Uttar Pradesh Rajarshi Tandon
Open University

DCEBCH-108
Plant Biochemistry

COURSE INTRODUCTION

The objective of this course is basic introduction of **Plant Biochemistry**. Because, plant biochemistry deals the chemical aspects of plant and its regulation and metabolic activities. The plant physiology that's governs by various chemicals in plants is studie by plant biochemistry. In this course, students will learn about different chemical and biological reactions such as photosynthesis, respiration, plant growth regulatory hormones and enzymes. The carbon and nitrogen assimilation and it synthesis process also discuss in plant biochemistry. This course contains the brief process of chemical reaction. The course is organized into following blocks:

Block 1 covers the basics of Electron Transport System (ETS) and nitrogen metabolism

Block 2 deals the nutrition of Photosynthetic process and carbon assimilation

Block 3 describes in brief the plant stress growth regulators

Uttar Pradesh Rajarshi Tandon
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DCEBCH-108
Plant Biochemistry

Block -I

Electron Transport system and Nitrogen Metabolism

ETH-036

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Introduction

This is the first block of Plant biochemistry consists of three units.

Unit-1: In the first unit that have general introduction of electron transport system. ETS system the Oxidative Phosphorylation is discussed in details. The oxidative phosphorylation is important pathway in the respiration system in plant and it provide passage to electron transport. The mitochondrial respiratory complexes are also discussed in this system.

Unit-2: This unit covers the basic introduction of nitrogen metabolism: the assimilation of nitrate and assimilation of ammonia into organic compounds also discussed in this unit. The enzyme of nitrate reduction and their regulation is highly discussed here.

Unit-3: This unit covers the Nitrogen fixation and assimilation. The Biological nitrogen fixation by free living and symbiotic association discussed in details. The structure and function of enzyme nitrogenase and nitrate assimilation is also discussed in this unit.

Unit-1.Electron Transport System in Plants

Contents

1.1. Introduction

The process in which electrons are transferred from NADH and $FADH_2$ to oxygen and the energy released in this oxidation- reduction reaction is used to synthesize ATP from ADP is known as Oxidative-phosphorylation. Oxidative phosphorylation, being a mitochondrial process, is studied by isolating and then fragmenting mitochondria. Oxidative Phosphorylation is the process whereby the free energy that is released when electrons are transferred along the electron transport (respiratory) chain is coupled to the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi). Oxidative phosphorylation is the main source of energy in aerobic cell. There are four complexes through which electrons pass to oxygen and several metal ions or prosthetic groups which are compactly and specifically connected with these complexes. The mitochondrial electron transport chain i.e. mitochondrial respiratory chain consists of five protein complexes:

1) Complex I : NADH-ubiquinone oxidoreductase
2) Complex II : succinate-ubiquinone oxidoreductase.
3) Complex III: Ubiquinone cytochrome- c oxidoreductase.
4) Complex IV: cytochrome-c oxidase o Complex V: ATP synthase

Objectives

- To understand the structural role of mitochondria in metabolic process

- To known how the oxidative phosphorylation take place in mitochondrial membrane
- To describe various components of electron transport chain
- To describe mechanism of pentose phosphate pathway in plants

1.2. Oxidative phosphorylation

The process (confined to the mitochondria of higher plant and animal cells) by which ATP is formed by the use of energy librated during the electron transport system known as oxidative Phosphorylation. The NADH + H^+ and $FADH_2$ formed in TCA and NADH + H^+ in glycolysis are an energy rich molecule. Each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. This is called oxidative phosphorlation or respiratory chain phosphorylation. In Oxidative phosphorylation, ATP is formed as a result of the transfer of electrons from NADH + H^+ or $FADH_2$ to O_2 by a series of electron carriers. This process, which takes place in mitochondria, is the major source of ATP in aerobic respiration.

1. It is a dehydrogenation reaction in which electron releases to form a molecules such electron cannot exist in free state and must received at once by hydrogen or electron acceptors.
2. The hydrogen atom and their electron are transferred to the first hydrogen acceptor, the NAD^+ , which is then reduced to NADH + H^+. NADH + H^+ oxidize in presence O_2 and 3 molecules of ATP are formed.
3. $NADH + H^+ + 1/2O_2 + 3ADP + 2Pi \longrightarrow NADH + 3ATP + H_2O$
4. From the reduced NAD or NADH + H^+ the hydrogen atoms and their electrons are passed on the FAD, which to reduced to $FADH_2$
5. In the next step, FADH2 is oxidized when it delivers the hydrogen atoms and their electrons transmit to the coenzyme Q. For example succinate dehydrogenase is an enzyme found in inner mitochondrial membrane. It also flavoprotein with FAD as the coenzymes.

6. Form reduced coenzyme Q hydrogen atoms are released in the cytoplasm and electron are now passed in a series of cytochromes (b, c, a and a_3). In this process cytochromes is alternatively reduced and oxidized.
7. In the last step the electrons are finally transferred from Cytochrome a_3 (also called Cytochrome oxidize) to the final acceptor or the molecular O_2.
8. The O_2 then units with hydrogen atoms (protons) to form water
9. 2 cytochromes a_3 (Fe^{++}) $+ 2H^+ + \frac{1}{2} O_2 \longrightarrow$ 2 Cytochromes a_3-$Fe^{+++} + H_2O$

The respiratory break down of simple carbohydrates in presence of oxygen is an oxidative process. During which many intermediates such as phosphoglyceraldehyde, pyruvic acid, iso-citric acid, α-ketoglutaric acid, succinic acid and malic acid are oxidised. The oxidation of all these is brought about by removal of a pair of hydrogen atoms (2H) from each one of them. The pair of hydrogen is usually picked from the substrate by NADH + H^+ or $FADH_2$ in the following manner.

NADH or $FADH_2$ released in glycolysis and Krebs cycle, finally reduce O_2 to H_2O. The transfer of H^+ and e^- from reduced NAD^+ or FAD to O_2 is not a simple process. The NADH gets oxidised at redox potential of -0.32V and O_2 is reduced at redox potential of +0.82V. Thus there is a gap of +1.14V in redox potential which is too much. Therefore, NADH and $FADH_2$ connot directly combine with O_2 to form H_2O. Many intermediate cytochromes and other carriers having intermediate redox potential are arranged in a series which transport electrons from reduced NAD^+ or FAD to O_2 and form electron transport system (ETS). As electron transport down to energy gradient through electron transport system results in the formation of ATP (Adenosine triphosphate) from ADP (Adenosine diphosphate) and inorganic phosphate. The ATP produced here is due to oxidation reduction reaction therefore, known as oxidative phosphorylation.

For example metabolic in the cytoplasmic oxloacetic acid which can be reduced to malic acid by the intramitochondrial reduced NAD^+ malic acid enter into where it is reoxidized by the intramitochondrial NAD in the present of malic dehydrogenase with the regeneration of oxalic acid. Thus the reduced

NAD^+ may be reoxidized by oxidised flavoproteins which accept the hydrogen from the NAD^+.

1.3.Electron Transport System (ETS)

The electron transport chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called chemiosmosis. Together, the electron transport chain and chemiosmosis make up oxidative phosphorylation. The electron transport chain involves a series of redox reactions that relies on protein complexes to transfer electrons from a donor molecule to an acceptor molecule. As a result of these reactions, the proton gradient is produced, enabling mechanical work to be converted into chemical energy, allowing ATP synthesis. In eukaryotes, the electron transport chain is located in the inner mitochondrial membrane. In prokaryotes, it is located within the plasma membrane. In electron transport, form organic subtract to molecular oxygen, four types of protein participate.

1.4. Steps of the Electron Transport Chain (ETC)

Electron transfer chain over four steps where electrons move along a series of proteins to generate an expulsion type force to move hydrogen ions, or protons, across the mitochondrial membrane. The electrons begin their reactions in Complex I, continuing onto Complex II, traversed to Complex III and cytochrome C yia coenzyme Q, and then finally to Complex IV.

Complex I

Complex is composed of flavin mononucleotide (FMN) and an enzyme containing iron-sulfur (Fe-S). FMN is one of several prosthetic groups or co-factors in the electron transport chain. In this reaction non-protein molecule is present as a prosthetic group required for the activity of a protein Prosthetic groups include co-enzymes. The enzyme in complex I is NADH dehydrogenase, a very large protein containing 45 amino acid chains. Complex

I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space; Complex I, also known as NADH dehydrogenase. It is in this way, the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

In this step another electron carrier and coenzyme like succinate is oxidize into fumarate. Where FADH2 revive from oxidation of FAD (flavin-adenine dinucleotide). The transport molecule, FADH2 is then reoxidized, donating electrons to Q (becoming QH2), while releasing another hydrogen ion into the cytosol. Q receives the electrons derived from NADH + H^+ from complex I and the electrons derived from FADH2 from complex II, including succinate dehydrogenase. While Complex II does not directly contribute to the proton gradient, it serves as another source for electrons.

Complex III

In this chain, the Q cycle is takes place and, this complex is also called Cytochrome oxidoreductases. Cytochrome proteins have a prosthetic heme group. The interaction between Q and cytochromes occurs where the molecules composed of iron continue transfer the electrons. This electron fluctuating between different oxidation states: Fe^{2+} (reduced) and Fe^{3+} (oxidized). Complex III pumps protons through the membrane and passes its electrons to cytochrome C for transport to the fourth complex of proteins and enzymes. The Cytochrome C that moves the electrons to the last complex. In the process, another hydrogen ion is released into the cytosol to further create the proton gradient.

Complex IV

The fourth complex is composed of Cytochrome proteins c, a, and a_3. Electrons are transferred one at a time into the complex from Cytochrome C. The electrons, in addition to hydrogen and oxygen, then react to form water in an irreversible reaction. The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The

reduced oxygen then picks up two hydrogen ions from the surrounding medium to produce water (H_2O). This is the last complex that translocates four protons across the membrane to create the proton gradient that develops ATP at the end. As the proton gradient is established, F1F0 ATP syntheses, sometimes referred to as Complex V, generates the ATP.

The structure is a series of proteins embedded in a membrane that pump hydrogen ions in one direction to create a concentration gradient - the function is to generate ATP. The electron transport proteins accept high energy electrons from the electron carriers NADH + H^+ (in photosynthesis) and NADH + H^+ and $FADH_2$ (in cellular respiration), and through the action of transporting them from one to the other in a series of electron exchanges, small units of energy are extracted and used to pump hydrogen ions.

In cellular respiration they are pumped from the matrix into the intermembrane space of the mitochondria - in photosynthesis they are pumped from the stroma into the lumen of the thylakoids.

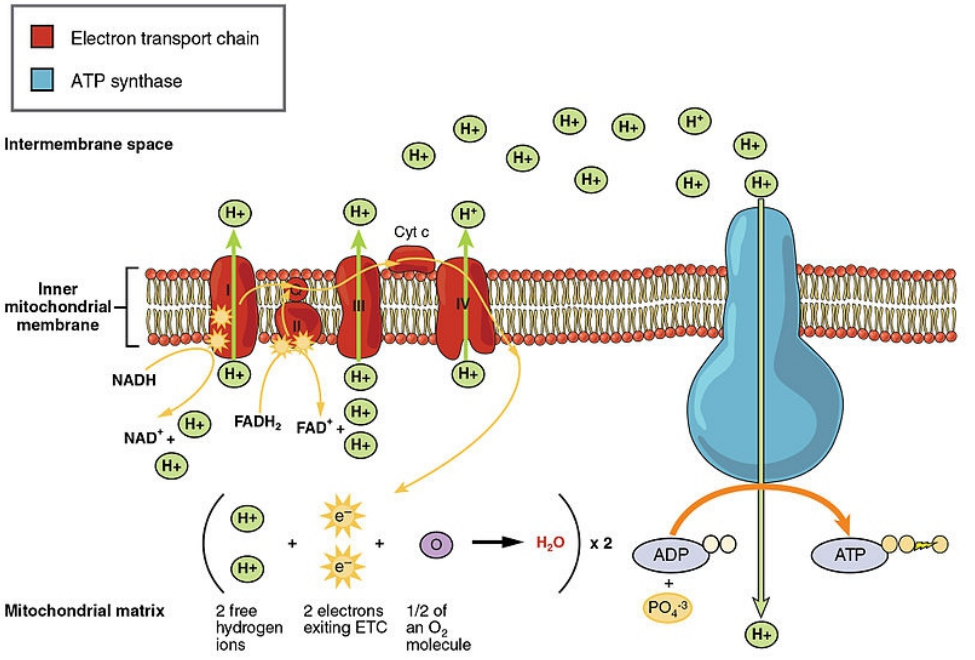


Fig. 1.1: Electron transport chain in mitochondria

Source: https://socratic.org/questions/what-is-the-structure-and-function-of-electron-transport-chain-in-chloroplast-an

In both cases, the high concentration of hydrogen ions can't cross the membrane (due to their charge), and place a great deal of osmotic pressure on the membrane. This pressure drives the hydrogen ions from [high] --> [low] through the enzyme ATP Synthase - using this energy to produce ATP molecules.

1.5.Mitochondria:

1.5.1. Structure and its functions

Mitochondria are ubiquitous organelles of eukaryotic organism found in constant number and covered by double unit membrane. Each of membrane is lipo-proteinic in nature. The outer membrane is about 60 to 75 A^0 thick while inner membrane is about 50 to 75^0 thick. The outer membrane of the mitochondria smooth and somewhat elastic and inner membrane has number of inward fold called cistae. The structure and number of cistae depends on the cell types. The outer surface of inner membrane called C face and inner surface called M-face. Generally, the metabolically active mitochondria have more matrixes like structure that contain about 50% protein The cristae are extended into mitochondrial matrix. Cristae may be branched or soft, complete or incomplete, strainght or zigzag, trubular or lamellar structure. These criste increase the surface are of inner membrane that is porn form more enzymatic activity. The inner membrane of mitochondria is consistes by diphosphatidly glycerol and has more protein. The two membranes are separated by an electron transparent region of about 80A wide that is called primitochondrial space. The primary function of membrane is to generate energy supplies in the form of ATP through oxidative Phosphorylation. The inner membrane of mitochondria is the site of electron transport system. The enzymes involved in fatty acid synthesis and ATP synthesizing complex are also located in the inner membranes. All enzymes of the Krebs cycle are found in the matrix, ensuring high enzyme concentration and reduced loss of intermediates. The inner mitochondrial membrane is the site of oxidative phosphorylation and contains the electron transport chain. It is folded into cristae, creating a large surface area for oxidative phosphorylation to occur, increasing the rate of respiration.

The selective permeability of the inner membrane prevents protons crossing the membrane, causing a high concentration of protons in the intermembrane space when they are pumped by the proton pump. The mitochondria are often preseont near the structure that require ATP or near source of fuel. Mitochondria are known as the "powerhouse" of the cell, generating ATP via oxidative phosphorylation (OXPHOS) complexes, which are present in the inner membrane of mitochondria.

Elementary or F_1 particles:

The electron microscopic image mitochondria reveal the structure of inner membrane mitochondria and cristae, both have knob like projections, which is known as elementary particles (EP), stalked particles or respiratory assemblies. These particles are a base, a stalk and a spherical head. The spherical head consist of a soluble protein is called as F1 coupling factor, which is considered as an enzyme responsible for ATP synthesis during oxidative Phosphorylation. The mitochondria are self replicating organelles that raise form pre existing ones by fragmentation, fission or by budding. The function of mitochondria is to oxidize dicarboxylic or tricarboxylic acids resulting into their oxygen products like CO_2, H_2O and ATP etc. the mitochondria matrix also contain all enzymes of Krebs cycle and electron transport chain also located in base piece of stalked particles where enzymes responsible for oxidative Phosphorylation. The oxidative Phosphorylation occurs in head piece.

The complexes are known as NADH: ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), ubiquinol–cytochrome c oxidoreductase (complex III, or cytochrome bc1 complex), cytochrome c oxidase (complex IV), and ATP synthase (complex V).

The NADH is first enzyme of respiratory system produced by Krebs cycle in the mitochondrial matrix. NADH is received two electrons and reduces ubiquinone to ubiquinol. After that the ubiquinol is further re-oxidize into cytochrome bc1 complex and transfers electrons to reduce molecular oxygen to water at complex IV. First complex (NADH) is consider as measure entering

part of respiratory chain and also know as rate limiting step in overall respiration.

Many electron carriers cytochromes are appeared in a definite sequence in inner membrane. They together formed electron transport system of energy rich molecules such as $NADH + H^+$ and $FADH_2$ for oxidation. Inner membrane has pin head partials called oxysomes, or elementary particles or F-F particles. These head of particles composed of ATPas enzymes and concern with oxidation of Phosphorylation.

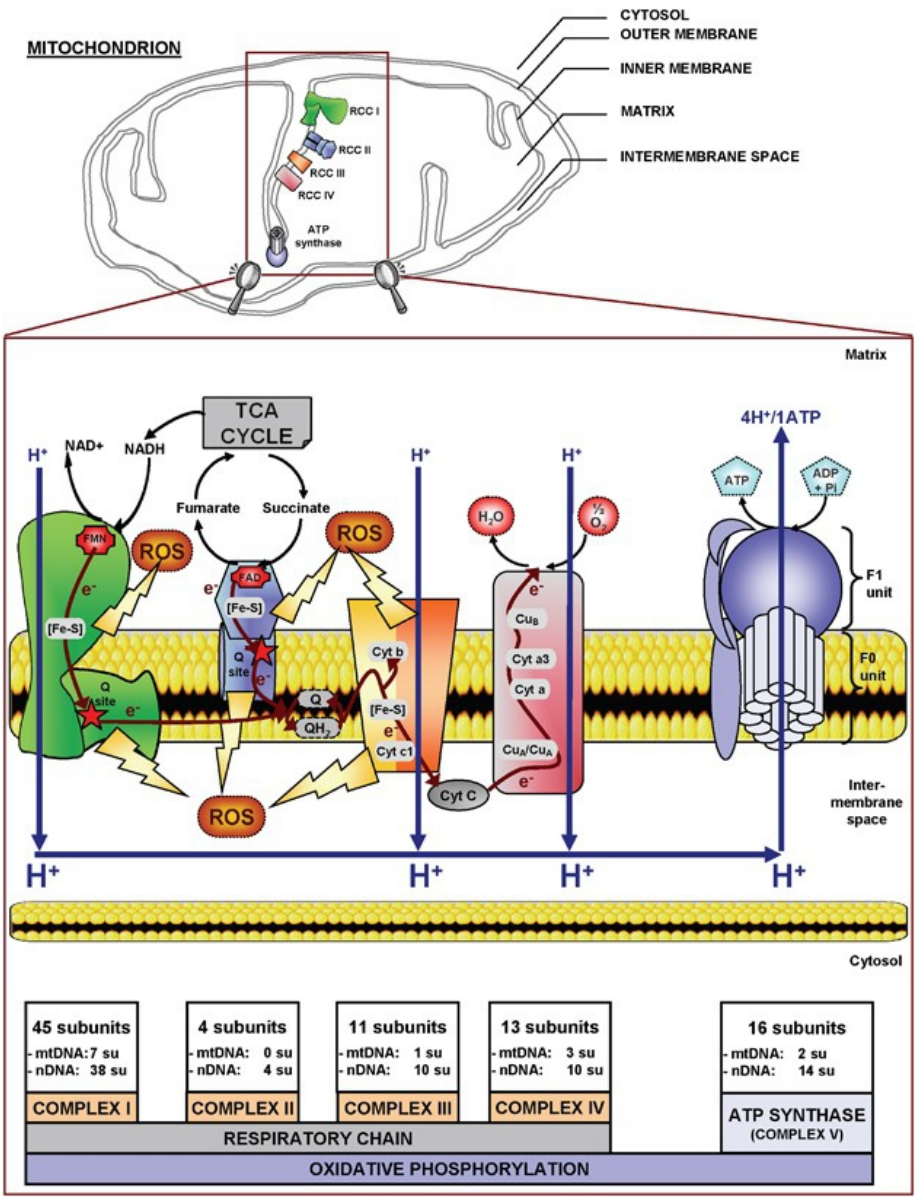


Fig.1.2: Oxidative Phosphorylation

Enzymes localizations in mitochondria

The outer, the inner, and the matrix have umber of compounds and enzymes. The inner membrane contains cytochromes b, c, c_1 a and a_3, the F_1 ATPhase associated with the mechanism of oxidative phosphorylation and certain dehydrogenase. The outer membrane have distinct monoamine oxidase, a flavoprotein that catalyze the oxidation of various monoamine. Outer membrane also contain 50% of lipids. Whereas, inner has 20% lipids. The enzymes like adenylate also found in space between membranes.

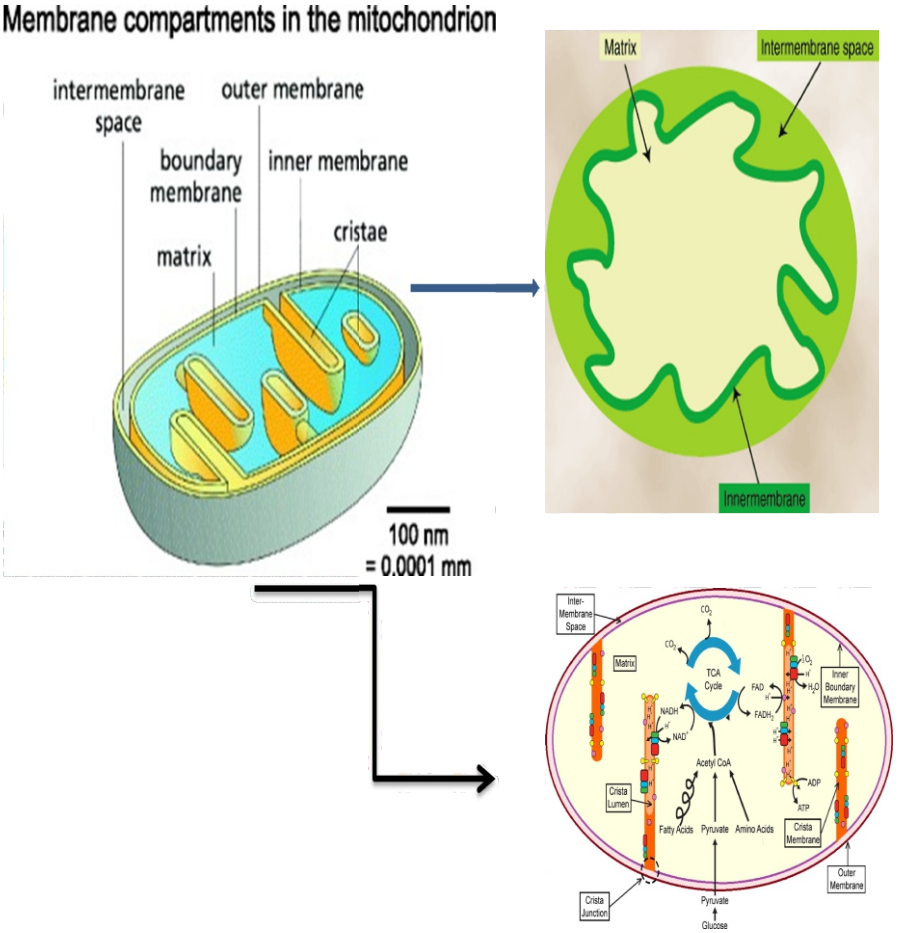


Fig.2.3: Structure of mitochondria

https://ars.els-cdn.com/content/image/1-s2.0-S0005273617300950-fx1_lrg.jpg

1.6. Summary

Mitochondria are organelles have double membranes. The inner membrane folds into cristae which divide the organelle into three compartments: the intermembrane space (between outer and inner membranes), cristae space (formed by infoldings of the inner membrane), and the matrix (within the inner membrane). The matrix of the mitochondria is the site of Krebs Cycle reactions. On oxidative phosphorylation, oxygen *must* be present to receive electrons from the protein complexes. This allows for more electrons and high energy molecules to be passed along, and maintains the hydrogen pumping that produces ATP. During glycolysis, only two ATP molecules are produced. $NADH + H^+$ is then oxidized to synthesized ATP. The pentose phosphate pathway takes place in the cytosol of the cell, the same location as glycolysis. It is special because no energy in the form of ATP, or adenosine triphosphate, is produced or used up in this pathway. The various components of electron transport system include. Cytochrome b, 2 types of cytochrome c, ubiquinone, flavo protein (FMN or FAD), iron sulphur protein (Fe-S) and enzyme cytochrome oxidase which is ultimately associated with cytochrome a and a_3. These components are arranged in a sequence in the inner Mitochondrial membrane.

1.7. Terminal Questions

Q.1. Define the structure and function of mitochondria and it role in oxidative phosphorylation ?

Answer:----------

Q.2. Define the electron transport chain in briefly ?

Answer:----------

Q.3. What is oxidative phosphorylation, define it ?

Answer:----------

Q.4. Briefly define the role of oxidative phosphorylation ?

Answer:----------

Q.5. Define the alternative respiration pathways in plants ?

Answer:----------

Q.6. Define the role of F_1 in iuito chandrei **?**

Answer:----------

1.8. Further suggested reading

1. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition
2. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016
3. Voet D and Voet J.G., Biochemistry", 4th Edition,2010
4. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.
5. Jeremy M. Berg, John L. Tymockzo and Luber Stryer, Biochemistry 7th Edition
6. Reginald H. Garrett, Charles M. Grisham, Biochemistry by Reginald H Garrett 5th Edition.

Unit-2: Nitrogen Metabolism

Contents

2.1. Introduction

Nitrogen is one of the important elements occurring in the entire living organism. In nature, about 78% by volume of nitrogen molecules exits in gaseous form. The plants contain about 1 or 2 percent nitrogen on a dry weight while other needed of nitrogen is fullfied by nitrogen fixation and assimilation. Nitrogen is the important part of protein and amino acids. Apart from that nitrogen also present in large number of other major components viz. nucleic acids, chlorophylls cytochromes, alkaloids, phytohormones and many vitamins. The atmospheric nitrogen is the source of nitrogen for much plant but some plant completed their nitrogen requirements only by soil. There are four kinds of components acts as source of nitrogen in soil such as, nitrate, nitrite, ammonium salt, and other organic nitrogenous compounds. In nature, the most effective source of nitrogen is nitrate ion (NO_3^-), however, nitrits (NO_2^-) is also absorbed by the plant.

2.2. About Nitrogen fixation

Nitrogen is essential to life because it is a key component of proteins and nucleic acids. Nitrogen occurs in many forms and is continuously cycled among these forms by a variety of bacteria. Although nitrogen is abundant in

the atmosphere as diatomic nitrogen gas (N_2), it is extremely stable, and conversion to other forms requires a great deal of energy. Historically, the biologically available forms NO_3^- and NH_3 have often been limited; however, current anthropogenic processes, such as fertilizer production, have greatly increased the availability of nitrogen to living organisms. The cycling of nitrogen among its many forms is a complex process that involves numerous types of bacteria and environmental conditions. Liver is main site of nitrogen metabolism′. Most of the conversions between organic and inorganic nitrogen are catalyzed by bacterial and archaeal enzymes. Nitrogen is essential for all organisms (in amino acids and nucleic acids). But unfortunately, most plants cannot utilize it in its elementary form. So they have to depend on the soil and they acquire nitrogen in inorganic form either as ammonium compounds or as nitrate. Plants require higher amounts of nitrogen as it is important in their structure and metabolism. Main source of nitrogen for the construction of nitrogenous organic compounds is the atmosphere. Nitrogen - most prevalent essential macro- elements which regulates plant growth, especially in agricultural systems. It is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure. The metabolism of nitrogen encompasses a number of topics, including nitrogen fixation, anabolism and catabolism of amino acids and purines, etc. Atmospheric nitrogen is the ultimate source of this element in biomolecule. The topic of nitrogen metabolism includes: - Nitrogen Fixation - Biosynthesis and Breakdown of amino acids.

Atmospheric nitrogen fixation

The atmospheric nitrogen fixation is carried out at certain atmospheric conditions through the electrical discharge in the troposphere and by cosmic radiation in the stratosphere. The atmospheric nitrogen fixation account about 10% of total nitrogen fixation.

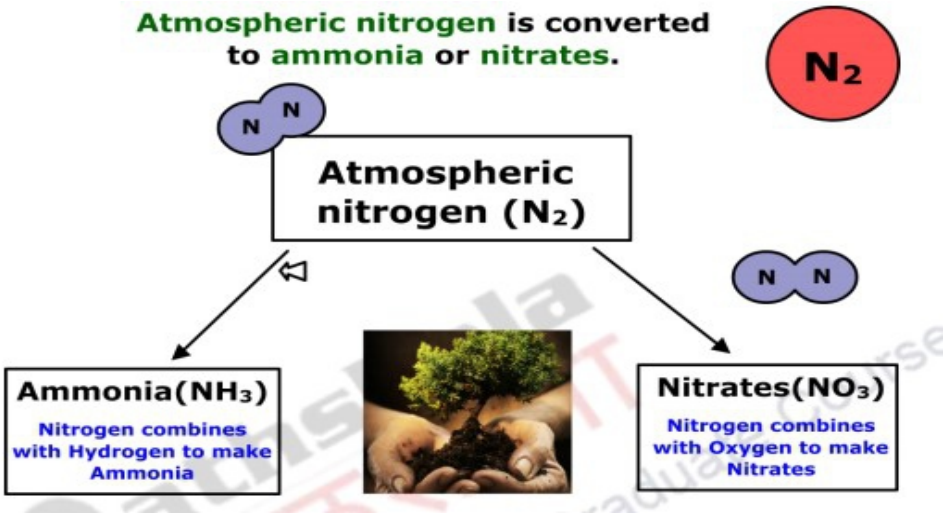


Fig.2.1: atmospheric nitrogen fixation

In atmosphere nitrogen molecules breaks down into nitrogen atoms, the nitrogen atoms combined with O_2 and formed nitrogen oxides. The nitrogen oxides when comes the contact with water, it forms nitrous acid (HNO_2) and nitric acid (HNO_3).

$$N_2 + O_2 \text{ (Lightning)} \rightarrow \text{Thunder } 2NO \text{ (Nitric Oxide)}$$

$$2NO + O_2 \rightarrow 2NO_2 \text{ Oxidation (Nitrogen peroxide)}$$

When nitrous acid or nitric fall on the soil along with rain water they react with the alkaline radicals of soil to form water soluble nitrates that are directly absorbed by plants.

