



Chapter 13

Unit V: Plant Physiology (Functional Organisation)

Photosynthesis



Learning Objectives

The learner will be able to,

- Learn the Ultra structure of Chloroplast .
- Realise the importance of solar energy and properties of light.
- Acquire knowledge of Quantum, Quantum yield and Quantum requirement.
- Develop curiosity for photosynthetic experiments like Red drop, Emerson Enhancement effect and Hill's Reaction.
- Analyse the pathway of electron- PS I and PS II.
- Recognise the Photo-Oxidative and Photo Chemical Pathway.
- Develop skill in Photosynthetic pathways and ability to draw C_3 , C_4 , C_2 and CAM cycle.

Chapter Outline

- 13.1 Definition, Significance and Site of photosynthesis
- 13.2 Photosynthetic pigments
- 13.3 Spectrum of electromagnetic radiation
- 13.4 Photosynthetic unit
- 13.5 Absorption spectrum and Action spectrum
- 13.6 Emerson's experiments & Hill's reaction
- 13.7 Modern concept of photosynthesis
- 13.8 Photo-oxidation phase of light reaction
- 13.9 Photochemical phase of light reaction
- 13.10 Photophosphorylation
- 13.11 Dark reaction or C_3 cycle
- 13.12 Hatch & Slack Pathway or C_4 Cycle

13.13 CAM cycle or Crassulacean Acid Metabolism

13.14 Photorespiration or C_2 Cycle

13.15 Factors affecting photosynthesis

13.16 Photosynthesis in bacteria

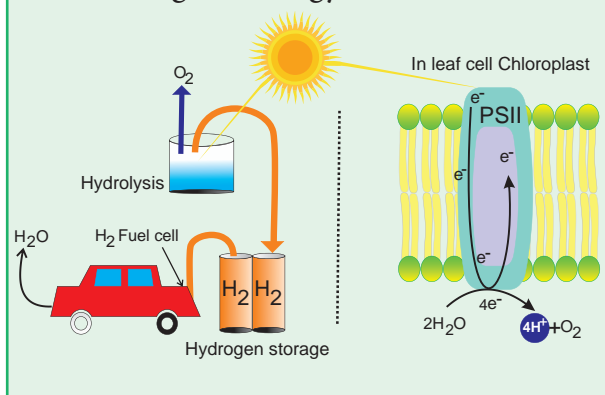
Life on earth is made up of organic compounds. How do we get these organic compounds? Ultimately, plants are the main source of all kinds of carbon compounds in this planet. We directly or indirectly depend on plants for this. Plants are the major machinery which produce organic compounds like carbohydrates, lipids, proteins, nucleic acids and other biomolecules.

Though man has reached the glory of achievements still he is not able to imitate the metabolic activities of plants which produces energy resources and other biomolecules.

The plants get energy from sun by converting solar or radiant energy into chemical energy by the process of Photosynthesis, which acts as a driving force for both biotic and abiotic world. Photosynthesis produces 1700 million tonnes of dry matter per year by fixing 75×10^{12} Kg of carbon every year. Photosynthetic organisms use only 0.2 % of incident solar light on earth. Carbohydrates produced by photosynthesis are the basic raw material for respiration and also to produce many organic compounds. It maintains atmospheric oxygen and carbon dioxide level. Photosynthesis consumes atmospheric carbon dioxide which is continuously added by the respiration of organisms. Photosynthesis is the

A quest for future energy

Hydrogen is considered as a promising energy vector for the next generation. It can be used for “green” electricity production or developing cogeneration systems such as fuel cells. The sustainability of its employment depends on the energy source used to synthesize it from hydrogen-rich compounds such as water or biomass. The splitting of water in hydrogen and oxygen by means of solar radiation in Photolysis is common in plants. Water splitting is not an easy process to mimic artificially but preliminary success is achieved so far. If young minds take up this as their research ambition a revolution can be made in green energy.

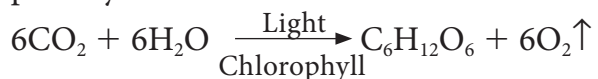


major endergonic reaction. In this chapter, we will study about the energy yielding process of photosynthesis and various types of energy utilization processes to produce carbohydrates.

13.1 Definition, Significance and Site of Photosynthesis

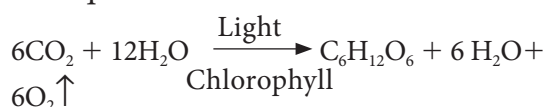
13.1.1 Definition of Photosynthesis

Photosynthesis is referred as photochemical oxidation and reduction reactions carried out with the help of light, converting solar energy into chemical energy. It is the most important anabolic process. Plants and photosynthetic bacteria use simple raw materials like carbon dioxide water and with the help of light energy synthesize carbohydrates and evolve oxygen. The overall chemical equation for photosynthesis is:



Ruben and Kamen (1941) demonstrated

six molecules of water as insufficient for the evolution of 6 molecules of O_2 and modified the equation as:



Photosynthesis is a collection of oxidation and reduction reactions (Redox reaction).

Oxidation- Water is oxidised into oxygen (loss of electrons).

Reduction – CO_2 is reduced into Carbohydrates (gain of electrons).

In some bacteria, oxygen is not evolved and is called as **non-oxygenic** and **anaerobic photosynthesis**. Examples: Green sulphur, Purple sulphur and green filamentous bacteria.

13.1.2 Significance of Photosynthesis

1. Photosynthetic organisms provide food for all living organisms on earth either directly or indirectly.
2. It is the only natural process that liberates oxygen in the atmosphere and balances the oxygen level.
3. Photosynthesis balances the oxygen and carbon cycle in nature.
4. Fuels such as coal, petroleum and other fossil fuels are from preserved photosynthetic plants.
5. Photosynthetic organisms are the primary producers on which all consumers depend for energy.
6. Plants provide fodder, fibre, fire wood, timber, useful medicinal products and these sources come by the act of photosynthesis.

13.1.3 Site of Photosynthesis

Chloroplasts are the main site of photosynthesis and both energy yielding process (Light reaction) and fixation of carbon di oxide (Dark reaction) that takes place in chloroplast. It is a double wall membrane bounded organelle, discoid or lens shaped, 4–10 μm in diameter and 1–33 μm in thickness. The membrane is a unit membrane and space between them is 100 to 200 \AA . A colloidal and proteinaceous matrix called stroma is present inside.

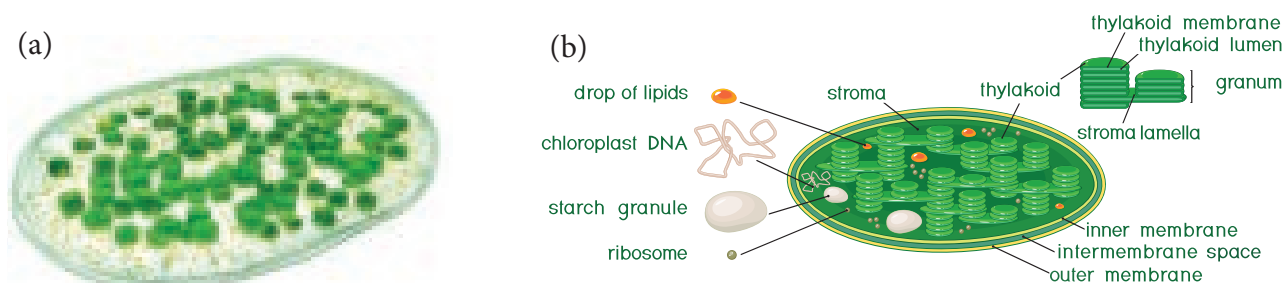


Figure 13.1: (a) 3D view of chloroplast (b) Sectional view of chloroplast

A sac like membranous system called **thylakoid** or **lamellae** is present in stroma and they are arranged one above the other forming a stack of coin like structure called **granum** (plural grana). Each chloroplast contains 40 to 80 grana and each granum consists of 5 to 30 thylakoids.

Thylakoids found in granum are called grana lamellae and in stroma are called stroma lamellae. Thylakoid disc size is 0.25 to 0.8 micron in diameter. A thinner lamella called Fret membrane connects grana. Pigment system I is located on outer thylakoid membrane facing stroma and Pigment system II is located on inner membrane facing lumen of thylakoid. Grana lamellae have both PS I and PS II whereas stroma lamellae have only PS I. Chloroplast contains 30–35 Proteins, 20–30% phospholipids, 5–10% chlorophyll,

4–5% Carotenoids, 70S ribosomes, circular DNA and starch grains. Inner surface of lamellar membrane consists of small spherical structure called as **Quantasomes**. Presence of 70S ribosome and DNA gives them status of semi-autonomy and proves endosymbiotic hypothesis which says chloroplast evolved from bacteria. Thylakoid contains pigment systems which produces ATP and $\text{NADPH} + \text{H}^+$ using solar energy. Stroma contains enzyme which reduces carbon di oxide into carbohydrates. In Cyanobacteria thylakoid lies freely in cytoplasm without envelope (Figure 13.1).

13.2 Photosynthetic Pigments

A photosynthetic pigment is a pigment that is present in chloroplasts or photosynthetic bacteria which captures the light energy necessary for photosynthesis (Table 13.1).

