PHOTOSYNTHESIS IN HIGHER PLANTS

Green plants carry out photosynthesis , a physico chemical process by which they use light energy to drive the synthesis of organic compounds. Ultimately all living forms on earth depend on sunlight for energy. The use of energy from sunlight by plants for doing photosynthesis is the basis of life on earth.

Photosynthesis is important due to two reasons :

(i) It is primary source of all food on earth.

(ii) It is responsible for the release of oxygen into the atmosphere.

Features of photosynthesis :

(a) Endergonic (Endothermic) process :
Reason: Require sunlight as a source of energy.

(b) Uphill process:
Reason : CO_2 is a weak electron acceptor.

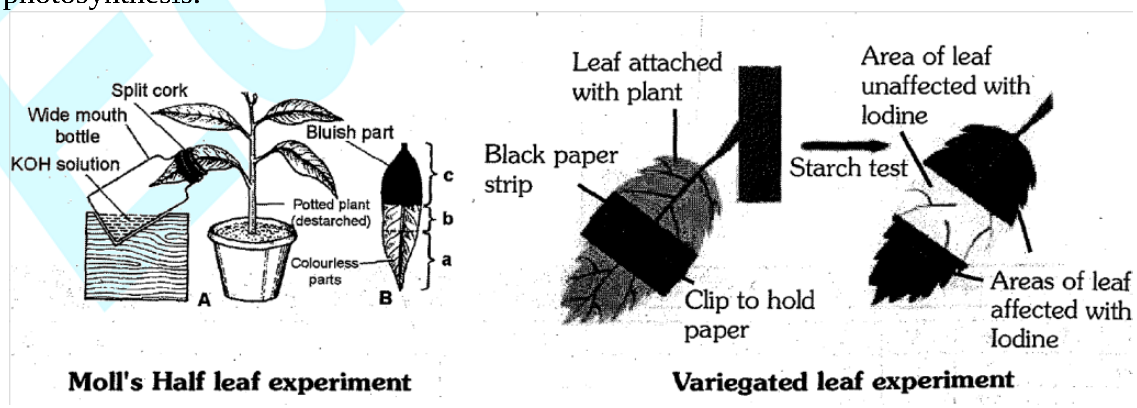
(c) Redox process:
Reason: Light reaction {splitting of water) is an oxidation and dark reaction ($C0_2$ fixation) is a reduction step.

(d) Physico chemical process:
Reason: Mechanism of photosynthesis convert light energy (physical form) into glucose/starch (chemical form).

WHAT DO WE KNOW

Some simple experiments shows that chlorophyll (green pigment of the leaf), light and CO_2 are required for photosynthesis to occur.
Look for starch formation in two leaves - a variegated .leaf or a leaf that was partially covered with black paper and one that was exposed to light. On testing these leaves for starch it was clear that photosynthesis occurred only in the green parts of the leaves in the presence of light.

Another experiment is the half-leaf experiment, where a part of a leaf is enclosed in a test tube (wide mouth bottle) containing some KOH soaked cotton (which absorbs CO_2), while the other half is exposed to air. The setup is then placed in light for some time. On testing for starch later in the two halves of the leaf, the exposed part of the leaf tested positive for starch while the portion that was in the tube, tested negative. This shows that CO_2 is required for photosynthesis.



Early experiments

Stephen Hales : He is credited for discovery of photosynthesis and known as father of plant physiology.

Joseph Priestley (1733-1804):

In 1770 he performed a series of experiments that revealed the essential role of air in the growth of green plants. He also discovered oxygen in 1774.

Priestley's observation :

(i) A candle burning in a closed space i.e. a bell jar, soon gets extinguished.

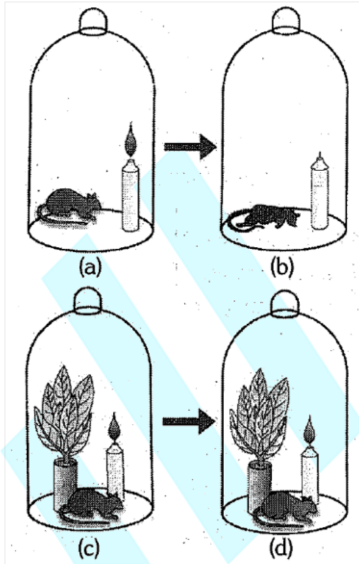
(ii) Similarly, a mouse would soon suffocate in a closed space.

Priestley's conclusion :

He concluded that a burning candle or an animal that breathe the air, both somehow, damage the air, But when he placed a mint plant in the same bell jar, he found that the mouse stayed alive and candle continued to burn.

Priestley's hypothesis :

Plants restore to "the air whatever breathing animals and burning candles remove.



Priestley's experiment

Jan Ingenuous (1730-1799) :

Using a similar setup as the one used by Priestley but by placing it once in the dark and once in the light, he showed that sunlight is essential to the plant process (photosynthesis) that somehow purifies the air fouled by burning candles or breathing animals.

In an another elegant experiment with an aquatic plant slowed that in bright sunlight small bubbles were formed around the green parts, while in the dark they did not.

Conclusion :

It is only the green parts of the plants that could release oxygen in the presence of sunlight.

Julius Von Sachs (1854) :

He provided evidences for production of glucose when plants grow. Glucose is usually stored as starch.

Glucose - Chemically reactive and soluble

Sucrose - Chemically inactive and soluble

Starch - Chemically inactive and insoluble

His later studies showed that the green substance in plants is located in special bodies within plant cells. (Today the green colour substance is known as chlorophyll and special bodies are chlordplasts)

By the middle of nineteenth century the empirical eguation representing the total process of photosynthesis for oxygen evolving organisms was then understood as :

$$CO_2 + H_2O \xrightarrow{\text{Light}} [CH_2O] + O_2$$

where $[CH_2O)$ represented a carbohydrate (eg. Glucose, a six carbon sugar)

Cornelius Van Niel (1891-1985)

A milestone contribution to the understanding of photosynthesis was made by him. He was a microbiologist. He based upon his studies of purple and green bacteria. demonstrated that "Photosynthesis is essentially a light dependent reaction in which hydrogen from a suitable oxidisable compound reduces carbon di-oxide to carbohydrates"

This can be expressed by :

$$2H_2A + CO_2 \xrightarrow{\text{Light}} 2A + CH_2O + H_2O$$

- In green plants and BGA. H_2O is the hydrogen donor and is oxidised to O_2.
- In purple and green sulphur bacteria H_2S is the hydrogen donor and is oxidised to sulphur or sulphate depending on the organism.

"He inferred that the O_2 evolved by the green plant comes from H_2O. not from CO_2"

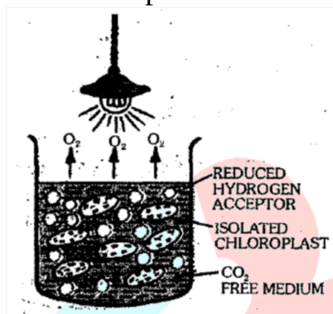
Robert Hill and Bendall :

They are credited for :

- Detailed study of light reaction and proposed Z scheme.

R. Hill:-

Detailed study of light reaction in isolated chloroplast of Stellaria plant. He illuminated the isolated chloroplasts of Stellaria media in the presence of hydrogen acceptors (ferricyanides) in the absence of carbon dioxide. The chloroplasts evolved oxygen



$$2A + 2H_2O \xrightarrow[\text{Hill reagent}]{\text{Sunlight}} 2AH_2 + O_2$$

A = Hydrogen acceptor (Hill reagent)

Hill reagent

Natural	Artificial
Eg. NADP	Eg. Potassium ferricyanide, Benzoquinone, Benzoquinone, chromate and DCPIP etc.

DCPIP (Dichlorophenol indophenols) is a blue colour dye, which become colourless on reduction.

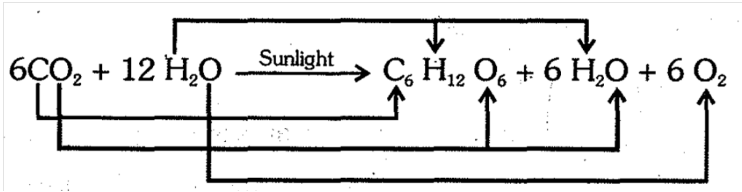
Ruben, Hasid and Kamen :

They proved that the O_2 evolved by the green plant comes from H_2O, not from CO_2 by using radio isotopic techniques.

