

8. JULIUS VON SACHS (1854): Showed that the process of photosynthesis takes place in chloroplasts and results in the synthesis of starch. He also showed that chlorophyll is confined to chloroplast.
9. GG STOCKS (1864): Obtained pure fraction of chlorophyll –a and b and detected the presence of chlorophyll – c.
10. ENGELMANN (1888): Plotted the action spectrum of photosynthesis.
11. FF BLACKMAN (1905): Noted that photosynthesis is a two step process. A dark reaction also occurs along with photochemical reaction. He also proposed the law of limiting factor.
12. WILLSTATTER AND STOLL (1913, 1918): Showed detailed account of chemical composition and functioning of chlorophyll.
13. WARBERG (1920): Flash light experiment with chlorella as useful material for photosynthesis experiments.
14. VAN NEIL (1931): Showed that the photosynthetic bacterial fixed CO₂ in the presence of H₂S. He postulated that the plants evolve O₂ by splitting H₂O not CO₂.
15. EMERSON AND ARNOLD (1932): Recognised light reaction consists of two distinct photochemical process. They showed that about 2500 chlorophyll molecules are required to fix one molecule of CO₂ in photosynthesis.
16. ROBIN HILL (1937): Isolated chloroplast suspended in water in presence of suitable hydrogen acceptor which evolve oxygen in presence of light. He demonstrated that the source of O₂ evolved during photosynthesis is water and not CO₂.
17. RUBEN AND KAMEN(1941): Used radioactive oxygen O¹⁸ and proved that oxygen evolved was part of water.
18. ARNON, ALLEN AND WHATLEY(1954): Demonstrated that fixation of CO₂ by chloroplast using C¹⁴O₂.
19. MELVIN CALVIN(1954): Traced the path of carbon in photosynthesis using unicellular algae chorella. Melvin calvin gave C₃-cycle and was awarded Nobel Prize in 1960 for the discovery
20. PARK AND BIGGINS(1961): Discovered quantosome 100 Angstrom thick and stated that it contains about 230 chlorophyll molecules.
21. HATCH AND SLACK(1967): Discovered C₄ pathway for fixation of CO₂
22. HUBER, MICHEL AND DISSENHOFER (1985): Crystallised photosynthesis reaction centre of bacterium Rhodobacter and got Nobel Prize in 1988.

RAW MATERIALS FOR PHOTOSYNTHESIS

- In green plants including algae, photosynthesis takes place in chloroplasts of the cells. During this process, solar energy is trapped and synthesis of carbohydrates takes place from carbon dioxide and water. This sunlight, carbon dioxide, water, chloroplast are important components necessary for plants to derive the process of photosynthesis.

SUNLIGHT

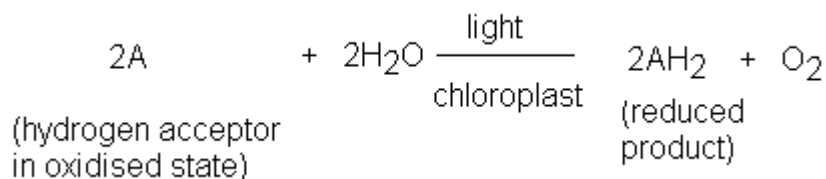
- Photosynthesis is a light dependent process. The literal meaning of word "Photosynthesis" is " the synthesis, with the help of light". To drive photosynthesis in plants, sunlight provides solar energy. Only 0.2% of the light energy, incident on earth is actually used by photo autotrophs.
- Light is the visible radiation which represents a very small portion of the total electromagnetic spectrum of radiation, emitted by the sun. Visible light (approx. between 400nm to 700 nm) causes the physiological sensation of vision of man. Visible light is actually a combination of several colours of different colours viz. Violet (400nm to 425 nm), blue (425nm to 490nm), green (490 – 550 nm), yellow (550 -585 nm), orange (585 – 640 nm) and red (640 – 700 nm)
- The most effective regions of visible light spectrum responsible for maximum photosynthesis in plants are blue and red regions of which red light is most effective. On the other hand, green light is least effective. Photosynthesis cannot take place beyond the range of visible spectrum.

CARBON DIOXIDE

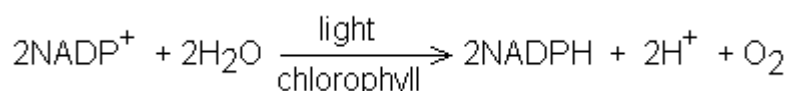
- In land plants, carbon dioxide is obtained from the atmosphere through the stomata. Small quantities of carbonates are also absorbed from soil through the roots. Hydrophytes get their carbon dioxide supply from the aquatic environment as bicarbonates. The latter are absorbed by hydrophytes through their general surface.

WATER

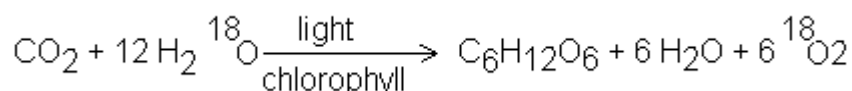
- In the process of photosynthesis, the source of liberated oxygen is water. Photosynthetic land plants absorb a large amount of water from the soil through the root hairs-present on their roots. But relatively very small amount of this absorbed water is used in the process of photosynthesis. Aquatic photosynthesis plants absorb water through their body surface.
- As mentioned earlier, Van Niel (1931) hypothesized that the photosynthetic organisms require a source of hydrogen. He proposed that oxygenic photosynthesis is an oxidation reduction reaction where hydrogen of water reacts with carbon dioxide to form organic compounds
- 1937, Robin Hill demonstrated that in absence of carbon dioxide, isolated chloroplasts of *Stellaria media* produced oxygen when they were illuminated in presence of hydrogen acceptor. Here ferricyanide is reduced to ferrocyanide by photolysis of water. This is Hill reaction and can be represented as



- The hydrogen acceptor is often called as Hill oxidant or Hill reagent. In plants, NADP⁺ (Nicotinamide adenine dinucleotide phosphate) acts as a hydrogen acceptor.



- In 1941, by using non-radioactive heavy isotope of oxygen (O¹⁸), Ruben and Kamen proved that during photosynthesis, oxygen comes from the water.



CHLOROPLASTS

- Chloroplasts (Chloros = green, plastos = moulded) are the green plastids which occur in all the green parts of the plants.
- They are the actual sites of photosynthesis.
- The chloroplasts contain chlorophyll and carotenoid pigments which are responsible for trapping light energy essential for photosynthesis.
- Majority of the chloroplasts of the green plants are formed in the mesophyll cells of the leaves.
- They are lens shaped, oval, spherical, discoid or even ribbon like organelles having variable length (5- 10 mm) and width (2 -4 mm).
- The chloroplasts are double membrane bound, each membrane are 9-10 nm in thickness. The space limited by the inner membrane of the chloroplast is called the stroma. It is the site of dark reaction.
- A number of organized flattened membranous sac called the thylakoids are arranged in stacks like piles of coins called grana. Thylakoids lying outside the grana are called stroma, thylakoids or the intergrana thylakoids
- Each granum may contain 20 to 50 thylakoid discs. There may be 40 – 60 grana per chloroplasts.
- The major function of thylakoids is to perform photosynthetic light reaction (photochemical reaction)
- The pigments and other factors of light reaction are usually located in thylakoid membranes.

