cholorophylls A and B the accessory pigments involved in photosynthesis are mostly carotenoids and xanthophylls. They appear to absorb wavelengths different form that of chlorophylls. The function of the accessory pigments is to absorb and transfer energy to the reaction centre. Furthermore, they help in quenching of excited free reactive species which would otherwise oxidize the chlorophyll molecules.

Various investigations across some decades have revealed the detailed structure of chloroplasts. The molecular structure of the photosystem Proteins have been deciphered for the better understanding of the photochemical process. Several pigments serve as antennae for light harvesting mechanisms. As mentioned earlier, the accessory pigments appear to function in photoprotection of chlorophylls during higher light intensity. The rate of photosynthesis can be measured by various parameters like Quantum yield, biomass

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accumulation, Oxygen yield, etc., the various parameters will be explained in the following sections and subsequent units. The rate of photosynthesis as a function of wavelength is termed as action spectrum. The action spectrum for chlorophyll is different from that of accessory pigments. The absorption spectrum explains the differential absorption capability of pigments necessary for optimizing the light harvesting capacity of plants. Investigations by Emerson and Arnold (1932) have revealed that a cluster of chlorophyll molecule (2500) to be responsible for producing a single molecule of Oxygen. Thus reaction centre of chloroplast constitute cluster of chlorophyll molecules which need to perform the electron process in repetition for evolving Oxygen molecules. The structural integrity of thylakoid membrane comprises of integral membrane Proteins with some domains expanding towards the stroma and lumen of thylakoid. The reaction centres are formed of Pigment-Protein complex. The two Photo Systems (PS-I and PS-II) are spatially separated in the membrane with a ratio of 1.5:1 in the thylakoid membrane. The relative proportion of the two photosystems varies depending upon the light conditions experienced by leaves. Non-oxygen evolving purple photosynthetic bacteria has been reported to possess reaction centre similar to that of PS-II in structure. Moreover the Protein sequences of PS-II components are similar to that of the bacterial reaction centre Proteins. Interestingly, the antennae pigments associated with the reaction centres possess variations in their composition across diverse plant groups. These molecules are present at a much higher concentration in plant groups of higher order. The antennae pigment molecules efficiently transfer and intensify the photo energy in chloroplasts. The antennae molecules are comprised of a large family of structural Proteins associated both with PS-I and PS-II to form the light harvesting complex of chloroplasts. The mechanism of electron transport and subsequent steps of Oxygen and ATP evolution associated with photochemical reaction. The thylakoid membrane is primarily associated with the photochemical reaction of photosynthesis. This process involves proton formation associated with photolysis of water. In the process of the electron transfer process H^+-ATPase mediated proton gradient is established across the thylakoid membrane.

Photochemical reactions of photosynthesis are regulated by coordinated action of electron carriers, their redox potential values and concentration of photon molecules received by chloroplasts. The current unit shall focus on the mechanism of photochemical reactions and associated photophosphorylation events occurring in the thylakoid membranes. The functional units called photosystems convert light energy into chemical energy. The activity of PS-II is associated with the photo-oxidation of H_2O into hydrogen and Oxygen. The H^+ liberated in the process is concentrated in the lumen of thylakoid. The process of photolysis is accompanied by transfer of excited electron across various electron carrier Proteins. The electron transfer process of PS-II is independent of PS-I, although both the photosystems are connected through non-cyclic electron transfer and photophosphorylation. At the terminal step of electron transfer cascade, $NADP^+$ is reduced to NADPH. The main assimilatory power produced through the photochemical reaction is $NADPH_2$ and ATP which are involved in the regulation of C_3 cycle. The energy stored within the photon molecules is termed as **Quantum**. The light energy received by chloroplasts appears in form of photon clusters or Quantum pockets. The photons which appear with wavelength in the visible range of spectrum (400 nm - 700 nm) are optimum for inducing the photochemical reaction in chloroplasts. The chlorophyll molecules which receive photons remain energetically in their ground state or unexcited condition. Excitation of chlorophyll molecules cause to liberate the excited electron which is transferred among the respective electron carriers. There are different ways by which excited chlorophyll molecules emit their energy and return to their ground state.

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Fluorescence is one the phenomenon exhibited by the chlorophyll molecules where the emitted energy is visualized in the form of fluorescent light. Differently enough, the chlorophyll molecules in their transient excited state can also liberate heat or transfer the excited electrons to adjacent carriers. The chlorophyll and carotenoid/xanthophylls molecules present in the chloroplasts exhibit different absorption spectra. Thus, optimum rate of photosynthesis followed by absorption of light by chlorophyll molecules has been observed to be at a range of 600 nm to 700 nm. Carotenoids are capable of optimum light absorption in the range of 400 nm - 500 nm. This feature provides them the characteristic yellowish orange colour. Higher plants usually possess Chlorophylls A and B while Chlorophyll (Chl) C and Chlorophyll (Chl) D are present in cyanobacteria and other algal members. However, Chl A is universally present in all photosynthetic organisms. Bacteriochlorophyll in comparison to plant chlorophyll exhibits certain differences in their chemical composition. The magnitude of action spectrum exhibited for photosynthesis clearly depicts the wavelength and pigments necessary for photochemical reactions. Physiological investigations based on the action spectra have led to the discovery of two photosystems associated with photosynthesis.

The photon molecules within the photosynthetically active wavelength possess almost similar efficiency in inducing electron transfer mechanism. In Eukaryotes photosynthesis is accomplished within specialized organelles called chloroplasts. However, in Prokaryotic organisms the process is carried in thylakoids scattered in the cytoplasm. The optimum rate of photosynthesis is measurable by the parameter of **Quantum yield** which depicts the number of Oxygen molecules yielded by the action of one photon molecule. Experiments in the year 1937 confirmed the formation of Oxygen by oxidation of water molecules. Thus process was associated with the reduction of Fe-salts in isolated chloroplasts. The process of photolysis of water occurs at the reaction centre of PS-II. PS-II produces both an oxidant and reductant necessary to continue the process of electron transfer to PS-I. PS-I also produces a strong reductant and a weak oxidant. The electrons ejected from PS-II travel in a Z scheme to complete the pathway of electron transfer across the two photosystems. During the process of electron transfer the protons liberated from PS-II are utilized for formation of electrochemical gradient. This energy driven process is utilized in the formation of ATP catalyzed by ATP synthase present in the thylakoid membrane. The redox-potential values of electron carriers provide the gradient for electron transfer across the thylakoid membranes. The process of electron transfer in PS-II may also operate even when PS-I is not functioning. This process of PS-II mediated electron flow is termed as cyclic electron flow as it returns back to the reaction centre of PS-II. Cyclic electron flow is accompanied by ATP production and termed as **Cyclic Photophosphorylation**. The process of non-cyclic electron flow from PS-I to PS-II accompanies **Non-Cyclic Photophosphorylation**. The water oxidation steps shall be discussed in the following section of the unit. It is important to understand the process of Oxygen formation by photolysis which involves redox reaction facilitated by the intermediate oxidation stages of Mn^{+}. The Mangano-Protein Complex present in the PS-II centre thus facilitates the process of water oxidation. Physiological investigations also revealed that Cl^{-} and Ca^{2+} are also involved in the process of water oxidation. The detailed explanation of each of the electron carriers and their structural features will be discussed in the following sections of the unit. In the context of learning the photochemical process of photosynthesis it is important to understand the repair mechanisms of the photosynthetic apparatus. The singlet Oxygen species generated can be deleterious by oxidizing various biomolecules including chlorophyll. Thus the carotenoids (accessory pigments) act as photochemical shield to chloroplast. These pigments by the virtue of their structural feature are able to quench the singlet Oxygen species formed within the chloroplasts. Another process of protection from reactive species is by non-photochemical quenching. The three important forms of xanthophylls mainly zeaxanthin, antheraxanthin and violaxanthin help in

the dissipation of heat energy. Thus, accessory or antennae pigments around the reaction centre protect the photosystem functioning in the thylakoid membrane.

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Carbon fixation is an important physiological process which regulates biomass accumulation, growth and productivity in plants. In this context it is important to understand that plants exhibit variations in the process of carbon fixation which is being modulated by CO_2 concentration, temperature and O_2 availability in the environment. It has been observed that a majority of plants (> 80%) exhibit C_3 pathway of carbon fixation associated with photosynthesis. Ribulose-1, 5- Bi-phosphate Carboxylase Oxygenase (RuBisCO) has been observed to be one of the most abundant proteins on the Earth. This regulatory enzyme is associated with carbon concentrating mechanisms in plants. The events of carbon fixation pathway have been found to be similar in most of the plants.

However, they might differ in some aspects like compartmentalization of sugars affected by diurnal fluctuations. The biochemical reactions occurring in the stroma of chloroplast are important components of the **Calvin cycle**. Preliminary investigations revealed that the Calvin cycle operates independent of the perception of light. Hence it was termed as Dark Reaction. Further experiments by physiologists revealed that the assimilatory powers produced during light reaction (ATP and $NADPH_2$) are the main driving force for biochemical steps of Calvin cycle. The Calvin cycle involves sequential steps associated with fixation and reduction of CO_2. The process is also termed as reductive pentose phosphate pathway. The other Carbon fixation pathways associated with the C_3 cycle are supplementary or dependent upon the products of the C_3 cycle. The Calvin cycle implies the molecules of ATP and $NADPH_2$ to accomplish the process of reductive Carbon fixation. This step involves conversion of RuBP (Ribulose-1, 5 BiPhosphate) to phosphogylceric acid which is necessarily catalyzed by RuBisCO. The first stable product of C_3 cycle is essentially a 3-Carbon compound known as 3-Phosphoglyceraldehyde. This biomolecule is also involved as an intermediate metabolite of other biochemical pathways. The Calvin cycle is broadly comprised of three phases namely carboxylation phase, reductive phase and regenerative phase. RuBP is the primary acceptor of CO_2 in the Calvin cycle which is essentially regenerated from xylulose or sedoheptulose sugar phosphates.

The detailed mechanisms and regulation of Calvin cycle steps shall be discussed in the following sections of the unit. The form of Carbon present in CO_2 is the most oxidized form which gets incorporated in PGALD as a reduced form. This molecule gets metabolized to Glyceraldehyde 3-Phosphate which contains Carbon in the most reduced state. Thus, incorporation of atmospheric Carbon into organic compounds within plants is a reductive process. However, the photosynthetic efficiency of plants largely depends upon the activity of RuBisCO. As the name suggests, RuBisCO is an enzyme which shows affinity both for CO_2 and O_2 present in the atmosphere. However, the K_m (substrate affinity) is higher for O_2. This results in diversion of RuBP metabolism towards a separate pathway of glycolate metabolism commonly known as photorespiration. Photorespiration is a metabolic adjunct to C_3 pathway in plants because it is unavoidable during high O_2/CO_2 ratios in the atmosphere. Thus, photorespiration results in the decreased rate of Carbon fixation, i.e., conversion of RuBP into PGALD.

Certain plants exhibit different mode of Carbon concentrating mechanisms where the inhibitory effect of Oxygen on RuBisCO is overcome by spatial compartmentalization of RuBisCO and Oxygen molecules. Phosphoenol pyruvate is one of the important intermediates of the C_4 pathway in plants which is metabolized to form Oxaloacetate. The first formed stable product in C_4 pathway is thus formed of 4-Carbon compound. This pathway is additional to that of Calvin cycle where there are negligible chances of photorespiration to occur. Thus, reduced rate of glycolate metabolism increases the photosynthetic efficiency of plants.

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Physiologists have reported that even algal and cyanobacterial members also exhibit certain CO_2 concentrating mechanisms like membrane pumps (HCO_3^-) which sequester inorganic form of Carbon. It has been observed that in marine habitats RuBisCO operates at a slower rate than its normal efficiency. The C_4 plants exhibit differences with C_3 plants in the terms of their leaf anatomy. The leaves of C_4 plants contain dimorphic chloroplasts localized in the mesophyll and bundle sheath cells. Most of the mesophyll cells are present in the vicinity of the bundle cells. This helps them to communicate the metabolites like oxaloacetate and malate to the bundle sheath cells. The cells are extensively connected symplastically by cytoplasmic extension of plasmodesmata. The C_4 cells typically represent a form known as Krantz anatomy. Malate and aspartate are the major carboxylation products of C_4 cycle. Thus the primary carboxylation reactions in this pathway is not catalyzed by RuBisCO, but by an enzyme called PEP Carboxylase. The C_4 metabolism which has been reported in more than 16 families of angiosperms is most pronounced in the poaceae family among the monocots. Another mode of CO_2 concentrating mechanism has been exhibited through Crassulacean Acid Metabolism (CAM) Pathway. Many desert plants or succulents undergo unique regulation of stomatal movement coinciding with diurnal fluctuations. The primary carboxylation step of CAM pathway is catalyzed by the enzyme PEP carboxylase. PEP carboxylase produces oxaloacetate during the night time. This OAA (OxAloAcelate) in turn gets converted into malate which is stored in the vacuole. Thus, the basic mechanism of C_4 and CAM pathway are not different.

However, CAM pathway exhibits a unique temporal regulation in terms of Carbon sequestration. During the day time the malate is transported to chloroplasts where it gets decarboxylated to liberate CO_2 necessary for Calvin cycle. Thus plants possess unique adaptations associated with Carbon fixation and regeneration of CO_2 acceptors in chloroplasts. A certain amount of CO_2 utilized in photosynthetic assimilation is obtained from the process of respiration in plants. Alternatively, the amount of O_2 liberated by photosynthesis may be required to carry out respiratory metabolisms. Thus, it is very important to understand the factors which govern the rate of Carbon fixation in plants. CO_2 concentration may appear to exhibit mechanisms of feedback inhibition on enzymes of Carbon fixation pathways. Moreover, breakdown and generation of ATP appear to be important regulator of various steps of Calvin cycle.

4.2.1 Wavelength Dependent Activation of Photosynthetic Pigments

The process of photosynthesis is largely comprised of biochemical steps categorized in two categories, i.e., the light dependent step (Light Reaction) and light independent step (Dark Reaction). The **Light Reaction** is accomplished by **Oxidation–Reduction Reactions** mediated by electron transfer between Pigment-Protein Complexes. Thus, Oxygen evolution and excitation /energy transfer process is a function of intensity and wavelength of light energy received by chloroplasts. Various physiological investigations have confirmed the presence of PS-I and PS-II photo centres associated with light reaction process. Experiments by Emerson have led to conclusions of the complementary effects of Red and Far Red wavelength in enhancing the rate of Oxygen evolution in light reaction (Quantum yield). The rate of photosynthesis within the range of optimum wavelength does not exhibit any variation associated with values of wavelength. This observation implies that the photon molecules within a fixed wavelength range possess equal efficiency. Emerson's experiments showed interesting results in terms of wavelength dependent regulation of photoreaction. Far Red wavelength (beyond 680 nm) applied in isolation exhibited a drop in the Quantum yield which could be complemented by Red wavelength (680 nm). This phenomenon (Red drop and Emerson enhancement effect) led to the conclusion of parallel existence of two Pigment Systems (PS-I and PS-II) associated with the light reaction.

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The chlorophyll molecules (Chl A and Chl B) in association with accessory pigments form reaction centre and light harvesting Complex in the two photosystems. The wavelength dependent activation of photosynthetic pigments can be better explained by the action spectrum represented by Quantum yield. This spectrum basically represents rate of photosynthesis (Quantum yield) obtained as a function of wavelength in the visible spectrum. Quantum yield drops at the wavelength near to 500 nm and further exhibits optimum response are red wavelength. However, the decrease in Quantum yield at far red wavelength is due to absence of PS-I functioning. It was further observed that the decrease in photosynthesis rate at 500 nm was attributed to the negligible absorbance of light by chlorophylls.

However, the absorption of light energy at a range of 500 nm - 600 nm is accomplished by accessory pigments of carotenoids and xanthophylls molecules. Thus, energy transfer from accessory pigments (Light harvesting complex) to reaction center alone does not appear sufficient to drive optimum photosynthesis.

The Ultrastructure of Chloroplast

The chloroplast is a membrane bound semi-autonomous organelle in photosynthetic Eukaryotic cells. In plants chloroplasts are largely evident in the mesophyll parenchyma of leaf tissue. However, it may also be present in the stem (phylloclade), fruit wall or roots in exceptional cases. The organelle is located in the cytoplasm and ranges form 4-10 μm in length. The process of biogenesis of chloroplast will be discussed in the following section of the unit. The most important parts of this organelle include the internal membrane system (**thylakoid**) and the matrix (**stroma**) which harbours majority of biochemical and light dependent reactions of photosynthesis. The organelle is surrounded by double membrane structure which is considered as the outer envelope. The internal structure of chloroplast mostly contains the network of thylakoid stacked in the form of **granum**. The internal matrix which holds the stacks or granum is termed as the stroma. The thylakoid membrane and stroma comprise of most of the Proteins, pigments and carrier molecules associated with the metabolic steps of photosynthesis (Refer Figure 4.1).

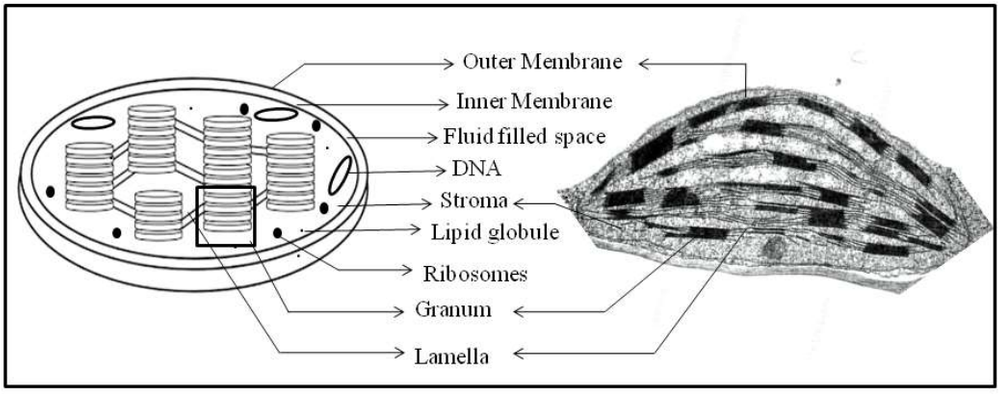

Fig. 4.1 *Structure of Chloroplast*

Chloroplastidial Membrane Properties

The outer envelope of chloroplast ranges from 8-10 nm in thickness. The outer and inner membrane of chloroplast shows considerable differences in their lipid compostion. The outer membrane contains a major amount of phospholipids (50%) associated with galactolipids (40%) and sulfolipids. However, the inner membrane mainly contains galactolipids (80%) associate with phospholipid and sulfolipids. The intermembrane space of 20 nm separates the two membranes. The inner membrane due to its typical nature is very selectively permeable

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to biomolecules. The inner membrane consists of carbohydrate transporter (triose phosphate transporter). The outer membrane due to its general phospholipidic nature is semi-permeable to ions and other metabolites like carbohydrates, reductants and coenzymes. The thylakoid membrane remains interconnected to form internal cisternae and has lipid composition similar to the inner membrane of envelope.

Internal Structure

The membrane bound internal region of chloroplast is comprised of the stroma and thylakoid stacks called **granum**. The stroma is a region formed of hydrophilic matrix containing proteinaceous molecules. More than fifty percent of Protein constituent of the stroma is formed by Ribulose 1, 5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) the enzyme responsible for Carbon fixation. The stroma exhibits dispersed distribution of lipid granules some of which contain plastoquinone in the vicinity of the membrane. Starch grains remain in the vicinity of the thylakoid membranes. In C_4 plants the bundle sheath chloroplasts show more abundance of starch grains. The ribosomes are of 70 S types and remain scattered in the stroma matrix. The plastidial DNA is circular in nature with a diameter ranging from 30-60 μm in algae and higher plants. The plastid DNA is devoid of histone Proteins and exhibit similarities to Prokaryotic DNA. The semi-autonomous nature of chloroplast is attained by the virtue of few Proteins being encoded by the plastidial DNA.

The **thylakoids** present in the chloroplast are stacked into granum. The thylakoids vary in size and are present in the form of 40-60 granum per chloroplast. The thylakoid membrane contains perforations to form connectivity between the inter-thylakoid space and stroma of chloroplast. The thylakoid membrane consists of majority of pigments and Proteins which act as electron carriers for photochemical reaction. In the early stage of development of chloroplast the thylakoids are formed by invagination from the inner membrane. In mature chloroplasts the thylakoids do not remain connected to the inner membrane. The components of the chloroplast form internal spaces at three regions, i.e., inter-membrane space between the two layers of envelope, the space of stroma between the inner membrane and thylakoid and the thylakoid lumen surrounded by the thylakoid membrane. The thylakoid lumen constitutes an important part of energy driven process of photophosphorylation. The lumen exhibits a low pH and acts as an internal reserve for proton molecules. The proton gradient formed across the thylakoid membrane thus helps in the process of photophosphorylation. The outer membrane of chloroplast is permeable to molecules smaller than 10 kDa size. Specialized pore like structures called porins function for transport of macro molecules across the outer membrane. The outer envelope of the chloroplasts also helps in recognition and entry of nucleus encoded Proteins into the inner matrix. The plastids may often remain connected by external tubular entensions called stromules. Algal chloroplasts in cyanobacteria and red algae possess phycobilisomes associated with the thylakoids. However, in higher plants due to absence of phycobilisomes the structure of thylakoid is much more complex.

Plastid Genome (Plastome)

The circular plastidial DNA remains scattered in the stroma in multiple copies. In active mesophyll cells of leaf the number of chloroplast ranges from 20-300 and the number of DNA copies in each chloroplast may exceed to 500 in number. The palstidical genome often termed as plastome comprises 20% of the total DNA obtained from leaf tissue. Proplastids prior to differentiation of chloroplast contain DNA organized in the form of nucleoids – similar to Prokaryotic cell. The plastidial DNA is devoid of Histone Proteins. Moreover, the plastid DNA exhibits various gene regulation features similar to the Prokaryotic DNA. The chloroplasts have been evolutionary related to the Prokaryotic symbionts or coacervates

which evolved as semiautonomous organelles within the Eukaryotic cells. Thus, the origin of chloroplast according to the endosymbiotic theory is explained in the light of its evolutionary relatedness to Prokaryotic organisms.

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Biogenesis of Chloroplast

The word plastid has been derived from the nature of plasticity of the organelle. Plastids are usually capable of movement and redifferentiation into various types. This plasticity is regulated by the requirement of the particular cell associated with external signals like light intensity. Various types of plastids like Etioplast, Amyloplast, Chromoplast, Leucoplast, Elaioplast and Chloroplast are usually interconvertible or undergo reversible changes among each other. The process of chloroplast formation initiates from the proplastids of shoot apical meristem. The proplastids are small organelles of 0.2-1.0 μm in diameter. Within the proplastids the precursor for thylakoids appears in the form of vesicles. The vesicles are structure derived from other young proplastids or by invagination of inner membrane of the plastid. The meristematic cells in the course of their differentiation into mesophyll cells undergo formation of chloroplast from the proplastids. The meristematic cells contain 10-20 proplastids in each of them. However, along with formation of mesophyll cells the number and volume of plastids increase to ten folds. The process of differentiation of chloroplasts from the proplastids is mostly regulated by the nuclear genome. The membrane mediated import of nuclear encoded Proteins into the organelle play a major role in chloroplast biogenesis. The process of chloroplast differentiation is regulated by the external factor like light intensity. During cell division the chloroplast or proplastids segregate into the daughter cells. The mature chloroplasts possess specific proteins and lipid molecules necessary for metabolic pathways. The mature chloroplasts appear as sites for phytohormone synthesis, starch metabolism and photosynthesis. In C_4 plants, the structure of chloroplasts in mesophyll and bundle sheath cells exhibit dimorphism in the terms of absence or presence of grana. This unique adaptation is regulated by nuclear genome and prevents Oxygen-mediated inhibition of RuBisCO.

Overview of Pigment and Protein Distribution in the Thylakoid

The photosynthetic pigments present in the chloroplast are usually chlorophylls A, B and carotenoids and xanthophylls. The pigments constitute the photosystems necessary for light harvesting and photochemical reactions. The reaction center, Antennae–Protein Complex and electron carrier proteins are essentially localized in the thylakoid membrane. The proteins present in the thylakoid membrane are usually integral membrane Proteins which also possess external hydrophilic domains protruding to the lumen or stroma of the chloroplast. The inner membrane associated domain of the proteins contains hydrophobic amino acids. The pigments necessary for light reaction remain associated in non-covalent interactions with the proteins in the thylakoids. The maturity of chloroplast is associated with the formation of photosynthetic apparatus within the organelles. The two photosystems and their antennae pigments are spatially separated from each other within the thylakoid membrane. The PS-II associated with light harvesting and photlysis of water is mostly aligned in the grana lamellae. The antennae pigments associated with PS-II also remains in its vicinity. The plastoquinone Proteins remain associated with the plastoglobulin vesicles scattered in the stroma matrix of the chloroplasts. The PS-I centre and its associated antennae pigments remain localized in the stroma lamellae and the edges of grana lamellae. The electron transferring Protein of **Cytochrome b_6/f Complex** is equally scattered both in the **Stroma** and **Grana** region of the **Chloroplast**. It usually connects the two photosystems in its vicinity. The spatial separation of the two photosystems is thus associated with diffusion of electron carrier Proteins between the grana and stroma region of the chloroplast. The protons generated by water oxidation are capable of diffusing into the stroma region of the chloroplast.