Table 13.1: Types of Photosynthetic pigments

Chlorophyll	Carotenoids	Phycobilins
1. Chlorophyll 'a' ($\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$) – Green plants and Cyanobacteria	1. Carotene ($\text{C}_{40}\text{H}_{56}$) – Lycopene (Red)	1. Phycocyanin – Cyanobacteria
2. Chlorophyll 'b' ($\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$) – Green algae and all higher plants	2. Xanthophyll ($\text{C}_{40}\text{H}_{56}\text{O}_2$ Yellow colour – Violaxanthin, Fucoxanthin (Brown Algae) and Lutein	2. Phycoerythrin – Red Algae
3. Chlorophyll 'c' ($\text{C}_{55}\text{H}_{32}\text{O}_5\text{N}_4\text{Mg}$) – Dinoflagellates, Diatoms and Brown Algae		
4. Chlorophyll 'd' – Red Algae		
5. Chlorophyll 'e' – Xathophycean Algae		
6. Bacteriochlorophyll 'a'		
7. Bacteriochlorophyll 'b'		
8. Chlorobium Chlorophyll 650		
9. Chlorobium Chlorophyll 666		

13.2.1 Chlorophyll

Chlorophyll 'a' is the primary pigment which acts as a reaction centre and all other pigments act as accessory pigments and trap solar energy and then transfer it to chlorophyll 'a'. Chlorophyll molecules have a tadpole like structure. It consists of Mg-Porphyrin head (Hydrophilic Head) and (Lipophilic tail) Phytol tail. The Porphyrin head consists of four pyrrole rings linked together by C-H bridges. Each pyrrole ring comprises of four carbons and one nitrogen atom. Porphyrin ring has several side groups which alter the properties of the pigment. Different side groups are indicative of various types of chlorophyll. The Phytol tail made up of 20 carbon alcohol is attached to carbon 7 of the Pyrrole ring IV. It has a long propionic acid ester bond. Long lipophilic tail helps in anchoring chlorophyll to the lamellae.

13.2.2 Carotenoids

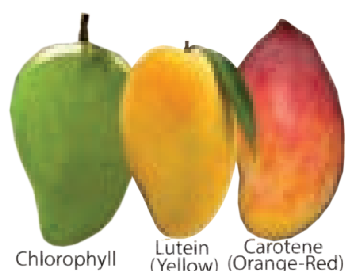


Figure 13.2: Changes in Fruit colour due to difference in pigmentation

Carotenoids are yellow to orange pigments, mostly tetraterpens and these pigments absorb light strongly in the blue to violet region of visible spectrum. These pigments protect chlorophyll from photo-oxidative damage. Hence, they are called as **shield pigments**. These pigments absorb light and transfer these to chlorophyll. Almost all carotenoid pigments have 40 carbon atoms. Ripening of fruits, floral colours and leaf colour change during autumn is due to Carotenoids (Carotene and Xanthophyll) (Figure 13.2).

i. Carotenes:

Orange, Red, Yellow and Brownish pigments, hydrocarbons (Lipids)

and most of them are tetraterpenes ($C_{40}H_{56}$). Carotene is the most abundant Carotene in plants and it is a precursor of Vitamin A. Lycopene is the red pigment found in the fruits of tomato, red peppers and roses.

ii. Xanthophylls:

Yellow ($C_{40}H_{56}O_2$) pigments are like carotenes but contain oxygen. Lutein is responsible for yellow colour change of leaves during autumn season. Examples: Lutein, Violaxanthin and Fucoxanthin.

13.2.3 Phycobilins

They are proteinaceous pigments, soluble in water, and do not contain Mg and Phytol tail. They exist in two forms such as 1. Phycocyanin found in cyanobacteria 2. Phycoerythrin found in rhodophycean algae (Red algae).

13.3 Spectrum of Electromagnetic Radiation

In the total electromagnetic spectrum, visible light is the smallest part. The entire life on earth depends on light and is the driving force for all organisms. Plants have natural potential to utilize solar energy directly. In the given picture electromagnetic radiation spectrum and components of visible spectrum are mentioned. The wavelength of solar radiation which reaches the earth

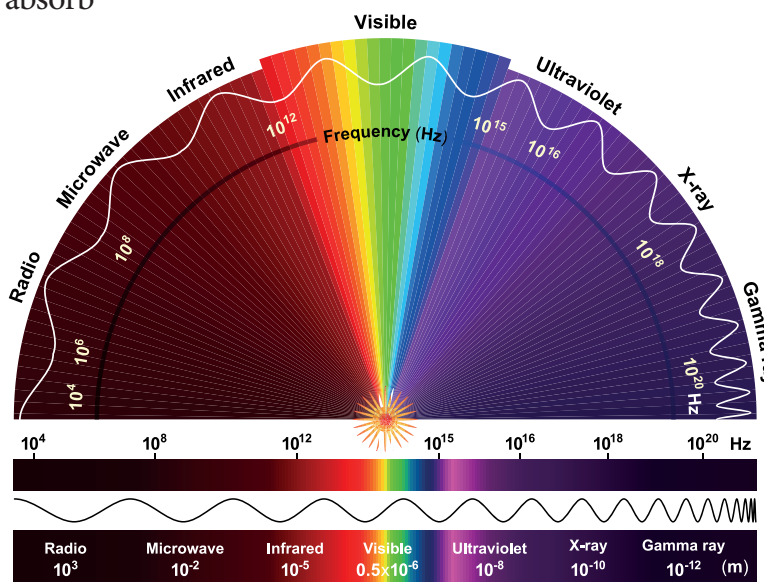


Figure 13.4: Electromagnetic Spectrum

is between 300 to 2600 nm. The visible spectrum ranges between 390 to 763 nm (3900 Å to 7630 Å). The colour of the light is determined by the wavelength. Energy of the quantum is inversely proportional to wavelength. Shorter wavelength has more energy than longer wavelength. Electromagnetic spectrum consists of 7 types of radiations such as gamma rays, X rays, U-V rays, Visible light spectrum, infrared rays, electric rays and radio rays (Figure 13. 4).



Light is extremely variable and if radiation is evenly distributed over the globe it is sufficient to melt 35 m thick ice layer.

Properties of Light

1. Light is a transverse electromagnetic wave.
2. It consists of oscillating electric and magnetic fields that are perpendicular to each other and perpendicular to the direction of propagation of the light.
3. Light moves at a speed of $3 \times 10^8 \text{ ms}^{-1}$
4. Wavelength is the distance between successive crests of the wave.
5. Light as a particle is called **photon**. Each photon contains an amount of energy known as **quantum**.
6. The energy of a photon depends on the frequency of the light (Figure 13.5).

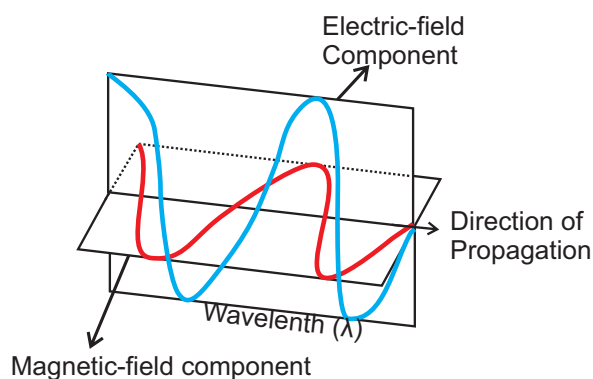


Figure 13.5: Oscillation of electric and magnetic vectors in light

Separation of Chlorophyll pigments by paper Chromatography method

Step 1. Extract chlorophyll pigment from the leaves using 80% Acetone.

Step 2. Allow to concentrate by evaporation.

Step 3. Apply few drops on one end above 2 cm from the edge of a chromatographic paper.

Step 4. A solvent with mixture of Petroleum ether and acetone in the ratio of 9:1 is prepared and poured into development chamber.

Step 5. Place the strip above the solvent by placing one end of the strip touching the solvent.

Observation

After one hour observe the chromatographic paper. You can find the pigments being separated into four distinct spots (Figure 13. 4).

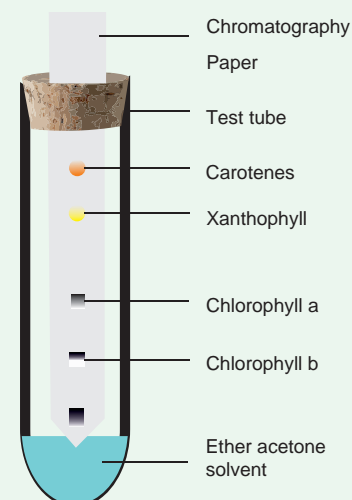


Figure 13.3: Paper Chromatography

13.4 Photosynthetic Unit (Quantasome)

Quantasomes are the morphological expression of physiological photosynthetic units, located on the inner membrane of thylakoid lamellae. Each quantasome measures about $180 \text{ Å} \times 160 \text{ Å}$ and 100 Å thickness. In 1952, **Steinman** observed granular structures in chloroplast lamellae under electron microscope. Later, **Park** and **Biggins** (1964) confirmed these granular structures as physiological units of photosynthesis and coined the term Quantasome. According to them one

quantasome contains about 230 chlorophyll molecules. A minimum number of chlorophyll and other accessory pigments act together in a photochemical reaction to release one oxygen or to reduce one molecule of CO_2 . It constitutes a photosynthetic unit. (Figure 13.6) **Emerson and Arnold** (1932) based on flashing light experiment found 2500 chlorophyll molecules are required to fix one molecule of CO_2 . However, the reduction or fixation of one CO_2 requires 10 quanta of light and so each unit would contain $1/10$ of 2500 i.e. 250 molecules. Usually 200 to 300 chlorophyll molecules are considered as a physiological unit of photosynthesis. According to Emerson 8 quanta of light are required for the release of one oxygen molecule or reduction of one Carbon dioxide molecule. The quantum yield is $1/8$ or 12 %.