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3;\ HNO_3 + \text{Ca or K salts} \rightarrow \text{Ca or K nitrates}$$

Industrial Nitrogen Fixation

The Haber-Bosch process: This process directly synthesizes ammonia from nitrogen and hydrogen. In 1909, the German chemist named Fritz Haber ascertained that atmospheric nitrogen could be combined with hydrogen under extremely high temperature and pressure condition which is catalyzed by an iron catalyst to yield an extremely high proportion of ammonia, which is the starting point for the production of a wide range of nitrogen compounds. This process was made commercially feasible by Carl Bosch and now called as the Haber-Bosch method or the synthetic ammonia

process. The Haber-Bosch process is now one of the largest and most-basic processes of the chemical industry throughout the world (figure 2.2).

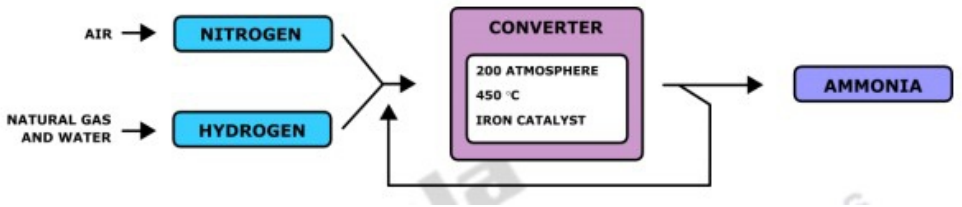


Fig.2.2: The Haber - Bosch process

Biological Nitrogen fixation

In the biological nitrogen fixation the atmospheric nitrogen is converting in to nitrogenous compound by microorganisms. It can be divided into non symbiotic and associative symbiotic nitrogen fixation. Biological nitrogen fixation (BNF) was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. BNF contributes about 60% of the total N_2 fixed in the biogeochemical nitrogen cycle. BNF is therefore called a key for sustenance of agriculture and reduction in soil fertility decline. These organisms utilize an enzyme called nitrogenase which catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Two kinds of nitrogen-fixing bacteria are known: free-living or non-symbiotic bacteria, including the cyanobacteria (or blue-green algae), Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia, and Clostridium and mutualistic or symbiotic bacteria such as Rhizobium, associated with leguminous plants and certain Azospirillum species, associated with cereal grasses. Biological nitrogen fixation can be represented by the equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions). Therefore, this fixation is costly process.

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

Non symbiotic nitrogen fixation

- Non symbiotic nitrogen fixation is done by free-living (nonsymbiotic) bacteria, includes the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*
- In the roots of grass and cereal plants no nodules are formed like symbiotic bacteria. The bacteria azospirillum bransilense, pseudomonas, Bacillus grow in the rizosphere in close contact with the root and involved in the other cortical regions of the roots and fix nitrogen

Symbiotic nitrogen fixation

- Nitrogen is also fixed by microbes symbiotically including such as *Rhizobium*, associated with leguminous plants (e.g., various members of the pea family); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses.
- Root nodules are also found in the certain non leguminous plants which also fix nitrogen e.g. In alder and alnus the actinomyceties is involved in nodules and fix nitrogen quite efficiently and play significant role on the nitrogen balance of some forest ecosystem.

  Associative symbiotic nitrogen fixation

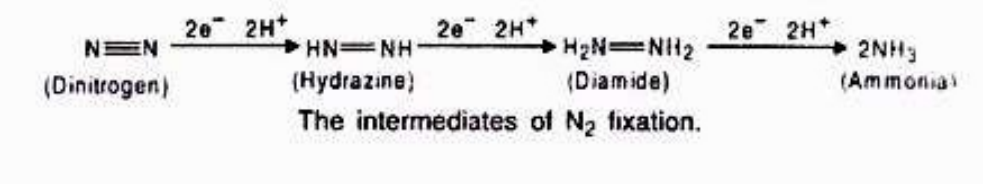
  - Nitrogen is also fixed by microorganism through non nodulation for example, the cyanobacteria, Anabaena azollae forms symbiotic association with Azolla Nostoc is found in the stem of Gunnera macrophylla. Azotobacctor paspali develops colonies below mucilaginous shealth of paspaum rotatum and fix atmospheric nitrogen.
  - The process of N_2 fixation occurs in nodule is mediated by the enzyme , called nitrogenase and leghaemoglobin (which mediates the reduction of N_2 to ammonia) firstly, this enzyme was extracted from the anaerobic di nitrogen fixer Clostridum pasteurianum.
  - The nitrogenase has 2 components i.e. Mo-Fe protein (molybdoferredoxin) and Fe-protein (azoferredoxin).The nitrogenase catalyzes the conversion of atmosphere di-nitrogen (N_2) to $2NH_3$. The ammonia is the first stable product of nitrogen fixation.

Latter, this enzyme has been isolated from most of other N_2 fixing bacteria.

The mechanism of N_2 fixation appears to be quite similar in most N_2 fixing prokaryotes. The enzyme has been fairly well characterized and the enzyme from these different systems share common properties allowing a unified single description of nitrogenase.

$$N_2 + 8_{e-} + 8H^+ \; 16\,ATP \xrightarrow[\text{nitrogenase}]{Mg^{2-}} 2NH_3 + H_2 \quad 16ADP + 16P_i$$

During nitrogen fixation, the free di-nitrogen first bound to MoFe protein and is not released until completely reduced to ammonia. The reduction of di-nitrogen is a stepwise reaction in which many intermediates are formed to form ammonia (NH_3) which is protonated at physiological pH to form $NH4^+$



The intermediates of N_2 fixation.

2.3. Assimilation of nitrate

Nitrogen assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment. In this process Nitrogen fixed by plant is converted into organic molecules such as protein, DNA, RNA etc. Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Ammonium ions are absorbed by the plant via ammonia transporters.

Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In aerobic soils where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed. However this is not always the case as ammonia can predominate in grassland and in flooded, anaerobic soils like rice paddies. Plant roots themselves can affect the abundance of various forms of nitrogen by changing the pH and secreting organic compounds or oxygen. This influences microbial activities like the inter-conversion of various nitrogen species, the release of ammonia from

organic matter in the soil and the fixation of nitrogen by non-nodule-forming bacteria.

Nitrogen assimilation requires the reduction of nitrate to ammonium, followed by ammonium assimilation into amino acids (Fig. 2A). Nitrate reduction takes place in both roots and shoots but is spatially separated between the cytoplasm where the reduction takes place and plastids/chloroplasts where nitrite reduction occurs.

In nitrogen assimilation, the N_2 fixation results is NH_4^+ formation, which reacts with organic acids and form amino acids which is mediated by ammonia assimilating enzyme.

GS–Glutaminesynthetase

GOGAT–Glutamatesynthese

GDH – Glutamate dehydrogenase

Protein and nucleic acid of dead plants and animal residue or excreted products of animal are degraded by microorganism with the liberation of ammonium. This is called ammonification. For this process, proteolysis and amino acid degradations are involved.

$$\text{Protein} \longrightarrow \text{Amino acids}$$

Amino acids are degraded by microbial activity and ammonia is released.

$$\text{Alanine} + \frac{1}{2}O_2 \xrightarrow[\text{deaminase}]{\text{analine}} \text{Pyrubic acid} + NH_3$$

However, the reduction of nitrate into ammonia is called nitrogen assimilation mostly occurs in root tissues and then transported to shoot through xylem. The ovell all summary of nitrate reduction is as

$$NO_2^- + 8\,\text{electrons} + 10H^+ \rightarrow NH_4 + 3H_2O$$

In first step the nitrate in converted in to nitrate; the reaction is catalyzed by enzyme- nitrate reductase. This steps occurs in cytosol outside of any organelles and requires NADH and electron donor.

$$NADH + H^+FAD \rightarrow NAD^+ FADH_2$$

$$FADH_2 + (Oxi)Mo \rightarrow (Red)Mo + 2H^+ + FAD$$

$$(\text{Red})\text{Mo} + 2\text{H}^+ + \text{NO}_3 \rightarrow (\text{Oxi})\text{Mo} + \text{N}O_2$$

In second step, the nitrite is reduced to ammonium. The reaction is catalyzed by enzyme nitrite reductase. The most probably electron donor in the reaction appears to be reduced Ferredoxin. The Ferredoxin is reduced in the light reaction of photosynthesis in green leaves. Nitrate reduction into nitrite is catalysed in the cytosol by the enzyme nitrate reductase. The overall reaction of nitrate reduction is as follows

$$\text{NO}_2 + 6\,e^- + 8\text{H}^+ \rightarrow + \text{H}_2\text{O}$$

2.4. Enzyme of nitrate reduction and their regulation

In plants, yeasts, algae, and fungi, nitrate reductase is a key enzyme of the nitrogen reduction and assimilation pathway. It catalyzes the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), which is itself reduced to ammonia ($NH4^+$) by nitrite reductase (NiR), before being assimilated into the amino acids and the nitrogen compounds of the cell. Nitrate reductase is an exceptionally short-lived protein. Its half-life time is only a few hours. The rate of *de novo* synthesis of this enzyme is very high. Thus, by regulating its synthesis, the activity of nitrate reductase in the tissue can be altered within hours. The involvement of Nitrate reductase activity in NO production has been evidenced in many plant organs and tissues over the past 20 years. NR was considered to produce directly NO via the reduction of NO_2^-, but another indirect mechanism of NO synthesis.

Various factors control the synthesis of the enzyme at the level of gene expression. Nitrate and light stimulate the enzyme synthesis. Part of the light effect is caused by carbohydrates generated by photosynthesis. The synthesis of the nitrate reductase protein is stimulated by glucose and other carbohydrates generated by photosynthesis, and are inhibited by NH_4^+, glutamine and other amino acids. Sensors seem to be present in the cell that adjusts via regulation of gene expression the capacity of nitrate reductase both to the demand for amino acids and to the supply of carbon skeletons from CO_2 assimilation for its synthesis. Nitrate reductase is regulated at the transcriptional and translational levels induced by light, nitrate, and possibly a negative feedback mechanism. First, nitrate assimilation is initiated by the

uptake of nitrate from the root system, reduced to nitrite by nitrate reductase, and then nitrite is reduced to ammonia by nitrite reductase. Ammonia then goes into the GS-GOGAT pathway to be incorporated into amino acids. When the plant is under stress, instead of reducing nitrate via NR to be incorporated into amino acids, the nitrate is reduced to nitric oxide which can have many damaging effects on the plant. Thus, the importance of regulating nitrate reductase activity is to limit the amount of nitric oxide being produced.

2.5. Nitorgenase system

The “nitrogen-fixing” bacteria contain an enzyme called nitrogenase which catalyzes the reduction of N2 to ammonia. The reaction is coupled with hydrolysis of 16 ATP molecules and production of H2. Nitrogenase can reduce nitrogen under normal conditions despite the unreactivity of dinitrogen, on the other hand, industrial production of ammonia is done at the conditions of high temperature and pressure. The production of this ammonia is extremely important for the bio-synthesis of amino acids. Till date, a total of four nitrogenase systems have been discovered in bacteria and archaea. Most of these systems are based on Mo and Fe whereas few of these systems have been identified to be based either on V and Fe or on Fe alone. Nitrogenase is a complex enzyme consisting of two types of proteins. The large protein is called the “MoFe protein” whereas the smaller one is called “Fe-protein”. The Fe-protein contains a [4Fe-4S] cluster which is coordinated with two cysteine residues and has a molecular weight of 57000-73000. The Fe-protein transfers electron to the MoFe protein by a known mechanism via ferredoxins to the nitrogenase where each electron transfer is accompanied by the hydrolysis of two ATP molecules bound to the Fe-protein

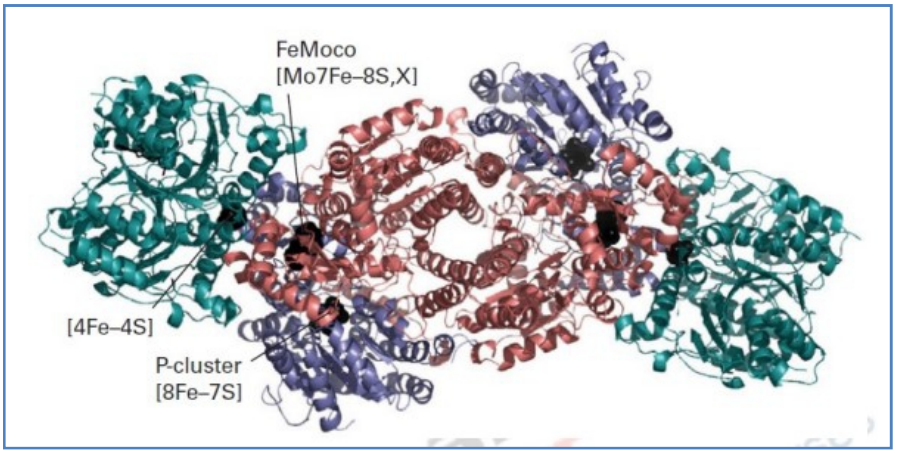
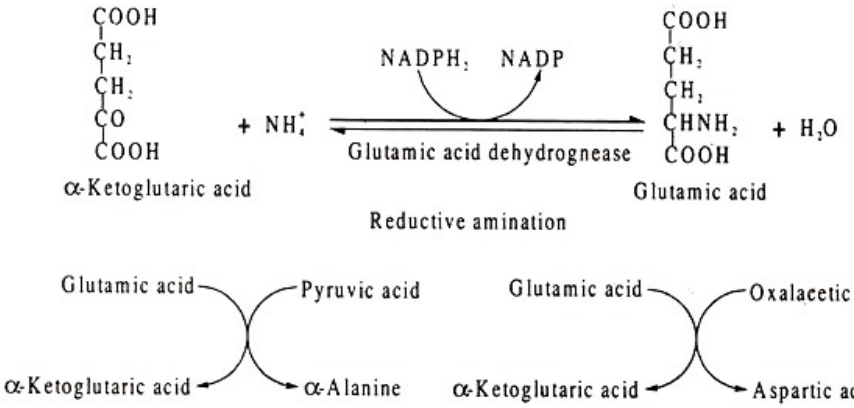


Fig.2.3: The structure of nitrogenase showing the Fe protein and the MoFe protein

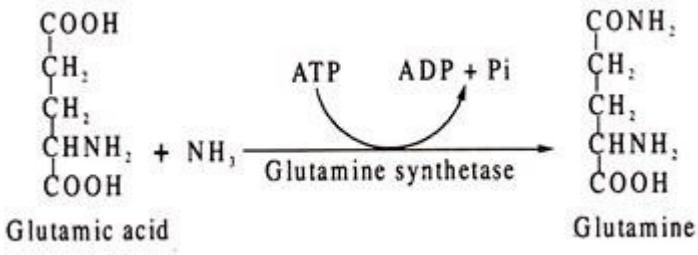
6. Assimilation of ammonia into organic compounds.

Nitrogen is an essential element for all living organisms and also viruses, coming next in importance to carbon. Nitrogen forms about 14% of the dry weight of the living matter, where it is primarily present as a constituent of proteins and nucleic acids. Although molecular nitrogen abounds in the earth's atmosphere, the biochemical mechanism for its utilization as a source of nitrogen is restricted to a small number of prokaryotic species, both photosynthetic and non-photosynthetic. All other living organisms -plants, animals and microorganisms of different kinds are solely dependent on combined nitrogen. Whereas plants and most bacteria and fungi can use inorganic nitrogenous salts, like ammonium or nitrate, animal organisms can utilize only organic nitrogenous compounds as source of nitrogen. Plants and microorganism which utilize nitrate as nitrogen source have to reduce it to the level of ammonia before incorporation into various organic compounds of their cells, because in their cellular constituents nitrogen is present in a reduced state. One of the most important routes of incorporations of ammonia is reductive amination of α-ketoglutaric acid in which $NADPH_2$ acts as H-donor. The enzyme catalyzing the reaction is glutamic acid dehydrogenase. Glutamic acid, produced by the reaction, can transfer the amino group to other keto acids by transamination:

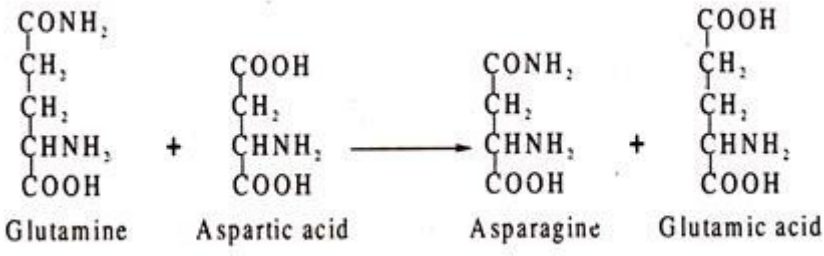


Transamination of keto acids by glutamic acid.

Glutamic acid can accept another molecule of NH_3 to produce an amide called glutamine. The reaction is catalysed by the enzyme glutamine synthetase which requires ATP:

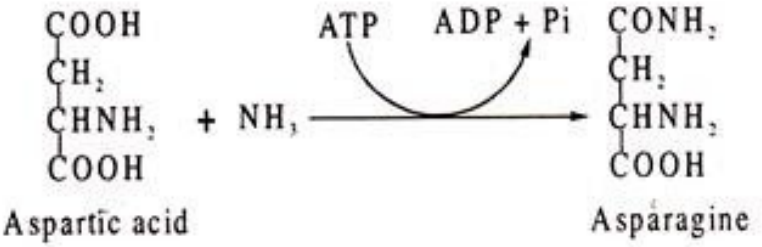


Glutamine may transfer its amido group to aspartic acid producing another amide, asparagine and glutamic acid:



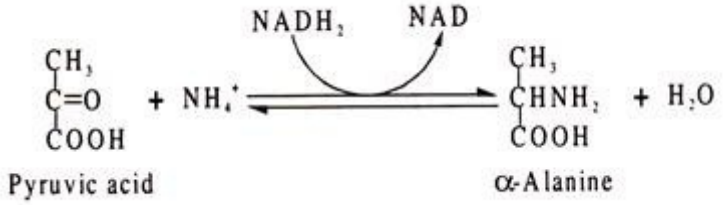
Asparagine may also be produced by direct amination of aspartic acid.

The reaction also requires ATP:

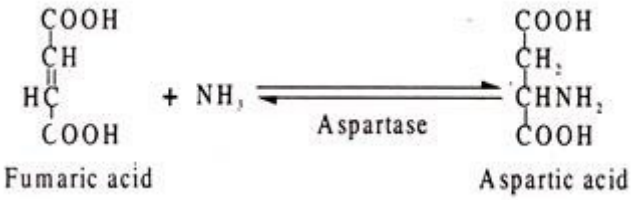


Although glutamic acid dehydrogenase reaction provides the main port of entry of ammonia into organic compounds, a few other reactions operate in specific organisms, by which ammonia is incorporated. For example, the members of the genus Bacillus lack the enzyme glutamic acid dehydrogenase and they employ a-alanine dehydrogenase reaction for amination of pyruvate.

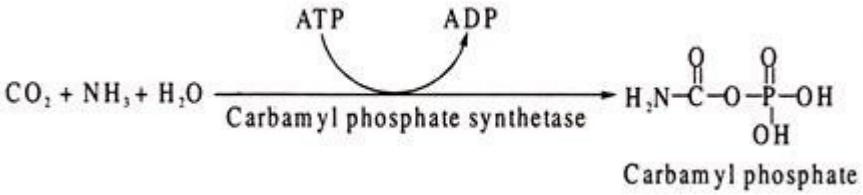
The enzyme is NAD-linked:



Another enzyme, aspartase, may incorporate ammonia into fumaric acid producing aspartic acid, though the enzyme is probably involved more in deamination of aspartic acid, rather than its formation.



A further route of entry of ammonia is catalyzed by the enzyme carbamy I phosphate synthesize. This enzyme uses CO2, NH_3 and ATP as substrates to form carbamyl phosphate which is an important intermediate in the synthesis of ornithine, arginine and pyrimidine bases of nucleic acid.



2.7. Summary

Nitrate is the principal nitrogen source for most crops. The fixation and assimilation of nitrate to is discussed in this unit. Nitrogen fixation is a chemical process by which molecular nitrogen is converted into organic molecules. Most of the chemical species involved in biological systems such as including proteins, nucleic acids, chlorophyll, various enzymes and vitamins, and many other cellular constituents contain nitrogen. Hence, a source of utilizable nitrogen is an essential requirement for all the living things which generally comes in the form of ammonia (NH_3) and nitrate (NO_3^-). The earth's atmosphere is the principal reservoir of nitrogen where it is present in the form of N_2 as the most abundant constituent of the atmosphere. Although the most abundant constituent of the atmosphere is N_2, the unreactivity of nitrogen limits its usefulness. The biological reduction of nitrogen is carried out exclusively by prokaryotes, including various bacteria, blue-green algae, yeasts and in symbiotic bacteria-legume associations under mild-conditions. The MeFo nitrogenase is the metalloenzyme responsible for the "fixation" of the atmospheric dinitrogen by reducing dinitrogen to ammonia. The reaction is coupled with hydrolysis of 16 ATP molecules and production of H_2. The ammonia is produced in plant tissues through a variety of processes as well as being taken up directly from the soil, for example, ammonia is generated through the fixation of atmospheric nitrogen by root nodules by photorespiring leaves and through the phenylpropanoid pathway.

2.8. Terminal questions

Q.1. What is nitrogen fixation? Discuss the roles of atmospheric nitrogen fixation.

Answer:----------

Q.2. What do you understand by biological nitrogen fixation? Descries in brief.

Answer:----------

Q.3. Write the nitrogen assimilation and role of enzymes in assimilation of nitrogen.

Answer:----------

Q.4. What is nitrogenase ? Discuss nitrogenase system and its role in nitrogen fixation.

Answer:----------

Q.5. Discuss about nitrate enzyme regulation.

Answer:----------

Q.6. Discuss about assimilation of ammonia into organic compound.

Answer:----------

2.9. Further readings

1. Plant physiology, by H.N. Sharma, Pradeep publication, Jalander.
2. Jeremy M. Berg, John L. Tymockzo and Luber Stryer, Biochemistry 7th Edition
3. Reginald H. Garrett, Charles M. Grisham, Biochemistry by Reginald H Garrett 5th Edition,
4. U. Satyanarayana, U. Chakrapani, Biochemistry by U. Satyanarayana. 3rd Edition.
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Unit-3: Nitrogen fixation and assimilation

Contents

3.1.Introduction:

Nitrogen is an essential nutrient in plant growth. The ability of a plant to supply all or part of its requirements from biological nitrogen fixation (BNF) thanks to interactions with endosymbiotic, associative and endophytic symbionts, confers a great competitive advantage over non-nitrogen-fixing plants. The biological nitrogen fixation, carried out by prokaryotes, leads to the reduction of molecular nitrogen to ammonia subsequently assimilated in amino acids. This is an event of capital importance allowing for the recovery of nitrogen irreversibly lost in ecosystems due to bacterial activities. The nitrogen fixation provides Earth's ecosystems with about 200 million tons N_2 per year. Rhizosphere associations between nitrogen-fixing microorganisms and plants have been a major driving force in allowing organisms to spread across the biosphere, occupy new niches, and adapt to a variety of environmental stresses. This review presents an overview and recent advances in the understanding of the associations between a wide range of diazotrophs and non-legumes.

Bacteria are large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil,

water, acidic hot springs, radioactive waste. Bacteria can be beneficial as well as detrimental to human health. Commensally, or "friendly" bacteria, share space and resources within our bodies and tend to be helpful. The human gut is a comfortable setting for bacteria, with plenty of nutrients available for their sustenance. However, some other causes infections are classified into as so called groups such as A Streptococcus, Clostridium perfringens (C. perfringens), E. coli and S. aureus etc. Biofertilizers are natural fertilizers that consist of live biomass or dormant cells of effective microbial strains. Bacteria, fungi, and algae are some of the beneficial microorganisms that help in improving the fertility of the soil. They are activated through seed or soil interactions with the rhizosphere, thereby increasing the availability of nutrients to the plants. These bacteria convert gaseous nitrogen into nitrates or nitrites as part of their metabolism, and the resulting products are released into the environment. Other denitrifying bacteria metabolize in the reverse direction, turning nitrates into nitrogen gas or nitrous oxide. When colonies of these bacteria occur on croplands, they may deplete the soil nutrients, and make it difficult for crops to grow.

3.2. Biological nitrogen

Biological nitrogen fixation (BNF) was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. BNF contributes about 60% of the total N_2 fixed in the biogeochemical nitrogen cycle. BNF is therefore called a key for sustenance of agriculture and reduction in soil fertility decline. These organisms utilize an enzyme called nitrogen's which catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Two kinds of nitrogen-fixing bacteria are known: free-living or non-symbiotic bacteria, including the cyanobacteria (or blue-green algae), Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia, and Clostridium and mutualistic or symbiotic bacteria such as Rhizobium, associated with leguminous plants and certain Azospirillum species, associated with cereal grasses.

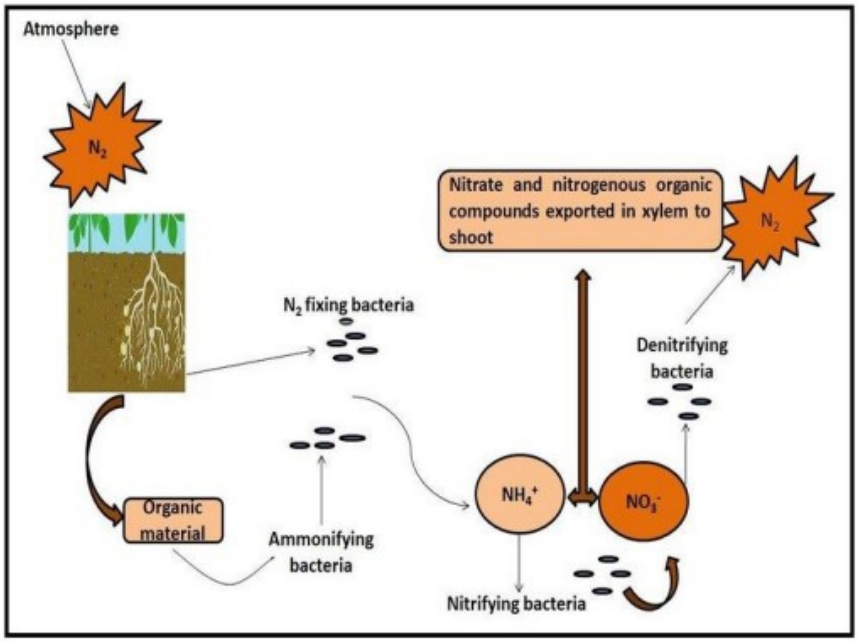


Fig.3.1: Diagrammatic representation of biological nitrogen fixation

Biological nitrogen fixation (BNF) is the term used for a process in which nitrogen gas (N_2) from the atmosphere is incorporated into the tissue of certain plants. Only a select group of soil microorganisms is able to obtain N this way, with the help of plants. The microbes are very helpful to incorporate N_2 form the air into tissues involves a host plant that is known as the macrosymbiont. For example microorganism that is associated with the host plant called symbiotic relationship or symbiosis. The bacteria that are most often involved with forage crops is popularly known as rhizobia, because it is classified as part of the bacterial genus known as *Rhizobium*. These soil bacteria infect the roots of the plant and form structures known as nodules. The chemical reactions, that are the process known as BNF, take place in the nodules. Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by a nitrogen's enzyme. The overall reaction for BNF is:

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

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the coformation of one equivalent of H_2 The conversion of N_2 into ammonia occurs at a metel cluster called FeMoco, an abbreviation for the

iron-molybdenum cofactor. The mechanism proceeds via a series of protonation and reduction steps wherein the FeMoco active site hydrogenates the N2 substrate. In free-living diazotrophs, nitrogenase-generated ammonia is assimilated into glutamate through the glutamine synthetase /glutamate synthase pathway. The microbial genes required for nitrogen fixation are widely distributed in diverse environments.

Biochemistry of nitrogen fixation: the nitrogen fixation require following items such as

- Nitrogenase and hydrogenase enzymes
- Protective mechanism against oxygen
- Ferrodoxin
- Hydrogen releasing system or electron donor
- Constants supply of ATP
- Coenzyme and co factor-TPP,CoA, inorganic phosphate and mg+
- Cobalt and modybdenum
- A carbon compound

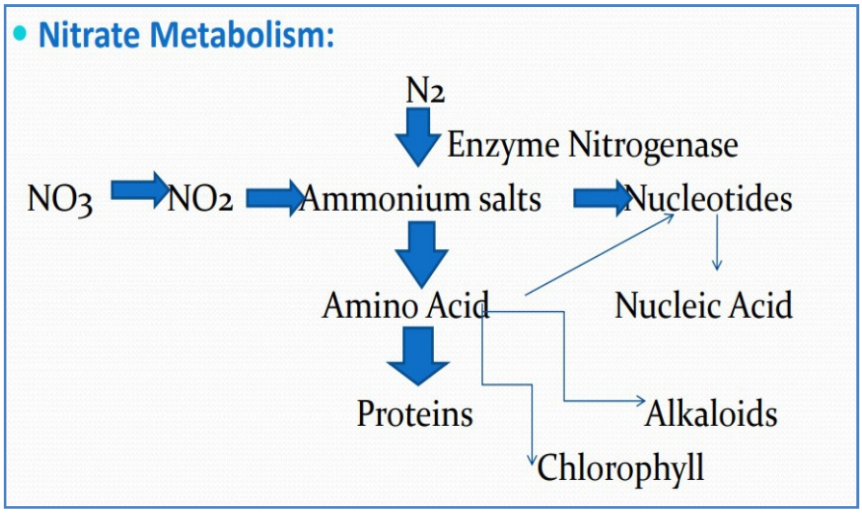


Fig.3.2: Biochemistry of nitrogen fixation

Biological Nitrogen fixation may be categorized into following types:

a) Non- Symbiotic/ asymbiotic Biological Nitrogen Fixation.

b) Associative Biological Nitrogen Fixation.

c) Symbiotic Biological Nitrogen Fixation.

