Photosynthesis in Higher Plants

CONTENTS

3. PHOTOSYNTHESIS

3.1 Introduction

- All animals including human beings depend on plants for their food.
- Life on earth is solar powered Green plants carry out 'photosynthesis', a physico-chemical process by which they use light energy to drive the synthesis of organic compounds.
- Photosynthesis is important due to two reasons: it is the primary source of all food on earth. It is also responsible for the release of oxygen into the atmosphere by green plants.
- Photosynthesis is anabolic, endothermic and reductive process.
- All the carbon which enters into the organic compounds of the plant cells comes from the assimilation of CO_2 gas during the photosynthesis.
- The atmosphere contains about 0.03% CO_2 (2200 billion or 2.2×10^{13}).

3.2 Definition

"Photosynthesis (from the Greek word [photo-], "light," and [synthesis], "putting together.", "composition") is a process that converts CO_2 into organic compound especially sugars using the energy from sunlight."

Or

Photosynthesis can be defined as a "series of processes in which solar electromagnetic radiation energy (SEMR) is captured, converted and conserved in the form of chemical energy by green plants".

Or

Biochemically this process can also be defined "as light sensitized Red-Ox process" for it involves light induced Oxidation-Reduction processes.

3.3 History of Photosynthesis

- 320 B.C. - Greek philosophers **Aristotle** and **Theophrastus** were of the view that plants absorb their food directly from the soil.
- 1648 - **Jan Baptista van Helmont** (1579-1644), by his experiment on willow shoots that water, not the soil provided the plants with its material for growth.
- 1674 -**Malphigii** who studied the anatomy of plant with the help of microscope stated that together with water coming from soil, plants also require air to breathe and stomata function in this process.
- 1727 -**Stephen Hales** was the first to mention that air and light are the factors for plant growth.
- 1772 - **Joseph Priestley** (1733-1804) in his interesting experiment in which a plant and a mice when kept inside separate air tight bell jars died shortly, but they survived if kept together in the same sealed chamber. The gas released by plants was called by him 'dephlogisticated air.' This was the term he used for oxygen which is one of the by-products of photosynthesis.
- 1779 - **Ingen Housz** performed a number of experiments and was the first to recognize the importance of light and chlorophyll.
- 1782 – **Senebier** was the first to classify the fact that fixed air CO_2, was an important factor in photosynthesis.
- 1783 – **Lavoisier** identified the gas released by plants as oxygen and the one produced by burning candle as carbon dioxide.
- 1804 – **de Saussure** stated that plants cannot live without CO_2 and O_2 he discovered that water also participates in photosynthesis.

- 1805 – **Julius Von Mayer** recognized that plants absorb light energy and convert it into chemical energy which is stored in organic compound.
- 1818 – **Pelletier** and **Caventon** gave the name 'chlorophyll' to the green chemical substance which could be extracted from plants by boiling in alcohol.
- 1837 – **Von Mohl** was first to describe the presence of chloroplast in plant tissue.
- 1845 – **Liebig** confirmed the inorganic salts (and not the organic matter) present in the soil were necessary for the growth of the plant.
- 1862 – **Sach** stated the product of photosynthesis is starch. He demonstrated the relationship between starch, chloroplast and photosynthesis.
- 1864 – **Stokes** purified chlorophyll and gave the names chlorophyll a, and chlorophyll b. He described the involvement of pigment, carotene and xanthophyll and fucoxanthin in brown algae in photosynthesis.
- 1888 – **Engelman** discovered the action spectrum of photosynthesis.
- 1913 - **Van Neil** concluded that in photosynthesis oxygen was released out of water and not from CO2
- 1905 – **Blackman** established the law of limiting factor and studied the interaction of different factors of photosynthesis.
- 1913 – **Richard Willstatter** confirmatively established the chemistry of chlorophyll in 1913 and another on photosynthetic process in 1918.
- 1920 – **Warburg** conducted photosynthetic experiment on unicellular alga, *Chlorella* and improved measuring methods.
- 1932 – **Emerson** and **Arnold** showed the existence of light and dark reactions through their flashing light experiments.
- 1937 - **Robin Hill** isolated chloroplast suspended in water in presence of a suitable hydrogen acceptor (e.g. ferricyanide, coenzyme NADP) which evolves oxygen in presence of light. He demonstrated that the source of O2 evolved during photosynthesis is water (photolysis of water) and not CO2 as was believed earlier.
- 1941 – **Ruben** and **Kamen** used radioactive ^{18}Oxygen and proved that oxygen evolved was part of water.
- 1954 - **Arnon, Allen and Whatley** by centrifugation separated the grana portion of chloroplast from stroma portion.
- 1954 – **Calvin** traced the path of carbon in photosynthesis using unicellular alga Chlorella . **Melvin Calvin** gave C_3 cycle and were awarded Nobel prize in 1960 for the discovery.
- 1961 – **Park** and **Biggins** discovered quantasome (180Å x 160 Å) 100 Å thick and stated that it contains about 230 chlorophyll molecules.
- 1965 – **Hatch and Slack** discovered C_4 cycle for CO2 fixation in certain tropical plants.

Photosynthesis is a 2-step process

- FIRST STEP: turns light energy into chemical energy (light-dependent reactions")
- SECOND STEP: uses that chemical energy to "fix" atmospheric carbon into sugar ("light-independent reactions, or the "Calvin Cycle")

3.4 Site of Photosynthesis (Where does photosynthesis take place)

Photosynthesis takes place in chloroplasts. (These are mainly plastids located in cells)

- In higher plants these are located primarily in leaf cells.
- There are about half a million chloroplasts per square millimeter of leaf surface.
- In chloroplast there is green pigment, **chlorophyll** along with some pigments.
- Chlorophyll plays an important role in the absorption of light energy during photosynthesis.

- Chloroplasts are found mainly in **mesophyll** cells forming the tissues in the interior of the leaf.
- O_2 exits and CO_2 enters the leaf through microscopic pores called **stomata** in the leaf.
- Veins deliver water from the roots and carry off sugar from mesophyll cells to nonphotosynthetic areas of the plant.