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This separation of PS-I and PS-II has been attributed to increased efficiency of the photosystems. Moreover, the spatial separation implies independent nature of electron transport possible in the two Photo Systems. In the absence of PS-I functioning, the PS-II can function by transferring electrons to the intermediate acceptor of plastoquinone. It has been observed that plant chloroplasts may possess higher amount of PS-II molecules in comparison with PS-I (1.5:1). This ration may, however, change with alteration in light intensity received by leaves. Purple photosynthetic non-oxygen evolving bacteria like *Rhodobacter* or *Rhodopseudomonas* has been reported to possess only a single photosystem.

Molecular Structure of Pigment-Protein Complex in Thylakoid

The molecular structure of photosynthetic pigment and Protein Complex associated in the thylakoid provides important insights to the distribution of electron transfer carriers. Various investigations have revealed the detailed structure of the two photosystems necessary for light reaction in the thylakoid.

PS-I is mostly composed of chlorophyll a associated with Chl B and β carotene molecules attached to Proteins. The PS-I complex is mostly concentrated at the stroma lamellae of the thylakid. The reaction center of PS-I is mostly formed of Chl A molecule capable of absorbing wavelength of 700 nm. The associated Chl B and β carotene molecules remaining in the vicinity of the reaction center transfer energy to the core region. PS-I also contain 2-4 molecules of heme containing Fe-S Proteins which function as the primary electron acceptor. The excited electrons form the reaction center of PS-I are immediately transferred to the Fe-S Proteins. The two major Proteins known as PsaA and PsaB are involved in the electron transfer process in the reaction center of PS-I. The intermediate forms of the Fe-S Proteins are designated as F_X, F_A and F_B. Thus, electrons are usually transferred from a series of Fe-S Proteins with intermediate forms and then received by Ferridoxin. The reduced plastcyanin is capable of donating electrons to the P 700 reaction center. The accessory Proteins associated with PS-I are designated as PsaF, PsaD and PsaE which help in association of soluble electron transfer complexes with the PS-I center.

The **PS-II** is mainly composed of Chl A and β carotene. Chlorophyll B is present in comparatively lesser amounts in PS-II. The PS-II component is held in a complex structure of multi-subunit Protein aggregate which bears two reaction centers and one antennae complex. The core region of the reaction centre contains two Proteins known as D_1 and D_2. The two Proteins D1 and D remain in association to each other. The core region of the reaction center in PS-II is composed of specialized Chl A molecules capable of absorbing light at the wavelength of 680 nm. The primary electron acceptor of PS-II is termed as phaeophytin which is a derivative of Chl A molecule without Mg^{2+}. The recation centre molecules along with phaeophytin, plastioquinone and carotenoids remain associated with the membrane Proteins D_1 and D_2. These membrane Proteins have been observed to exhibit sequence similarity to the L and M peptides of Purple Bacteria. Some accessory Proteins like $cytb_{559}$ is associated with the protective function of PS-II. The Quinine Protein (Q) is capable of accepting excited electron from the reaction centre of PS-II. The reaction centre of PS-II is important for its photolytic activity of water. The Proteins associated with PS-II are manganese bound Proteins which form a moiety of four manganese ions bridged by Cl^- in between them. The mangano-Proteins are thus important components of the PS-II structure present in the inner side of thylakoid membrane. These Proteins are generally associated with water oxidation in the presence of light. The manganese ions perform the function of intermediate redox molecules which form a part of the water oxidation clock. The Light Harvesting Complexes (LHC) associated with PS-I and PS-II are usually comprised of Chl A, Chl B, Xanthophylls and cartenoid molecules. They remain in vicinity of the two

photosystems and usually function to transfer energy to the respective reaction centres. The process of PS-I and PS-II mediated electron flow occurs from H_2O to NADP. The electron transfer carriers are usually plastocyanin, $cytb_6$, cytf and Fe-S Protein. The F_1-ATPase is an important component of the thylakoid membrane which involves in ATP hydrolysis and couples proton transfer across the membrane of the thylakoid.

Antennae Pigments are clusters of molecules which belong to the component of LHC I and LHC II associated with intensification of light energy received by chloroplasts. These molecules are also known as Chl A/B Antennae Proteins. Electron microscopic analysis has revealed the molecular structure of the LHC molecules. The Protein of LHC contains three **α helical** region which binds to 15 Chl A, Chl B and carotenoid molecules. The Proteins of both LHC I and LHC II have similarities in their Protein sequences and show evolutionary relatedness. The LHC polypeptides remain associated with each other and the pigment molecules remain in vicinity with each other. This aggregation of molecules in the LHC helps in improving the efficiency of electron transfer. The LHC is comprised of both inner and outer regions in the thylakoid membrane. The outer region is associated with the functioning of light energy harvest and the inner region remains in vicinity of the reaction centres of the two photosystems. The process of energy transfer occur form the outer part of LHC, through the inner integral part of LHC associated with the reaction centre. There lays considerable differences among the Protein and pigment composition of LHC I and LHC II. In PS-I the core region of LHC is composed of 100-120 Chl A and 15-20 β carotene molecules. This association remains in the vicinity of P 700 centre. The PS-II associated LHC II contains two major Pigment-Protein Complexes known as CP4 and CP47. Interestingly, the core region of LHC has similarities in their Protein composition while the outer region exhibits variations. The composition of LHC outer region is largely regulated by the intensity of light received by chloroplasts. The algal and higher plant chloroplasts contain two classes of LHC I and LHC II. The detailed analysis of LHC II complex has revealed the presence of four complexes LHCA, LHCB, LHCC and LHCD. The core component of this complex has been reported to be LHCIIb which exists in form of a Trimeric Membrane Protein. Each of the monomers of this LHCIIb is composed of polypeptides associated with lutein. The activity of LHC molecules remaining in association with reaction centre can reversibly dissociate from PS-II when not required. This process is accomplished by Threonine-Phosphory lation reactions catalyzed by Protein Kinase Activity. In the antennae complex of PS-II the peripheral Proteins used for energy transduction are commonly CP29, CP26, CP24. These components transfer excited electrons to main core region of antennae and then to PS-II reaction centre.

Structure of F_1-ATPase in Thylakoid Membrane

The F_1- ATPase is localized at the thylakoid membrane which energizes the transfer of proton across the membrane. The proton efflux activity of the ATPase results in the formation of electrochemical gradient across the thylakoid lumen. The ATPase is a transmembrane Protein comprised by CF1-F_0 particles. The enzyme is comprised of nine separate polypeptides organized into two subunits. The F_0 complex is a membrane integrated subunit further composed of domains/subunits I, II, III and IV. This membrane bound subunit of F_0 particle functions for proton transport and also contains specific sites for the binding of F_1 catalytic domain. The extrinsic domain of F_1 catalytic subunit is also composed of five smaller subunits commonly designated as **α**, **β**, **γ**, **δ** and **ε**. Electron microscopic analysis have revealed further structural details of the enzyme. The F_1 particle is represented by a hexagonal symmetrical ring of alternating α and β subunits. The δ was reported to be important in mediating the interaction between the F_0 and F_1 particles. The other subunits were found to be associated with the core region of α and β subunits. The catalytic and binding sites present in the F_1 particle has

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been revealed by fluorescence studies. The α and β subunits also possess nucleotide binding sites necessary for maintaining the catalytic activity of the enzyme. The α-and β subunits of the F_1 particle share about 50-60% similarities with their mitochondrial counterparts. However, interesting observations have revealed that the CF_1 particle is more sensitive to the Fungal Pathotoxins. Magnesium ion has been designated to play a crucial role in facilitating nucleotide binding to the F_1 particle.

Structural Features of Chlorophyll and Carotenoids

The chlorophyll molecules are universally present in both Eukaryotic and Prokaryotic photosynthetic organisms. The biosynthesis of chlorophylls in the cells initiate from two precursors AminoLevulinic Acid (ALA) and PorphoBilinoGen (PBG). Chlorophyll is thus a derivative of porphyrin which is represented by a cyclic tetrapyrrole consisting of four pyrrole rings joined to one another. The centre region of the tetrapyrrole contains a Mg atom bonded to Nitrogen atoms of the four pyrroles. The isocyclic ring present in the structure contains an acid side chain on the C10 which is esterifed with a methyl group. The C7 of the isocyclic ring contains a long phytol tail. There is a structural difference in the II pyrrole ring among chlorophyll A and chlorophyll B. The methyl (CH_3) group in the C3 atom of Chl A is replaced by a –CHO group present in Chl B. Thus, the presence of –CHO group in Chl B renders it to be more polar in nature than Chl A. Certain differences in the physical and chemical properties also exsist between Chl A and Chl B (Refer Figure 4.2). The absorption maxima of Chl A are 430 nm and 662 nm, respectively. Chl B exhibits absorption maxima at 430 nm and 620 nm, respectively. Chl A exhibits better solubility in presence of petroleum ether. This difference is attributed to the differences in the polarity of Chl A and Chl B. Bacteriochlorohyll differs from phyto-chlorophyll in the presence of acetyl group in the C2 of IInd and IVth pyrrole rings. The formation of chlorophyll from AminolevuLinic Acid (ALA) proceeds through various pathways. α-ketoglutaric acid (keto analog of glutamate) undergoes reduction of C1 atom to produce Di-Oxo-Valeric Acid (DOVA). DOVA produces ALA by the process of transamination reactions. Alternately, ALA may also be produced directly from Glutamate. This step is accomplished by the formation of a metabolic intermediate called Glutamate Semialdehyde. ALA is further metabolized to Porotogen which then forms protoporphyrin (immediate precursor of chlorophyll).

The carotenoids are accessory pigments involved in photosynthesis. They remain associated with the chlorophyll molecules and help in intensifying the photosynthetic efficiency (Refer Figure 4.3). The carotenoids are terpenoid compounds mainly comprised of xanthophylls and carotenes. Xanthophylls are the Oxygen derivatives of carotenes and are also termed as carotenols. The major carotene in higher plants is β-carotene. A small amount of α carotene may also be present in the chloroplasts. The xanthophylls are mainly formed of Lutein, Violaxanthin and Neoxanthin.

However, lutein is the most abundant form of xanthophylls. Various other forms of xanthophylls are present in different classes of algal members. The absorption maxima exhibited by the carotenoids are at 430 nm - 480 nm. The biosynthetic pathway of carotenoid initiates form condensation of two trans-geranyl-geranyl (C_{20} terpenal) pyrophosphate to form Phytoene. Phytoene (C_{40}) undergoes desaturation and produced various forms of carotenoids. The desaturase enzymes are localized in the membranes of the plastid and are responsible for inter conversion of various types of carotenoids. The formation of xanthophylls molecules are regulated by environmental factors, such as light intensity and wavelength. Wavelengths of blue light induce optimum biosynthesis of various forms of xanthophylls in

the plastids. Phycobilins or bile pigments (Phycocyanin and Phycoerythrin) are another class of photosynthetic pigments present in some classes of Red and Blue-Green Algae.

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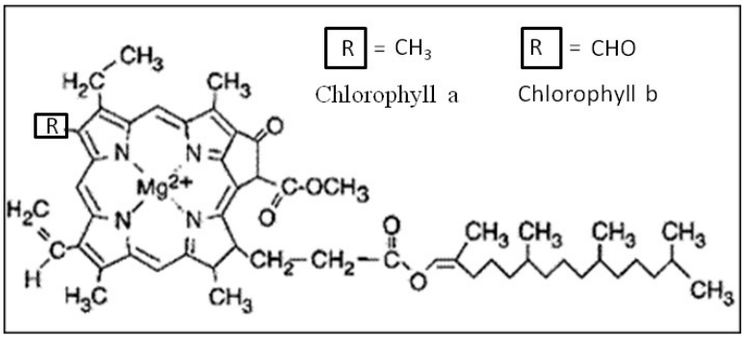

Fig. 4.2 *Structure of Chlorophyll A and Chlorophyll B*

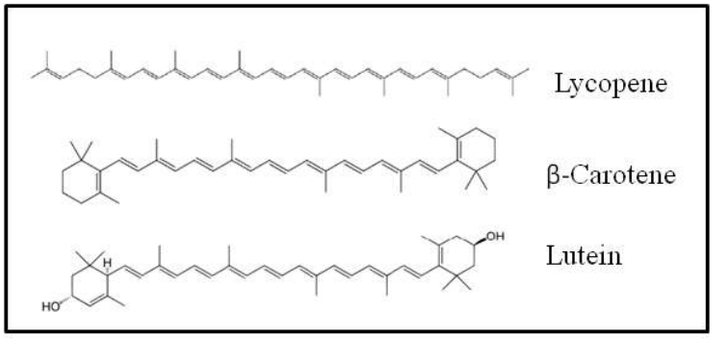

Fig. 4.3 *Structure of Carotenoids*

Concept of Quantasome and Quantum Yield

The photosynthetic unit is comprised of smallest group of pigment molecules which can efficiently induce a photochemical reaction. The effective mechanism of electron ejection from the photosystem is mediated by these pockets of energy or photosynthetic units. Investigations by Arnold and Emerson in the year 1932 revealed that one molecule of O_2 are evolved by the combined action of every 2500 chlorophyll molecules. The energy units of light wavelength trapped within photon molecules are termed a **Quantum.** Thus physiological investigations have reported that about eight quanta of light energy is required for the reduction of one CO_2 molecule during Photosynthesis.

Thus, the concept of **Quantasome** explains the association of 300 molecules of Chlorophyll in each of the photosystems which function as Photosynthetic Units. Each Quantasome is capable of absorbing one Quantum of light energy. Gaffron and Wohl (1936) proposed that the Quantum received by the photosynthetic pockets is transduced to the core region of the reaction centre in each of the photosystems. Kok (1956) stated that the intermediate electron carriers (Cytochrome, Plastocyanin and Plastoquinone) exist in a ratio of one per 300 molecules of Chlorophyll. The number of Oxygen atoms produced by one Quantum of light energy received in the photosystem is termed as **Quantum yield**. Alternately, the number of Quanta required to liberate one molecule of Oxygen is termed as **Quantum requirement**.

Observations have revealed that the absorption spectrum of chlorophyll does not completely coincide with the action spectrum of photosynthesis. This is attributed to the fact that accessory pigments with different absorption maxima (500 nm - 600 nm) are also involved

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in the process of photosynthesis. In early 1940 experiments conducted by Emerson and his group revealed wavelength dependent variation in the rate of photosynthesis. The Quantum yield measured in isolated chorolasts of *Chlorella* was examined separately with lights of wavelength 680 nm and > 700 nm. It was observed that wavelength higher than 680 nm resulted in a drop in the Quantum yield. This effect was termed as **Red Drop Effect.** The Far Red region of the visible spectra was found to be responsible for this Red Drop Effect. Subsequently, later investigations in 1957 revealed that supplementation of far red light with wavelengths near to 680 nm could recover the Quantum yield or photosynthetic efficiency. This phenomenon of combined effect of Red and Far Red wavelengths in producing optimum magnitude of Quantum yield was termed as **Emerson Enhancement Effect**. Thus this observation implies the simultaneous functioning of two photosystems in the chloroplasts.

Mechanism of Light Absorption and Energy Transfer During Photosynthesis

The light energy received by chloroplast appears in the form of Quantum which induces energy driven excitation of electron from the reaction centre. The effect of Quantum absorption depends upon the frequency of light energy. The visible spectra of light fall in between the UV (UltraViolet) and infrared zone which ranges from 400 nm to 700 nm. The absorption of Quantum results in excitation of the outer orbital electrons which initiates the photochemical reaction. The chlorophyll molecules which initially remain in their ground state (S_0) pass through intermediate oxidized stages (S_1) and (S_2). The excited forms of Chl molecule remain in the singlet or triplet stage for a transient time period. The return of Chl molecules into their ground state is accomplished by either emission of fluorescence or phosphorescence. In the later case, the return of excited molecule from triplet to ground state is accompanied by emission of low energy light for longer durations (Phosphorescence). The intensity of fluorescence is higher than that of phosphorescence and is emitted for shorter durations. Fluorescence involves return of electron from its singlet stage to ground state. Among all the types of photosynthetic pigments Chlorophyll A is directly involved in the process of photosynthesis. The other pigments essentially transfer their energy to Chl A molecules present in the reaction centre. The process of energy transfer from the antennae pigments to the reaction centre occurs by the phenomenon of Fluorescence Resonance Energy Transfer (FRET). In this mechanism the fluorescence emission of one molecule appears to be the excitation wavelength of the adjacent molecule. Thus, the excited electron is transmitted across the thylakoid membrane. The efficiency of energy transfer from Chl B to Chl A has been reported to be nearly 100%. However, the transfer of energy from Phycoerythrin to Chl A possess 80-90% efficiency and lesser for Carotenoids to Chlorophyll A (20-50%).

Light Reaction

The physiological experiments performed by Emerson and Rabinowitch (1960) confirmed the physiological functioning of chloroplasts in response to light wavelength. The process of photochemical reaction is accomplished by electron transport between the two photosystems PS-I and PS-II. The two photosystems contain core reaction centre associated with the functioning of Chl A. The wavelength associated with Chl A in the two photosystems vary as 680 nm and 700 nm in PS-II and PS-I, respectively. The differences thus lie in the efficiency to absorb longer and shorter wavelength of Red Light. Light reaction occurring in the Thylakoids involves Oxidation of Water to Oxygen and transfer of electron to $NADP^+$. The protons liberated from oxidation of water result in the formation of $NADPH_2$. The Chl molecules in P 700 centre are capable of optimum photon absorption in its reduced state. The connecting molecules which transfer electrons between PS-I and PS-II are mainly Copper containing Protein plastocyanin and a Quinone group of carrier termed as

Plastoquinone. The cytochrome Proteins involved in this process are mainly cytochrome B6 and cytochrome F.

The Water Oxidation Mechanism of Light Reaction

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The most important component of PS-II mediated part of light reaction is the oxidation of Water and liberation of Oxygen (Refer Figure 4.4). PS-II centre receives photons of wavelength 680 nm and upon excitation transfers electron to a chlorophyll derivative known as Pheophytin. Additionally the chlorophyll molecules should essentially return to reduced ground date in order to refunction by absorbing new photon molecules. Thus, the electron is obtained by oxidation of water molecules, which in turn reduces the Chl A molecules. Two water molecules are oxidised by Chl A to liberate four hydrogen ions and four electrons as shown in the given reaction.

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

The water oxidation mechanism accomplished in the PS-I reaction centre is facilitated by intermediate oxidation stages of Mn^+ designated as S_0, S_1, S_2, S_3 and S_4. The oxidation-reduction cycle occurring by the help of these four stages of Mn^+ was reported by Kok *et al* (1970) and is commonly mentioned as the **Water Oxidation Clock** (Refer Figure 4.4)**.** Every step of transition between S_0 and S_4 is a photon-mediated redox reaction. The last step of this water oxidation reaction series involves transformation of S_4 to S_0 accompanied by liberation of Oxygen. The process of water oxidation results in the liberation of four protons from two water molecules. The reaction centre possesses the mangano-protein complex which together with Ca^{2+} and Cl^- forms the catalytic **Oxygen Evolving Complex (OEC)**. The protons liberated are initially stored in the lumen of thylakoid. The OEC is localized towards the inner membrane of the thylakoid and attached to the D_1 and D_2 Proteins present in PS-II which facilitates the entry of protons into the lumen. These protons are later transferred to the stromal region by the activity of F_1 – ATPase. The proton motive force generated due to electrochemical gradient thus allows proton movement across the thylakoid membrane. Detailed discussion of the process of proton mediated ATPase activity will be discussed in the following section of the unit. A single PS-II reaction centre containing OEC must be excited four times in order to release a single O_2 from oxidation of two molecules of water. The four electrons obtained from water oxidation are transferred to the P680 site. In this context it is important to understand that an intermediate complex of electron carrier exists between the OEC and P680 centre and is termed as Z and Tyr_Z. Tyr_z transfers the electron to water and again obtains it by oxidizing the cluster of Mn ions. Thus the process of electron transfer from water to Mn ion is completed after four electrons are obtained from water. These electrons are transferred by Mn^+ to Tyr_Z complex.

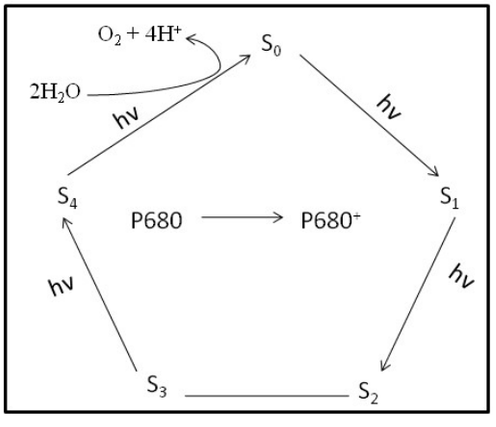

Fig. 4.4 *Water Oxidation Clock*

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Pheophytin and Quinone Mediated Electron Transfer Occurs from PS-II

The pheophytin is a specialized molecule of chlorophyll where the central magnesium ion is replaced by two Hydrogen atoms. This molecule by the virtue of its structural differences possesses different spectral properties than chlorophyll. Physiological evidences have confirmed that pheophytin is the immediate electron acceptor of PS-II centre. Furthermore the two quinone molecules present in the electron transfer complex obtain electrons from the pheophytin molecules. The quinine molecules designated as plastoquinone (PQ_A and PQ_B) remains in the vicinity of PS-II reaction centre. The process of oxidation and reduction of plastoquinone occurs in the presence of protons and electrons. The PQ form of plastoquinone is reduced to Plastohydroquinone (PQH_2) by obtaining two electrons from phaeophytin and also two protons from the stromal side of thylakoid membrane. The dynamic nature of PQH_2 results in its dissociation from the reaction centre complex and moves towards the inner part of the membrane. In this region, PQH_2 is reoxidized by transferring the electrons to $Cytb_6f$ complex. The non-polar nature of the quinone Protein results in its diffusible nature across the membranes. The protons of PQH_2 released combine with other protons obtained from water oxidation. This process decreases the pH of the thylakoid lumen. PQH_2 mediated transfer of proton results in the formation of ATP catalysed by the activity of ATPase. Usually two molecules of ATP are generated for each pair of electron passing through PQH_2.

Electron Transfer through Cytochrome B_6-F and Plastocyanin Connect to PS-I Centre

The process of electron transfer from PQH_2 to PS-I centre is mediated by two intermediate electron carriers called $cytb_6f$ and plastocyanin. The cytochromeb_6f complex is a multi-subunit Protein with prosthetic group attached to it. This complex Protein possess heme group attached to it to the peptide part. Furthermore the cyt Protein complex also possesses the Fe-S centre commonly termed as **Rieske centre**. The mechanism of electron flow from the PQH_2 to cyt complex has been explained by Q cycle. Investigations have revealed that the two electrons released by plastohydroquinone are involved in different pathways. The linear electron transfer chain accounts for the electron which is received by FeS_R (Rieske centre) and forwarded to the cytf complex. The other electron obtained from PQH_2 travels through a cyclic process and is associated with proton pumping activity across the membrane. The electron received by cytf complex is further transferred to Plasto-Cyanin (PC) which is a Blue coloured Copper Containing Protein. The $cytb_6f$ complex is larger in size than quinone and plastocyanin. It is also localized across the stroma and grana region of thylakoid. This result in its immobile nature compare to the other electron carriers (PQ and PC) present in the thylakoid membrane. plastocyanin is a small water soluble copper containing Protein of around 11 kDa in size. It transfers electron between the cytochrome b_6f complex and PS-I centre. The biosynthesis of this Protein is partially regulated by the copper availability of the plant tissue. Plastocyanin is localized in the lumen of the chloroplast. In certain organisms like Cyanobacteria and Algae c-type cytochrome may be present as a substitute of Plastocyanin.

The PS-I centre is a complex aggregation of Proteins which include the reaction centre surrounded by the light harvesting complex or antennae pigment. The core region of antennae pigment in PS-I is comprised of 100 Chl A molecules. The core antennae pigments and PS-II reaction centre are associated with PsaA and PsaB Proteins which are of molecular weight in the range of 65-70 kDa in size. The electron carriers associated with the PS-I

centre are strong reductants in nature. Physiological evidences reveal the presence of quinone and phylloquinone associated with Chl A molecule which serves as electron carriers. The Fe-S centre of the reaction centre form an efficient electron acceptor/carrier with three forms FeS_x, FeS_A and FeS_B, respectively. The electron receiving centres of PS-I are designated as A and B which transfer electrons to the water soluble Fe-S Protein called Ferridoxin. Furthermore, Ferridoxin-NADP reductase reduces $NADP^+$ to NADPH. This process terminates the process of electron transfer from PS-II to PS-I. The reduced form of ferridoxin associated with the photochemical reaction also serves in other metabolic pathways like nitrate metabolism and regulation of Carbon Fixation Pathways.