3.3.Fixation by free living

- Non symbiotic nitrogen fixation is done by free-living (nonsymbiotic) bacteria, includes the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*
- In the roots of grass and cereal plants no nodules are formed like symbiotic bacteria. The bacteria azospirillum bransilense, pseudomonas, Bacillus grow in the rizosphere in close contact with the root and involved in the other cortical regions of the roots and fix nitrogen

Symbiotic nitrogen fixation

Symbiotic nitrogen fixation is the type of biological nitrogen fixation. In this, the atmospheric nitrogen is converted into the nitrogenous compounds through living organisms. It is of 2 types, symbiotic nitrogen fixation and non-symbiotic nitrogen fixation.

- Nitrogen is also fixed by microbes symbiotically including such as *Rhizobium*, associated with leguminous plants (e.g., various members of the pea family); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses.
- non-symbiotic nitrogen fixation is occurs by bacteria such as Azospirillum, Azotobacter, BGA.

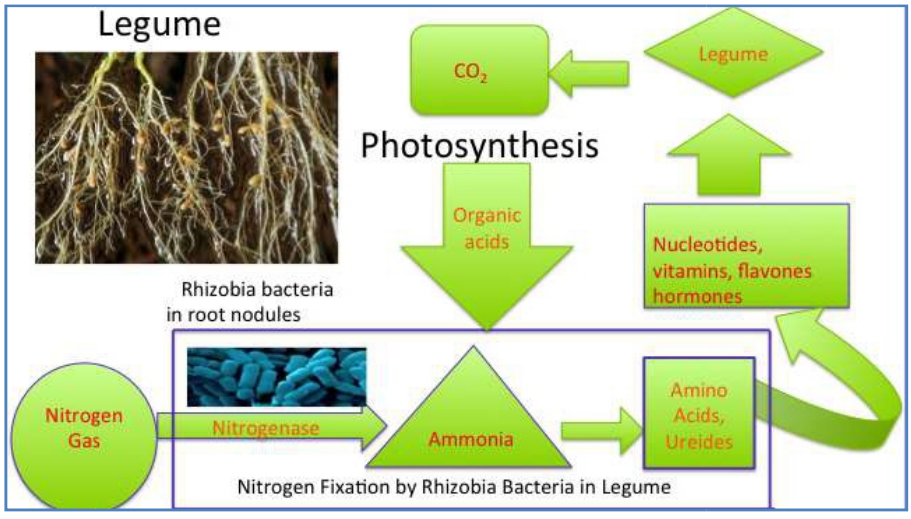


Fig.3.3: symbiosis relationship between Legumes and Rhizobia bacteria

Associative symbiotic nitrogen fixation

- Nitrogen is also fixed by microorganism through non nodulation for example, the cyanobacteria, Anabaena azollae forms symbiotic association with Azolla, Nostoc is found in the stem of Gunnera macrophylla. Azotobacctor paspali develops colonies below mucilaginous shealth of paspaum rotatum and fix atmospheric nitrogen.
- The process of N_2 fixation occurs in nodule is mediated by the enzyme , called nitrogenase and leghaemoglobin (which mediates the reduction of N_2 to ammonia) firstly, this enzyme was extracted from the anaerobic di nitrogen fixer Clostridum pasteurianum.
- The nitrogenase has 2 components i.e. ***Mo-Fe protein (molybdoferredoxin) and Fe-protein (azoferredoxin)***. The nitrogenase catalyzes the conversion of atmosphere di-nitrogen (N_2) to $2NH_3$. The ammonia is the first stable product of nitrogen fixation.

Latter, this enzyme has been isolated from most of other N_2 fixing bacteria.

The mechanism of N_2 fixation appears to be quite similar in most N_2 fixing prokaryotes. The enzyme has been fairly well characterized and the enzyme from these different systems share common properties allowing a unified single description of nitrogenase.

$$N_2 + 8_{e-} + 8H^+ \; 16\,ATP \xrightarrow[\text{nitrogenase}]{Mg^{2-}}$$

$$2NH_3 + H_2 \quad 16ADP + 16P_i$$

During nitrogen fixation, the free di-nitrogen first bound to MoFe protein and is not released until completely reduced to ammonia. The reduction of di-nitrogen is a stepwise reaction in which many intermediates are formed to form ammonia (NH_3) which is protonated at physiological pH to form NH_4^+

3.4. Symbiotic association

Symbiotic nitrogen fixation is the type of biological nitrogen fixation. In this, the atmospheric nitrogen is converted into the nitrogenous compounds through

living organisms. It is of 2 types, symbiotic nitrogen fixation and non-symbiotic nitrogen fixation.

- Nitrogen is also fixed by microbes symbiotically including such as *Rhizobium*, associated with leguminous plants (e.g., various members of the pea family); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses.
- non-symbiotic nitrogen fixation is occurs by bacteria such as Azospirillum, Azotobacter, BGA.

Symbiotic nitrogen fixation in legumes can occur in both natural and agricultural ecosystems and contribute substantial N that is cheap, sustainable, and environmentally friendly, in that it is less prone to leaching and volatilization and hence to environmental pollution. BNF is therefore an alternative to the use of N fertilizers which are costly and inaccessible to resource-poor farmers.

3.5.Structure and function of enzyme nitrogenases

Structure:

Nitrogenases are enzymes that are produced by certain bacteria, such as cyanobacteria (blue-green bacteria). These enzymes are responsible for the reduction of nitrogen (N_2) to ammonia (NH_3). Nitrogenases are the only family of enzymes known to catalyze this reaction, which is a key step in the process of nitrogen fixation. Nitrogen fixation is required for all forms of life, with nitrogen being essential for the biosynthesis of molecules (nucleotides, amino acids) that create plants, animals and other organisms. They are encoded by the Nif genes or homologs. They are related to protochlorophyllide reductase.

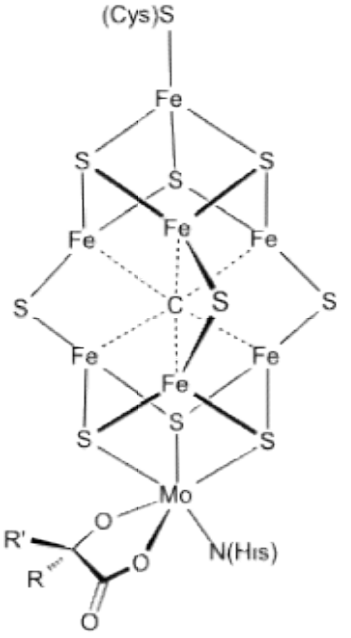


Fig.3.4

Function

Nitrogenase (NASE) is an enzyme that fixes atmospheric nitrogen (N_2) into ammonia. Though abundantly present in the atmosphere, most organisms cannot utilize N_2 directly, and must instead take it in through other forms, like ammonia or nitrate. The triple bond in N_2 is highly resistant to changes in oxidation state, and nitrogenases, found only in nitrogen-fixing bacteria, are the only proteins capable of reducing N_2 to ammonia.

Nitrogenase catalyzes the following reaction:

$$N_2 + 8\ H+ + 16\ MgATP + 8\ e- \rightarrow 2NH3 + H_2 + 16\ Mg\ ADP + 16\ Pi$$

Two different proteins comprise the nitrogenase complex. The FeMo protein binds substrate and reduces H^+ and N_2 to H_2 and ammonia, while the Fe protein receives electrons from ferredoxin, hydrolyzes ATP, and reduces the FeMo protein. To the right is shown a crystal structure where two complexes of FeMo protein bound to Fe protein were crystallized together.

The Fe protein is here bound to two ADP x AlF4-, an analog for the planar transition state of ATP hydrolysis. The motif that binds ATP is a conserved nucleotide binding motif called Walker's motif A. Coloring by evolutionary conservation, the nucleotide binding pocket is clear. At the bottom of the protein, where the Fe protein comes into contact with the FeMo

protein, is a 4Fe:4S cluster, held in place by cysteines. This cluster accepts electrons from ferredoxin and gives electrons to the FeMo protein.

When the Fe protein is bound to the FeMo protein (zoom out), ATP is hydrolyzed and electrons that were transferred to the 4Fe:4S cluster of the Fe protein by ferredoxin are transferred to the FeMo protein. The crystal structure of the complete nitrogenase complex reveals how the binding of the Fe and FeMo proteins, the hydrolysis of ATP, and the transfer of electrons are all coupled. The figure below summarizes how these processes are linked.

3.6.Nitrate assimilation

Nitrate assimilation is one of the two major biological processes by which inorganic nitrogen is converted to ammonia and thence to organic nitrogen. Nitrogen assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment. In this process Nitrogen fixed by plant is converted into organic molecules such as protein, DNA, RNA etc. Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium ($NH4^+$). Ammonium ions are absorbed by the plant via ammonia transporters.

Ammonification

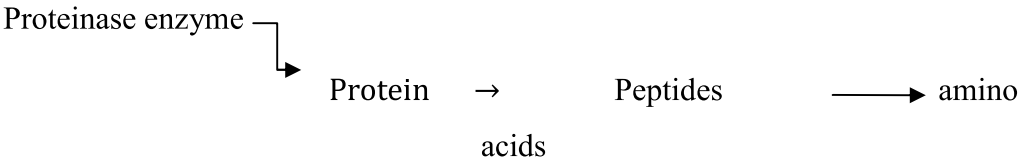
N_2 fixation results in NH_4^+ formation which reacts with organic acids and form amino acids which is mediated by ammonia assimilating enzyme.

- GS–Glutaminesynthetase
- GOGAT–Glutamatesynthes
- GDH – Glutamate dehydrogenase

Protein and nucleic acid of dead plants and animal residue or excreted products of animal are degraded by microorganism with the liberation of ammonium. This is called ammonification. For this process, two steps are involved.

a. Proteolysis

Protein $\longrightarrow$ Amino acids



Clostridium, actinomycities, pseudomonas and many fungi cause proteolysis.

b. Amino acid degradation

Amino acids are degraded by microbial activity and ammonia is released.

$$\text{Alanine} + \frac{1}{2}O_2 \xrightarrow[\text{deaminase}]{\text{analine}} \text{Pyrubic acid} + NH_3$$

Nitrification:

Plants cannot take ammonia directly therefore; ammonia is converted rapidly (biological oxidation) to nitrate or nitrite by microbial activity by the process of nitrification. In the process of nitrification ammonia is firstly converted into nitrite by *nitrosomonas.* The nitrite is oxidized into nitrate by nitrogenous bacteria. However, different microbes are responsible for each steps of nitrification in marine environment.

Denitrifications

Transformation of nitrate to nitrogen gas by microorganism is called denitrification. A denitrification bacterium (micrococcus denitrificans) lives in deep in the soil and they like to live in oxygen free medium. Denitrification is reverse of nitrogen fixation. It decreases soil fertility and reducing agriculture productivity.

Factors limiting Biological Nitrogen Fixation

There are three main factors effecting the process of biological nitrogen fixation these are:

- Edaphic factors for example extensive soil moisture, drought, salinity and Deficiency of P, Ca, Mo.
- Climatic factors factors for example Extreme temperature and availability of sun light
- Biotic factors factors for example Excessive defoliation of host plant, Crop competition, Insects and nematodes

Photosynthetic organisms, with the possible exception of some blue-green algae and plants that have a symbiotic association with rogen-fixing bacteria, derive most of their nitrogen from nitrate1. Nitrate assimilation is a key process for nitrogen (N) acquisition in green microalgae. Among Chlorophyte algae, Chlamydomonas reinhardtii has resulted to be a good model system to unravel important facts of this process, and has provided important insights for agriculturally relevant plants. In this work, the recent findings on nitrate transport, nitrate reduction and the regulation of nitrate assimilation are presented in this and several other algae. Latest data have shown nitric oxide (NO) as an important signal molecule in the transcriptional and posttranslational regulation of nitrate reductase and inorganic N transport.

3.7. Summary

Nitrogen is highly inert gas thus why it cannot be used directly by the higher plants, and therefore has to be fixed. The phenomenon of conversion of free Nitrogen (molecular & elemental) into Nitrogenous compounds which become available to the plants for absorption is known as Nitrogen Fixation. Nitrogen fixation is essential to life because fixed inorganic nitrogen compounds are required for the biosynthesis of all nitrogen-containing organic compounds, such as amino acids and proteins, nucleoside triphosphates and nucleic acids. Biological nitrogen fixation was discovered by German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by a nitrogenase enzyme. The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the coformation of one equivalent of H_2 The conversion of N_2 into ammonia occurs at a metel cluster called FeMoco, an abbreviation for the iron-molybdenum cofactor. The mechanism proceeds via a series of protonation and reduction steps wherein the FeMoco active site hydrogenates the N_2 substrate. Transformation of nitrate to nitrogen gas by microorganism is called denitrification. A denitrification bacterium (micrococcus denitrificans) lives in deep in the soil and they like to live in oxygen free medium.

3.8. Termined Questions

Q.1. Discuss about nitrogen fixation by living being

Answer:----------

Q.2. Define the role of nitrgenase and hydrogenase

Answer:----------

Q.3. What is biological nitrogen fixation discuss it.

Answer:----------

Q.4. Briefly structure and function of nitrogenase.

Answer:----------

Q.5. Define nitrogen cycle.

Answer:----------

Q.6. Discuss the factor effecting on biological nitrogen fixation**.**

Answer:----------

3.9.Further readings

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4. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.
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DCEBCH-108
Plant Biochemistry

Block -2

Photosynthetic Process and Carbon Assimilation

Introduction

This is the second block of Plant biochemistry. This consists of three units.

Unit-4: This unit covers the process of photosynthetic in plants. The various components of photosynthesis such as Chlorophylls, membranes and organelles, z scheme, are discussed. The photoperiodism and photosynthetic light dependant reactions discuss in this unit. Apart from that the photosynthetic apparatus and pigments involved in photosynthesis are mentioned in details. The various reactions such as Hill reaction, generation of NADPH and ATP reaction discuss in details. The light harvesting complexes are also discussed here.

Unit-5: This unit covers the synthesis of photochemical. The source, types of applications of terpenes, lignins, waxes and alkaloids are discussed in details. The process of their biosynthesis and mentioned in brief.

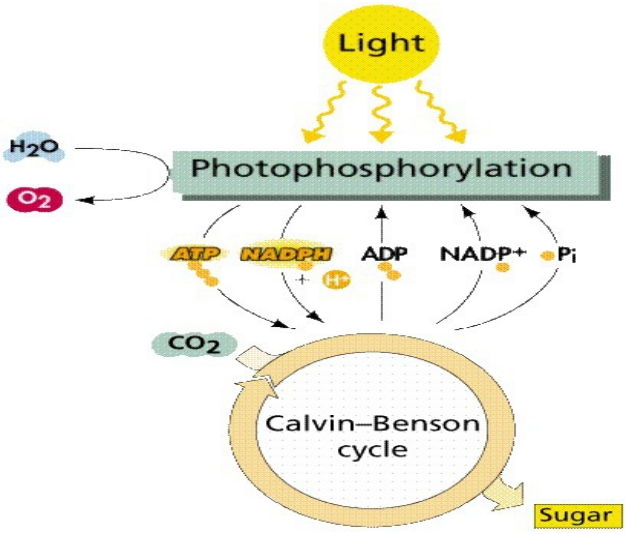
Unit-6: This unit covers the carbon assimilation. The carbon assimilation is the impartment in energy production and plant structure. The cyclic and non cyclic photophosphorylation, Calvin cycle, and photorespiration discussed in this unit.

Unit-4.: Photosynthetic process

Contents

4.1. Introduction

All photosynthetic organisms used water as electron and hydrogen H^+ donor except bacteria. The 90% of the world's photosynthesis in carried out by marine and fresh water algae. Thus the photosynthesis is a very important metabolic process because it supplies food to the biological world and purifies the atmosphere through taking up Co_2 from the atmosphere and releasing O_2 in the atmosphere.



Photosynthesis is the synthesis of carbohydrates from Co_2 and H_2o in presence of sunlight on chlorophyll.

$$6Co_2 + 12H_2o \xrightarrow[Chlorophyll]{Light} C_6H_{12}o_6 + 6o_2 + 6H_2o$$

In this equation carbohydrate is produced as a end product of photosynthesis. However, this equation does not apply to the entire photosynthetic organism. Instead of water, some other organism used other compounds as electron donors. For example green and purple bacterial used hydrogen sulfide that show in equations.

$$Co_2 + 2H_2S \xrightarrow{Light} CH_2O + H_2O + 2S$$

Photosynthesis taking place in water labed with oxygen isotope does not fact yield labeled O_2.

Imagine photosynthesis occurring in two connecting factories. The product of first factory is energy carrying molecules ATP (adenosine triphosphate) and NADPH $+H^+$ (reduced nicotinamide adenine dinucleotide phosphate) and second factory to make sugar, the final product. All organisms, including human, need energy to fuel the metabolic reactions of growth, development and reproduction. But organisms cannot use light energy directly for their metabolic needs. The green plants have chloroplast and can synthesize their own food by the process of photosynthesis. The entire humanity depends

on plants for food. Every year about 200 billion tons of carbon is utilized in the process of photosynthesis. Thus the photosynthesis is the most massive chemical event going on the earth. The plants take up 7×10^{11} tons of Co_2 to produce roughly 5×10^{11} tons of solid plant material.

Objectives

After studying this unit you should be able to :

- Know the structure and functions of chloroplast.
- To discuss Photoperiodism
- To discuss light reaction of photosynthesis.
- To known about dark reaction of photosynthesis.

4.2. Chlorophylls

Chlorophyll is a pigment that gives plants their green color, and it helps plants create their own food through photosynthesis. Chlorophyll is located in a plant's chloroplasts, which are tiny structures in a plant's cells. This is where photosynthesis takes place. Phytoplankton, the microscopic floating plants that form the basis of the entire marine food web, contain chlorophyll, which is why high phytoplankton concentrations can make water look green. Chlorophyll's job in a plant is to absorb light—usually sunlight. The energy absorbed from light is transferred to two kinds of energy-storing molecules. Through photosynthesis, the plant uses the stored energy to convert carbon dioxide (absorbed from the air) and water into glucose, a type of sugar. Chlorophyll gives plants their green color because it does not absorb the green wavelengths of white light. That particular light wavelength is reflected from the plant, so it appears green.

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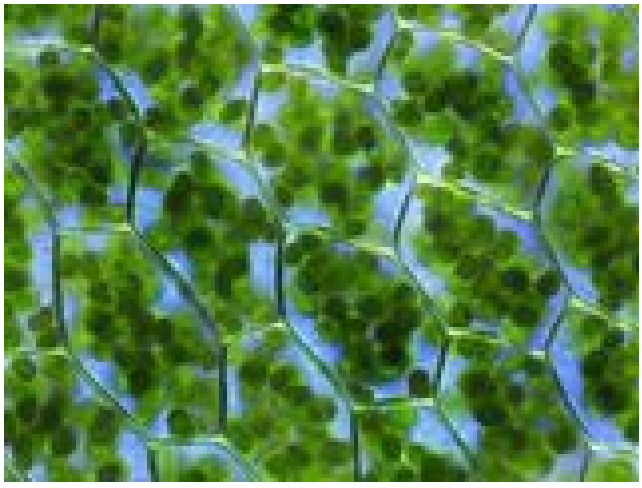


Fig 4.1: Image of chlorophyll

Chlorophyll occurs in several distinct forms: chlorophylls a and b are the major types found in higher plants and green algae; chlorophylls c and d are found, often with a, in different algae; chlorophyll e is a rare type found in some golden algae. The chlorophyll molecule consists of a central magnesium atom surrounded by a nitrogen-containing structure called a porphyrin ring.

Chloroplasts are organelles specializing in the conversion of radiant energy to chemical energy. These organelles are only found in plant cells and some protists such as algae. Animal cells do not have chloroplasts. Chloroplasts work to convert solar energy into sugars that can be used by cells. The entire process is called photosynthesis and it all depends on the little green chlorophyll molecules in each chloroplast. Two membranes contain and protect the inner parts of the chloroplast. They are appropriately named as the outer and inner membranes. The inner membrane surrounds the stroma and the grana (stacks of thylakoids). Single thylakoid stack is called a granum.

Chlorophyll is a naturally occurring molecule that gives plants their green color and is responsible for facilitating one of the most incredibly miraculous processes on earth – the process of photosynthesis. It is found in the chloroplasts of green plants cells. The chemical energy stored by photosynthesis in carbohydrates drives biochemical reactions in nearly all living organisms. As the chlorophyll in leaves decays in the autumn, the green colour fades and is replace by the oranges and reds of carotenoids. The basic

structure of a chlorophyll molecule is a porphyrin ring, co-ordinated to a central atom. The chlorophyll is mixture of two compounds, chlorophyll-a and chlorophyll-b (Figure 4.2): There are five different types of chlorophyll molecules that are naturally present in photosynthetic organisms: Chlorophyll *a*, *b*, *c*, *d,* and *f.* All of the chlorophyll molecules have similar chemical structures. The small differences in the chemical structures of the chlorophyll molecules cause variations in their light absorption properties.

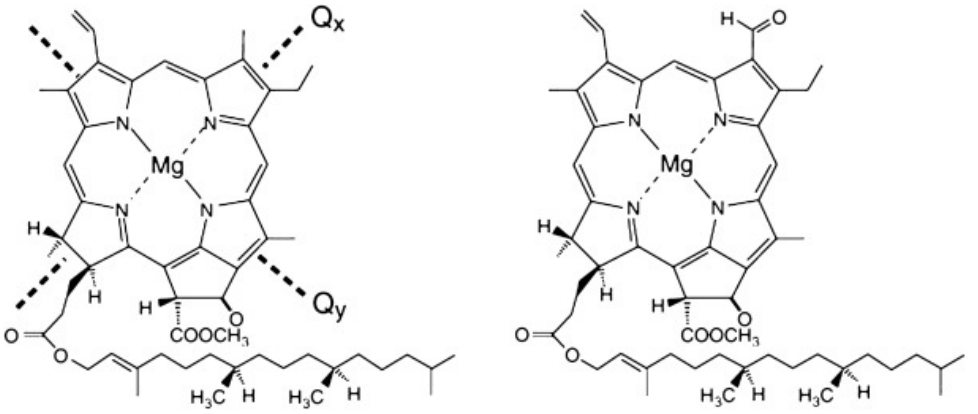


Fig.4.2: The molecular structure of (A) chlorophyll a and (B) chlorophyll b.
Source: https://www.sciencedirect.com/topics/earth-and-planetary-sciences/chlorophyll

In natural chlorophyll there is a ratio of 3 to 1 (of a to b) of the two components. Both of these two chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalise stabilising the structure. Such delocalised molecules have very strong absorption bands in the visible regions of the spectrum, allowing the plant to absorb the energy from sunlight. Due to presence of different side group, chlorophyll absorbs different wavelength of light. so that light that is not significantly absorbed by chlorophyll a, at, say, 460nm, will instead be captured by chlorophyll b, which absorbs strongly at that wavelength.

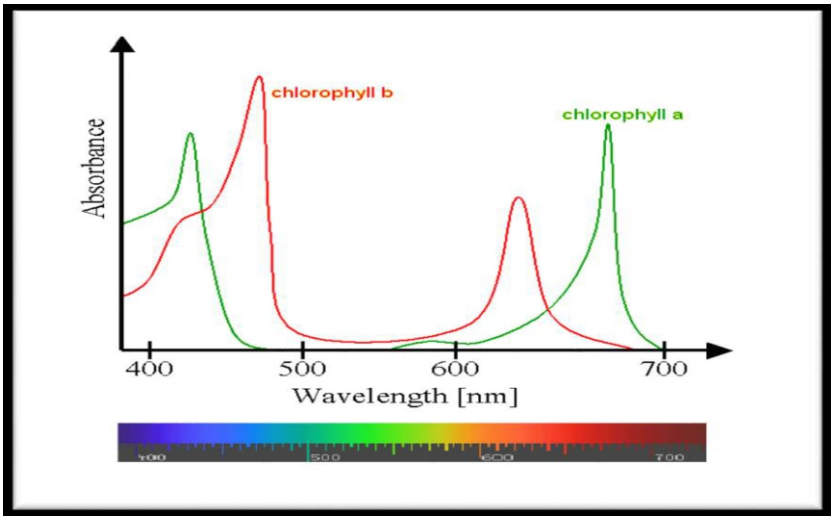


Fig.4.3: Absorption spectra of Chlorophyll a and b

In the photosynthetic reaction electrons, are transferred from water to carbon dioxide i.e. carbon dioxide is reduced by water. Chlorophyll assists this transfer as when chlorophyll absorbs light energy, an electron in chlorophyll is excited from a lower energy state to a higher energy state. In this higher energy state, this electron is more readily transferred to another molecule. This starts a chain of electron-transfer steps, which ends with an electron being transferred to carbon dioxide. The chlorophyll have numerous health benefits such as

- Cleanses and oxygenates, and builds the blood
- A powerful detoxification effect on the body
- Skin healing
- Rich in enzymes that promote quick rejuvenation of our cells
- High in Amino acids
- A natural deodorant

4.4: Photoperiodism

Plants require light for growth and development. Through photosynthesis, plants are able to convert light energy into chemical energy, which is used for their survival and growth. Light also plays an important role in regulating important developmental processes in plants. Plants can sense and respond to the duration, quality, intensity and direction of light and respond to it. Photoperiod is the duration of daily illumination received by an organism. One of the most striking developmental processes that depend on the photoperiod is

flowering. We see different flowering plants burst into blossom at specific periods of the year. For example, many of the temperate trees bloom when the daylength increases and the temperatures are warmer during the spring season. Many tropical plants especially trees are known to flower when the daylength reduces during post-rainy season. Such seasonality in flowering time probably helps in restricting the reproductive phase to a period that is suitable for pollination or seed dispersal by appropriate agents. Whatever the purpose, the mechanism by which plants are able to perceive and respond to photoperiod is by itself a fascinating process.

The photoperiod is perceived by photoreceptors called phytochromes and cryptochromes together with circadian clock components, which measure the duration of light or darkness in a day. The photoperiod signal is then transduced to bring about expression of genes involved in transition of vegetative meristems to floral meristems.

a. Photoreceptors

1. Phytochromes

These photoreceptors perceive light in the red region (λ = 600-700 nm) of the spectrum and modulate important physiological processes in plants, which include seed germination, seedling photomorphogenesis, shade avoidance and flowering induction. For example, when seeds germinate, they require daylight for attaining photosynthetic competence. Availability of daylight can be sensed by the seeds through phytochromes, which then induce germination and make the seedlings green and photosynthetically competent. If daylight is not available, the seedlings show etiolation, in which the long slender shoots appear to search for light availability. A phytochrome consists of a soluble protein dimer and a chromophore called phytochromobilin (a linear tetrapyrrole compound) (Fig 1) binds to each subunit towards the amino terminal. There are 5 types of phytochromes seen in plants (PHY A, PHY B, PHY C, PHY D and PHY E). The PHY A protein can only form a homodimer and the Pr isomer is light labile, while the other PHY proteins can form heterodimers and both isomers are light stable.

2. Cryptochromes

These are blue light receptors in plants, which like phytochromes regulate a number of light responses including germination and seedling growth morphology. However, cryptochromes, along with another type of blue light receptors called phototropins, also bring about the regulation of some responses that lead to stomatal guard cell movements and tropic or nastic leaf movements. Cryptochromes are products of three genes, Cry1, Cry2 and Cry3. The CRY proteins are nuclear, where they associate with other accessory proteins and bring about regulation of light-induced genes.

b. Circadian clock components

Besides photoreceptors that sense light, the duration or time of the light period is measured by a mechanism called the circadian clock. The clock consists of input pathways, central oscillators and the output pathways (Fig 4.4).

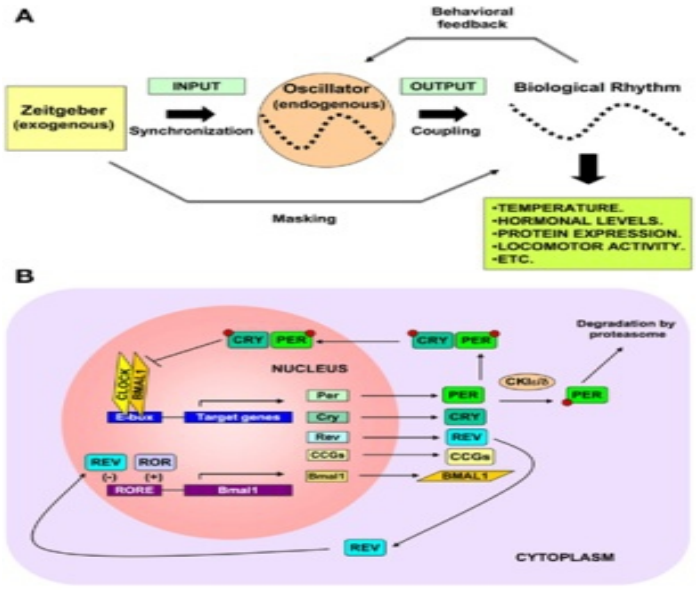


Fig.4.4: Circadian clock components

In the morning, two clock proteins, which show an oscillatory behavior in expression during a 24 h period, namely the late elongated hypocotyls (LHY) and circadian clock associated1 (CCA1), are induced by phytochromes and cryptochromes .These clock proteins, in turn, induce two regulatory proteins

called pseudoresponse regulators (PRR9 and PRR7) that negatively regulate CCA1 and LHY to form a feedback loop. So, the duration of expression of these oscillatory proteins is modified by PHY and CRY to synchronise the internal clocks to the period of light availability. In the evening, the clock proteins LHY and CCA1 are degraded due to accumulation of inactive forms of PHY and CRY. This allows expression of two other regulatory proteins, gigantea (GI) and timing of cab expression (TOC1), which together form a feedback loop. Another protein, zeitlupe (ZTL), which degrades TOC1 in light, is in an inactive form in the dark, which allows TOC1 expression. TOC1, like CCA1 and LHY is an oscillatory protein.

