PHOTOSYNTHESIS IN HIGHER PLANTS

- Photosynthesis (Gk. *photos* – light, *synthesis* – formation) is an enzyme regulated anabolic process of manufacturing organic compounds inside the chlorophyll containing cells from carbon dioxide and water with the help of sunlight as a source of energy. A simple equation of photosynthesis is as follows :

$$6CO_2 + 6H_2O \xrightarrow[\text{Radiant Energy (686 kcal)}]{\text{Chlorophyll, Enzymes}} C_6H_{12}O_6 + 6O_2 \uparrow$$

HISTORY OF PHOTOSYNTHESIS

- However, the function of water is to provide hydrogen for the synthesis of organic compounds. All the liberated oxygen comes from it.

Table : History of photosynthesis

Stephen Hales (1727)	Discovered that green plants require sun- light for their nutrition
Joseph Priestley (1770)	Demonstrated that green plants purify the foul air (*i.e.*, phlogiston), produced by burning of candle, and convert it into pure air (*i.e.*, dephlogiston).
Jan Ingehousz (1779)	Concluded by his experiments that green plants purify noxious air only during the day in presence of light.
Julius Von Sachs (1854)	Demonstrated that first visible product of photosynthesis is starch. He also showed that chlorophyll is confined to the chloroplasts.
Engelman (1884, 1888)	Showed that chloroplast was the seat of photosynthesis in the cell. Discovered the role of different wavelengths of light on photosynthesis and plotted the first action spectrum.
Blackmann (1905)	Discovered two steps, *i.e.*, light and dark reactions in photosynthesis. He further proposed the 'law of limiting factors'.
Warburg (1919)	Performed flashing light experiment using green alga *Chlorella* as a suitable material for the study of photosynthesis.
van Niel (1931)	Demonstrated that some bacteria use H_2S instead of H_2O in the process of photosynthesis.

- It was **van Niel** (1931), a graduate student, who observed that during bacterial photosynthesis hydrogen sulphide was broken down to hydrogen and sulphur. The hydrogen reduces carbon dioxide to synthesize carbohydrate and sulphur is accumulated as a waste product. He also propounded that oxygen is evolved from water.

$$12H_2S + 6CO_2 \xrightarrow[\text{Bacterio-chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 12S + 6H_2O$$

- **Robin Hill** (1937) illuminated the isolated chloroplasts of *Stellaria media* in the presence of leaf extract or hydrogen acceptors and in the absence of CO_2. The chloroplasts evolved oxygen (O_2).

$$12A + 2H_2O \xrightarrow[\text{Chloroplast}]{\text{Light}} 2AH_2 + O_2$$

where A represents hydrogen acceptor.

- These hydrogen acceptors (*e.g.*, ferricyanides, chromates, benzoquinones, dichlorophenol indophenol etc) are also called **Hill oxidants.** The reaction involving the production of oxygen by the illuminated chloroplast in the absence of CO_2 fixation is called **Hill reaction.**
- **Ruben** and **Kamen** (1941) and **Ruben** *et al* (1941) suspended *Chlorella* in water having heavy isotope of oxygen ^{18}O, instead of natural oxygen ^{16}O. The suspension was illuminated. Oxygen evolved was tested by means of mass spectrometer. It was found to be heavy isotope, ^{18}O. It proves that oxygen evolved during photosynthesis comes from splitting of water.

$$16CO_2 + 12H_2{}^{18}O \xrightarrow[\text{Chlorophyll, Enzymes}]{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6^{18}O_2 \uparrow$$

- Part of the spectrum used in photosynthesis has a wavelength between 400-700 nm. It is called **photosynthetically active radiation** (PAR). The wavelengths of light most efficient in photosynthesis are those of **red light. Green light** is least effective in photosynthesis.

PHOTOSYNTHETIC ORGANELLE

- Chloroplasts are the green plastids which occur in all green parts of the plants. They are the actual sites of photosynthesis. They occur mostly in the chlorenchymatous cells (particularly in mesophyll) of leaves and young stems. Each chloroplast of a higher plant is an organelle consisting of a double membrane.
- The chloroplast envelope encloses a liquid proteinaceous matrix called **stroma.** Stroma is hyaline, slightly electron dense, granular matrix and contains all the necessary enzymes of photosynthesis. The stroma is the site of **dark reaction** of photosynthesis. The lamellar system within the stroma forms flattened sac like lamellae called **thylakoids** (Gk. *thylakos* = sac, *oid* = like). These thylakoids are stacked in some places to form **grana.**
- The grana stacks are interconnected by membranous lamellae called **stroma lamellae** or **fret channels.** 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. Thylakoids possess four types of major complexes; photosystem I, photosystem II, Cyt b_6-f complex and coupling factor (ATP synthetase). Photosystem II is thought to occur mostly in the appressed or partition regions of granal thylakoids while photosystem I lies in the non-appressed parts as well as stroma lamellae.