Electron Transport Pathway in Chloroplast

In the earlier sections various electron carriers associated with the two photosystems were discussed in details. The process of electron transfer from PS-II to PS-I operates through various intermediate carrier Proteins and is commonly termed as the **Non-Cyclic Electron Flow**. This process does not involve the return of electron into the PS-II reaction centre. However, another pathway of PS-I mediated electron transfer occurs simultaneously to the non-cyclic transfer and hence is termed as **Cyclic Electron Transfer (CET) Pathway**. This pathway involves plastocyanin mediated return of electron to the PS-I centre of chloroplast. Interestingly the formation of $NADPH^+$ occurs at the end of the non-cyclic pathway. However, both the electron transport pathways are associated with ATP formation catalyzed by the enzyme ATPase. This process of ATP formation coupled to photochemical reaction is termed as **Photophosphorylation.** The process of photophosphorylation is associated with the cyclic and non-cyclic electron transport pathways and hence termed and **Cyclic and Non-Cyclic Photophosphorylation**. The brief process of electron transport pathways shall be discussed in the following sections of the unit.

Non-Cyclic Electron Transport Pathway

The Magano-Protein associated with the Oxygen Evolving Complex (OEC) has been observed to exhibit enzymatic activity which catalyzes the splitting of water (**Hill reaction**). Mn^+, Ca^{2+} and Cl^- are associated with the OEC complex. Various compounds like hydroxylamine, ammonia, Carbonyl Cyanide 3-Chloro Phenylhydrazone (CCCP) or heavy metals have been reported to inhibit the process of electron transfer from the PS-II region. The Mn-Protein of OEC mediates the electron transfer from water to the PS-II region. The electron from PS-II region is transferred to pheophytin molecule. The process of electron transport further advances to Quinone and Plastoquinone. The PQH_2-PQ interconversion event associated with the electron transport pathway is coupled to ATP formation. This process is termed as the **Non-Cyclic Photophosphorylation**. A commonly implied inhibitor of electron transport from Quinone to other carriers is **Di-Chlorophenyl-DiMethyl-Urea (DCMU)**. PQH_2 transfers the electrons to the Rieske Center (Fe-S). This Fe-S center is further oxidized by cytochrome (cyt F) followed by electron transfer to the Plasto-Cyanin (PC) complex. The plastocyanin Protein acts as the electron donor for the PS-I center. The PS-I centre transports electrons to the ferridoxin and finally to $NADP^+$. This terminates the process of non-cyclic electron transport pathway of chloroplasts.

Cyclic Electron Transport Pathway

The cyclic pathway of electron transport is associated with the PS-I center which transmits the electron across Ferridoxin, Plastoquinone-Fd Complex and Plastocyanin Protein. The cyclic process of electron transport does not involve oxidation of water molecules and it

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essentially operates independent of the non-cyclic process. Photon-mediated excitation of the P 700 molecules results in transfer of electrons to ferridoxin. The redox reaction associated with electron transfer is further mediated by the activity of Fd-PQ oxido-reductase enzyme and cytochrome b_6f complex. The electron returns to the P 700 center via the plastocyanin molecule. **Paraquat** is a well known inhibitor which blocks the electron transfer from PS-I to $NADP^+$. The process of ATP formation in this pathway is energised by proton mediated electrochemical gradient across the **Thylakoid Lumen**. Thus ATP formation in this pathway is termed as the **Cyclic-Photophosphorylation** (Refer Figure 4.5)**.**

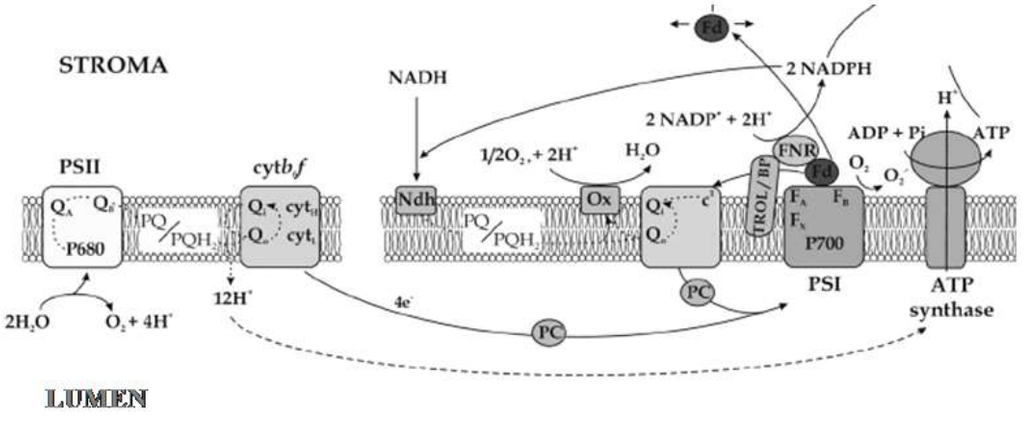

Fig. 4.5 *Mechanism of Light Reaction*

Proton Transport across Thylakoid Membrane: Chemiosmotic Theory

The process of proton energised phosphorylation in the thylakoids was initially reported by Daniel Arnon amd co-workers in 1950. The process of light dependent ATP formation in the chloroplasts is thus termed as Photophosphorylation. Physiological investigations in the next decade reported the formulation of **Chemiosmotic Theory** by Peter Mitchell (1960s). Similar kind of mechanism is also associated with aerobic respiration and electron transport chain occurring in the mitochondria. The higher proton concentration in the thylakoid lumen occurs due to proton liberation from water oxidation and plastohydroquinone oxidation. This results in the formation of a proton motive force manifested as a transmembrane electrochemical gradient across the thylakoid membrane. According to the chemiosmotic theory the difference in ion concentration across the membrane is utilized in the form of free energy. ATPase dependent proton transfer thus couples to ATP formation. According to the Second Law of Thermodynamics, 'Any asymmetric or unequal distribution of energy results in driving a forward reaction'. The thylakoid membranes exhibit a difference in the chemical potential obtained due to concentration gradient of protons. The asymmetric nature of photosynthetic membrane in the chloroplast results in such electrochemical gradient. Higher accumulation of protons in the thylakoid lumen results in its acidic pH, while the stroma region remains alkaline due to fewer protons present there. Earlier investigations before 1960 were performed with isolated chloroplasts in buffer solutions of pH 4.0 and pH 8.0. These buffers equilibrated the pH across the membranes of chloroplast. However, difference in the pH across the thylakoid membrane was associated with ATP generation independent of light perception or electron transport.

According to Mitchell the **Proton Motive Force (*p*)** generated in the thylakoid membrane is the sum total of **Proton Chemical Potential** and **Electric Potential** across the membrane. During the operation of light reaction in the chloroplast the protons

accumulating in the lumen result in formation of electrochemical gradient mostly due to the difference in the pH. Physiologists have referred to the process of ATP synthesis where four protons are transported across the membrane coupled to synthesis of one ATP molecule. ATP synthase is mostly localized in the stroma lamellae and grana. The ATPase synthase is a 400 kDa Protein with two major components called CF_0-CF_1. The CF_0 particle is a hydrophobic membrane spanning domain which provides the channel for passage of protons. The CF_1 particle is composed of various polypeptides which comprise of catalytic domains associated with proton efflux and ATP synthesis. CF_0 region of the enzyme rotates to provide a motor like action associated with proton efflux and ATP synthesis. The polypeptide nature and catalytic sites are mostly similar for ATP synthase associated with chloroplast and mitochondria. However, certain structural differences may exist. The basic mechanism of proton mediated ATP production is similar in both the cases.

4.2.2 Calvin Cycle

The process of Carbon fixation in plants primarily occurs through Calvin cycle (C_3) which has been named after the workers Melvin Calvin, A.A. Benson, J. Bassham and their co-workers (1961). The experimental evidence of the Calvin cycle appeared after the successful implication of radioactive tracer technique in the dark reaction steps of chloroplasts from unicellular alga *Chlorella*. The form of Carbon used in the experiment was C^{14} isotope incorporated in CO_2. The reactions were separately terminated at different time span to isolate the various metabolites produced in the sequential steps of the cycle. Chromatographic separation of the products (reaction intermediates) detected by radioactive signal revealed the sequential steps associated with the cyclic regeneration of RuBP required for combining with CO_2. Thus Calvin cycle is associated with the process of Carbon fixation occurring with the use of ATP and $NADPH_2$ produced during the light reaction. The first stable product of Calvin cycle is a 3-C compound called 3-phosphoglyveraldehyde. The primary acceptor of CO_2 in this cycle is a 5–C compound called Ribulose 1, 5 BiPhosphate (RuBP). The various steps of Calvin cycle has been divided into three main phases.

Enzymatic Steps of Calvin Cycle

Following are the steps included in enzymatic steps of Calvin cycle:

- **Carboxylation Step**

This step involves CO_2 mediated carboxylation of the primary acceptor RuBP, which liberates the stable metabolic intermediate termed as 3-phosphoglycerate. Ribulose, 1, 5-BisPhosphate or RuBP is regenerated in the process of Calvin cycle and remains in the cells. This molecule combines with CO_2 to produce an unstable 6-C compound (2-Carboxy-3-Keto arabinitol 1, 5 Bisphosphate) which is hydrolysed into two molecules of 3-Phosphoglyceric Acid (3 PGA). The enzyme which catalyzes the reaction is Ribulose-1, 5 Bisphosphate Carboxylase Oxygenase (RuBisCO). This enzyme is abundantly present in the stroma region of chloroplast.

- **Reduction Step**

The formation of 3PGA is followed by its reduction to form 3-Phosphoglyceraldehyde (3PGALD). This reaction requires the involvement of assimilatory powers produced in the light reaction, i.e., ATP and $NADPH_2$. The process of 3PGALD reduction is catalyzed by the enzyme triose phosphate dehydrogenase. This process proceeds in two steps where

3PGA is phosphorylated into 1,3-biphospho glyceric acid which is further metabolized to 3-phosphoglyceraldehyde. The later step is catalyzed by the enzyme triose phosphate dehydrogenase.

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- **Regeneration of RuBP**

Formation of 3 PGALD is followed by the sequential steps of hexose sugar production and the cycle restarts with the formation of RuBP regeneration. The form of Carbon present in CO_2 is present in its oxidized form which gets reduced to combine with RuBP and forms 3-PGALD. Thus the process of Carbon fixation is also known as reductive pentose phosphate pathway. Here RuBP is carboxylated to form 3PGALD. In order to maintain the process of Calvin cycle 3 molecules of RuBP are regenerated at the ultimate step of Calvin cycle. This is important for preventing depletion in the levels of Carbon Fixation occurring during photosynthesis. The sequential steps involved in the process of RuBP regeneration is as follows:

- One molecule of 3-phosphoglyceraldehyde isomerizes to form DiHydroxyAcetone Phosphate (DHAP) catalyzed by the enzyme triose phosphate isomerase.
- DHAP formed in the earlier step now undergoes aldol condensation with one molecule of 3-PGALD to form fructose, 1,6-bisphosphate. This step is catalyzed by the enzyme aldolase.
- Fructose, 1,6-bisphosphate formed in the earlier step is involved as an important metabolite in various physiological processes. This 6-C compound is dephosphorylated by the enzyme phosphatase to form fructose-6 phosphate. A part of the hexose sugar (fructose-6 phosphate) formed is utilized for the synthesis of glucose, starch and sucrose. The compartmentalization of sucrose and starch biosynthesis occurs in the cytosol and chloroplast, respectively.
- A third molecule of 3-PGALD formed in the first step recombines with a 2C unit of fructose 6-phosphate (C_1 and C_2) catalyzed by the enzyme transketolase to form a sugar called erythrose 4 –phosphate. Similar reaction with the other Carbon units of fructose 6-phosphate results in the formation of xylulose phosphate.
- Erythrose 4-phosphate combines with dihydroxyacetone phosphate catalyzed by aldolase to form a seven-C compound sedoheptulose 1,7 -bisphosphate.
- Sedoheptulose 1, 7-bisphosphate is dephosphorylated to sedoheptulose 7- phosphate by the action of specific phosphatase.
- Sedoheptulose 7- phosphate combines with 3 PGALD, catalyzed by transketolase to form xylulose -5 phosphate and ribose- 5 phosphate.
- Two molecules of xylulose -5 phosphate are converted to ribulose -5 phosphate catalyzed by the enzyme ribulose-5-phosphate epimerase. Another molecule of ribulose-5-phosphate is synthesized from ribose-5-phosphate catalyzed by ribose-5-phosphate isomerise.
- At the final step of the cycle, RuBP, i.e., Ribulose 1, 5 Bisphosphate is produced by kinase mediated phosphorylation of ribulose-5-phosphate. This molecule is thus replenished in the cycle and acts as an acceptor of CO_2.

Carbon Balance in Calvin Cycle

The reaction proportion or chemical equivalent of Carbon atom in the entire Calvin cycle can be summarized briefly (Refer Figure 4.6). Six molecules of CO_2 are required to produce one

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molecule of hexose (Carbon fixation). In total six molecules of RuBP combine with six molecules of CO_2 to form a total of twelve molecules of 3PGALD. Five molecules of 3 PGALD convert into five molecules of DHAP. Three molecules of 3 PGALD condense with three of the five molecules of DHAP to produce three molecules of 6-C Fructose 1, 6-Bp. This molecule is later dephosphorylated to form F, 6P. One molecule of F, 6P is diverted towards hexose sugar, sucrose and starch formation. The other two molecules reenter the Calvin cycle. Two molecules of F, 6P combine with nonreactive 3 PGALD to form 2 molecules of Xu-5P and two molecules of E-4P. The two molecules of E-4P now combine with remaining 2 molecules of DHAP to yield 2 molecules Su-7P. Su-7P (2 molecules) now reacts with 3PGALD (2 molecules) to produce Xu-5P (2 molecules) and R-5P (2 molecules). At the final step there are 4 molecules of Xu-5P which produce 4 molecules of Ru-5P. The other two molecules of Ru-5P are produced from R-5P. Thus a total of 6 molecules of Ru-5P is produced at the penultimate step of Calvin cycle which gets phosphorylated to regenerate six molecules of RuBP.

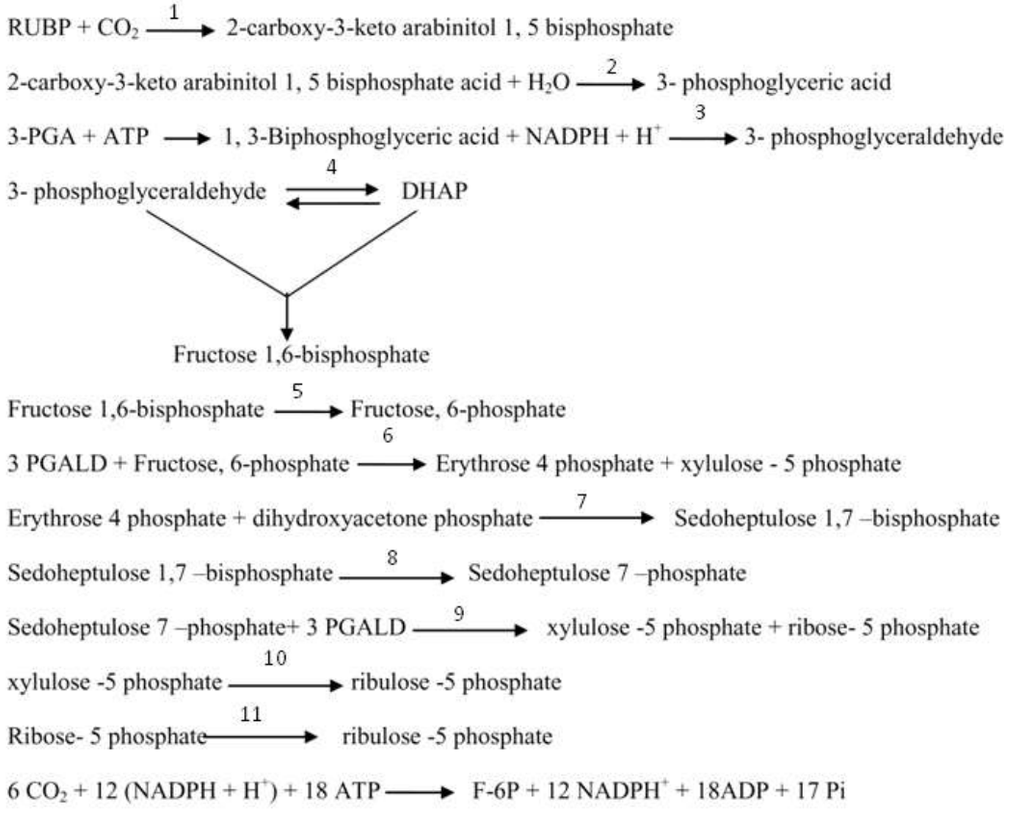

Fig 4.6 *Schematic Representation of Calvin Cycle (Enzymes)*

Experimental Evidences for Calvin Cycle

The experiments performed by Melvin Calvin and his group involved two green algae *Chlorella* and *Scenedesmus*. Each of the Algal suspensions was allowed to photosynthesise in constant temperature and illumination. The photochemical reaction was followed by the event of dark reaction. In this case exogenous CO_2 was supplied which contained C^{14} radioisotope incorporated within it. The separate suspensions were allowed to undergo steps of dark reaction and each of the set up was terminated in boiling methanol at variable time intervals. This led to formation of different metabolic intermediates in each of the set up. Methanolic medium was also used to extract the sugar intermediates from each of the

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setup which were separated by par chromatography and detected for radioactive signal. It was observed that the radioactive C^{14} present in CO_2 got incorporated in different sugar molecules produced in the metabolic pathway. Moreover the intensity of radiation was proportional to the time of exposure of radioactive CO_2. The smallest time exposure (5 sec) provided to the suspensions resulted in the formation of 3 PGA as the first stable compound in the pathway. Increase in the time interval to 30 sec led to detection of radioactive signal 3 PGALD, triose phosphates and hexose phosphates. Furthermore it was observed that pentose and heptose sugars were also detected with radioactive signal. Thus, experiments confirmed that the initial stable product of C_3 cycle was a 3-C intermediate called 3-PGA.

Interestingly a light dependent relation was observed for this metabolic pathway which exhibited higher accumulation of 3PGA and lower rates of RuBP regeneration in the absence of light. This was attributed to the fact that a drop in the light reaction in thylakoids resulted in lower production of the assimilatory powers, namely ATP and $NADPH_2$. These assimilatory powers are required in the dark reaction for the conversion of 3 PGA into 3 PGALD. Thus in the absence of these molecules 3 PGA was unable to be converted to 3 PGALD and subsequently the pathway could not proceed further leading to slower regeneration of RuBP. The conditions were reversed by further exposure to light conditions.

Regulation of Calvin Cycle

The initial steps of Calvin cycle produce three molecules of fructose-6-phosphate of which one molecule is diverted towards sugar and starch synthesis in the cytosol and chloroplast respectively. In total twelve molecules of triose phosphate are initially produced from RuBP and CO_2. Thus (6/36) one sixth of the triose phosphate is utilized in the process of sugar metabolism. The rest of the fructose molecules get re-utilized in the Calvin cycle. It has been observed that more than 80% of the energy required to carry out the reactions of Calvin cycle is obtained from the reductant $NADPH_2$. The levels of metabolites in various steps of the cycle regulate the catalytic activity of the regulatory enzymes.

This in turn modulates the rate of Calvin cycle in variable light and dark conditions. The expression of enzymatic Proteins associated with Carbon fixation is regulated at the post transcriptional levels and also through modulation of nuclear and chloroplastdial gene expression. Physiologists refer that the binding of Calvin cycle enzymes to the thylakoid membrane provide protection of substrate from getting metabolized into other pathways. Moreover the nature of reactions prevents backward or reverse reactions to occur in most of the cases. This prevents wastage of resources. Certain enzymes associated with the Calvin cycle (RuBisCO, NADP-glyceraldehyde-3-phosphate dehydrogenase, Fructose-1,6-bisphosphatase, Sedoheptulose-1,7-bisphosphatase and Ribulose-5-phosphate kinase) contain more than one disulphide groups (S-S) present in their structure. These sulphide groups remain in the oxidized state during dark conditions which is reduced to form sulphydryl derivatives in the presence of light.

Thus, presence of –SH group results in light-dependent activation of the enzymes during Calvin cycle. Light-dependent activation of RuBisCO is facilitated by pH and Mg^{2+} concentration in the stroma. This result in the formation of RuBisCO-CO_2 complex associated with Mg^{2+}. Various light-dependent enzymes including RuBisCO have been suggested to be regulated by Ferridoxin-Thioredoxin System. Another important enzyme called RuBisCO activase is involved in the diurnal regulation of active RuBisCO in the chloroplast.

4.2.3 Photorespiration in Plants

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A competitive relation exists between the carboxylation and oxygenation reaction of RuBisCO which acts upon the substrate ribulose-1, 5 bisphosphate. Interestingly, RuBisCO possess both carboxylation and oxygenation properties. The process of oxygenation of RuBP mediated by RuBisCO results in CO_2 loss from Calvin cycle. This results in a drop in the rate of Carbon fixation occurring during photosynthesis. Thus, the C_2 oxidative cycle which involves oxygenation of RuBP to form glycolate is referred to as the pathway of photorespiration. Earlier investigations revealed C_3 pathway as the only photosynthetic reaction to be operative among plant members.

Initial experiments by Krotkov in 1963 revealed that green leaves exposed to bright illumination thereafter exhibited higher respiratory CO_2 yield. This observation of post-illumination CO_2 burst was termed as photorespiration. The CO_2 yield increased due to higher light intensity was thus differentiated from normal respiratory CO_2 generation. Thus the process of photorespiration possesses certain differences with normal aerobic respiration in the fact that the former is a light dependent phenomenon occurring only in photosynthetic tissues. Photorespiration is mostly dependent upon the operation of Calvin cycle. Green plants which possess high CO_2 compensation point are susceptible to the process of photorespiration. C_4 plants (Maize, Sugar Cane) usually possess low Carbon compensation point and therefore possess insignificant chances of photorespiration. In the process of photorespiration it has been reported that both CO_2 and O_2 compete for binding with RuBisCO at its active site. Experiments have revealed that in the presence of equal concentrations of CO_2 and O_2, RuBisCO in angiosperms bind to CO_2 with a 80 fold higher efficiency. The Oxygen sensitivity of RuBisCO has also been observed from Anaerobic Autotrophic Bacteria.

Enzymatic Steps Involved in Photorespiration

The process of photorespiration is accomplished in three organelles namely Chloroplast, Peroxisome and Mitochondria. The formation of glycolate is a major step in photorespiration followed by the formation of Glycine and Serine (Figure 4.7).

- The initial step of photorespiration involves the formation of glycolate from the combination of ribulose bisphophate and Oxygen. In this reaction two metabolites are produced, i.e., PhosphoGlyceric Acid (PGA) and Phosphoglycolic Acid. PGA enters the Calvin cycle while phosphoglycolic acid is hydrolyzed and dephosphorylated to glycolate. This step is catalyzed by the enzyme phosphatase. This event occurs in the chloroplast and the form of RuBisCO acting in the presence of Oxygen is termed as RuBP oxygenase.
- The glycolate (C_2) formed in the chloroplast is transported to the peroxisomes where it is oxidized to glyoxylate catalyzed by the enzyme glycolic acid oxidase. In this step Hydrogen peroxide produced as a byproduct is further degraded by catalase.
- Followed by the formation of glyoxylate, a transamination reaction occurs by combining glyoxylate with L- glutamate which results in the formation of glycine and α-ketoglutarate.
- The glycine molecules are transported to the mitochondria where it combines with Oxygen to form serine, CO_2 and Ammonia. Thus, CO_2 liberated at this stage results due to post-illumination burst called photorespiration. This reaction is catalyzed by serine hydroxymethyl transferase.
- Serine produced in the earlier reaction is transported back to the peroxisomes where it undergoes transamination to form hydroxypyruvate catalyzed by serine-glyoxylate amino transferase.

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- Hydroxypyruvate present in the peroxisomes is further reduced to glyceric acid. This step is catalyzed by hydroxypyruvate reductase and co-factor $NADH_2$.
- The glycolate or C_2 cycle is completed with the migration of glyceric acid to chloroplast. The cycle terminates in the formation of 3-phosphoglyceric acid from glyceric acid which is catalyzed by the enzyme glycerate kinase. 3 PGA is an important intermediate in Calvin cycle which is replenished partly by C_2 cycle.