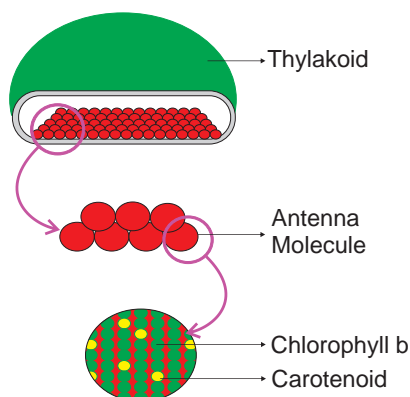


Figure 13.6: Quantasome

13.5 Absorption Spectrum and Action Spectrum

13.5.1 Absorption Spectrum

The term absorption refers to complete retention of light, without reflection or transmission. Pigments absorb different wavelengths of light. A curve obtained by plotting the amount of absorption of different wavelengths of light by a pigment is called its **absorption spectrum**.

- Chlorophyll 'a' and chlorophyll 'b' absorb quanta from blue and red region
- Maximum absorption peak for different forms of chlorophyll 'a' is 670 to 673, 680 to 683 and 695 to 705nm.

- Chlorophyll 'a' 680 (P680) and Chlorophyll 'a' 700 (P700) function as trap centre for PS II and PS I respectively.

13.5.2 Action Spectrum

The effectiveness of different wavelength of light on photosynthesis is measured by plotting against quantum yield. The curve showing the rate of photosynthesis at different wavelengths of light is called **action spectrum**. From the graph showing action spectrum, it can be concluded that maximum photosynthesis takes place in blue and red region of the spectrum. This wavelength of the spectrum is the absorption maxima for Chlorophyll (a) and Chlorophyll (b). The Action Spectrum is instrumental in the discovery of the existence of two photosystems in O_2 evolving photosynthesis (Figure 13.7).

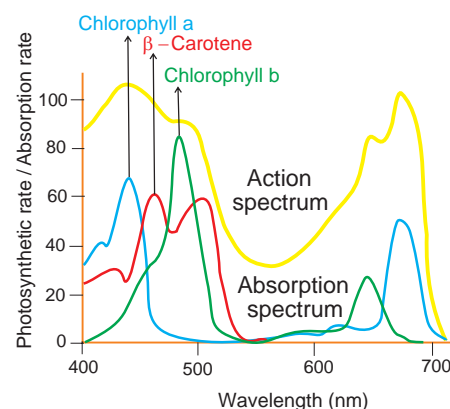


Figure 13.7: Absorption and action spectrum

13.6 Emerson's Experiments and Hill's Reaction

13.6.1 Red Drop or Emerson's First Effect

Emerson conducted experiment in *Chlorella* using only one wavelength of light (monochromatic light) at a time and he measured quantum yield. He plotted a graph of the quantum yield in terms of O_2 evolution at various wavelengths of light. His focus was to determine at which wavelength the photochemical yield of oxygen was maximum. He found that in the wavelength of 600 to 680 the yield was constant but suddenly dropped in the region above 680 nm (red region). The fall in the photosynthetic yield beyond red region of the spectrum is referred as **Red drop or Emerson's first effect**.



13.6.2 Emerson's Enhancement Effect

Emerson modified his first experiment by supplying shorter wavelength of light (red light) along with longer wavelength of light (far red light). He found that the monochromatic light of longer wavelength (far red light) when supplemented with shorter wavelength of light (red light) enhanced photosynthetic yield and recovered red drop. This enhancement of photosynthetic yield is referred to as Emerson's Enhancement Effect (Figure 13.8).

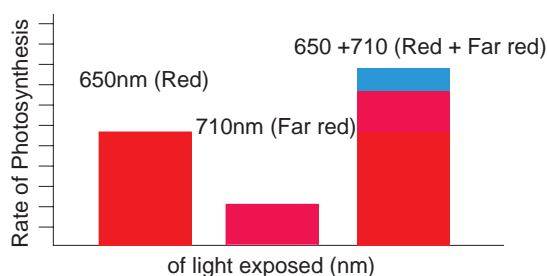


Figure 13.8: Emerson's Enhancement Effect

Photosynthetic rate at far red light (710 nm) = 10

Photosynthetic rate at red light (650 nm) = 43.5

Photosynthetic rate at red + far red (650 + 710 nm) = 72.5 (Enhancement effect).

13.6.3 Hill's Reaction

R. Hill (1937) isolated chloroplasts and when they were illuminated in the presence of suitable electron acceptors such as ferricyanide, they were reduced to ferrocyanide and oxygen is evolved. Hill's Reaction is now considered to be equivalent to Light Reaction.

Conclusions of Hill's Reaction:

1. During photosynthesis oxygen is evolved from water.
2. Electrons for the reduction of CO_2 are obtained from water.
3. Reduced substance produced, later helps to reduce CO_2



A is the Hydrogen acceptor, the common *in vitro* hydrogen acceptors are ferricyanide,

benzoquinone and Di Chloro Phenol Indole Phenol (DCPIP).

13.7 Modern Concept of Photosynthesis

Photosynthesis is an Oxidation and Reduction process. Water is oxidised to release O_2 and CO_2 is reduced to form sugars. The first phase requires light and is called **light reaction or Hill's reaction**.



1. Light reaction: It is a photochemical reaction whereas dark reaction is a thermochemical reaction.

Solar energy is trapped by chlorophyll and stored in the form of chemical energy (assimilatory power) as ATP and reducing power $\text{NADPH} + \text{H}^+$. $\text{NADPH} + \text{H}^+$ alone are known as **reducing powers**. This reaction takes place in thylakoid membrane of the chloroplast. Oxygen is evolved as a result of splitting of water molecules by light.

Light reaction is discussed in two phases:

i. Photo-oxidation Phase:

- Absorption of light energy.
- Transfer of energy from accessory pigments to reaction centre.
- Activation of Chlorophyll 'a' molecule.

ii. Photo Chemical Phase:

- Photolysis of water and oxygen evolution
- Electron transport and synthesis of assimilatory power.

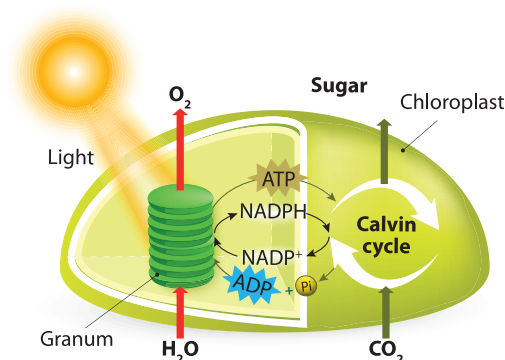


Figure 13.9: Light and Dark Reaction

2. Dark reaction (Biosynthetic phase): Fixation and reduction of CO_2 into carbohydrates with the help of assimilatory power produced during light reaction. This reaction does not require light and is not directly light driven.

Hence, it is called as **Dark reaction or Calvin-Benson cycle** (Figure 13.9).

13.8 Photo-Oxidation Phase of Light Reaction

The action of photon plays a vital role in excitation of pigment molecules to release an electron. When the molecules absorb a photon, it is in excited state. When the light source turned off, the high energy electrons return to their normal low energy orbitals as the excited molecule goes back to its original stable condition known as **ground state**. When molecules absorb or emit light they change their electronic state. Absorption of blue light excites the chlorophyll to higher energy state than absorption of Red light, because the energy of photon is higher when their wavelength is shorter. When the pigment molecule is in an excited state, this excitation energy is utilised for the phosphorylation. Phosphorylation takes place with the help of light generated electron and hence it is known as **photophosphorylation**.

13.8.1 Photosystem and Reaction Centre

- Thylakoid membrane contains Photosystem I (PS I) and Photosystem II (PS II).
- PS I is in unstacked region of granum facing stroma of chloroplast.
- PS II is found in stacked region of thylakoid membrane facing lumen of thylakoid.



- Each Photosystem consists of central core complex (CC) and light harvesting Complex (LHC) or Antenna molecules (Figure 13.10).
- The core complex consists of respective reaction centre associated with proteins, electron donors and acceptors.
- PS I – CC I consists of reaction centre P700 and LHC I.
- PS II – CC II consists of reaction centre P680 and LHC II (Table 13.2).
- Light Harvesting Complex consists of several chlorophylls, carotenoids and xanthophyll molecules.
- The main function of LHC is to harvest light energy and transfer it to their respective reaction centre.

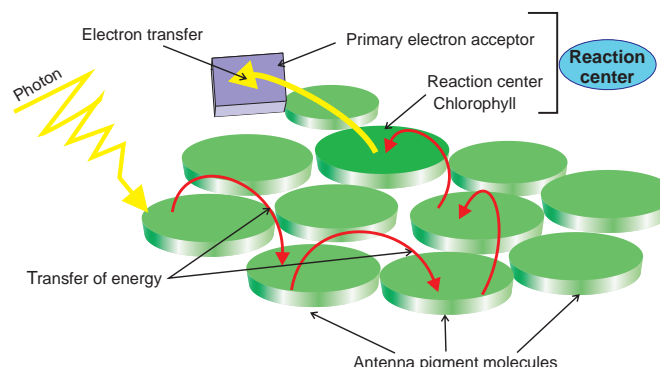


Figure 13.10: Photosystem

13.9 Photo chemical phase of light reaction

In this phase electrons pass through electron carrier molecules and generate assimilatory powers ATP and NADPH + H⁺. Splitting of water molecule generates electrons replacing electrons produced by the light.