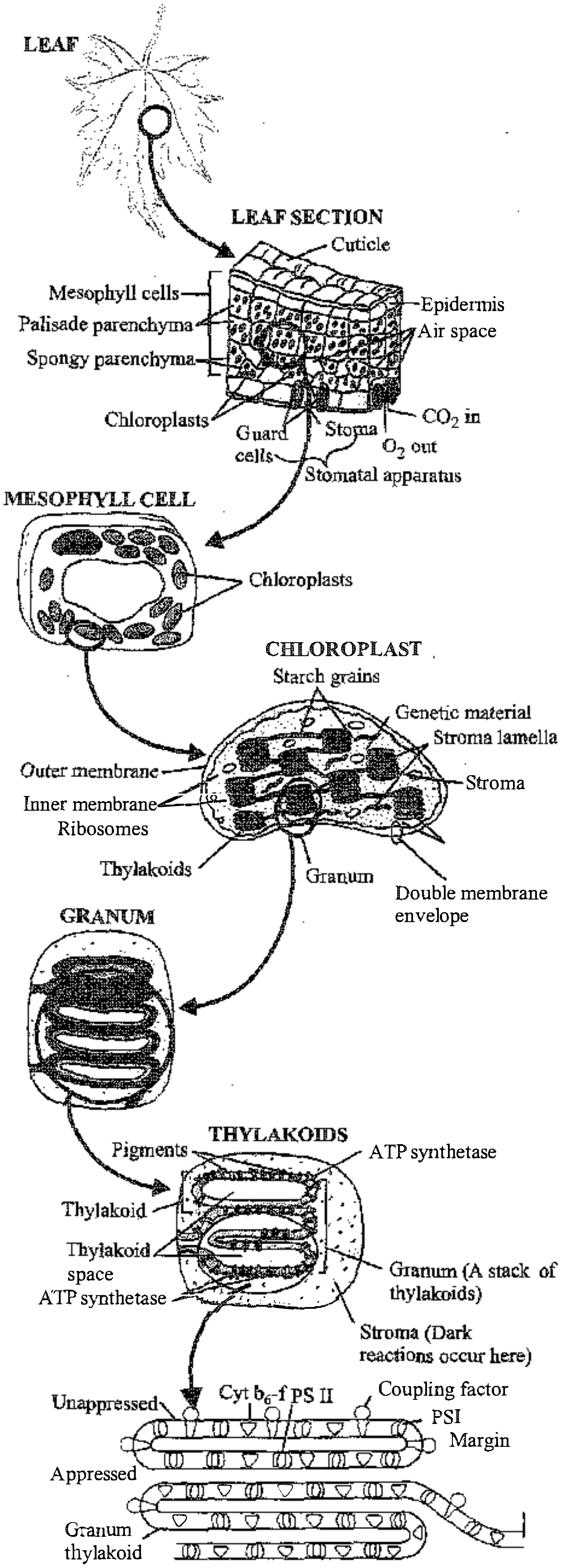

Fig.: Chloroplast position and its structures

PHOTOSYNTHETIC PIGMENTS

- Those pigments which occur on photosynthetic thylakoids of chloroplasts and take part in absorption of light energy for the purpose of photosynthesis are known as photosynthetic pigments. Photosynthetic pigments are of two types – chlorophylls and carotenoids.
- **Chlorophylls** are the green photosynthetic pigments. Five types of chlorophylls occur in plants other than bacteria – *a*, *b*, *c*, *d* and *e*. Bacteria possess two types of related pigments – bacteriochlorophyll (further of several subtypes) and bacterioviridin (chlorobium chlorophyll). **Chlorophyll *a*** is bluish-green in the pure state. It has an empirical formula of $C_{55}H_{72}O_5N_4Mg$. **Chlorophyll *b*** is olive green in the pure state with an empirical formula of $C_{55}H_{70}O_6N_4Mg$.

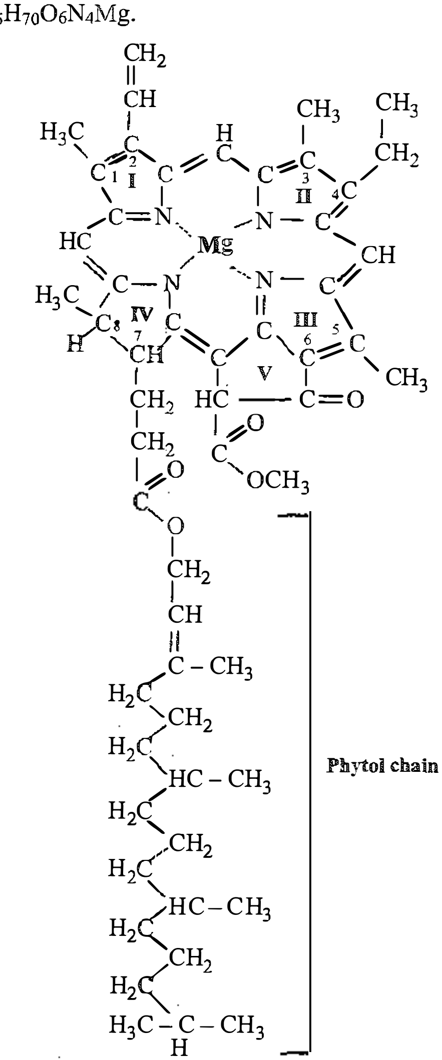

Fig.: Structure of chlorophyll *a*

- Chlorophyll structure was first studied by **Wilstatter, Stoll** and **Fischer** in 1912. It has a tadpole like configuration with a head called **porphyrin** and a tail made up of long chain alcohol called **phytol.** Porphyrin head is made up of four pyrrole rings which are linked by **methine bridges** (— CH =). The skeleton of each pyrrole ring is made up of 5 atoms – 4 carbon and one nitrogen. A nonionic

magnesium atom is held in the **centre of porphyrin head** by nitrogen atoms of pyrrole rings (through two covalent and two coordinate bonds). Phytol is an insoluble long chain of carbon and hydrogen atoms with a formula of $C_{20}H_{39}OH$. Chlorophyll *b* differs from chlorophyll *a* in having formyl group (— CHO) instead of a methyl (— CH_3) group at carbon atom 3.

- **Carotenoids** are a group of **yellow, brown to reddish** pigments which are associated with the chlorophylls inside the chloroplasts but occur alone inside the chromoplasts. Alongwith cholorophyll *b* the carotenoids are also called **accessory pigments** because they hand over the energy absorbed by them to chlorophyll *a*. Carotenoids have **conjugate double bonds** (— C = C — C = C —). They are of two types, **carotenes** and **xanthophylls.**

PHOTOSYNTHETIC UNIT

- A photosynthetic unit is the smallest group of pigment molecules which takes part in a photochemical act of conversion of light energy into chemical energy. It has a **photocentre** or **reaction centre** which is fed by about 200 harvesting pigment molecules. The photocentre consists of a dimer of special chlorophyll *a* molecules, P_{700} and P_{680}.
- The harvesting molecules form a protein based complex called **light harvesting complex (LHC).** In green plants, photosynthetic units occur in the form of two distinct groups called **photosystem** or **pigment systems I** and **II.** Each photosystem contains 250-400 pigment molecules.

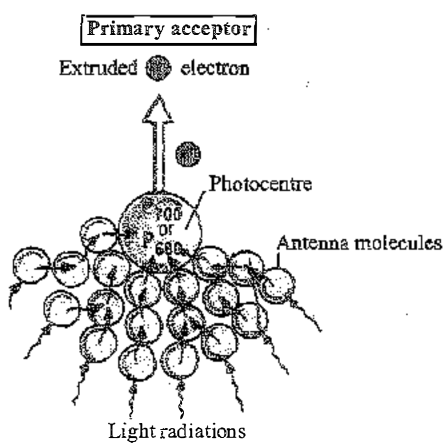

Fig.: Harvesting of light by a photosynthetic unit.