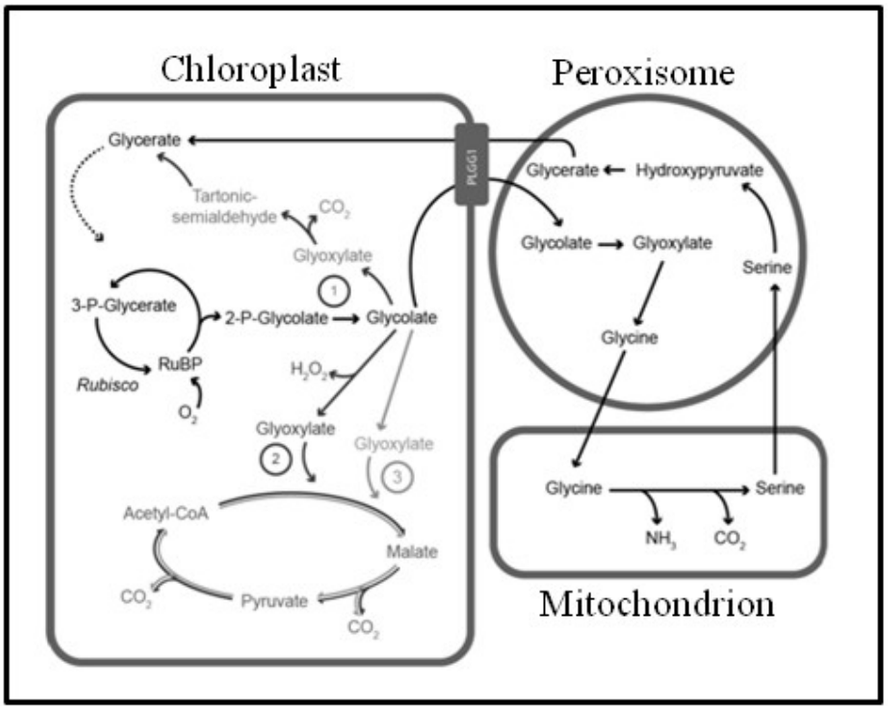

Fig. 4.7 *Pathway of Photorespiration*

Significance of Photorespiration

There lies questionable facts as to what benefit does the plant obtain from the process of photorespiration. The atmospheric condition where high light intensity is accompanied by high O_2/CO_2 ratio is ideal for photorespiration. This pathway thus promotes the oxygenation ability of RuBisCO. According to Hatch and Slack (1970) photorespiration has been termed as a metabolic adjunct to the Calvin cycle. This means that this metabolic pathway coupled top Calvin cycle is unavoidable and reduces the Carbon fixing efficiency of plants to lower than 60%. Moreover increasing temperature results in lower amount of CO_2 present in the air in comparison with O_2. This condition triggers the oxygenation ability of RuBisCO. It has been proposed by physiologists that under high temperature and light intensity the stomata remain closed. During such conditions the process of photorespiration consumes the molecules of ATP and $NADH_2$ which may otherwise damage the photosynthetic apparatus. However, during the normal operation of Calvin cycle photorespiration decreases the ATP and $NADH_2$ count in the chloroplasts. Inhibitors of the metabolic steps of photorespiration like α hydroxysulphonate are known to inhibit the activity of glycolic acid oxidase. Further transgenic approach and by modulating environmental conditions it is possible to check the Carbon loss due to photorespiration. This may prove beneficial in terms of increasing agricultural productivity in major crops.

4.2.4 C_4 Cycle

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Certain group of plants possesses adaptive features to avoid the consequences of photorespiration and subsequent Carbon loss during photosynthesis. Preliminary investigations in sugarcane leaves performed by Kortschak, Hartt and Burr (1965) reported 4-C containing organic acids namely malate and aspartate to possess radioactive signals. Later experiments by Hatch and Slack confirmed the presence of C^{14} radioactivity to be detected in sugars like Oxaloacetate, Malate and Aspartate. This was obtained as a result of exposure of radioactive CO_2 for few seconds in sugar cane leaves. Radioactive Carbon Dioxide exposure for longer durations also resulted in the appearance of radioactive signal in hexose mono-phosphates and sucrose. Thus the C_4 pathway was revealed to produce C-4 compounds as the earliest stable products namely oxaloacetate and malate (Refer Figure 4.8).

The property of C_4 pathway is more pronounced in plant members of Poaceae family. However, different other Angiospermic families have also been reported to possess similar features associated with photosynthetic pathway. The major adaptation of C_4 plants are essentially

- Complete absence or negligible rate of photorespiration.
- Tolerance to higher temperature in arid tropical regions.
- Specialized bundle sheath cells in leaves (Krantz anatomy).

These adaptive features allow these plants like sugarcane, maize, etc. to survive in temperature adversities.

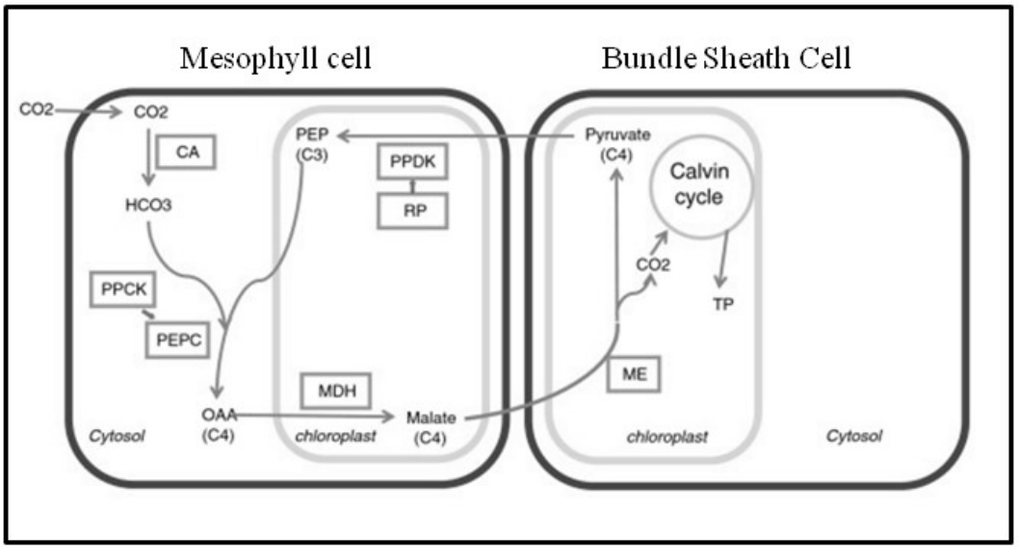

Fig. 4.8 *C_4 Pathway*

Krantz Anatomy in C_4 Leaves

The C_4 plants possess specialized features of enlarged parenchymatic bundle sheath cells which are larger than the adjoining mesophyll cells of the leaves. The large sized bundle sheath cells appear to be radial in arrangement surrounding the vascular bundles. This provides them a ring or wreath like appearance. Thus, Krantz in German means wreath and hence the name Krantz anatomy. The bundle sheath cells are connected to adjoining mesophyll cells by symplastic connection of plasmodesmata. Interestingly the mesophyll and bundle sheath cells of C_4 leaves represent dimorphic chloroplasts. The chloroplasts in the mesophyll cells contain grana while those in bundle sheath cells lack the presence of grana. Thus the

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later does not contain Oxygen levels arising from the light reactions. Thus the presence of RuBisCO and CO_2 in the C_4 leaves has been associated with minimum Oxygen exposure. This phenomenon prevents the occurrence of photorespiration. The organic acids produced in the mesophyll cells are carried to the bundle sheath cells where they appear as a major source of CO_2 to carry out Calvin cycle. This compartmentalization of RuBisCO and Oxygen prevents the Carbon loss during Calvin cycle.

Mechanism of C_4 Cycle

The sequential steps of the C_4 cycle are associated with two consecutive carboxylation reactions occurring in the chloroplasts of mesophyll and bundle sheath cells:

- PhosphoEnol Pyruvic (PEP) Acid present in the chloroplasts of mesophyll cells is carboxylated in the presence of CO_2 to form oxaloacetate. This reaction is catalyzed by the enzyme phosphoenol pyruvate decarboxylase. The process of PEP carboxylation occurs by the HCO_3^- formed by dissolution of CO_2 in cytoplasm.
- The oxaloacetate formed in the chloroplasts is converted to malate catalyzed by the action of $NADP^+$ specific malate dehydrogenase. Transamination reaction may also result in the formation of aspartate acted by the enzyme transaminase.
- Malic acid produced in the chloroplast of mesophyll cells is transferred to the chloroplast of bundle sheath cells where it is decarboxylated to produce CO_2 and pyruvic acid. The reaction is catalyzed by $NADP^+$ specific malic enzyme. The malate molecules thus form a CO_2 source required for operation of Calvin cycle within the chloroplast of bundle sheath.
- The pyruvic acid produced in the earlier step is transferred to the chloroplasts of mesophyll cells where it undergoes phosphorylation to regenerate phosphenol pyruvate. This step is being catalyzed by the enzyme pyruvate Pi kinase.
- The CO_2 liberated in the bundle sheath chloroplast combines with RUBP and enters in the Calvin cycle
- The pathway of C_4 cycle has been reported to be less efficient than the C_3 cycle. This I because the fixation of one CO_2 molecule through C_4 cycle requires 2NADPH + 5ATP molecules which is lesser (3ATP) for C_3 cycle. Alternatively, the ATP loss by photorespiration lowers the Carbon fixing efficiency in C_3 plants.

Variations in the Mechanism of C_4 Cycle

C_4 plants exhibit considerable variation in the type of enzyme and organic acid formed in the chloroplast of mesophyll cells which migrates to the bundle sheath cell chloroplast.

- **$NADP^+$ ME Type:** This type of pathway employs $NADP^+$ specific malic enzyme for the process of decarboxylation. In this case the OAA molecules are metabolized to malate which is transferred to chloroplast of bundle sheath cells, for example, *Zea mays*, *Saacharum officnarum* and *Sorghum sudanense* (Refer Figure 4.9).
- **NAD^+ ME Type:** In this kind of pathway the malic enzyme active requires the presence of NAD^+. Moreover, in this type of pathway OAA formed in the chloroplast of mesophyll cells get transaminated to aspartate. Aspartate gets transferred to the mitochondrian of bundle sheath where it reforms OAA and malate, for example, *Amaranthus edulis, Atriplex spongiosa* and *Portulaca oleracea*.
- **PCK Type:** Certain C_4 plants employ the use of enzyme PEP-carboxykinase to produce phospheonol pyruvate from OAA within the cytoplasm of bundle sheath cells. CO_2 liberated in this process is utilized in Calvin cycle. In this pathway the

process of malate formation is substituted by direct decarboxylation of OAA into PEP. Moreover in this pathway the metabolic process in bundle sheath cells occurs in cytoplasm and chloroplast instead of mitochondria, for example, *Panicum maximum and Chloris gayana*.

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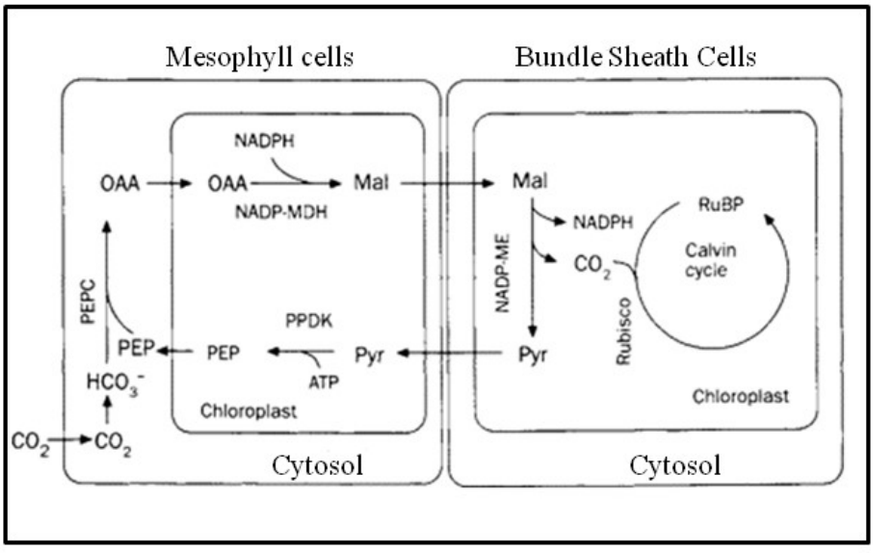

Fig. 4.9 *NADP-ME Type of C_4 Pathway*

Significance of C_4 Pathway

It has been observed that the nature of C_4 pathway coincides with the position of chloroplasts in the bundle sheath cells of leaves. The leaves of plants exhibiting NAD^+ ME type of pathway possess agranal chloroplast which are centrifugal in nature and situated towards the outer part of Krantz cells. PCK type of pathway has been observed to be associated with chloroplast which possess grana and are centrifugal in position. However, in NAD^+ ME type of pathway the chloroplast although contain grana are centripetal in position, i.e., they are present towards the inner sides of the Krantz cells.

Thus the C_4 pathway appears to be a modification of Calvin cycle associated with CO_2 concentrating mechanisms which appear to be advantageous to tropical plants. The evolutionary origin of C_4 plants have been related with the decrease in the atmospheric CO_2 concentration across several ages. Furthermore, absence of photorespiration maintains the Carbon fixation efficiency of C_4 plants in comparison with C_3 plants.

Crassulacean Acid Metabolism (CAM)

Certain plants exhibit diurnal regulation in the organic acid metabolism in their aerial organs associated with the regulation of stomatal movement. Such mechanism was reported in *Bryophyllum* sp. (Crassulaceae) and hence termed as Crsuulacean Acid Metabolism (CAM). Various other plant members like *Kalanchoe*, *Sedum, Crassula* and *Opuntia* have also been reported to possess similar kind of metabolism. These plants have unique adaptations for water use efficiency by which they can thrive in dry arid climates. Most of them are succulent in nature often with reduced leaves and photosynthetic stem. CAM pathway occurs in the photosynthetic organs of these plants. Stomatal movement in these plants coincides with the regulation of organic acid metabolism. In CAM plants stomata remains open during the night when organic acid content increases. However, in the day time the malate is decarboxylated to liberate CO_2 necessary for operating Calvin cycle. This results

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in the decrease in osmolytes which results in stomatal closing. Furthermore, stomatal closure during the day time leads to decreased water loss due to transpiration. There are various biological and external factors which affect the diurnal fluctuation in acid metabolism. The growth rate and age of foliar tissue affects acid formation during the night phase. The duration of the day and night periods also affect the intensity of acid formation (Refer Figure 4.10).

Steps in CAM Pathway

- The source of carbohydrates, i.e., starch is hydrolyzed to glucose which metabolizes to form malic acid. Moreover, pyruvate or phospheonol pyruvate is also converted to oxaloacetate and malate. These reactions are catalyzed by the enzymes PEP carboxykinase, PEP carboxylase and malic enzyme. This event preferably occurs during the night time when Malic Acid is stored in the vacuole.
- Light exposure during the day time results in decarboxylation of malate into pyruvate and CO_2 catalyzed by malic enzyme. In certain CAM plants malate dehydrogenase may catalyze formation of oxaloacetate from malate. OAA in turn is converted into PEP and CO_2 catalyzed by PEP caboxykinase.
- Pyruvate and phospheonol pyruvate produced in the cells is utilized in the Calvin cycle.

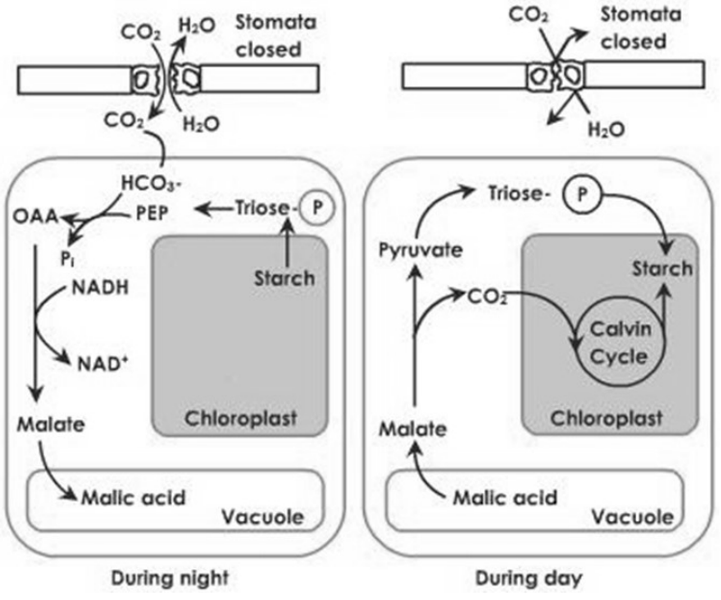

Fig. 4.10 *CAM Pathway*

Warburg Effect

The phenomenon of inhibition of photosynthesis induced by high levels of Oxygen prevalent in saturating light conditions is termed as Warburg's effect. This effect was observed in green algae *Chlorella* and reported by Warburg in 1920. Later similar observations were confirmed in wheat. The basic mechanism of Warburg effect associates with photorespiration mediated inhibition of Carbon fixation. During high Oxygen levels generated from photosynthesis followed by saturating light intensity RuBisCO mediated glycolate metabolism may appear to be more pronounced than C_3 cycle. Plants which do not exhibit photorespiration are also been reported to be insensitive for Warburg effect.

Carbon-Dioxide Compensation Point

The amount of CO_2 present in the atmosphere at which the rate of photosynthesis becomes equal or compensates to the rate of respiration is termed as Carbon Dioxide compensation point. The CO_2 evolved in respiration is utilized in photosynthesis while the O_2 obtained from photosynthesis is alternately used in respiration. C_4 plants which utilize optimum CO_2 due to negligible amount of photorespiration exhibit low Carbon Dioxide compensation point in the range of 2-5 ppm. However, C_3 plants exhibit photo respiratory pathway and subsequent high Carbon dioxide compensation point of around 50 ppm. This increases even more at higher temperatures due to high rate of photorespiration.

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'Check Your Progress'

1. What is FRET?
2. Define reaction centre.
3. What is fluorescence?
4. To what the molecular structure of photosynthetic pigment and Protein complex associated with?
5. What happens in carboxylation step?

4.3 SUMMARY

- The most abundant Protein on Earth (RuBisCO) is interestingly involved in the Carbon fixation process in green autotrophic organisms like Algae, Bryophyte, Pteridophyte Gymnosperms and Angiosperms.
- Chloroplasts are semi-autonomous organelles within photosynthetic cells and are capable of transient movement in response to light.
- Light energy necessary for photosynthesis is available in form of photons designated to form Quanta (pockets of energy).
- The energy harnessing and transfer process of photosynthesis is accomplished by Fluorescence Resonance Energy Transfer (FRET) method.
- The chlorophyll molecules are the primary pigments associated with photosynthesis. They form cluster of molecules known as Quantasomes.
- Emerson and Arnold (1932) have revealed that a cluster of chlorophyll molecule (2500) to be responsible for producing a single molecule of Oxygen.
- Experiments by Emerson have led to conclusions of the complementary effects of Red and Far Red wavelength in enhancing the rate of Oxygen evolution in light reaction (Quantum yield).
- The internal structure of chloroplast mostly contains the network of thylakoid stacked in the form of granum.
- The thylakoid membrane remains interconnected to form internal cisternae and has lipid composition similar to the inner membrane of envelope.
- The stroma is a region formed of hydrophilic matrix containing proteinaceous molecules.
- The stroma exhibits dispersed distribution of lipid granules some of which contain plastoquinone in the vicinity of the membrane.

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- In the early stage of development of chloroplast the thylakoids are formed by invagination from the inner membrane.
- Algal chloroplasts in Cyanobacteria and Red Algae possess Phycobilisomes associated with the thylakoids.
- Various types of plastids like Etioplast, Amyloplast, Chromoplast, Leucoplast, Elaioplast and Chloroplast are usually interconvertible or undergo reversible changes among each other.
- The process of differentiation of chloroplasts from the proplastids is mostly regulated by the nuclear genome.
- The mature chloroplasts appear as sites for phytohormone synthesis, starch metabolism and photosynthesis.
- The reaction center, antennae–Protein Complex and Electron Carrier Proteins are essentially localized in the thylakoid membrane.
- Plant chloroplasts may possess higher amount of PS-II molecules in comparison with PS-I.
- PS-I is mostly composed of chlorophyll a associated with Chl B and β carotene molecules attached to Proteins.
- PS-II is mainly composed of Chl A and β carotene. The core region of the reaction centre contains two Proteins known as D_1 and D_2.
- The primary electron acceptor of PS-II is termed as Phaeophytin which is a derivative of Chl A molecule without Mg^{2+}.
- The Light Harvesting Complexes (LHC) associated with PS-I and PS-II are usually comprised of Chl A, Chl B, Xanthophylls and Cartenoid molecules.
- The F_1- ATPase is localized at the thylakoid membrane which energises the transfer of proton across the membrane.
- The ATPase is a transmembrane Protein comprised by CF_1-F_0 particles.
- The functional units called photosystems convert light energy into chemical energy.
- The electron transfer process of PS-II is independent of PSI, although both the photosystems are connected through non-cyclic electron transfer and photo-phosphorylation.
- The energy stored within the photon molecules is termed as Quantum.
- The photons which appear with wavelength in the visible range of spectrum (400 nm-700 nm) are optimum for inducing the photochemical reaction in chloroplasts.
- Fluorescence is one the phenomenon exhibited by the chlorophyll molecules where the emitted energy is visualized in the form of fluorescent light.
- Bacteriochlorophyll in comparison to plant chlorophyll exhibits certain differences in their chemical composition.
- The optimum rate of photosynthesis is measurable by the parameter of Quantum yield which depicts the number of Oxygen molecules yielded by the action of one photon molecule.
- Cyclic electron flow is accompanied by ATP production and termed as cyclic photophosphorylation. The process of non-cyclic electron flow from PS-I to PS-II is accompanies non Cyclic photophosphorylation.

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- The Mangano-Protein Complex present in the PS-II centre facilitates the process of water oxidation.
- The biosynthesis of chlorophylls in the cells initiate from two precursors AminoLevulinic Acid (ALA) and PorphoBilinoGen (PBG).
- The absorption maxima of Chl A are 430 nm and 662 nm, respectively. Chl b exhibits absorption maxima at 430 nm and 620 nm, respectively
- The carotenoids are accessory pigments involved in photosynthesis.
- Quantasome explains the association of 300 molecules of chlorophyll in each of the photosystems which function as Photosynthetic Units. Each Quantasome is capable of absorbing one Quantum of light energy.
- The number of Quanta required to liberate one molecule of Oxygen is termed as Quantum requirement
- The phenomenon of combined effect of Red and Far Red wavelengths in producing optimum magnitude of Quantum yield was termed as Emerson enhancement effect.
- The excited forms of chlorophyll molecule remain in the singlet or triplet stage for a transient time period.
- The process of energy transfer from the antennae pigments to the reaction centre occurs by the phenomenon of Fluorescence Resonance Energy Transfer (FRET).
- The connecting molecules which transfer electrons between PS-I and PS-II are mainly copper containing Protein plastocyanin and a quinone group of carrier termed as Plastoquinone.
- The oxidation-reduction cycle occurring by the help of these four stages of Mn^{+} was reported by Kok *et al*, (1970) and is commonly mentioned as the Water Oxidation Clock.
- The reaction centre possesses the Mangano-Protein Complex which together with Ca^{2+} and Cl^{-} forms the catalytic Oxygen Evolving Complex (OEC).
- Pheophytin is the immediate electron acceptor of PS-II centre.
- The PQ form of plastoquinone is reduced to Plastohydroquinone (PQH_2) by obtaining two electrons from phaeophytin and also two protons from the stromal side of thylakoid membrane.
- The non-polar nature of the quinone Protein results in its diffusible nature across the membranes.
- The linear electron transfer chain accounts for the electron which is received by FeS_R (Rieske centre) and forwarded to the cytochrome f complex.
- A commonly implied inhibitor of electron transport from quinone to other carriers is Di-Chlorophenyl-diMethyl-Urea (DCMU).
- Paraquat is a well known inhibitor which blocks the electron transfer from PS-I to $NADP^{+}$.
- Physiological investigations in the 1960s reported the formulation of Chemiosmotic Theory by Peter Mitchell.
- According to the chemiosmotic theory the difference in proton concentration across the membrane is utilized in the form of free energy.
- It has been observed that a majority of plants (> 80%) exhibit C_3 Pathway of Carbon fixation associated with photosynthesis.