Table 13.2: Differences between Photosystem I and Photosystem II

Photosystem I	Photosystem II
1. The reaction centre is P700	1. Reaction centre is P680
2. PS I is involved in both cyclic and non-cyclic.	2. PS II participates in Non-cyclic pathway
3. Not involved in photolysis of water and evolution of oxygen	3. Photolysis of water and evolution of oxygen take place.
4. It receives electrons from PS II during non-cyclic photophosphorylation	4. It receives electrons by photolysis of water
5. Located in unstacked region granum facing chloroplast stroma	5. Located in stacked region of thylakoid membrane facing lumen of thylakoid.
6. Chlorophyll and Carotenoid ratio is 20 to 30:1	6. Chlorophyll and Carotenoid ratio is 3 to 7:1

13.9.1 Photolysis of Water

The process of Photolysis is associated with **Oxygen Evolving Complex** (OEC) or water splitting complex in pigment system II and is catalysed by the presence of Mn^{++} and Cl^- . When the pigment system II is active it receives light and the water molecule splits into OH^- ions and H^+ ions. The OH^- ions unite to form water molecules again and release O_2 and electrons (Figure 13.11).

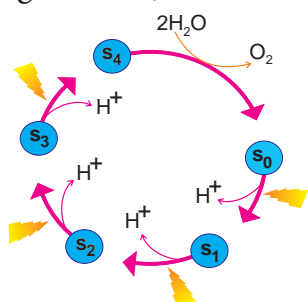
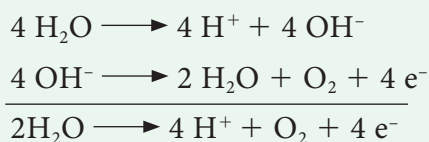


Figure 13.11: Oxygen Evolving Complex (OEC)



13.9.2 Electron Transport Chain of Chloroplast

Electron Transport Chain in each photosystem involves four complexes:

- **Core Complex (CC):** CC I in PS I the reaction centre is P700, CC II in PS II the reaction centre is P680
- **Light Harvesting Complex or Antenna complex (LHC):**
- Two types: LHC I in PS I and LHC II in PS II.

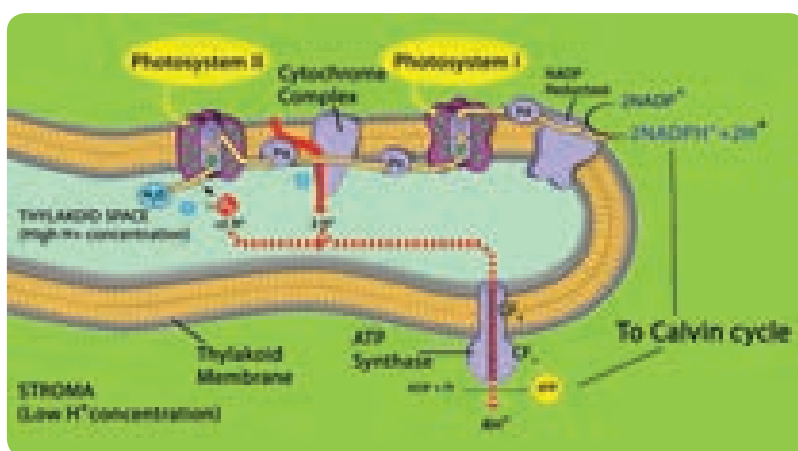


Figure 13.12: Electron Transport Chain in Chloroplast

- **Cytochrome b6 f complex:** It is the non-pigmented protein complex connecting PS I and PS II. Plastoquinone (PQ) and Plastocyanin (PC) are intermediate complexes acting as mobile or shuttle electron carriers of Electron Transport Chain. PQ acts as shuttle between PS II and Cytochrome b₆-f complex and PC connects
- **Cytochrome b₆-f and PS I complex.**
- **ATPase complex or Coupling factor:** It is found in the surface of thylakoid membrane. This complex is made up of CF₁ and CF₀ factors. This complex utilizes energy from ETC and converts ADP and inorganic phosphate (P_i) into ATP (Figure 13.12).

13.10 Photophosphorylation

Phosphorylation taking place during respiration is called as **oxidative phosphorylation** and ATP produced by the breakdown of substrate is known as **substrate level phosphorylation**. In this topic, we are going to learn about phosphorylation taking place in chloroplast with the help of light. During the movement of electrons through carrier molecules ATP and NADPH + H^+ are produced. Phosphorylation is the process of synthesis of ATP by the addition of inorganic phosphate to ADP. The addition of phosphate here takes place with the help of light generated electron and so it is called as **photophosphorylation**. It takes place in both cyclic and non-cyclic electron transport.

13.10.1 Cyclic Photophosphorylation

Cyclic photophosphorylation refers to the electrons ejected from the pigment system I (Photosystem I) and again cycled back to the PS I. When the photons activate P700 reaction centre photosystem II is activated. Electrons are raised to the high energy level. The primary electron acceptor is Ferredoxin Reducing Substance (FRS) which transfers electrons to Ferredoxin

(Fd), Plastoquinone (PQ), cytochrome b6-f complex, Plastocyanin (PC) and finally back to chlorophyll P700 (PS I). During this movement of electrons Adenosine Di Phosphate (ADP) is phosphorylated, by the addition of inorganic phosphate and generates Adenosine Tri Phosphate (ATP). Cyclic electron transport produces only ATP and there is no $\text{NADPH} + \text{H}^+$ formation. At each step of electron transport, electron loses potential energy and is used by the transport chain to pump H^+ ions across the thylakoid membrane. The proton gradient triggers ATP formation in ATP synthase enzyme situated on the thylakoid membrane. Photosystem I need light of longer wave length ($> 700 \text{ nm}$). It operates under low light intensity, less CO_2 and under anaerobic conditions which makes it considered as earlier in evolution (Figure 13.13).

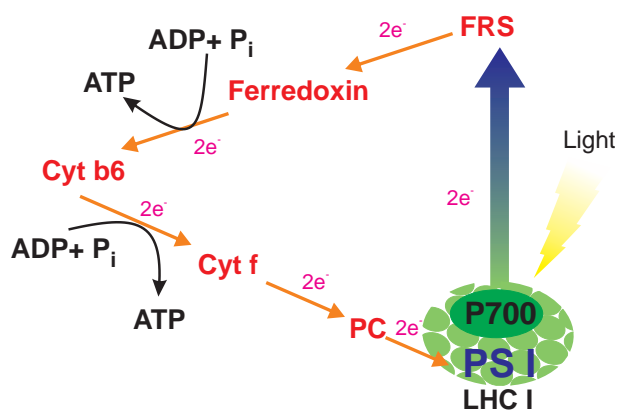


Figure 13.13: Cyclic Photophosphorylation

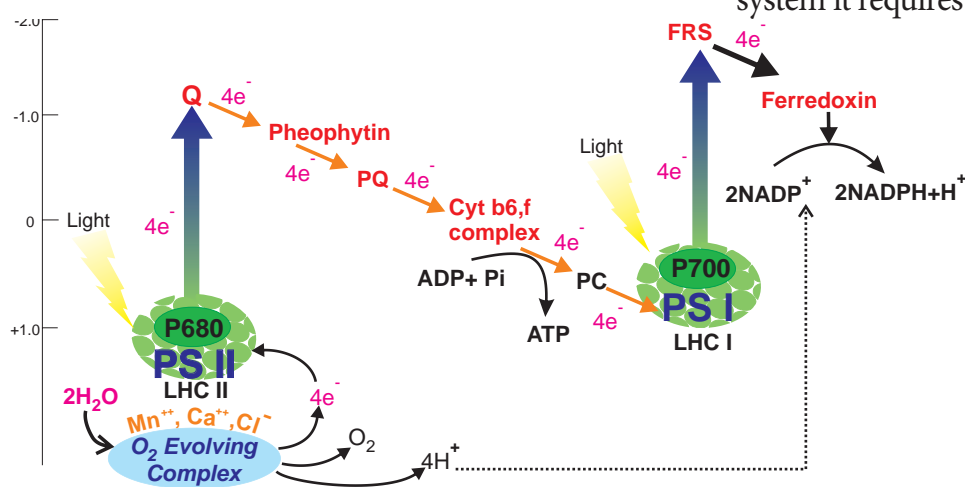


Figure 13.14: Non-Cyclic Photophosphorylation

13.10.2 Non-Cyclic Photophosphorylation

When photons are activated reaction centre of pigment system II (P680), electrons moved to the high energy level. Electrons from high energy state passes through series of electron carriers like pheophytin, plastoquinone, cytochrome complex, plastocyanin and finally accepted by PS I (P700). During this movement of electrons from PS II to PS I ATP is generated (Figure 13. 16). PS I (P700) is activated by light, electrons are moved to high energy state and accepted by electron acceptor molecule ferredoxin reducing Substance (FRS). During the downhill movement through ferredoxin, electrons are transferred to NADP^+ and reduced into $\text{NADPH} + \text{H}^+$ (H^+ formed from splitting of water by light).