- Cyanobacteria and other photosynthetic bacteria do not possess chloroplasts. However, the photosynthetic pigments which lie freely in the cytoplasm. These photosynthetic pigments are also different from those of eukaryotes.
- Thylakoids possess four types of major complexes; photosystem I, photosystem II, cy b₆ – f complex and coupling factor (ATP synthetase)
- Photosystem II is thought to mostly occur in the appressed or partition to mostly occur in the appressed or partition regions of granal thylakoids while photosystem I lies in the non-appressed parts as well as stroma thylakoids.

PHOTOSYNTHETIC PIGMENTS

(i) Chlorophylls

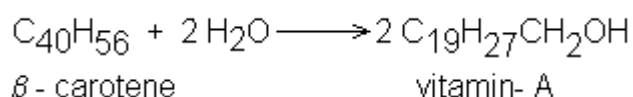
It is a green pigment which traps solar radiation and convert light energy to the chemical energy. Generally, it is of two types.

- (a) Chlorophyll –a (C₅₅H₇₂O₅N₄Mg): It participates directly in the light reactions of photosynthesis has a head called a porphyrin ring with a magnesium atom at its centre. Attached to the porphyrin is a hydrocarbon tail, which interacts with hydrophobic regions of proteins in the thylakoid membrane.
- (b) Chlorophyll-b (C₅₅H₇₀O₆N₄Mg): It differs from chlorophyll-a only in one of the functional group bonded to porphyrin. This diagram simplifies by placing chlorophyll at the surface of the membrane; most of the molecules are actually immersed in the hydrophobic core of the membrane.

(ii) Carotenoids

These are yellow, brown and orange pigments, which absorb light strongly in blue-violet range. These are called shield pigments, because they protect chlorophyll from photo oxidation by light intensity and also from oxygen produced during photosynthesis. Along with chlorophyll-b, the carotenoids are also called as accessory pigments, because they absorb energy and give it to chlorophyll-a. carotenoids are two types:

- (a) Carotenes: Carotenes consists of an open chain conjugated double bond system ending on both the sides with ionone rings. They are hydrocarbons with molecular formula C₄₀H₅₆ carotenes are orange in colour. The red colour of tomato and chillies is, because of carotene call lycopen. The common carotene is β-carotene which is converted to vitamine-A by animals and humans



- (b) Xanthophylls: Also known as carotenols. These are similar to carbon, but differ in having two oxygen atoms is the form of hydroxyl, carboxyl group attached to the ionone rings. Their molecular formula is C₄₀H₅₆O₂. The yellow colour of autumn leaves is due to lutein and a characteristics xanthophylls of brown algae is fucoxanthin.

(iii) Phycobilins

Phycobilins consist of four pyrrol rings and lack Mg and phytol tail. The phycobilin pigments are of two types.

(a) Blue – Phycocyanin, allophycocyanin

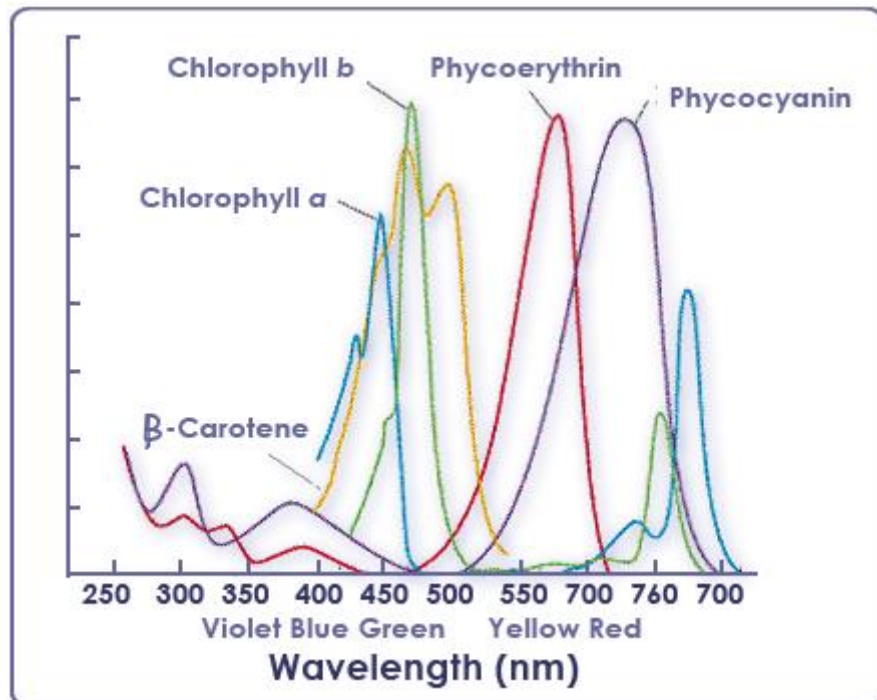
(b) Red – phycoerythrin

These pigments are useful in chromatic adaptations. Phycoerytherin transfer energy to phycocyanin which in turn transfer energy to carotenoids which is ultimately received by chlorophyll –a.

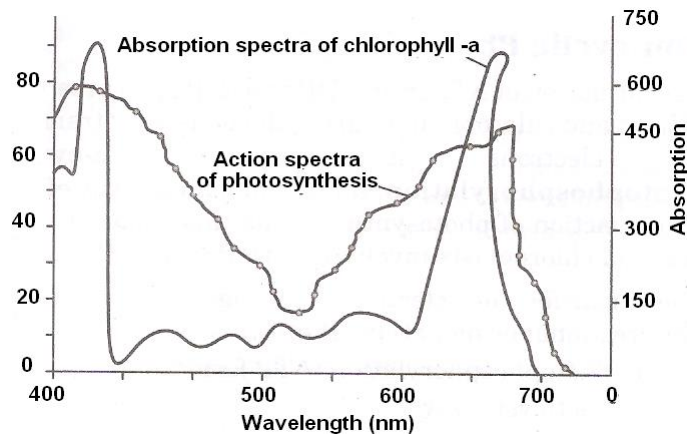
- The chlorophylls, carotenoids and phycobilins together form a complex of pigment in thylakoid membrane. These complexes work for the absorption of light and its transfer to a reaction center. These complexes are called photosynthetic unit or photosystem or pigment system. These system show clear division of labour. Some pigments called as accessory pigments such as carotenoids act to receive the light. They basically harvest the light molecules towards a reaction center thus, also called as Light Harvesting complexes (LHC). Chlorophyll-a act as reaction center and perform further reaction of photosynthesis.

ABSORPTION SPECTRUM AND ACTION SPECTRUM

- The graphic representation of curve depicting the various wavelength of light absorbed by a substance is known as absorption spectrum. Chlorophyll mostly absorb light radiations in blue (more) and red parts of light spectrum (430 to 662 nm for chlorophyll a, 455 and 604 nm for chlorophyll b)



- Action Spectrum: It is a graphical representation of curve depicting the rate of photosynthesis in various wavelengths of light.
- Fluorescence: It is property of almost immediate emission of long wave radiation by substances after attaining excited state on receipt of light energy e.g. Chlorophyll
- Phosphorescence: the delayed emission of long-wave radiations from an activated molecule is called phosphorescence. It continues for some time after removal of irradiation source.