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- Ribulose-1, 5- Bis-phosphate Carboxylase Oxygenase (RuBisCO) has been observed to be one of the most abundant Proteins on Earth.
- The Calvin cycle implies the molecules of ATP and $NADPH_2$ to accomplish the process of reductive Carbon fixation.
- The form of Carbon present in CO_2 is the most oxidized form which gets incorporated in PGALD as a reduced form.
- Photorespiration is a metabolic adjunct to C_3 pathway in plants because it is unavoidable during high O_2 CO_2 ratios in the atmosphere.
- Phospheonol pyruvate is one of the important intermediates of the C_4 pathway in plants which is metabolized to form oxaloacetate.
- The leaves of C_4 plants contain dimorphic chloroplasts localized in the mesophyll and bundle sheath cells.
- Malate and aspartate are the major carboxylation products of C_4 cycle.
- A competitive relation exists between the carboxylation and oxygenation reaction of RuBisCO which acts upon the substrate Ribulose-1, 5 BisPhosphate (RuBP). Interestingly, RuBisCO possess both carboxylation and oxygenation properties.
- Inhibitors of the metabolic steps of photorespiration like α hydroxysulphonate are known to inhibit the activity of glycolic acid oxidase.
- The evolutionary origin of C_4 plants have been related with the decrease in the atmospheric CO_2 concentration across several ages. Furthermore, absence of photorespiration maintains the Carbon fixation efficiency of C_4 plants in comparison with C_3 plants.

4.4 KEY TERMS

- **Quantasomes**: Cluster of chlorophyll molecules which form energy pockets and associated with Quantum yield.
- **Granum:** The internal structure of chloroplast mostly contains the network of thylakoid stacked in the form of granum**.**
- **Photophosphorylation**: The process of ATP production in photochemical process.
- **Plastome:** The chloroplastidial genome comprised of 2-100 single stranded DNAl molecules.
- **Etioplast**: Colourless or yellowish plastids formed due to skotopmorphogenesis in dark grown plants.
- **Antennae pigments:** Clusters of accessory pigment molecules which belong to the component of LHC II associated with intensification of light energy received by chloroplasts.

4.5 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Fluorescence Resonance Energy Transfer (FRET) is the process of transducing energy by transmitting excited electron across Protein carriers during photosynthesis. It involves transfer of energy to adjacent molecules by the phenomenon of resonance transfer.

2. Reaction centre is the energy receiving sites of photosystem I and II comprised of Chl A molecules which receive light wavelengths of 680 nm and 700 nm, respectively.
3. Fluorescence is one the phenomenon exhibited by the chlorophyll molecules where the emitted energy is visualized in the form of fluorescent light.
4. The molecular structure of photosynthetic pigment and Protein complex associated in the thylakoid provides important insights to the distribution of electron transfer carriers.
5. The Carboxylation step involves CO_2 mediated carboxylation of the primary acceptor RuBP, which liberates the stable metabolic intermediate termed as 3-phosphoglycerate. Ribulose, 1, 5-Bisphosphate is regenerated in the process of Calvin cycle and remains in the cells. This molecule combines with CO_2 to produce an unstable 6-C compound (2-Carboxy-3-Keto Arabinitol 1, 5 Bisphosphate) which is hydrolysed into two molecules of 3-Phospho-glyceric Acid (3 PGA). The enzyme which catalyzes the reaction is Ribulose-1, 5 Bisphosphate Carboxylase Oxygenase. This enzyme is abundantly present in the stroma region of chloroplast.

4.6 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Brief a note on resonance.
2. Describe the features of chloroplast membrane.
3. Explain the ultrastrurture of chloroplasts in plants.
4. What are proplastids and etioplasts? Describe the biogenesis of chloroplast.
5. Mention the functions of accessory pigments in photosynthesis.
6. Define photophosphorylation.
7. What do you mean by crassulacean acid metabolism? Mention its significance.
8. Differentiate between the C_3 and C_4 pathway in plants.

Long-Answer Questions

1. Define resonance. What do you mean by fluorescence resonance energy transfer? Mention its implication in photosynthesis.
2. What do you mean by light harvesting complex? Explain its significance in photosynthesis.
3. Describe the molecular structure of PS-I and PS-II in the thylakoid membrane.
4. Explain the structure of F_1 ATPase present in thylakoid membrane.
5. Describe the pathway of Calvin cycle also provide related diagram.
6. Differentiate between photophosphorylation and oxidative phosphorylation.
7. Give a detailed account on the mechanisms of Hill reaction.
8. Explain schematically the process of light reaction.
9. Describe the chemiosmotic theory in the light of chloro-plastidial ATP synthesis.

4.7 FURTHER READING

NOTES

Hopkins, William G. and Norman P.A. Huner. 2013. *Introduction to Plant Physiology*, 4th Edition. Noida: Wiley India Pvt. Ltd.

Verma, S K and Mohit Verma. 1995. *A Textbook of Plant Physiology, Biochemistry and Biotechnology*. New Delhi: S Chand & Company Ltd.

Blackstock, James C. 2014. *Guide to Biochemistry*. Oxford: Butterworth-Heinemann.

Park, K. H. 2008. *Carbohydrate-Active Enzymes: Structure, Function and Applications*. Amsterdam: Elsevier.

Fromm, Herbert J. and Mark Hargrove. 2012. *Essentials of Biochemistry*. Berlin: Springer.

Fearon, William Robert. 2014. *An Introduction to Biochemistry*. Amsterdam: Elsevier.

UNIT 5 FAT METABOLISM AND PROTEIN SYNTHESIS

NOTES

Structure

5.0 INTRODUCTION

Plant tissues store lipids as the main source of Carbon in the cells. Fats and oils serve as the major stored form of reduced Carbon in plants. Oil seeds like soybean, groundnut, canola, sunflower and linseed are the major source of stored lipids. Biochemical connection between the lipid and organic acid pathway provide cues for the source of energy in germinating seedlings incapable of carrying out photosynthesis. Major plant organs associated with lipid storage are seeds and fruits like Palm Oil, Olive and Avocados. The present unit will deal with the aspects of lipid biosynthesis and their degradation through α and β-oxidation pathways. The various types of phospholipids present in the plant cell membrane determine their biophysical properties. The membrane lipids and their derivatives serve as signaling intermediates in various pathways.

The process of biosynthesis of fatty acids in plants is primarily accomplished in the plastids. The main precursor Acetyl-CoA is involved in the cyclic condensation of two Carbon atoms. The main enzyme involved in the process is fatty acid synthase. This enzyme complex catalyzes the sequential steps necessary for fatty acid biosynthesis. The growing acyl molecules are covalently attached to Acyl-Carrier Protein (ACP) to form a complex called Acyl-ACP.

In this unit, you will study about fat metabolism, structural features of fats, classification of lipids, phospholipids, Protein synthesis in Prokaryotes and Eukaryotes, structure and biogenesis of ribosomes.

5.1 UNIT OBJECTIVES

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

- Understand what fat metabolism is
- Discuss about the structural features of fats
- Explain the classification of lipids
- Discuss about phospholipids

- Explain how Protein synthesis occurs in Prokaryotes and Eukaryotes
- Analyse the structure and biogenesis of ribosomes

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5.2 FAT METABOLISM

Plant tissues store lipids as the main source of Carbon in the cells. Fats and oils serve as the major stored form of reduced Carbon in plants. Oil seeds like soybean, groundnut, canola, sunflower and linseed are the major source of stored lipids. Biochemical connection between the lipid and organic acid pathway provide cues for the source of energy in germinating seedlings incapable of carrying out photosynthesis. Major plant organs associated with lipid storage are seeds and fruits like Palm Oil, Olive and Avocados. The present unit will deal with the aspects of lipid biosynthesis and their degradation through α and β-oxidation pathways. The various types of phospholipids present in the plant cell membrane determine their biophysical properties. The membrane lipids and their derivatives serve as signaling intermediates in various pathways. The biosynthesis of triacylglycerol and polar glycerolipids are important pathways regulating the process of lipid metabolism in plants. The chloroplasts and Endoplasmic Reticulum are the main organelles associated with lipid biosynthesis. Fats and oils are structurally diverse in nature and soluble in organic solvents. These biomolecules are insoluble in water. Since lipids represent a more reduced form of Carbon, the complete oxidation of 1 gm lipid molecule liberates 40 kJ or 9.3 kilo Cal of energy. This process produces larger number of ATP molecules in comparison with the oxidation of 1 gm starch which liberates around 3.8 kilo Cal energy. Thus, lipids represent a much better source of stored energy in plants. Similarly, the biosynthesis of membrane lipids, mainly phospholipids, requires a higher amount of metabolic energy. Structural lipids like wax compounds are involved in maintaining the structural integrity of the plant tissues. Certain groups of lipids function as metabolic precursors for terpenoid and carotenoid pathways. Plant fats and oils mostly exist in the form of triacylglycerols or triglycerides. In this case the fatty acid molecules are linked with ester bonds to the three –OH groups of glycerol molecules. The plant-derived fatty acid molecules are usually straight chain even numbered Carbon molecules varying from 12-20 in number. The oils are liquid at room temperature and vary in their Oxygen reactivity. The oils are unsaturated lipids with kinks and twists in their structure formed due to a larger number of double bonds. This results in their lower melting point and liquid form at room temperature. The fats are saturated lipids which remain solid at the room temperature. Most plant-derived fats are unsaturated in nature (Oleic Acid, Linoleic Acid and Linoleneic Acid).

However, certain amount of saturated fats (Stearic Acid, Palmitic Acid) may also be present in the plant tissues. Lipid storing organelles or oleosomes are the major sites of storage of triacylglycerol. This organelle is a single membrane bound structure which separates the triglycerides from the aqueous environment of the cytoplasm. The seeds are the major organs which exhibit the presence of olesomes in the cytoplasm of cotyledons or in endosperms. The membrane of oil body are comprised of phospholipids and various hydrophobic Proteins namely Oleosins, Steroleosins and Caleosins. The oleosins help in stabilizing the membrane of oil bodies. The phospholipid membrane contains the hydrophobic acyl group inserted into the inner side while the polar hydrophilic group remains aligned towards the outer side. The phospholipid membrane of two adjacent oil bodies is capable of fusing to each other. The biosynthesis of triacylglycerol occurs in the membrane of Endoplasmic Reticulum. The process of oil body biogenesis occurs after fat assimilation which buds off from the ER membrane. The alignment of the lipid molecules in aggregation provides amphiphatic nature to the biological membranes. The hydrophobic ends of fatty

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acid molecules are formed of Acyl groups which remain aligned towards the inner side of the membrane. The polar side chains remain arranged towards the aqueous side of the cytosol. Polar glycerolipids form the major constituent part of the membranes. The polar glycerolipids are formed of C-16 or C-18 molecules which are formed of esterification to the –OH group of glycerol at the first or second molecule of glycerol. According to the structure the polar glycerolipids are usually of two types, i.e., Glyceroglycolipids and Glycerophospholipids. In glycolipids the head group is constituted of sugar molecules while in the case of phospholipids the head group is formed of phosphate group. The plant membranes also contain sphingolipids and sterols as minor constituents. Various other lipidic molecules are present in the plants tissues which are involved in photosynthesis, photoprotection and antioxidative functions. These are usually chlorophyll, carotenoids and tocopherols present abundantly in the leaf tissue. In photosynthetic cells the chloroplast membranes are usually formed of glyceroglycolipids while other membranes of non-photosynthetic type are mainly composed of glycerophospholipids.

The process of biosynthesis of fatty acids in plants is primarily accomplished in the plastids. The main precursor Acetyl-CoA is involved in the cyclic condensation of two Carbon atoms. The main enzyme involved in the process is fatty acid synthase. This enzyme complex catalyzes the sequential steps necessary for fatty acid biosynthesis. The growing acyl molecules are covalently attached to Acyl-Carrier Protein (ACP) to form a complex called Acyl-ACP. The main regulatory enzyme participating in the initial step of fatty acid biosynthesis is Acetyl-CoA carboxylase which catalyzes the reaction of Acetyl-CoA with CO_2 to form malonyl-CoA. This molecule now reacts with ACP to form malonyl-ACP. The fatty acids synthesized in the chloroplast are further utilized to synthesize glycerolipids in the Endoplasmic Reticulum (ER). These glycerolipids produced are integrated in the membranes. This process involves transfer of fatty acids from ACP-Acyl complex to Glycerol-3-Phosphate to form Glycerophospholipds (Phosphatidic Acid). The formation of Diacyl-Glycerol (DAG) from phosphatidic acid is catalyzed by the activity of a specific phosphatase. The formation of phosphatidic acid is carried out by two separate pathways in the chloroplast and ER. These are referred to as the Prokaryotic and Eukaryotic pathways, respectively. Jasmonates and Phosphatidyl-Inositol Triphosphates (IP_3) are two of the major signaling lipids formed from fatty acids. These molecules are involved in signaling cascade between the membrane and cytoplasmic components. The conversion of lipids into soluble sugars in the germinating seeds is essential to provide respiratory substrate. The process of breakdown of fatty acids into Acetyl-CoA occurs in specialized organelles called glyoxysomes. The formation of Acetyl-CoA is followed by formation of succinate and malate in the Mitochondria and Cytosol.

Structural Features of Fats

The **fats** or **glycerides** are esters of long chain fatty acids bound to the trihydroxyalcohol called glycerol. Most glycerides in nature exist in the form of esterified molecules of fatty acids along with the 3–OH groups of glycerol attached with it. Tripalmitin, for example is a glyceride ester of glycerol and palmitic acid. Plant-based fatty acids usually possess even number of C atoms. Mono and diglycerides usually do not exist as structural fats, but may function as stable metabolic intermediates. Lipids are referred to as a group of organic compounds which are soluble in organic solvent namely chloroform, benzene and hexane. Fats, waxes, phospholipids, glycolipids and sterols fall in the class of major lipids. The fatty acids existing in plants appear as saturated or unsaturated fatty acids. The numbering of C atoms in fatty acids starts from the C atom bearing the–COOH group. In this respect the, C atom adjacent to the –COOH group is referred to as the α-Carbon atom. The next is called-β-Carbon. The number of C atoms and its total number of double bonds are referred

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by numbers. As an example, 18:2 indicates an unsaturated fatty acid with 18 C atoms and 2 double bonds. The unsaturated fatty acids possess a number of double bonds which provide kwinks or folds in its chain conformation. Thus the unsaturated fats (oils) by the virtue of its structural folds exhibit lower melting point and are mostly liquid at room temperature. The saturated fats do not possess double bonds. In addition to triglycerides plant based fats and oils also possess other types of lipids. Fats and waxes are known as neutral lipids. These molecules are readily soluble in common organic solvents.

Classification of Lipids

According to Bloor (1943) the lipid molecules are classified into the following types based on their chemical composition.

Simple Lipids: These molecules represent as esters of fatty acids with various alcohols.

- **Fats and Oils**: Trigylcerides, i.e., esters of fatty acid with trihydroxy alcohol or glycerol. These molecules appear to be the most abundant type among all lipids. It is the main storage form of fat in plants which remains stored as a source of energy.
- **Waxes**: Esters of fatty acids with high molecular weight monohydroxy alcohols. They vary on Carbon number from C14 to C36. They are inert saturated molecules present as outer covering of epidermis and exodermis of plant organs. Their purpose is to protect plants from desiccation and pathogen attack.

Compound Lipids or Hetero Lipids: These are esters of fatty acids with alcohol but also possess additional groups, as follows:

- **Phospholipids (Phosphatids):** These lipid molecules contain phosphate group in the form of phosphoric acid, nitrogen and other components.
- **Glycolipids:** They contain carbohydrate groups attached to the esterified forms. They do not possess phosphoric acid group. They contain complex sugar residues. Glycolipids form an integrative part of the cell membrane. The external surface of membranes often possesses glycolipids as major signaling molecules.

Derived Lipids: These molecules are derived from hydrolysis of simple and compound Lipids. These include Fatty Acids, Alcohols, Mono and Diglycerides, Sterols and Terpenes.

5.2.1 Phospholipids

The formation of esterified glycerol-lipids may often involve the presence of phosphate groups as a polar head in their structure. These molecules are referred to as phospholipids. The phospholipids are usually amphiphatic molecules which contain polar phosphate groups (hydrophilic) and acyl chain (hydrophobic) aligned accordingly in the membranes. The phospholipids are the major constituent of biological membranes and they also remain associated with various structural Proteins (Refer Figure 5.1). Certain structural phospholipids like lecithin are involved in the process of ion transport across the membrane. The phospholipids are primarily composed of a glyceride group attached with two fatty acid chain (esterifed) along with a phosphate group. Further investigations are required to decipher the pathway of phospholipid biosynthesis in plants. Unlike neutral lipids the alignment of phospholipids helps in the orientation of a membrane in a cell. The breakdown of phospholipids is accomplished by the activity of phosphatidases which catalyze the hydrolysis of fatty acids and phosphoric acid ester bonds. In plants palmitic acid, linoleic acid and linolenic acid are the main functioning phospholipids. The phospholipids possess crucial role in cell signaling and stress tolerance in plants. The various components of phospholipids vary in their component present in the phosphorylated group. The phospholipids are a good source of phosphate groups in the cells.

Phosphatidylcholine and phosphatidyl inositol are the chief components associated with primary root growth and proliferation of root hairs. The enzyme Phospholipase D plays a vital role associated with breakdown of phospholipids and abiotic stress signaling. Exogenously applied phospholipids and lysophospholipids have been found to modulate plant growth and development in response to abiotic stress. Phospholipids help in forming the permeability of the membrane and easy passage of amphiphatic molecules across it. Based upon the functional group or component present in the phosphorylated moiety the phospholipids are categorized as follows:

- **Phosphatidylcholine (Lecithin):** The phosphorylated moiety of the phopsholipid contains choline group present as a main component. It functions in cell signaling and root growth.
- **Phosphatidylinositol:** The presence of a hexahydric alcoholic group called inositol in the phosphorylated moiety of phospholipid. The phosphorylated form of this molecule (IP_3) acts as a signaling molecule or secondary messenger.
- **Phosphatidylethanolamine:** Presence of ethanolamine group in the phosphorylated moiety.
- **Phosphatidylglycerol:** It contains glycerol in its phosphorylated moiety.
- **Diphosphatidylglycerol:** Two glycerol molecules are linked to the fatty acid chains in form of esterified bonds.

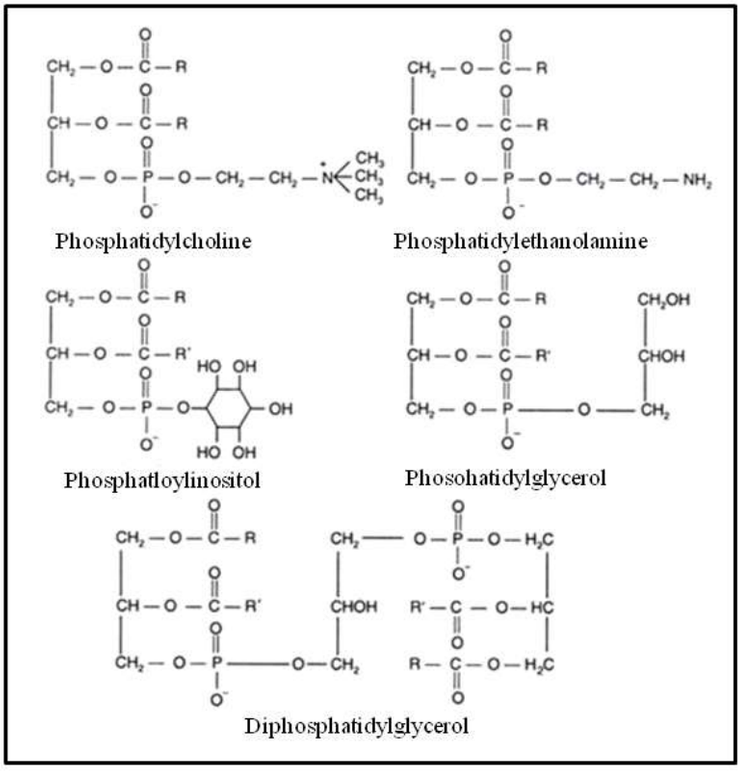

Fig. 5.1 *Structural Features of Phospholipids*

Membrane Functioning and Lipid Composition

The membrane composition and its chemical nature are largely influenced by the composition of the phospholipids present in it. The fluidity of the membrane is largely influenced by the composition and specific complements of lipids. The diversity in the lipid components is

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important for temperature regulation and maintaining functionality of the membrane. The ability of the plants to respond to various environmental effects and temperature fluctuations is adjusted by various lipid groups. The phosphatidylcholine component of phospholipid provides mobility to the membrane. The abundance of unsaturated phospholipids in the membrane determines the tolerance levels associated with lower temperatures. In chilling conditions it is necessary to maintain the fluidity of the membrane. Investigations in *Arabidopsis* and *Nicotiana* have revealed chilling stress tolerance associated with unsaturated lipid molecules. Recent investigations by physiologists have revealed that diphosphatidylglycerol and phosphatidylcholine are important regulators of the membrane properties in response to chilling stress. The membrane phospholipids serve as the precursor for various signaling molecules in plants, animals and microbes. Jasmonic Acid (18:3) and Linolenic Acid help in the process of pathogen defense. IP_3 is an important signaling molecule derived from PIP_2 which helps in releasing Ca^{2+} from the tonoplast and orgnellar reserves. Advancements in the genetic and molecular studies of various forms of phospholipases have resulted in better understanding of phospholipid signaling in plants.

5.2.2 Biosynthesis of Fatty Acids

The main sites of lipid biosynthesis in plant cell are chloroplast and Endoplasmic Reticulum. The process of fatty acid biosynthesis is accomplished by a sequential process of condensation of 2 Carbon units. The formation of acyl chains occur by covalent bond formation with acyl-Carrier Protein (ACP). The enzymes involved in this pathway remain in association to form fatty acid synthase. The main regulatory enzyme of the pathway appears to be Acetyl-CoA carboxylase which forms Malonyl-CoA from Acetyl-CoA and CO_2.

The sequential steps of fatty acid biosynthesis are as follows:

Synthesis of Glycerol: The process of glycerol synthesis in plants is accomplished by various pathways. However, the common pathway involves formation of glycerol from DiHydroxy Acetone Phosphate (DHAP) which is produced as an intermediate of glycolysis. At the initial step of the reaction DHAP is reduced to α-glycerophosphate catalyzed by the enzyme Glycerol-3-Phosphate dehydrogenase. In this reaction the co-enzyme $NADH_2$ is oxidized. In the following step α-glycerophosphate is hydrolyzed by glycerophosphatase to produce phosphoric acid and glycerol.

Synthesis of Fatty Acids: The process of fatty acid biosynthesis is accomplished by a sequential process of condensation of 2 Carbon units. The first step of fatty acid synthesis involves the formation of malonyl-CoA from Acetyl-CoA. In the following step Malonyl-CoA combines with ACP to form ACP-Malonyl-CoA.

- In the first stage the acetate group is transferred from Acetyl-CoA to cysteine group of an enzyme called 3-Keto-Acyl-ACP synthase which then combines with Malonyl-ACP to form Aceto-Acetyl ACP.
- The Keto group in the 3-C position is now reduced by the activity of three enzymes to form a 4-C new Acyl compound called Butyryl-ACP.
- The reaction of Acetyl-CoA carboxylase requires the presence of Mn^{++} and biotin as cofactors. Fatty acid synthetase catalyzes the reaction of one molecule of Acety-CoA to Malonyl-CoA to form Butyryl CoA. In this reaction one molecule of CO_2, H_2O and CoA are released. Butyryl CoA produced in the reaction further combines with Malonyl-CoA to form 6-C compound. The reaction continues until it forms a derivative of 16-18C Acyl chain.
- The enzyme fatty acid synthetase is a complex assemblage of multienzyme complex in association with a Acyl-Carrier Protein (ACP). The detailed understanding of the

reaction of formation of fatty acid from Acetyl-CoA and Malonyl-CoA can be grouped into three categories.

Malonyl-CoA is grouped into three categories:

- Initiation Reaction
- Chain Elongation Reaction
- Termination Reaction

Initiation Reaction: As described earlier, in this first reaction the acetyl group is transferred from the Acetyl-CoA molecule to the –SH group of multienzyme complex called fatty acid synthetase. The ACP molecule is similar in nature to CoA in possessing a phospho- pantetheine as a functional unit in its structure. In case of CoA the functional group is esterified to the ADP molecule while in ACP it is bound to serine of the Protein chain.

Chain Elongation Reaction: In this step six types of reactions operate to produce long chain Acyl ends of fatty acids. The sequential reactions in the chain elongation process include:

- Malonyl Transfer
- Condensation
- Reduction
- Dehydration
- Reduction and Acyl Transfer

The process of chain elongation involves the transfer of malonyl group from Malonyl-CoA to the –SH group of the multi enzyme complex. This step is followed by condensation which in turn produced a 4-C compound. Followed by this step reduction, dehydration and reduction produces a saturated 4-C unit called Butyryl-CoA. In the process of Acyl transfer reaction the fatty acid residue is transferred to the –SH group. This process is repeated in a cyclic manner until a 16-18 C compound is liberated. In each cycle of the chain elongation step the fatty acid molecule increases by 2C atoms. $NADPH_2$ is required as a coenzyme in this step of chain elongation.

Termination Reaction: In the termination step when a desired length of fatty acid has been generated the cyclic process of chain elongation stops. The Acyl group instead of being transferred to –SH group now associates with the Coenzyme CoA to form CoASH. The CoA derivative can further be utilized in fatty acid biosynthesis. The enzyme is liberated free and is capable of catalyzing a new reaction.