This can be expressed as :

$$6CO_2^{16} + 12H_2O^{18} \xrightarrow[\text{Pigments}]{\text{Sunlight}} C_6H_{12}O_6^{16} + 6H_2O^{16} + 6O_2^{18}$$

“"The correct equation that would represent the overall process of photosynthesis is therefore"



Q. Can you explain why twelve molecules of water as substrate are used in the following equation?

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

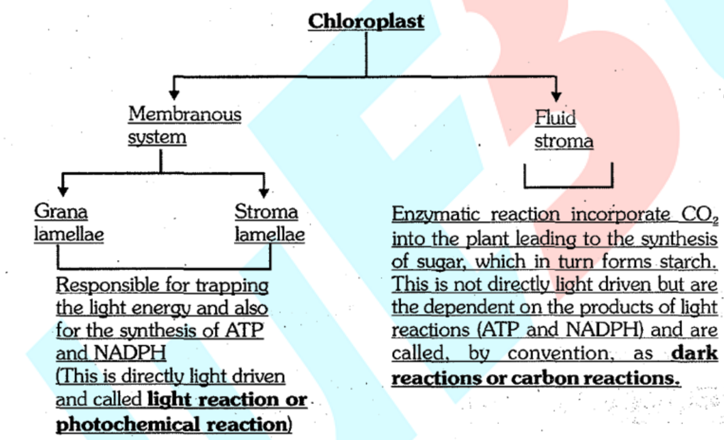
Ans. To make one molecule of glucose total six turns of Calvin cycle are required. Per Calvin cycle there is a need of 2NADPH(H^+) and to fulfit this need 2 molecules of H_2O splits. Therefore splitting of total 12 molecules of H_2O occur.

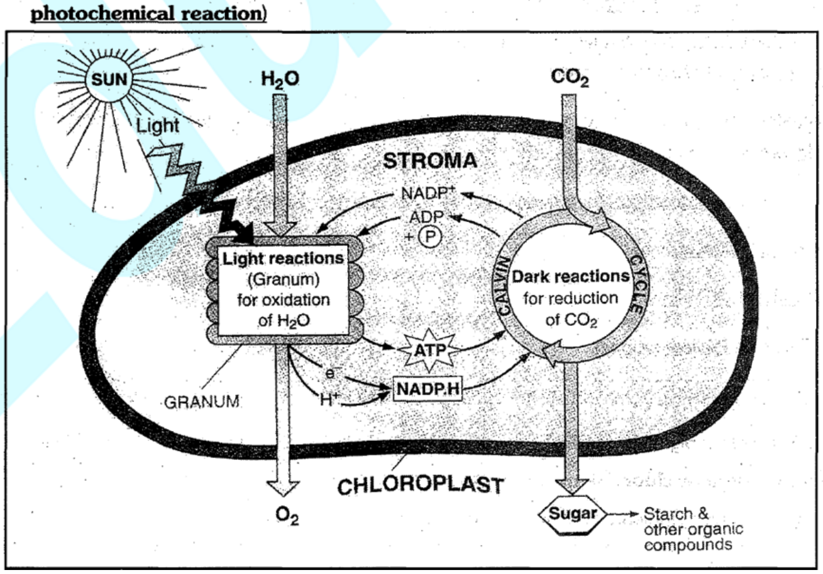
Where does photosynthesis take place?

Photosynthesis does take place in the green leaves of plants but it does so also in other green parts of the plants. There is a clear cut division of labour within the chloroplast.

Q. Can you name some parts of plants other than leaves, where photosynthesis may occur ?

Ans. Sepals, herbaceous stem and unripe green fruits (green chilly, green tomato) etc.

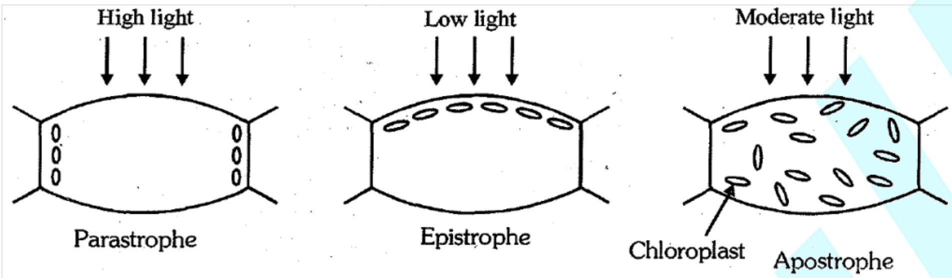




Alignment of chloroplasts :

Usually the chloroplasts align themselves along the walls of the mesophyll cells. Such that they get the optimum quantity of the incident light.

Condition	Alignment of chloroplasts
High light intensity	Parallel to the incident light/Lateral wall (Parastrophe)
Low light intensity	Perpendicular to the incident light (Epistrophe)
Moderate light intensity	Random (Apostrophe)



Various kinds of alignment of chloroplasts

Q. When do you think the chloroplasts will be aligned with their flat surface parallel to the walls ?
Ans. Under high light conditions, this is called parastrophe.

Q. When do you think chloroplast will be perpendicular to the incident light ?
Ans. Under low light conditions, this is called epistrophe.

How many pigments are involved in Photosynthesis

Leaf pigments can be separated from any green plant through paper chromatography, and the picture obtained is called chromatogram.
Chromatographic separation of the leaf pigments shows that the colour we see in leaves is not due to a single pigment but due to four pigments :
(i) Chi - a (Bright green or blue green)
(ii) Chi - b !Yellow green)
(iii) Xanthophylls (Yellow)
(iv) Carotene (Yellow orange)

Bacteriochlorophyll or Bacteriopurpurin :
It is purple colour pigment, molecular formula is C55H740 6N4Mg. It occurs inside purple bacteria.
Bacterioviridin or chlorobium chlorophyll :
It is a green colour pigment, occur inside green bacteria.

Types of pigments :
(A) Chlorophylls (B) Carotenoids (C) Phycobillins

(A) Chlorophylls :
They are green colour pigments and occur inside chloroplast. These pigments are lipid in nature, insoluble in water and soluble in organic solvents.

Types of chlorophyll :

(1) Chlorophyll-a

- Its a bluish green or bright green pigment with molecular formula $C_{55}H_{72}O_5N_4$ Mg.
- Chlorophyll-a is a universal photosynthetic pigment
  Reason : Present in every photosyntehtic organism (except eubacteria)
- Chlorophyll-a is a primary photosynthetic pigment.
  Reason : Primary reaction of photosynthesis which involve conversion of light energy into chemical energy (ATP and NADPH) is mediated by chi-a molecule (Reaction centre)
- Chlorophyll-a is most abundant photosynthetic pigment.

Synthesis:

Glycine + Succinyl CoA $\longrightarrow$ Protochlorophyll (protochlorophyllide) $\xrightarrow[2H]{Light}$ Chlorophyll-a

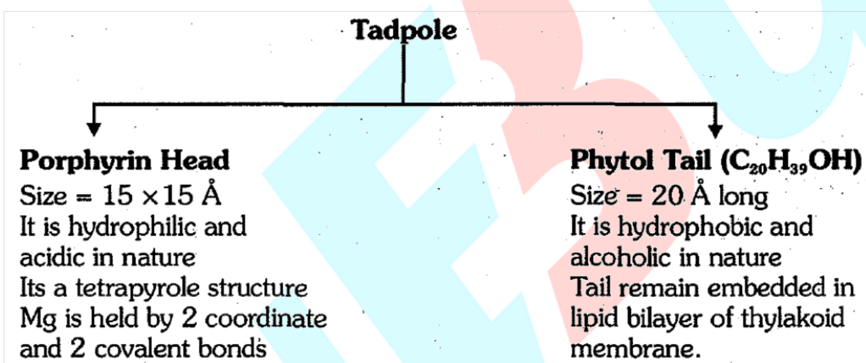
Glutamic acid $\rightarrow$ Chlorophyll-a

Three minerals are essentail for chlorophyll synthesis namely Mg, Fe and N (Mg and N are structural constituent)

Light is compulsory for chlorophyll synthesis in Angiosperms.

Structure of chlorophyll-a :

Structure of chi-a look like tadpole.



(2) Chlorophyll-b

It is yellow green pigment, molecular formula is $C_{55}H_{70}O_6N_4$ Mg.

It is structurally similar to chi-a, except it has –CHO group in place of $–CH_3$ at III position of II pyrrole ring.

(3) Chlorophyll-c

Chlorophyll-c lacks phytol tall.

(4) Chlorophyll-d

(5) Chlorophyll-e

(B) Carotenoids :

Carotenoids are yellow to yellow orange colour pigments occur alone inside chromoplast and occur along with chlorophylls inside chloroplast. These pigments are universal in occurence (except eubacteria) and insoluble in water.

Chemically they are terpenes and considered as most stable pigments. Light is not necessary for, their synthesis.

They are hydro carbons with conjugated double bonds (–CH = CH – CH = CH–)

Carotenoids

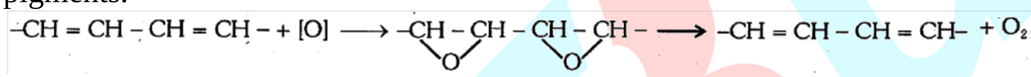
Carotenes
- They are yellow orange coloured purely hydrocarbon and empirical formula is $C_{40}H_{56}$
- They are of three types : (a) α - carotene (b) β - carotene (c) γ - carotene
- β Carotene are common in plants and also responsible for colour of carrot.