PHOTOSYNTHETIC UNIT

- It is the smallest group of photosynthetic pigment molecules which can pick up light energy and convert it into chemical form. A photosynthetic unit has 250-400 pigment molecules. It has a photocentre of chlorophyll a molecules surrounded by harvesting molecules differentiated into core molecules and antenna molecules
- Antenna molecules are meant for absorbing radiation energy of different wavelengths. On absorbing a photon of light, the pigment molecule enters excited state. In this state the electrons move into outer orbital. The excited state lasts for 10^{-9} seconds. In this period the excited antenna pigment molecule transfer its energy to a core molecule through resonance. If this does not happen, the energy is lost as fluorescence. The core molecules pass over their energy to trap centre or photocentre. The frequency of excitation is very high. It is met by collaboration of core and antenna molecules. Each time the trap centre or photocentre gets excited, it expels an electron and becomes oxidized. An electron is required to convert it to normal state.

PHOTOSYSTEM I (PS I)

- It is a photosynthetic pigment system along with some electron carriers that is located on both the nonappressed part of grana thylakoids as well as stroma thylakoids.
- PS-I has more of chlorophyll a
- Chlorophyll b and carotenoids are comparatively less.

- Photosystem I has a reducing agent X which is special chlorophyll P_{700} molecule, FeS centre B or ferredoxin, plastoquinone, cytochrome complex and plastocyanin.
- It takes part in both cyclic and non-cyclic photophosphorylation.
- PS-I can carry on cyclic phosphorylation independently.
- Normally it drives an electron from photosystem II to $NADP^+$

PHOTOSYSTEM II (PS II)

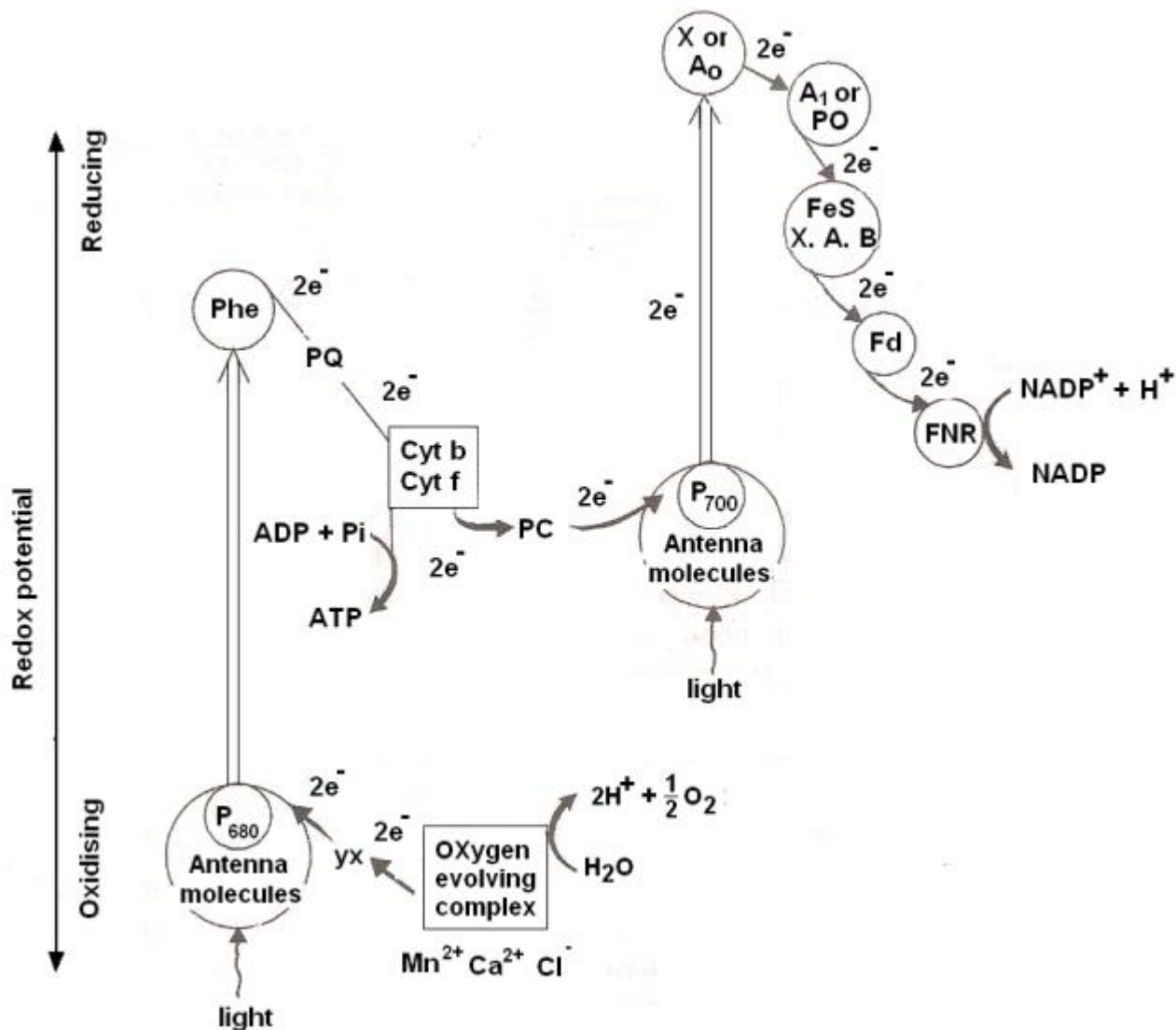
- It is a photosynthetic pigment system alongwith some electron carriers that is located in the appressed part of grana thylakoids.
- PS –II has chlorophyll a,b and carotenoids.
- Chl a and Chl b contents are equal.
- Carotenoid content is higher as compared to that of PS I
- The photocentre is a special chlorophyll a molecule called P_{680}
- It is surrounded by other chlorophyll a molecules, chlorophyll b and carotenoid molecules
- PS II also contains Mn^{2+} , Cl^- , quencher molecules Q, plastoquinon (PQ), cytochrome complex and plastocyanin.
- It picks up electron released during photolysis of water.
- The same is extruded on absorption of light energy.
- As the extruded electron passes over cytochrome complex, sufficient energy is released to take part in the synthesis of ATP from ADP and inorganic phosphate.
- This photophosphorylation is non-cyclic.
- PS II can operate only in conjugation with PS I

NON – CYCLIC PHOTOPHOSPHORYLATION

- It is the normal process of photophosphorylation in which the electron expelled by the excited photocentre does not return to it.
- Non-cyclic photophosphorylation is carried out in collaboration of both photosystem I and II.
- Electron released during photolysis of water is picked up by photocentre of PS II called P_{680} .
- The same is extruded out when the photocentre absorbs light energy.
- The extruded electron has an energy equivalent to 23 kcal / mole
- It passes through a series of electron carriers phaeophytin, PQ. Cytochrome $b_6 - f$ complex and plastocyanin.
- While passing over cytochrome complex, the electron loses sufficient energy for the synthesis of ATP.