- **Photosystem I (PS I)** is a photosynthetic pigment system alongwith 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. It consists of a photocentre, light harvesting complex (LHC I) and some electron carriers. Photocentre has a dimer of special chlorophyll *a* molecules called P_{700}. PSI can carry on cyclic photophosphorylation independently.
- **Photosystem II (PS II)** is a photosynthetic pigment system alongwith some electron carriers that is located in the appressed part of the **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.
- Photosystem II consists of a photocentre, oxygen evolving complex, light harvesting complex (LHC II) and some electron carriers. All are connected to membrane proteins. Photocentre has a dimer of special chlorophyll *a* molecules called P_{680}. PS II can operate only in conjunction with PS I and carries out noncyclic photophosphorylation.

MECHANISM OF PHOTOSYNTHESIS

- Photosynthesis occurs in two phases – photochemical and biosynthetic. Photochemical phase is also called **light or Hill reaction** (after the name of the scientist Robert Hill who discovered its details). Biosynthetic phase is also termed as **dark or Blackman's reaction** (after the name of scientist who first postulated it).

Photochemical phase (Light reaction)

- It occurs inside the thylakoids, especially those of grana region. Photochemical step is **dependent upon light.** The function of this phase is to produce assimilatory power consisting of reduced coenzyme NADPH and energy rich ATP molecules. Photochemical phase involves photolysis of water and production of assimilatory power (NADPH and ATP).
- The phenomenon of breaking up of water into hydrogen and oxygen in the illuminated chloroplast is called **photolysis** or photocatalytic splitting of water.
- The enzyme has four Mn ions. Light energised changes in Mn (Mn^{2+}, Mn^{3+}, Mn^{4+}) removes electrons from OH^- component of water forming oxygen.
- It requires two other ions Ca^{2+} and Cl^-

$$4OH^- \xrightarrow[Mn^{2+}, Ca^{2+}, Cl^-]{\text{oxygen evolving complex}} 2H_2O + O_2\uparrow + 4e^-$$

- The electrons released during photolysis of water are picked up by P_{680} photocentre of photosystem II and either follows Z scheme (non-cyclic photophosphorylation) or cyclic photophosphorylation.

Photophosphorylation

- The term phosphorylation was coined by Arnon and others in 1954. Formation of ATP in the chloroplast by photosynthesis is called photophosphorylation.

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 680 nm and when CO_2 fixation is inhibited.
- Cyclic photophosphorylation is performed by photosystem I only. Its photocentre P_{700} extrudes an electron after absorbing a photon of light. The expelled electron passes through a series of carriers including P_{700} chlorophyll molecule. Plastoquinone (PQ), FeS complex, ferredoxin (Fd), cyt b_6–f and plastocyanin before returning to photocentre. Over the cytochrome complex (cyt b_6–f), the electron creates a proton gradient for synthesis of ATP from ADP and inorganic phosphate.

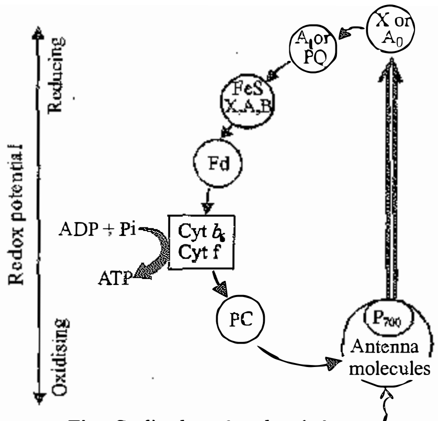

Fig.: Cyclic photophosphorylation.

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.

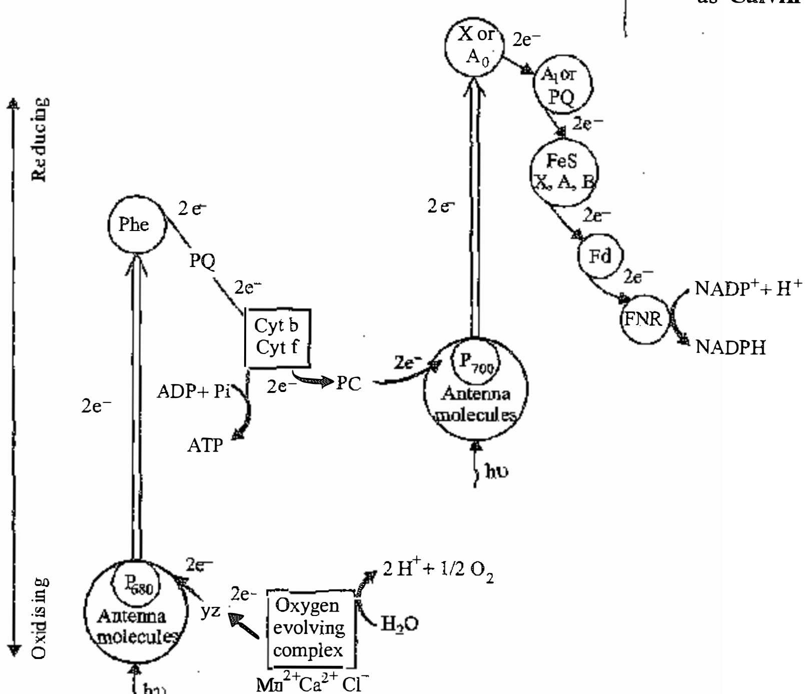

Fig.: Non-cyclic photophosphorylation.

- 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 (hυ). 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.
- The electron is handed over to photocentre P_{700} of PS I by plastocyanin. P_{700} extrudes the electron after absorbing light energy. The extruded electron passes through special chlorophyll P_{700} molecule, Fe-S ferredoxin, to finally reach $NADP^+$. The latter then combines with H^+ (released during photolysis) with the help of NADP-reductase to form **NADPH.** This is called **Z scheme** due to its characteristic zig-zag shape based on redox potential of different electron carriers.