Xanthophyll
- They are yellow coloured oxygen containing derivative of carotenes. ($C_{40}H_{56}O$, $C_{40}H_{56}O_2$ etc.)
- **Lutein :** It is common in plants and responsible for **autumn colour of leaf**
- Diatoxanthin (Dinoflagellates), Fucoxanthin (Brown algae)

Function of carotenoids :

(1) They are accessory pigments and make photosynthesis more efficient by absorbing different wavelengths of light.

(2) They protect chi-a from photo oxidation and they also protect photosynthetic machinery by converting lethal nascent oxygen into unharmful molecular oxygen, thus called shield pigments.



(3) β-carotene is acts as a precursor of vitamin-A

$$C_{40}H_{56} + 2H_2O \longrightarrow 2C_{19}H_{27}CH_2OH$$

β-carotene　　　　vitamin-A

(4) They help in entomophilly and zoochory.

(C) Phycobillins:

- They are hot water solubie, open tetrapyrrole pigments which are associated with proteins.
- They lack Mg and .phytol tail.

Types:

(i) Phycocyanin - Blue　　(ii) Phycoerythrin - Red　　(iii) Allophycocyanin - Light blue

They occur exclusively in BGA and Red algae as an accessory pigments.

Special points :

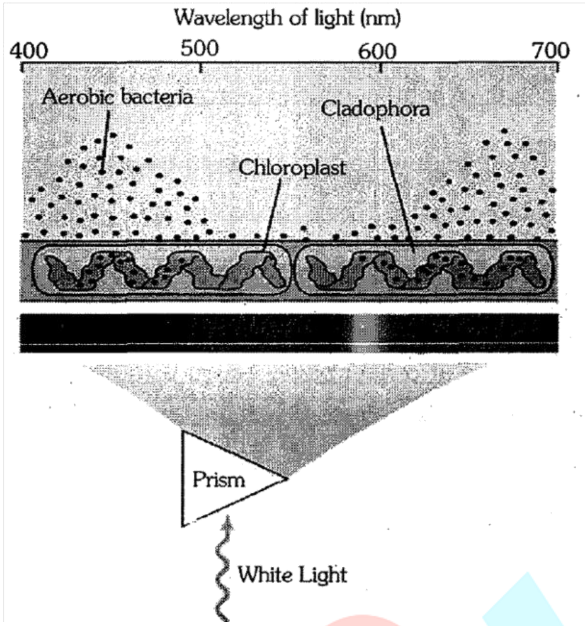
- **Etiolation** : Yellowing of leaves due to deficiency of light.
- **Phytochrome** : It is a proteinaceous (chromoprotein) pigment in angiosperms and responsible for seed germination and flowering.

Absorption spectrum :

It is a graphic representation of absorption of different wavelength of light by various pigment molecules. (chl-a, chl-b and carotenoids

T.W. Engelmann (1843-1909):

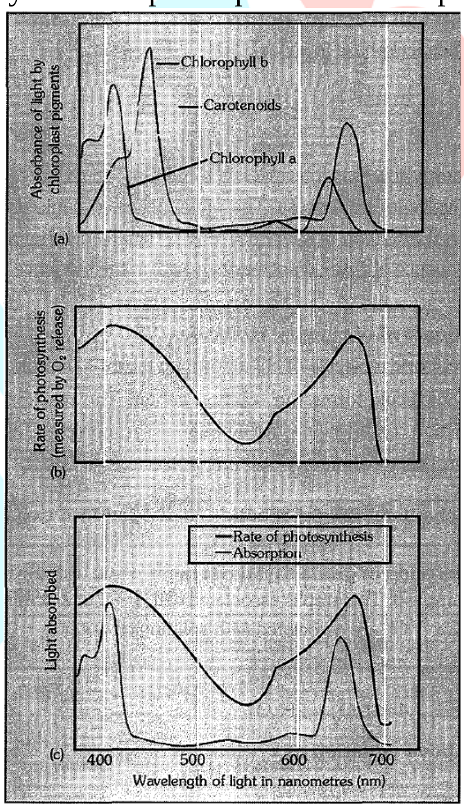
He described the first action spectrum of photosynthesis. By using a prism he split sunlight into its spectral components MBGYOR) and then illuminated a green algae. Cladophora placed in a suspension of aerobic bacteria. "The bacteria were used to detect the sites of O_2 evolution"



Observation :

(i) Bacteria accumulated mainly in the region of blue and red light of the split spectrum.

(ii) It resembles roughly the absorption spectra of chlorophyll a and b.



Q. Which is the most abundant plant pigment in the world ?

Ans. Chlorophyll a

Q. Determine the wavelength (colour of light) at which chlorophyll a shows the maximum absorption ?

Ans. Wavelength = approx 430 nm

Colour = Blue

Q. Can you say that there is a complete one to one overlap between the absorption spectrum of chlorophyll-a and the action spectrum of photosynthesis.

Ans. No. there is no complete one to one overlap between the absorption spectrum of chlorophyll a and the action spectrum of photosynthesis because at certain points action is more than aborption because wavelengths not absorbed by chlorophyll a are absorbed by accessory pigments and transferred to reaction center (chlorophyll a).

Mechanism of photosynthesis :

Photosynthesis accomplish under two stages :

(1) Light reaction (2) Dark reaction

(1) Light reaction :

What is light reaction?

It is an oxidation reaction (H_2O oxidised) occurs at grana of chloroplast. This reaction is also called photochemical phase or Hill reaction.

Light reaction or photochemical phase includes :

- Light absorption
- Water splitting
- Oxygen release
- Formation of high-energy chemical intermediates. ATP and NADPH

Several complexes are involve in the process. The pigments are organised into two discrete photochemical light harvesting complexes (LHC) with in the photo system-I (PS-I) and photosystem-II (PS-II).

They are named in the sequence of thier discovery and not in the sequence in which they function during the light reaction.

The LHC are made up of hundred of pigment molecules (250-400) bound to proteins. Each photosystem has all the pigments (except one molecule of chi-a) forming a light harvesting system also called antennae. The single chlorophyll-a molecule forms the reaction center. The reaction center is different in both the photosystem. In PS-I reaction center chlorophyll-a has an absorption peak, at 700 nm, hence is called P 700, while in PS-II It has absorption maximum at 680 nm, and is called P 680.

For ease of study, mechanism of light reaction is divided into two parts:

(I) Photolysis of water :

Occurs at grana i.e. lumen side of grana thylakoid membrane with the help of water splitting complex or OEEC (oxygen evolving complex.) This step is associated with PS-II of Z-scheme.

Three minerals Mn ion, Ca^{++}, Cl^{-} are associated with splitting of water.

$$4H_2O \xrightarrow{OEC} 4H^+ + 4OH^-$$

$$4H^- \xrightarrow{OEC} 4e^- + O_2 + 2H_2O$$

We can summarized above equation as :

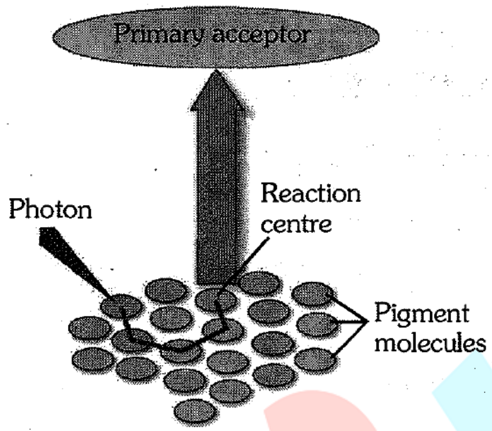
$$2H_2O \xrightarrow{OEC} 4H^+ + O_2 + 4e^-$$

Q. Where are the protons and O_2 formed likely to be released?

Ans. Protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids while O_2 released outside.

(II) Formation of high energy intermediates :

ATP and NADPH are produced through non-cyclic photo phosphorylation or z-scheme.



Plastocyanin : It is a copper containing blue colour protein.

Phaeophytin : It is derived from chlorophyll a where, Mg is replaced by 2 H. Its acts as a primary electron acceptor of Z scheme.

Emerson effect and Red drop :

Emerson and Arnold experimented upon Chlorella.