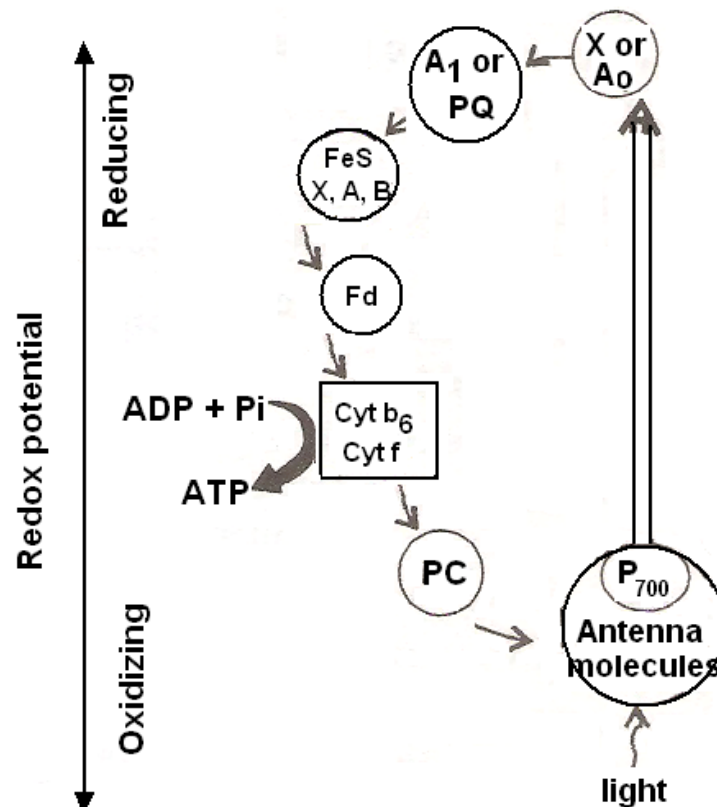
PHOTOSYNTHESIS

- The electron is handed over to photocentre P₇₀₀ of PS I by plastocyanin. P₇₀₀ extrudes the electron after absorbing light energy. The extruded electron passes through special chlorophyll P₆₈₀ molecules, Fe-S, ferredoxin, to finally reach NADP⁺
- The latter then combines with H⁺ with the help of NADP – reductase to form NADPH.
- This is called Z scheme due to its characteristics zig-zag shaped based on redox potential of different electron carriers.
- Non-cyclic photophosphorylation or Z-scheme is inhibited by CMU and DCMU.
- DCMU (Dichlorophenyldimethyl urea) is a herbicide which kills the weed by inhibiting CO₂ fixation as it is strong inhibitor of PS II



CYCLIC PHOTOPHOSPHORYLATION

- It is a process of photophosphorylation in which an electron expelled by the excited photocentre is returned to it after passing through a series of electron carriers.
- It occurs under conditions of low light intensity, wavelength longer than 680nm and when CO₂ fixation is inhibited.
- Absence of CO₂ fixation results in non-requirement of electrons for formation of NADPH
- Cyclic photophosphorylation is performed by photosystem I only.
- Its photocentre P₇₀₀ extrudes an electron with gain of 23 kcal/mol of energy after absorbing a photon of light.
- After losing the electron the photocentre becomes oxidized.
- The expelled electron passes through a series of carriers including P₇₀₀ chlorophyll molecules, plastoquinone (PQ), FeS complex, ferredoxin (Fd) cyt b₆ – f and plastocyanin before returning to photocentre.
- Over the cytochrome complex (cyt b₆-f), the electron creates a proton gradient for synthesis of ATP from ADP and inorganic phosphate.
- Halobacteria or halophile bacteria also perform photophosphorylation but ATP thus produced is not used in synthesis of food. These bacteria possess purple pigment bacteriorhodopsin attached to plasmamembranes. As light falls on the pigment, it creates a proton pump which is used in ATP synthesis.
- Cyclic photophosphorylation is the most effective anaerobic phosphorylation mechanism.



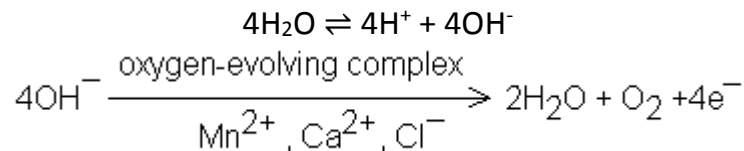
CHEMIOSMOTIC HYPOTHESIS OF ATP FORMATION

- The view was propounded by Peter Mitchell in U.K. in 1961 in the case of mitochondria and chloroplast.
- Mitchell's chemiosmotic theory was confirmed by G.Hind and Andre jagendorf at cornell university in 1963.
- According to this view, electron transport, both in respiration and photosynthesis produces a proton gradient (pH gradient)
- The gradient develops in the outer chamber or inter-membrane space of mitochondria and inside the thylakoid lumen in chloroplasts.
- Lumens of thylakoid becomes enriched with H^+ ion due to photolytic splitting of water.
- Primary acceptor of electron is located on the outer side of thylakoid membrane.
- It transfer its electrons to a H-carrier. The carrier removes a proton from matrix while transporting electron to the inner side of membrane.
- The proton is released into the lumen while the electron passes to the next carrier.
- NADP reductase is situated on the outside of thylakoid membrane.
- It obtains electron from PS I and protons from matrix to reduce $NADP^+$ to $NADP + H^+$ state.
- The consequences of the three events is that concentration of proton decreases in matrix or stroma region while their concentration in thylakoid lumen rises resulting in decrease in pH.
- A proton gradient develops across the thylakoid.
- The proton gradient is broken down due to movement of protons through transmembrane channels, cF_0 of ATPase ($cF_0 - F_1$ particle).
- The rest of the membrane is impermeable to H^+ , cF_0 provides facilitated diffusion of H^+ or protons.
- As the protons move to the other side of ATP, they bring about conformational changes in cF_1 particle of ATPase or coupling factor.
- The transient cF_1 particles of ATPase enzyme from ATP from ADP and inorganic phosphate.
- Therefore, ATP synthesis through chemiosmosis requires a membrane, a proton pump, a proton gradient and $cF_0 - cF_1$ particle or ATP-ase
- One molecule of ATP is formed when $3H^+$ used by the ATP synthase.

LIGHT REACTION (Photochemical phase).

- It occurs inside the thylakoids, especially those of grana regions.
- Photochemical step is dependent upon light. The function of this phase is to produce assimilatory power consisting of reduced co-enzyme NADPH and energy rich ATP molecules.
- Photochemical phase involves photolysis of water and production of assimilatory power.
- The phenomenon of breaking up of water into hydrogen and oxygen in the illuminated chloroplast is called photolysis of water.

- Light energy, an oxygen evolving complex (OEC) and an electron carrier are required.
- Oxygen evolving complex was formerly called Z-enzyme
- It is attached to the inner surface of thylakoid membrane.
- The enzyme has four Mn ions. Light energized changes in Mn (Mn^{2+} , Mn^{3+} , Mn^{4+}) removes electrons from OH^- component of water forming oxygen.
- Liberation of O_2 requires two other ions Ca^{2+} and Cl^- .
- Electron carrier transfer the released electrons to P_{680}



- The electron released during photolysis of water are picked up by P_{680} photocentre of photosystem II.
 - On receiving a photon of light energy the photo-centre expels an electron with a gain of energy (23 kcal/mole).
 - It is the primary reaction of photosynthesis which involves the conversion of light energy into chemical form.
 - The phenomenon is also known as quantum conversion.
 - The electron extruded by the photocentre of photosystem II is picked up by the quencher phaeophytin.
 - From here the electron passes over a series of carriers in a downhill journey losing its energy at every step.
 - The major carriers are plastoquinone (PQ) cytochrome b-f complex and plastocyanine (PC).
 - While passing over cytochrome complex, the electron loses sufficient energy for the creation of proton gradient and synthesis of ATP from ADP and inorganic phosphate by the process of photophosphorylation.
 - From plastocyanin the electron is picked up by the trap centre P_{700} of photosystem I.
 - On absorbing a photon of light energy, P_{700} pushes out the electron with a gain of energy.
 - The electron passes over carriers, FeS, ferredoxine and NADP-reductase.
 - The latter gives electron to $NADP^+$ for combining with H^+ ions to produce NADPH.
- $$NADP^+ + 2e^- + H^+ \xrightarrow{\text{NADP reductase}} NADPH$$
- NADPH is a strong reducing agent. It constitutes the reducing power which is also contains a large amount of chemical energy.