Biosynthetic phase (Dark or Blackman's reaction)

- Dark reaction does not require the presence of light but requires assimilatory power (ATP + NADPH) produced during the photochemical reaction. It **occurs in stroma** or matrix of chloroplast. All the enzymes required for the process are present in the matrix or stroma of the chloroplast. There are two main pathways for the biosynthetic or dark phase – **Calvin cycle (C_3 cycle) and C_4 (dicarboxylic acid) cycle.**

Calvin cycle (C_3 cycle)

- Carbon dioxide fixation in the presence of ATP and NADPH and its conversion to glucose, through a series of reaction, catalysed by specific enzymes is termed as **Calvin cycle.** The cycle was discovered by Calvin, Benson and their colleagues in California, U.S.A. They fed *Chlorella* and *Scenedesmus* with carbon dioxide containing radioactive ^{14}C. Radioactive carbon ^{14}C has a half life of 5568 years. Therefore, the path of CO_2 fixation can be easily traced with its help. Phosphoglyceric acid is, the first stable product of photosynthesis. Calvin cycle is divided into three phases : carboxylation, reduction and regeneration of RuBP.
  - **Carboxylation** - Photosynthetic carboxylation requires ribulose-1, 5-biphosphate or **RuBP** as acceptor of carbon dioxide and RuBP carboxylase-oxygenase or **RuBisCO** as enzyme. The enzyme was previously called **carboxydismutase.** RuBisCO is the most abundant protein of the biological world. Carbon dioxide combines with ribulose-1, 5 biphosphate to produce a transient intermediate compound called 2-carboxy 3-keto 1,5-biphosphoribotol. The intermediate splits up immediately in the presence of water to form two molecules of **3-phosphoglyceric acid** or PGA. It is the first stable product of photosynthesis.
- **Reduction or glycolytic reversal -** These are a series of reactions that lead to the formation of glucose. The processes involved in this step or phase are reversal of the processes found during glycolysis part of respiration, thus named glycotic reversal.
- 3-Phosphoglyceric acid or 3-PGA is further phosphorylated by ATP with the help of enzyme **triose phosphate kinase** (phosphoglycerate kinase).
- It gives rise to 1, 3-bisphosphoglyceric acid (2 molecules).

- The steps involve utilization of 2 molecules of ATP for phosphorylation and two of NADPH for reduction per CO_2 molecule fixed. The fixation of six molecules of CO_2 and 6 turns of the cycle are required for the removal of one molecule of glucose from the pathway.
- These reactions can be summarised as follows:

$$\underset{\text{(2 mols)phosphoglyceric acid}}{\text{3-PGA}} + \underset{\text{(2 mols)}}{\text{ATP}} \xrightarrow[\text{kinase}]{\text{Phosphoglycerate}} \underset{\text{(2 mols) 1-3 bisphosphoglyceric acid}}{\text{(1,3-bis PGA)}} + \underset{\text{(2 mols)}}{\text{ADP}}$$

$$\underset{\text{(2 mols)}}{\text{1,3-bis PGA}} + \underset{\text{(2 mols)}}{\text{NADPH}} \xrightarrow[\text{dehydrogenase}]{\text{Glyceraldehyde 3-phosphate}} \underset{\text{(2 mols) 3-phosphoglyceraldehyde}}{\text{3-PGAld}} + \underset{\text{(2 mols)}}{H_3PO_4} + \underset{\text{(2 mols)}}{NADP^+}$$

$$\underset{\text{(1 mol)}}{\text{3 PGAld}} \xrightarrow[\text{isomerase}]{\text{phosphotriose}} \underset{\text{(1 mol) dihydroxyacetone-3-phosphate}}{\text{3-DHAP}}$$

$$\underset{\text{(1 mol)}}{\text{3 PGAld}} + \underset{\text{(1 mol)}}{\text{DHAP}} \xrightarrow{\text{aldolase}} \underset{\text{(1 mol) Frutose 1,6 bisphosphate}}{\text{F-1, 6-BP}}$$

$$\text{F-1, 6-BP} + H_2O \xrightarrow{\text{Frutose 1, 6-bisphosphatase}} \underset{\text{Frutose-6-phosphate}}{\text{F-6-P}} + H_2PO_3$$

$$\text{F-6-P} \xrightarrow{\text{Isomerase}} \underset{\text{Glucose 6-phosphate}}{\text{G-6-P}}$$

$$\text{G-6-P} \xrightarrow{\text{Phosphatase}} \text{Glucose} + H_3PO_4 + H_2O$$

- **Regeneration-** Regeneration of the CO_2 acceptor molecule RuBP is crucial if the cycle is to continue uninterrupted. The regeneration steps require one ATP for phosphorylation to form RuBP. Steps of this process can be summarised as follows:

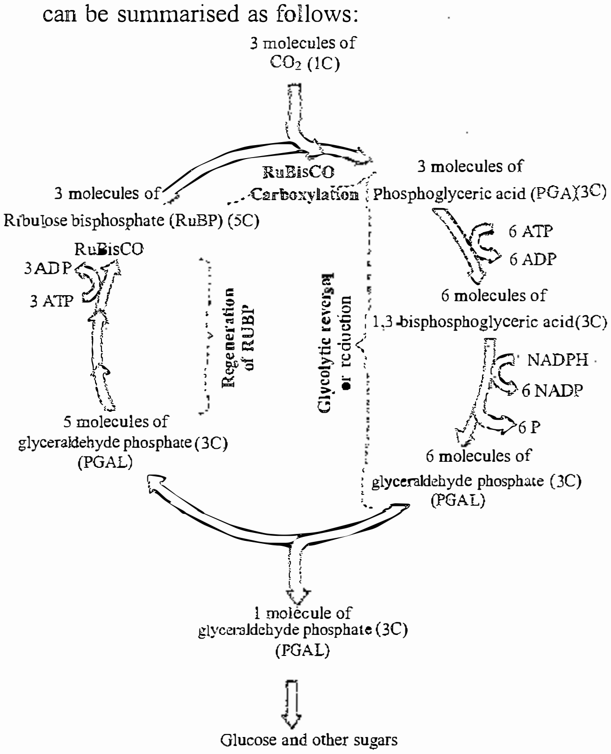

Fig.: Schematic representation of Calvin cycle or C_3 cycle.