Condensation of Fatty Acid and Glycerol: The process of fat or triglyceride synthesis does not occur directly from fatty acids and glycerol but from α-glycerophosphate and CoA derivatives. In the first step of this process acylation of α-glycerophosphate by two molecules of fatty Acyl-CoA liberates phosphatidic acid. The phosphatidic acid formed in the earlier step is dephosphorylated by phosphatase to form diglyceride. The acylation of the free –OH group of diglyceride produces triglyceride molecule in combination with fatty Acyl-CoA.

Fatty acids formed in the earlier step may undergo modification and combine with glycerol to form glycerolipids. The double bonds are placed in the 16:0 and 18:1 fatty acids catalyzed by desaturase enzymes. This enzyme appears as an integral membrane Proteins present in the membranes of chloroplast and endoplasmic reticulum. The function of the desaturase enzyme is to insert a double bond at specific positions of the fatty acids.

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Plastids and ER are Sites for Glycerolipid Synthesis

Fatty acids are synthesized in the plastid and further utilized for biosynthesis of glycerolipids required for components of membrane and oleosomes. The process of glycerolipid synthesis follows two pathways, i.e., the chloroplastidal pathway and the ER pathway. In the initial phase of the reaction the fatty acid molecules are transferred from the Acyl-ACP or Acyl-CoA complex to glycerol-3-phosphate. This reaction produces phosphatidic acid. This step is followed by the activity of a specific phosphatase which catalyzes the conversion of phosphatidic acid to form DiAcylGlycerol (DAG). The other types of phospholipids mainly phosphatidylethanolamine and phosphatidylcholine are produced from DAG. Phosphatidic acid may also directly produce phosphatidylinositol or phosphatidylglycerol. Each of these molecules produced in the process act as specific signaling molecule in membranes and cytoplasmic compartments.

Briefly, the process of glycerolipid synthesis follows two pathways respectively:

- **The Prokaryotic (Chloroplastidial) Pathway:** In this pathway the products of chloroplastidial fatty acid synthesis (16:0 and 18:1 ACP products) are utilized to form phosphatidic acid. The fatty acids may also be released in the cytoplasm in the form of CoA Esters.
- **The Eukaryotic (Cytoplasmic-ER) Pathway:** In this pathway the reactions are accomplished by a set of acyltransferases (from ER) which utilize the fatty sacids to produce phosphatidic acid and its derivatives.

The operation of the two pathways may occur simultaneously or in separation in different plant species. It has been reported that in *Arabidopsis* and *Spinacea* both the pathways contribute to the formation of chloroplastidial lipids. However, in many angiosperms phosphatidylglycerol appears to be the only product of Prokaryotic pathway. The other types of lipid components are produced through the Eukaryotic pathway. Similar mechanisms are operative in the oil seeds where the key enzymes responsible for the biosynthesis of Triacylglycerol (TAG) are Acyl-CoA:DAG Acyltransferase and PC:DAG Acyltransferase. The TAG accumulates in the specialized unit membrane organelles called oleosomes. Thus, oil seeds possess abundance of this structure scattered in the cytoplasm of cotyledon and endosperm cells. The oil bodies are surrounded by unit membrane which contains specialized Proteins called Oleosins, Caleosins and Steroleosins. It has been observed that the oleosins help in stabilizing the membrane of oil bodies. During germination the oil bodies are degraded and the TAG is mobilized by Glyoxylate Cycle to produce Sucrose and other organic acids. These molecules are mobilized into the growing seedling and provide respiratory substrate.

Distribution of Fatty Acids in the Plant Tissues

Lipid molecules are widely distributed in the plant kingdom and they function as storage, structural and signaling molecules. Although starch and other complex polysaccharides are some of the stored product in plants nevertheless, lipids serve as good sources of energy. The number of ATP molecules released from oxidation of lipid is higher than that of carbohydrates. In higher plants lipids are mainly stored in the reproductive tissues, i.e., fruits and seeds. Certain members of oil yielding palms like *Elaeis* sp. or Avocados store considerable amount of lipid in their mesocarp. In oil seeded plants lipids serve as important reserve material in cotyledons, for example Sunflower, Canola, Castor, Linseed, Flax, Groundnut, soybean, etc. Certain cereals like Wheat may contain certain amount of lipids in the aleurone layer. The primary lipid storing organelle present in plant tissue is known as spherosomes or oleosomes which remain scattered in the cytoplasm.

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Biochemical Mechanism of Fat Degradation

The process of lipid metabolism is very dynamic and it depends upon the growth stage of the specific plant tissues. Lipids biosynthesized and stored are degraded during its requirement of energy substrate for respiration. During seed germination of fatty seeds the degradation of fat liberates Acetyl-CoA which combines with other organic acid to produce sucrose. The sucrose produced is mobilized to various parts of the growing seedling and participate in the process of Krebs' cycle. In certain plants carbohydrate sources may decline due to certain reasons which trigger the catabolic metabolism of lipids.

In the initial stage of fat breakdown lipase catalyzes the conversion of triglycerides to glycerol and fatty acids. The process occurs through a hydrolytic reaction which occurs in three steps. The first two steps are reversible which produce diglycerides and monoglycerides. However, the final step of hydrolysis is irreversible. The final step of conversion of monoglycerides to fatty acids and glycerol occur in the glyoxysomes. Ca^{2+} is required as a cofactor for the final step of reaction.

Oxidation of Glycerol

The glycerol formed in the previous step is likely to react with ATP to form glycerol-3-phosphate. This step is further catalyzed by the enzyme glycerol-3- phosohate dehydrogenase and NAD^+ to produce dihydroxyacetone phosphate. This molecule (DHAP) is capable of entering glycolysis. DHAP is further converted to pyruvic acid by the steps of glycolysis. This process occurs in the cytoplasm and it yields 2 molecules of ATP. This step is termed as substrate level phosphorylation. Furthermore, 2 NADH are produced in this step which generate 4ATP molecules by the process of terminal oxidation. The downstream oxidation of pyruvic acid in TCA cycle produces around 15 ATP along with CO_2 and H_2O. Thus glycerol molecules are capable of liberating 21(2 + 4 + 15) ATP in the process of respiration. However, the enzyme glycerol kinase requires the consumption of one ATP molecule. Thus the net gain is 20 ATP.

Oxidative Breakdown of Fatty Acids

α-Oxidation Pathway

Long chain fatty acids are broken down by the process of α-oxidation or β-oxidation which produces 2-C units of Acetyl-CoA. In the α-oxidation pathway the fatty acids are broken down until it is reduced to 12 C atoms. Fatty acids less than 13 C atoms are not affected by this process. The complete process of α-oxidation liberates one molecule of CO_2 obtained from the –COOH group of the fatty acid. In this process the α-C atom number 2 is oxidized. Hence it is termed as α-oxidation. The process of α-oxidation takes place as follows:

- The initial step of the pathway involves Oxidative Decarboxylation of Fatty Acids to form Aldehyde compound. The reaction is catalyzed by the enzyme fatty acid peroxidase. The α-C atom gets oxidized and CO_2 is liberated as a byproduct.
- The aldehyde produced is further oxidized to a new fatty acid which has C number lesser than the initial one. The step is catalyzed by aldehyde dehydrogenase. This step implies NAD^+ as a cofactor which gets reduced to $NADH^+ + H^+$.

Investigations have been reported that the α-oxidation of fatty acids and $NADH^+$ production in plants may not be directly associated to ATP formation. However, the aldehyde compounds generated are likely to be reduced to produce long chain alcohol. This process may contribute to wax formation in plants. The α-D fatty acids produced are utilized in the formation of cerebrosides in higher plants.

β-Oxidation Pathway

The β-oxidation pathway has been reported to be more common for saturated fatty acid breakdown in plants. However, the mechanism has not been clearly revealed in the case of unsaturated fats. The process operates in mitochondria and glyoxysome where 2 C atoms are removed from the fatty acid to form Actyl-CoA. The process involves oxidation of β-C in the fatty acids. Thus it is named as β-oxidation pathway (Refer Figure 5.2). The various steps of the β-oxidation pathway are as follows:

- The first step involves activation of the fatty acid by the presence of ATP and enzyme thiokinase. In this process CoASH is consumed and CoA derivative of fatty acid is formed. This reaction involves the requirement of Mg^{2+} as a cofactor. The AMP molecule liberated combines with ATP to form two molecules of ADP. The product formed in the first reaction is termed as Fatty-Acyl-CoA.
- The second step of the pathway is catalyzed by Acyl-CoA dehydrogenase which catalyzes the removal of two Hydrogen atoms from the α and β C atoms of the fatty Acyl-CoA molecule. This results in the formation of Trans α, β unsaturated Fatty-Acyl-CoA molecule. The reaction is energised by FAD^+ molecule.
- The third step involves addition of a water molecule across the double bond to form β-Hydroxy-Acyl-CoA. The step is catalyzed by the enzyme Enoyl Hydrase.
- Followed by this the β-Hydroxy-Acyl-CoA molecule is dehydrogenated by the presence of NAD^+ specific β-Hydroxy-Acyl-CoA dehydrogenase enzyme. This results in the removal of two hydrogen atoms from the β-C which now bears a Carbonyl group. The molecule thus formed is termed as β-Keto-Fatty-Acyl-CoA.
- The last step of the pathway thio-cleavage of β-Keto-Fatty-Acyl-CoA molecule. The process is atalyzed by the enzyme β-Keto-Acyl-Thiolase. This results in the formation of two molecules of active Acetyl-CoA and a Fatty Acyl-CoA. The later in comparison with the first initiating molecule appears shorter by two C atoms.

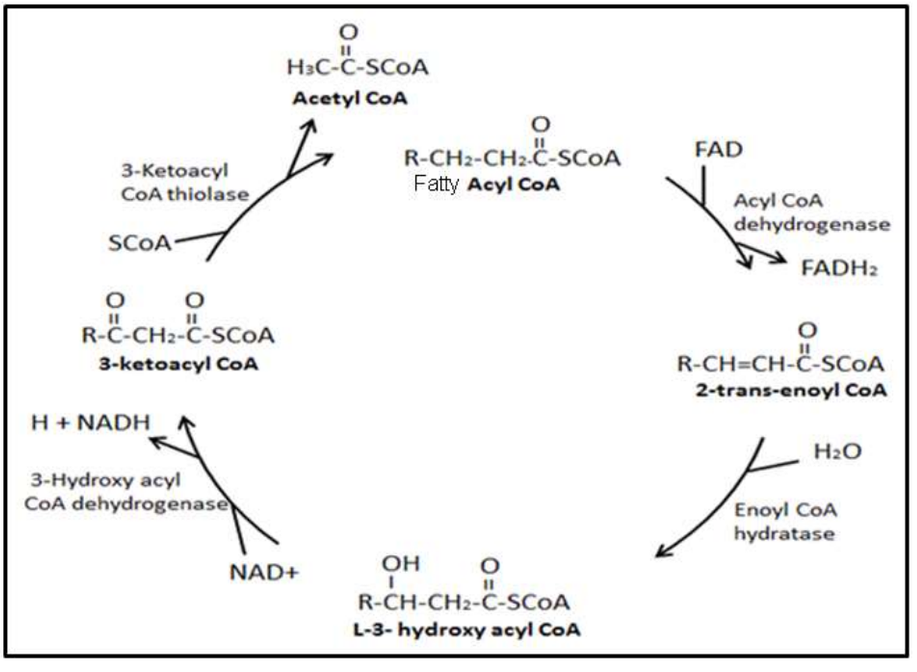

Fig. 5.2 *The β-Oxidation Pathway*

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Energy Balance in β-Oxidation Pathway

The Fatty-Acyl-CoA molecule liberated at the end of the β-oxidation pathway renters the pathway at its second step. In this case the first step is omitted as it is already being activated. The Fatty-Acyl-CoA molecule re-entered in the pathway further liberates two Carbon atoms to form Acetyl-CoA. Thus in each turn of β-oxidation pathway one $FADH_2$ is liberated at the second step, one NADH+ H^+ in the fourth step and one Acetyl-CoA molecule in the last step. The terminal oxidation of the $FADH_2$ and $NADH_2$ molecules will liberate 2 and 3 ATP molecules, respectively. Thus in total 5 ATP molecules are likely to be generated in a single cycle of this pathway. Thus, the process of β-oxidation pathway coupled to TCA cycle mediated by acetyl-CoA will liberate a considerably significant amount of energy in the form of ATP. As an example, it can be understood that the complete oxidation of palmitic acid (C-16) through β-oxidation pathway and TCA cycle will liberate around 129 molecules of ATP. Thus the fats serve as high energy source respiratory substrate in plants.

5.2.3 The Glyoxylate Cycle

Preliminary investigations in seed germination physiology revealed that the decrease in fat content during stages of germination showed coincidence with the increase in sugar content in certain germinating oil seeds. Later on in 1957 Kornberg and Krebs reported that a particular strain of *Pseudomonas* sp. was capable of converting ^{14}C-labelled Acetic Acid into Malic Acid and Citric Acid. The observations were elaborated with reaction details where acetic acid combined with CoA to form Acetyl-CoA. The Acetyl-CoA formed combined with oxaloactetate or glyoxylic acid to produce other organic acids like malate and citrate. Malate was produced by the activity of malate synthetase. Glyxoylic acid appeared as a breakdown product of Iso-Citric Acid catalyzed by the Enzyme Isocitratase. Thus, investigations by Kornberg and Krebs provided the first evidence of glyoxylate cycle or glyoxylic cycle through which the fats are converted into sugars (sucrose or other carbohydrates) during the germination of fatty seeds. The conversion of lipids to sugars is triggered during the germination process. The process initiates with the breakdown of triacyl glycerol localized in the oil bodies called Oleosins. The fatty acids are oxidized in a specialized peroxisome known as glyoxysomes. Glyoxysomes are double membrane organelles abundant in the oil storage tissues like seeds and cotyledons. Briefly, the Acetyl-CoA formed is metabolized in the glyoxysome to produce succinate. The succinate formed is transported to mitochondria where it forms two metabolic intermediates, i.e., Oxaloacetate and Malate. Finally the malate produced is transported to the cytoplasm. In the final step of this pathway malate produces glucose by a process called Gluconeogenesis. In certain seeds, the fatty acids may be diverted to other metabolic pathways. However, in certain oil seeds like Castor Beans, 1 gm of Lipid stoichiometrically produces 1 gm carbohydrate. This utilizes 40% energy efficiency. The initial degradation of the triacylglycerols occurs by the activity of lipases. Lipases are hydrolytic enzymes localized in the membrane of the oil bodies. The lipases hydrolyze TAGs into three molecules of fatty acids and glycerol. While certain plants exhibit lipase activity associated with the oil bodies, peanut and cucumber exhibit lipase activity in the glyoxysomes. During the process of lipid breakdown the oil bodies remain in vicinity to the glyoxsomes. The glyoxylate cycle is intimately associated with the Kreb's cycle and has been reported in various species of bacteria, yeasts, molds and higher plants. The pathway is briefly completed in glyoxysomes, mitochondria and cytosol (Refer Figure 5.3).

Mechanism of Glyoxylate Cycle

Following are the reactions involved in glyoxylate cycle:

Reactions in Glyoxysome

- In the initial step of the Alyoxylate pathway the Acetyl-CoA formed due to β-oxidation of fatty acids undergoes condensation with Oxaloacetic Acid to form Citric Acid. the reaction is catalyzed by the Enzyme Citrate Synthase
- Citric Acid produced is dehydrated to produce Cis-Aconitic Acid in the presence of the Enzyme Aconitase. The Cis-Aconitic Acid produced reacts with one molecule of water to form Isocitric Acid.
- Iso-Citric Acid is metabolized into Glyoxylic Acid and Succinic Acid. The step is catalyzed by the Enzyme Isocitratase.
- Glyoxylic acid now associates with Acetyl-CoA in the presence of the Enzyme Malate synthetase to produce Malic Acid.
- In the final step of the reaction series occurring in glyoxysomes the Malic Acid produced is oxidised into Oxaloacetic Acid. This step is catalyzed by the presence of the Enzyme Malic Dehydrogenase and Coenzyme NAD.
- OAA produced in the previous reaction combines with Acetyl-CoA to produce Citric Acid.

Reactions in the Mitochondria

- The Succinic Acid produced in the Glyoxysome usually moves to the Mitochondria where it is converted to Oxaloacetic Acid. This reaction resembles that of Krebs cycle pathway. The reaction of conversion of succinic acid to oxaloacetic acid does not occur in the glyoxysome. This is due to the absence of two enzymes in glyoxysomes namely succinic dehydrogenase and fumarase. These enzymes are localized in the mitochondria.
- Oxaloacetic acid formed in the previous step undergoes decarboxylation in the presence of ATP to form Phospheonol Pyruvic (PEP) Acid. This reaction is ATP dependent phosphorylation process which releases ADP along with PEP.

Reactions in the Cytosol

- Phospheonol Pyruvic Acid produced in the Mitochondria is transported to the Cytosol. In this step, PEP undergoes metabolic conversion by the process of reverse Glycolysis to produce Fructose and Glucose Phosphates.
- Fructose and glucose formed now combine enzymatically to produce sucrose. Sucrose is the non-reactive transportable form of sugar mobilized into the growing seedling. With the formation of carbohydrates from the lipids the glyoxysomes disappear from the cell. The oil bodies are completely degraded at the mature stage of seedling emerged from the seeds.

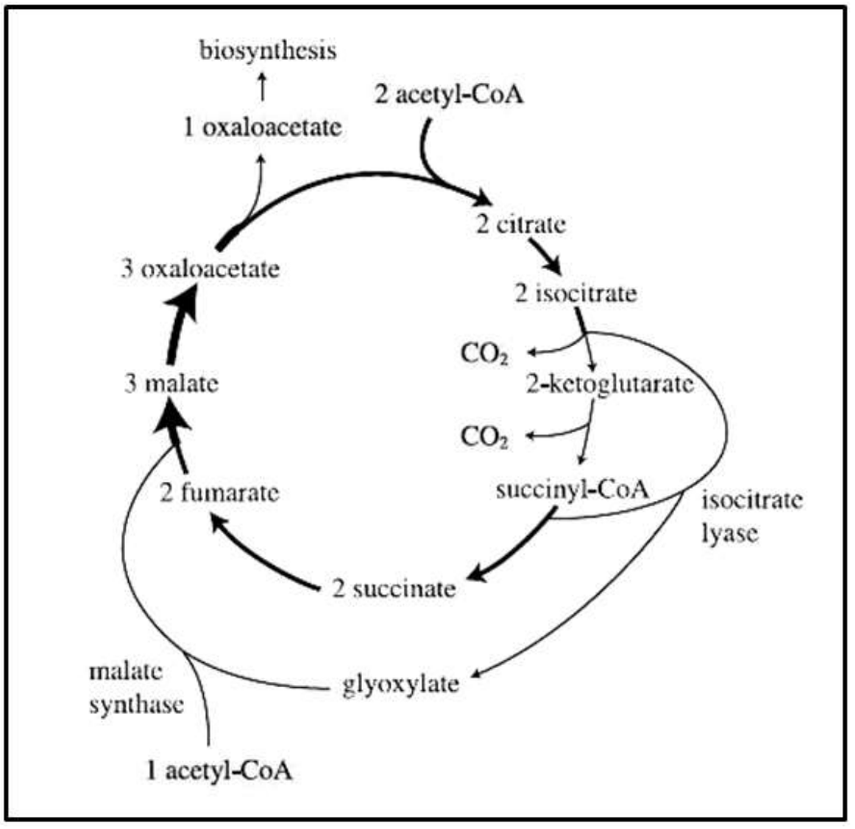

Fig. 5.3 *Glyoxylate Pathway*

Significance of Glyoxylate Cycle

The pathway of glyoxylate cycle is carried out mostly in the case of fatty or oil seeds during the process of germination and seedling growth. Lipids are a good source of energy stored in the reduced form of Carbon. However, since the fats are insoluble in nature it cannot participate as a substrate in aerobic respiration directly. The emerging seedlings possess non-green cotyledons or emerging leaf primordia. In such a situation, it is necessary to provide respiratory substrate to the non-photosynthetic seedling for its growth and establishment. Thus fatty acids are degraded by the process of β-oxidation pathway to produce acetyl-CoA. This molecule is further involved in the process of sucrose biosynthesis through the glyoxylate cycle. Thus, a part of the Glyoxylate Cycle exhibits reactions of reverse Glycolysis and is thus termed as Gluconeogenesis. In certain Microorganisms (Bacteria) which are capable of growing in the presence of Ethanol or acetate as a source of Carbon utilize the pathway of Glyoxylate cycle to produce Sugars.

'Check Your Progress'

1. What is phosphatidylcholine?
2. Define phosphatidylinositol.
3. What is the significance of ethanolamine group in the phosphorylated moiety?
4. Give a function of desaturase enzyme.

5.3 PROTEIN SYNTHESIS IN PROKARYOTES AND EUKARYOTES

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Proteins are largely involved in all metabolic pathways. The requirement of Proteins in cells depends upon its functional role in a tissue or organ. Plants exhibit dynamic signaling mechanisms associated with Protein-Protein interaction and cross talk pathways. In Eukaryotes the process of translation involves the association of 70 different ribosomal Proteins, 20 enzymes to activate the Amino Acid precursors and 15 auxiliary enzymes. Translation is an efficient process with high accuracy. It has been reported that *Escherichia coli (E. coli)* cells at 37°C are capable of synthesizing around 100 Amino Acid long peptide in 5 minutes. Cells maintain coordination between the Protein synthesis and degradation process which helps in balancing the turnover rate of enzymes and hormones. Various mechanisms control the process of transcription and translation. The phenomenon of RNA silencing prevents the process of mRNA binding to ribosome and its translation. The spatial and temporal separation of various isozymes is often associated with alternate splicing and regulation of translation. In the middle of the 19th century Paul Zamecnik and his group investigate the possible site of translation in rat liver cells. They implied radioactive tracer technique using radioactive Amino Acids which revealed that the nascent Proteins were associated with the ribonucleoprotein particles. Fractionation method confirmed that the ribosomes were the probable site for Protein synthesis. Later on in 1955 Mahon Hoagland identified the activated form of the Amino Acid necessary for translation. The process of activation of Amino Acid was found to be an ATP dependent active process occurring in the cytosol. Apart from the core process of translation, activation of Amino Acid and regulation of post-transitional modification are essential factors associated with Protein synthesis. Protein synthesis is a regulative Process in Prokaryotes and Eukaryotes. The process of Protein synthesis and its elongation occurs in the ribosome-mRNA complex which then gets transported to the Endoplasmic Reticulum. The final process of Protein folding, post-translational modification and sorting occurs from ER to dictyosomes. The process of translation is an enzymatic process which occurs by the help of Codon-Anticodon complementary recognition of Amino Acid bearing tRNA molecules. Ribosomes are present both in the Prokaryotic and Eukaryotic cells which differ in their structure of subunits and RNA molecules. The association of the ribosomal subunits help in its association with the mRNA molecule. In this process of recognition specific nucleotide sequences in the mRNA and ribosomal RNA help in formation of ribosome-mRNA complex. The process is largely similar for both Prokaryotes and Eukaryotes.

However, there lie certain differences in the sequences of nucleotides. The specificity of the genetic code is maintained by the triplet codons aligned in the mRNA chain. The initiation and termination codons regulate the process of translation. The Amino Acids specific to each codon are carried by the tRNA molecules which possess the specific anticodons. The tRNA-amino acid conjugation is also an enzymatic process catalyzed by Amino-Acyl-tRNA synthase enzyme. This enzyme performs two main functions activation of Amino Acid for peptide bond formation and attachment of Amino Acid to the respective carrier tRNA. The Amino-Acyl-tRNA synthases are highly specific in recognizing their Amino Acids. Thus, the enzyme deals with the proofreading function which controls the fidelity of Protein synthesis. Improper recognition by the enzyme will lead to the incorporation of wrong Amino Acid in the peptide chain. The process of Protein synthesis in Prokaryotes and Eukaryotes possess significantly lower chances of errors. The charged tRNA molecules carrying the specific amino acids are carried to the initiation site of ribosome-mRNA complex. The A, P and E sites in the ribosome are associated with initiation, elongation and exit of uncharged tRNA molecules. The process of Protein synthesis proceeds with sequential

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peptide bond formation between the –COOH and NH_2 groups of the adjacent Amino Acids. The process of peptide bond formation is thus a dehydration reaction. The steps of initiation, elongation and termination of translation are regulated by various Protein factors. These Protein factors undergo temporal association or dissociation from the translational complex. Prokaryotic and Eukaryotic translation exhibits certain differences in reference to the initiating Amino Acid, Protein factors and elongation process. The semi-autonomous organelles of chloroplast and mitochondria also possess specific apparatus for accomplishing the translational mechanisms. These organelles possess specific ribosomes which are sites for translation. Prokaryotic and Eukaryotic translation has been found to be sensitive to a different set of inhibitors. Interestingly, the process of chloroplastidial and mitochondrial translation differs from the Eukaryotic mechanisms but is similar to the Prokaryotic process. Prokaryotic ribosomes are smaller in size in comparison with Eukaryotic ribosomes. Various plants (like Tobacco, Maize, Spinach and Barley) and Algal members (*Chlamydomonas* sp.) have been investigated for deciphering the process of chloroplastidial translation. In 1975 R.J. Ellis from U.K. reported the process of Protein synthesis in isolated chloroplast of pea. The **Shine-Dalgarno sequence** present in the prokaryotic system helps in the recognition of the mRNA and Ribosomes. Similar sequences have also been reported in the chloroplastidial ribosomes. In the mid 1990s *in-vitro* systems of tobacco chloroplast were developed for the study of chloroplastidial translation. The other components associated with translational machinery are major Proteins or cofactors helping in the process of translation. The components regulating elongation and termination of translation are similar in chloroplast and Prokaryotes. In Prokaryotes the process of mRNA synthesis is tightly coupled to translation. The direction of synthesis of messenger RNA and translation are similar. In this case the nascent mRNA is not transported from the nucleus to cytoplasm. The half-life of bacterial mRNA is less than 1.5 minutes after which they are susceptible to degradation by the activity of the nucleases. Thus, in order to maintain a good rate of Protein synthesis the two processes are highly coupled to each other. The group of ribosomes together bound to the mRNA molecule is known as **polyribosome** or **ergosomes.** During optimum conditions it has been found that a single ribosome is associated with 80 nucleotide long fragment of mRNA. The Eukaryotic mRNA is monocistronic in nature and therefore associates with around ten to fifteen ribosomes. However, the prokaryotic mRNA being polycistronic may contain several ribosomes attached to it. The present unit shall discuss the detailed mechanism of protein synthesis in prokaryotes and eukaryotes.