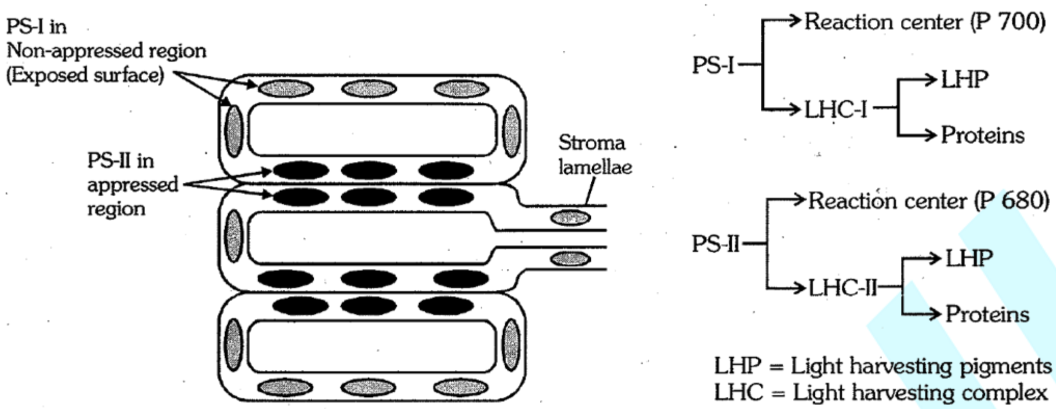
Observation :

- Reported minimum photosynthetic yield, when supplied monochromatic beam of 700 nm or > 680 nm only. (Red drop)
- Reported enhancement in photosynthetic yield, when both 700 nm and 680 nm supplied together (Enhancement effect or Emerson effect)

Conclusion :

Two types of photosystems (PS-I and PS-II) exist in photosynthetic units. They operate simultaneously and their operation I activation required 700 nm and 680 nm radiation.

	PS-I		**PS-II**
(1)	Located at non-oppressed parts of grana thylakoid and stroma lamellae	(1)	Usually located at appressed parts of grana thylakoids.
(2)	Its reaction center is P 700	(2)	Its reaction center is P 680
(3)	It has reducing nature (reduce NADP)	(3)	It has oxidizing nature (oxidise H_2O)
(4)	It participates in both cyclic and non-cyclic photophosphorylation	(4)	It participates only in non-cyclic photophosphorylation



Photophosphorylation

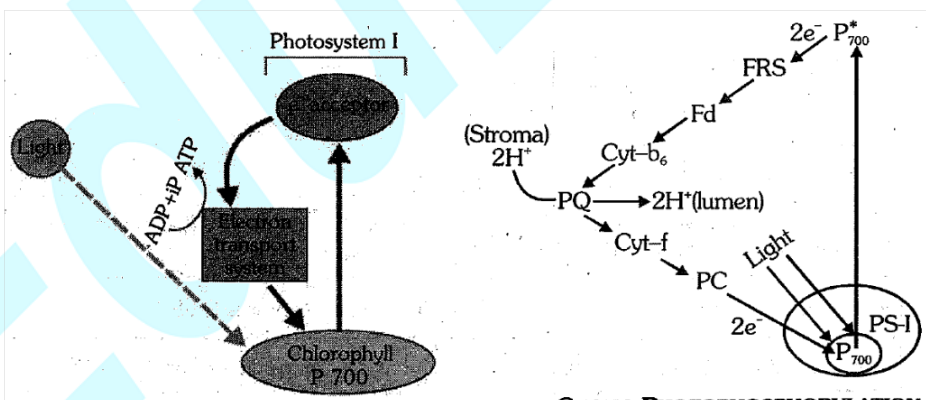
ATP synthesis by cells On mitochondrial and chloroplasts) is named phosphorylation. Photophosphorylation is the synthesis of ATP from ADP and inorganic phosphate in the presence of light. It is of two types (according to Arnon et al)

(a) Cyclic photophosphorylation :

When only PS-I is functional, the electron is circulated within the photosystem-1 and the phosphorylation CATP synthesis) occurs due to cyclic flow of electrons.

A oossible location where this could be happening is in the stroma lamellae because the stroma lamellae membranes lack PS-D as well as NADP reductase enzyme. Cyclic photophosphorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

During cyclic photophomhorylation the excited electrons does not pass on to $NADP^+$ bufis cycled back to the PS-I complex through the electron transport chain/system (ETS). The cyclic flow, hence, nisults only in the synthesis of ATP but not of NADPH.



CYCLIC PHOTOPHOSPHORYLATION

(b) Non-cyclic photophosphorylation :

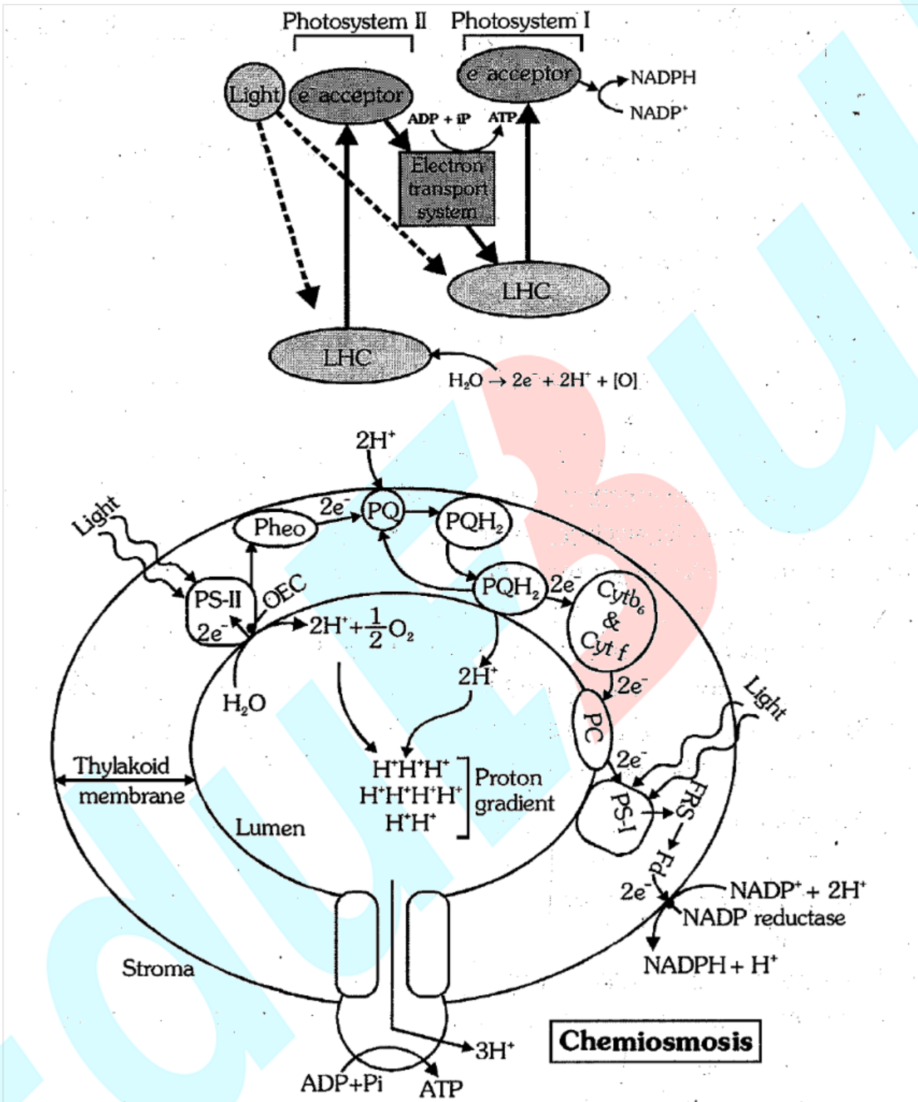
When the two photosystems CPS I and PS IT) work together the process called non-cyclic photophosphorylation. The two photosystems are connected through an electron transport chain.

This whole scheme of transfer of electrons, starting from the PS II, uphill to the acceptor, down the electron transport chain to PS-II, excitation of electrons, transfer to another acceptor, and finally down hill to $NADP^{\pm}$. reducing it to NADPH + $H^{\pm}$. It is called the Z scheme, due to its characteristic shape. This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

Z scheme was proposed by Hill and Bendall.

Redox potential :

If is the measure of the tendency of a chemical molecule to acquire electrons and thereby be reduced. It is also called oxidation - reduction potential. It is measured in volts (v) or mili volts (mv).



Quantum requirement -

The number of light Quanta or photons required for the evolution of 1 mol. of O_2 in photosynthesis. Emerson calculated that the quantum requirement is 8.

Quantum Yield –

The number of oxygen molecule evolved by one quantum of light in photosynthesis is called as Quantum yield. Hence the quantum yield is 0.125 or 12.5%

Chemiosmetic hypothesis

This hypothesis has been put forward by Peter Mitchell to explain the mechanism of ATP synthesis in respiration (oxidative phosphorylation) and in photoswthesis (Photophosphorylation).

This hypothesis (ATP synthesis) based I linked to development of a proton gradient across a membrane.