$$\text{F-6-P} + \text{3PGAld} \xrightarrow{\text{Transketolase}} \underset{\text{(Erythrose 4-phosphate)}}{\text{E-4-P}} + \text{X-5-P}$$

$$\text{E-4-P} + \text{3-DHAP} \xrightarrow{\text{Aldolase}} \underset{\text{(Sedoheptulose 1, 7 biphosphate)}}{\text{Se-1, 7-BP}}$$

$$\text{Se-1, 7 BP} + H_2O \xrightarrow[Mg^{2+}]{\text{Sedoheptulose 1,7-bisphosphatase}} \underset{\text{(Sedoheptulose – 7 phosphate)}}{\text{Se-7-P}} + H_3PO_4$$

$$\text{Se-7-P} + \text{3-PGAld} \xrightarrow{\text{Transketolase}} \underset{\text{Xylulose 5-phosphate}}{\text{X-5-P}} + \underset{\text{Ribose 5-phosphate}}{\text{R-5-P}}$$

$$\text{X-5-P} \xrightarrow{\text{Ribulose 5-phosphate epimerase}} \underset{\text{Ribulose-5-phosphate}}{\text{Ru-5-P}}$$

$$\text{R-5-P} \xrightarrow{\text{Ribulose 5-phosphate isomerase}} \text{Ru-5-P}$$

$$\text{Ru-5-P} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Phosphoribulose kinase}} \underset{\text{(RuBP)}}{\text{Ru-1,5-BP}} + \text{ADP}$$

- RuBP starts the cycle again. This process of carbon dioxide is also called C_3 cycle as first stable product of it is a 3 carbon compound, **phosphoglyceric acid**.
- The net reaction of C_3 dark fixation of carbon dioxide is

$$6RuBP + 6CO_2 + 18\ ATP + 12\ NADPH \rightarrow 6RuBP + C_6H_{12}O_6 + 18\ ADP + 18Pi + 12\ NADP^+$$

Hatch and Slack's cycle or C_4 cycle

- For a long time, Calvin cycle (C_3 cycle) was considered to be the only photosynthetic pathway for reduction of CO_2 into carbohydrates. **Kortschak, Hartt** and **Burr** (1965) reported that rapidly photosynthesising sugar cane leaves produced a 4-C compound like aspartic acid and malic acid as a result of CO_2-fixation.
- This was later supported by **M.D. Hatch** and **C.R. Slack** (1966) and they reported that a 4-C compound oxaloacetic acid (OAA) is the first stable product in CO_2 reduction process. This occurs only after 1 sec of photosynthesis, although on longer exposures, 3-PGA is formed. This led to an alternative pathway of CO_2 fixation, which is known as **Hatch and Slack's cycle** or C_4 cycle (as 4-C compound is first stable product).
- These C_4 plants have a characteristic leaf anatomy called **Kranz anatomy.** Here vascular bundles are surrounded by sheaths of large parenchymatous cells called **bundle sheaths**, which are surrounded by **mesophyll cells.** So here **two types of chloroplasts** are present, *i.e.*,
  - **Bundle sheath chloroplasts:** Larger in size, lack grana (agranal chloroplasts) and contain starch grains.
  - **Mesophyll chloroplasts:** Smaller in size, contain grana and lack starch grains.

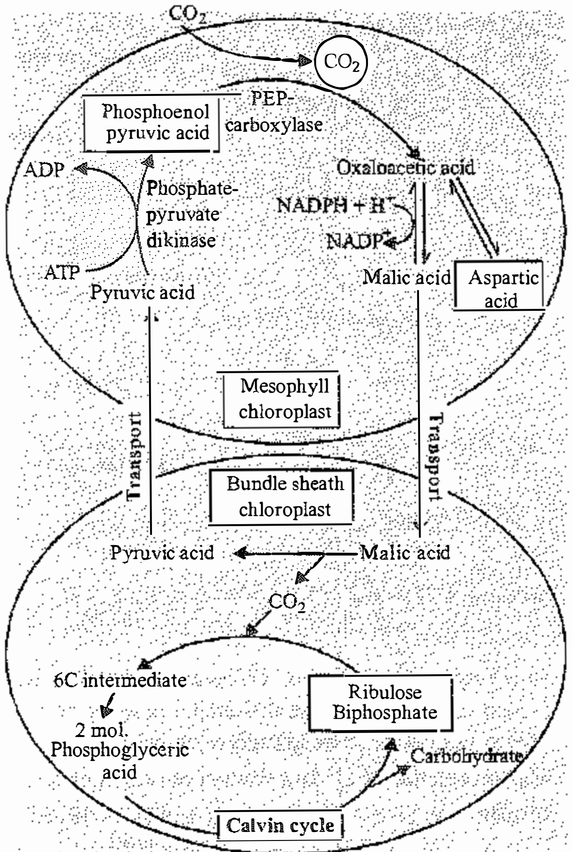

Fig.: Hatch and Slack cycle

- Here in C_4 plants, there occur 2 carboxylation reactions, first in mesophyll chloroplast and second in bundle sheath chloroplast. CO_2 acceptor molecule here is **PEP (phosphoenol pyruvate)** and not RuBP. Further, **PEP-carboxylase** is the key enzyme (RuBP-carboxylase enzyme is negligible or absent in mesophyll chloroplast, but is present in bundle sheath chloroplast). In C_4 plants, for formation of one mole of hexose (glucose), **30 ATP** and **12 $NADPH_2$** are required.

CAM plants (Crassulacean acid metabolism plants)

