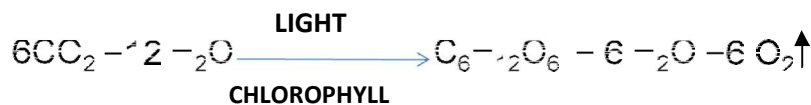


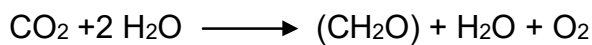
PHOTOSYNTHESIS

9.1 DEFINITION: Photosynthesis is the process by which organisms convert light energy into chemical energy in the form of reducing power as NADPH and ATP. This reducing power is used to fix carbon dioxide as carbohydrates (sugars). In oxygenic photosynthetic organisms, including higher plants, the source of reducing equivalents is H₂O, releasing O₂ as a by product.

Thus, the overall reaction of oxygenic photosynthesis can be represented as.

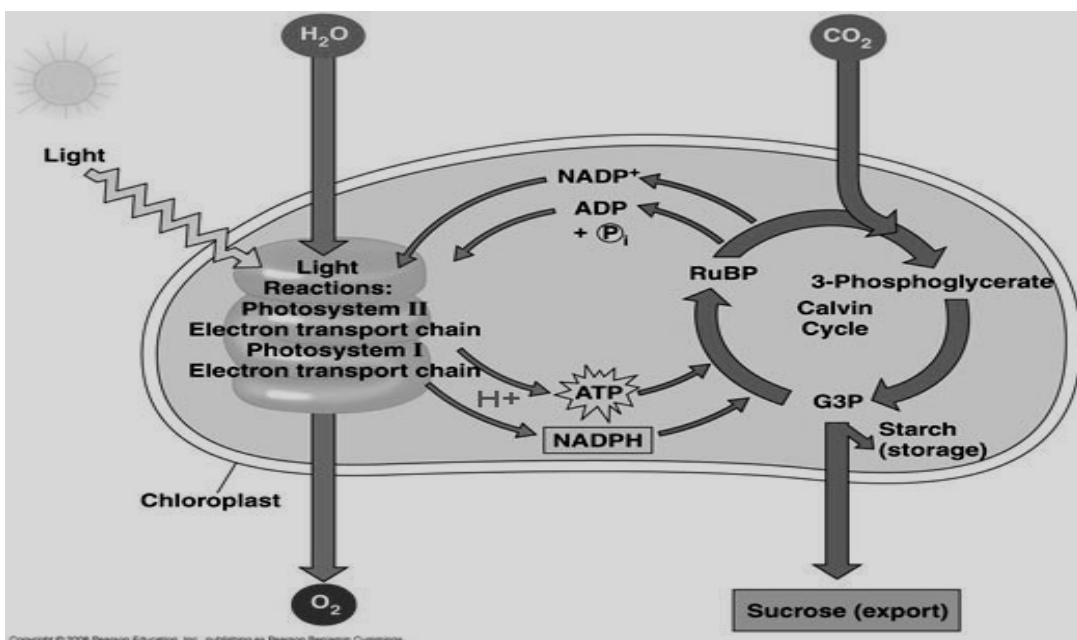


This equation is frequently represented by the simplified form:



Reactions of Photosynthesis:

During the normal functioning of the photosynthetic system, light serves to reduce nicotinamide-adenine dinucleotide phosphate (NADP). This in turn serves as a reducing agent for carbon fixation in the carbon reduction cycle. ATP is also formed during the electron flow from water to NADP, and it too used in carbon reduction. The chemical reactions in which water is oxidized to oxygen, NADP is reduced, and ATP is formed are generally known as the **light reactions** while the carbon reduction reactions are called the **dark reactions**.