	Photophosphorylation		Oxidative phosphorylation
(1)	Membranes through which proton graident develop. are the membranes of thylakoid	(1)	Membrane through which proton gradient develop. is the inner membrane of mitochondria
(2)	Protons accumulate towards the inner side of the embrane. i.e., in the lumen.	(2)	Protons accumulate towards the outerside of the membrane i.e. in the iiltermembrane space.
(3)	Light energy is utilised for the production of proton gradient required for phosphorylation	(3)	Energy of oxidation reduction utilised for the same process.

Mechanism for development of proton gradient :

Steps that cause a proton gradient to develop :

(a) Since splitting of the water molecule takes place on the inner side of the thylakoid membrane. The protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids.

(b) As electrons move through the photosystems, protons are transported across the meml:irane. This happens because the primary acceptor of electron (Phaeophytin) which is located towards the outer side of the membrane transfers its electron not to an electron carrier but to an H carrier (Plastoquinone), Hence. this molecule (plastoquinone) removes a proton &om the stroma where transporting an electron. When this molecule passes on its electron to the - electron carrier (Cytochrome f) on the inner side of the membrane,· the proton is released into the inner side or the lumen side of the membrane.

(c) The NADP reductase enzyme is located on the stroma side of the membrane. Alongwith electrons that come from the acceptor of electrons of PS-I, protons are necessary for the reduction of $NADP^+$ to NADPH+ H^+. These protons are also removed from the stroma.

Hence, within the chloroplast proton in the stroma decrease in number, while in the lumen there is accumulation of protons. This create a proton gradient across the thylakoid membrane as well as a measurable decrease in pH in the lwnen.

Significance of proton gradient :

This gradient is important because it is the breakdown of this gradient that leads to synthesis of ATP. The gradient is broken down due to the movement of protons across the membrane to the stroma through the transmembrane channel of the CF_0 of the ATP synthase.

ATP synthase enzyme Macbinary :

The ATP SYnthase enzyme consists of two parts :

(a) **CF_0 :** It is embedded in the membrane and forms a transmembrane channel that carries out facilitated diffusion of protons across the membrane.

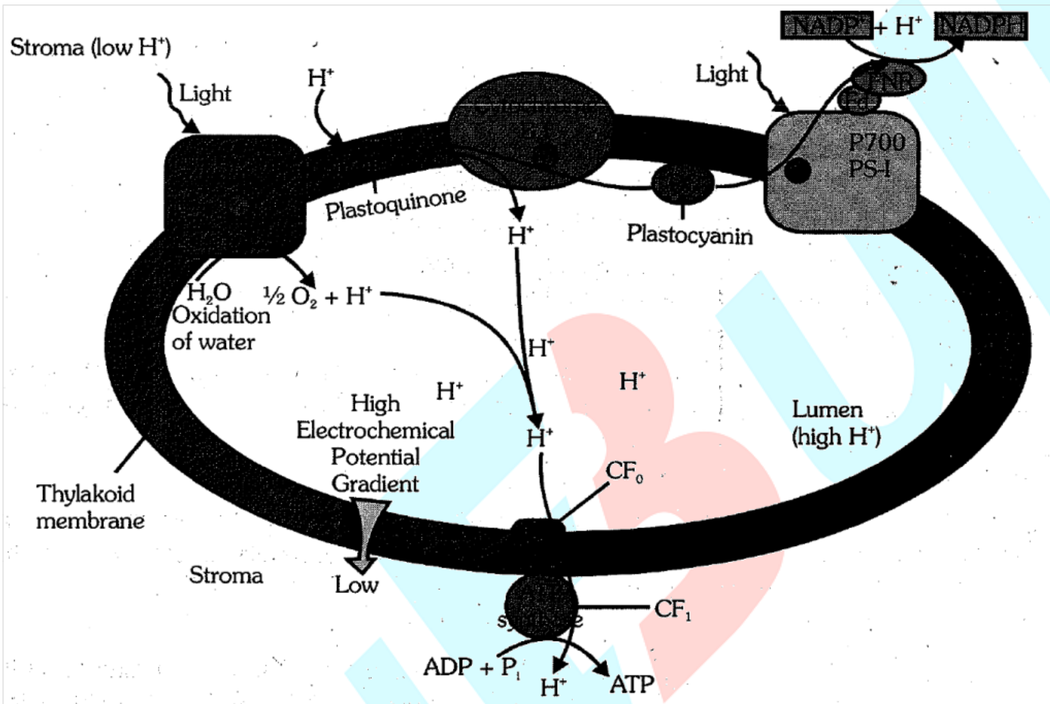
(b) **CF_1 :** It is protrude on the outer surface of the thylakoid membrane on the side that faces the stroma.

Requirements of chemiosmosis :

(i) A membrane (ii) A proton pump (iii) A proton gradient (iv) ATP synthase enzyme

Mechanism of ATP synthase action :

- The breakdown of the proton gradient provides enough energy to cause a confonnational change in the CF_1 particle of the ATP synthase, which makes the enzymes synthesize several molecules of energy packed ATP.
- Radiation or light energy is used to pump protons across a membrane, to create a gradient or high concentration of protons within the thylakoid lumen.



ATP synthesis through chemiosmosis

BEGINNER'S BOX-1

1. The electrons, that are removed from photo system-II and photo system-I during z-scheme, must be replaced.
(1) By photo system-I and $NADP^+$ (2) By splitting of water and NADPH
(3) By ATP and photo system-II (4) By splitting of water and photo system-II

2. This is achieved by electrons available respectively :-
(1) 700 nm and 680 nm (2) 430 nm and 680 nm
(3) 700 nm and 430 nm (4) 680 nm and 700 nm

3. The reaction centres of photosystem-11 and photosystem-1 have absorption peak respectively at :-
(1) Photosystem with reaction centre P680 work after P700 containing photosystem so it is called PS-II
(2) Photosystem with reaction centre P700 starts the photosynthesis so it is called P&I

(3) Photosystem with reaction centre P680 was discovered after P700 containing photosystem so it is called P&II
(4) Photosystem with reaction centre P700 absorb high wavelengths of light so it is called P&I

4. Process of photosynthesis that occur in the stroma lamellae membranes, lack :-
(1) Photosystem-1 (2) Electron transport system
(3) NADP reductase enzyme (4) ATP synthase

5. Regarding photosynthesis, Jan Ingenuous established the essentiality of sunlight and green colour respectively by using:-
(1) Bell jar setup and an aquatic plant
(2) An aquatic plant and radio isotopic technique
(3) Cladophora algae and purple sulphur bacteria
(4) Bell jar setup and green sulphur bacteria

Where are the ATP and NADPH used (Bark reaction)

(2) Dark reaction :
The products of light reaction are ATP, NADPH and O_2. Of these O_2 diffuses out of the chloroplast while ATP and NADPH are used to drive the process leading to synthesis of food, (more accurately sugars). This is biosynthetic phase of photosynthesis. This process does not directly depend on the presence of light but is dependent on the product of the light reaction i.e. ATP and NADPH, besides CO_2 and H_2O.

Q. Does the term dark reaction for biosynthetic phase of photosynthesis, is a misnomer?
Ans. Yes, dark reaction term is a misnomer because this reaction not occurs in dark. Instead this reaction is dependent on the product of the light reaction i.e, ATP and NADPH, so dark reaction process also occurs in presence of light.
Dark reaction occurs at fluid of stroma and also called Blackman's reaction or Biosynthetic phase. It is a reductive step (CO_2 reduced) of photosynthesis. Dark reaction operates in different photosynthetic organisms through three different ways :
[A] C_3 pathway [B] C_4 pathway [C] CAM pathway
C_3 pathway consists of only C_3 cycle or Calvin cycle while C_4 pathway and CAM pathway consists of both C_3 cycle and C_4 cycle. Therefore, Calvin cycle occurs in all photosynthetic plants ; it does not matter wheather they have C_3 or C_4 (or any other) pathways.