3.4.1 Major photosynthetic pigments

- Pigments are substances that have an ability to absorb light, at specific wavelengths.
- They are pigments located in thylakoid membranes that take part in harvesting light energy for photosynthesis.
- Chloroplasts have two types of photosynthetic pigments-Chlorophylls and Carotenoids.
- Bacteria possess Bacterio-chlorophylls instead of chlorophylls.
- Phycobillins occur in some algae.
- The ability of chlorophyll a pigment to absorb light of different wavelength is maximum.
- The wavelength at which maximum photosynthesis occurs in a plant.
- The wave length at which there is maximum absorption by chlorophyll 'a', i.e., in blue and red regions, also show higher rate of photosynthesis.
- Hence we can conclude that Chlorophyll 'a' is the chief pigment associated with photosynthesis.
- Though chlorophyll is the major pigment responsible for trapping light, other thylakoid pigments like Chlorophyll 'b', Xanthophylls and Carotenoids, which are called accessory pigments, also absorb light and transfer the energy to chlorophyll 'a'.
- Indeed they not only enable a wider range of wavelength of incoming light to be utilised for photosynthesis but also protect Chlorophyll 'a' from photo-oxidation.

3.4.1.1 Chlorophylls

- Chlorophylls are green in colour.
- Chlorophylls are of five types –Chlorophyll a, Chlorophyll b, Chlorophyll c, Chlorophyll d, Chlorophyll e.
- All the Chlorophylls are soluble in organic solvents like ether, acetone, carbon disulphide, benzol, etc.
- Chlorophyll 'a' (bright or blue green in the chromatogram)
  - It has empirical formula of $C_{55}H_{72}O_5N_4Mg$ and molecular weight of 893.
  - It is bluish-green in colour.
  - It occurs in all oxygenic photosynthetic organisms.
  - Therefore, it is called universal photosynthetic pigment.
  - Chlorophyll 'a' is also involved in photoconversion or primary reaction of photosynthesis in which light energy is changed into chemical energy. So it is also called primary photosynthetic pigment.
- Chlorophyll 'b ' (yellow green)
  - It has empirical formula of $C_{55}H_{70}O6_5N_4Mg$ and molecular weight of 907.
  - It is Olive-green in colour.
  - It occurs in along with Chlorophyll a in all green plants including green algae and Euglenophyceae.
  - It is more soluble in 90% methyl alcohol.
- Chlorophyll 'c'
  - It has empirical formula of $C_{35}H_{32}O_6N_4Mg$ and molecular weight of 613.
  - It occurs in brown algae, diatoms and dinoflagellates.
  - It is more soluble in aqueous solution.
- Chlorophyll 'd '
  - It has empirical formula of $C_{54}H_{70}O6_5N_4Mg$ and molecular weight of 895.

- It occurs in red algae.
- It is more soluble in both polar and non-polar alcohols.

- Chlorophyll 'e '
  - It is not properly characterised.
  - It occurs in Xanthophyceae along with Chlorophyll 'a'.

3.4.1.2 Carotenoids

- Carotenoids are a group of yellow and orange coloured lipid soluble pigments which occur in chloroplasts as well as specialised plastids called chromoplasts.
- They are tetraterpenes and bear conjugate double bonds.
- The ends may be open or bear cyclic groups.
- Carotenoids are accessory photosynthetic pigments.
- They absorb radiations in the middle part of light spectrum.
- They provide colouration to many fruits and flowers.
- Carotenoids are accessory pigments absorbing light in middle part of spectrum.
- Carotenoids are of two types- carotenes and Xanthophylls.

❖ **Carotenes (orange to orange-red)**

- It has empirical formula of $C_{40}H_{56}$.
- It is orange or orange-red pigments in colour.
- β- Carotene is the most common carotene.
- It's both end are cyclised.
- Lycopene is carotene found in tomato fruit. It has open ends.
- Other less common carotenes are α–carotene and γ–carotene absorbs light mostly between 400 to 500 nm.
- β- Carotene is a source of vitamin A.
- Carotenes protect chlorophylls from destruction by oxygen radicals by absorbing the same and forming xanthophylls.
- In human beings and other animals it is converted to vitamin A.

$$C_{40}H_{56} + 2H_2O \rightarrow 2C_{19}H_{27}CH_{2O}H$$

Vitamin A

❖ **Xanthophylls (yellow)**

- It has empirical formula of $C_{40}H_{56}O_x$.
- It is yellow in colour.
- The number of oxygen atoms may be 1 to 8.
- Lutein and zeaxanthin (both $C_{40}H_{56}O_2$) are hydroxylated forms of α–carotene and γ–carotene.
- Autumnal colouration of leaves is mostly due to xanthophylls.
- They are little soluble in carbon disulphide in which carotenes are quite soluble.
- Absorption spectrum is similar to β-Carotene but extends on both sides , i.e., below 400 nm and above 500 nm.
- A xanthophyll cycle seems to operate for photoprotection of chloroplast constituents.
- Dissipation of excess energy by changing violoxanthin into zeaxanthin.
- The reverse reaction occurs under low light conditions as well as in darkness.

3.4.1.3 Phycobillins (water soluble)

- Phycobillins constitute a major group of photosynthetic pigments occuring in blue-green and red algae.
- These consist of an open conjugated system of four pyrrol rings and lack Mg and the phytol chain.
- They are red Phycoerythrin or blue Phycocyanin and allophycocyanin in colour.
- They are water soluble and heat labile.