Structure of Ribosomes

Ribosomes are cell organelles which facilitate in the process of Protein synthesis. They are the sites of Protein synthesis which involves translation of mRNA into Proteins. In Prokaryotic cells ribosomes not functioning in translation may appear free in the cytoplasm. However, in Eukaryotes the ribosomes are clustered and often remain attached with the outer membrane of Endoplasmic Reticulum. The sedimentation coefficients of each subunit of the ribosomes are designated by Svedberg unit. Bacterial 70S has been reported to possess a diameter of 23 nm. This Prokaryotic ribosome possess two subunits, i.e., smaller 30S subunit and larger 50S subunit. The ribosomes present in the chloroplast and mitochondria possess resemblance to Prokaryotic ribosomes. Eukaryotic ribosomes are larger in size (80S) and possess a smaller 40S subunit and a larger 60S subunit. The association of the smaller and larger subunits is regulated by the concentration of Mg^{2+}. In the presence of lower Mg^{2+} concentration the subunits of ribosomes dissociate from each other.

Prokaryotic Ribosome

The detailed structure of Prokaryotic 70S ribosome has been investigated. The ultra-structural details of the rRNA have also been reported in Prokaryotes. Electron microscopic imaging

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has been performed to investigate the structure of ribosomal subunits. The smaller subunit of ribosome possesses a head, base and a platform. The platform separates the head from the base by forming a cleft or shoulder. The larger subunit possesses a ridge like structure, central protuberance and a stalk (Refer Figure 5.4).

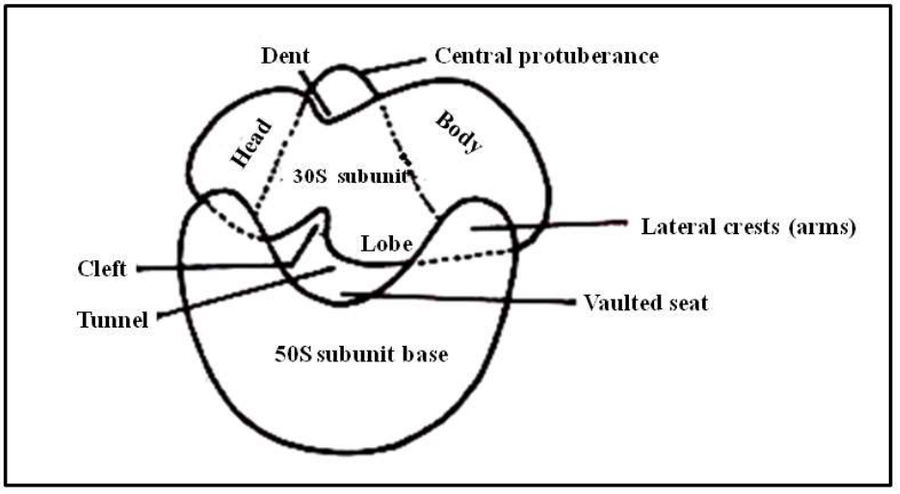

Fig. 5.4 *Structure of Prokaryotic Ribosome*

Chemical Composition

The chemical composition and organization of bacterial ribosome involves three types of ribosomal RNA (rRNA) and more than 50 different ribosomal Proteins. The rRNA comprises of 60-70% of the entire ribosomal composition. The 30S subunit is composed of 16S rRNA and 21 different Proteins. The 50S subunit contains 23S rRNA, 5S rRNA and 32 different types of ribosomal Proteins. The organisation of rRNA appears similar in Eukaryotes where an additional 5.8S rRNA species is present. Investigations have been successful in dissociating the rRNA and r-Protein component from ribosomal subunits. In the time period of 1970-1980 X-ray crystallographic analysis and Nuclear Magnetic Resonance (NMR) studies have revealed the structural details of each of the rRNA species and the r-Proteins. It has been found that the rRNA molecules possess double stranded regions present due to intra strand complementation of nucleotide sequences. The 16S rRNA possess four double stranded domains, i.e., 5′ domain, central domain, 3′ major domain and 3′ minor domain. The 23S rRNA possess six double stranded domains in it. There are certain double helical regions present in the structure of ribosomal RNA which may often contain certain unpaired bases. In the last few decades of 1980-2005 cryoelectron microscopic analysis along with X-ray crystallography has revealed the structure of ribosomal Proteins. Various microorganisms like *E.coli*, *Thermus thermophilus*, and *Haloarcula marismortui* have been implied for the investigations of the structure of r-Protein. These studies further revealed the localization of various functional domains present in the ribosomes. The 30S-0S interface and the active site for peptidyl transferase are mostly determined by the rRNA components of the ribosome. The 23S rRNA forms around six domains while the 5S rRNA forms the seventh domain. These seven domains interact among each other to form a compact mass of RNA in the 50S subunit. The domains do not exhibit separate identity when found together in the 50S subunit. The protuberances of this subunit are attached to the rest of the part by the help of RNA stem. In the smaller 30S subunit the 16S rRNA forms four domains. The 3′ minor domain present in this subunit forms the interface region between the 30S-50S subunits. The smaller subunit (30S) of Prokaryotic ribosome exhibits better structural flexibility.

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Ribosomal Proteins

The r-Proteins are mainly concentrated in the surface of ribosome and are associated with Protein-RNA crosstalk and Protein-Protein interactions. This process helps in the stabilization of various domains of rRNA inside each subunit of ribosomes. The 30S-50S interface of ribosome is largely free of Proteins. In the large subunit the Proteins are localized within the regions of protuberance tips confined by RNA molecules. The association of the RNA and Protein molecules occur by loops and Protein tails being inserted between RNA helices. The globular domains of the r-Proteins remain in the exterior side of the particle. This structural association of Proteins and RNA help in stabilizing the structure of ribosome. Mg^{2+} present in the vicinity of the RNA-Protein complex help in stabilizing the tertiary structure of 23S and 5S rRNA molecules.

Biogenesis and Function of Ribosomal Subunits

In bacteria the genes for 5S, 23S and 16S rRNAs are clustered in a single region of the operon which works as a functional unit. In Eukaryotes the organization of rRNA and r-Protein genes are more complex in nature. The 18S, 5.8S and 28S rRNA genes are present in bigger regions of nucleolus organizer zone. They possess multiple copies in their DNA fragments. Interestingly, the r-Proteins of eukaryotic ribosomes are also widely scattered in the chromosomes. They possess multiple duplications in the genome. The formation of subunit rRNA molecules in Eukaryotes initiates by formation of 45S precursor molecule. This molecule of rRNA is split into 41S and 20S fragments catalyzed by the activity of endonucleases. The subunits of 41S and 20S thus formed further produce 28S and 18S molecules respectively. This reaction is catalyzed by exonuclease activity. The 5S rRNA genes are present in multiple copies in the Eukaryotic DNA. The process of rRNA synthesis occurs in the nucleolus simultaneously with r-Protein synthesis in the cytoplasm. The processing of ribosomal subunits, however, occurs in the nucleolus before their delivery into the cytoplasm. Thus nucleolus possess important role in the process of rRNA synthesis and ribosome biogenesis.

Ribosomes function as the main site for Protein synthesis carried out by the translation of mRNA. The process is accomplished by acceptance of correct Amino-Acyl tRNA molecule to the mRNA, peptide bond formation between two adjacent Amino Acids and lastly exit of deacylated tRNA from the ribosome. The function of the 30S subunit is to decode the mRNA and helps in translocation of tRNA. This subunit functions in association with the large subunit. Interestingly, the 50 S subunit exhibits autocatalytic properties and thus function as a ribozyme. This subunit helps in the process of peptide bond formation in the Proteins. the three sites present in between the two subunits form the Amino-Acyl (A) site, peptidyl-transferase (P) site and exit site €. These sites provide areas of interaction between the tRNA and ribosomal particles. The anticodon part of the tRNA binds to the 30S-mRNA complex and the 3′ CCA end binds to the 50S subunit. Thus the tRNA-ribosomal interaction occurs in the interface region of 30S-50S subunits of the ribosome. The Eukaryotic 80S ribosome possess certain similarities to Prokaryotic ribosome but is, however, more complex. There are considerable differences in the RNA: Protein ratio present in Prokaryotic and Eukaryotic ribosomes. The smaller subunit of Eukaryotic ribosome, i.e., 40S exhibits constancy in its size. However, there lies considerable variation in the larger subunit (60S) and its component - 28S rRNA molecule.

Association of Ribosome and Endoplasmic Reticulum (ER)

The membrane of ER forms extensive network of cisternae connecting the nuclear region with other parts of the cell in the cytoplasm. The ER membrane forms an internal lumen and carries ribosomes on its external surface. Based upon the presence and absence of ribosomes

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the ER are classified as smooth ER and rough ER. The meristematic or secretary cells involve in Active Protein Synthesis. Such cells exhibit the presence of rough ER which show clusters of ribosomes attached to its membrane. The part of the cytoplasm which contains rough ER appears granular or basophilic in nature. The surface area of ER is increased due to the complex network of cisternae. The smooth ER is different in morphology form the rough ER. This type of ER does not associate ribosomes in its surface and are involved in transport of Protein and lipid vesicles to the Golgi bodies.

Structure of transfer RNA (tRNA)

The tRNA molecules play a significant role in the process of translation. The fidelity of the Protein synthesis process is maintained by recognition of correct Amino Acid and its attachment to the tRNA which is catalyzed by the enzyme Amino-Acyl tRNA synthase. The process of recognition of Amino Acid cannot be accomplished without tRNA molecules. The enzyme aminoacyl tRNA synthase possess its corresponding types specific to each Amino Acid. This enzyme helps the tRNA molecules to recognise the Amino Acids which are followed by its interaction with the ribosome-mRNA complex. In 1964, R.W. Holley and his group reported the detailed structure of tRNA from yeast cells. The molecule was an Alanyl-tRNA. In Prokaryotes (*E. coli*) there are around 55 tRNAs reported for 20 Amino Acids. The tRNA molecules possess around 74-95 bases which form a complex secondary structure. Most of the tRNAs possess unusual bases like Pseudouridine, Inosine and Dihydroxy Uridine. The process of formation of unusual bases occurs by methylation of normal bases. The ratio of A:U and G:C in tRNA is near to unity. There are intra-strand DNA like double helical segments present in the structure of tRNA. In the helical regions G:C bonds have been found to be more common than the A:U pairs. All tRNA molecules possess guanine residue at the 5′ terminal end and a CCA′ sequence at the 3′ end. The number of modified bases in the tRNA may exceed up to 50 in number.

Clover Leaf Model of tRNA (Secondary Structure)

The secondary conformation of tRNA is commonly termed as the clover leaf model. The structure forms due to intra-strand complementary nucleotide bases. The A:U and G:C pairing results in the formation of several stem loops in the structure of the tRNA. The tRNA molecule comprises of four double helical regions each of which possess a loop. The anticodon is present in the loop of the second helical region which recognizes its corresponding codon in the mRNA. The number of tRNA molecules is not as high as the number of codons. Thus, one tRNA molecule can recognize more than one, i.e., several codons necessary for the synthesis of Amino Acid. Thus, single Amino Acid can be accepted by more than one tRNA species. These tRNA molecules are called isoaccepting tRNAs. The acceptor arm of the tRNA consists of a double helical arm that carries a single stranded region of 3′ acceptor end of tRNA. The base sequence of this region is represented by 5′CCA3′ which links to an Amino Acid. The T-arm of tRNA is named due to the triplet sequence of the loop carried in this arm. The DHU arm of tRNA possesses dihydroxyuridine in the loop associated with the arm. The extra arm or the variable loop differs in different tRNA. This region lies between the T arm and the anticodon arm. The size of variable loop of tRNAs form two classes of tRNA, i.e., Class I tRNA and Class II tRNA. The Class I tRNA consist of a small variable loop which comprises of 3-5 bases. The Class II tRNA possesses a large variable loop with 13-21 bases along with 5 base pairs in the variable stem region (Refer Figure 5.5).

The three dimensional structure of tRNA was characterized from its crystalline form in 1968. A. Klug in 1982 reported the three dimensional structure of tRNA. The tertiary conformation of the tRNA molecule is derived from the secondary structure of clover leaf

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model. The L shaped tertiary structure is formed by condensing four arms of the clover leaf conformation. The L shaped tertiary structure possess following two major domains:

- The Acceptor Arm
- Anticodon Arm-DHU Arm Biloop

The additional arm length varies in various tRNA molecules. The stem with CCA end projects from the L shaped structure in different orientations. The two arms of the L shaped tRNA orient in variable angles to each other.

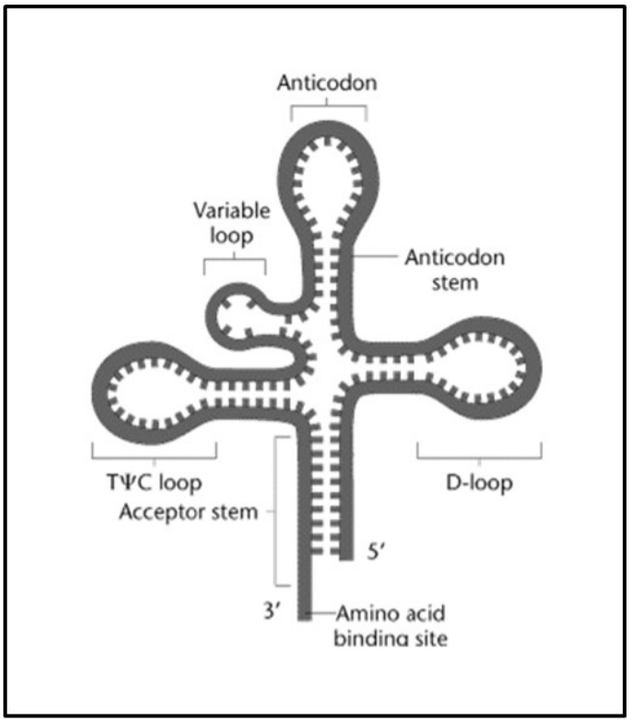

Fig. 5.5 *Clover Leaf Model of tRNA*

Aminoacyl-tRNA Synthases

The tRNA molecules recognize the specific Amino Acids with the help of the enzyme amino acyl-tRNA synthase. The tRNA molecule further interacts with the Proteins and Nucleic Acids. The capability of the tRNA molecules to recognize aminoacyl tRNA synthase is crucial in maintaining the efficiency of translation. This results in the formation of aminoacyl tRNAs. Detailed investigations have been performed to decipher the interaction between the Amino Acids, Amino Acyl tRNA synthase and the tRNA molecules. Different synthase enzymes have been investigated for the process of recognition of their corresponding Amino Acid molecules. The synthase enzyme is comprised of individual subunits of 40 kD to 110 kD in size. The enzymes are monomeric, dimeric or tetrameric units. The active site of the enzyme constitutes a smaller part of the Protein. Based on the structural type of the enzyme it has been classified into two groups each comprised of ten enzymes.

Class I-Synthetases: They possess an N-terminal catalytic domain consisting of two conserved sequences which are called **signature sequence**. It also possesses a nucleotide binding fold which consist of alternating β strand and α-helices. Interestingly, the signature sequences function for binding of ATP and Amino Acids. The C-terminus end of the Protein binds to the anticodon of tRNA. The intermediate region of the enzyme between the N and C terminal domain interacts with the acceptor arm of the tRNA.

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Class II Synthetases: This type of synthetase enzyme possess a C-terminal catalytic domain which is formed of a large antiparallel β sheet covered by α-helices. In between the regions of the catalytic domain a region binds to the acceptor arm of the tRNA molecule. The N terminal domain in this class of enzyme functions for anticodon binding.

The process of recognition of different aminoacyl tRNA synthase enzymes by their respective tRNA molecules is referred to as the second genetic code. The anticodon and 73rd base in the acceptor arm are universal sites of recognition. This is applicable for iso-accepting group of tRNAs which recognize the same Amino Acid). At least one of the three available anticodon nucleotides helps in the process of recognition.

Mechanism of Translation

The process of translation occurs through sequential steps of Amino Acid activation and its recognition by the aminoacyl tRNA synthetase enzyme. The main steps associated with the process of translation are as follows:

Preinitiation of Translation: The process of translation in initiated by the formation of Amino-Acyl tRNA complex. The process of activation of Amino Acid is obtained by binding of the Amino Acid (aa_1) to ATP. The process is mediated by the enzyme amino acyl tRNA synthase. This results in the formation of aminoacyl-tRNA synthase-AMP-Amino Acid complex formation.

$$aa_1 + ATP + \xrightarrow{\text{Amino-AcyltRNA}} \text{Synthase (aRS)} \quad \text{aa-AMP-aRS Complex} + Pi$$

The complex formed reacts with a particular tRNA and transfers the Amino Acid to the tRNA. The reaction is accompanied by liberation of AMP and the enzyme is liberated for a new reaction.

Initiation of Translation: The synthesis of polypeptide chain is initiated by the Amino Acid methionine.

This Amino Acid is coded by AUG. GUG also rarely codes for methionine. In Prokaryotes formylated form of methione is used as an initiating Amino Acid. Thus in *E. coli* $tRNA_f^{met}$ and $tRNA_m^{met}$ both exist for binding to the two forms of methionine. In Prokaryotes the formylation of methionine is an important requirement to initiate translation. Thus, methionine is incorporated at the intermediate position of the peptide. In Eukaryotic system, the enzyme transformylase is usually absent. In plants, $tRNA_f^{met}$ is absent and incapable of accepting formylated methionine. In Prokaryotes the recognition of ribosomes with the mRNA occurs with the help of Shine-Delgrano sequence while in Eukaryotes it occurs at the 5′ capped end of the mRNA. The ribosome then reaches the AUG codon by scanning the mRNA. In Prokaryotes there is no requirement of initiation factor or cofactors necessary for initial complex formation between the ribosome and mRNA. However, in Eukaryotes ATP and a number of cofactors are necessary for the formation of the ribosome-mRNA complex. Moreover, in Prokaryotes the 30S subunit initiates the recognition of mRNA even prior to the binding of $tRNA_f^{met}$. In Eukryotes the 40S binds to mRNA only after the interaction of $tRNA_m^{met}$ with the ribosome.

Translation Initiation in Prokaryotes

The 30S subunit of the ribosome facilitates the formation of ribosome-mRNA complex. The initiation complex formed is 70S ribosome which receives the $tRNA_f^{met}$ at the AUG site of the mRNA. The initiation factors called IF1, IF2 and IF3 facilitate the formation of 70S ribosome complex. The initiation codon functions in coordination with the Shine

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Delgarno sequence of the mRNA which binds to the complimentary sequence present in the 3′ end of 16S rRNA. This sequence is known as the **anti-Shine-Delgarno Sequence**. In the mRNA the **Shine-Delgarno sequence** is located upstream to the translation initiation codon and it appears to be a ribosome binding site. Collectively this region of mRNA is termed as the **Translation Initiation Region.** Thus the 16S rRNA regulates the process of ribosome-mRNA interaction. The tRNA-ribosome interaction is facilitated by the certain bases in the 16S rRNA molecules. These rRNA bases are important for binding of the anticodon stem loop of tRNA to the P site of small subunit of ribosome. Furthermore, the tRNA interacts with the 23S rRNA of the larger subunit of the ribosome. This interaction occurs by the CCA terminus of the tRNA at the P site. The process of formylation of methionine is catalyzed by the transformylase enzyme in the presence of formyl-tetrahydrofolic acid.Various initiating factors contribute in the process of 70S ribosome formation. The cofactor IF2 associates with the Formylated Amino-tRNA complex present in the vicinity of the ribosome. This results in the formation of the 30S initition complex which is also known as the primary-ternary complex. The two cofactors IF1 and IF2 facilitate the irreversible association of the 30S-50S complex which forms the 70S unit of ribosome. Followed by this step, IF2 is released by hydrolysis of GTP. The $tRNA_f^{met}$ is now associated with the P site. In the aminoacyl site the Amino Acid forms peptide bond with the next adjacent Amino Acid. Thus, formation of a peptide bond results in the elongation of peptide.

Translation Initiation in Eukaryotes

Eukaryotes exhibit certain differences in the process of initiation of translation, although the basic mechanism remains similar in nature. The Eukaryotic system contains a large number of initiation factors associated with the assembly of ribosome. The initiation Amino Acid, i.e., methionine is not formylated in case of Eukaryotes. The smaller subunit of 40S rRNA interacts with the $tRNA_m^{met}$ irrespective of or prior to the ribosome-mRNA complex formation. The various Eukaryotic initiation factors are designated as eIF2, eIF2B, eIF3, etc. The process of translation initiation occurs with formation of a eIF2-GTP Binary Complex. This Complex associates with $tRNA_m^{met}$ to form a ternary complex. Furthermore, followed by the initiation process eIF2B facilitates the recycling of GDP to GTP and release of eIF2-GTP Complex. The ternary complex formed in the earlier step binds to the 40S subunit in the ribosome. The initiation factors eIF1, eIF1A and eIF3 facilitate to form the 43S Complex. Other factors like eIF3, eIF4B and eIF4F help in the association of mRNA to the 43S Complex. The 43S complex is bound to the 5′ end of the mRNA and scans the nucleotide sequence from the 5′-3′ direction. This process involves ATP hydrolysis. The two factors eIF1 and eIF1A guide in the process of scanning of mRNA and searching of initiation codon. The 43S Complex interacts with the AUG initiation codon. The AUG codon remains in the vicinity of **Kozak's sequence**. The first codon-anticodon base pairing takes place in the intermediate region of initiation codon and initiator tRNA in the Ternary Complex. During transfer of $tRNA_m^{met}$ into the P site GTP is hydrolyzed by eIF2. The reaction is facilitated by GTPase-Activating Protein. Along with the event of GTP hydrolysis eIF5B-GTP binds to the Complex. The other initiation factors dissociate and this is accompanied by the association of the 60S subunit to the 40S subunit-mRNA-tRNA complex. The dissociation of eIF5B marks the end of the initiation phase of translation. The Eukaryotic translation has been widely investigated in mammalian Red Blood Cells. Variations in the intermediate steps are likely to occur in various plant systems (Refer Figures 5.6 and 5.7).