Electrons released from the photosystem II are not cycled back. It is used for the reduction of NADP^+ in to $\text{NADPH} + \text{H}^+$. During the electron transport it generates ATP and hence this type of photophosphorylation is called **non-cyclic photophosphorylation**. The electron flow looks like the appearance of letter 'Z' and so known as **Z scheme**. When there is availability of NADP^+ for reduction and when there is splitting of water molecules both PS I and PS II are activated (Table 13.3). Non-cyclic electron transport PS I and PS II both are involved co-operatively to transport electrons from water to NADP^+ (Figure 13.14).

13.10.3 Bio energetics of light reaction

- To release one electron from pigment system it requires two quanta of light.
- One quantum is used for transport of electron from water to PS I.
- Second quantum is used for transport of electron from PS I to NADP^+
- Two electrons are required to generate one $\text{NADPH} + \text{H}^+$.

Table 13.3 Differences between Cyclic Photophosphorylation and Non-Cyclic Photophosphorylation

Cyclic Photophosphorylation	Non-Cyclic Photophosphorylation
1. PS I only involved	1. PS I and PS II involved
2. Reaction centre is P700	2. Reaction centre is P680
3. Electrons released are cycled back	3. Electron released are not cycled back
4. Photolysis of water does not take place	4. Photolysis of water takes place
5. Only ATP synthesized	5. ATP and NADPH + H ⁺ are synthesized
6. Phosphorylation takes place at two places	6. Phosphorylation takes place at only one place
7. It does not require an external electron donor	7. Requires external electron donor like H ₂ O or H ₂ S
8. It is not sensitive to di chloro di methyl urea (DCMU)	8. It is sensitive to DCMU and inhibits electron flow

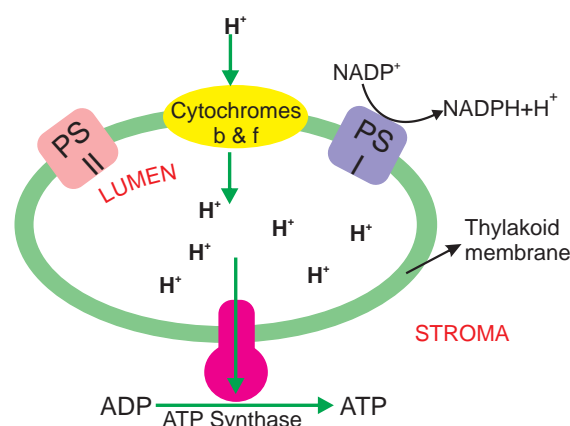
- During Non-Cyclic electron transport two NADPH + H⁺ are produced and it requires 4 electrons.
- Transportation of 4 electrons requires 8 quanta of light.

Check your grasp!

Name the products produced from Non-Cyclic photophosphorylation?

Why does PS II require electrons from water?

Can you find the difference in the Pathway of electrons during PS I and PS II?

**Figure 13.15: Chemiosmotic Theory****13.10.4 Chemiosmotic Theory**

Chemiosmosis theory was proposed by **P. Mitchell** (1966). According to this theory electrons are transported along the membrane through PS I and PS II and connected by Cytochrome b6-f complex. The flow of electrical current is due to difference in electrochemical potential of protons across the membrane. Splitting of water molecule takes place inside the membrane. Protons or H⁺ ions accumulate within the lumen of the thylakoid (H⁺ increase 1000 to 2000 times). As a result, proton concentration is increased inside the thylakoid lumen. These protons move across the membrane because the primary acceptor of electron is located outside the membrane. Protons in stroma less in number and creates a proton gradient. This gradient is broken down due to the movement of proton across the membrane to the stroma through CF₀ of the ATP synthase enzyme. The proton motive force created inside the lumen of thylakoid or chemical gradient of H⁺ ion across the membrane stimulates ATP generation (Figure 13.15).

The evolution of one oxygen molecule (4 electrons required) requires 8 quanta of light. C₃ plants utilise 3 ATPs and 2 NADPH + H⁺ to evolve one Oxygen molecule. To evolve 6 molecules of Oxygen 18 ATPs and 12 NADPH + H⁺ are utilised. C₄ plants utilise 5 ATPs and 2 NADPH + H⁺ to evolve one oxygen molecule. To evolve 6 molecules of Oxygen 30 ATPs and 12 NADPH + H⁺ are utilised.

13.11 Dark Reaction or C₃ Cycle or Biosynthetic Phase or Photosynthetic Carbon Reduction (PCR) Cycle

Biosynthetic phase of photosynthesis utilises assimilatory powers (ATP and NADPH + H⁺) produced during light reaction are used to fix and reduce carbon di oxide into carbohydrates. This reaction does not require light. Therefore, it is named Dark reaction. Ribulose 1,5 bisphosphate (RUBP) act as acceptor molecule of carbon di oxide and fix the CO₂ by RUBISCO enzyme. The first product of the pathway is a 3- carbon compound (Phospho Glyceric Acid) and so it is also called as C₃ Cycle. It takes place in the stroma of the chloroplast.

M. Melvin Calvin, A.A. Benson and their co-workers in the year 1957 found this path way of carbon fixation. Melvin Calvin was awarded Nobel Prize for this in 1961 and this pathway named after the discoverers as **Calvin-Benson Cycle**. Dark reaction is temperature dependent and so it is also called thermo-chemical reaction.

Check your grasp!

What will be the quanta requirement for complete light reaction which releases 6 oxygen molecules?

Solution: Complete light reaction releases 6 oxygen molecules. If one molecule of oxygen evolution requires 8 quanta means, for 6 oxygen molecules $6 \times 8 = 48$ quanta of light required for complete light reaction.

Dark reaction consists of three phases: (Figure 13.16).

1. Carboxylation (fixation)
2. Reduction (Glycolytic Reversal)
3. Regeneration

Phase 1- Carboxylation (Fixation)

The acceptor molecule Ribulose 1,5 Bisphosphate (RUBP) a 5 carbon compound

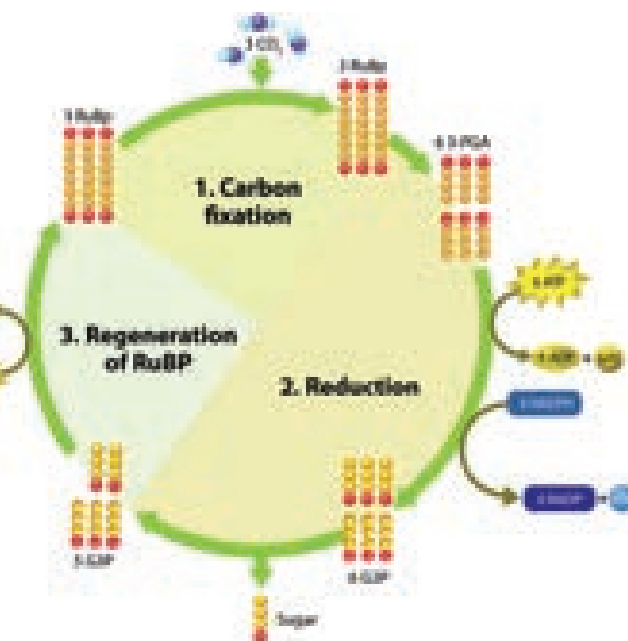


Figure 13.16: Phases of Calvin Cycle

with the help of RUBP carboxylase oxygenase (RUBISCO) enzyme accepts one molecule of carbon dioxide to form an unstable 6 carbon compound. This 6C compound is broken down into two molecules of 3-carbon compound phospho glyceric acid (PGA) (Figure 13.17).

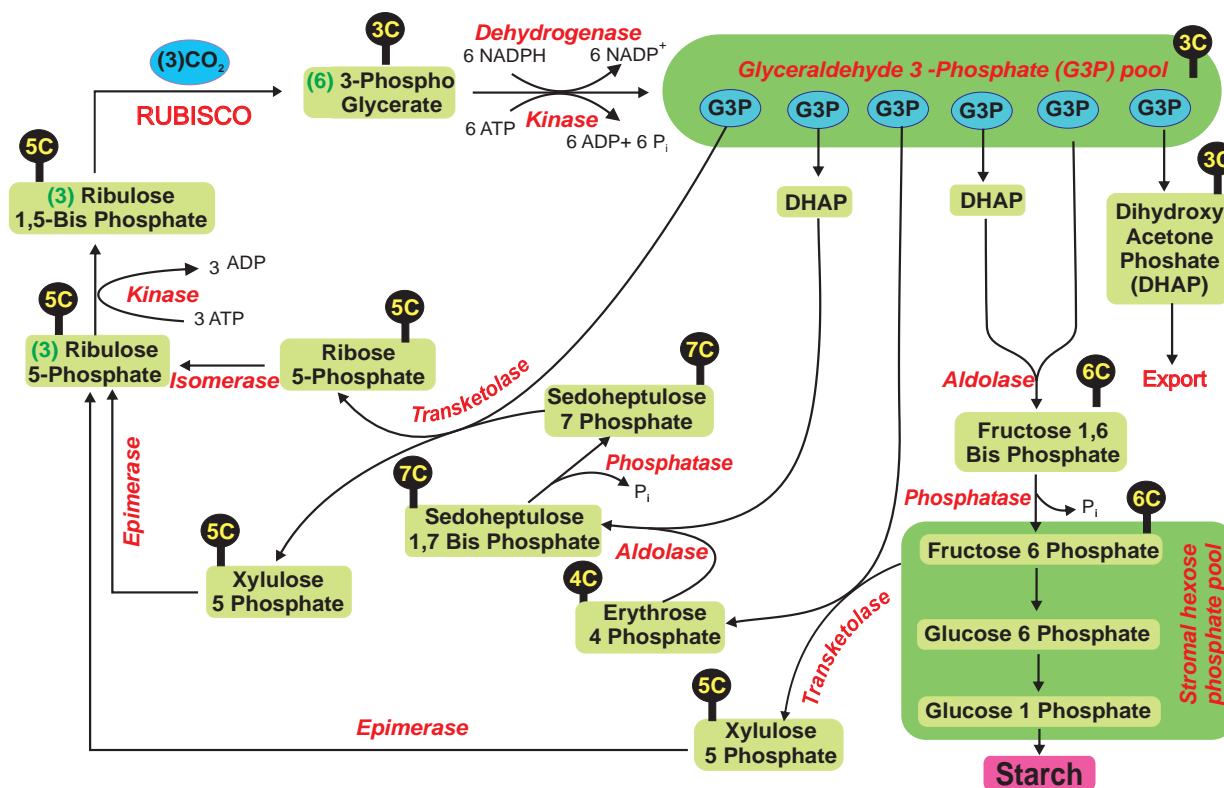
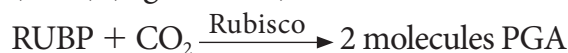
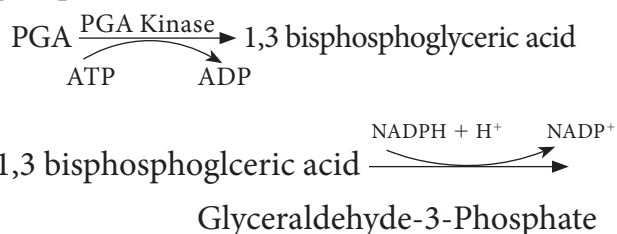