Day length and flowering Some plants especially in the temperate regions flower when the days get longer in early summer and are called long-day plants. Others flower when the day length decreases in early winter and are called short-day plants

Long-day plants require a day length greater than the critical day length and can flower even if grown in continuous light. Short-day plants require a shorter day length than the critical day length, but more importantly respond to the long dark exposure rather than the light exposure. If the dark period is interrupted by a short period of illumination, they do not flower. Phytochromes and cryptochromes, along with the clock components play an important role in regulating the daylength dependent flowering response.

Induction of flowering

Long-day plants

Plants of the genus Arabidopsis flower under long-day conditions.Red light is known to inhibit and far red light is known to promote their flowering. Under inductive conditions, PHY A is known to be involved in day length perception by interacting with the circadian clock pathway.When the day length is favourable, it stabilizes aprotein CONSTANS (CO) present in leaves, which is involved in transmitting the day length signal to the shoot apical meristem via the mobile signalling protein FLOWERING LOCUS T (FT). In the shoot apical meristem, FT interacts with a transcription factor FLOWERING

LOCUS D (FD) and induces the expression of genes that transform the vegetative meristem to a reproductive or floral meristem. PHY B on the other hand is not involved in day length perception and leads to degradation of CO when high red: far red ratios prevail during mornings.

Short-day plants

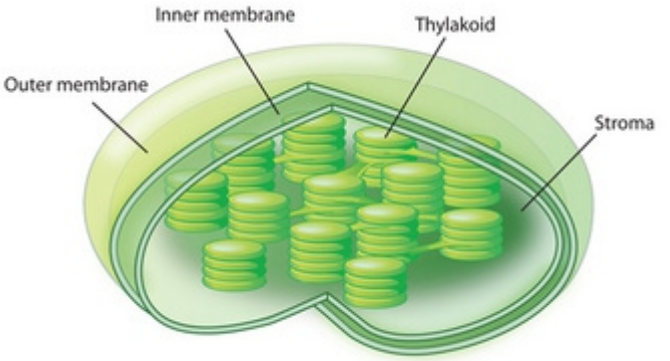
Rice is a short-day plant and flowers when the day length reduces during post rainy or early winter season. It shares the flowering induction components with Arabidopsis. For example, a gene Heading date 3a (Hd3a) is an ortholog of the FT gene in Arabidopsis and Heading date 1 (Hd 1) is an ortholog of CO.

Day neutral plants

In day-neutral plants like tomato, an ortholog of FT called single flower truss (SFT) is known to induce flowering in the absence of any day length signal.Over-expression of CO ortholog in tomato did not affect flowering time, suggesting that CO is not linked to flowering in tomato.

4.4. Photosynthetic membranes and organelles

Most living things depend on photosynthetic cells to manufacture the complex organic molecules they require as a source of energy. Photosynthetic cells are quite diverse and include cells found in green plants, phytoplankton, and cyanobacteria. During the process of photosynthesis, cells use carbon dioxide and energy from the Sun to make sugar molecules and oxygen. These sugar molecules are the basis for more complex molecules made by the photosynthetic cell, such as glucose. Then, via respiration processes, cells use oxygen and glucose to synthesize energy-rich carrier molecules, such as ATP, and carbon dioxide is produced as a waste product. Therefore, the synthesis of glucose and its breakdown by cells are opposing processes.



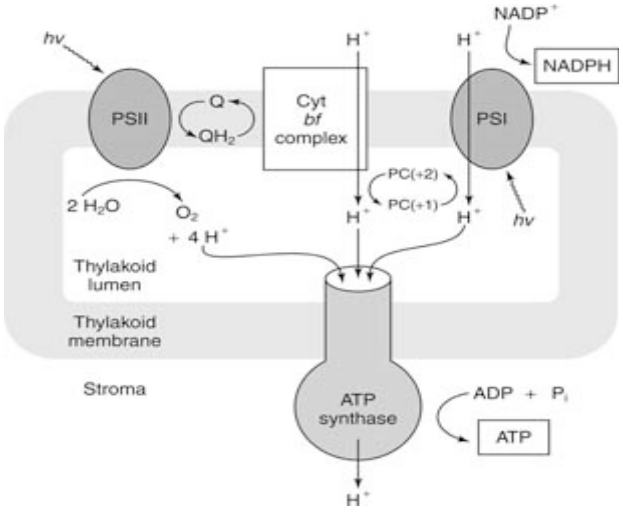
Photosynthetic cells contain special pigments that absorb light energy. Different pigments respond to different wavelengths of visible light. Chlorophyll, the primary pigment used in photosynthesis, reflects green light and absorbs red and blue light most strongly. In plants, photosynthesis takes place in chloroplasts, which contain the chlorophyll. Chloroplasts are surrounded by a double membrane and contain a third inner membrane, called the thylakoid membrane, that forms long folds within the organelle. In electron micrographs, thylakoid membranes look like stacks of coins, although the compartments they form are connected like a maze of chambers. The green pigment chlorophyll is located within the thylakoid membrane, and the space between the thylakoid and the chloroplast membranes is called the stroma.

Chlorophyll A is the major pigment used in photosynthesis, but there are several types of chlorophyll and numerous other pigments that respond to light, including red, brown, and blue pigments. These other pigments may help channel light energy to chlorophyll A or protect the cell from photo-damage. For example, the photosynthetic protists called dinoflagellates, which are responsible for the "red tides" that often prompt warnings against eating shellfish, contain a variety of light-sensitive pigments, including both chlorophyll and the red pigments responsible for their dramatic coloration. Photosynthesis consists of both light-dependent reactions and light-independent reactions. In plants, the so-called "light" reactions occur within the chloroplast thylakoids, where the aforementioned chlorophyll pigments reside. When light energy reaches the pigment molecules, it energizes the electrons within them, and these electrons are shunted to an electron transport chain in

the thylakoid membrane. Every step in the electron transport chain then brings each electron to a lower energy state and harnesses its energy by producing ATP and NADPH.

4.5. z scheme

During photosynthesis, the"Z-scheme" describes change of oxidation or reduction. The vertical axis in the figure represents the reduction potential of a particular species, the higher the position of a molecular species, the more negative its reduction potential, and the *more easily it donates electrons*. In the Z-scheme, electrons are removed from water (to the left) and then donated to the lower (non-excited) oxidized form of P680. Absorption of a photon excites P680 to P680*, which "jumps" to a more actively reducing species. P680* donates its electron to the quinone-cytochrome bf chain, with proton pumping. The electron from cytochrome bf is donated to PSI, converting P700 to P700*. This electron, along with others, is transferred to NADP, forming NADPH. Alternatively, this electron can go back to cytochrome bf in cyclic electron flow.



4.6. Light dependant reactions

We know the green plant obtained their energy form sunlight where radiant energy of sunlight is converted into chemical energy. This process completed into two steps- light and dark reaction. The light reaction is initiated

when specific wavelength of light absorbed by antenna chlorophylls. It is well known that photosynthesis light reaction in green plant involved in participation of two separate photo systems such as PS-I and PS-II.

In the light reaction following events occur -

1. Photooxidation of water take peace and O_2 is released.
2. Synthesis of ATP taking place by photo phosphorylation.
3. NADP+ is reduced to NADPH+H^+ Nicotinamide adenine dinucleotide phosphate.

Ruben et al-used radioactive O_2 in water supplied to the plant and found that O_2 released in photosynthesis comes from water-

$$6Co_2 + 12H_2{}^{18}o \rightarrow C_6H_{12}O6 + 6H_2O + 6^{18}O_2$$

The photophosphorylation is of two types:

- Non cyclic photophosphorylation or scheme or Z scheme.
- Cyclic photophosphorylation.

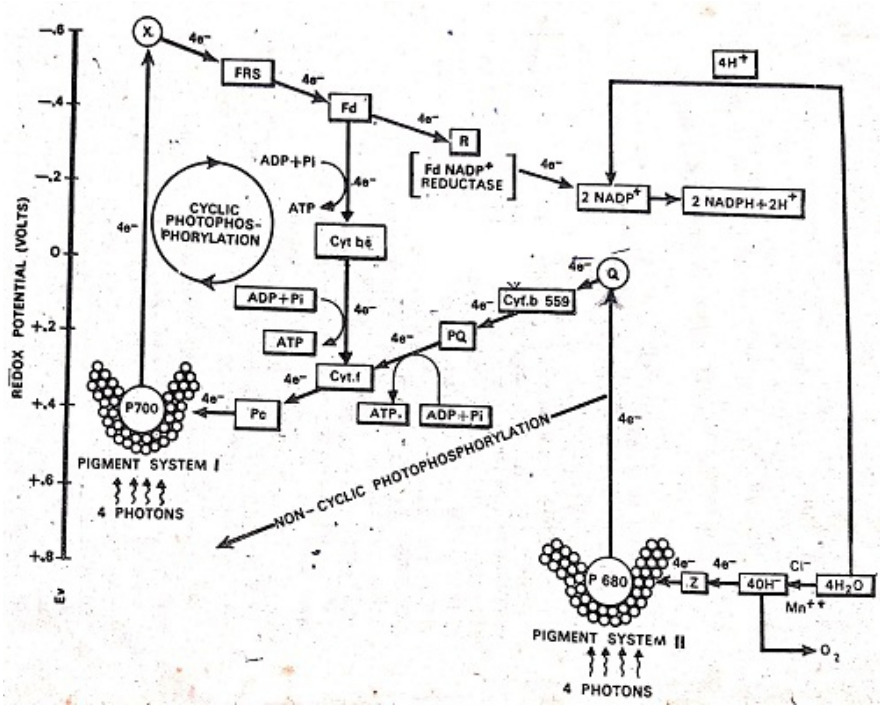


Fig. 4.5: Cyclic and Non Cyclic phoyophosphorylation.

Oxygenic photosynthesis or Non cyclic photophosphorylation

Oxygenic photosynthesis or Non-cyclic photophosphorylation occurs as a result of an interaction between photosystem I and photosystem II. Non-cyclic photophosphorylation helps in the formation of ATP as a result of electron flow from water to NADP. As this is a unidirectional flow, and does not follow any cyclic procedure, is called as non-cyclic photophosphorylation. The electron released from one particular pigment system does not return back to same pigment system. So electron deficiency of this pigment system satisfied by other pigment system.

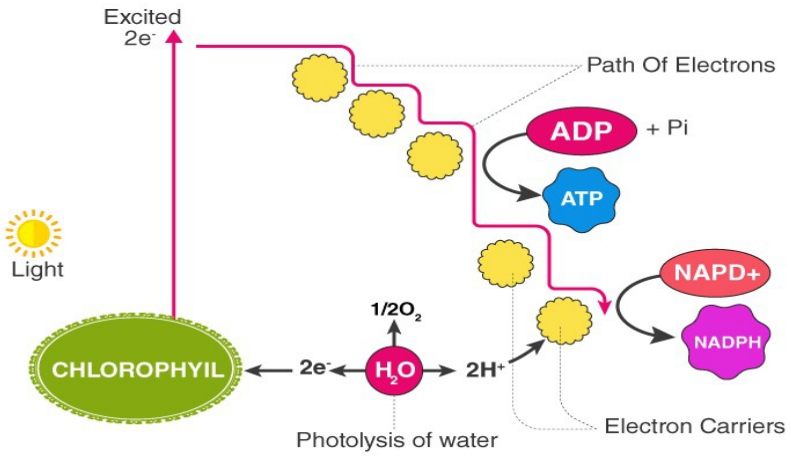


Fig.4.6: Non-cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

Light of longer wave length hits pigment of photosystem-I as a result P700 gets excited and releases electrons which are accepted by an unknown primary electron acceptor A (Fe-5) believed to be an iron sulphur protein complex and gets reduced. From reduced A (Fe-S) the electrons are accepted by a nonhemeiron protein called ferrodoxin (Fd) and from reduced Fd. The electrons from reduced Fd are transferred to $NADP^+$ and $NADP^+$ get reduced to $NADPH+H^+$. This causes deficit of electron in photo-system I. This deficit is made up by photo excitation of P680 of Photosystem I. Electron deficit created in photosystem II is filled by electrons derived by the photo-oxidation of water in presence of Mn^{++} and cl^- ions.

When a lower wave length of light is received by PS II, P680 looses an electron which is accepted by quinone. The electron then travels down the hill through a series of electron carriers B, PQ, cytf and plastocyanin. The energy released in the transfer of electron from PQ to cytf is utilised to convert ADP into ATP and then electron goes to PS I. At this stage water dissociates into H^+ and OH^- ions. The hydroxyl ion (OH^-) looses electrons and transferred to PS II. The H^+ are taken up by NADP+ which get reduced to $NADPH+H^+$.

Thus, in non-cyclic photophosphorylation the electron is not cycled back. Therefore, it is called non cyclic photophosphorylation.

Anoxygenic photosynthesis or cyclic photophosphorylation

Anoxygenic photosynthesis or cyclic photophosphorylation takes place under certain conditions. It operates when Co_2 assimilation is curtailed and $NADPH +H^+$ starts accumulating. The cyclic transport is for more production of ATP which is needed by chloroplast.

In this process, only photosystem I operates. So no photoxidation of water take place. Therefore, no evolution of O_2 and no formation of $NADPH+H^+$ occurs.

The electron flows from P_{700} to A (Fe-s), then to Fd which is unable to pass electron to NADP+. The electron passes to $cytb_6$ and cytf and then to PS I. Thus, here electron is cycled back. In this only one PSI operates. Therefore no $NADPH+H^+$ is formed and Co_2 fixation is curtailed. This results in decline in quantum yield.

But when shorter wave length is given simultaneously PS II also comes in operation and photoxidation of water relases H+ which reduces NADP+ to $NADPH+H^+$ and photosynthetic enhancement takes place.

4.7. Hill reaction

The Hill reaction is the light-driven transfer of electrons from water to Hill reagents (non-physiological oxidants) in a direction against the chemical potential gradient as part

of photosynthesis. Robin Hill discovered the reaction in 1937. He demonstrated that the process by which plants produce oxygen is separate from the process that converts carbon dioxide to sugars. Hill's finding was that the origin of oxygen in photosynthesis is water (H_2O) not carbon dioxide (CO_2) as previously believed. Hill's observation of chloroplasts in dark conditions and in the absence of CO_2, showed that the artificial electron acceptor was oxidized but not reduced, terminating the process, but without production of oxygen and sugar. This observation allowed Hill to conclude that oxygen is released during the light-dependent steps (Hill reaction) of photosynthesis. Process by which plants, and some bacteria use the energy from sunlight to produce sugar. Also, it is termed "Carbon fixation".

The overall equation is

6H2O + 6CO2 ----------> C6H12O6+ 6O2

-It include two major stages

1. Light reactions -it generate energy carrier molecules ATP and NADPH

-it occur in the thylakoids (Grana) - Hill Reaction

2. Dark reactions or Calvin-Benson cycle

- it generate C-C covalent bonds of carbohydrates

-it occur in the stroma of the chloroplast

4.8: Generation of NADPH and ATP

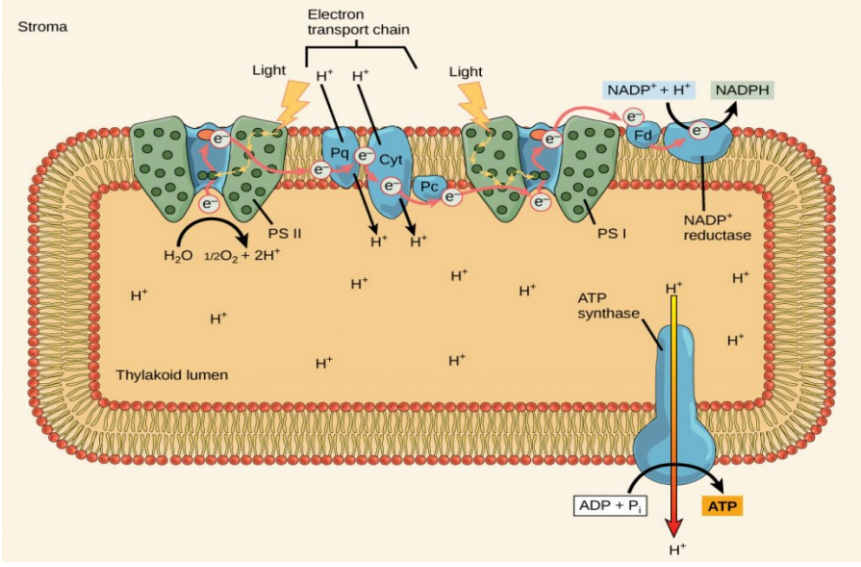
Photosynthesis takes place in two stages: the light-dependent reactions and the Calvin cycle. In the **light-dependent reactions**, which take place at the thylakoid membrane, chlorophyll absorbs energy from sunlight and then converts it into chemical energy with the use of water. The light-dependent reactions release oxygen as a byproduct as water is broken apart. In the Calvin cycle, which takes place in the stroma, the chemical energy derived from the light-dependent reactions drives both the capture of carbon in carbon dioxide molecules and the subsequent assembly of sugar molecules. The two reactions

use carrier molecules to transport the energy from one to the other. The carriers that move energy from the light-dependent reactions to the Calvin cycle reactions can be thought of as "full" because they bring energy. After the energy is released, the "empty" energy carriers return to the light-dependent reactions to obtain more energy. You should be familiar with the energy carrier molecules used during cellular respiration: NADH and FADH2. Photosynthesis uses a different energy carrier, **NADPH,** but it functions in a comparable way. The lower energy form, **NADP+**, picks up a high energy electron and a proton and is converted to NADPH. When NADPH gives up its electron, it is converted back to $NADP^+$.

The overall purpose of the light-dependent reactions is to convert solar energy into chemical energy in the form of NADPH and ATP. This chemical energy will be used by the Calvin cycle to fuel the assembly of sugar molecules.

The light-dependent reactions begin in a grouping of pigment molecules and proteins called a **photosystem**. There are two photosystems (Photosystem I and II), which exist in the membranes of thylakoids. Both photosystems have the same basic structure: a number of antenna proteins to which chlorophyll molecules are bound surround the reaction center where the photochemistry takes place. Each photosystem is serviced by the light-harvesting complex, which passes energy from sunlight to the reaction center. It consists of multiple antenna proteins that contain a mixture of 300–400 chlorophyll *a* and *b* molecules as well as other pigments like carotenoids. A photon of light energy travels until it reaches a molecule of chlorophyll pigment. The photon causes an electron in the chlorophyll to become "excited." The energy given to the electron allows it to break free from an atom of the chlorophyll molecule. Chlorophyll is therefore said to "donate" an electron (**Figure 1**).The absorption of a single **photon** or distinct quantity or "packet" of light by any of the chlorophylls pushes that molecule into an excited state. In short, the light energy has now been captured by biological molecules but is not stored in any useful form yet. The energy is transferred from chlorophyll to chlorophyll until eventually (after about a millionth of a second), it is delivered to the reaction center. Up to this point, only energy has

been transferred between molecules, not electrons. o replace the electron in the chlorophyll, a molecule of water is split. This splitting releases two electrons and results in the formation of oxygen (O_2) and 2 hydrogen ions (H^+) in the thylakoid space. The replacement of the electron enables chlorophyll to respond to another photon. The oxygen molecules produced as byproducts exit the leaf through the stomata and find their way to the surrounding environment. The hydrogen ions play critical roles in the remainder of the light-dependent reactions. Keep in mind that the purpose of the light-dependent reactions is to convert solar energy into chemical carriers (NADPH and ATP) that will be used in the Calvin cycle. In eukaryotes and some prokaryotes, two photosystems exist. The first is called **photosystem II** (PSII), which was named for the order of its discovery rather than for the order of the function. After a photon hits the photosystem II (PSII) reaction center, energy from sunlight is used to extract electrons from water. The electrons travel through the **chloroplast electron transport chain** to photosystem I (PSI), which reduces NADP+ to NADPH.



GENERATING AN ENERGY MOLECULE: ATP

In the light-dependent reactions, energy absorbed by sunlight is stored by two types of energy-carrier molecules: ATP and NADPH. The energy that these molecules carry is stored in a bond that holds a single atom to the molecule.

For ATP, it is a phosphate atom, and for NADPH, it is a hydrogen atom. Recall that NADH was a similar molecule that carried energy in the mitochondrion from the citric acid cycle to the electron transport chain. When these molecules release energy into the Calvin cycle, they each lose atoms to become the lower-energy molecules ADP and NADP+.

The buildup of hydrogen ions in the thylakoid space forms an electrochemical gradient because of the difference in the concentration of protons (H^+) and the difference in the charge across the membrane that they create. This potential energy is harvested and stored as chemical energy in ATP through chemiosmosis, the movement of hydrogen ions down their electrochemical gradient through the transmembrane enzyme ATP synthase, just as in the mitochondrion.

The hydrogen ions are allowed to pass through the thylakoid membrane through an embedded protein complex called ATP synthase. This same protein generated ATP from ADP in the mitochondrion. The energy generated by the hydrogen ion stream allows ATP synthase to attach a third phosphate to ADP, which forms a molecule of ATP in a process called photophosphorylation. The flow of hydrogen ions through ATP synthase is called chemiosmosis (just like in cellular respiration), because the ions move from an area of high to low concentration through a semi-permeable structure.

GENERATING ANOTHER ENERGY CARRIER: NADPH

The remaining function of the light-dependent reaction is to generate the other energy-carrier molecule, NADPH. As the electron from the electron transport chain arrives at photosystem I, it is re-energized with another photon captured by chlorophyll. The energy from this electron drives the formation of NADPH from NADP+ and a hydrogen ion (H+). Now that the solar energy is stored in energy carriers, it can be used to make a sugar molecule.

The photosynthetic apparatus of purple bacteria consists of two types of pigment-protein complexes: the reaction centers and the light-harvesting complexes. The main function of the light-harvesting complexes is to gather

light energy and to transfer this energy to the reaction centers for the photo-induced redox processes. In most purple bacteria, the photosynthetic membranes contain two types of light-harvesting complexes: light harvesting complex I (LH-I) and light harvesting complex II (LH-II) [1]. While LH-I is tightly bound to the photosynthetic reaction centers, LH-II is not directly associated with the reaction centers, but transfers energy to the reaction centers via LH-I [1].

4.9: Light harvesting complexes

Light-harvesting systems are present in the photosynthetic apparatus of all photosynthetic organisms. These systems are not necessary for the photosynthetic reaction but they increase the capacity for harvesting light and, in addition, have important regulatory functions. Light-harvesting systems typically have a very high pigment/protein ratio and are thus a cost-efficient way of maximizing light-harvesting capacity.

The light absorption processes associated with photosynthesis occur in large protein complexes present in thylakoid membrane known as photosystems. The photosystem consists of two pigment protein such as antenna complex and core complex. The antenna complex also known as light harvesting complex (LHC). The two definite types of photosystem like photosystem-I (PS I plastocyanin-ferredoxin oxidoreductase) and Photosystem II (PS II, water-plastoquinone oxidoreductase) are found in nature. The PS I consists of LHC I and core complex I (CC I) located in the stroma lamella of thylakoids. The PS II consists of LHC II and CC II located in stacked grana domain. The PS I uses the absorbed energy to transfer an electron to low potential acceptor that via intermediates reduces NAD+ (Nicotinamide adenine dinucleotide phosphate). The PS II oxidizes water releasing O2, H+ and e_ and reduce PS I reaction centres (P700).

Photosystem I

Photosystem I is the light-driven plastocyanin-ferredoxin oxidoreductase present in the thylakoid membranes of cyanobacteria and chloroplasts. PSI is involved in the cyclic and non-cyclic photophosphorylation. PS I receive the electrons from photosystem II. This system produces a strong reductant which

reduces NADP+ to NADPH + H^+. The reaction center of this photosystem contains chlorophyll a molecules (P700) that absorb light of 700 nm wavelength. Molecular oxygen is not evolved in this system. The structure of photosystem I in a cyanobacterium has been provided in Fig. 4.7. It is a homotrimer with each subunit in the trimer containing 12 different protein molecules bound to 96 molecules of chlorophyll a, 22 molecules of carotenoid, 4 lipid molecules, 3 clusters of Fe_4S_4 and 2 phylloquinones.

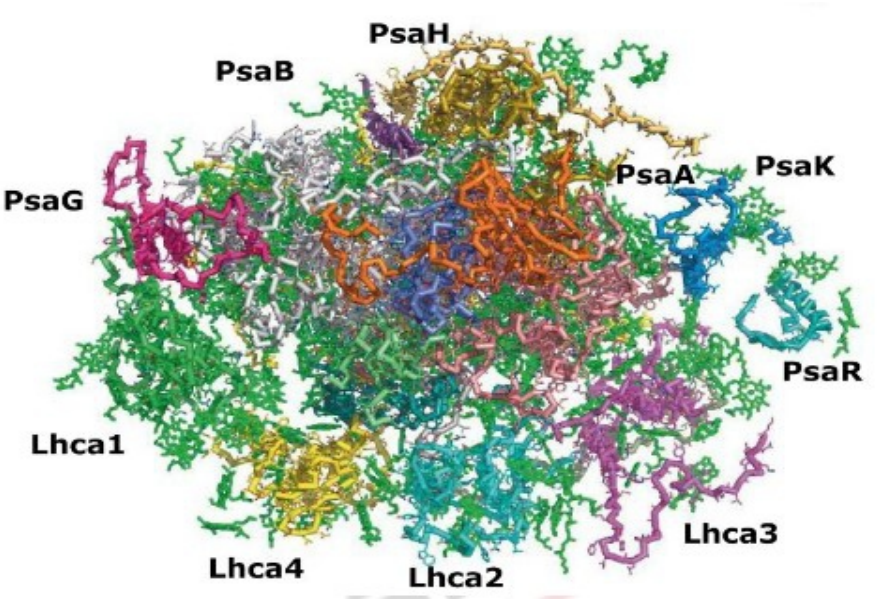


Fig.4.7: Structure of Photosystem I

The function of PS I is as followings:

- **Excitation of electron in PS I:** Photoexcited electrons enter photosystem I via an electron transport chain set in the thylakoid membrane where it waits until the electron is excited by another photon.
- **Chemiosmosis:** The energy fall is harvested to transport hydrogen (H+) through the membrane, into the thylakoid lumen to generate ATP
- **Conversion of NADP + to NADPH:** The excited electrons in photosystem I oxidize NADP + to NADPH which will be needed in the Calvin Cycle.

Photosystem II

Photosystem II" is the first link in the photosynthesis chain is a multi subunit pigment- protein complex (water-plastoquinone oxidoreductases)

embedded in the lipid environment of the thylakoid membranes of plants, algae and cyanobacteria. At the heart of this photosytem, is a reaction center (RC) core containing chlorophyll a molecules (P 680) that absorbs light of 680 nm. Driven by light, this enzyme catalyzes the chemically and thermodynamically demanding reaction of water splitting. While doing so, it harnesses solar irradiation to oxidize two molecules of water to molecular oxygen, liberating electrons which provide the reducing equivalents required for the conversion of CO_2 into the organic molecules of life. Photosystem II (PS II) is involved only in non-cyclic photophosphorylation. Photosystem II (PS II) donates electrons to photosystem I where NADP+ is reduced. This system is responsible for the photolysis of water and involves the evolution of molecular oxygen. Photosystem II (Fig. 4.8) is also a complex assembly of more than 20 different protein molecules bound to: 50 or more chlorophyll a molecules, Some half dozen carotenoid molecules and 2 molecules of plastoquinone.

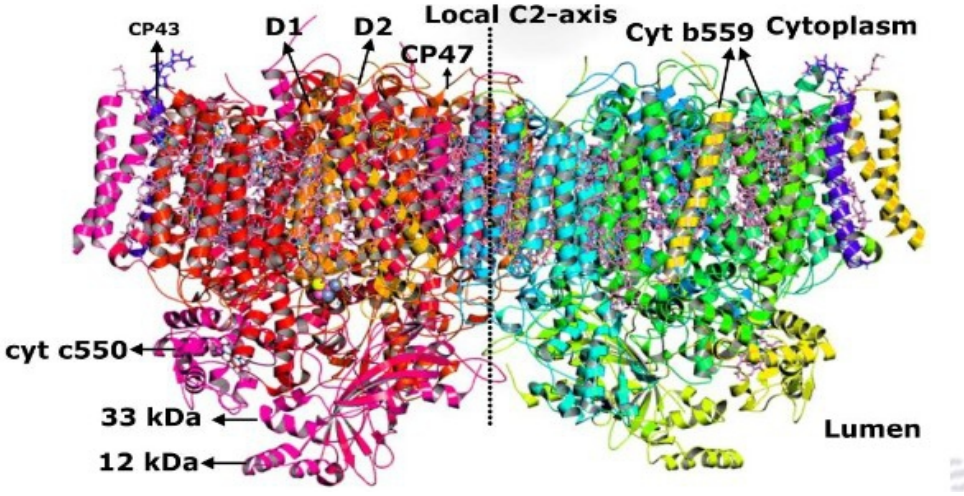


Fig.4.8: Structure of Photosystem II

4.10: Summary

- Photosynthesis is a process used by plants and other organisms to convert light energy, normally from the sun, into chemical energy that can be used

to fuel the organisms' activities. Carbohydrates, such as sugars, are synthesized from carbon dioxide and water.