DARK REACTION (Biosynthetic phase)

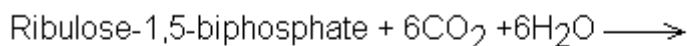
- Dark reaction of photosynthesis occurs in presence of or absence of light i.e. independent of light.
- Dark reaction occurs in stroma fraction of the chloroplast.
- Dark reaction is purely enzymatic reaction and is slower than light reaction of photosynthesis.
- Dark reaction was first of all established in detail by Dr. Calvin, Benson and J. Bassham and for this work they were given Nobel prize (1961).
- The techniques used for studying different steps were radioactive tracer technique using ^{14}C chromatography and autoradiography and the material used were chlorella and scenedesmus. These are microscopic, unicellular algae and can be easily maintained in laboratory.
- Dark reaction is also named as Blackman's reaction.

C_3 - PATHWAY OR CALVIN CYCLE

- The details of the step involved in the dark reaction were discovered by Professor M. Calvin and hence the dark reaction known to be called as Calvin cycle.
- This is the major pathway for the fixation of carbon dioxide in green plants. It represents phase II i.e. dark reaction. It takes place in the stroma of the chloroplasts.
- The reactions are enzyme. Controlled and temperature dependent. After the fixation of carbon dioxide, the first stable compound formed is 3-carbon phosphoglyceric acid (PGA). Hence, it is also called the C_3 – pathway.
- Calvin cycle can be described under three stages:

(a) Carboxylation of RUBP:

- In this process there is fixation of atmospheric CO_2 into a stable organic compound with the help of enzyme RuBP, Carboxylase-oxygenase or RuBisCO



3 phosphoglyceric acid (3PGA)

(b) Reduction of CO_2

- The 3-C PGA then undergoes reduction with the help of the assimilatory power to form 3-c phosphoglycerldehyde (PGAL). NADPH_2 provides the hydrogen and ATP supplies energy for the reduction. Enzyme triphosphate dehydrogenase catalyses the reaction.
- Some molecules of PGAL are converted into another triosephosphate called Dihydroxy Acetone Phosphate (DHAP) in presence of enzyme phosphor triose isomerase.

- The formation of sugars (end products of photosynthesis), the 3-C triose phosphates (PGAL 3-C and PHAP 3-C) to form 6-C hexose sugar fructose 1,6-biphosphate in the presence of enzyme aldolase.
- Fructose biphosphate is the diphosphorylated first to fructose monophosphate and then to fructose (6-C) in the presence of enzyme phosphatase. Some fructose monophosphate molecules may be isomerised into glucose monophosphate by the enzyme isomerase and then into glucose (6-C). The hexose sugar may be further converted to sucrose (C₁₂H₂₂O₁₁) or to starch (C₆H₁₀O₅)_n and are stored in storage cells.

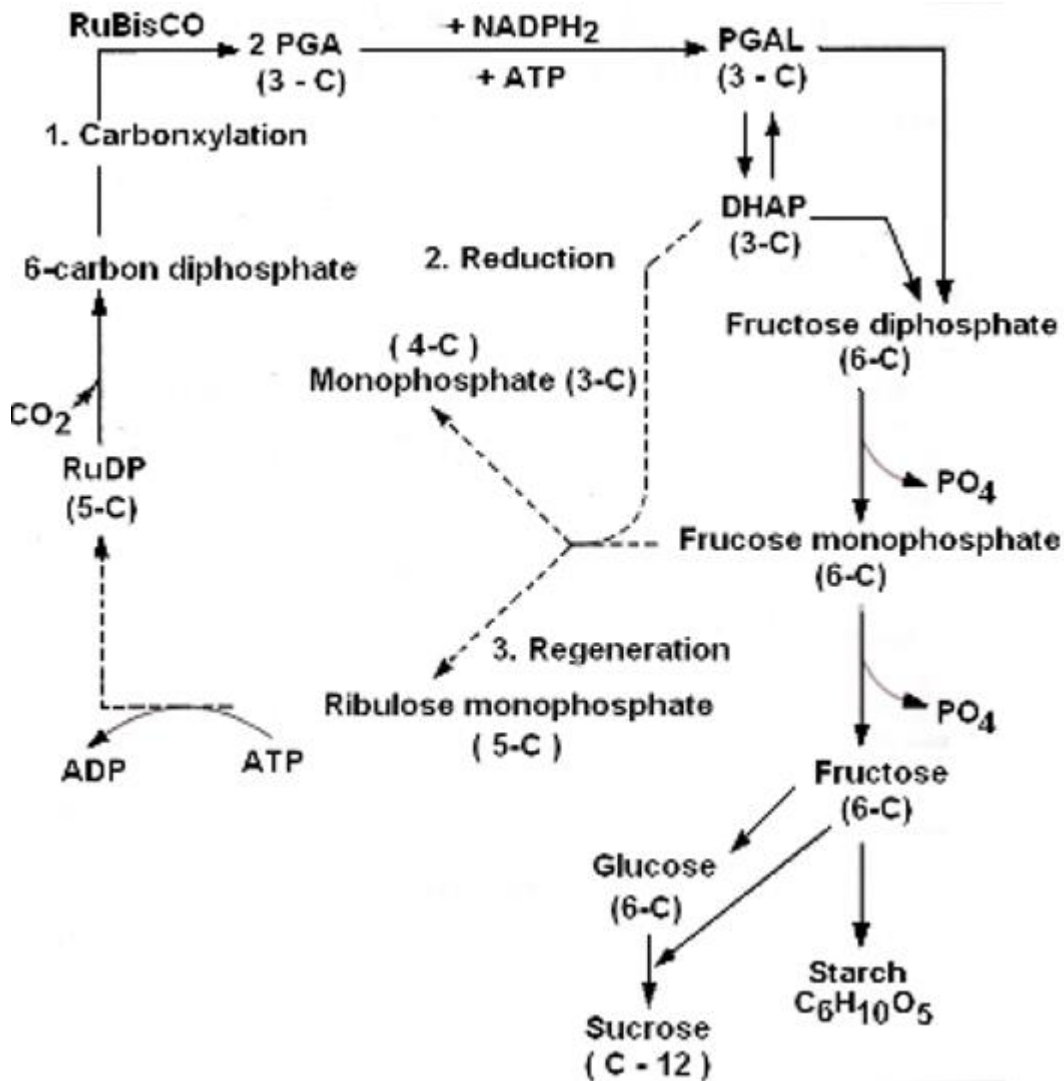
(c) Regeneration of RuBP

- The 5-C RuBP is constantly required for the fixation of CO₂ in the calvin cycle. It is regenerated through another chain of reactions.
- Some molecules of triosephosphate and fructose monophosphates are used from the calvin cycle for the formation of RuBP to be used again to combine with CO₂
- The net reaction of calvin cycle can be represented by



Balnce sheet of calvin cycle

IN	OUT
6CO ₂	1 glucose
18 ATP	18 ADP
12 NADPH	12 NADP



C₄ – PATHWAY OR HATCH AND SLACK PATHWAY

- In some plants, the first stable product, after the fixation of CO₂, is 4-C dicarboxylic acid called oxaloacetic acid (OAA), such plants are called C₄ plants and path of carbon (dark reaction) is called C₄ – pathway.
- It was first noticed by Kortschak (1964) in the photosynthesis of sugarcane leaves. However details of the C₄ – pathway, were worked out by Hatch and Slack (1966). Therefore, it is called Hatch and slack pathway.