Figure 13.17: Calvin cycle

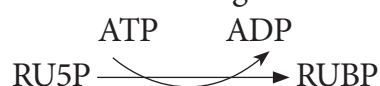
Phase 2 – Glycolytic Reversal / Reduction

Phospho glyceric acid is phosphorylated by ATP and produces 1,3 bis phospho glyceric acid by PGA kinase. 1,3 bis phospho glyceric acid is reduced to glyceraldehyde 3 Phosphate (G-3-P) by using the reducing power NADPH + H⁺. Glyceraldehyde 3 phosphate is converted into its isomeric form di hydroxy acetone phosphate (DHAP).



Phase 3 – Regeneration

Regeneration of RUBP involves the formation of several intermediate compounds of 6-carbon, 5-carbon, 4-carbon and 7- carbon skeleton. Fixation of one carbon dioxide requires 3 ATPs and 2 NADPH + H⁺, and for the fixation of 6 CO₂ requires 18 ATPs and 12 NADPH + H⁺ during C₃ cycle. One 6 carbon compound is the net gain to form hexose sugar.



Overall equation for dark reaction:



DO YOU KNOW? RUBISCO – RUBP Carboxylase Oxygenase enzyme, is the most abundant protein found on earth. It constitutes 16 % of the chloroplast protein. It acts as carboxylase in the presence of CO₂ and oxygenase in the absence of CO₂.

13.12 Hatch & Slack Pathway or C₄ Cycle or Dicarboxylic Acid Pathway or Dicarboxylation Pathway

Till 1965, Calvin cycle is the only pathway for CO₂ fixation. But in 1965, **Kortschak**, **Hart** and **Burr** made observations in sugarcane and found C₄ or dicarboxylic acid pathway. Malate and aspartate are the major labelled

products. This observation was confirmed by **Hatch & Slack** in 1967. This alternate pathway for the fixation of CO₂ was found in several tropical and sub-tropical grasses and some dicots. C₄ cycle is discovered in more than 1000 species. Among them 300 species belong to dicots and rest of them are monocots. C₄ plants represent about 5% of Earth's plant biomass and 1% of its known plant species. Despite this scarcity, they account for about 30% of terrestrial carbon fixation. Increasing the proportion of C₄ plants on earth could assist biosequestration of CO₂ and represent an important climate change avoidance strategy.

C₄ pathway is completed in two phases, first phase takes place in stroma of mesophyll cells, where the CO₂ acceptor molecule is 3-Carbon compound, phospho enol pyruvate (PEP) to form 4-carbon Oxalo acetic acid (OAA). The first product is a 4-carbon and so it is named as C₄ cycle. oxalo acetic acid is a dicarboxylic acid and hence this cycle is also known as **dicarboxylic acid pathway** (Figure 13.18). Carbon dioxide fixation takes place in two places one in mesophyll and another in bundle sheath cell (di carboxylation pathway). It is the adaptation of tropical and sub tropical plants growing in warm and dry conditions. Fixation of CO₂ with minimal loss is due to absence of photorespiration. C₄ plants require 5 ATP and 2 NADPH + H⁺ to fix one molecule of CO₂.

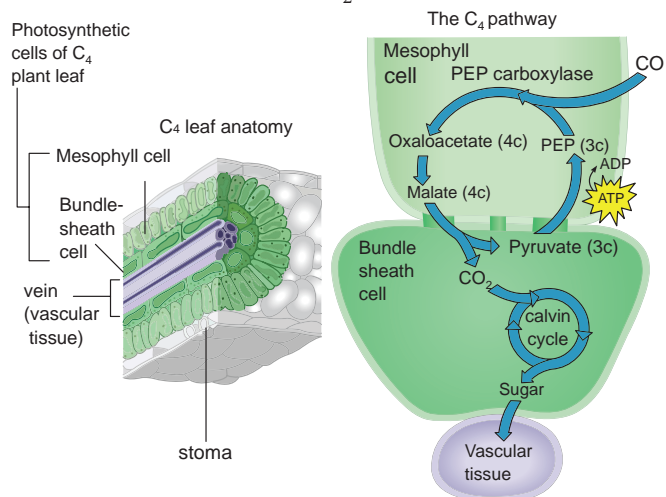


Figure 13.18: C₄ Cycle

Phosphoenol Pyruvate + CO₂
(PEP) (3C) |

Oxaloacetic acid (OAA) (4C) 

$$\text{RUBP} + \text{CO}_2 \xrightarrow{\text{Rubisco}} 2 \text{PGA}$$

(5C) (3C)

Check your grasp!

C_4 plants requires 30 ATPs and 12 NADPH + H^+ to synthesize one glucose, but C_3 plants requires only 18 ATPs and 12 NADPH + H^+ to synthesize one glucose molecule. If then, how can you say C_4 plants are more advantageous?

Solution: C_4 plants are more advantageous than C_3 plants because most of the energy lost during photo respiration in C_3 plants.

13.13 Crassulacean Acid Metabolism or CAM cycle

It is one of the carbon pathways identified in succulent plants growing in semi-arid or xerophytic condition. This was first observed in crassulaceae family plants like *Bryophyllum*, *Sedum*, *Kalanchoe* and is the reason behind the name of this cycle. It is also noticed in plants from other families Examples: *Agave*, *Opuntia*, Pineapple and Orchids. The stomata are closed during day and are open during night (Scotoactive). This reverse stomatal rhythm helps to conserve water loss through transpiration and will stop the fixation of CO_2 during the day time. At night time CAM plants fix CO_2 with the help of Phospho Enol Pyruvic acid (PEP) and produce oxalo acetic acid

(OAA). Subsequently OAA is converted into malic acid like C_4 cycle and gets accumulated in vacuole increasing the acidity. During the day time stomata are closed and malic acid is decarboxylated into pyruvic acid resulting in the decrease of acidity. CO_2 thus formed enters into Calvin Cycle and produces carbohydrates (Figure13.19).

Significance of CAM Cycle

1. It is advantageous for succulent plants to obtain CO_2 from malic acid when stomata are closed.
2. During day time stomata are closed and CO_2 is not taken but continue their photosynthesis.
3. Stomata are closed during the day time and help the plants to avoid transpiration and water loss.

13.14 Photorespiration or C_2 Cycle or Photosynthetic Carbon Oxidation (PCO) Cycle

Respiration is a continuous process for all living organisms including plants. Decker (1959) observed that rate of respiration is more in light than in dark. Photorespiration is the excess respiration taking place in photosynthetic cells due to absence of CO_2