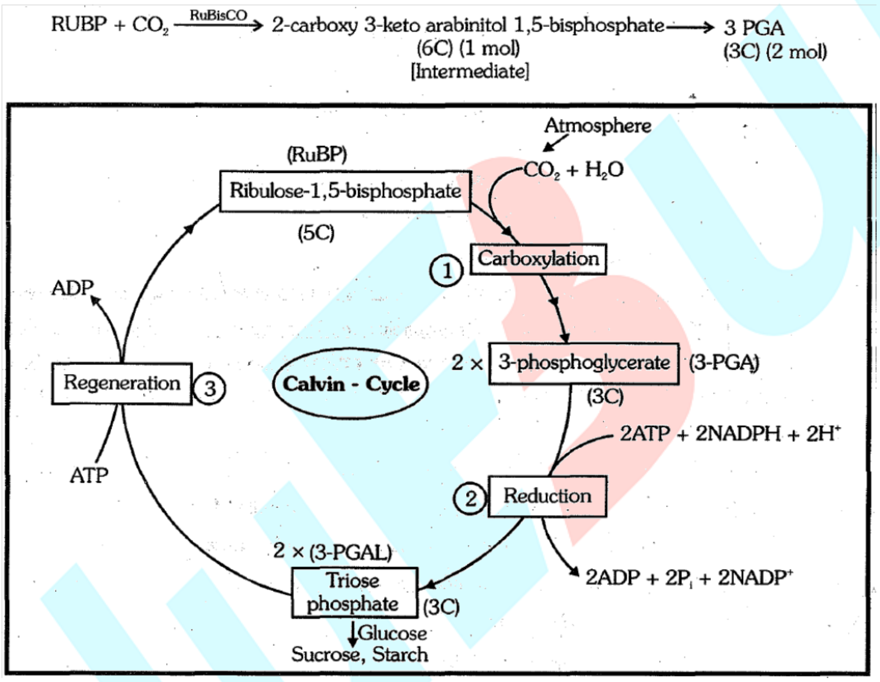
[A] The Calvin cycle :
The use of radioisotope ^{14}C by Calvin in alga (Chiarella) photosynthesis studies led to the discovery that the first CO_2 fixation product is a 3 carbon organic acid called 3 phosphoglyceric acid or in short 3 PGA. It has a 3 carbons thus cycle named C_3 cycle.
Melvin Calvin and his co-workers then worked out the whole pathway and showed that the pathway operated in cyclic manner ; the RUBP was regenerated.
Calvin cycle can be described under three stages :
(1) Carboxylation **(2) Reduction** **(3) Regeneration**

(1) Carboxylation:
It is the most crucial step (because RuBisCO has dual nature and this step will determines wheather Calvin cycle run or photorespiration). In this step CO_2 is utilised for the carboxylation

of RUBP. This reaction is catalysed. by the enzyme RuBisCO which results in the formation of two molecules of 3-PGA.

RuBisCO

- Its full name is Ribulose 1,5-bisphosphate carboxylase oxygenase enzyme, Its former name, was carboxydismutase.
- RuBisCO is considered as a most abundant protein on the earth.
- It has dual nature, thus capable to bind with CO_2 (carboxylase) as well as O_2 (oxygenase). Although the enzyme has more affinity with CO_2.
- Binding of CO_2 or O_2 is competitive with active site. It is the relative concentration of O_2 and CO_2 in the stroma of chloroplast that determines which of the two will bind to the enzymes.
- Mg and Red light are necessary for activation of enzyme RuBisCO.



(2) Reduction :

These are a series of reactions that lead to the formation of glucose. (glycolytic reversal)

The fixation of six molecules of CO_2 and for this fixation 6 turns of the Calvin cycle are required for removal of one molecule of glucose from the pathway.

(3) Regeneration :

Regeneration of the CO_2 acceptor molecule RUBP is crucial if the cycle is to continue uninterrupted.

Steps of calvin cycle	Number of ATP and NADPH required per CO_2
Carboxylation	Zero ATP and Zero NADPH + H^+
Reduction	Two ATP and two NADPH + H^+
Regeneration	One ATP and Zero NADPH + H^+

Hence for every CO_2 molecule entering the Calvin cycle, 3 molecules of ATP and 2 of NADPH are required.

Input	Output
6 CO_2	$C_6H_{12}O_6$
18 ATP	18 ADP
12 NADPH	12 $NADP^+$

[B] The C_4 pathway or Hatch and Slack pathway:

Plants that are adapted to dry tropical regions have the C_4 pathway. Such plants are called C_4 plants.

C_4 plants: Sugarcane, Maize, Sorghum, Amaranthus, Sa/sola, Atriplexetc.

Special features of C_4 plants :

(i) They have a special type of leaf anatomy i.e., Kranz anatomy.

(ii) They tolerates higher temperatures.

Reason: Pyruvate phosphate dikinase (PPDK) a low temperature sensitive enzyme of C_4. Thus, C_4 plants show poor rate of photosynthesis at low temperature.

(iii) They show a response to high light intensities.

(iv) They lack of process called photorespiration. Thus, they have a greater productivity of biomass.

(v) The evolution of the C4 photosynthetic system is probably one of the strategy for maximising the availability of CO_2 while minimising water loss. C_4 plants are twice as efficient as C_3 plants in terms of fixing carbon (making sugar). C_4 plants loses only half as much water as a C_3 plant for the same amount of CO_2 fixed.

Features of Kranz Anatomy :

(i) Mesophyll is not differentiated into palisade and spongy tissue.

(ii) Cells of bundle sheath arranged in concentric rings around vascular bundle. (Wreath manner)

(iii) Cells of mesophyll and bundle sheath are interconnected by plasmodesmata.

(iv) Bundle sheath cells may form several layers around the vascular bundle ; they are characterised by :

  (a) Having a large number of chloroplast

  (b) Thick walls impervious to gaseous exchange

  (c) No intercellular spaces.

C_4 plants are also characterised by Dimorphic chloroplast.

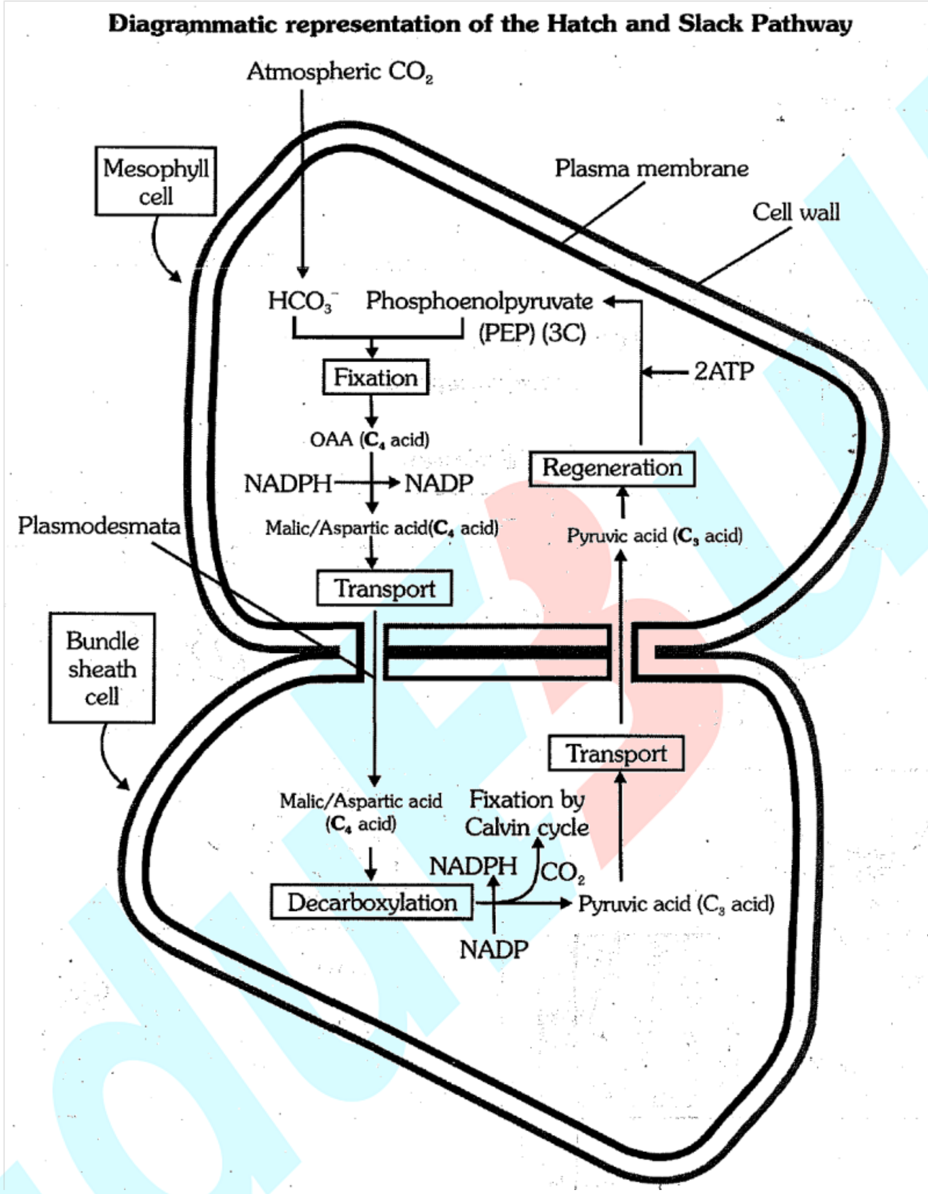
  (a) Chloroplasts of mesophyll cells are small in size and granal (with grana)

  (b) Chloroplasts of bundle sheath cells are large in size and agranal (without grana)

Mechanism of C_4 pathway:

- This pathway that has been named the Hatch and Slack pathway, is again a cyclic process.
- The primary CO_2 acceptor is a 3-carbon molecule phosphoenol pyruvate (PEP) and is present in the mesophyll cells. The enzyme responsible for this fixation is PEP carboxylase or PEP case. It is important to register that the mesophyll cells in C4 plants lack RuBisCO enzyme. The C4acid 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 bundle sheath cells. In bundle sheath cells these C_4, acids are broken down to release CO_2, and 3-carbon molecule (pyruvic acid)
- 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 cycle (a cycle common to all plants)

- The bundle sheath cells in C_4 plants are rich in an enzyme RuBisCO, but lack PEPcase .
- Thus the basic pathway that results in the formation of sugars (Calyin cycle), is common to the C_3 and C_4 plants.