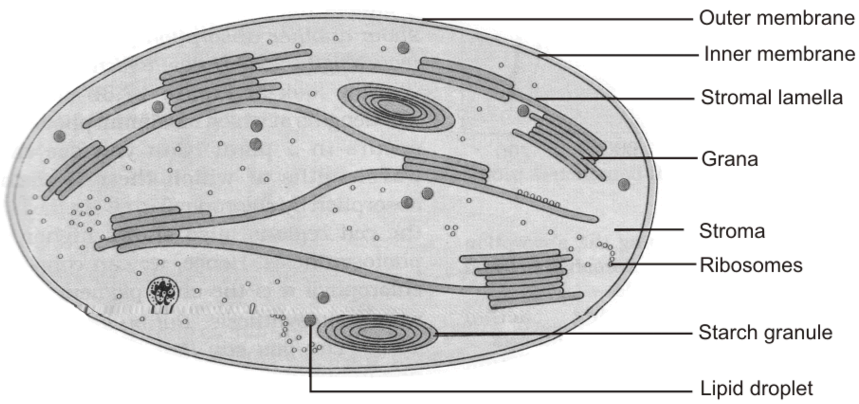
- They are covalently linked to proteins to form complexes called billiproteins.
- They are attached to thylakoids.
- Phycobillins have two main functions-
  - ❖ Absorption of light at different wavelength and handling over the same to chlorophylls
  - ❖ Chromatic adaptation.

3.4.2 Distribution of Photosynthetic Pigments

	Pigment	Distribution in Plants
(a)	Chlorophyll	
1	Chlorophyll a	All plants except bacteria
2	Chlorophyll b	Angiosperms and Algae
3	Chlorophyll c	Diatoms and Brown Algae
4	Chlorophyll d	Red Algae
5	Chlorophyll e	Xanthophyta
6	Bacterio-chlorophyll - a	Purple and Green Bacteria
7	Bacterio-chlorophyll - b	Rhodopseudomonas
8	Bacterioviridin	Chlorobium
(b)	Phycobillins	
	Phycoerythrin	Blue green Algae and Red Algae
	Phycocyanin	Blue green Algae and Red Algae
(c)	Carotenoids	
	Carotene	Mostly in Algae and Higher Plants
	Xanthophyll	Mostly in Algae and Higher Plants

3.4.3 Structural organization of Chloroplast

- In a typical mesophyll cell there are 30–40 chloroplasts, each about 2–4 microns by 4–7 microns long.
- These are lens-shaped, oval, spherical, discoid or even ribbon-like organelles having variable length (5-10mm) and width (2-4mm).
- Each chloroplast has two membranes around a central aqueous space, the **stroma.**
- In the stroma is an elaborate system of interconnected membranous sacs, the **thylakoids.**
  - The interior of the thylakoids forms another compartment, the *thylakoid space.*
  - Thylakoids may be stacked into columns called *grana.*
- Chlorophyll the main light absorbing pigment of chloroplasts is located in the thylakoids.
- Photosynthetic prokaryotes like lack chloroplasts.
  - Their photosynthetic membranes arise from infolded regions of the plasma membranes, folded in a manner similar to the thylakoid membranes of chloroplasts.



Diagrammatic representation of an electron micrograph of a section ofChloroplast

3.5 Mechanism of Photosynthesis [Photochemical and biosynthetic phases]-

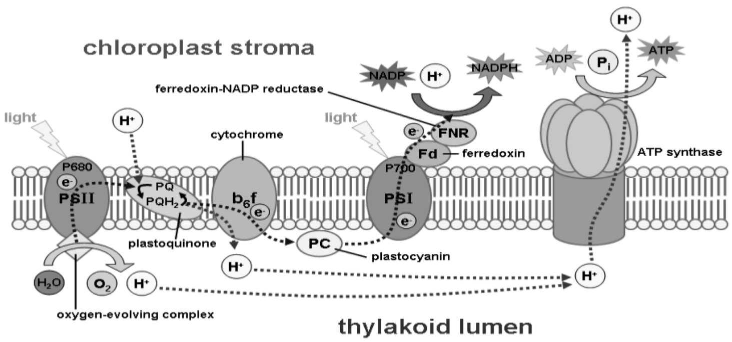
- Photosynthesis is two processes, each with multiple stages. Warburg (1919) and Arnon (1954) studied the mechanism of photosynthesis and concluded that it occurs in two steps-
    - Light reaction (Photochemical phase)
    - Dark reaction (Biosynthesis phase)
        - The **light reactions** *(photo)* convert solar energy to chemical energy. The **Calvin cycle** *(synthesis)* uses energy from the light reactions to incorporate CO_2 from the atmosphere into sugar.
        - In the light reactions, light energy absorbed by chlorophyll in the thylakoids drives the transfer of electrons and hydrogen from water to **$NADP^+$** (nicotinamide adenine dinucleotide phosphate), forming NADPH.
        - NADPH, an electron acceptor, provides reducing power via energized electrons to the Calvin cycle.
        - Water is split in the process, and O_2 is released as a by-product.
        - The light reaction also generates ATP using chemiosmosis, in a process called **photophosphorylation.**
        - Thus light energy is initially converted to chemical energy in the form of two compounds: NADPH and ATP.
        - The Calvin cycle is named for Melvin Calvin who, with his colleagues, worked out many of its steps in the 1940s.
        - The cycle begins with the incorporation of CO_2 into organic molecules, a process known as **carbon fixation.**
        - The fixed carbon is reduced with electrons provided by NADPH.
        - ATP from the light reactions also powers parts of the Calvin cycle.
        - Thus, it is the Calvin cycle that makes sugar, but only with the help of ATP and NADPH from the light reactions.
        - The metabolic steps of the Calvin cycle are sometimes referred to as the light-independent reactions, because none of the steps requires light *directly*
        - Nevertheless, the Calvin cycle in most plants occurs during daylight, because that is when the light reactions can provide the NADPH and ATP the Calvin cycle requires.

- While the light reactions occur at the thylakoids, the Calvin cycle occurs in the stroma.