- Photosynthetic cells contain chlorophyll and other light-sensitive pigments that capture solar energy. In the presence of carbon dioxide, such cells are able to convert this solar energy into energy-rich organic molecules, such as glucose. These cells not only drive the global carbon cycle, but they also produce much of the oxygen present in atmosphere of the Earth. Essentially, nonphotosynthetic cells use the products of photosynthesis to do the opposite of photosynthesis: break down glucose and release carbon dioxide.
- Photoperiodic responses of plants relate to duration of light and flowering is the most striking. Two important photoreceptors – phytochromes and cryptochromes sense light, while the circadian clock components measure it duration. These two act jointly to regulate flowering induction. The components which signal flowering response involve Constans (CO) and Flowering locus T (FT) and are similar in long day, short day and day neutral plants, but the mechanism by which the expression of these components is regulated differs.
- The plant photosynthetic reactions occur in two stages namely "light reactions" involving electron-proton transfer processes and dark reactions involving the reduction of CO_2 for the biosynthesis of carbohydrates. During the light reactions, the solar energy is converted into ATP and NADPH with the help of multi-pigment protein complexes known as photosystem I and photosystem II.

4.11. Terminal questions

Q.1. Discuss structure and function of chlorophyll.

Answer:----------

Q.2. Write difference between light and dark photosynthesis ?

Answer:----------

Q.3. What is photosynthesis? discuss the role photosynthesis in green plants.

Answer:----------

Q.4. Discuss about non-cyclic photophosphorylation.

Answer:----------

Q.5. What mean about Photoperiodism? Discuss effect on plant growth and flowering.

Answer:----------

Q.6. Discuss about z scheme in photosynthesis.

Answer:----------

4.12. Further readings

1. Plant physiology by H.N. Srivastava, Pradeep Publication, Jalendher
2. General biochemistry: J.H. Weil, New Age International (P) Limited, 5th edition, 2013,
3. Principles of Biochemistry: Lehninger, Nelson and Cox. Student edition, CBS 1439 Publishers and Distributors, Delhi.
4. Elements of biochemistry: J.L. Jain, S. Chand publication, Seventh Edition.
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Unit-5. Synthesis of phytochemicals

5.1. Introduction

Naturally occurring compounds, known as phytochemicals are thought to be largely responsible for the protective health benefits of these plant-based foods and beverages, beyond those conferred by their vitamin and mineral contents. Phytochemicals, also referred to as phytonutrients, which are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds, and are classified according to their chemical structures and functional properties. These phytochemicals, which are part of a large and varied group of chemical compounds, are also responsible for the color, flavor, and odor of plant foods, such as blueberries' dark hue, broccoli's bitter taste, and garlic's pungent odor The phytochemicals are used in functional food, soft drinks, and many other food items, which are having good nutrient value and significant importance, economically. Phytochemicals have great antioxidant potential and are of great interest due to their beneficial effects on health of human beings, and they give immense health benefits to the consumers. In India, phytochemicals, as well as medicinal plants, have remained the most abundant source of health care and life improvement since very long. India is the richest source of traditional herbal plants with their prescriptions. In India, Ayurvedic, Unani and Siddha medico-therapeutics are playing a very important role in the society since ancient time,

Objectives:

- To understand the phytochemicals
- To discuss about lignin's and its types
- To discuss about terpenes and waxes
- To know about alkaloids and its structure and functions

5.2. Terpenes

Terpenes is comes under the category of phytochemicals. It is the aromatic organic compounds produced mostly from aromatic plants. This organic compound release special types of fragrance and also have the characteristic scent of many plants, such as cannabis, pine, and lavender, as well as fresh orange peel. This terpenes also used in other oils to produce fragrance. In nature, these terpenes protect the plants from animal grazing or infectious germs. The common plant sources of terpenes are tea, thyme, cannabis, Spanish sage, and citrus fruits (e.g., lemon, orange, mandarin). Inadition, terpenes are produced by aromatic plants and herbs, such as rosemary and lavender, as well as some animals. Terpenes play a vital role in plants. In some plants, terpenes attract pollinators, while in other plants; they cause a strong reaction to repel predators, such as insects or foraging animals. Terpene has many health benefits so they are used in various kinds of medicines. It used in antiplasmodial activity that is notable as its mechanism of action is similar to the popular antimalarial drug in use-chloroquine. Monoterpenes specifically are widely studied for their antiviral property. Certain terpenes were widely used in natural folk medicine such as curcumin that used as anti-inflammatory, antioxidant, anticancer, antiseptic, antiplasmodial, astringent, digestive, diuretic medicine. Terpenes are also responsible for the fragrance, taste, and pigment of plants. However, the terpenes are categorizes on the basis of their organization and number of isoprene units it contains. An isoprene unit is a building block of terpenes that is a gaseous hydrocarbon with the molecular formula C_5H_8.

5.2.1. Classification of terpenes

On the number of terpenes units they are

a) Monoterpenes: 2 isoprene units, 10 carbon atoms.

b) Sesquiterpenes: 3 isoprene units, 15 carbon atoms.

c) Diterpenes: 4 isoprene units, 20 carbon atoms.

d) Triterpenes: 6 isoprene units, 30 carbon atoms.

e) Tetraterpenes: 8 isoprene units, 40 carbon atoms.

Each isoprene molecule contains five carbon atoms with double bonds. The simplest terpenes are monoterpenes that contain two isoprene molecules in which 10 carbon are present. As such another isoprene is sesquiterpenes contain 3 units that have 15 carbons and next isoprene's so on. Terpenes can be subdivided into groups *acyclic* or *cyclic* which indicate their structure. Acyclic terpenes are linear, like the monoterpene β-myrcene. Cyclic terpenes form a ring, like the monoterpene p-cymene. Terpenes are found in different plant family such as

- Lamiaceae (Labiatae): Melissa officinalis (lemon balm), Lavandula officinalis (lavender), Mentha spp. (mint, Bo He).
- Coniferae: Thuja occidentalis .
- Leguminoseae: Glycyrrhizae spp. (Gan Cao).
- Taxaceae: Taxus spp. (yew)

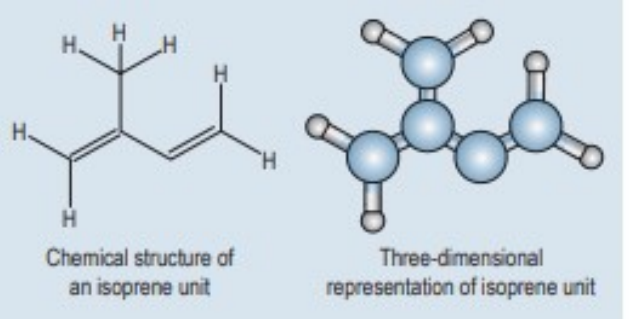


Fig.5.1: The chemical structure of the isoprene building block of terpenes

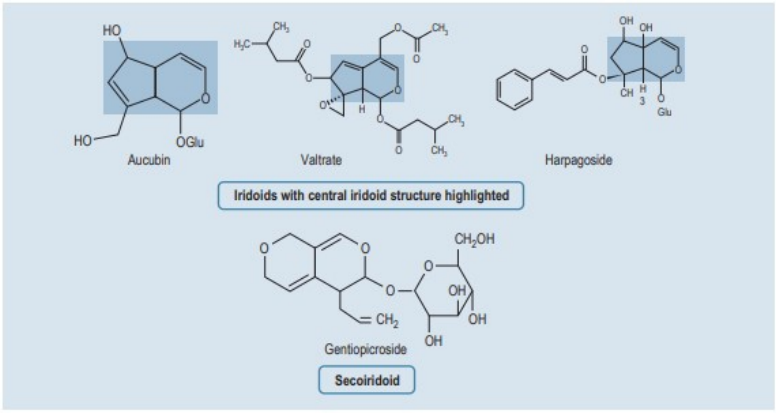


Fig.5.2: Examples of iridoids and secoiridoids.

Table: Different types of terpenes and their properties

Classification	Carbon atoms	Species produced from	Medicinal uses
Monoterpenes	C_{10}	*Quercus ilex*	Fragrances, repellent
Sesquiterpenes	C_{15}	*Helianthus annuus*	Treat malaria, treat bacterial infections, and migraines
Diterpenes	C_{20}	*Euphorbia, salvia miltiorrhiza*	Anti-inflammatory, cardiovascular diseases
Triterpenes	C_{30}	*Centella asiatica*	Wound healing, increases circulation

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7120914/

Monoterpenes

Monoterpenes are the basic units of terpenes that contain two isoprene units. The monoterpenes are volatile in nature and have very characteristic odours and are used in perfumes. Due to nonpolar and light weight they easily penetrate membrane. Monoterpenes generally have antibacterial, antifungal and antiseptic in nature. Menthols, Iridoids and Secoiridoids are the example of monoterpenes.

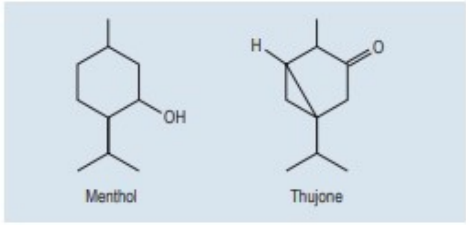


Fig.5.3: Menthol and thujone

Sesquiterpenes

This unit contains three isoprene units and it is not volatile as monoterpenes. Sesquiterpenes, containing the chemical formula $C_{15}H_{24}$, are much larger compounds than monoterpenes and are much more stable in comparison, it is used as antiprotozoal and antitumor. Sesquiterpenes are naturally occurring and found in plants, fungi, and insects, and act as a defensive mechanism or attract mates with pheromones in insects. The some plant family are also have it as

- Cannabaceae: Humulus spp. (hops).
- Compositae: Atractylodes spp. (Cang Zhu , Bai Zhu).
- Magnoliaceae: Magnolia offi cinalis (Hou Po).
- Cyperaceae: Cyperus rotundus (Xiang Fu)

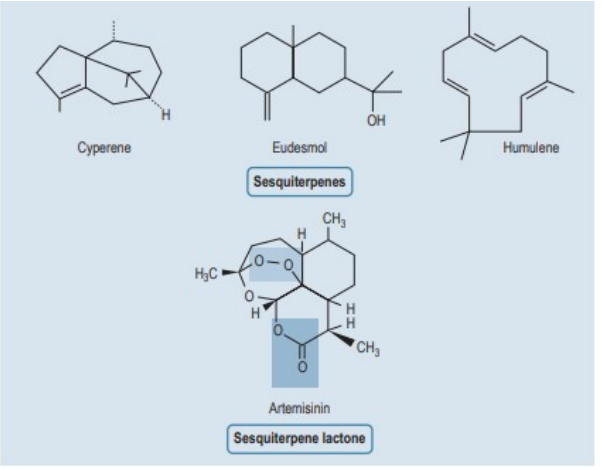


Fig.5.4: Various sesquiterpenes

Diterpenes

Diterpenes contain four isoprene units and have 20 carbon atoms. It contains the molecular formula, $C2_0H_{32}$. Diterpenes have physiologically active groups such as vitamin A activity well as plant growth hormones that regulate germination, flowering and switch reproductive cycles. Diterpenes have many therapeutic benefits such as antitumor, cytotoxic, and anti-inflammatory. Diterpenes is mostly found in

- Euphorbaceae: Croton spp. (croton, Ba Dou)
- Lamiaceae (Labiatae): Rosmarinus offi cinalis (rosemary),
- Taxaceae: Taxus (yew)

Triterpenes

It contains six isoprene units. It is widely distribution including animals. It plays a role as precursors to steroids in animal and plant organisms, and is derived from mevalonic acid. It is used as anticancer, antioxidant, antiviral, and anti-atherosclerotic activities. It is two main types: steroidal (usually tetracyclic triterpenoids, saponins) and the pentacyclic. When present as glycosides, triterpenes are referred to as saponins.

Teraterpenes

It contains eight isoprene unit Tetraterpenes are also known as carotenoids that have the molecular formula $C_{40}H_{56}$ and can be in the category of terpenes because they are made from isoprene units. They are found in all different types of fungi, bacteria, and plants. Carotenoids can be divided into:

- Carotenes.
- Xanthophylls (carotenoids containing oxygen).

Table: Medicinal Properties of terpenes from different sources

Terpene	**Medicinal properties**
Tea tree	Contains the active ingredient to treat cutaneous infections
Thyme	Possesses powerful antibacterial and antifungal properties
Cannabis	Possesses psychoactive properties and used against many infectious diseases
Spanish sage	Enhances memory and is used in anti-dementia drugs
Citrus fruits	Drugs against pediculosis
Citral	Antibacterial and antifungal effects
Lemongrass	Insect repellent

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7120914/

5.3. Lignins

Lignin is the most abundant aromatic polymer in nature and the second most abundant biomass on earth. In paper production and other processes, lignin is obtained as a side product and mainly used energetically. The use of lignin in wood adhesives or for wood modification has received a lot of scientific attention. Lignin is one of the main components of plant cell wall and it is a natural phenolic polymer. Lignin has high molecular weight, complex composition and structure. Lignin is a highly heterogeneous polymer derived from a handful of precursor lignols that crosslink in diverse ways Lignin was first mentioned in 1813 by the Swiss botanist A. P. de Candolle, who described it as a fibrous, tasteless material, insoluble in water and alcohol but soluble in weak alkaline solutions, and which can be precipitated from solution using acid. Lignins are particularly important in the formation of cell walls, especially in wood and bark, because they lend rigidity and do not rot easily. Chemically, lignins are polymers made by cross-linking phenolic precursors Lignin biosynthesis extensively contributes to plant growth, tissue/organ development, lodging resistance and the responses to a variety of biotic and abiotic stresses. After a series of steps involving deamination, hydroxylation, methylation and reduction, lignin monomers are produced in cytoplasm and transported to the apoplast Lignin biosynthesis is a very complex network that is divided into three processes:

(i) biosynthesis of lignin monomers,

(ii) transport and

(iii) polymerization.

The lignin content shows a large variability between species: in general, in monocotyledons, it ranges between 5 and 12%, in softwoods between 25 and 35% and in hardwoods between 18 and 30%. The structural arrangement of lignin also differs between these three groups. Lignin is the second most abundant biopolymer in nature and accounts for almost 30% of the plants. Its deposition in the cell wall is of great importance for plant development:

i. it provides rigidity and strength to the cell wall, giving mechanical support for the plant organs;

ii. it presents hydrophobicity favoring the transport of water and solutes in the vascular system and

iii. it protects the cell against pathogens

The chemical structure of lignin varies significantly depending on the lignin source. Thus, upon considering lignin for any application, the characteristics of the different lignins have to be known, and the right type of lignin has to be carefully selected based on the target application. The lignin is constituted by the three precursors: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. In this precursor of lignin the hydroxyl group is linked to the C4 and substitutions with one or two methoxyl groups. Due to present of three phinolic groups it called called p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) The side-chain carbons are designated as α-, β- and γ-, with Cα attached to the aromatic C1.

For application in wood material treatment G- and H-lignin units and thus softwood or grass lignin is widely considered most promising. The reason for this can be found in the structure of the monomers; G- and H-lignin units have more reactive sites at the benzene ring, making them more reactive toward other binding material or chemical treatment.

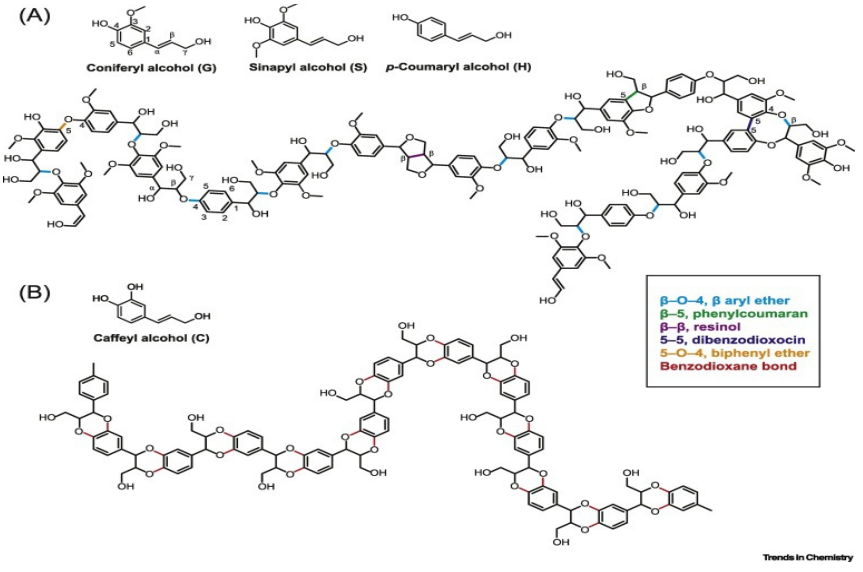


Fig.5.5: Model chemical structures of Lignin.

The main linkages found in lignin are shown in different colors. The main and most important bond found in hardwood, softwood, and grass lignins is the β–O–4 unit (light blue), which normally constitutes 40–85% of the total

linkages depending on the wood species. The other types of bonds present in lignin include the β–β (violet), 5–5 (dark purple), β–5 (green), and 5–O–4 (orange) that together account for the remaining 15–60% of interunit linkages (A). Exceptions to these standard linkages can be found in nature. For example, lignin in vanilla seed coats is formed almost exclusively by caffeyl alcohol units held connected together through benzodioxane linkages (red) (B)

Classification of lignin

Lignins have been generally classified into three major groups, based on the chemical structure of their monomer units: softwood lignin, hardwood lignin, and grass lignin. Lignin differs naturally in content and composition between biomass materials at various levels, e.g., between species, within species and between components.

a) softwood lignin,

b) hardwood lignin,

c) grass lignin

5.4. Waxes

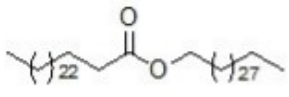
Waxes are considered as simplest fatty acid esters in nature. Structurally, they are considered as esters of long-chain (C_{14}-C_{36}) saturated and unsaturated fatty acids with long-chain (C_{16}-C_{30}) alcohols. They comprise a diverse mixture of aliphatics, triterpenoids, flavonoids and/or phenolic lipids, such as, alkylresorcinols. These molecules are completely water-insoluble and generally solid at biological temperatures. Waxes are a second group of neutral lipids that are of physiological importance, though they are a minor component of biological systems. Waxes are found in protective coating on skin, fur, and feathers. wax is found on leaves and fruits of higher plants and on the skeleton of many insects. Plant waxes are generally the waterproofing components found in an amorphous layer on the outer surface of the plants. They are essential for plants as barrier protection against environmental stress. Typical plant waxes include candelilla wax, carnauba wax, rice bran wax as well as sunflower wax, etc

Candelilla wax is mainly obtained from the leaves of plant *Euphorbia antisyphilitica Zuccarini* native to northern Mexican and

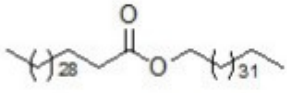
south-westren united states. Candelilla wax contains approximately 42% hydrocarbons, 39% wax, resin and sitosteroyl esters, 8% free wax and resin acids, 6% lactones, and 5% free wax and resin alcohols

Carnauba wax is obtained from the Brazilian palm *Coernicia cerifera Martius*, also known as carnauba wax palm. It is found on the upper and lower surface of the palm leaves. Carnauba wax is one the hardest plant waxes, with melting temperature ranging from 82.5 to 83 °C.

Rice bran wax is another high melting vegetable wax found in husks of rice *O. sativa* (Feuge and Cousins, 1957). It is obtained as a by-product from the de-waxing of rice bran oil. The composition of rice bran wax is relatively homogeneous, with the major components being even-numbered aliphatic acids (wax acids) and higher alcohol esters



a major component of beeswax



a major component of carnauba wax

Characteristics of wax

- They are the main source of energy in planktons.
- The melting point of waxes is higher than that of TAGs.
- Waxes are considered water-repellant due to which certain skin glands secrete them to protect hair and skin by keeping them lubricated.
- The leaves of plants like rhododendrons, poison ivy and several tropical plants are shiny in appearance due to wax coating on them. This prevents excess evaporation of water and provides protection from the parasites.
- The wax prevents the plant from losing excessive amounts of water

5.5. Alkaloids

Alkaloid, which means alkali-like substances, is basic nitrogenous compounds of plant or animal origin and generally possessing a marked

physiological action on man or animals. The nitrogen is usually contained in a heterocyclic ring system and it mainly derived from amino acids. Alkaloids are a group of molecules with a comparatively large occurrence in nature around the world. They are very diverse chemicals and biomolecules, but they are completely secondary compounds and they are derivative of amino acids. The term alkaloid was coined in 1819 by the pharmacist W. Meisner and meant simply, alkali like. Typical alkaloids are derived from plant sources, they are basic, they comprehend one or more nitrogen atoms, generally existing in a heterocyclic ring, and they habitually have a noticeable physiological action on human or other animals. The term 'proto-alkaloid' or 'amino-alkaloid' is occasionally applied to compounds such as hordenine, ephedrine and colchicine which lack one or more of the properties of typical alkaloids.

Maximum alkaloids are well-defined crystalline substances which bond with acids to form salts. In the plant they may exist in the free- state, as salts or as N -oxides. In addition to the elements carbon, hydrogen and nitrogen, most alkaloids contain oxygen. Alkaloid is a powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties.

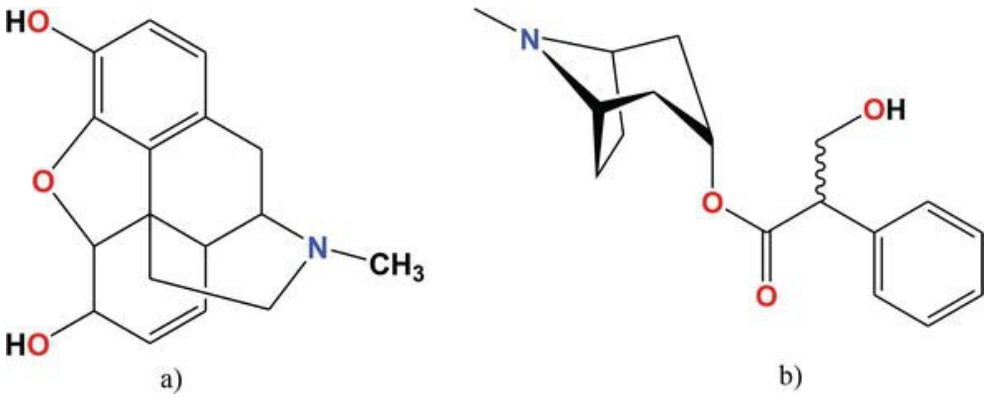


Fig.5.6: Structures of alkaloids: (a) morphine and (b) atropine

5.5.1. Types of Alkaloids

Alkaloids can be classified by their mutual molecular precursors, founded on the biological pathway used to build the molecule. From a structural perspective, alkaloids are separated according to their shapes and origins. There are three main types of alkaloids:

a) True alkaloids

b) Protoalkaloids

c) Pseudoalkaloids

True alkaloids

True alkaloids are derived from amino acids and they share a heterocyclic ring with nitrogen. These alkaloids are extremely reactive substances with biological activity even in small doses. All true alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine which is a brown liquid. True alkaloids form water-soluble salts. Furthermore, most of them are crystalline substances which bond with acids to form salts.

Protoalkaloids

Protoalkaloids are of a closed ring, being impeccable but structurally simple alkaloids. Protoalkaloids are compounds, in which the N atom derived from an amino acid is not a fragment of the heterocyclic ring. Such kinds of alkaloid include compounds derived from ltyrosine and l-tryptophan.

Pseudoalkaloids

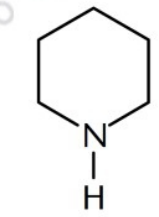
Pseudoalkaloids can be acetate and phenylalanine derived or terpenoid, as well as steroidal alkaloids. Examples of pseudoalkaloids include such compounds as coniine, capsaicin, ephedrine, solanidine, caffeine, theobromine and pinidine. they are compounds of elementary carbon skeletons of which are not derived from amino acids.

Classification Based Upon the Ring System

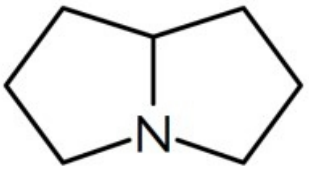
It is perhaps the most extensively established and mutual approach of classification of alkaloids for which the principal condition is the presence of the basic heterocyclic nucleus. According to this concept, the alkaloids are classified on the basis of the ring system present in them. For example

- Pyrrolidine alkaloids have a pyrrolidine (C_4N skeleton) nucleus. The structural α of these alkaloids is l-ornithine (in plants) and l-arginine (in animals). e.g., Hygrine.

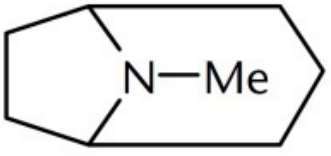
- Piperidine alkaloids contain the piperidine nucleus. True piperidine alkaloids have onecycle compounds with the C_5N nucleus.
- The pyrrolizidine nucleus is characteristic of this group of alkaloids. e.g., Senecionine.
- Tropane alkaloids have a tropane (C4N skeleton) nucleus. Structurally, these alkaloids synthesize as post-cursors of pyrrolines.
- Quinoline alkaloids containing principally the 'quinoline' nucleus comprise a series of alkaloids obtained exclusively from the Cinchona bark.
- The Isoquinoline alkaloids are a huge class of medicinally active alkaloids whose properties are variable.
- Imidazole alkaloids contain an imidazole ring structure in them. This group of alkaloids is an exception in the transformation process of structures, because the imidazole nucleus is already made at the stage of the precursor.



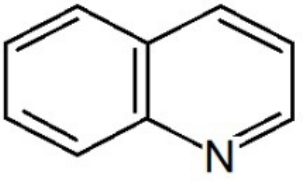
Piperidine Ring System



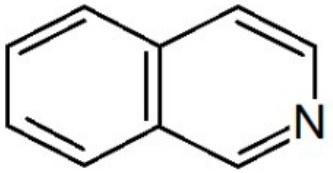
Pyrrolizidine Ring System



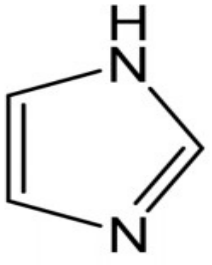
Tropane Ring System



Quinoline Ring System



Isoquinoline Ring System



Imidazole Ring System

Function of alkaloids in plants

a) They may act as protective against insects and herbivores due to their bitterness and toxicity.

b) They are, in certain cases, the final products of detoxification in metabolic reactions, therefore considered as waste products of metabolism.

c) They may provide nitrogen to the plant organs in case of nitrogen deficiency (source of nitrogen).

d) They, sometimes, act as growth regulators in certain metabolic systems.

e) They may be utilized as a source of energy in case of deficiency in carbon dioxide assimilation, especially those alkaloids containing a sugar moiety.

Methods of isolation of Alkaloids

Extracts of plants containing alkaloids were known and used because of their diverse activity by people from ages. But ages ago people did not know direct methods to isolate pure compounds from specified plant species. Alkaloids in plants usually exist as aqueous solution in tissues. To isolate them the method called extraction is usually used. For commercially useful alkaloids, special extraction methods were developed. In general mixture containing alkaloid should be dissolved with some solvent with reagents. Extraction method allows recovery of alkaloids from solution. Then, each alkaloid can be separated from mixture and be obtained in pure form. To obtain crystalline form of alkaloids, certain solvents should be used. Another method is chromatography. It uses differences in degrees of adsorption of different alkaloids in some solvent system on solid materials such as silica or alumina.

5.6. Summary

Phytochemicals, referred to as phytonutrients, are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds. They are classified according to their chemical structures and functional properties. Thousands of phytochemicals have been identified, and researchers speculate that there are likely many more they haven't yet discovered in the foods we eat..Lignin is of interest for various applications in wood products. Most research has been carried out on the application of Lignin as an adhesive. Adhesives are used for both solid wood and wood materials, as well as in

engineered wood products such as fiberboards. The alkaloids are defined as the basic nitrogenous complex organic compounds available naturally in the plant and animals. Generally, Alkaloids contains a heterocyclic ring in their molecular structure but nonheterocyclic structures have also been reported. Morphine and codeine are natural products of Papaver somniferum. However, the codeine is naturally produced in small amounts. Alkaloids are found essentially in the plants of the Dicotyledonous families. They occur in certain parts of the plants like leaves, fruits, seeds, bark and roots.

5.7. Terminal questions

Q.1: What are the phytochemicals? Discuss it.

Answer:----------

Q.2: Write the role of phytochemicals in human being.

Answer:----------

Q.3: What is wax? Discuss the role of wax in green plants.

Answer:----------

Q.4: Discuss about lignin and its classification.

Answer:----------

Q.5: What do you mean about Alkaloids? Discuss its types and use.

Answer:----------

Q.6: Discuss about terpenes.

Answer:----------

5.8. Further readings

1. Plant physiology by H.N. Srivastava, Pradeep Publication, Jalendher
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4. Elements of biochemistry: J.L. Jain, S. Chand publication, Seventh Edition.
5. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

Unit-6.Carbon assimilation

6.1. Introduction

Carbon assimilation is the process by which inorganic carbon is converted to organic compounds by living organisms. These compounds are then used to store energy and as structure for other biomolecules. The Calvin cycle fixes carbon in the chloroplasts of plants and algae, and in the cyanobacteria. It also fixes carbon in the anoxygenic photosynthesis in one type of proteobacteria called purple bacteria, and in some non-phototrophic proteobacteria. The reverse Krebs cycle is an alternative to the standard Calvin-Benson cycle for carbon fixation. It has been found in strict anaerobic or microaerobic bacteria (as Aquificales) and anaerobic archea. Photophosphorylation involves the oxidation of H_2O to O_2, with NADP+ as electron acceptor. Therefore, the oxidation and the phosphorylation of ADP are coupled by a proton gradient across the membrane. Photorespiration is the process of light-dependent uptake of molecular oxygen (O_2) concomitant with release of carbon dioxide (CO_2) from organic compounds which are take place in green plant tissue. Photorespiration is also known as the oxidative photosynthetic carbon cycle means C_2 cycle.