Input	Output
6 CO_2	$C_6H_{12}O_6$
30 ATP	30 ADP + 30 iP
12 NADPH	12 NADP

Photosynthesis in C_4 plants is relatively less limited by atmospheric CO_2 levels because CO_2 effectively pumped into bundle sheath cells. Therefore,

(a) Little or no chance of photorespiration.

(b) CO_2 is not a limiting factor for C_4 plants.

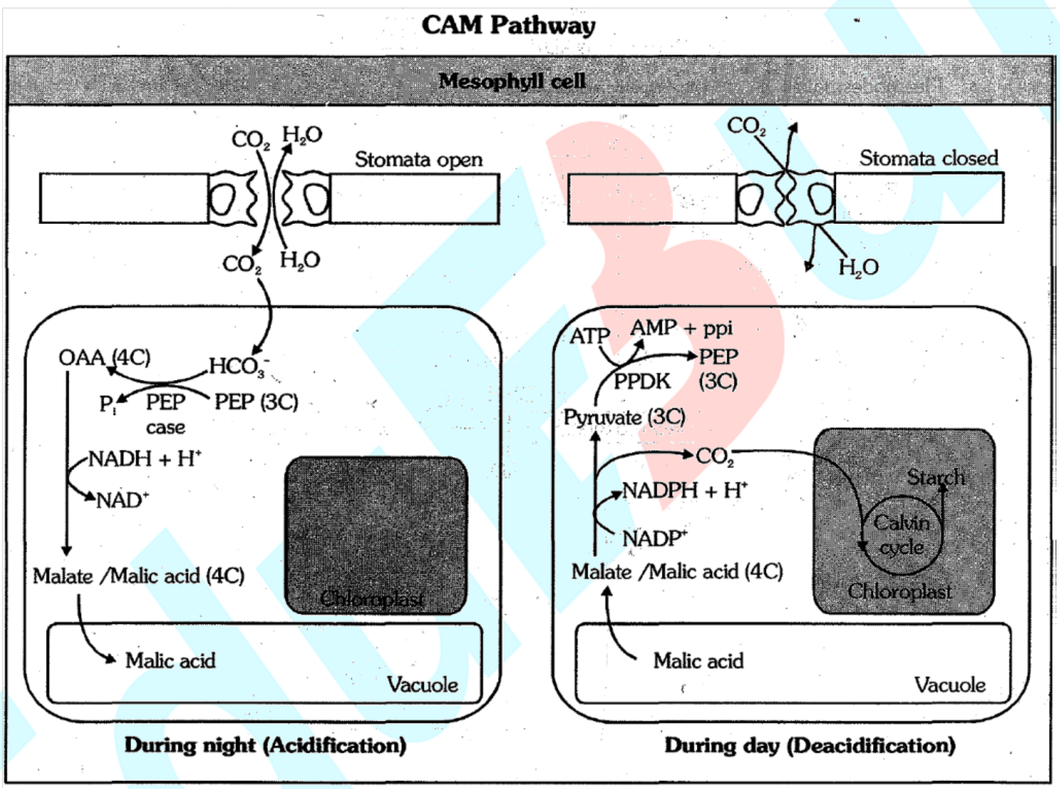
CAM Pathway:

- CAM pathway (Crassulacean acid metabolism) was discovered by Oleary and Rouhani.
- They observed that CO_2 fixation ocrurs during night in members of Crassulaceae family (succulent xerophytes).
  Succulents or CAM plants are characterised by scotoactive stomata (stomata, open during night and remain closed during day time)

CAM Plants:

Kalanchoe, Bryophyllum, Opuntia. Agave, Aloe, Euphorbia, Pineapple, Welwitschia (GYmnosperm)

- These plants are adapted to water conservation.
- Primary acceptor of C02 is PEP and OAA is the first product.
- PEP case and RuBisCO both are present in mesophyll cells.



Photorespiration

- It is also called PCO (photosynthetic carbon oxidation) cycle or C_2-cycle or glycolate metabolism.
- This cycle was discovered by Decker and Tio in tobacco (a C_3 plant)
- Photorespiration is a wasteful process because there is neither synthesis of sugars nor of ATP and NADPH. Rather it results in the release of CO_2 with the utilisation of ATP.
- Approximately 25% carbon is lost during this process.
- Photorespiration is a characteristic of C_3 plants.
- Three cell organelles are required to complete a turn of PCO cycle, namely ; chloroplast, peroxisome and mitochondria.

Conditions favour photorespiration :

(a) High light intensities : It is considered as main condition for photorespiration. This leads to following two conditions (b & c).

(b) Higher concentration of O_2 and lower concentration of CO_2 inside mesophyll cells. (under such condition RuBisCO binds with O_2 and acts as an oxygenase enzyme)

(c) High temperatures.

Warburg effect :

The Warburg's effect is the decrease in the rate of photosynthesis by high oxygen concentrations. Oxygen is a competitive inhibitor of the carbondioxide fixation by RuBisCO. Furthermore, oxygen promotes photorespiration which reduces photosynthetic output.

Bacterial Photosynthesis		**Plant Photosynthesis**	
(1)	Pigment containing structures are chromatophores.	(1)	Pigment containing structures are thylakoids insode chloroplasts
(2)	Pigments are bacteriochlorophyll and bacterioviridin	(2)	Pigments are chlorophylls and carotenoids
(3)	Its anoxygenic because PS II is absent whose photocenter is B-890 (Ps-II absent)	(3)	Its oxygenic because PS II is present which can photolyse the H_2O.
(4)	any one pigment system is present whose photocenter is B-890 (PS-II absent)	(4)	Two pigments system PS-I (P 700) and PS-II (P 680) are present.
(5)	Action spectrum us infer red.	(5)	Action spectrum is blue-red
(6)	Duringn light reaction NAD^+ being reduced to NADH	(6)	During light reaction $NADP^+$ being reduced to NADPH.

Characteristics	C_3 Plants	C_4 Plants	Choose from
Cell type in which the Calvin cycle takes place	Mesophyll	Bundle sheath	Mesophyll/Bundle sheath/both
Cell type in which the initial carboxylation reaction occurs	Mesophyll	Mesophyll	Mesophyll/Bundle sheath /both
How many cell types does the leaf have that fix CO_2 ?	One	Two	**One:** Mesophyll **Two:** Bundle sheath and mesophyll. **Three:** Bundle sheath, palisade, spongy mesophyll
Which is the primary CO_2 acceptor?	RuBP	PEP	RuBP/PEP/PGA
Number of carbons in the primary CO_2 acceptor	5	3	5 / 4 / 3
Which is the primary CO_2 fixation product ?	PGA	OAA	PGA/OAA/RuBP/PEP
No. of carbons in the primary CO_2 fixation product	3	4	3 / 4 / 5
Does the plant have RuBisCO?	Yes	Yes	Yes/No/Not always
Does the plant have PEP Case?	Yes	Yes	Yes/No/Not always
Which cells in the plant have Rubisco?	Mesophyll	Bundle sheath	Mesophyll/Bundle sheath/none
CO_2 fixation rate under high light conditions	Medium	High	Low/ high/ medium
Whether photorespiration is present at low light intensities ?	Negligible	Negligible	High/negligible/sometimes
Whether photorespiration is present at high light intensities ?	High	Negligible	High/negligible/sometimes
Whether photorespiration would be present at low CO_2 concentrations ?	High	Negligible	High/negligible/sometimes
Whether photorespiration would be present at high CO_2 concentrations ?	Negligible	Negligible	High/negligible/sometimes
Temperature optimum	20-25°C	30-40°C	30-40 C/20-25C/above 40 C
Examples	Wheat Rice	**Maize** **Sugarcane** **Sorghum**	

Factors affecting photosynthesis :

The rate of photosynthesis is vezy important in determining the yield of plants including crop plants. Photosynthesis is under the influence of several factors. Both internal (plant) and external.

(A) Plant factors :

Plant or internal factors are depends on the genetic predisposition and the' growth of the plant.