3.5.1 Diversity in photosynthetic pathways

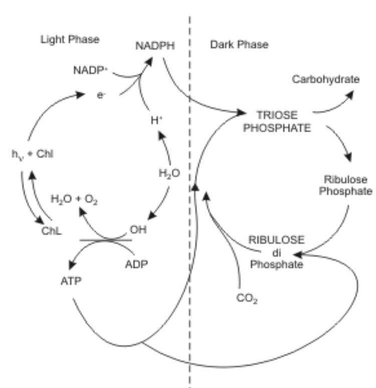
3.5.2 Light Reaction/Hill's Reaction

- Light reactions or the 'Photochemical' phase include light absorption, water splitting, oxygen release, and the formation of high-energy chemical intermediates, ATP and NADPH.



Light-dependent reactions of photosynthesis at the thylakoid membrane

- In light reaction, solar energy is trapped by chlorophyll and stored as chemical energy of ATP and as reducing power in NADPH.
- When a chlorophyll molecule absorbs photon of light; the chlorophyll molecule is raised from a ground state to an excited state. This results in ejection of an electron. This is called primary photochemical reaction.
- The light absorbing pigments are located in thylakoids membranes. These include chlorophyll molecules P680 and P700 which form the reaction centres of photo centres. The accessory pigments harvest solar energy and pass them over to light harvesting molecules which absorb light energy and transmit it over to reaction centre where the photochemical act occurs.



Schematic representation of interrelations between two phases of photosynthesis

3.5.3 Absorption of Light Energy/Red drop or EMERSON EFFECT [Photosynthetic electron transport]

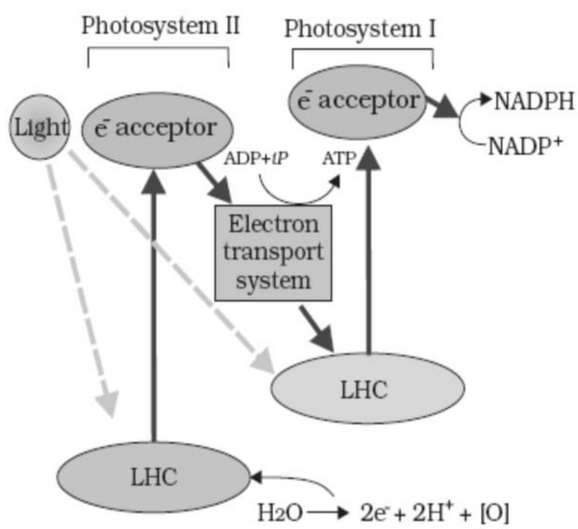
- The thylakoids convert light energy into the chemical energy of ATP and NADPH.
- Light is a form of electromagnetic radiation.

- Like other forms of electromagnetic energy, light travels in rhythmic waves.
- The distance between crests of electromagnetic waves is called the **wavelength.**
  - Wavelengths of electromagnetic radiation range from less than a nanometer (gamma rays) to more than a kilometer (radio waves).
- The entire range of electromagnetic radiation is the **electromagnetic spectrum.**
- The most important segment for life is a narrow band between 380 to 750 nm, the band of **visible light.**
- While light travels as a wave, many of its properties are those of a discrete particle, the **photon.**
  - Photons are not tangible objects, but they do have fixed quantities of energy.
- The amount of energy packaged in a photon is inversely related to its wavelength.
  - Photons with shorter wavelengths pack more energy.
- While the sun radiates a full electromagnetic spectrum, the atmosphere selectively screens out most wavelengths, permitting only visible light to pass in significant quantities.
  - Visible light is the radiation that drives photosynthesis.
- When light meets matter, it may be reflected, transmitted, or absorbed.
  - Different pigments absorb photons of different wavelengths, and the wavelengths that are absorbed disappear.
  - A leaf looks green because chlorophyll, the dominant pigment, absorbs red and blue light, while transmitting and reflecting green light.
- A **spectrophotometer** measures the ability of a pigment to absorb various wavelengths of light.
  - It beams narrow wavelengths of light through a solution containing the pigment and measures the fraction of light transmitted at each wavelength.
  - An **absorption spectrum** plots a pigment's light absorption versus wavelength.
- The light reaction can perform work with those wavelengths of light that are absorbed.
- There are several pigments in the thylakoid that differ in their absorption spectra.
  - **Chlorophyll *a*,** the dominant pigment, absorbs best in the red and violet-blue wavelengths and least in the green.
  - Other pigments with different structures have different absorption spectra.
- Collectively, these photosynthetic pigments determine an overall **action spectrum** for photosynthesis.
  - An action spectrum measures changes in some measure of photosynthetic activity (for example, O_2 release) as the wavelength is varied.
- The action spectrum of photosynthesis was first demonstrated in 1883 in an elegant experiment performed by Thomas Engelmann.
  - In this experiment, different segments of a filamentous alga were exposed to different wavelengths of light.
  - Areas receiving wavelengths favorable to photosynthesis produced excess O_2.
  - Engelmann used the abundance of aerobic bacteria that clustered along the alga at different segments as a measure of O_2 production.

- The action spectrum of photosynthesis does not match exactly the absorption spectrum of any one photosynthetic pigment, including chlorophyll *a*.
- Only chlorophyll *a* participates directly in the light reaction, but accessory photosynthetic pigments absorb light and transfer energy to chlorophyll *a*.
    - **Chlorophyll *b*,** with a slightly different structure than chlorophyll *a*, has a slightly different absorption spectrum and funnels the energy from these wavelengths to chlorophyll *a*.
    - **Carotenoids** can funnel the energy from other wavelengths to chlorophyll *a* and also participate in *photoprotection* against excessive light.
    - These compounds absorb and dissipate excessive light energy that would otherwise damage chlorophyll.
    - They also interact with oxygen to form reactive oxidative molecules that could damage the cell.
- When a molecule absorbs a photon, one of that molecule's electrons is elevated to an orbital with more potential energy.
    - The electron moves from its ground state to an excited state.
    - The only photons that a molecule can absorb are those whose energy matches exactly the energy difference between the ground state and excited state of this electron.
    - Because this energy difference varies among atoms and molecules, a particular compound absorbs only photons corresponding to specific wavelengths.
    - Thus, each pigment has a unique absorption spectrum.
- Excited electrons are unstable.
- Generally, they drop to their ground state in a billionth of a second, releasing heat energy.
- Some pigments, including chlorophyll, can also release a photon of light in a process called fluorescence.
    - If a solution of chlorophyll isolated from chloroplasts is illuminated, it will fluoresce and give off heat.
- Chlorophyll excited by absorption of light energy produces very different results in an intact chloroplast than it does in isolation.
- In the thylakoid membrane, chlorophyll is organized along with proteins and smaller organic molecules into **photosystems.**
- A photosystem is composed of a reaction center surrounded by a light-harvesting complex.
- Each **light-harvesting complex** consists of pigment molecules (which may include chlorophyll *a*, chlorophyll *b*, and carotenoid molecules) bound to particular proteins.
- Together, these light-harvesting complexes act like light-gathering "antenna complexes" for the reaction center.
- When any antenna molecule absorbs a photon, it is transmitted from molecule to molecule until it reaches a particular chlorophyll *a* molecule, the **reaction center.**
- At the reaction center is a **primary electron acceptor,** which accepts an excited electron from the reaction center chlorophyll *a*.