Objectives

- To discuss about carbon assimilation
- To discuss about photophosphorylation
- To discuss about photorespiration

6.2. Photophosphorylation

We know about photosynthesis occurs in plants that is the biological process of converting light energy into chemical energy. In this process, light energy is captured and used for converting carbon dioxide and water into glucose and oxygen gas. The complete process of photosynthesis is carried out by light reaction and by dark reaction. The photosynthesis of light reaction is associated with presence of phosphate molecules. The phosphate in the presence of light or the synthesizing of ATP play significant role in photosynthesis. Thus, the phosphorylation can be defined as the process of converting the energy of light into the energy of chemical bonds. It takes place in the chloroplast of cells, specifically in the thylakoid membrane. In the process of phosphorylation, light is absorbed by the chlorophyll and convert ADP into ATP molecule. Phosphorylation is an important process occurring in the living cell because by this process energy-rich molecule called ATP is formed. In this process, the phosphate group is added to ADP to form ATP by the enzyme kinases and phosphorylases.

Photophosphorylation involves the oxidation of H_2O to O_2, with NADP+ as electron acceptor. Therefore, the oxidation and the phosphorylation of ADP are coupled by a proton gradient across the membrane. Photophosphorylation process may be occurs either by cyclic process or non cyclic process.

6.2.1. Cyclic Photophosphorylation

Cyclic Photophosphorylation occurs where same electron is recycled. The chlorophyll absorbed the light energy that stimulates the electrons. The electron is then passed towards an electron acceptors protein which passes it along with an electron transport channel. Cyclic photophosphorylation involves the use of only **one** photosystem (PS I) and does **not** involve the reduction of NADP+. As the electron is passed along the transport channel, the electron loses energy, which is then used to make ATP from ADP and Pi. The electron is then recycled and again enters into the photosystem again. As the electron returns to the Photosystem (PSI), NADP+ is not reduced and water is not needed to replenish the electron supply.

In this Photophosphorylation process, only photosystem I operates. So no photoxidation of water take place, Therefore, no evolution of O_2 and no formation of NADPH + H^+ occurs. But when shorter wave length is given simultaneously, PS II also comes in operation and photoxidation of water relases H^+ which reduces $NADP^+$ to NADPH + H^+ and photosynthetic enhancement takes place. In cyclic photophosphorylation, the high energy electron is free from P700 to ps1 flow down to a cyclic pathway.

In bacterial photosynthesis, a single photosystem is present, and therefore it involved in cyclic photophosphorylation. It is favored in anaerobic conditions and conditions of high irradiance and CO_2 compensation points.

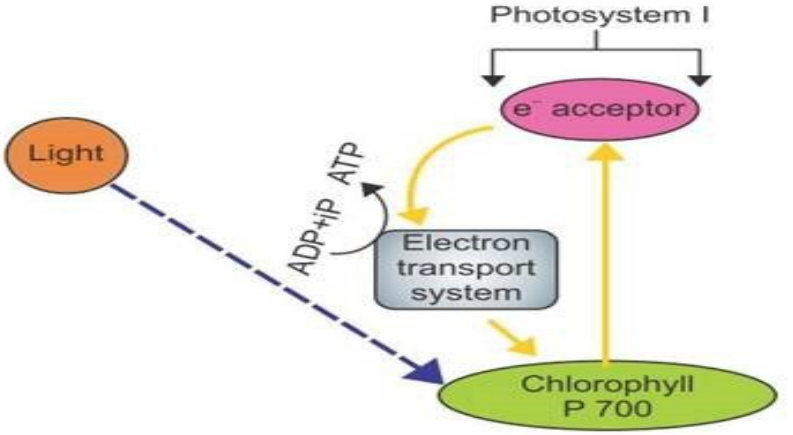

Fig.6.1: Cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

6.2.2. Non cyclic photophosphorylation

Non-cyclic photophosphorylation is two step process occurs as a result of an interaction between photosystem I and photosystem II. Non-cyclic photophosphorylation helps in the formation of ATP as a result of electron flow from water to $NADP^+$. Being a light reaction, non-cyclic photophosphorylation happens in the thylakoid membrane. Light of longer wave length hits pigment of photosystem-I as a result P700 gets excited and releases electrons which are accepted by an unknown primary electron acceptor and are finally passed on to NADP . Here, the electrons combine with the

protons (H^+) which is produced by photo oxidation (splitting up) of the water molecule and reduces $NADP^+$ to $NADPH + H^+$. Where a water molecule is broken down into $2H^+ + 1/2\ O_2 + 2e^-$ by a procedure called photolysis (light splitting).

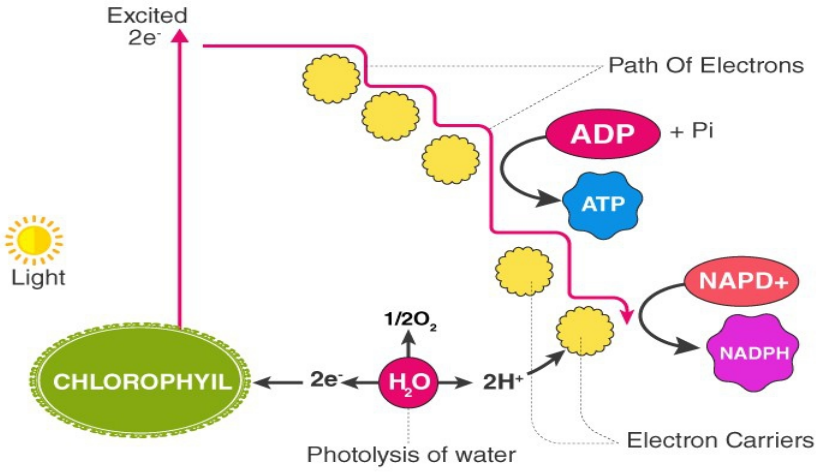

Fig.6.2: Non-cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

When a lower wave length of light is received by PS II, P680 looses an electron which is accepted by quinone. The electron then travels down the hill through a series of carriers B, PQ, cytf and plastocyanin. The energy released in the transfer of electron from PQ to cytf is utilised to convert ADP into ATP and electron goes to PS I. At this stage water dissociates into H^+ and OH^- ions. The hydroxyl ion (OH) looses electrons and transferred to PS II. The H^+ are taken up by NADP+ which get reduced to $NADPH+H^+$. Thus, in non-cyclic photophosphorylation the electron is not cycled back to same pigment system. Therefore, it is called non cyclic photophosphorylation.

Table 6.1 : Difference between Cyclic and non Cyclic

Photophosphorylation

Cyclic Photophosphorylation	Non-Cyclic Photophosphorylation
Only Photosystem I involved in the process.	Both Photosystem I and II are involved in the process
In cyclic photophosphorylationP700 is the active reaction center.	In non-cyclic photophosphorylationP680 and P700 are the active reaction center.
Electrons passes in a cyclic manner.	Electrons passes in a non -cyclic manner.
ATP molecules are generated.	Both NADPH + H^+ and ATP molecules are formed.
NADPH + H^+ is not produced.	NADPH + H^+ is produced.
This process is ideal only for bacteria.	This process is ideal in all green plants.

6.3. Calvin Cycle or C_3 cycle

It was discovered by Calvin. He used 14C and green alga chlorella and scendesmus and discovered C_3 cycle of Co_2 fixation using radioactive tracer technique. Sixmolecules of Co_2 combine with six molecules of ribulose 1,5 diphosphate in presence of water to form 12 molecules of 3-phosphoglyceric acid in presence of enzyme carboxydismutase. 3-phosphoglyceric acid (PGA) is first stable and detectable compound of calvin cycle which is 3 carbon compound. Therefore, this cycle commonly known as C_3 cycle. The 12 molecules of PGA react with 12 ATP molecules to produce 12 molecules of 1, 3-Diphosphoglyceric Acid in presence of Phosphoglycerokinase.

The 12 molecules of diphosphoglyceric acid is reduced to 12 molecules of phosphoglyceraldehyde by 12 molecules of NADPH $+H^+$. 12 NADPH $+H^+$ and 12 H_3Po_4 are regenerated in the process in presence of enzyme 3-phosphoglyceraldehyde dehydrogenate.

The 5 molecules of 3-phosphoglyceraldehyde get isomerised to form dihydroxy acetone phosphate. The 3 molecules of dihydroxy acetone phosphate combines with 3 molecules of 3-phosphoglyceraldehyde to form 3 molecules of Fructose 1, 6 diphosphate in presence of enzyyme aldolase.

Each molecules of Fructose 1, 6-diphosphate loses one phosphate in presence of enzyme phosphatase to form 3 molecules of Fructose-6 phosphate. One molecule of Fructose 6-phosphate forms one molecule of Hexose sugar.

2 molecules of frustose 6- phosphate react with 2 molecules of 3-phosphoglyceraldehyde to produce 2 molecules of xylulose 5-phosphate and 2 molecules of erythrose-4-phosphate in presence of enzyme Transketolase.

2 molecules of Erythrose-4-phosphate combines with 2 molecules of dihydroxy acetone phosphate to produce 2 molecules of sedoheptulose-1, 7, diphosphate in presence of transaldolase enzyme.

Each molecule of sedoheptulose-1, 7 diphosphate loses one phosphate group in presence of phosphate enzyme to form sedoheptulose-7 phosphate.

2 molecules of sedoheptulose-7 phosphate react with 2 molecules of 3-phosphoglyceraldehyde in presence of enzyme transketolase to produce 2 molecules of Ribose-5-phosphate and 2 molecules of xylulose-5-phosphate.

2 molecules of ribose-5-phosphate are converted to two molecules of ribulose-5-phosphate in presence of phosphoribose isomerase enzyme.

4 molecules of xylulose-5-phosphate are isomerised to 4 molecules of ribulose-5-phosphate in presence of enzyme ribulose phosphate isomerase.

At the end of cycle all the six molecules of ribulose-5-phosphate get converted to ribulose-1, 5- diphosphate.

Thus, the whole process of calvin cycle begins with the absoption of 6molecules of Co_2 by 6 molecules of RUBP and ends with the formation of 1 molecule of hexose sugar with the regeneration of 6 mol. of RUBP.

The energy required in this reaction is supplied by 12 $NADPH+H^+$ and 18 ATP, formed in the light reaction of photosynthesis.

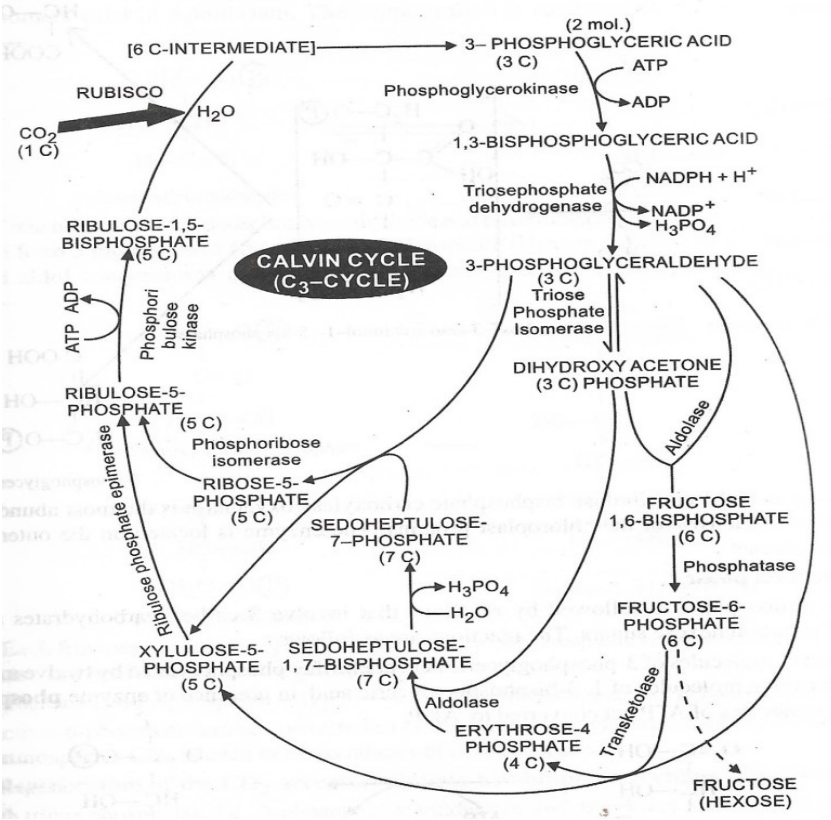

Fig.6.3: Calvin Cycle (C3 cycle)

6.4. Photorespiration

Photorespiration is the light-dependent evolution of CO_2. This photorespiratory pathway is initiated by the fixation of O_2 by Rubisco producing phosphoglycolate, which is metabolized in the photorespiratory pathway to form CO_2 and NH_3. Photorespiration is the process of light-dependent uptake of molecular oxygen (O_2) concomitant with release of carbon dioxide (CO_2) from organic compounds. Photorespiration is also known as the oxidative photosynthetic carbon cycle means C_2 cycle. In the process of photorespiration, the enzyme RuBisCO oxygenates into RuBP, wasting some of the energy produced by photosynthesis.

The inhibition of photosynthesis by high O_2 level was term as Warburge effects. However, the inhibition is mostly observed inC3 plants. The plants were hardly affected by varying O_2 concentration. It is generally believed that respiration of light is approximately equal to respiration in darkness. But, during 1955 and 1959 Decker indicated that respiration of plants such as

tobacco, is much more in the in the light than dark. It is estimated at this time respiration in light in green leaves and algae is may be 3-5 times more than the rate of respiration. Thus the respiration, that occurs only in light in green cells and responsible for release of extra CO_2 has been termed as photorespiration. In process of photo respiration, temperature and oxygen concentration play an important role. Photorespiration is very high when the temperature is between 25 and 30 ºC. The oxygenation of ribulose bisphosphate synthesizing glycolic acid and subsequent metabolism of glycolic acid releasing CO_2. At high CO_2 concentration, the photorespiration process is decreases while higher concentration of O_2 in atmosphere inhibits photosynthesis and accelerates photorespiration.

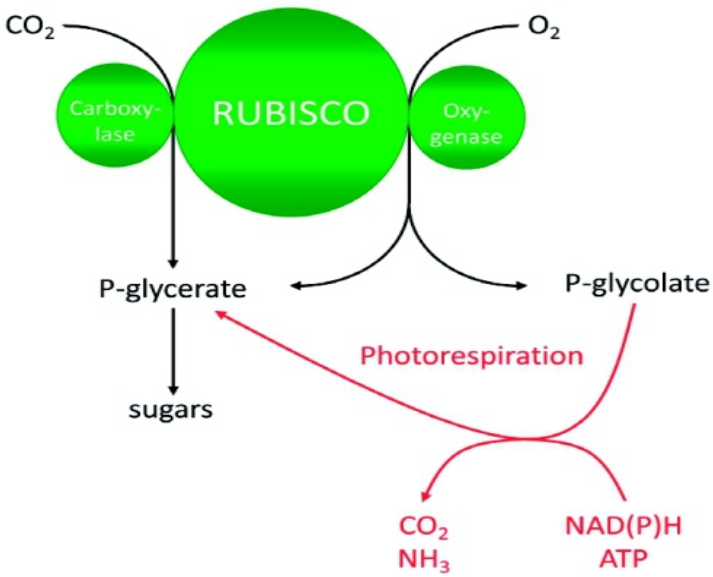

Fig.6.4: Photorespiration

Photorespiratory metabolism generates metabolites, like glycine, serine etc. for other plant processes. Genetically engineering Rubisco made by selectively removing the oxygenase enzyme activity, to reduce photorespiration, does not necessarily lead to improved plant performance, especially under unfavorable growth conditions. Photorespiration has been reduced at the site of rubisco by reducing the O2 concentration between 1% and 2%. At these O2 concentrations the Mehler reaction is still active, but due

to higher Km, the oxygenase reaction of rubisco is strongly reduced. Photorespiration is important for energy dissipation to prevent photoinhibition.

Mechanism of photorespiration

1. In the presence of excess oxygen and low CO_2, ribulose 1,5 diphosphate produced in the chloroplast during photosynthesis is split into 2 phospho glycolic acid and 3 phospho glyceric acid by the enzyme, ribulose 1,5 diphosphate oxygenase.
2. The 3 phospho glyceric acid enters the Calvin cycle.
3. In the next step, phosphate group is removed from 2 phosphoglycolic acid to produce glycolic acid by the enzyme, phosphatase.
4. Glycolic acid then it come out of chloroplast and enter the peroxisome. Here, it combines with oxygen to form glyoxylic acid and hydrogen peroxide. This reaction is catalyzed by the enzyme, glycolic acid oxidase. Hydrogen peroxide is toxic and it is broken down into water and oxygen by the enzyme, Catalase. Photorespiration is an oxidation process. In this process, glycolic acid is converted into carbohydrate and CO2 is released as the by product. As glycolic acid is oxidized in photorespiration, it is also called as glycolate metabolism.
5. The glyoxylic acid converted into glycine by the addition of one amino group with the help of the enzyme, amino transferase.
6. Now, the glycine is transported from the peroxisome into the mitochondria. In the mitochondria, two molecules of glycine condense to form serine and liberate carbon dioxide and ammonia.
7. Amino group is removed from serine to form hydroxyl pyruvic acid in the presence of the enzyme, transaminase.
8. Hydroxy pyruvic acid undergoes reduction with the help of NADH to form glyceric acid in the presence of enzyme alpha hydroxyl acid reductase.
9. Finally, regeneration of 3 phosphoglyceric acid occurs by the phosphorylation of glyceric acid with ATP. This reaction is catalyzed by the enzyme, Kinase.

10. The 3 phosphoglyceric acid is an intermediate product of Calvin cycle. If it enters the chloroplast, it is converted into carbohydrate by photosynthesis and it is suppressed nowadays with the increased CO2 content in the atmosphere.

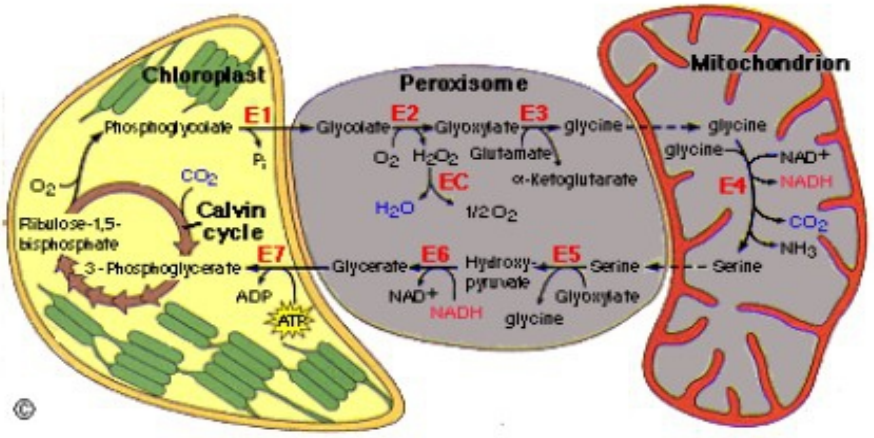

Fig 6.5: Photorespiration

Source: http://eagri.org/eagri50/PPHY261/lec11.pdf

6.5. Summary

Carbon fixation or carbon assimilation is the conversion process of inorganic carbon (carbon dioxide) to organic compounds by living organisms. The most prominent example is photosynthesis, although chemosynthesis is another form of carbon fixation that can take place in the absence of sunlight. Photosynthesis is the process by which are green plants prepare their food in the presence of sunlight using water and carbon dioxide as the raw materials. Photorespiration helps in classifying the plants, generally, photorespiration is found in C_3 plants and absent in C_4 plants. Carbon dioxide is evolved during the process and it prevents the total depletion of CO_2 in the vicinity of chloroplasts. This process causes oxidation of glycolic acid which arises as an unwanted byproduct of photosynthesis. The glycolic acid after oxidation is converted into carbohydrate but the remainder is converted into CO_2. Cyclic Photophosphorylation occurs where same electron is recycled. The chlorophyll absorbed the light energy that stimulates the electrons. The electron is then

passed towards an electron acceptors protein which passes it along with an electron transport channel. Cyclic photophosphorylation involves the use of only **one** photosystem (PS I) and does **not** involve the reduction of $NADP^+$.

6.6.Terminal question

Q.1. Discuss the carbon assimilation or fixation in living organism.

Answer:----------

Q.2. Write about the photophosphorylation.

Answer:----------

Q.3. Short Notes:

(a) Calvin Cycle (b) Lyclic Phosphorylation

Answer:----------

Q.4. Describe the non cyclic photophosphorylation.

Answer:----------

Q.5. Discuss about photorespiration.

Answer:----------

Q.6. Describe the significance of photorespiration.

Answer:----------

6.7. Further suggested readings

1. The Cycle of Photosynthesis by Arnold Ringstad
2. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition

3. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016
4. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.
5. TMH-Instant Notes of Biochemistry-2nd Edition
6. Reginald H. Garrett, Charles M. Grisham, Biochemistry by Reginald H Garrett 5th Edition.

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DCEBCH-108
Plant Biochemistry

Block -III

Plant Stress Growth Regulators

Introduction

This is the Third block of Plant biochemistry. This consists of three units.

Unit-7: The Stress metabolism in plants is mentioned in this unit. Mostly in this unit covers the abiotic and biotic stress in details. The various abiotic stress such as salinity, water stress, chilling, heating and heavy metal stress briefly discussed here. The pathogenesis, heavy metals and their impact on plant growth and metabolism is also mentioned in brief.

Unit-8: This unit covers the process of respiration. The Regulation of plant glycolysis, regulation of plant glycolysis, translocation of metabolites across mitochondrial membrane, TCA cycle is discussed in details.

Unit-9: Plant growth regulator is covers in this last unit of chapter. Phytohormones and its effect on plant growth and development are mentioned in this unit. The morphogenetic and regulation processes by light in plant are discussed in briefly.

Unit-7.Stress Metabolism in Plants

7.1. Introduction

Plants are subjected to a wide range of environmental stresses which reduces and limits the productivity of agricultural crops. Two types of environmental stresses are encountered to plants which can be categorized as (1) Abiotic stress and (2) Biotic stress. The abiotic stress causes the loss of major crop plants worldwide and includes radiation, salinity, floods, drought, extremes in temperature, heavy metals, etc. On the other hand, attacks by various pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores are included in biotic stresses. As plants are sessile in nature, they have no choice to escape from these environmental cues. Plants have developed various mechanisms in order to overcome these threats of biotic and abiotic stresses. They sense the external stress environment, get stimulated and then generate appropriate cellular responses.

Objectives

- To understand the plant stress
- To discuss about biotic and a biotic stress
- To know how to reduced plant stress
- To know role of cell organelles in plant stress

7.2. Plant Stress

The change in optimal condition of any factor essential for its growth will lead to aberrant change in physiological processes and due to this plant body will experience tension and this state referred as plant stress. The plants are subjected to a wide range of environmental stresses which reduces and limits the productivity of agricultural crops. Reduction in growth, yield and death of the plant or plant part. Stress may be caused due to biotic or abiotic factors. Thus the plant stresses can be categorized as (1) Abiotic stress and (2) Biotic stress.

There are some basic concepts of plant stress

The stress in physical term is defined as mechanical force per unit area applied to an object.

- In response to the applied stress, an object change in the dimension, this is also khown as strain.
- A biological condition which may be stress for one plant may be optimum for another plant. As plants are sessile, it is thought to measure the exact force exerted by stress, and therefore, in biological term, it is difficult to define stress

7.2.1. Abiotic stress:

Abiotic stresses such as drought (water stress), excessive watering (water logging), extreme temperatures (cold, frost and heat), salinity and mineral toxicity, negatively impact growth, development, yield and seed quality of crop and other plants. In future, it is predicted that fresh water scarcity will increase and ultimately intensity of abiotic stresses will increase. Hence, there is an urgency to develop crop varieties that are resilient to abiotic stresses to ensure food security and safety in coming years. A plants first line of defense against abiotic stress is in its roots. The chances of surviving stressful conditions will be high if the soil holding the plant is healthy and biologically diverse. One of the primary responses to abiotic stress such as high salinity is the disruption of the Na+/K+ ratio in the cytoplasm of the plant cell. The measure cause of abiotic stress is following

- Drought and Flooding
- Physical stress

- Chemical stress Heavy Metals
- Air Pollution
- Wind
- Radiation
- Light
- Temperature Competition
- Allelopathy
- Alkanity
- Soil pH
- Pesticides

7.2.2. Biotic stress

Biotic stress in plants is caused by living organisms, specially viruses, bacteria, fungi, nematodes, insects, arachnids and weeds. The agents causing biotic stress directly obtain their nutrients from host which can lead to death of plants. Biotic stress can become major because of pre- and postharvest losses. Despite lacking the adaptive immune system, plants can counteract biotic stresses by evolving themselves to certain sophisticated strategies. The defense mechanisms which act against these stresses are controlled genetically by plant's genetic code stored in them. Stresses Plants are constantly exposed to a variety of potential microbial pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores. In order to defend themselves, plants have developed a variety of defense responses many of which are induced by pathogen attack. However, pathogenic microbes have evolved the means to suppress PTI by secreting specialized proteins, called as effectors, into the plant cell cytosol that alter resistance signaling or manifestation of resistance responses.

Bacteria: Metabolomic and transcriptomic analysis of rice, in response to bacterial blight pathogen Xanthomonas oryzae pv. oryzae revealed global metabolic and transcriptomic changes in leaf tissues.

Fungi: On the basis of their lifestyles, plant pathogenic fungi have been divided into two classes: the biotrophs and the necrotrophs. Biotrophs feed on living host tissue, whereas necrotrophs first kill the host tissue and then feed on the dead tissues.

Biotrophic Fungi: For resistance against biotrophs, gene-for-gene mechanism is important. According to gene-for-gene hypothesis, given by Flor, for every gene in the plant that confers resistance, there is a corresponding gene in the pathogen that confers avirulence.

Necrotrophic Fungi: Transcript profiling of various plant-pathogen systems suggest differential regulation of a large number of transcripts in response to pathogen attack.

7.3. Salinity stress

The presence of salt in water or soil is called salinity. When, this salinity brings about change in plant metabolism, it is called salinity stress. High concentrations of salts in soils account for large decreases in the yield of a wide variety of crops all over the world. The problem is huge; almost 1000 million ha of land is affected by soil salinity. The surface stress of water leads to gradual increase in salinity of the soil, which is a major hurdle in growth of vegetation and known as salinity stress. Millions across the land have gone about production as result of salt loading through the irrigation water. All salts can affect plant growth, but not all inhibit growth. In addition, salts do not act alone in the soil, but interact in their effects on plants; some of these interactions are simple (e.g. interactions between Na^+ and Ca^{2+}), whereas some are complex (e.g. carbonates, and their effects via increased soil pH). Saline solutions impose both ionic and osmotic stresses on plants. These stresses can be distinguished at several levels. In salt sensitive plants, shoot and to a lesser extent root growth is permanently reduced within hours of salt stress and this effect does not appear to depend on Na^+ concentrations in the growing tissues, but rather is a response to the osmolarity of the external solution.

Classification of salinity stress

1. Primary salinity
2. Secondary salinity

Primary salinity

This type of salinity stress causes a strain when the salt lies in the vicinity of the cell but not inside it. This give rise to two conditions.

i. Osmotic stress

ii. Oxidative stress

Osmotic stress

When the concentration of salt increase initial the cell to a level higher than inside the cell, then a hypertonic solution is found outside the cell. The vicinity of the cell develops a negative osmotic potential and it becomes impossible for the plants to draw water in it. Besides being hypertonic, the external surrounding draws water out of the cell. The cell loses water and its protoplasm shrinks. Thus the cell gets plasmolysis.

1. Oxidative stress

The soil has sodium ion in excess due to salinity. These sodium ions mimic oxidative stress that is they produced by the O^{-2} radicals. For example bleaching or loss of cofactor (corrosion of sulfur). Besides sodium ion (Na^{+}) competes with calcium and potassium ions to enter the cell thus the electrolytic balance of the cell along with its biochemistry is attracted.

2. Secondary salinity

Its cause stress when salt enters in the cell. The secondary stress has following effects such as

Membrane permeability

This is affected adversely due to damage caused to the glycoprotein. Actually, the Na+ react with water of hydration around the proteins, the water of hydration is removed, the confirmation of protein that is tertiary and quaternary structure is disrupted, and protein lose their functional character. The membrane becomes permeable this the cell membrane get disrupted, and cytoplasmic granule also disappear when the salt enter the cytosol. Due to disappearance of cytoplasmic granular the cytoplasmic density is lowered.

Dilitariases effects of salinity stress

- The first plant response to salinity stress is a decrease in leaf growth with associated reduction in leaf area available for photosynthesis.
- Excessive accumulation of salts can lead to death of tissue
- Reduction of H_{2O} uptake by roots due to present of salt in the soil solution, its osmotic potential become negative and it becomes impossible to draw water out of it thus drought like condition prevail.

- In extreme conditions, the root not only fails to draw water from the soil solution but also lose their own water as in the case of wheat.
- Inhibit plastic extensibility of cell walls
- Foliage scotch, netting necroses and reduced photosynthesis
- Na^+ and Cl^- ions inhibit photosynthesis and carbohydrate assimilation and hence it not rectified result in marginal yields of thoughtful condition.

Cl^- induced damage

- The typical Cl^- stress magnification in mild leaf scarcity and foliage of thoughtful economic value.
- Tipburn and scorching are proceeds by marginal chlorosis.
- In advance stages, the necrosis tissues may cover 50% of more of the leaf thus causing a great reduction in the photosynthetic activity of the leaf and eventually causing complete collapse.

Na^+ induced damage

- The inter organ distribution of Na^+ in various plant appears to be of great importance, higher Na+ concentration are commonly found in roots while concentration of the ions in leaves are much lower.
- Sodium ions may also alter the crystalline structure of soil may become dispersed or flocculated thereby causing poor aeration and lower water availability. Under such condition Ca^{2+} is removed from the roots and this calcium deficiency cause immense damage.
- Metabolic toxicity of Na^+ is largely a result of its ability to compete with K^+ for binding sites essential for cellular function. More than 50 enzymes are activated by K^+, and Na^+ cannot substitute in this role.
- K^+ is found in very low concentration amount, compared to Na+ is saline condition. Na+ competes with K^+ by a low affinity mechanism. Moreover, protein synthesis requires high concentrations of K^+, owing to the K^+ requirement for the binding of ^{t}RNA to ribosomes
- Reduction in cell division, cell expansion, leaf size and overall plant functioning
- The formation of protein is also inhibited hence in pH of the cystol which adversely affects the electrolytic balance of the cell.