- This metabolism was first of all reported in *Bryophyllum*, a member of Family **Crassulaceae** and hence is called crassulacean acid metabolism. This occurs in mostly **succulents** (xerophytes) like *Opuntia, Agave, Aloe, Sedum, Kalanchoe,* etc.
- The most characteristic feature of these plants is that their stomata remain open at night (in dark) but closed during the day (in light). Thus, CAM is a kind of adaptation in succulents to carry out photosynthesis without much loss of water.
- In theseplants, there is no kranz anatomy, but there occurs **dark acidification,** *i.e.*, during night malic acid is formed. This malic acid breaks up into CO_2 and pyruvic acid in day time and CO_2 released is utilized in C_3 cycle.
- In CAM plants **OAA** (oxaloacetic acid) is formed due to carboxylation as in C_4 plants. Like C_4 plants, OAA is reduced to **malic acid** in CAM plants and is accumulated in the vacuole. Stomata are open at night. CO_2 is absorbed from outside. With the help of PEP carboxylase, it is immediately fixed. The acceptor is phosphoenol pyruvate or PEP. Absorption of CO_2 during night and its storage as organic acid (malic acid) is called **acidification.**

$$PEP + HCO_3^- \xrightarrow{\text{PEP carboxylase}} OAA + H_3PO_4$$

$$OAA + NADPH \xrightarrow{\text{Dehydrogenase}} \text{Malic acid} + NADP^+$$

Table : Differences between C_3 plants and C_4 plants

	C_3 Plants	C_4 Plants
1.	Plants are adapted mainly for the tropical and subtropical climates and also for colder climate.	Plants are adapted for relatively hotter climatic regions of the equator and of the tropics.
2.	It involves Calvin cycle or C_3 cycle.	It involves Hatch-Slack cycle or C_4 cycle.
3.	Leaf anatomy is of common type.	Leaf anatomy is of special type (Kranz anatomy).
4.	First stable product is PGA during photosynthesis, where RuBP acts as the CO_2 acceptor.	First stable product is OAA during photosynthesis where PEP acts as the CO_2 acceptor.
5.	Photorespiration is present.	Photorespiration is slightly present.
6.	Utilise 18 molecules of ATP for the production of 1 molecule of glucose.	Utilise 30 molecules of ATP for the production of 1 molecule of glucose.

Table : Differences between C_3 cycle and C_4 cycle

	C_3 Cycle	C_4 Cycle
1.	Known as Calvin cycle and the first stable compound is a 3 carbon compound (PGA). The CO_2 acceptor is RuBP.	Known as Hatch-Slack pathway and the first stable compound is a 4 carbon compound (OAA). The CO_2 acceptor is PEP.
2.	Occurs in mesophyll cells only.	Occurs in mesophyll cells and bundle sheath cells as a continuous event.
3.	Chloroplasts are monomorphic (*i.e.*, of one type).	Chloroplasts are dimorphic (*i.e.*, of two types).
4.	Less energy consuming process.	High energy consuming process.
5.	This cycle operates in all green plants.	This cycle is restricted to some plants.

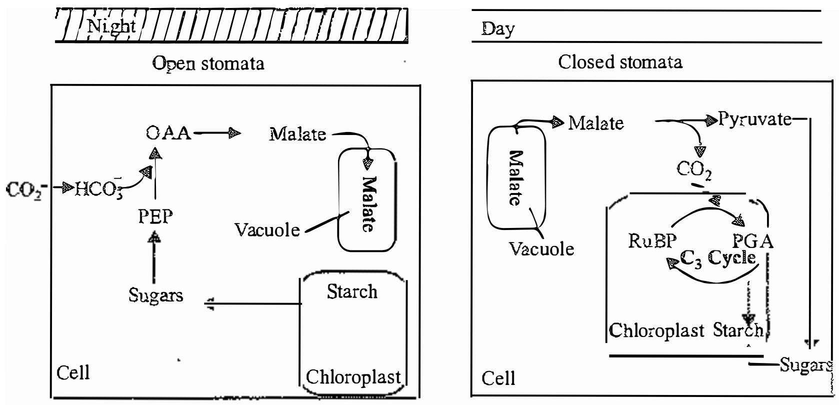

Fig.: An outline of CAM pathway.

- **Malic acid is end product of dark fixation of CO_2.** It is stored inside cell vacuoles.
- During day time malic acid undergoes oxidative decarboxylation and CO_2 is released. Liberation of CO_2 from an organic acid during day time is called deacidification.

$$\text{Malic acid (malate)} + NADP^+ \xrightarrow[\text{enzyme}]{\text{Malic}} \text{Pyruvate} + CO_2 + NADPH$$

- The diurnal acidification and de-acidification during the night and day time respectively is called **CAM (Crassulacean Acid Metabolism).**
- In C_4 plants initial carboxylation and final carboxylation are separated by space but in CAM plants initial carboxylation and final carboxylation are separated by time. All reactions of CAM occur in mesophyll cells. Chloroplasts are absent in bundle sheath cells of CAM plants. CAM pathway is important for the survival of succulents.

FACTORS AFFECTING PHOTOSYNTHESIS

- For a study of the affecting factors it is essential to have a knowledge of **Blackman's law of limiting factors.**
- Before 1905 it was customary to study the effect of individual factors on the rate of photosynthesis in terms of minimum (at which photosynthesis will start), optimum (at which photosynthesis is at its best) and maximum (above which photosynthesis does not occur).
- Many workers obtained different values of these **cardinal points** under different conditions, *e.g.*, they found optimum CO_2 concentration to be greater at high intensity than at low intensity
- F.F. Blackman (1905) stated that, when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factors (*i.e.*, factor present in minimum amount).

External or environmental factors

Carbon dioxide

- **CO_2 concentration** of the atmosphere is 0.036% or 360 ppm.
- Increase in its concentration upto 0.05% increases the rate of photosynthesis in most C_3 plants. A decline is observed beyond 0.1%. When CO_2 concentration is reduced, there comes a point at which illuminated plant parts stop absorbing carbon dioxide from their environment. **It is known as CO_2 compensation point or threshold value.** At this value CO_2 fixed in photosynthesis is equal to CO_2 evolved in respiration and photorespiration.
- The value is 25-100 ppm in C_3 plants and 0 -10 ppm in C_4 plants.
- The reason for low compensation value for C_4 plants is the **greater efficiency of CO_2 fixation through PEP carboxylase.**

Light

- Plants are broadly classified into two groups depending upon their inability or ability to tolerate high **light intensity** : shade plants (sciophytes, *e.g.*, *Oxalis*) and sun plants (heliophytes, *e.g.*, *Dalbergia*).
- At low light intensity the rate of photosynthesis is reduced.
- There is a point in light intensity where there is no gaseous exchange in photosynthesis. It is called **light compensation point.**
- A plant cannot survive for long at compensation point because there is a net loss of organic matter due to respiration of nongreen organs and respiration in dark.
- The **light intensity** at which a plant can achieve **maximum amount of photosynthesis** is called **light saturation point.**
- Maximum photosynthesis occurs in blue-violet and red regions of the light spectrum where most of the absorption is carried out by chlorophylls.
- **Red light** favours **carbohydrate accumulation** while **blue** light stimulates **protein synthesis.**

Temperature

- **Temperature** does not influence photochemical part of photosynthesis (light reaction) but affects the biochemical part (dark reactions). The optimum temperature is 10-25°C for C_3 plants and 30-45°C for C_4 plants.