- The solar-powered transfer of an electron from a special chlorophyll *a* molecule to the primary electron acceptor is the first step of the light reactions.

- Each photosystem—reaction-center chlorophyll and primary electron acceptor surrounded by an antenna complex—functions in the chloroplast as a light-harvesting unit.
- There are two types of photosystems in the thylakoid membrane.
    - **Photosystem I (PS I)** has a reaction center chlorophyll *a* that has an absorption peak at 700 nm.
    - **Photosystem II (PS II)** has a reaction center chlorophyll *a* that has an absorption peak at 680 nm.
    - The differences between these reaction centers (and their absorption spectra) lie not in the chlorophyll molecules, but in the proteins associated with each reaction center.
    - These two photosystems work together to use light energy to generate ATP and NADPH.

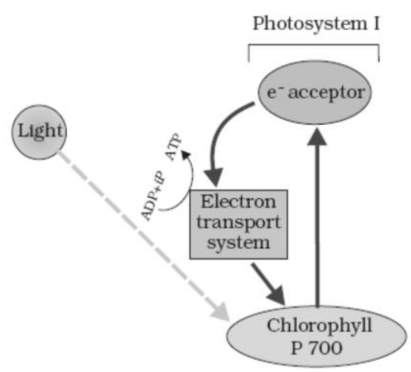


Z Scheme of Light Reaction

- During the light reactions, there are two possible routes for electron flow: cyclic and noncyclic.
- **Noncyclic electron flow,** the predominant route, produces both ATP and NADPH.
    - Photosystem II absorbs a photon of light. One of the electrons of P680 is excited to a higher energy state.
    - This electron is captured by the primary electron acceptor, leaving the reaction center oxidized.
    - An enzyme extracts electrons from water and supplies them to the oxidized reaction center. This reaction splits water into two hydrogen ions and an oxygen atom that combines with another oxygen atom to form O2.
    - Photoexcited electrons pass along an electron transport chain before ending up at an oxidized photosystem I reaction center.

- As these electrons "fall" to a lower energy level, their energy is harnessed to produce ATP.
- Meanwhile, light energy has excited an electron of PS I's P700 reaction center. The photoexcited electron was captured by PS I's primary electron acceptor, creating an electron "hole" in P700. This hole is filled by an electron that reaches the bottom of the electron transport chain from PS II.
- Photoexcited electrons are passed from PS I's primary electron acceptor down a second electron transport chain through the protein ferredoxin (Fd).
- The enzyme $NADP^+$ reductase transfers electrons from Fd to $NADP^+$. Two electrons are required for $NADP^+$'s reduction to NADPH. NADPH will carry the reducing power of these high-energy electrons to the Calvin cycle.

- The light reactions use the solar power of photons absorbed by both photosystem I and photosystem II to provide chemical energy in the form of ATP and reducing power in the form of the electrons carried by NADPH.
- Under certain conditions, photoexcited electrons from photosystem I, but not photosystem II, can take an alternative pathway, **cyclic electron flow.**
  - Excited electrons cycle from their reaction center to a primary acceptor, along an electron transport chain, and return to the oxidized P700 chlorophyll.
  - As electrons flow along the electron transport chain, they generate ATP by **cyclic photophosphorylation.**
  - There is no production of NADPH and no release of oxygen.



Cyclic Phosphorylation

- Noncyclic electron flow produces ATP and NADPH in roughly equal quantities.
- However, the Calvin cycle consumes more ATP than NADPH.
- Cyclic electron flow allows the chloroplast to generate enough surplus ATP to satisfy the higher demand for ATP in the Calvin cycle.
- Chloroplasts and mitochondria generate ATP by the same mechanism: chemiosmosis.
  - In both organelles, an electron transport chain pumps protons across a membrane as electrons are passed along a series of increasingly electronegative carriers.
  - This transforms redox energy to a proton-motive force in the form of an H^+ gradient across the membrane.
  - ATP synthase molecules harness the proton-motive force to generate ATP as H^+ diffuses back across the membrane.
- Some of the electron carriers, including the cytochromes, are very similar in chloroplasts and mitochondria.