- Due to susceptibility of PS I to Na+ there is an increase in PS II; PS I ratio due to reduction in electron transport chain.
- The phosphonol pyruvate activity is lowered
- There is reduced nitrate reductase and nitrate reductase activity.
- Some time, increase in glutamine synthetage activity due to an increase in the demand for nitrogen.
- There is always increase in respiration rate to generate more energy to compete the stress.

Parameter describing water status of plants

i. Water potential

ii. Relatives water control

7.4. Water stress

The availability of excessive (flooding) or inadequate (drought) supply of water is called the water stress. Condition of water stress occurs when supply of water is limited for plant roots and it also occurs when transpiration rate becomes intense. Water stress is primarily caused by the water deficit, i.e. drought or high soil salinity. However, In case of high soil salinity, drought flooding and low soil temperature, water exists in soil solution but plants cannot uptake it for metabolic process. Drought occurs in many parts of the world every year, frequently experienced in the field grown plants under arid and semi-arid climates. Each year, water stress on arable plants in different parts of the world disrupts agriculture and food supply. Drought is a situation that lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological functions. The uptake of water by cells generates a pressure known as turgor.

Water stress affects the plant as such in many ways:

- **Photosynthesis**

In photosynthesis, the water deficiency leads to different water metabolic changes along with functional and structural rearrangements of photosynthesizing apparatus.. Photosynthesis of higher plants decreases with the reduction in the relative water content (RWC) and leaf water

potential. The photosynthesis rate of leaves in both C3 and C4 plants decrease under the drought conditions.

- **Protein synthesis**

During water stress, the drought conditions alter process of protein synthesis by quantitative and qualitative changes. Due to suppressed synthesis of protein, plant leave decrease in more pronouncedly in C3 than in C4 plants. Water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs. Heat-shock proteins (Hsps) and late embryogenesis abundant (LEA)-type proteins are two major types of stress-induced proteins during different stresses including water stress.

- **Lipids:**

Water stress can lead to a disturbance of the association between membrane lipids and proteins as well as enzymes activity and transport capacity of membranes

- **Morphological changes:**

Water stress creates number of morphological changes such as changes in leaf anatomy, shrinkage in the size of leaves, decrease in the number of stomata; thickening of leaf cell walls, cutinization of leaf surface, and underdevelopment of the conductive system. However, the other changes such as formation of tube leave in cereals and induction of early senescence is the other reported morphological changes.

- **ABA accumulation:**

ABA, plant hormones, accumulate more in water stress condition that leads to response and tolerance to dehydration. The amount of ABAs in xylem saps increases substantially under reduced water availability in the soil, and this results in an increased ABA concentration in different compartments of the leaf.

- **Mineral nutrition:**

Water stress also affects plant mineral nutrition and disrupts ion homeostasis. Some studies showed the reduction of nitrate uptake and decrease in nitrate reductase activity under water stress.

7.5. Heat stress

Plants have various mechanisms for photo protection which regulate absorption and dissipation of light energy. They have a limited ability to regulate the amount of sunlight that they absorb. When light temperature induces a metabolic change in a living being is called high temperature stress or heat stress. To protect themselves from light stress plants have evolved mechanisms at morphological, physiological and molecular levels. Light regulation can be done at the morphological level by a thick cuticle, wax coated epidermis, multilayered epidermis, epidermal hair system, sunken stomata, leaf rolling, adjustable leaf angles, sclerophylly, leaf specific mass, leaf specific area and root: shoot ratio. Many acid zones around the world have plants which have ineffective transpiration in the plants to significantly higher than ambient environment. Three types of adjustment are expected in temperature related stress condition. At the physiological and molecular levels plants have D1 damage repair cycle, state transition, carotenoids, xanthophylls cycle, antioxidant system, water water cycle, cyclic electron transport, photorespiration etc. In this module, we study the damage and acclimation/adaptation in plants due to photoinhibition, and the physiological and molecular mechanisms that regulate photo inhibition

a. Once excess light has been absorbed, it can be dissipated via several routes, including thermal dissipation of excess excitation energy.

b. Macromolecules such as proteins are denoted at higher temperature, these are to be removed and their replacements synthesized.

c. Metabolic pathways are affected by temperature disturbance causing the accumulation or depletion of certain metabolites.

d. Some physical properties of membrane such as lipid fluidity are inflamed by temperature stress. These membranes are to be repaired.

e. The changes in photosynthesis were related to stomatal aperture, while a similar study on well-watered heat-stressed oaks and pines found no change in stomatal conductance but instead a down regulation of the photosynthetic apparatus

Effects of heat stress

- Loss of water
- Disturbed transpiration
- Disturbed respiration
- Disturbed protein structure
- Oxidative stress
- Disturbed photosynthesis
- Increases membrane permeability
- Effects on cell division

Loss of water

High temperature cause increased evaporation form the soil surface as a result of which the moisture level in the soil drops below the wilting point, beside high temperature also tends to increase transpiration form leaf surface. When the water level drops below permanent wilting point. Due to increase in high temperature, the increase in soil water evaporation and transpiration occurs. Resulting soil moisture decreases and permanent wilting point increases. Thus the plant desiccation and plant death occurs.

Disturbed transpiration

High temperature causes drought like condition which may cause hydro passive closure of stomata.

Disturbed respiration

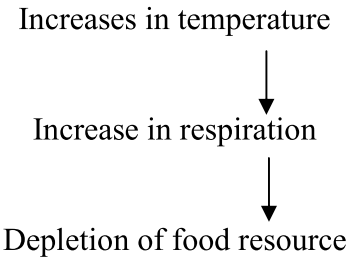
The respiration coefficient is initially influenced positively by change in the temperature but at later stages of the anaerobic mode of respiration states.

$$\text{RQ} = \frac{\text{increasase in temperature}}{\text{Rat of respiration}}$$

It result is depletion of food reserved in cell, and then during the starvation, C3 plant more affected than the C4 and CAM plants.

Disturbed Photosynthesis

A temperature at which rate of respiration of CO_2 release and photosynthesis or CO_2 absorbed are equal is termed as compensation point. When the temperature goes beyond this, the rate of CO_2 fixation is reduced and constant increase in respiration causes a depletion in starch and glycogen levels reading to starvation and ultimate cell death.



At extremely high temperature, the coupling between transport and oxidative Phosphorylation is impaired in the mitochondria.

- This leads to loss of cellular reserve of ATP molecules essential for various biosynthesis processes.
- Photosynthesis activity in C3 mesophiles is impaired and O2 resolution steps
- Ribulose-1-5-biphasphate carboxylase, NADP glyceraldehydes dehydrogenase and phosphoenol pruvate carboxylase are unstable at high temperature.
- Due to change in heat induced membrane fluidity, the oxidative distribution if light energy between the two photosynthesis is severely disturbed.
- The structural injury to the chloroplast cause the cessation of the change separation property
- Overall rate of photosynthesis decline

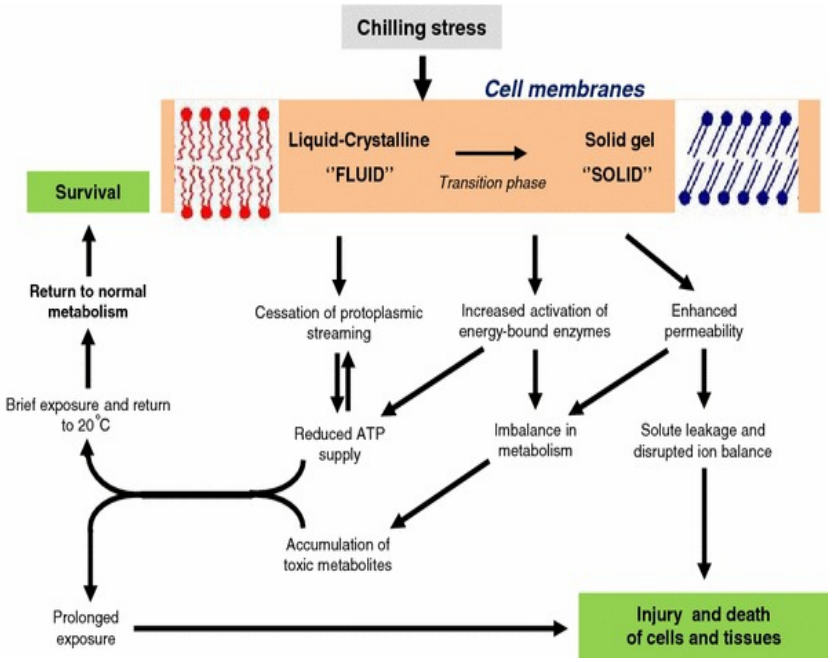
Increase membrane permeability

The membrane dynamics is the direct function of semi-liquid crystalline state normal rotation and translational moments of proteins. If there is an increase in temperature, the lipids become fluid. The membrane losses structure and become permeable for all organic and inorganic compounds. Many cytoplasmic contents leaks out of it. There is a formation of hydrophobic chandelles which facilitates the transpiration of ions.

7.6. Chilling stress

The stress occurs due to low temperature from 0 to 15°C is called chilling stress. The low temperature limit at which plants can grow is about 5°C, independent of the growth form, life cycle or geographic location. The temperature less then 0°C causes freezing stress. Plants which grow in tropical

or subtropical areas are susceptible to low temperature whereas temperate plants survive even at -300^{0}C. Cold-adapted plants show genotypic changes that resist local low temperature extremes and enable their survival over generations. On the other hand plants showing cold acclimation are able to reversibly adjust their metabolism to cold temperature stress. The potential cold stress symptoms include surface lesions on leaves, a water-soaked appearance of the tissue, discoloration, desiccation, embolisms in xylem vessels, frost-burn, tissue break down, accelerated senescence, delayed transition to flowering and pollen sterility.



Chilling stress causes water from cell to flow outside, causing dehydration-induced damage to cells. Therefore, the main cause of frost damage to plants in nature is extracellular ice crystal formation that causes secondary water stress to the surrounding cells. Chilling is a common environmental stress in nature that can directly affect the physiological functions of chloroplasts. First, chilling can change the lipid membrane state and enzyme activities in chloroplasts. Then, the efficiency of photosynthesis declines, and excess reactive oxygen species (ROS) are produced.

Adaptive mechanisms to low temperature stress

Morphological and life cycle adaptations

Evergreen conifers and broad leafed evergreens have wax coated leaves so that they are protected in winter from desiccation. The conifers have small, narrow, needle-like leaves in spruces, pines, firs, or scaled leaves as in case of cedar and cypress, which reduces the surface area to reduce transpiration of water and the risk of freezing They also have fewer stomata in the needle leaves.

Physiological mechanisms for acclimation to low temperature

Low temperature causes dehydration stress as mentioned earlier. Some of the physiological processes that protect cells against dehydration stress, like osmotic adjustment and antioxidant metabolism have been covered in the module on dehydration and salinity. Photo inhibition of photosynthesis takes place under low temperature because of low temperature induced desiccation. Conifers show acclimation to cold stress induced photo inhibition by reducing antenna size, a partial loss of photosystem II, and nonphotochemical quenching of absorbed light as heat

7.7. Stress resistance mechanism:

Various approaches have been so far been tested to produce stress tolerant plants using classical genetic methods as well as improved plant breeding techniques. One approach to improve plant resistance and crop performance in water-limited environments is to select genotypes that have improved yield in dry environments. Plant modification for enhanced tolerance is mostly based on the manipulation of genes that protect and maintain the function and structure of cellular components. Apart from that some other approaches and techniques are also adopted for stress resistance.

- Avoidance mechanism: prevent exposure to stress.
- Tolerance mechanism: permit the plant to withstand stress
- Acclimation: alter their physiology and response stress.

- Regulation of plant stress responses
- Abscises acid (ABA)
- Jasmonic acid
- Ethylene
- Calcium

7.8. Summary

In the environment, plants are constantly being exposed to a number of adverse conditions. Being immobile and deprived of highly specialized immune system, they have developed intricate mechanisms to adapt and survive under various types of abiotic and biotic stresses. On the perception of certain stimuli various signaling cascades are stimulated generating appropriate responses. This result in massive transcriptional reprogramming that makes the plant tolerant against the stress. Recent advances in the field of genomics and proteomics approach have widened our view regarding plant signal transduction and gene regulation. All the plants are not equally capable in withstanding water stress and their response to the stress also varies. Even in the highly tolerant species of plants, tolerance comes through changes in the molecular and physiological mechanisms that make plants morphologically adaptable to water deficits.

7.9.Termination questions

Q.1. What is the plant stress? Discuss briefly.

Answer:----------

Q.2. Define the drought stress.

Answer:----------

Q.3. What is the salinity stess?

Answer:----------

Q.4. Briefly define the effects on water stress in plant.

Answer:----------

Q.5. Define the effects of heat stress in plants.

Answer:----------

Q.6. Define the plant tolerance capacity**.** How to plant can tolerate different stress

Answer:----------

7.10. Suggested Further readings

1. Plant physiology by H.N. Srivatava, Pradeep Publication, Jalender.
2. Plant physiology by vince ördög ey al.,
3. Textbook of Plant Physiology by V. Verma, published by ANE Books
4. A Textbook Of Plant Physiology by *by A. S. Gontia*
5. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016
6. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.
7. Jeremy M. Berg, John L. Tymockzo and Luber Stryer, Biochemistry 7th Edition

Unit-8.Respiration

8.1. Introduction

Glycolysis is the first step in the breakdown of glucose to extract energy for cellular metabolism. Glycolysis consists of an energy-requiring phase followed by an energy-releasing phase. During this process each glucose molecules splits and converted in to two 3 carbon unit (pyruvate) by sequential reaction and some free energy released. In this process, the glucose is conserved in the form of ATP and NADH. Thus we can say that glycolysis is a series of reactions that extract energy from glucose by splitting it into two three-carbon molecules called pyruvates. Glycolysis is an almost universal central pathway of glucose catabolism. The process of glycolysis regulate a number of path way.

Objectives

- To explain plant glycolysis and their regulation
- To discuss the Translocation of metabolites
- To explain TCA cycle in brief

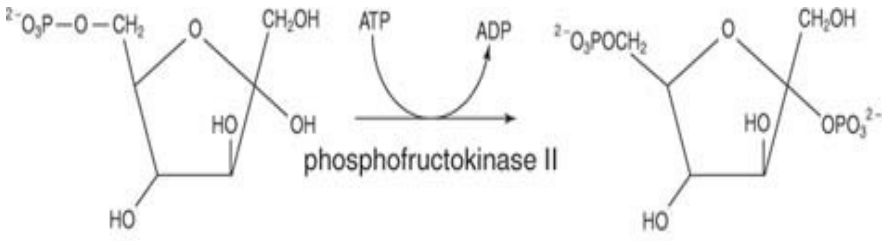
8.2. Regulation of plant glycolysis

In glycolysis, enzymes hexokinase is directly inhibited by its product, glucose-6-phosphate, and pyruvate kinase is inhibited by ATP itself. The main control point for the glycolytic pathway is phosphofructokinase (PEK), which is allosterically inhibited by high concentrations of ATP and activated by high concentrations of AMP. Phosphofructokinase is regulated by the energy charge of the cell that is the fraction of the adenosine nucleotides of the cell that contain high energy bonds. The inhibition of PFK by ATP is unusual, since ATP is also a substrate in the reaction catalyzed by PEK. Thus, when energy is required, glycolysis is activated. The glycolysis regulation occurs in the three reactions for obtaining equilibrium. In every step, the free energy change depends on two factors:

- The free energy difference between the products and reactants in the standard state and the concentration of the products and reactants.
- Reactions at equilibrium have a free energy change of zero.

1. Synthesis of glucose -6- phosphate

This reaction is also known as kinase reaction, where the glucose-6-phosphate is formed. This reaction, added a phosphate to glucose immediately when glucose enter in the cell, due this phosphorylation, glucose transport out of cell prevented and reactivity of oxygen is also increase. The phosphorylation of glucose in all cell body is catalyzed by several enzymes called the hexokinases. ATP is complexed with Mg^{+2} which a co- substrate in this reaction. The Hexokinase is inhibited by its product, so the phosphorylation of glucose is inhibited if there is a buildup of glucose-6-phosphate. In mammalian cells, the breakdown of glycogen is regulated by covalent modification of glycogen phosphorylase. This regulation reduces the rate of formation of glucose-6-phosphate.



2. Fructose-6-phosphate ➪ fructose-1,6-bisphosphate

Glucose-6-phosphate has other metabolic fates than simply leading to pyruvate. For example, it can be used to synthesize ribose for DNA and RNA nucleotides. The most important regulatory step of glycolysis is the phosphofructokinase reaction. Phosphofructokinase is regulated by the **energy charge** of the cell—that is, the fraction of the adenosine nucleotides of the cell that contain high-energy bonds. Energy charge is given by the formula:

$$ATP + \frac{1}{2}[ADP]/([ATP] + [ADP] + [AMP])$$

The energy charge of a cell can vary from about 0.95 to 0.7. *ATP inhibits* the phosphofructokinase reaction by raising the K m for fructose-6-phosphate. AMP activates the reaction. Thus, when energy is required, glycolysis is activated. When energy is plentiful, the reaction is slow down. Finally, phosphofructokinase is *inhibited by citrate*. Citrate is the TCA cycle intermediate, where 2-carbon units enter the cycle. A large number of compounds—for example, fatty acids and amino acids, can be metabolized to TCA cycle intermediates.

3. Phosphoenol pyruvate → pyruvate:

The third big step in the free-energy diagram is the Pyruvate-kinase reaction, where ATP is formed from phosphoenol pyruvate. *ATP inhibits* pyruvate kinase, similar to the inhibition of PEK. Pyruvate kinase is also *inhibited by acetyl-Coenzyme A*, the product of pyruvate metabolism that enters the TCA cycle. Fatty acids also allosterically inhibit pyruvate kinase, serving as an indicator that alternative energy sources are available for the cell.

If glycolysis is activated, then the activity of pyruvate kinase must also be increased in order to allow overall carbon flow through the pathway. Feed-forward activation ensures that the enzymes act in concert to the overall goal of energy production.

Glycolysis produces short but high bursts of energy. However, glycolysis produces energy at a high rate but for a short duration. Biopsies of animal muscle indicate two types of tissue; the two types have different metabolic activities. The flight muscles in the breasts of chickens and turkeys, for example, are light, while the leg and other muscles are dark. The color of the dark meat comes from the iron present in the cytochromes involved in oxygen consuming respiration.

8.2.1. Allosteric Regulation of Glucose Metabolism

There are three enzymes which allosterically control the complete glycolytic pathway. Phosphofructokinase is principle rate limiting enzyme, whose activity is inhibited by high amounts of ATP and activated by AMP. This leads to accumulation of glucose-6- phosphate, which acts as inhibitor to hexokinase. Therefore, when the cell has ample amount of energy of ATP, the glucose catabolism is inhibited. In step 9, Pyruvate Kinase is also inhibited by ATP. These three glycolytic enzymes catalyse allosteric reactions with high negative ΔG o values that are predominantly irreversible under normal conditions. The further control is employed by glyceraldehyde-3-phosphate dehydrogenase, catalyses the reduction of NAD^+ to NADH. If NADH amount is increased in cytosol, mitochondrial oxidation is slows down. In this pathway, the molecules of ATP are generated by substrate-Level Phosphorylation- Direct transfer of phosphate group from substrate to ADP.

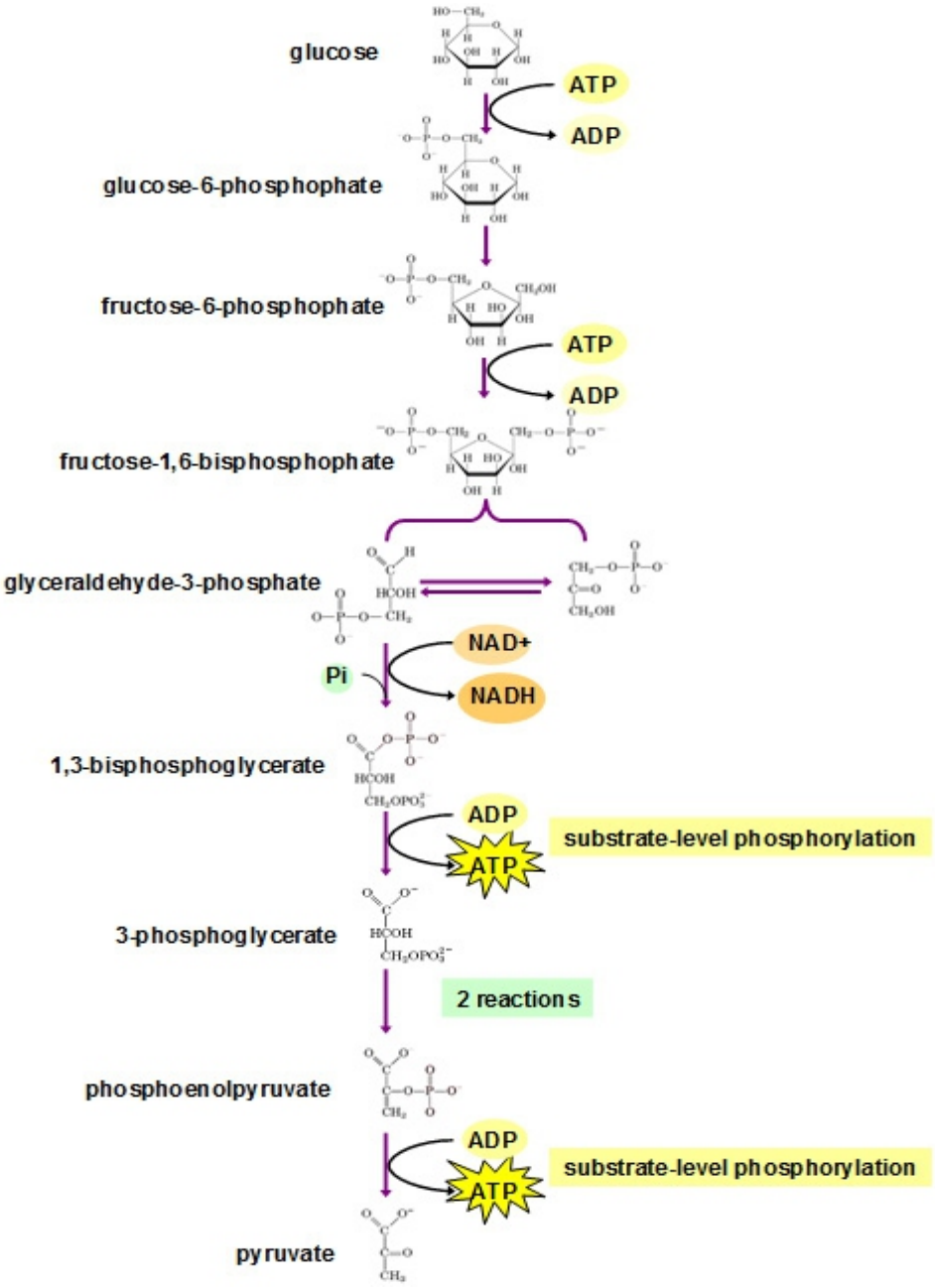


Fig.8.1 : Steps involved in Glycolysis

Source:http://www.nature.com/scitable/content/ne0000/ne0000/ne0000/ne0000/14897204/f3_dapoian_v2.jpg

It is catalyzed by water soluble enzymes whereas membrane and ion gradients are not required. It ensues twice during the pathway. The first reaction is catalysed by phosphoglycerate kinase which transfers the high-energy phosphate group of 1, 3-bisphosphoglycerate to ADP, generating 3-

phosphoglycerate and ATP. The second reaction involves transferring of high-energy phosphate group in phosphoenolpyruvate (PEP) to ADP, yielding pyruvate and ATP. This reaction is catalyzed by pyruvate kinase and strongly exergonic ($\Delta G^{\circ\prime} = -7.5$ kcal/mol). Oxidation of NADH produced during glycolysis to NAD^{+}. In this reaction, 3- phosphoglyceraldehyde is converted to 1, 3- bisphophoglyceric acid and the reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase. NAD+ is reduced to NADH and later it generates ATP by oxidative phosphorylation in mitochondria.

8.3. Translocation of metabolites

Translocation is the process within plants that functions to deliver nutrients and other molecules over long distances throughout the organism is called translocation of metabolites. The translocation process is carried out by both plants and animals. In the plant, the moments of nutrients from leaves to other tissues are proceeds. Translocation occurs within a series of cells known as the phloem pathway or phloem transport system. The nutrients that are translocated in the phloem as solutes in a solution called phloem sap. The example of phloem sap is sugars, amino acids, and minerals that are predominant nutrients follow the phloem transport system. Apart of nutrient, different types of substance such as hormones, proteins, and nucleic acids are also moved throughout the plant via translocation. Because translocation is responsible for the delivery of nutrients and hormones to developing seeds and fruits, this process is critical to the achievement of optimal crop yield. It also accounts for the ultimate nutritional composition of plant foods important to humans.

8.3.1. Pathway of Translocation

The movement of sugars and other molecules generally follows a path that originates in plant organs where sugars (the primary solute) are made and terminates in regions where these nutrients are utilized. The organs where the pathway begins are called source regions, or sources, and the ends of the pathway are referred to as sink regions, or sinks. The plant structures that lie

between terminal source and sink tissues, such as the stem of an herbaceous plant, the trunk and branches of a tree, or the petiole of a leaf, make up the translocation pathway. All of these structures contain numerous living cells that require nourishment and, thus, these pathway tissues can also function as sinks. The translocation of molecules via the phloem pathway is dependent on the specialized functioning.

8.3.2. Role of mitochondrial membrane in translocation of metabolites

The mitochondrial inner membrane harbors a large number of metabolite carriers. It has the functional barrier to the passage of small molecules between the cytosol and the matrix and maintains the proton gradient that drives oxidative phosphorylation. The precursors of carrier proteins are synthesized in the cytosol and imported into mitochondria by the translocase of the outer membrane and the carrier translocase of the inner membrane. Molecular chaperones in the cytosol and intermembrane space bind to the hydrophobic precursors to prevent their aggregation. In mitochondrial inner membrane, a high energy electron is passed along an electron transport chain. By the energy released pumps, hydrogen out of the matrix space. The gradient created by this drives and hydrogen back through the membrane, through ATP synthase. Translocation into mitochondria is driven by both ATP hydrolysis and an electrochemical H^+ gradient across the inner membrane, whereas translocation into chloroplasts is driven solely by the hydrolysis of GTP and ATP. To be translocated across the mitochondrial membrane, proteins must be at least partially unfolded.

All other proteins are synthesized in the cytosol from where they are imported into mitochondria by a translocase in the outer membrane of mitochondria and translocases in the inner membrane of mitochondria Proteins of the matrix and the inner membrane, each making up about 40% of all mitochondrial proteins. Proteins imported into the matrix of mitochondria are usually taken up from the cytosol within seconds or minutes of their release from ribosomes. These proteins include many of the enzymes that catalyze the major biochemical functions of the organelle in

metabolic conversions and respiration, in the biosynthesis of lipids, iron-sulfur clusters, heme, and amino acids, or in the expression of mitochondrial genes (Fig.8.2. Proteins of the outer membrane and the IMS, each making up about 10% of the mitochondrial proteome, use a diversity of strategies to make their way into mitochondria. Outer membrane and IMS proteins play crucial roles in the communication with the cytosol and with other mitochondrial compartments, in the uptake of metabolites, lipids, or metal ions as well as with the regulation and execution of apoptosis. Most of the mitochondrial precursor proteins have a signal sequence at their N terminus that is rapidly removed after import by a protease (the signal peptidase) in the mitochondrial matrix. The signal sequences are both necessary and sufficient for import of the proteins that contain them.

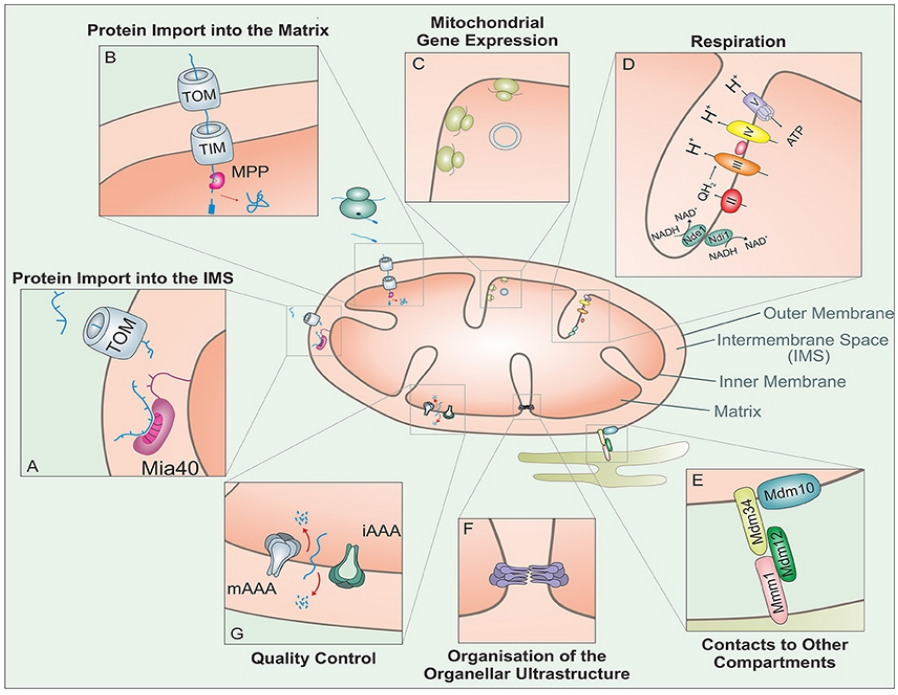


Fig.8.2: Mitochondria carry out a large variety of different biological activities.