Oxygen

- Small quantity of **oxygen** is essential for photosynthesis except in some anaerobic bacteria. C_3 plants show optimum photosynthesis at low oxygen concentration. It is assumed that oxygen takes part in oxidation of photosynthetic pigments, intermediates and enzymes in the presence of strong light **(photo-oxidation)**. O_2 competes with CO_2 for reducing power. At a very high oxygen content the rate of photosynthesis begins to decline in all plants. The phenomenon is called **Warburg effect.**

Water

- The amount of **water** used in photosynthesis is very small. The rate of photosynthesis falls in water deficient soils.

Air pollutants

- **Air pollutants** like dust and smoke particles present in the atmosphere reduce photosynthesis by reducing light penetration and forming a layer over the plants.

Internal or plant factors

- Photosynthesis does not occur in the absence of **chlorophyll.** Therefore, variegated leaves produce less organic food than the completely green leaves.
- **Leaf age** also affects rate of photosynthesis.
- As a leaf develops, the rate of photosynthesis rises with the age till it becomes maximum at full maturity.
- Afterwards the rate of photosynthesis begins to decline as ageing or senescence brings about deactivation of enzymes and degeneration of chlorophyll.
- **Phytohormones** like cytokinins and gibberellins increase the rate of photosynthesis but abscisic acid reduces the same.
- Slow rate of translocation causes **accumulation of photosynthetic end products** during afternoon. This reduces the rate of photosynthesis.

Emerson's enhancement effect

- **Robert Emerson** in 1950 at the University of Illinois observed that the red light of wavelengths longer than 680 or 690 nm was not effective in photosynthesis. He concluded that simultaneous application of light of shorter and longer wavelengths, increases the rate of photosynthesis. This is called **Emerson's enhancement effect.** Emerson (1957) found a sharp reduction in the rate of photosynthesis when monochromatic light of more than 680 nm wavelength (Red region of the spectrum) was used alone. It is called **red drop. Quantum yield** is the number of O_2 molecules which are released per quantum of light absorbed.

Absorption and action spectrum

- A graph showing amount of energy of different wavelengths of light absorbed by a substance is called **absorption spectrum.** It is measured with the help of **spectrophotometer.** The absorption spectra of chlorophylls *a* and *b* show that they absorb maximum light in the blue-violet and red wavelengths.
- The graphic curve depicting the relative rates of photosynthesis at different wavelengths of light is called **action spectrum.** It shows that maximum photosynthesis occurs in **blue-violet** and **red** parts of the light.

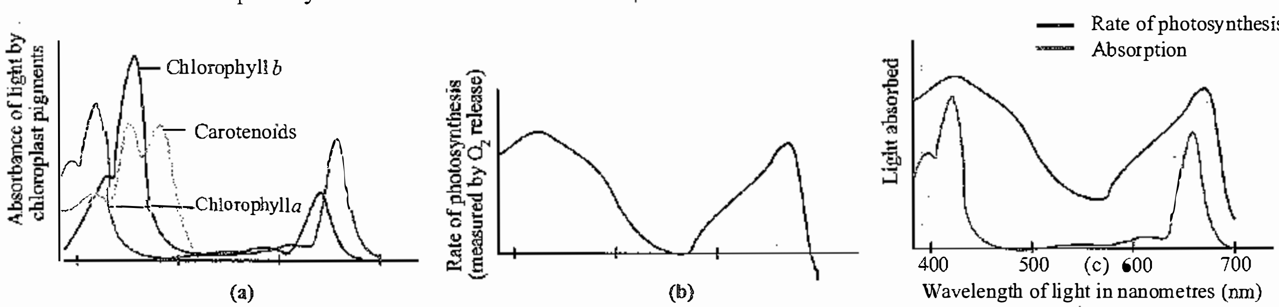

Fig.: (a) Graph showing the absorption spectrum of chlorophyll, *a, b* and the carotenoids; (b) Graph showing action spectrum of photosynthesis; (c) Graph showing action spectrum of photosynthesis superimposed on absorption spectrum of chlorophyll *a*

PHOTORESPIRATION

- The photorespiration is defined by **Krotkov (1963)** as an extra input of O_2 and extra release of CO_2 by green plants in light. The process occurs in **chloroplast, peroxisome** and **mitochondria.**
- Ribulose biphosphate carboxylase (RuBisCO), the main enzyme of Calvin cycle that fixes CO_2, acts as ribulose biphosphate oxygenase under low atmospheric concentration of CO_2 and increased concentration of O_2.
- In presence of high concentration of O_2 the enzyme RuBP oxygenase splits a molecule of Ribulose-1,5 biphosphate into one molecule each of **3-Phosphoglyceric acid** and **2-phosphoglycolic acid.**

$$\text{Ribulose-1, 5bisphosphate} \xrightarrow{O_2} \text{2-Phosphoglycolic acid} + \text{3-Phosphoglyceric acid}$$

- The 2-Phosphoglycolic acid loses its phosphate group in presence of enzyme **phosphatase** and is converted into **glycolic acid**

  2 Phosphoglycolic acid + H_2O → Glycolate + Phosphate
- The glycolic acid, **synthesised in chloroplast** as an early product of photosynthesis, is **then transported to the peroxisome.**

$$\text{Glycolic acid} + O_2 \rightarrow \text{Glyoxylic acid} + H_2O_2$$
$$\text{Glyoxylic acid} + \text{Glutamic acid} \rightarrow \text{Glycine} + \alpha\text{-ketoglutarate.}$$

- The **glycine is transported out of peroxisomes into mitochondria.**

$$2\ \text{Glycine} + H_2O + NAD^+ \rightarrow \text{Serine} + CO_2 + NH_3 + NADH.$$

- The **CO_2 is then released in photorespiration from mitochondria.** The NH_3 released during glycine decarboxylation is transported to cytoplasm or chloroplast, where it is incorporated into synthesis of glutamic acid.