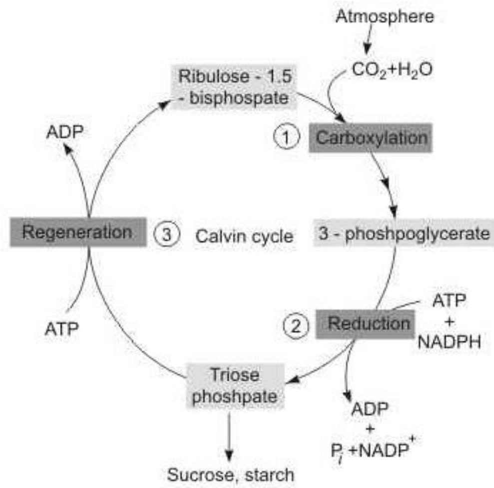
- The ATP synthase complexes of the two organelles are also very similar.
- There are differences between oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts.
- Mitochondria transfer chemical energy from food molecules to ATP; chloroplasts transform light energy into the chemical energy of ATP.
- The spatial organization of chemiosmosis also differs in the two organelles.
- The inner membrane of the mitochondrion pumps protons from the mitochondrial matrix out to the intermembrane space. The thylakoid membrane of the chloroplast pumps protons from the stroma into the thylakoid space inside the thylakoid.
- The thylakoid membrane makes ATP as the hydrogen ions diffuse down their concentration gradient from the thylakoid space back to the stroma through ATP synthase complexes, whose catalytic knobs are on the stroma side of the membrane.
- The proton gradient, or pH gradient, across the thylakoid membrane is substantial.
    - When chloroplasts are illuminated, the pH in the thylakoid space drops to about 5 and the pH in the stroma increases to about 8, a thousand fold different in H^+ concentration.
- The light-reaction "machinery" produces ATP and NADPH on the stroma side of the thylakoid.
- Noncyclic electron flow pushes electrons from water, where they have low potential energy, to NADPH, where they have high potential energy.
    - This process also produces ATP and oxygen as a by-product.

3.5.5 Difference between Cyclic and Non-cyclic Photophosphorylation

	Cyclic Photophosphorylation	**Non-cyclic Photophosphorylation**
1	It is related to photoact I.	It is related to both photoact I and II.
2	Electrons released from chlorophyll return back.	The electrons released from chlorophyll are never drained back to it but are replaced by electrons from OH^- ions originating in the dissociation of water.
3	There is no photolysis of water and release of oxygen.	Oxygen is released during photolysis of water.
4	NADP is not oxidized.	NADP is oxidized to NADPH.
5	Photophosphorylation takes place at two places.	Photophosphorylation takes place at one place.

3.5.6 Dark Reaction- Calvin cycle

- The Calvin cycle regenerates its starting material after molecules enter and leave the cycle.
- The Calvin cycle is anabolic, using energy to build sugar from smaller molecules.
- Carbon enters the cycle as CO_2 and leaves as sugar.
- The cycle spends the energy of ATP and the reducing power of electrons carried by NADPH to make sugar.
- The actual sugar product of the Calvin cycle is not glucose, but a three-carbon sugar, **glyceraldehyde-3-phosphate (G3P).**
- Each turn of the Calvin cycle fixes one carbon.
- For the net synthesis of one G3P molecule, the cycle must take place three times, fixing three molecules of CO_2.
- To make one glucose molecule requires six cycles and the fixation of six CO_2 molecules.
- The Calvin cycle has three phases.



The Calvin Cycle

Phase 1: Carbon fixation-[Carboxylation]

- In the **carbon fixation** phase, each CO_2 molecule is attached to a five-carbon sugar, Ribulose bisphosphate (RuBP).
    - This is catalyzed by RuBP carboxylase or **rubisco.**
    - Rubisco is the most abundant protein in chloroplasts and probably the most abundant protein on Earth.
    - The six-carbon intermediate is unstable and splits in half to form two molecules of 3-phosphoglycerate for each CO_2.

Phase 2: Reduction-Glycolytic reversal

- During **reduction,** each 3-phosphoglycerate receives another phosphate group from ATP to form 1,3-bisphosphoglycerate.
- A pair of electrons from NADPH reduces each 1,3-bisphosphoglycerate to G3P.
    - The electrons reduce a carboxyl group to the aldehyde group of G3P, which stores more potential energy.
- If our goal was the net production of one G3P, we would start with $3CO_2$ (3C) and three RuBP (15C).
- After fixation and reduction, we would have six molecules of G3P (18C).
    - One of these six G3P (3C) is a net gain of carbohydrate.
        - This molecule can exit the cycle and be used by the plant cell.

Phase 3: Regeneration-Regeneration of RuBP

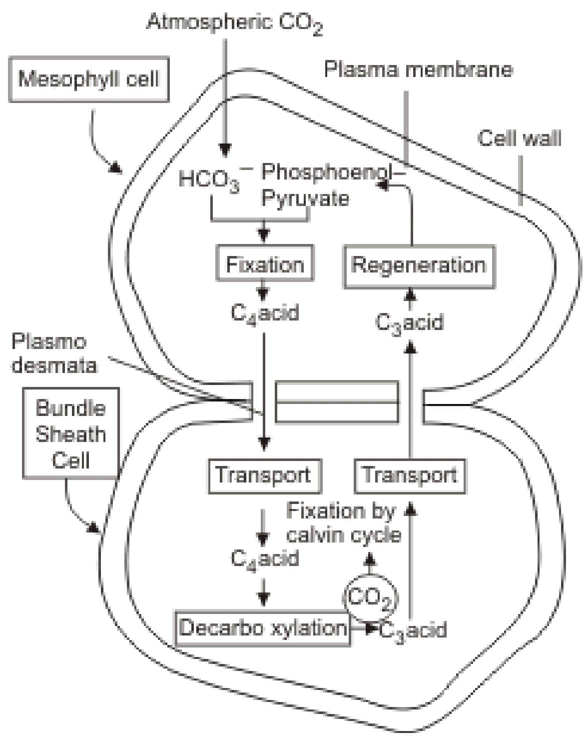
- The other five G3P (15C) remain in the cycle to **regenerate** three RuBP. In a complex series of reactions, the carbon skeletons of five molecules of G3P are rearranged by the last steps of the Calvin cycle to regenerate three molecules of RuBP.
- For the net synthesis of one G3P molecule, the Calvin cycle consumes nine ATP and six NADPH.
- The light reactions regenerate ATP and NADPH.
- The G3P from the Calvin cycle is the starting material for metabolic pathways that synthesize other organic compounds, including glucose and other carbohydrates.
- Hence for every CO_2 molecule entering the Calvin cycle, 3 molecules of ATP and 2 of NADPH are required.
- It is probably to meet this difference in number of ATP and NADPH used in the dark reaction that the cyclic phosphorylation takes place.