8.4. The TCA Cycle

The TCA cycle is a central pathway into which many metabolites are involved. It consists of a number of reactions which generate NADH and

FADH2 which can in turn be used by the oxidative phosphorylation pathway to generate ATP. The TCA cycle occurs in the **matrix** of the mitochondria.

Steps of the citric acid cycle

A preview of the molecules produced during the citric acid cycle already has been given. But how, exactly, are those molecules made. The cycle show the following steps for production of NADH, 2FADH2, ATP, GTP release of carbon dioxide molecules (Fig. 8.3).

Step 1. In the first step of the citric acid cycle, acetyl CoA joins with a four-carbon molecule, oxaloacetate, releasing the CoA group and forming a six-carbon molecule called citrate.

Step 2. In the second step, citrate is converted into its isomer, isocitrate. This is actually a two-step process, involving, first the removal and then the addition of a water molecule, that is why the citric acid cycle is sometimes described as having nine steps—rather than the eight listed here.

Step 3. In the third step, isocitrate is oxidized releasing a molecule of carbon dioxide, leaving behind a five-carbon molecule α-ketoglutarate. During this step, NAD^+, is reduced to NADH. The enzyme catalyzing these steps is **isocitrate dehydrogenase,** which is important in regulating the speed of the citric acid cycle.

Step 4. The fourth step is similar to the third. In this case, the α-ketoglutarate is oxidized and reducing NAD+, to NADH, and releasing a molecule of carbon dioxide in the process. The remaining four-carbon molecules pick up Coenzyme A, forming the unstable compound succinyl-CoA. The enzyme catalyzing these steps **is α-ketoglutarate dehydrogenase** which is important in the regulation of the citric acid cycle.

Step5. In step five, the CoA of succinyl CoA is replaced by a phosphate group, which is then transferred to ADP to make ATP. In some cells, GDP guanosine diphosphate is used instead of ADP, forming GTP (guanosine triphosphate) as a product. The four-carbon molecule produced in this step is called succinate.

Step 6. In step six, succinate is oxidized, forming another four-carbon molecule called fumarate. In this reaction, two hydrogen atoms with their

electrons are transferred to FAD, producing $FADH_2$. The enzyme that carries out this step is embedded in the inner membrane of the mitochondrion, so $FADH_2$ can transfer its electrons directly into the electron transport chain.

Step 7. In step seven, water is added to the four-carbon molecule fumarate, converting it into another four-carbon molecule called malate.

Step 8. In the last step of the citric acid cycle, oxaloacetate, the starting four-carbon compound is regenerated by oxidation of malate. Another molecule of NAD^+ is reduced to NADH in the process.

It is important to be aware that the primary role of the TCA cycle is production of NADH + H^+ and $FADH_2$ It also produces various molecules that are supplied to various biosynthetic processes, which can enter or exit the cycle at various points depending on the demand on different reactions for example, alpha-ketoglutarate can leave the cycle to be converted into amino acids or **succinate** can be converted to them.

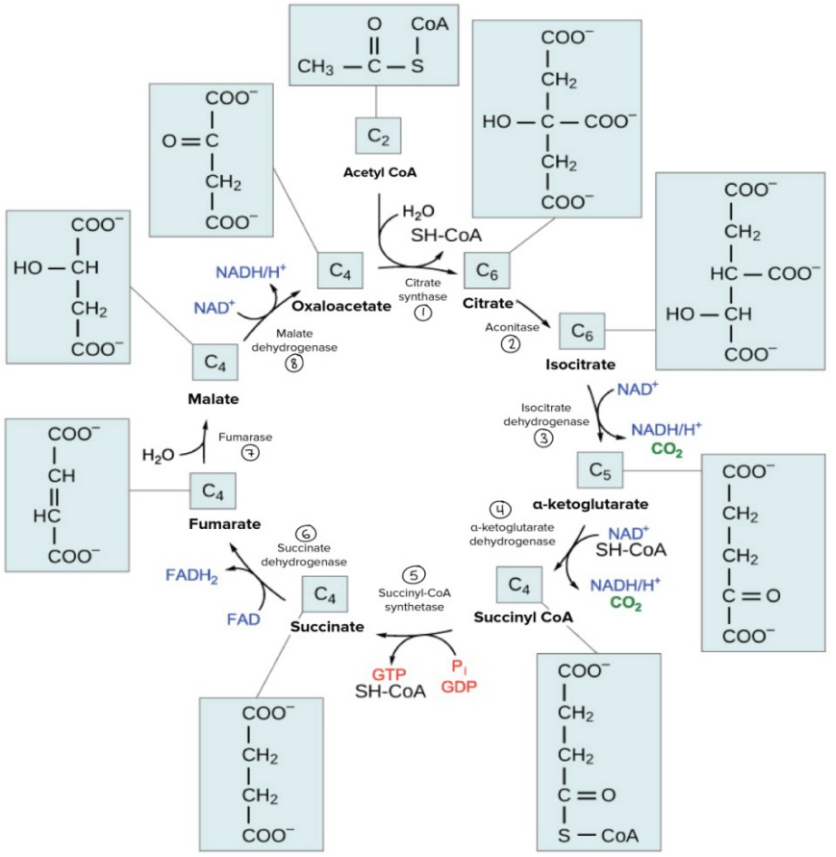


Fig. 8.3: Steps of the citric acid cycle

Each cycle produces two molecules of carbon dioxide, three molecules of NADH + H^+, three hydrogen ions, one molecule of $FADH_2$ and one molecule of GTP. As such each molecule of glucose produces double of this (2 carbon dioxide, 6 NADH, 6 hydrogen ions, 2 $FADH_2$ and 2 GTP).

8.4.1. Regulation of the TCA Cycle

The TCA Cycle is regulated in a variety of ways. As already mentioned, isocitrate dehydrogenase regulates step 3 of the TCA cycle, making it the rate limiting step of the cycle. In addition to this, energy availability also regulates the cycle, so low energy signals, such as ADP activate the cycle and high levels of NADH + H^+ (a product of the cycle) inhibit it.

8.5. Summary

This is attained by feedback inhibition or stimulation of insulin also increases the rate of glucose uptake into several types of target cells, but not liver or brain cells. Emotional or physical stress releases the hormone epinephrine from the adrenal medulla. Epinephrine stimulates glycogenolysis and inhibits glycogenesis. In emergency conditions, when epinephrine is released in relatively large quantities, enormous production of glucose provides the energy required to manage the situation.

8.6. Terminal Questions

Q.1. What is Glycolysis? Explain it

Answer:----------

Q.2. What do you mean by regulation of glycolysis .

Answer:----------

Q.3. Discuss steps required for glycolysis?

Answer:----------

Q.4. Why TCA cycle is found only in aerobic organism? Explain with stepwise reactions of TCA cycle.

Answer:----------

Q.5. What do you mean by traslocation? Discuss it briefly.

Answer:----------

Q.6. Discuss the role of mitochondria in translocation of proteins molecules.

Answer:----------

8.7. Further Readings

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Unit-9: Plant growth regulator

Contents

9.1. Introduction

Plant growth regulators (PGRs) are chemicals used to modify plant growth such as increasing branching, suppressing shoot growth, increasing return bloom, removing excess fruit, or altering fruit maturity. The importance of PGRs was first recognized in the 1930s. Since that time, natural and synthetic compounds that alter function, shape, and size of crop plants have been discovered. Today, specific PGRs are used to modify crop growth rate and growth pattern during the various stages of development, from germination through harvest and post-harvest preservation. There are five groups of plant-growth-regulating compounds: auxin, gibberellin (GA), cytokinin, ethylene, and abscisic acid (ABA). For the most part, each group contains both naturally occurring hormones and synthetic substances. These growth-regulating substances most often are applied as a spray to foliage or as a liquid drench to the soil around a plant's base. PGRs, indirectly effect the plant yield. The indirect role of PGR is to (1) preventing lodging in cereals, (2) preventing preharvest fruit drop, (3) synchronizing maturity to facilitate mechanical

harvest, (4) hastening maturity to decrease turnover time, and (5) reducing labor requirements. Plant hormones are important biotic factors to regulate root growth. Among the seven kinds of plant hormones, auxin and gibberellin (GA) are strong accelerators of shoot growth, but these are not always accelerators for root growth.

Objectives

- To discuss the role of plant growth regulator and its types
- To know the plant hormones and its role in plant growth
- To discuss the regulation of plant morphogenetic processes by light

9.2. Phytohormones

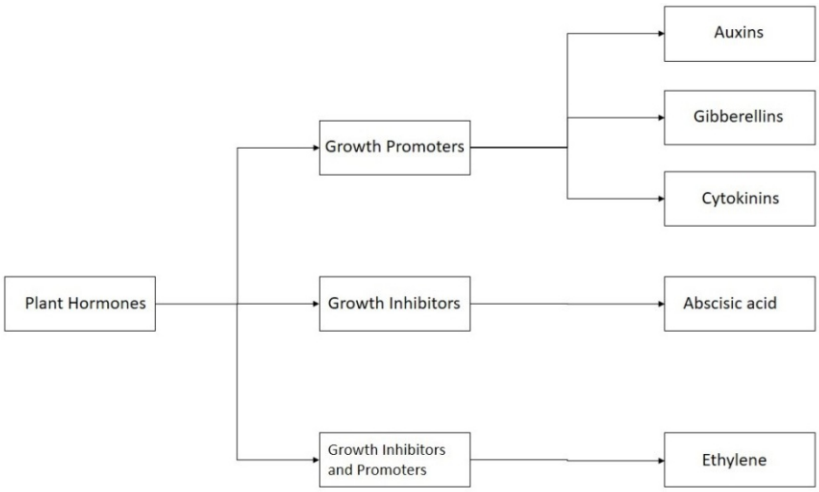
Plant hormones are also known as phytohormones. The phytohormones are signal molecules, produced within plants, in extremely low concentrations. Plant hormones control all aspects of plant growth and development, from embryogenesis, the regulation of organ size, pathogen defense, stress tolerance and through to reproductive development. The phytohormones play vital role in gene expression and transcription. Hormones are not nutrient but naturally occurring chemicals found in plant in small amounts, which promote and influence the growth. However, the very similar chemicals also produce by fungi and bacteria, that can also effect the plant growth. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized.

The plants coordinate their behavior against environmental changes by using hormones. The functions of control and coordination in plants are performed by certain chemical substances. These chemical substances are called plant hormones or phytohormones. There are four major types of plant hormones. Which are involved in the control and coordination in plants. These hormones are following four types such as:

a. Auxins
b. Gibberellins
c. Cytokinins

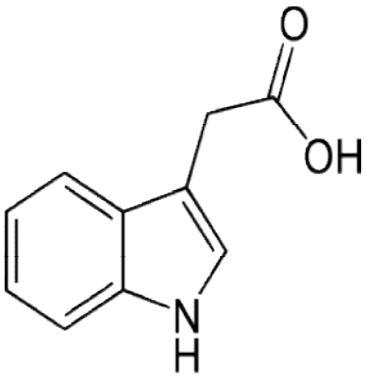
d. Abcisic acid

e. Ethylene



9.2.1. Auxins

Auxin is the first plant hormones, which was identified and named by Dr. Fritz Went in 1926. Chemically, the auxin was identified as Indole acetic acid or IAA and was seen to be involved in diverse growth regulatory responses like cell elongation, cambial cell division, adventitious root formation, apical dominance, gravitropism. Another naturally occurring auxin was identified as indole butyric acid (IBA). While 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA (Naphthalene acetic acid) is the synthetic plant hormones. The major site of auxin production is apical meristem of plants. Auxin hormone is responsible for phototropic and geotropic responses of plants. Auxin is synthesized, stored and metabolized by plants, and transported over short and long distances. The precursor for IAA biosynthesis is the structurally similar compound Tryptophan, which is an amino acid and a constituent of proteins. There are some bacterial species produce auxin are Bradyrhizobium, Azospirillum, Rhizobium and Enterobacter cloacae, and cyanobacteria etc.



β-indolylacetic acid (**IAA**)

Functions of auxin

- It promotes elongation of young shoots (Indole acetic acid)
- Affects secondary cell growth by inducing vascular cambium and secondary xylem
- Promotes fruit growth
- Promote Cell elongation of stems and roots
- Apical dominance, IAA in apical bud suppresses the growth of lateral buds
- Induces parthenocarpy i.e. development of fruit without fertilization e.g. in tomatoes
- It also help in cell division and xylem differentiation

9.2.2. Gibberellins

Gibberellins (GAs) are named after the fungus *Gibberella fujikuroi*, which causes excessive growth and poor yield in rice plants. GAs are different kinds that known as GA_1, GA_2, GA_3….etc. It is produced in the meristem of apical buds and roots, young leaves, embryo. GAs abundant in seeds, are also formed in young leaves and in roots. It is a tetracyclic, diterpenoid hormones. Gibberellins were discovered by their ability to induce elongation of shoots. GAs regulates metabolic and developmental processes. In 1950s, gibberellins were also isolated from plants, and shown to play an important role in the induction of seed germination and flowering. In plant more than 125 GAs identified but they are vary in different plant. However, not more than 12-15 different GAs may be present in a particular species. Gibberellins are

synthesized by the terpenoid biosynthesis pathway, in which the 5 carbon isopentyl diphosphate molecules serve as building blocks for formation of larger terpenoid molecules. Some bacterial species produced GAs are *Azotobacter spp. , Rhizobium spp. , Bacillus subtilis.*

Functions of GAs

a. Stem and leaf elongation
- stimulates cell division, growth of leaves
- causes bolting- the rapid growth of floral stems
- promote the growth of dwarf peas and are involved in the bolting

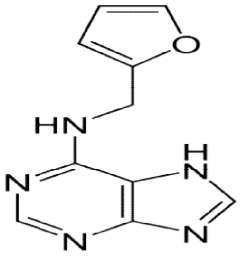
b. Fruit growth
- controlled by Gibberellins and auxin
- Grapes are sprayed to grow bigger

c. Germination
- Break seed dormancy and causes seed germination
- Induces the formation of hydrolytic enzymes such as lipase, amylase in the endosperm of germinating cereal grains and barley seeds

9.2.3. Cytokinins

The Cytokinins plant hormone, play an important role in cytokinesis process during cell division. It discovered due to their ability to induce cell division in cultured cells. The first cytokinin identified in 1955 was kinetin, which was purified from autoclaved DNA isolated from herring sperm and calf thymus, and named kinetin, because of its ability to promote cell division (cytokinesis).It stimulates cytokinesis produced in roots by plants and transported to other organs. There are three biologically active cytokinins that are widespread in plants, namely isopentenyl adenine (iP), zeatin (Z), dihydrozeatin (DZ). Cytokinins are naturally synthesised in the plants where rapid cell division occurs e.g. root apices, shoot buds, young fruits, etc. Cytokinins are synthesized from the ribonucleotide precursor, adenosine

monophosphate (AMP), which can exist as a free nucleotide or as a part of RNA. Cytokinins act in conjunction with auxin to retard senescence. Cytokinins such as 6-furfurylaminopurine (kinetin) are used commercially in the storage of green vegetables to reduce yellowing. *Azotobacter spp., Rhizobium spp., Bacillus subtilis* are used in production of cytokinins.



Kinetin (6-furfurylaminopurine)

Functions of cytokinins

a. Cell division and cytokinesis
- Moves in xylem sap
- Stimulates RNA and protein synthesis
- Works in conjunction with auxin
- Promotes nutrient mobilization and delay leaf senescence

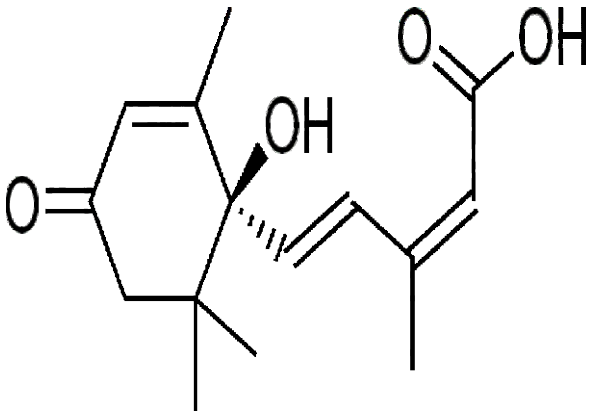
b. Apical dominance
- Cytokinins and auxin are antagonistic- auxin from terminal bud causes the shoot to lengthen.
- Cytokinins from roots stimulate Axillary bud
- Auxin stimulates lateral root formation cytokinins to restrain it.

c. Anti-aging
- slows leaf deterioration

9.2.4. Abcisic acid

Abcisic acid (ABA) is a growth-inhibiting hormone so that it called negative regulators hormones in plant growth. However, auxin gibberellins and cytokinins also inhibit growth in high concentration. ABAs act as an antagonist to GAs. Because it promote abscission and shedding of fruits, so that it name is considered as abcisic acid. It inhibits plant metabolism and regulates abscission

and dormancy. It is also called “stress hormone” as it increases the tolerance of plants. ABA synthesized in vascular tissues on perception of water deficit was shown to accumulate in guard cells and bring about stomata closure, thus protecting the plant from excessive water loss and wilting. ABA has been detected in most living organisms, including cyanobacteria, algae, plant pathogenic fungi, mosses, sponges, protozoa and even human granulocytes, indicating that it is an ancient signalling molecule with diverse functions.



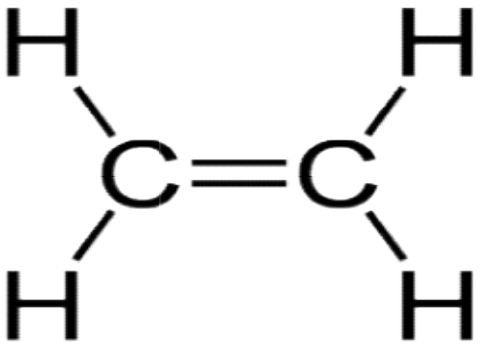
Abcisic acid (ABA)

Functions of ABS

- inhibits cell division in the vascular cambium
- the onset of seed dormancy
- stress hormone – closes stomata
- Accelerates dormancy in seeds that is useful for storage purpose
- Stimulates closure of stomata to prevent transpiration under water stress

9.2.5. Ethylene

Ethylene is a colorless, flammable and gaseous organic compound with a sweet and musky odor. It is naurally occurring plant hormones that acts as a growth promoter as well as an inhibitor. Occurs in gaseous form. In higher plants ethylene is synthesized in all the plant parts namely, roots, tubers, stems, leaves, buds, flowers, and fruits. Ethylene is biosynthesized from methionine via S-adenosyl-Lmethionine (S-AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). It regulates many physiological processes and one of the most widely used hormones in agriculture for fruit ripening.



Ethylene

Functions of Ethylene

- It hastens the ripening of fruits
- Controls epinasty of leaves
- Breaks seed and bud dormancy
- Stimulates rapid elongation of petioles and internodes
- Promotes senescence and abscission of leaves and flowers
- Induces root growth and root hair formation thereby increasing the absorption surface

9.3. Effect of Phytohormones on plant growth and development

Plant growth and development involves the integration of many environmental and endogenous signals. It induces the intrinsic genetic program and determines plant form. There are some substance which are use as growth regulators collectively called the plant hormones or phytohormones. This group includes auxin, cytokinin, the gibberellins (GAs), abscisic acid (ABA), ethylene, the brassinosteroids (BRs), and jasmonic acid (JA), each of which acts at low concentrations to regulate many aspects of plant growth and development. A single hormone can regulate an amazingly diverse array of cellular and developmental processes, while at the same time multiple hormones often influence a single process. The concept of plant hormones originates from a classical experiment on phototropism, the bending of plants toward light, carried out by Charles Darwin and his son Francis in 1880. Ethylene and cytokinin are both perceived by receptors sharing similarity to bacterial two-component regulators.

1. Physiological effects of ABA

Stomatal closure: GTG_1 and GTG_2 (G protein coupled receptor [GPCR]-type G protein) are two ABA receptors that bind ABA and hydrolyze GTP. This regulates Ca^{2+} channel activity that results in cytosolic Ca^{2+} accumulation from internal pools. S1P (sphingosine-1-phosphate) is a determinant of the G protein signaling pathway that activates anion channels, causing anion efflux and membrane depolarization. This change in membrane potential results in K+ efflux from guard cells.

Seed dormancy: During seed maturation, ABA levels are seen to increase during two phases. The first ABA peak precedes the maturation phase. This is important for preventing premature germination or vivipary at the end of the cell division phase of embryogenesis. ABA also plays a role in the induction of dormancy or germination inhibition in mature seeds.

ABA signaling: Using ABA-specific antibodies, proteins that bound to ABA in a cell extract were identified. These included diverse proteins like Flowering time control protein A (FCA), magnesium–protoporphyrin IX chelatase subunit H (CHLH), G-protein-coupled receptor 2 (GCR2), which were situated in different cellular compartments.

2. Physiological effects of Cytokinins

An important role of cytokinins is in regulation of the cell cycle. The cell cycle consists of 4 phases, with two gap phases separating the S phase during which DNA synthesis occurs and the M phases where mitosis occurs. Cytokinin levels are seen to increase transiently during the beginning of S and the M phase, suggesting their role in regulating progression of the cell cycle. Cytokinins and auxin together regulate the path of differentiation of meristematic cells in the shoot apical meristem. Cytokinin signaling has been shown to induce the expression of knotted (KNOX) genes that play a role in shoot apical meristem identity. Cytokinins also play a role in root meristem identity. Higher levels of cytokinins inhibit root development, while cytokinin signaling mutants like ahk3 show longer roots and better lateral root development. Cytokinins are also known to play a role in retarding leaf

senescence. Externally applied cytokinins show this effect as also plants over expressing the cytokinin synthesis gene IPT.

3. Physiological effects of Gibberellins

Gibberellins play important role in signaling. Biosynthesis of GA and the DELLA protein RGL2 is induced in imbibed seeds. GA then down regulates the transcript levels of RGL2 and leads to onset of the germination program. In addition, GA inhibits the dormancy effects imposed by abscisic acid (ABA). After germination, bioactive GA plays a role in cell elongation and expansion associated with an increase in plant height and leaf expansion. Gibberellins are known to play an important role in the transition of plants from vegetative to reproductive stage. GA synthesis in response to photoperiod under inductive conditions.GA is thought to move to the shoot apical meristem.

4. Physiological Effects of Ethylene

It influences plant processes such as seed germination, growth, diageotropism, senescence, flowering, abscission, fruit ripening, abiotic and biotic stress responses. Seed dormancy prevents germination during the unfavorable period. This allows the seed to survive after being dispersed from the mother plants. The breaking of dormancy is accompanied by several physiological changes that affect subsequent germination response. The ethylene is produced during germination and the production of ethylene is stimulated by the factorsthat break dormancy. Generally, exogenous ethylene stimulates the germination of both dormant and non-dormant seed. Ethylene inhibits growth by impairing cell division, DNA synthesis and cell expansion in the meristems of roots, shoots and axillary buds. In germinating seedlings, ethylene production is localized in the apical hook region. The major cause for this growth inhibition is ceasation or retardation of mitosis in root and shoot meristem and in axillary buds. In the root apex, ethylene inhibits mitosis by 60%. Ethylene plays a significant role in flower initiation. However, it is evident that in some species ethylene inhibits flower induction and enhances flower bud abortion; on the contrary in some species ethylene promotes flower bud induction. In response to variety of stresses, plant stimulates ethylene

production. For example, ethylene production is enhanced after exposure of plant tissues to low-temperature, water stress, osmotic stress, chemical stress, mechanical stress, gravitational stress, wounding, radiation, ozone and heavy metals, herbivory, and pathogen attack. Ethylene induced physiological responses namely, growth inhibition, epinasty, stomatal closure, and senescence and abscission of leaves, flowers, and fruitsmay help plants to maintain normal function and successfully adapt to stressful conditions.

5. Physiological effects of auxins

Auxins are principally involved in plant growth and elongation. They promote apical dominance - where the apex / tip of a plant grow while the lateral buds don't develop. Auxins also induce adventitious rooting from shoot cuttings. They increase the rate of cell elongation, which is thought to arise by auxin-induced acidification of the apoplast that leads to a loosening of cellulose fibers and expansion of the cell due to turgor pressure. Auxins play a role in regulating growth in response to directional stimuli, and are thus important in tropic responses (e.g. gravitropism). At high concentrations auxins inhibit growth and some synthetic auxins are used as herbicides.

9.4. Regulation of plantmorphogenetic processes by light

Photomorphogenesis is referring to the response of plant to light, which is the central theme in plant development. In other words the photomorphogenesis is light-mediated development, where plant growth patterns respond to the light spectrum. However, it is completely separate process from photosynthesis where light is used as a source of energy. Thus, any change in the structure and function of an organism in response to changes in light intensity is known as photomorphogenesis.

Morphogenesis comprises the integration of growth and differentiation, mediated by cell division and specialization as a result of a complex spatial and temporal hormonal control, which occurs through regulation and expression of multiple gene systems, correlative action of meristems and their derivatives and environmental variations. However, in plant tissue culture, this endogenous links are disrupted. Tissues are exposed to exogenous conditions, represented

by plant growth regulators, nutrients from the culture medium and controlled conditions of temperature and light. Therefore, morphogenesis seems to be modulated by the interaction of these factors, and also by other signaling agents, that act directly or indirectly on genetic level, triggering specific processes of synthesis that interfere with various biochemical pathways.

Phytochromes, cryptochromes, and phototropins are photochromic sensory receptors that restrict the photomorphogenic effect of light to the UV-A, UV-B, blue, and red portions of the electromagnetic spectrum. There are at least three stages of plant development where photomorphogenesis occurs: seed germination, seedling development, and the switch over from the vegetative to the flowering stage.

The morphogenic process is modulated not only by a series of cell intrinsic factors, but also by extrinsic factors, whether biotic or abiotic. These factors will act by modulating cellular activity to a particular development into a specific direction, or by cell reprogramming with the restoration of its totipotency characteristics. There are two important stages of photomorphogenesis:

- Pattern specification where the cells and tissues develop the ability to respond to light during some developmental stage.
- Pattern realization during which the photoresponse occurs.

The plant responds to light signals in the following two ways:

- Phytochrome-mediated photoresponse
- Blue-light response or cryptochrome-mediated photoresponse

Photo receptor reads the information contained in the light by selectively absorbing different wave length of light. Photoreceptors perceives light in the red region (λ = 600-700 nm) of the spectrum and modulate important physiological processes in plants, which include seed germination, seedling photomorphogenesis, shade avoidance and flowering induction. For example, when seeds germinate, they require daylight for attaining photosynthetic competence. Absorption of light causes conformational change in the pigment or associated protein causes photochemical oxidation reduction.

A group of wavelength-specific photoreceptors, E3 ubiquity ligases, and various transcription factors work together to regulate these two critical processes. Phytochromes are the main photoreceptors in plants for perceiving red/far-red light and transducing the light signals to downstream factors that regulate the gene expression network for photomorphogenic development. Phytochromes are a class of photoreceptors that sense red and far-red light. Photomorphogenic is mediated by phytochrome. Phytochrome is a proteinaceous pigment that acts as a photoreceptor and absorbs red and far-red light. It also absorbs blue light. A plant has multiple phytochromes that sometimes act independently of one another and sometimes are dependent either at the same time or at different times in the process of development.

Phytochrome exists in two forms-

- Pfr
- Pr

Pfr is in a biologically active form and absorbs far-red. Pfr is converted to Pr when far-red light is absorbed. The red wavelengths are absorbed by Pr. when the red light is absorbed, Pr is converted to Pfr.

The phytochrome-mediated response can be divided into three categories depending upon the amount of light absorbed.

- **Very Low Fluence Responses**– These responses are non-photo reversible and are initiated by very low fluences.
- **Low Fluence Responses-** These are photo reversible. It includes most of the red and far-red photoresponses, including the lettuce seed germination.
- **High Irradiance Responses-** These require prolonged exposure to light of high irradiance. These saturate at much higher influences than low fluence responses and are non-photo reversible.

Most photomorphogenic responses in higher plants appear to be under control of one (or more) of four classes of photoreceptors:

- Phytochromes (red and far-red)
- Cryptochrome (blue and UV-A): seedling development and flowering

- Phototropin (blue and UV-A): differential growth in a light gradient
- UV-B receptors: unknown
- The phytochromes absorb red (660 nm) and far red light (735 nm)
- Have a major role in every stage of development from seed germination to flowering
- Cryptochromes and phototropin detects both blue (400-450nm) and UV –A light (320-400nm).
- Cryptochrome have a major role during seedling development, flowering and resetting the biological clock
- Phototropin mediates phototropic responses

9.5. Summary

The phytochemicals are also known as plant hormones. The plant hormones play important role in embryogenesis, the regulation of organ size, pathogen defense, stress tolerance and through to reproductive development. The plant hormones such as Auxins, Gibberellins, Cytokinins, Abcisic acid and Ethylene regulate the plant growth and development. The major site of auxin production is apical meristem by plants. Auxin hormone is responsible for phototropic and geotropic responses of plants. Gibberellins (GAs) were discovered by their ability to induce elongation of shoots. GAs regulates metabolic and developmental processes. Cytokinins play a role in root meristem identity. Higher levels of cytokinins inhibit root development, while cytokinin signaling mutants like ahk3 show longer roots and better lateral root development. Abcisic acid (ABA) is a growth-inhibiting hormone so that it called negative regulators hormones in plant growth. The process of morphogenic process is governs and modulated by intrinsic and extrinsic factors. These factors will act by modulating cellular activity to a particular development into a specific direction, or by cell reprogramming with the restoration of its totipotency characteristics.

9.6. Terminal Questions

Q.1: What are phytochemicals? Discuss its role in plant growth and development.

Answer:----------

Q.2: Define the plant hormones.

Answer:----------

Q.3: Which pigment is associated with photomorphogenesis.

Answer:----------

Q.4: Briefly define the role of Auxin hormone in plant growth.

Answer:----------

Q.5: Define the nature of Gibberellins (GAs).

Answer:----------

Q.6: Discuss the plant morphogenetic processes by light.

Answer:----------

9.7. Further readings

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Notes