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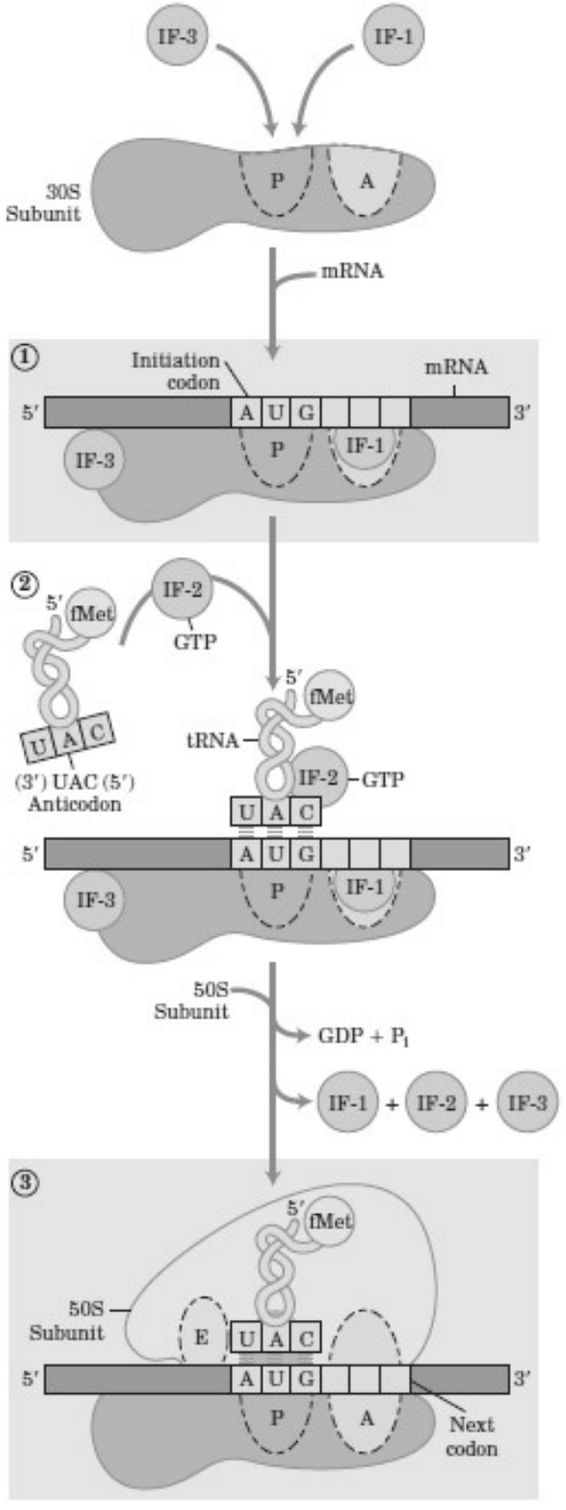

Fig. 5.6 *Formation of Intiation Complex in Prokaryotes (Adapted from Lehninger Biochemistry)*

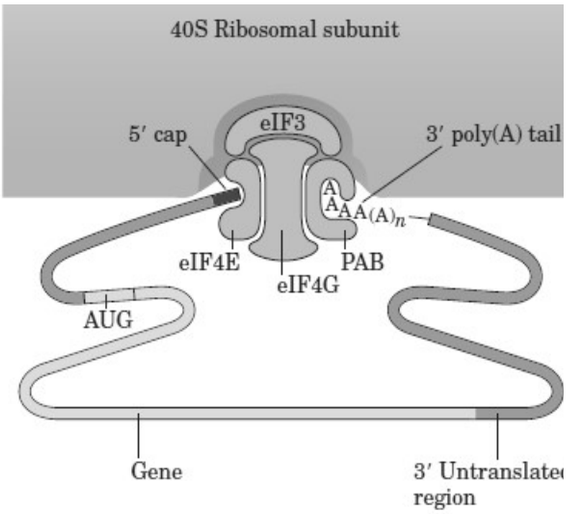

***Fig. 5.7** The Eukaryotic Initiation Complex (Adapted from Lehninger Biochemistry)*

Elongation of Polypeptide Chain: The process of elongation of polypeptide chain facilitated by peptide bond occurs through almost similar mechanisms in Prokaryotes, Eukaryotes and in Archaea. There are certain minor differences among the processes.

- **Elongation of Peptide in Prokaryotes**: The Prokaryotic translation involves peptide bond formation among the adjacent Amino Acids necessary for elongation of peptide. The process occurs in the 70S ribosome which carries the mRNA and tRNA complex. During ambient conditions around 15-20 Amino Acids are joined by peptide bond within the duration of a second. The rate of elongation is comparatively slower in Eukaryotes. Investigations have revealed that the ribosomes possess A, P and E sites necessary for translation. The AA-tRNA appears at the P site and renders the A site free for the next Amino Acid. After the formation of the 70S initiation complex the new aminoacyl tRNA enters the A site of ribosome. The proper binding of the new aminoacyl tRNA requires the presence of elongation factor called EF-Tu. This elongation factor is a G Protein which combines with GTP and forms a binary complex called EF-Tu-GTP. This binary complex now binds to the aminoacyl tRNA to form a ternary complex. The ternary complex is EF-Tu-GTP-aminoacyl tRNA which binds in the A/P hybrid state. Followed by this step the GTP gets hydrolysed to release EF-Tu from the ternary complex. This results in the movement of CCA end of the tRNA into the A site. The EF-Tu is later dissociated to accept new GTP molecules which help in starting a new cycle.

  The process of peptide bond formation is a catalytic process which involves the reaction between the –COOH and $–NH_2$ group of the adjacent Amino Acids. The process is catalyzed by the activity of peptidyl transferase enzyme which helps in translocation of aa-tRNA. The aa-tRNA possesses its CCA end with Amino Acid moves for the formation of peptide bond. After the formation of peptide bond the tRNA at the P site is deacylated and the tRNA in A site remains attached with the nascent polypeptide. The peptidyl-tRNA is translocated from the A site to the P site. The aminoacyl end of the tRNA remains bound to the A site and later moves to the P site on the 50 S subunit. The newly formed peptidyl tRNA remains in the hybrid state. The anticodon of the tRNA gets shifted from the A site to P site. The elongation factor EF-G gets attached to the ribosome and is released during GTP hydrolysis. Since the EF-Tu and

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EF-G factors are unable to bind to the ribosome simultaneously, the entry of aminoacyltRNA at the A site and the movement of peptidyl tRNA at the P site has to be undertaken sequentially (Refer Figure 5.8).

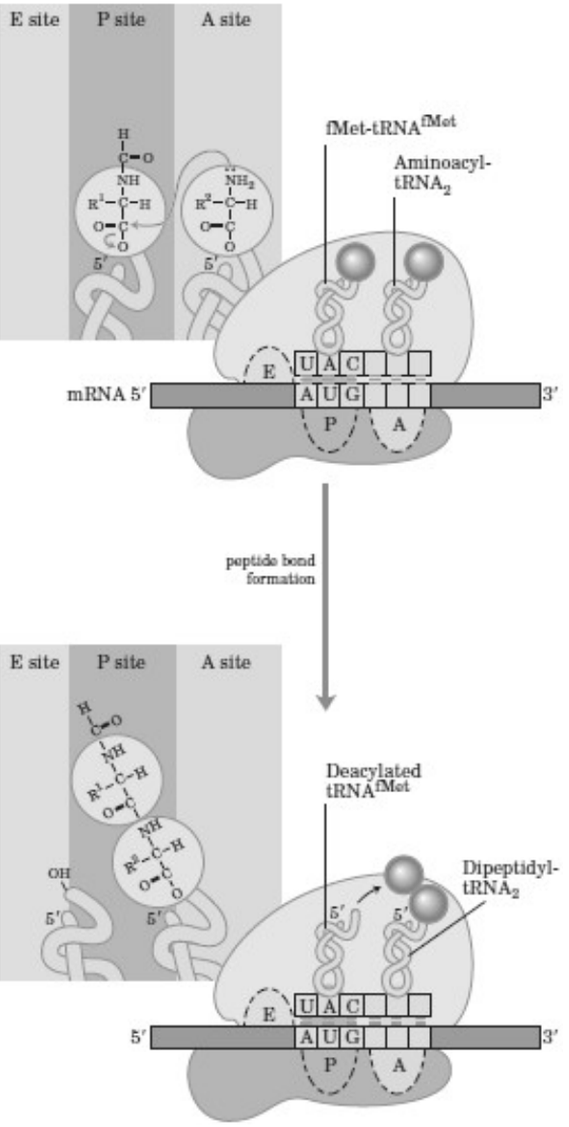

Fig. 5.8 *Elongation Step in Prokaryotes (Adapted from Lehninger Biochemistry)*

- **Elongation of Polypeptide in Eukaryote:** The process of elongation in Eukaryotes does not exhibit any difference in the mechanism. However, the additional elongation factor operative here is the eEF1 and eEF2. Another important elongation factor has been reported in Yeast, .i.e., eEF3.

  Termination of Polypeptide Chain: The process of termination of peptide occurs in the presence of UAA, UAG and UGA termination codons. This phenomenon is common in both the Eukaryotes and Prokaryotes.

- **Termination in Prokaryotes**: The release factors mainly RF1 and RF2 recognize the termination codons. RF1 is capable of recognizing the UAA and UAG codons while the RF2 recognizes UAA and UGA. UGA is recognized by RF2 only. The

release factors facilitate the ribosome at the A site to recognize the Stop Codon. RF3 facilitates the action of RF1 and RF2 in a GTP dependent process. The polypeptidyl tRNA in order to get released must be present in the P site. The release factors help in the splitting of the –COOH group of the polypeptide and the last tRNA carrying the polypeptide chain (Refer Figure 5.9).

- **Termination in Eukaryotes**: The process of termination on Eukaryotes involves the usage of release factors, i.e., eRF1and eRF3 which are regulated by GTP. This cofactor corresponds to the RF1 of Prokaryotes and recognizes the three termination codons.

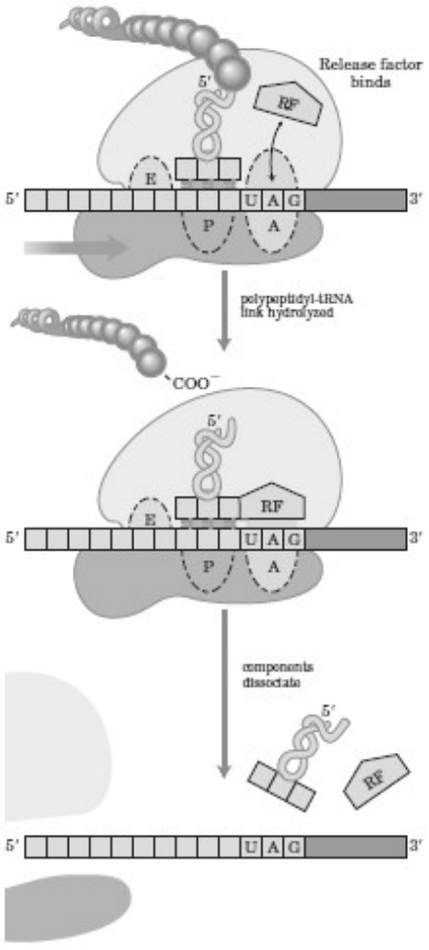

Fig. 5.9 *Termination Step in Prokaryotes (Adapted from Lehninger Biochemistry)*

Dissociation of Ribosomes

Followed by the release of polypeptide chain, Ribosome Recycling Factor (RRF) in association with EF-G and IF3 helps in recycling of ribosome. RRF in association with EF-G binds to the empty A site of the ribosome and stimulates the release of the uncharged tRNA molecules. After the release of the tRNAs the RRF and EF-G factors dissociate from the ribosomes. IF3 contributes to the release of the mRNA and ribosomal subunits from each other.

• Translation in Semi-Autonomous Organelles (Chloroplast and Mitochondria)

The two semi-autonomous organelles, i.e., Chloroplast and Mitochondria possess their own components of translation. The genes for some of these components are present in the nuclear genome and transported from cytosol to the organelles after their synthesis. The bacterial translation inhibitor **chloramphenicol** also inhibits the process in these organelles. In Eukaryotic translation **cycloheximide** is an important inhibitor. The bacterial translation is inhibited by other antibiotic agents like linomycin and erythromycin. Interestingly, the translatin process in these organelles is operated through 70S ribosome. Thus the process appears to be similar to the eubacterial type of translation. The Shine-Delgarno sequence which is common in Prokaryotic mRNA-ribosome recognition is also evident in chloroplast mRNAs. The mitochondrial translation involves synthesis of complete or partial subunits of Protein necessary for the process of respiration and electron transport chain. The rRNA of mitochondria is exclusively transcribed from the mtDNA.

'Check Your Progress'

5. What is Class I-synthetases?
6. Give the funcrtions of Class II-synthetases.
7. Define Shine Delgarno sequence.
8. Define translation initiation region.

5.4 SUMMARY

- The fats or glycerides are esters of long chain fatty acids bound to the trihydroxyalcohol called Glycerol.
- Most glycerides in nature exist in the form of esterified molecules of fatty acids along with the 3 –OH groups of glycerol attached with it.
- Oil seeds like Soybean, Groundnut, Canola, Sunflower and Linseed are the major source of stored Lipids.
- Biochemical connection between the lipid and organic acid pathway provide cues for the source of energy in germinating seedlings incapable of carrying out photosynthesis.
- The membrane lipids and their derivatives serve as signaling intermediates in various pathways.
- The biosynthesis of triacylglycerol and polar glycerolipids are important pathways regulating the process of lipid metabolism in plants.
- Lipids represent a more reduced form of Carbon, the complete oxidation of 1 gm lipid molecule liberates 40 kJ or 9.3 kilo Cal of energy.
- The biosynthesis of membrane lipids, mainly phospholipids, requires a higher amount of metabolic energy.
- Plant fats and oils mostly exist in the form of triacylglycerols or triglycerides. In this case the fatty acid molecules are linked with ester bonds to the three –OH groups of glycerol molecules.

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- The fats are saturated lipids which remain solid at the room temperature. Most plant-derived fats are Unsaturated in nature (Oleic Acid, Linoleic Acid and Linoleneic Acid). However, certain amount of Saturated fats (Stearic Acid, Palmitic Acid) may also be present in the plant tissues.
- The seeds are the major organs which exhibit the presence of olesomes in the cytoplasm of cotyledons or in endosperms.
- The membrane of oil body are comprised of phospholipids and various hydrophobic Proteins namely Oleosins, Steroleosins and Caleosins. The oleosins help in stabilizing the membrane of oilbodies.
- The alignment of the lipid molecules in aggregation provides amphiphatic nature to the biological membranes.
- The process of biosynthesis of fatty acids in plants is primarily accomplished in the plastids.
- The conversion of lipids into soluble sugars in the germinating seeds is essential to provide respiratory substrate. The process of breakdown of fatty acids into Acetyl-CoA occurs in specialized organelles called glyoxysomes.
- The formation of esterified glycerol-lipids may often involve the presence of phosphate groups as a polar head in their structure. These molecules are referred to as phospholipids.
- The breakdown of phospholipids is accomplished by the activity of phosphatidases which catalyze the hydrolysis of fatty acids and phosphoric acid ester bonds.
- The fluidity of the membrane is largely influenced by the composition and specific complements of lipids.
- The process of fatty acid biosynthesis is accomplished by a sequential process of condensation of 2 Carbon units. The formation of acyl chains occur by covalent bond formation with Acyl-Carrier Protein (ACP).
- The process of fatty acid biosynthesis is accomplished by a sequential process of condensation of 2 Carbon units. The first step of fatty acid synthesis involves the formation of Malonyl-CoA from Acetyl-CoA. In the following step Malonyl-CoA combines with ACP to form ACP-Malonyl-CoA.
- The enzyme fatty acid synthetase is a Complex assemblage of multienzyme Complex in association with a Acyl-Carrier Protein (ACP).
- The process of fat or triglyceride synthesis does not occur directly from fatty acids and glycerol but from α-glycerophosphate and CoA derivatives.
- The process of glycerolipid synthesis follows two pathways, i.e., the chloroplastidal pathway and the ER pathway.
- Long chain fatty acids are broken down by the process of α-oxidation or β-oxidation which produces 2-C units of Acetyl-CoA.
- The β-oxidation pathway has been reported to be more common for saturated fatty acid breakdown in plants.

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- The mechanism has not been clearly revealed in the case of unsaturated fats. The process involves oxidation of β-C in the fatty acids. Thus it is named as β-oxidation pathway.
- Translation is an efficient process with high accuracy. It has been reported that E. coli cells at 37°C are capable of synthesizing around 100 Amino Acid long peptide in 5 minutes.
- In Eukaryotes the process of translation involves the association of 70 different ribosomal Proteins, 20 enzymes to activate the Amino Acid precursors and 15 auxillary enzymes.
- The phenomenon of RNA silencing prevents the process of mRNA binding to ribosome and its translation.
- In the middle of the 19th century Paul Zamecnik and his group investigate the possible site of translation in rat liver cells.
- The tRNA-aminoacid conjugation is also an enzymatic process catalyzed by Amino-Acyl-tRNA synthase enzyme. This enzyme performs two main functions ie. activation of Amino Acid for peptide bond formation and attachment of Amino Acid to the respective carrier tRNA.
- The process of Protein synthesis in Prokaryotes and Eukaryotes possess significantly lower chances of errors.
- The A, P and E sites in the ribosome are associated with initiation, elongation and exit of uncharged tRNA molecules.
- The steps of initiation, elongation and termination of translation are regulated by various Protein factors.
- The semi-autonomous organelles of chloroplast and mitochondria also possess specific apparatus for accomplishing the translational mechanisms.
- Various plants (like Tobacco, Maize, Spinach and Barley) and algal members (*Chlamydomonas* sp.) have been investigated for deciphering the process of chloroplastidial translation.
- The group of ribosomes together bound to the mRNA molecule is known as polyribosome or ergosomes.
- The Eukaryotic mRNA is monocistronic in nature and therefore associates with around ten to fifteen ribosomes.
- The Prokaryotic mRNA being polycistronic may contain several ribosomes attached to it.
- The sedimentation coefficients of each subunit of the ribosomes are designated by Svedberg unit. Bacterial 70S has been reported to possess a diameter of 23 nm.
- Eukaryotic ribosomes are larger in size (80S) and possess a smaller 40S subunit and a larger 60S subunit. The association of the smaller and larger subunits is regulated by the concentration of Mg^{2+}.
- The rRNA comprises of 60-70% of the entire ribosomal composition.
- The 30S subunit is composed of 16S rRNA and 21 different Proteins.
- The 50S subunit contains 23S rRNA, 5S rRNA and 32 different types of ribosomal Proteins.

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- Various microorganisms like *E.coli*, *Thermus thermophilus*, and *Haloarcula marismortui* have been implied for the investigations of the structure of r-Protein.
- The association of the RNA and Protein molecules occur by loops and Protein tails being inserted between RNA helices.
- Mg^{2+} present in the vicinity of the RNA-Protein complex help in stabilizing the tertiary structure of 23S and 5S rRNA molecules.
- In Bacteria the genes for 5S, 23S and 16S rRNAs are clustered in a single region of the operon which works as a functional unit.
- The ER membrane forms an internal lumen and carries ribosomes on its external surface. Based upon the presence and absence of ribosomes the ER are classifed as smooth ER and rough ER.
- In 1964, R.W. Holley and his group reported the detailed structure of tRNA from Yeast cells.
- All tRNA molecules possess guanine residue at the 5′ terminal end and a CCA′ sequence at the 3′ end.
- The Class I tRNA consist of a small variable loop which comprises of 3-5 bases. The Class II tRNA possesses a large variable loop with 13-21 bases along with 5 base pairs in the variable stem region.
- The tertiary conformation of the tRNA molecule is derived from the secondary structure of clover leaf model. The L shaped tertiary structure is formed by condensing four arms of the clover leaf conformation.
- The capability of the tRNA molecules to recognize Amino-Acyl tRNA synthase is crucial in maintaining the efficiency of translation. The enzymes are monomeric, dimeric or tetrameric units. The active site of the enzyme constitutes a smaller part of the Protein.
- Based on the structural type of the enzyme it has been classified into two groups each comprised of ten enzymes.
- The process of recognition of different aminoacyl tRNA synthase enzymes by their respective tRNA molecules is referred to as the second genetic code.
- The process of translation in initiated by the formation of Amino-Acyl tRNA complex.
- The synthesis of polypeptide chain is initiated by the Amino Acid Methionine. This Amino Acid is coded by AUG. GUG also rarely codes for Methionine.
- In Prokaryotes formylated form of Methione is used as an initiating Amino Acid.
- In Eukaryotic system the enzyme transformylase is usually absent. In plants $tRNA_f^{met}$ is absent and incapable of accepting Formylated Methionine.
- The initiation complex formed is 70S ribosome which receives the $tRNA_f^{met}$ at the AUG site of the mRNA.
- The initiation factors called IF1, IF2 and IF3 facilitate the formation of 70S ribosome complex.
- The initiation codon functions in coordination with the Shine-Delgarno sequence of the mRNA which binds to the complimentary sequence present in the 3′ end of 16S rRNA.

- In the mRNA the Shine Delgarno sequence is located upstream to the translation initiation codon and it appears to be a ribosome binding site.
- The Eukaryotic system contains a large number of initiation factors associated with the assembly of ribosome. The process of translation initiation occurs with formation of a eIF2-GTP binary complex.
- The process of peptide bond formation is a catalytic process which involves the reaction between the –COOH and $–NH_2$ group of the adjacent Amino Acids.
- The peptidyl-tRNA is translocated from the A site to the P site. The aminoacyl end of the tRNA remains bound to the A site and later moves to the P site.
- The process of termination of peptide occurs in the presence of UAA, UAG and UGA termination codons. This phenomenon is common in both the Eukaryotes and Prokaryotes.
- Followed by the release of polypeptide chain, Ribosome Recycling Factor (RRF) in association with EF-G and IF3 helps in recycling of ribosome.

5.5 KEY TERMS

- **Glycerides:** Glycerides are esters of long chain fatty acids bound to the trihydroxyalcohol called glycerol.
- **Waxes:** Waxes are Esters of fatty acids with high molecular weight monohydroxy alcohols.
- **Phospholipids:** Phospholipids (phosphatids) lipid molecules contain phosphate group in the form of phosphoric acid, nitrogen and other components.
- **Glycolipids:** Glycolipids contain carbohydrate groups attached to the esterified forms.
- **Spherosomes:** Oil bodies or specialized organelles called oleosomes.
- **Glyoxysome:** Specialized organelles with double membrane (specialized peroxysomes) carrying out glyoxylate cycle in fatty seeds and cotyledons.
- **Polyribosome or ergosomes:** The group of ribosomes together bound to the mRNA molecule.
- **Pseudouridine, inosine and dihydroxy uridine:** Modified bases in tRNA formed by methylation.
- **Clover leaf model:** Secondary structure of tRNA showing intra-strand complementary sequences.
- **Kozak's sequence:** Specific mRNA sequence necessary for ribosome-mRNA recognition in Eukaryotes.

5.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Phosphatidylcholine (lecithin) are the phosphorylated moiety of the phopsholipid contains choline group present as a main component.
2. Phosphatidylinositol is the presence of a hexahydric alcoholic group called inositol in the phosphorylated moiety of phospholipid.

3. Phosphatidylethanolamine is the presence of ethanolamine group in the phosphorylated moiety.
4. Desaturase enzymes function is to desaturase enzyme is to insert a double bond at specific positions of the fatty acids.
5. Class I-synthetases possess an N-terminal catalytic domain consisting of two conserved sequences which are called signature sequence.
6. Class II synthetases is the type of synthetase enzyme possess a C-terminal catalytic domain which is formed of a large antiparallel â sheet covered by α-helices.
7. Shine-Delgarno sequences of the mRNA upstream to the translation initiation region which binds to the complimentary sequence present in the 3(phosphatids) end of 16S rRNA. The sequence is present in Prokaryotic mRNA and help in formation of ribosome-mRNA complex
8. Translation initiation region can be defined as the mRNA the Shine-Delgarno sequence is located upstream to the translation initiation codon and it appears to be a ribosome binding site. Collectively this region of mRNA is termed as the TIR.

5.7 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Define glycerides. How lipids are classified?
2. Why fats are considered as high energy sources?
3. Germinating seeds exhibit a decrease in fats accompanied by an increase in sugar content. Why?
4. Define spherosomes and oleosins. What are their functions?
5. How many ATPs are liberated by the complete β-oxidation of a C18 fatty acid?
6. Define the termination step of translation phase.
7. Explain the secondary structure of tRNA. Name three antibiotics used as translational inhibitors in Prokaryotes and Eukaryotes.

Long-Answer Questions

1. Explain briefly the pathways of fatty acid synthesis in plants.
2. Discuss the various types of phospholipids found in plants.
3. Explain the process of glycerol synthesis in plants.
4. What do you mean by α-oxidation and β-oxidation of fatty acids? Schematically explain the pathway of β-oxidation of fatty acids. Mention the significance of the pathway.
5. How many ATPs are produced in a single cycle of β-oxidation of fatty acids? Explain giving examples
6. Define Shine-Delgarno sequence with the help of examples. Explain the significance of Kozaks sequence.
7. Explain a comparative account of translation mechanism in Prokaryotes and Eukaryotes.
8. Give a detailed note on second genetic code.

NOTES

5.8 FURTHER READING

Hopkins, William G. and Norman P.A. Huner. 2013. *Introduction to Plant Physiology*, 4th Edition. Noida: Wiley India Pvt. Ltd.

Verma, S K and Mohit Verma. 1995. *A Textbook of Plant Physiology, Biochemistry and Biotechnology*. New Delhi: S Chand & Company Ltd.

Blackstock, James C. 2014. *Guide to Biochemistry*. Oxford: Butterworth-Heinemann.

Park, K. H. 2008. *Carbohydrate-Active Enzymes: Structure, Function and Applications*. Amsterdam: Elsevier.

Fromm, Herbert J. and Mark Hargrove. 2012. *Essentials of Biochemistry*. Berlin: Springer.

Fearon, William Robert. 2014. *An Introduction to Biochemistry*. Amsterdam: Elsevier.