(1) Number of leaves:
Rate of photosynthesis is directly proportional to number of leaves.

(2) Size of leaves :
Rate of photosynthesis is direetly proportional to size of leaves.

(3) Orientation of leaves:
Orientation of leaf must be in such a way which favour optimum light absorption,

(4) Mesophyll :
For optimum rate of photosynthesis the mesophyll (cortex of leaf) should be well qeveloped.

(5) Number of chloroplast:
Rate of photosYothesis is directly proportional to number of chloroplast.

(6) Amount of chlorophyll :
Rate of photosynthesis is directly proportional to chlorophyll amount.

(7) Age of leaves :
Until leaf attain maturity the rate of photosynthesis increases but along with Senescence its decreases.

(8) Internal CO_2 concentration:
Upto a limit the rate of photosynthesis is directly proportional to internal C02 concentration.

(B) External factors :

The external factors would include :

(1) Sunlight
(2) Temperature
(3) Atmospheric CO_2 concentration
(4) Water

As a plant photosynthesises, all these factors will simultaneously affect its rate. Hence, though several factors interact and simultaneously affect its rate but usually one factor is the major cause or is the one that limits the rate. (Limiting factor)

Law of limiting factor : This law was proposed by Blackman. (1905)

"If a chemical process is affected by more than one factor then its rate will be determined by the factor which is at suboptimal level or nearest to its minimal value : It is the factor which directly affects the process if its quantity is changed."

(1) Light:

Light affect photosynthesis by three different ways :

(i) Light quality **(ii) Light intensity** **(iii) Duration of exposure of light**

(i) Light quality :

Plant capture and utilize sunlight ranging from 400 to 700 nm. This range of incident sunlight termed as photosynthetically active radiation (PAR). PAR has spectral components i.e. VIBGYOR. Most effective spectral components are blue and red while least effective is green.

(ii) Light intensity :

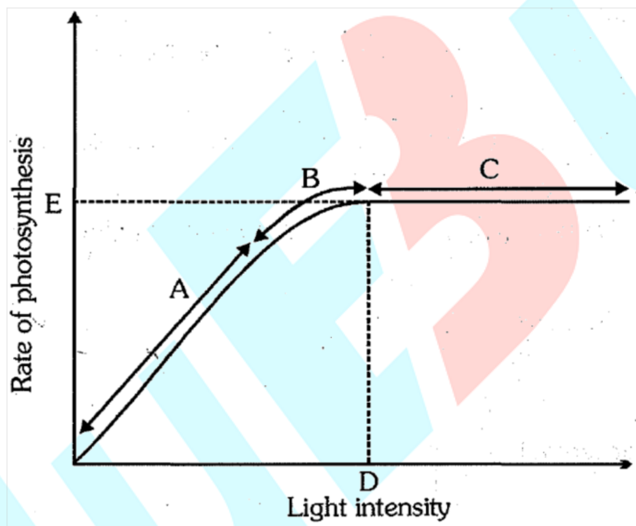
- There is a linear relationship between incident light and cog fixation rates at low light intensities because in this condition light become the limiting factor.
- At higher light intensities, gradually the rate does not show further increase as other factors become limiting.
- Rate of photosynthesis saturates at 10 percent of the full sunlight. At very high light intensity solarisation may occur.

Solarisation includes :

(a) Photo oxidation of pigments, enzymes and photosynthetic carbon (RUBP)

(b) Photo inhibition (Reduction in hydration)

Intensity of light, at which rate of photosynthesis, becomes equal (or compensate) with the rate of respiration in plants is known as light compensation point. (Net photosynthesis or NPP at this- point is zero and no gaseous exchange between plant and atmosphere).



Above graph showing the effect of light on the rate of photosynthesis. Based on the graph. answer the following questions :

Q. At which point/s CA. B or C) in the curve is light a limiting factor ?

Ans. A

Q. What could be the limiting factor Is in region A ?

Ans. Light

Q. What do C and D represent on the curve ?

Ans. C is the point where rate of photosynthesis does not show further increase as other facto' become limiting. D is the point where light saturation occurs.

(iii) Light duration. : ;

Duration of light never affects rate of photosynthesis. (If quality/wavelength and intensity of light are constant).

Note : Plants photosynthesise optimumly in intermittent light.

Although light is a rare limiting factor but under following circumstances light acts as a limiting factor :

- Plants in shade
- In dense forest
- Cloudy sky
- Along with depth in a water body.

(2) Carbon dioxide concentration :

- Carbon-di-oxide is the major limiting factor for photosynthesis. The concentration of CO_2 is very low in the atmosphere i.e.. 0.03 to 0.04 percent or 300 to 400 PPM.
- plants show saturation at about 360 μL^{-1} while C_3 responds to increased CO_2 concentration and saturation is seen only beyond 450 μL^{-1}. Thus, current availability of CO_2 level (0.03 to 0.04%) is limiting to the C_3 plants.
- Increased in CO_2 concentration upto 0.05% can cause an increase in CO_2 fixation rates but beyond this the levels can become damage in over longer periods. (Green house effect).

Green house crops/C_3 plants such as tomatoes and bell pepper show higher photoswthetic rates under CO_2 enriched atmosphere that leads to higher yield. This phenomenon is called CO_2 fertilizing effect.

CO_2 compensation point: It is the point where rate of photosynthesis become equal to the rate of respiration (NPP is zero).

CO_2 compensation point for C_4 plants is 0-10 ppm.

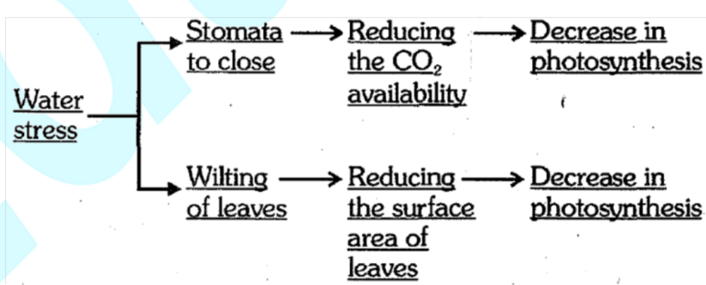
CO_2 compensation point for C_3 plants is 25-100 ppm.

(3) Temperature:

- The dark reaction being enzymatic, is temperature controlled. Though the light reaction is also temperature sensitive but they are affected to a much lesser extent.
- The C_4 plants respond to "higher temperature (30- 40"C) and show higher rate of photos thesis while C_3 plants have a much lower temperature optimum (20-25°C)
- Optimum temperatures also depends on the habitat. Tropical plants have a higher temperature optimum than the plants adapted to temperate climates.

(4) Water:

Water is one of the reactant in the light reaction, the effect of water as a factor is more through its effect on the plant, rather than directly on photosynthesis.



Inhibitors :

They are used as weedicides or herbicides.

- DCMU (Dichlorophenyl Dimethyl urea) I Diuron, CMU I Monouron and PAN (peroxy acetylnitrates) inhibit photosynthesis by blocking PS-II as they stop electron flow between P 680 and PQ.

- Diquat, Paraquat, (viologen dyes) inhibit cyclic photophosphorylation by blocking electron flow between P 700 and Fd.

BEGINNER'S BOX-2

1. In Calvin cycle for the fixation of 5 molecules of CO_2, how many ATP and NADPH are required in reduction step?
(1) 18 ATP and 12 NADPH (2) 15 ATP abd 10 NADPH
(3) 10 ATP and 10 NADPH (4) 3 ATP and 2 NADPH

2. Dark reactions of photosynthesis :
(1) Occur in darkness
(2) Are not light dependent
(3) Are not directly light driven but are dependent on light
(4) Occur in both presence of light and in darkness

3. There is a linear relationship between light and C02 fixation rates at .... intensities. At .... intensities the rate does not show further increase as other factors become limiting. Choose the correct terms for blanks respectively-
(1) high, light low light (2) low, light, low light
(3) high, light, high light (4) low light, high light

4. In photorespiration –
(1) Neither sugar nor ATP is synthesised (2) Sugar is synthesised
(3) ATP is synthesised but not sugar (4) Both ATP and sugar are synthesised

5. In the biosynthetic phase of photosynthesis the first C02 fixation product is a 3-carbon organic acid. This fact was discovered by Melvin Calvin :-
(1) By the use of O^{18} in bacterial photosynthesis
(2) By the use of ^{14}C in the 'photo' Synthesis of C_3 angiosperm plant
(3) By the use of N^{15} in the photosynthesis of maize plant
(4) By the use of ^{14}C in the algal photosynthesis

ANSWER KEY

BEGINNER'S BOX-1

1.	(4)	**2.**	(4)	**3.**	(3)	**4.**	(3)	**5.**	(1)

BEGINNER'S BOX-2

1.	(3)	**2.**	(3)	**3.**	(4)	**4.**	(1)	**5.**	(4)