ANATOMICAL PECULIARITIES OF C₄ – PLANTS

- (a) The leaf mesophyll consists of compactly arranged cells.
- (b) It is not differentiated into palisade and spongy mesophyll as in C₃ plants
- (c) The vascular bundles (veins) in the leaves are surrounded by a distinct bundle sheath of radially enlarged parenchyma cells.
- (d) The chloroplast in leaf cells are dimorphic i.e. granal and agranal chloroplast

- Chloroplasts in mesophyll cells are smaller and possess grana.
- Chloroplasts in the bundle sheath cells are larger and without grana.

This type of leaf anatomy in C_4 -plants is called as Kranz anatomy

IMPORTANT STEPS IN C_4 – PATHWAY

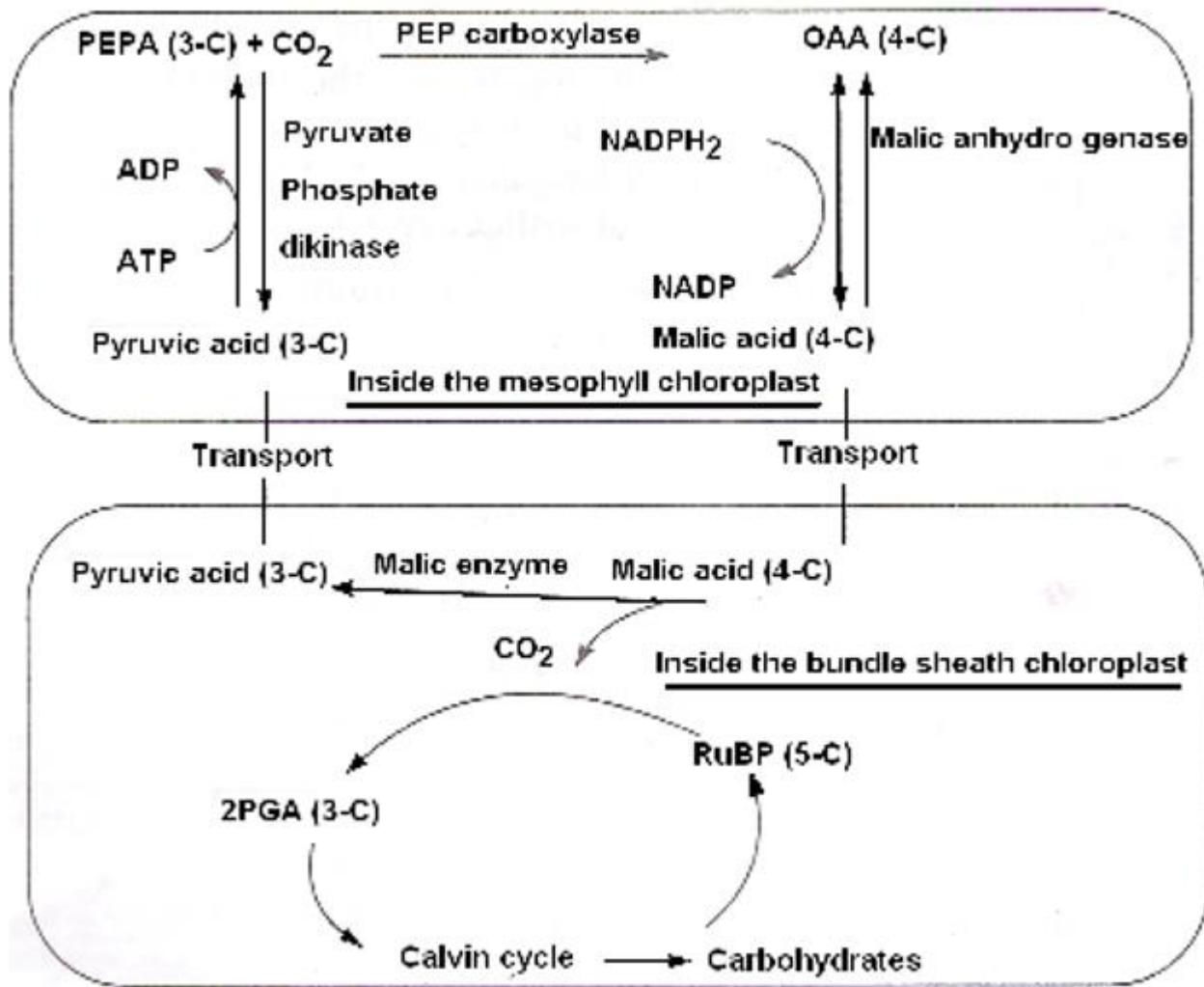
- (a) First part reactions are completed in the stroma of the chloroplasts in mesophyll cells.
(b) Second part, reactions are completed in the stroma of the chloroplasts in bundle sheath cells.

Part I (in mesophyll cells)

- First CO_2 fixation: In this pathway, the first CO_2 acceptor is 3-C phosphoenol Pyruvate (PEP), CO_2 first combines with 3-C PEP to form 4-C OAA (oxaloacetic acid). As OAA is a dicarboxylic acid pathway.
- 4-C OAA is converted into 4-C malic acid or 4-C aspartic acid and transported to bundle sheath cells.

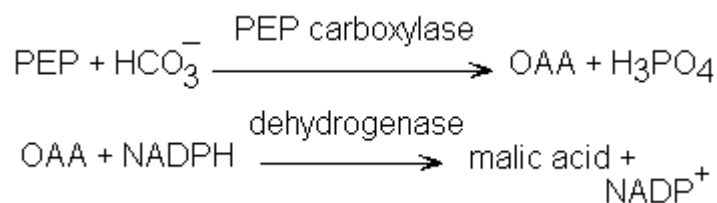
Part II (in bundle sheath cells)

- In the chloroplasts of bundle sheath cells, 4-C malic acid undergoes decarboxylation to form CO_2 and 3-C pyruvic acid.
 - Second CO_2 fixation: The CO_2 released in decarboxylation of malic acid combines with 5-C RuBP (Ribulose 1,5-biphosphate) to form 2 molecules of 3-C PGA. Further, the conversion of PGA to sugar is the same as in the calvin cycle.
 - The pyruvic acid produced in decarboxylation of malic acid is transported back to the mesophyll cells. Here, it is converted to phosphoenol pyruvic acid (PEPA) and again made available for the C_4 -pathway.
- In C_4 pathway when carbon dioxide fixation take place, an additional 2 molecules of ATP per molecule of CO_2 fixed are also required to convert pyruvic acid to phosphoenol pyruvic acid. Thus in C_4 cycle in all 30ATPs are required for fixing 6 molecules of carbon dioxide.