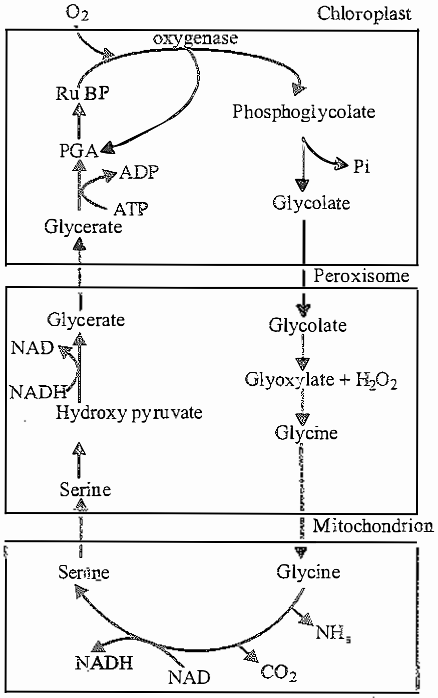

Fig.: Schematic representation of photorespiratory pathway

- The amino acid serine returns to peroxisome where it is deaminated and reduced to **hydroxypyruvic** acid and finally to **glyceric acid.**

  Serine + α-ketoglutarate → Hydroxypyruvate + Glutamate

  Hydroxypyruvic acid → Glyceric acid

- The glyceric acid finally enters the chloroplast where it is phosphorylated to 3-Phosphoglyceric acid, which enters into C_3 cycle.

  Glyceric acid + ATP → 3-Phosphoglyceric acid + ADP

CHEMIOSMOTIC HYPOTHESIS OF ATP FORMATION

- This 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 intermembrane space of mitochondria and inside the thylakoid lumen in chloroplasts. Lumen 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 transfers its electrons to an 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 outerside of thylakoid membrane. It obtains electron from PS I and protons from matrix to reduce $NADP^+$ to NADP + H^+ state. The consequences of these events is that concentration of protons 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). As protons move to the other side of ATPase, they bring about conformational changes in CF_1 particle of ATPase or coupling factor. The transient CF_1 particle of ATPase enzyme form 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 $3H^+$ are used by the ATP synthase.

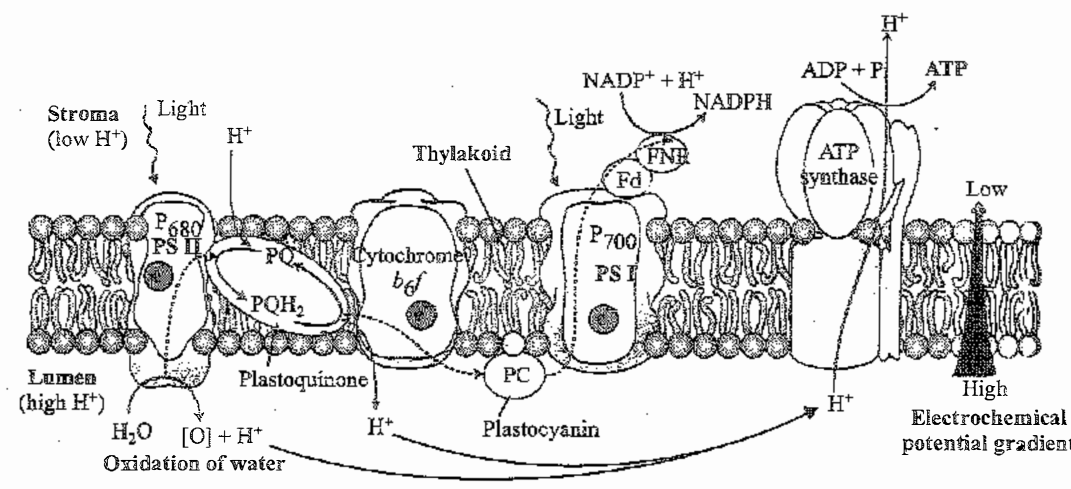

Fig.: Mechanism of chemiosmotic synthesis of ATP in chloroplast
(FNR = Flavoprotein ferredoxin - NADP reductase).

CONCEPT MAP

Photosynthesis

Formation of glucose from CO_2 and water in presence of sunlight and chlorophyll.

Factors affecting photosynthesis

- **External factors**: Light, CO_2 conc., temperature, water, O_2 etc.
- **Internal factors**: Chlorophyll conc., leaf age, phytohormones etc.

Site of photosynthesis

Photosynthesis occurs in double membrane bound chloroplasts that consist of stroma, grana stacks, thylakoids and stroma lamellae. Thylakoids contain photosynthetic pigments.

Photosynthetic pigments

They include chlorophylls, carotenes and phycobilins. Chlorophylls are the major pigments while other two are accessory ones. They may be of various types and form two major pigment systems.

- **PS I** → Includes P_{700} as photocentre and LHC (light harvesing complex) with electron carriers.
- **PS II** → Includes P_{680} as photocentre and LHC.

Photosynthetic mechanism

An enzymatic anabolic reaction involving many intermediates.

Photochemical Phase

Light dependent phase occurring in thylakoids.

- **Photolysis**: Water molecule is broken down into O and H by absorbing light energy. It produces e^-.
- **Photophosphorylation**: Electrons released in photolysis of water are used for production of ATP through photophosphorylation. It may be of two types:
  - **Cyclic**: Involves only PS I and produces only ATP.
  - **Non-cyclic**: Involves both PS I and PS II and produces both ATP and NADPH.

Biosynthetic Phase

Light dependent reaction that occurs in stroma. It can be of three types.

- **C_3 cycle**: CO_2 combines with RuBP and produces glucose *via* many intermediate steps. Also called as Calvin cycle. 3C-phosphoglyceric acid is first stable product.
- **C_4 cycle**: Glucose is formed by CO_2 fixation but initial acceptor is PEP in mesophyll cells and first stable product is 4C compound oxaloacetic acid. C_4 plants have kranz anatomy and are more efficient than C_3 plants.
- **CAM cycle**: In xerophytes, CAM cycle is observed where CO_2 is fixed at night. Similar to C_4 plants, but two steps are separated by time (day and night) not in space. It helps in reducing transpiration.

Photorespiration

Light dependent oxygenation of RuBP and release of CO_2 in photosynthetic cells of plant. It involves chloroplasts, peroxisomes and mitochondria. It consumes energy and wastes the work of photosynthesis.