- To make one molecule of glucose 6 turns of the cycle are required.

IN	OUT
Six CO_2	One Glucose
18 ATP	18 ADP
12 NADPH	12NADP

3.5.7 Hatch and Slack pathway

- This pathway that has been named the Hatch and Slack Pathway is again a cyclic process.
- The pathway has following steps-
    - The primary CO_2 acceptor is a 3-carbon molecule Phosphoenol pyruvate (PEP).
    - The enzyme responsible for this fixation is PEP carboxylase or PEPcase.
    - It is important to register that the mesophyll cells lack RuBisCO enzyme.
    - The C_4 acid OAA is formed in the mesophyll cells.
    - It then forms other 4-carbon compounds like malic acid or aspartic acid in the mesophyll cells itself, which are transported to the bundle sheath cells.
    - In the bundle sheath cells these C_4 acids are broken down release CO_2 and a 3-carbon molecule.
    - The 3-carbon molecule is transported back to the mesophyll where it is converted to PEP again, thus, completing the cycle.
    - The CO_2 released in the bundle sheath cells enters the C_3 or the Calvin pathway, a pathway common to all plants.
    - The bundle sheath cells are rich in an enzyme Ribulose biphosphate carboxylase-oxygenase (RuBisCO), but lack PEPcase.
    - Thus, the basic pathway that results in the formation of the sugars, the Calvin pathway is common to the C_3 and C_4 plants.



Hatch and Slack Pathway

3.5.7.1 Significance of Hatch and Slack pathway

- This pathway is found in sugarcane, maize, sorghum and other graminaceous plants and some dicot and in some succulents where CO_2 fixation occurs.

3.5.8 Difference between C_3 and C_4 Plants

C_3 Plants	C_4 Plants
Algae (*Chlorella*), sunflower, Beans, Mustard , etc.	Maize, sorghum, sugarcane and other graminaceous plants.
Plants have Calvin cycle.	Plants have Hatch and Slack cycle.
The first stable product of photosynthesis is 3-C compound, Phosphoglyceric acid(PGA).	The first stable product of photosynthesis is 4-C compound, Oxaloacetic acid or malic acid succulents.
There is only one CO_2 acceptor example-ribulose diphosphate.	There are two CO_2 acceptor e.g., Phosphoenol pyruvic acid and RuBP.
Kranz anatomy is absent.	The leaves have Kranz type of anatomy.
The optimum temperature lies between 10-25˚C.	The optimum temperature lies between 35-45˚C.
Photorespiration occurs which reduces the photosynthetic yield.	No Photorespiration.
Chloroplast has PS I and PS II.	Chloroplast lack photosystem II, it therefore, depends upon mesophyll for supply of $NADPH^+$ H^+.

3.5.9 Blackman's Law of Limiting Factor

- It is based on Liebig's law of minimum. It was proposed by Blackman (1905).
- According to him, when a process is conditioned as to its rapidity by a number of separate factors, its rate is limited by the pace of the slowest factor. In other words, at one time only one factor (limiting factor) limits the rate of the process.
- A limiting factor is that factor which is deficient to such an extent that increase in its value directly increases the rate of the process.

3.6 Factors affecting Photosynthesis

- The rate of photosynthesis is under the influence of several factors, both internal (plant) and external.
- The plant factor includes the number, size, age and orientation of leaves, mesophyll cells and chloroplasts, internal CO_2 concentration and the amount of chlorophyll.
- The plant or internal factors are dependent on the genetic predisposition and the growth of the plant.
- The external factors would include the availability of sunlight, temperature, CO_2 concentration and water.
- As a plant photosynthesises, all these factors will simultaneously affect its rate.
- Hence, though several factors interact and simultaneously affect photosynthesis or CO_2 fixation, usually one factor is the major cause or is the one that limits the rate.
- Hence, at any point the rate will be determined by the factor available at sub-optimal levels.
- When several factors affect any bio-chemical process, Blackman's (1905) Law of Limiting Factors comes into effect.
- This states the following- If a chemical process is affected by more than one factor, then

its rate will be determined by the factor which is nearest to its minimal value: it is the factor which directly affects the process if its quantity is changed.

- Example, despite the presence of a green leaf and optimal light and CO_2 conditions, the plant may not photosynthesises if the temperature is very low.
- This leaf, if given the optimal temperature, will start photosynthesizing.

3.6.1 External Factors

3.6.1.1 Light

- Light provides energy for photosynthesis. Plants obtain light energy from the natural source, the sun.

  (a) Light Quality- Photosynthesis is successfully accomplished only in the visible light (3800 Åwavelength-7600 Å wavelength) of spectrum. Rate of photosynthesis is maximum in red light, average in blue light and minimum in green light.

  (b) Light Intensity-Chlorophyll utilizes only 3% of the total light absorbed. Photosynthesis initiates at very low intensity and the rate of the process increases with the increase in light intensity. Light intensity decreases the rate of photosynthesis.

  (c) Light Duration- Photosynthesis is significantly affected by the duration of light. Long continuous periods of light increase the rate of photosynthesis.

3.6.1.2 CARBON DIOXIDE (CO_2)

- Generally 0.03% CO_2 is present in the atmosphere.
- As the CO_2 concentration increases upto 0.9%, the rate of photosynthesis also increases. But the concentration of CO_2 above 0.9% is harmful and decreases the rate of photosynthesis.
- Under normal conditions of light, temperature and CO_2 act as a limiting factor for photosynthesis.

3.6.1.3 Temperature

- Under favorable conditions of light and carbon dioxide, photosynthesis increases with rise of temperature till it becomes maximum 25-30˚C which is the optimum temperature for photosynthesis.
- In *Opuntia*, it takes place even at 55˚C.
- In Lichens - 20˚C, and in Conifers-35˚C are the initiating temperatures or minimum temperatures for photosynthesis.
- The effect of temperature varies from plant to plant.
- C_4 plants have higher optimum temperature than C_3 plants.