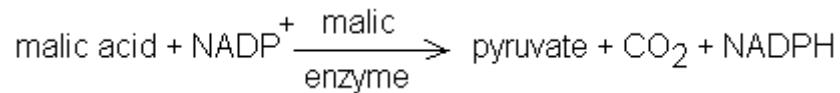
CAM (Crassulacean Acid Metabolism) PATHWAY

- In the member of crassulaceae, cactaceae, agavaceal, orchidaceae, CO₂ fixation occurs during night only.
- In succulents belonging to the above families the stomata remain closed during day time in order to reduce transpiration and the stomata open during night.
- In CAM plants OAA is formed due to carboxylation as in C₄ plants.
- Like C₄ plants, OAA is reduced to make malic acid in CAM plants and is accumulated in the vacuole.
- Absorption of CO₂ during night and its storage as organic acid (malic acid) is called acidification.

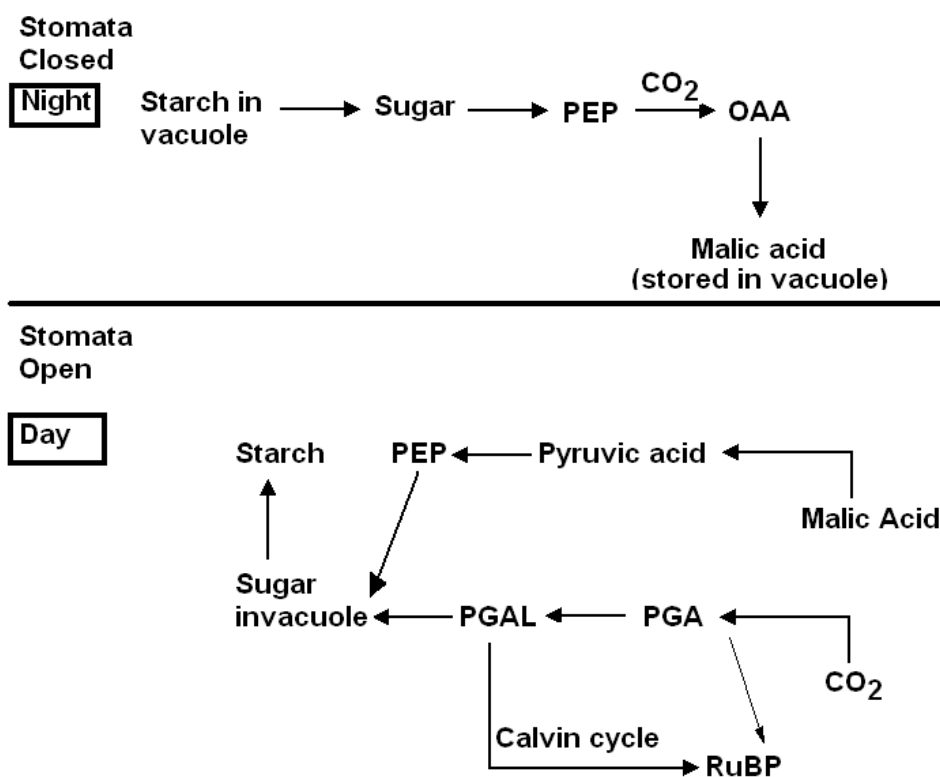


- During day time malic acid undergoes oxidative decarboxylation nad CO₂ is released.

- Liberation of CO₂ from an organic acid during day time is called deacidification.



- The diurnal acidification and deacidification during the night and day time respectively is called CAM
- In C₄ plants, initial carboxylation and final carboxylation is separated by space but in CAM plants, they are separated by time.
- All reactions of CAM occurs in mesophyll cells.
- Chloroplasts are absent in bundle sheath cells of CAM plants
- CAM pathway is important for the survival of succulents



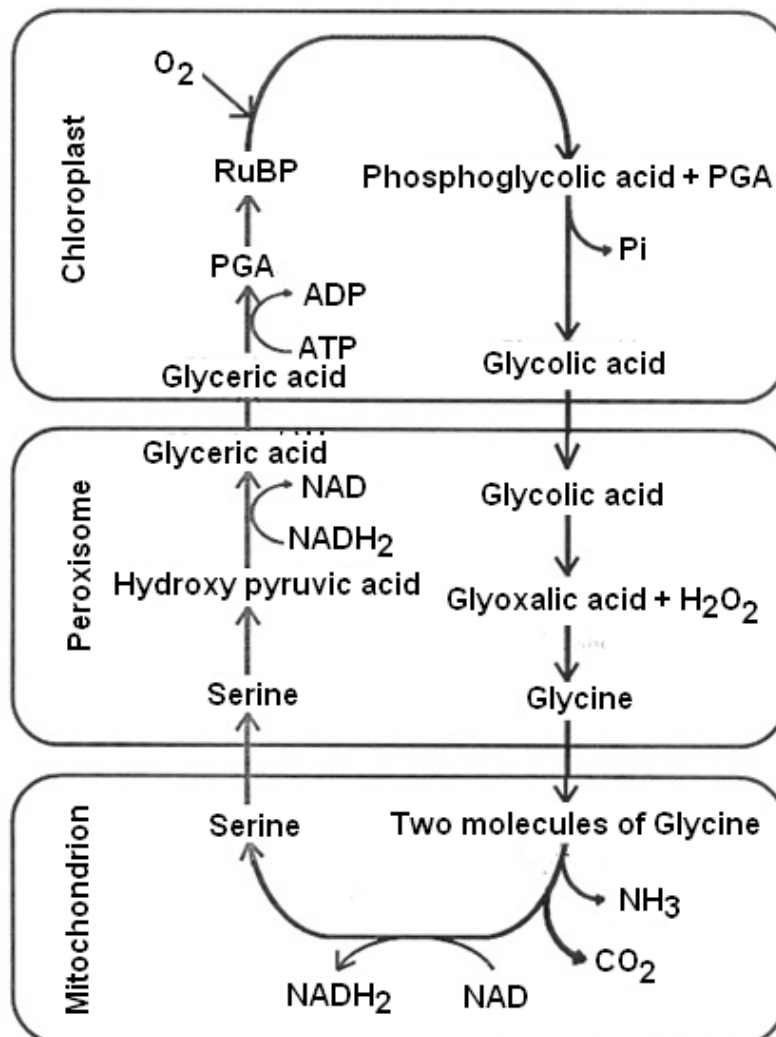
NUMBER OF ATP AND NADPH REQUIRED FOR 1CO₂ FIXATION

PATHWAY	ATP	NADPH
C ₃ cycle	3	2
C ₄ cycle	5	2
CAM	6.5	2

PHOTORESPIRATION

- It was first observed by Otto Warburg (1920) that presence of high O₂ concentration and high temperature decreases the rate of photosynthesis. Later it was demonstrated by Dicker and Tijo (1959) in tobacco.