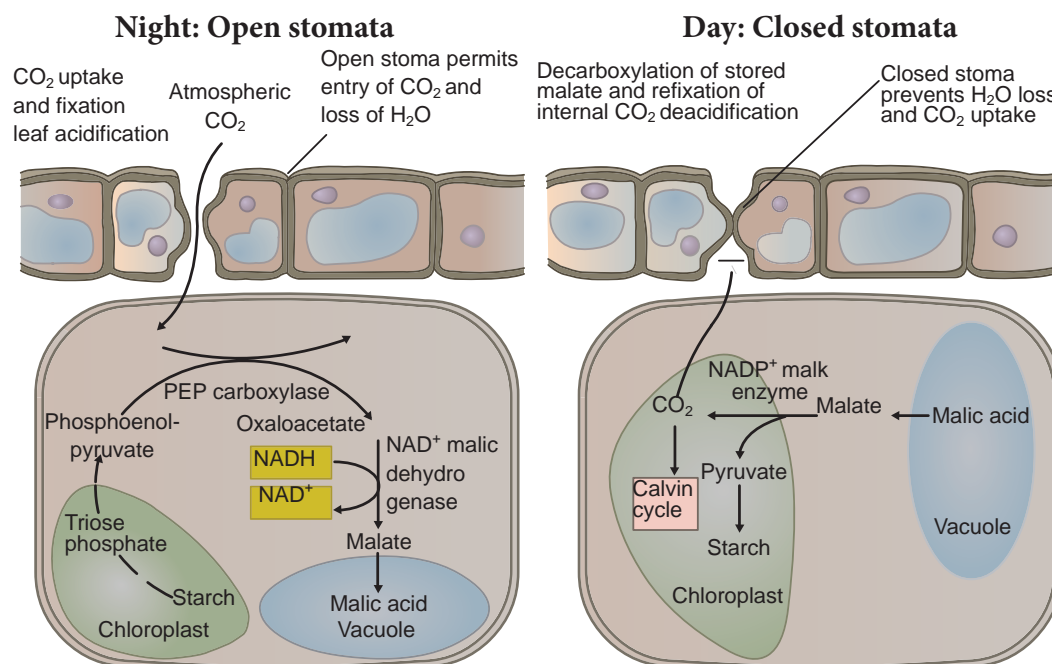


Figure 13.19: CAM cycle

and increase of O_2 (Table 13.5). This condition changes the carboxylase role of RUBISCO into oxygenase. C_2 Cycle takes place in chloroplast, peroxisome and mitochondria. RUBP is converted into PGA and a 2C-compound phosphoglycolate by Rubisco enzyme in chloroplast. Since the first product is a 2C-compound, this cycle is known as C_2 Cycle. Phosphoglycolate by loss of phosphate becomes glycolate. Glycolate formed in chloroplast enters into peroxisome to form glyoxylate and hydrogen peroxide. Glyoxylate is converted into glycine and transferred into mitochondria. In mitochondria, two molecules of glycine combine to form serine. Serine enters into peroxisome to form hydroxy pyruvate. Hydroxy pyruvate with help of $NADH + H^+$ becomes glyceric acid. Glyceric acid is cycled back to chloroplast utilising ATP and becomes Phosphoglyceric acid (PGA) and enters into the Calvin cycle (PCR cycle). Photorespiration does not yield any free energy in the form of ATP. Under certain conditions 50% of the photosynthetic potential is lost because of Photorespiration (Figure 13.20).

13.15.1 Significance of photorespiration

1. Glycine and Serine synthesised during this process are precursors of many biomolecules like chlorophyll, proteins, nucleotides.
2. It consumes excess $NADH + H^+$ generated.
3. Glycolate protects cells from Photo oxidation.

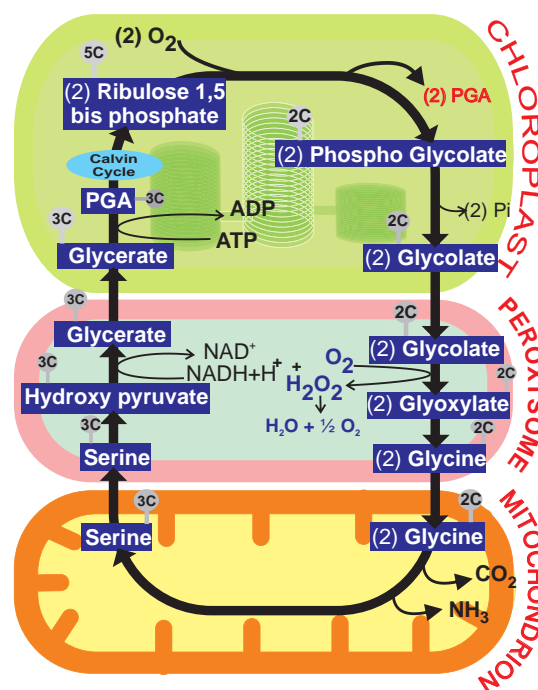


Figure 13.20: Photorespiration

13.15.2 Carbon Dioxide Compensation Point

When the rate of photosynthesis equals the rate of respiration, there is no exchange of oxygen and carbon dioxide and this is called as carbon dioxide **compensation point**. This will happen at particular light intensity when exchange of gases becomes zero. When light is not a limiting factor and atmospheric CO_2 concentration is between 50 to 100 ppm the net exchange is zero.

13.15 Factors affecting Photosynthesis

In 1860, **Sachs** gave three cardinal points theory explaining minimum, optimum and maximum factors that control photosynthesis.

Table 13.5: Differences between Photorespiration and Dark Respiration

Photorespiration	Dark respiration
1. It takes place in photosynthetic green cells	1. It takes place in all living cells
2. It takes place only in the presence of light	2. It takes place all the time
3. It involves chloroplast, peroxisome and mitochondria	3. It involves only mitochondria
4. It does not involve Glycolysis, Krebs Cycle, and ETS	4. It involves glycolysis, Krebs Cycle and ETS
5. Substrate is glycolic acid	5. Substrate is carbohydrates, protein or fats
6. It is not essential for survival	6. Essential for survival
7. No phosphorylation and yield of ATP	7. Phosphorylation produces ATP energy
8. $NADH_2$ is oxidised to NAD^+	8. NAD^+ is reduced to $NADH_2$
9. Hydrogen peroxide is produced	9. Hydrogen peroxide is not produced
10. End products are CO_2 and PGA	10. End products are CO_2 and water



In 1905, **Blackman** put forth the importance of smallest factor. **Blackman's law of limiting factor** is actually a modified Law proposed by **Liebig's Law of minimum**. According to Blackman, "*When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the lowest factor*". To conclude in an easy way "*at any given point of time the lowest factor among essentials will limit the rate of photosynthesis*". For example, when even sufficient light intensity is available, photosynthesis may be low due to low CO_2 in the atmosphere. Here, CO_2 acts as a limiting factor. If CO_2 is increased in the atmosphere the rate of photosynthesis also increases. Further increase in photosynthesis is possible only if the available light intensity is also increased proportionately (Figure 13.21).

Factors affecting photosynthesis are further grouped into External or Environmental factors and Internal factors.

- I. **External factors:** Light, carbon dioxide, temperature, water, mineral and pollutants.
- II. **Internal factors:** Pigments, protoplasmic factor, accumulation of carbohydrates, anatomy of leaf and hormones.

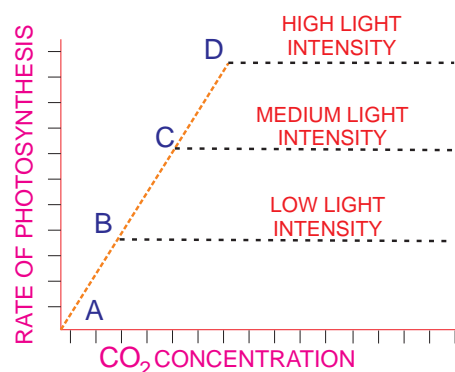


Figure 13.21: Blackman's Law of Limiting Factors

13.15.1. External factors

1. Light

Energy for photosynthesis comes only from light. Photooxidation of water and excitation of pigment molecules are directly controlled by light. Stomatal movement leading to diffusion of CO_2 is indirectly controlled by light.

a. Intensity of Light:

Intensity of light plays a direct role in the rate of photosynthesis. Under low intensity the photosynthetic rate is low and at higher intensity photosynthetic rate is higher. It also depends on the nature of plants. Heliophytes (Bean Plant) require higher intensity than Sciophytes (*Oxalis*).

b. Quantity of Light:

In plants which are exposed to light for longer duration (Long day Plants) photosynthetic rate is higher.

c. Quality of light:

Different wavelengths of light affect the rate of photosynthesis because pigment system does not absorb all the rays equally. Photosynthetic rate is maximum in blue and red light. **Photosynthetically Active Radiation (PAR)** is between 400 to 700 nm. Red light induces highest rate of photosynthesis and green light induces lowest rate of photosynthesis.

2. Carbon dioxide

CO_2 is found only 0.3 % in the atmosphere but plays a vital role. Increase in concentration of CO_2 increases the rate of photosynthesis (CO_2 concentration in the atmosphere is 330 ppm). If concentration is increased beyond 500ppm, rate of photosynthesis will be affected showing the inhibitory effect.

3. Oxygen

The rate of photosynthesis decreases when there is an increase of oxygen concentration. This Inhibitory effect of oxygen was first discovered by **Warburg** (1920) using green algae *Chlorella*.

4. Temperature

The optimum temperature for photosynthesis varies from plant to plant. Temperature is not uniform in all places. In general, the optimum temperature for photosynthesis is 25°C to 35°C . This is not applicable for all plants. The ideal temperature for plants like *Opuntia* is 55°C , Lichens 20°C and Algae growing in hot spring photosynthesis is 75°C . Whether high temperature or low temperature it will close the stomata as well as inactivate the enzymes responsible for photosynthesis (Figure 13.22).



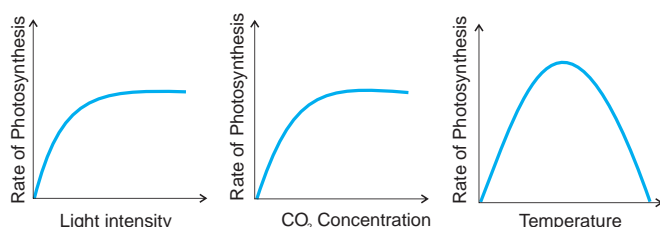


Figure 13.22: Factors affecting Photosynthesis

5. Water

Photolysis of water provides electrons and protons for the reduction of NADP, directly. Indirect roles are stomatal movement and hydration of protoplasm. During water stress, supply of NADPH + H⁺ is affected.