3.6.1.4 Water

- Of the total water absorbed by the plant approximately 1.0% is used in photosynthesis that is why water usually does not become a limiting factor directly.
- According to some scientist in water deficient soil there is approximately, 87% reduction in the rate of photosynthesis.

- The reason for this reduction is that due to deficiency of water within soil a deficiency of water is caused in cytoplasm and the stomata get closed due to which CO_2 does not enter into the plants.
- Also due to deficiency of water photosynthetic enzyme do not work properly.

3.6.2 Internal Factors

3.6.2.1 Chlorophyll

- Chlorophyll is essential for Photosynthesis.
- Photosynthesis does not take place in etiolated plants and plants without chlorophyll.
- In variegated leaves photosynthesis occurs in green parts of the leaf.
- Willstatter studied the effect of amount of chlorophyll on the rate of photosynthesis.
- He studied photosynthesis/Chlorophyll unit in leaves of different ages of plants.
- This is known as assimilatory number.
- This number decreases with the age of the leaf.

3.6.2.2 Protoplasmic Factor

- Photosynthesis does not start immediately after exposure of plant to light and immediately after development of chlorophyll.

3.6.2.3 Anatomy of Leaf

- The amount that increases the chloroplast depends on structural features of the **leaves** like the size, position and behaviour of the stomata and the amount of intercellular spaces.
- Some other characters like thickness of cuticle, presence of epidermal hairs, amount of mesophyll tissue, etc. influence the intensity of light reaching in the chloroplast.

3.6.2.4 Amount of food stored in Leaf

- If the synthesized food by photosynthetic cells is allowed to accumulate in those cells the rate of photosynthesis falls.
- If this food is removed from the cells the rate of photosynthesis increases.

3.7 Photorespiration

- When rubisco adds O_2 to RuBP, RuBP splits into a three-carbon piece and a two-carbon piece in a process called **photorespiration.**
    - The two-carbon fragment is exported from the chloroplast and degraded to CO_2 by mitochondria and peroxisomes.
    - Unlike normal respiration, this process produces no ATP.
  - In fact, photorespiration *consumes* ATP.
    - Unlike photosynthesis, photorespiration does not produce organic molecules.
  - In fact, photorespiration *decreases* photosynthetic output by siphoning organic material from the Calvin cycle.
- A hypothesis for the existence of photorespiration is that it is evolutionary baggage.
- When rubisco first evolved, the atmosphere had far less O_2 and more CO_2 than it does today.
    - The inability of the active site of rubisco to exclude O_2 would have made little difference.

- Today it does make a difference.
  - Photorespiration can drain away as much as 50% of the carbon fixed by the Calvin cycle on a hot, dry day.
- Certain plant species have evolved alternate modes of carbon fixation to minimize photorespiration.

3.8 The importance of photosynthesis

- In photosynthesis, the energy that enters the chloroplasts as sunlight becomes stored as chemical energy in organic compounds.
- Sugar made in the chloroplasts supplies the entire plant with chemical energy and carbon skeletons to synthesize all the major organic molecules of cells.
  - About 50% of the organic material is consumed as fuel for cellular respiration in plant mitochondria.
  - Carbohydrate in the form of the disaccharide sucrose travels via the veins to nonphotosynthetic cells.
    - There, it provides fuel for respiration and the raw materials for anabolic pathways, including synthesis of proteins and lipids and formation of the extracellular polysaccharide cellulose.
    - Cellulose, the main ingredient of cell walls, is the most abundant organic molecule in the plant, and probably on the surface of the planet.
- Plants also store excess sugar by synthesis of starch.
  - Starch is stored in chloroplasts and in storage cells in roots, tubers, seeds, and fruits.
- Heterotrophs, including humans, may completely or partially consume plants for fuel and raw materials.
- On a global scale, photosynthesis is the most important process on Earth.
  - It is responsible for the presence of oxygen in our atmosphere.
  - Each year, photosynthesis synthesizes 160 billion metric tons of carbohydrate.

3.9 Points to Remember

- Green plants make their own food by photosynthesis.
- During this process carbon dioxide from the atmosphere is taken in by leaves through stomata and used for making carbohydrates, principally glucose and starch.
- Photosynthesis takes place only in the green parts of the plants, mainly the leaves.
- Within the leaves, the mesophyll cells have a large number of chloroplasts that are responsible for CO_2 fixation.
- Within the chloroplasts, the membranes are sites for the light reaction, while the chemosynthetic pathway occurs in the stomatal aperture.
- Photosynthesis has two stages: the light reaction and the carbon fixing reactions.
- In the light reaction the light energy is absorbed by the pigments present in the antenna, and funneled to special chlorophyll *a* molecules called reaction centre chlorophylls.
- There are two photosystems, PS I and PS II. PS I has a 700 nm absorbing chlorophyll *a* P700 molecule at its reaction centre, while PS II has a P680 reaction centre that absorbs red light at 680 nm.
- After absorbing light, electrons are excited and transferred through PS II and PS I and finally to NAD forming NADH. During this process a proton gradient is created across the membrane of the thylakoid. The breakdown of the protons gradient due to movement through the F0 part of the ATPase enzyme releases enough energy for synthesis of ATP. Splitting of water molecules is associated with PS II resulting in the release of O_2, protons and transfer of electrons to PS II.
- In the carbon fixation cycle, CO_2 is added by the enzyme, RuBisCO, to a 5-

carbon compound RuBP that is converted to 2 molecules of 3-carbon PGA.

- This is then converted to sugar by the Calvin cycle, and the RuBP is regenerated. during this process ATP and NADPH synthesised in the light reaction are utilised. RuBisCO also catalyses a wasteful oxygenation reaction in C3 plants: photorespiration.
- Some tropical plants show a special type of photosynthesis called C4 pathway.
- In these plants the first product of CO_2 fixation that takes place in the mesophyll, is a 4-carbon compound. In the bundle sheath cells the Calvin pathway is carried out for the synthesis of carbohydrates.