- RuBisCO is most abundant enzyme and it has affinity to both CO_2 and O_2 . In C_3 – plants, when there is higher O_2 concentration and temperature, O_2 binds with RuBisCO instead of CO_2 and form one molecule of phosphoglycerate and phosphoglycolate in pathway called photorespiration, so there is neither synthesis of sugars, nor of ATP. Instead it results in the release of CO_2 with the utilization of ATP. In photorespiratory pathway there is no synthesis of ATP and NADPH.
- The process can be understood in the following steps.
 1. Oxygen binds with RuBP oxygenase to form phosphoglycolate in chloroplast which gets converted to glycolate and transported to peroxisomes.
 2. In peroxisome it forms glyoxylate and then glycine.
 3. Glycine then enters mitochondria and loses NH_4 and CO_2 in a reaction and it form serine.
 4. Serine is transported to perioxisomes and in a series of reaction it form glycerate which gets converted to PGA and then RuBP in the chloroplast.
 5. So, here we can see, there is no fixing of CO_2 instead CO_2 is given off along with NH_4 . Thus it reduces the rate of photostntthesis in C_3 plants



PRINCIPLE OF LIMITING FACTORS (Blackman, 1905)

- When a process is conditioned as to its rapidity by number of separate factors, the rate of process is limited by the pace of the slowest factor. In other words, at one time only one factor limits the rate of the process. It is called limiting factor. A limiting factor is that factor which is deficient to such a extent that increase in its value directly increases the rate of the process.

FACTORS AFFECTING PHOTOSYNTHESIS

- The light reaction totally depends on the availability of light, water, pigments etc and the dark reaction depends on the temperature and available CO₂

EXTERNAL FACTORS

- Light: In photosynthesis light is converted to chemical energy in the food formed.
 - (i) Light intensity – Light intensity required to get the optimum value differs with different species. Usually with increase in light intensity increase in rate is noticed. The value of light saturation at which further increase in photosynthetic rate is not accompanied by an increase in CO₂ uptake is called light saturation point.
 - (ii) Light quality- Blue and red light of the spectrums is said to be the best for the photosynthesis. The maximum photosynthesis is shown to occur in the red part of the spectrum with the next peak in blue part. The green light has inhibitory effect.
 - (iii) Light duration – Generally photosynthesis is independent of light duration. It is more in intermittent light than continuous light.
- Carbon dioxide: Carbon dioxide is present in low concentration and form about 0.03% of total atmosphere CO₂ is natural limiting factor of photosynthesis. If the concentration of CO₂ is increased from 0.03% to 1%, the rate of photosynthesis increases, If concentration of CO₂ exceeds 1% rate of photosynthesis decreases due to closer of stomata.
- Water: Water deficiency may decrease the rate. Less availability of water may further check the rate by closing the stomata there by affecting the entry of CO₂.
- Temperature: The optimum temperature for photosynthesis is 15°C to 35°C. if the temperature is increased too high, the rate of photosynthesis is reduced due to denaturation of enzymes involved in the process. Photosynthesis occurs in conifers at high altitude at 35°C. Some algal in host springs can undergo photosynthesis even at 75°C. When other factors are not limiting rate of photosynthesis gets doubled for every 10°C rise in temperature until an optimum is reached.
- Oxygen: Excess of O₂ may become inhibitory for the process. Enhanced supply of O₂ increase the rate of respiration simultaneously decreasing the rate of

photosynthesis. An increase in oxygen concentration decreases photosynthesis and the phenomenon is called Warbrug effect.

- Mineral elements: Some mineral elements like Fe, Mg, Cu, Mn, Cl etc are associated with synthesis of chlorophyll and important reactions in photosynthesis like photolysis of water. So, absence of these elements decreases the rate of photosynthesis.

INTERNAL FACTORS

- Chlorophyll: Chlorophyll is an important internal factor for photosynthesis since it absorbs the radiant energy of light. Light initiates the mechanism of photosynthesis by transferring its electrons and getting excited. Emerson (1929) found direct relationship between the chlorophyll content and the rate of photosynthesis. The chlorophyll deficient mutants are albinos. They can't synthesize carbohydrates by photosynthesis, so they cannot survive.
- Leaf anatomy: Photosynthesis also depends upon the anatomy of leaf. If the assimilatory surface by palisade parenchyma is extensive there will be increased photosynthesis.
- Leaf age: In immature leaf the rate of photosynthesis is at minimum level. A mature leaf shows photosynthetic rate at maximum. When leaf becomes old, the rate decreases.
- End products: The end products of photosynthesis are carbohydrates. Accumulation of carbohydrates decreases the rate of photosynthesis. If the carbohydrates are translocated rapidly the rate of photosynthesis increases.
- Protoplasmic factors: These factors include the hydration of protoplasm and also the enzymatic activity. If there is an appreciable decrease in the hydration of the protoplasm the process of photosynthesis is inhibited because the enzymes get denatured.

BACTERIAL PHOTOSYNTHESIS

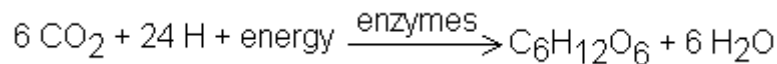
- It is an oxygenic (without evolution of O_2) because water is not employed as hydrogen donor. Instead H_2 H_2S and other compounds are employed. Trap centre is usually B_{890} of bacterio-chlorophyll a. It absorbs radiations between 870-890 nm of infra-red range. Though both cyclic and non-cyclic photophosphorylations occur there is only one photosystem. Assimilatory power consists of ATP and NADH.

CHEMOSYNTHESIS

- It is the manufacture of organic food from inorganic raw materials like carbon dioxide and a hydrogen donor with the help of energy obtained from exergonic reactions.

Chemosynthesis is performed by certain bacteria. They are able to manufacture food in the absence of light.

- The organism carrying out chemosynthesis are called chemoautotrophs. Many of the chemoautotrophs are also able to obtain nourishment as saprotrophs and are thus actually facultative chemoautotrophs. They oxidize the inorganic substances present in their substrate. The energy is trapped and used in synthesis of organic compounds from inorganic raw materials. Chemoautotrophs do not have a light trapping mechanism. They, however perform Calvin cycle reactions of carbon assimilation.



Some common chemoautotrophs are nitrifying bacteria, sulphur bacteria, iron bacteria, methane bacteria, hydrogen bacteria and carboxy bacteria.

TRANSLOCATION OF ORGANIC NUTRIENTS

- It is the movement of organic nutrients from the region of source or supply to the region of sink or utilisation. Phloem (sieve tubes / sieve cells) is the pathway for this translocation as found out by
 - (i) Steam girdling.
 - (ii) Stem girdling
 - (iii) Sieve tube puncturing
 - (iv) Radio autography
 - (v) Sieve tube analysis
- Important theories about the mechanism of translocation of organic nutrients are:
 - (a) Cytoplasmic / Protoplasmic Streaming Hypothesis
In a sieve tube element, organic solutes pass to all parts by cytoplasmic streaming while they pass from one element to another through diffusion.
 - (b) Transcellular streaming hypothesis
Sieve tubes possess tubular transcellular strands which show peristalsis and hence take part in translocation of organic nutrients.
 - (c) Mass flow hypothesis
Organic region of high osmotic concentration to the region of low concentration in a mass flow due to occurrence of pressure gradient. It is most widely accepted theory.