6. Minerals

Deficiency of certain minerals affect photosynthesis e.g. mineral involved in the synthesis of chlorophyll (Mg, Fe and N), Phosphorylation reactions (P), Photolysis of water (Mn and Cl), formation of plastocyanin (Cu).

7. Air pollutants

Pollutants like SO₂, NO₂, O₃ (Ozone) and Smog affects rate of photosynthesis.

13.15.2 Internal Factors

1. Photosynthetic Pigments

It is an essential factor and even a small quantity is enough to carry out photosynthesis.

2. Protoplasmic factor

Hydrated protoplasm is essential for photosynthesis. It also includes enzymes responsible for Photosynthesis.

3. Accumulation of Carbohydrates

Photosynthetic end products like carbohydrates are accumulated in cells and if translocation of carbohydrates is slow then this will affect the rate of photosynthesis.

4. Anatomy of leaf

Thickness of cuticle and epidermis, distribution of stomata, presence or absence of Kranz anatomy and relative proportion of photosynthetic cells affect photosynthesis.

5. Hormones

Hormones like gibberellins and cytokinin increase the rate of photosynthesis.

13.16 Photosynthesis in bacteria

Though we study about bacterial photosynthesis as the last part, bacterial photosynthesis formed first and foremost in evolution. Bacteria does not have specialized structures like chloroplast. It has a simple type of photosynthetic apparatus called chlorosomes and chromatophores (Table 13.6). Van Neil (1930) discovered a bacterium that releases sulphur instead of oxygen during photosynthesis. Here, electron donor is hydrogen sulphide (H₂S) and only one photosystem is involved (PS I) and the reaction centre is P₈₇₀. Pigments present in bacteria are bacteriochlorophyll a, b, c, d, e and g and carotenoids. Photosynthetic bacteria are classified into three groups:

1. Green sulphur bacteria. Example: *Chlorobacterium* and *Chlorobium*.

Table 13.6: Difference between photosynthesis in plants and photosynthesis in bacteria

Photosynthesis in Plants	Photosynthesis in Bacteria
1. Cyclic and non-cyclic phosphorylation takes place	1. Only cyclic phosphorylation takes place
2. Photosystem I and II involved	2. Photosystem I only involved
3. Electron donor is water	3. Electron donor is H ₂ S
4. Oxygen is evolved	4. Oxygen is not evolved
5. Reaction centres are P700 and P680	5. Reaction centre is P ₈₇₀
6. Reducing agent is NADPH + H ⁺	6. Reducing agent is NADH + H ⁺
7. PAR is 400 to 700 nm	7. PAR is above 700 nm
8. Chlorophyll, carotenoid and xanthophyll	8. Bacterio chlorophyll and bacterio viridin
9. Photosynthetic apparatus – chloroplast	9. It is chlorosomes and chromatophores



2. Purple sulphur bacteria. Example: *Thiospirillum* and *Chromatium*.
3. Purple non-sulphur bacteria. Example: *Rhodospseudomonas* and *Rhodospirillum*.

Test tube funnel experiment or Experiment to prove oxygen evolved during Photosynthesis

1. Place *Hydrilla* plant at the bottom of a beaker containing water.
 2. Cover the plant with an inverted funnel.
 3. Invert a test tube over the funnel.
 4. Keep this setup in sunlight.
- Note your observations (Figure 13.23).

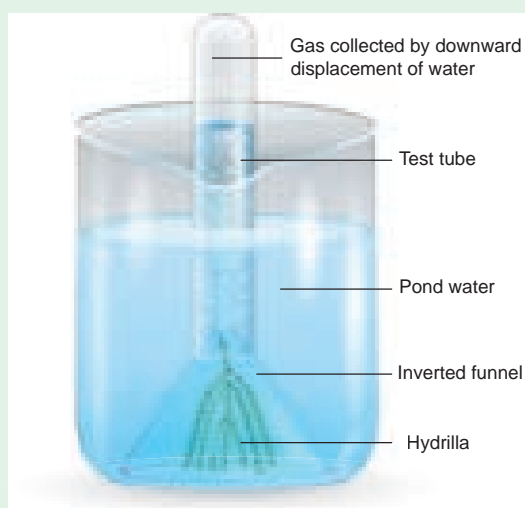


Figure 13.23: Test tube funnel experiment

Summary

Photosynthesis is an oxidation and reduction process. It has two phases: the light reaction and dark reaction. During light reaction water is oxidised to release O_2 and during dark reaction CO_2 is reduced to form sugars. Solar energy is trapped by pigment system I and pigment system II. P700 and P680 act as reaction centres for PS I and PS II respectively. Splitting of water molecule (Photolysis) produces electrons, protons and oxygen. Photophosphorylation takes place through cyclic and non-cyclic mechanisms and generates energy and reducing power. Dark reaction or biosynthetic phase of photosynthesis use the products of light energy (ATP and $NADPH + H^+$) and carbon dioxide is reduced to Carbohydrates. Carbon pathway in C_3 cycle has RUBP as

the acceptor molecule and the first product is PGA (3C). Carbon pathway in C_4 plants involves mesophyll and bundle sheath cells, Kranz anatomy. Dimorphic chloroplast, no photorespiration, acceptor molecule as PEP and first product as OAA (4C) are some of the unique characters of C_4 cycle. C_2 Cycle or photorespiration is operated when less amount of CO_2 is used for reduction and O_2 increases. Rubisco starts to play oxygenase role. Succulent and xerophytic plants show reverse stomatal rhythm as they open during night time and close during day time and follow CAM cycle. Night time produces malic acid and during day time malate is converted into pyruvate and produces CO_2 which is reduced to carbohydrates. Photosynthesis is affected by internal and external factors. Bacterial photosynthesis is the primitive type of photosynthesis and it involves only photosystem I.



Evaluation

1. **Assertion (A):** Increase in Proton gradient inside lumen responsible for ATP synthesis
Reason (R): Oxygen evolving complex of PS I located on thylakoid membrane facing Stroma, releases H^+ ions
 - a. Both Assertion and Reason are True.
 - b. Assertion is True and Reason is False.
 - c. Reason is True and Assertion is False.
 - d. Both Assertion and Reason are False.
2. Which chlorophyll molecule does not have a phytol tail?
 - a. Chl- a
 - b. Chl- b
 - c. Chl- c
 - d. Chl- d
3. The correct sequence of flow of electrons in the light reaction is
 - a. PS II, plastoquinone, cytochrome, PS I, ferredoxin.
 - b. PS I, plastoquinone, cytochrome, PS II ferredoxin.
 - c. PS II, ferredoxin, plastoquinone, cytochrome, PS I.
 - d. PS II, plastoquinone, cytochrome, PS II, ferredoxin.

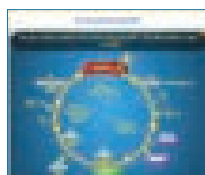
4. For every CO_2 molecule entering the C_3 cycle, the number of ATP & NADPH required
 - a. $2\text{ATP} + 2\text{NADPH}$
 - b. $2\text{ATP} + 3\text{NADPH}$
 - c. $3\text{ATP} + 2\text{NADPH}$
 - d. $3\text{ATP} + 3\text{NADPH}$
5. Identify true statement regarding light reaction of photosynthesis.
 - a. Splitting of water molecule is associated with PS I.
 - b. PS I and PS II involved in the formation of $\text{NADPH} + \text{H}^+$.
 - c. The reaction center of PS I is Chlorophyll a with absorption peak at 680 nm.
 - d. The reaction center of PS II is Chlorophyll a with absorption peak at 700 nm.
6. Two groups (A & B) of bean plants of similar size and same leaf area were placed in identical conditions. Group A was exposed to light of wavelength 400-450nm & Group B to light of wavelength of 500-550nm. Compare the photosynthetic rate of the 2 groups giving reasons.
7. A tree is believed to be releasing oxygen during night time. Do you believe the truthfulness of this statement? Justify your answer by giving reasons.
8. Grasses have an adaptive mechanism to compensate photorespiratory losses-Name and describe the mechanism.
9. In Botany class, teacher explains, Synthesis of one glucose requires 30 ATPs in C_4 plants and only 18 ATPs in C_3 plants. The same teacher explains C_4 plants are more advantageous than C_3 plants. Can you identify the reason for this contradiction?
10. When there is plenty of light and higher concentration of O_2 , what kind of pathway does the plant undergo? Analyse the reasons.



ICT Corner

Photosynthesis

Let's play
photosynthesis

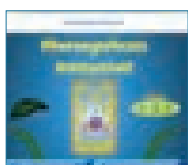


Steps

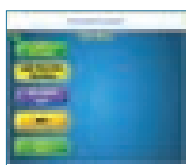
- Scan the QR code
- Start a new game and tap
- Click light dependent reaction and follow the steps
- After completion – move back and Click Calvin cycle reaction and follow the steps

Activity

- Observe the cycle and record it
- Check your grasp by click the Quiz tap
- Conclude your observations.



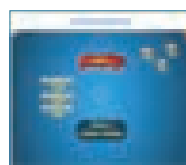
Step 1



Step 2



Step 3



Step 4



Web URL:

<https://biomanbio.com/HTML5GamesandLabs/PhotoRespgames/photointeractivehtml5page.html>

* Pictures are indicative only