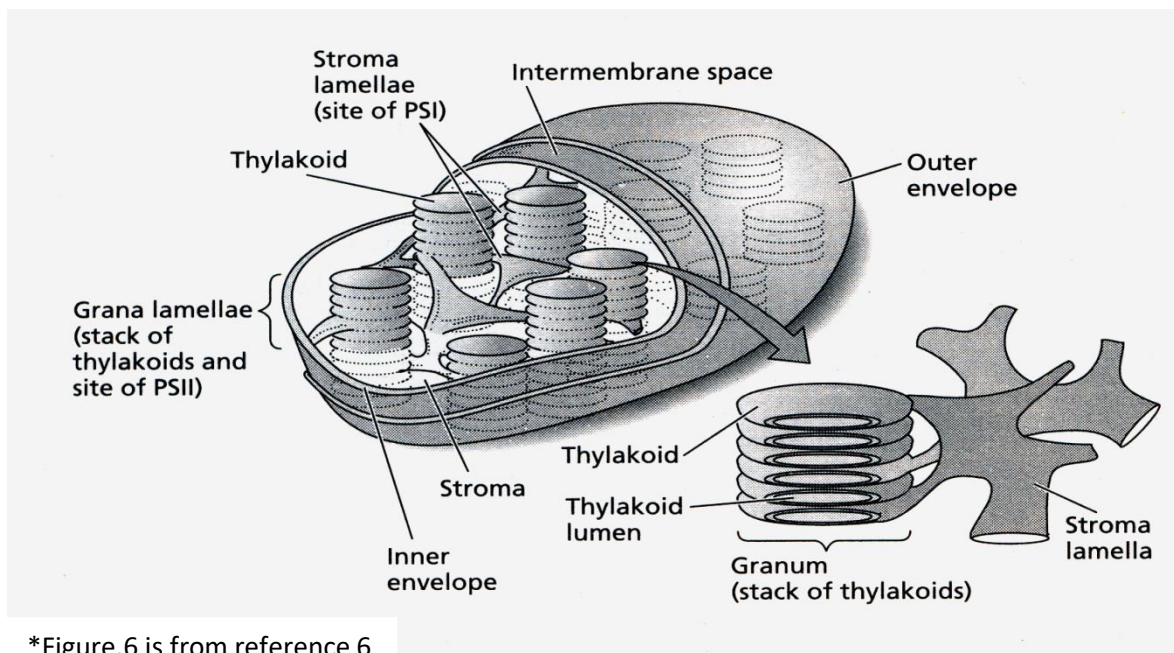
All most all reactions up to NADP reduction takes place with in the thylakoids, while the carbon reduction rections take place in the aqueous region of chloroplast, the stroma. The following figure explains about the link between light reaction and dark reaction.

9.2 ENERGY SYNTHESIS:

Energy synthesis is the prime function of photosynthesis. It involves the role of various aspects like chloroplast and its structure, pigments present in chloroplast, Electro magnetic spectrum, photosynthetically active radiation, light interception by canopy, Capturing of light by pigment molecules, Light reaction (photolysis of water, electron transport between photo systems, generation of reducing power) and utilization of this reducing power in fixation of CO₂ to carbohydrates (dark reaction). Each of this aspect is briefly explained here.

9.2.1 CHLOROPLAST AND ITS STRUCTURE:

In photosynthetic eukaryotes, photosynthesis takes place in the sub cellular organelle known as chloroplast.(Fig.6)



*Figure.6 is from reference 6

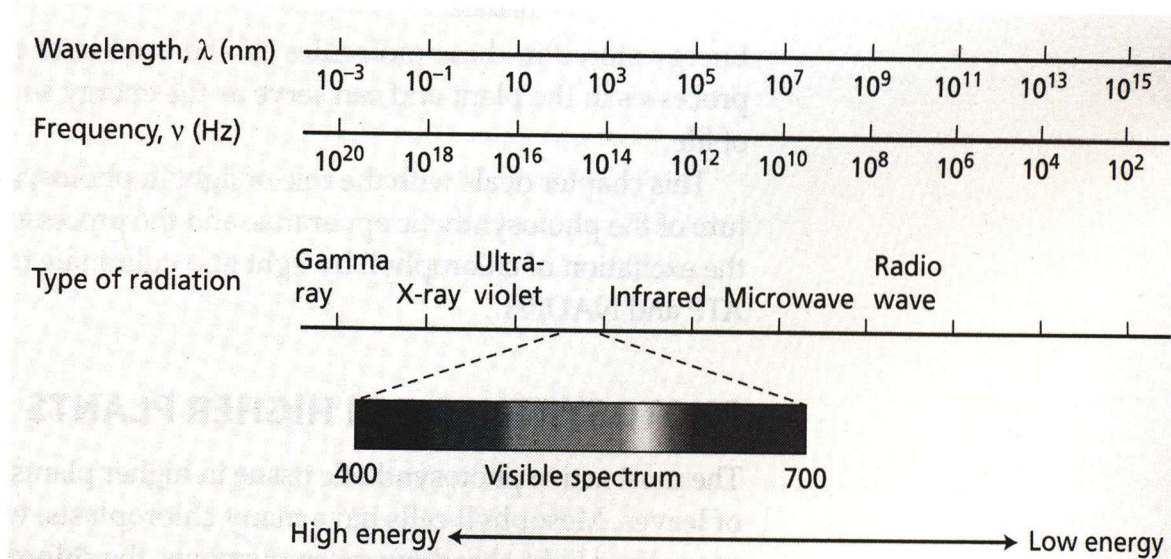
The most striking aspect of the structure of the chloroplast is this extensive system of the internal membranes known as **thylakoids**, (On these thylakoid bodies some round shaped photosynthetic units called **quintasomes** are present.) All the chlorophyll is contained within this membrane system, which is the **site of the light reactions** of photosynthesis. The carbon reduction reactions or **dark reactions** which are catalyzed by water soluble enzymes, takes place in the **stroma**, the region of the chloroplast outside the thylakoids. Stroma forms the matrix of the chloroplast. In this portion of chloroplast, lamellae are loosely arranged. The lamellae which are found in this region are called stroma lamellae.

9.2.2 PHOTOSYNTHETIC PIGMENTS:

There are four different pigments in higher plants- two chlorophylls (Chl. A and Chl. B) and two carotenoids (carotene and xanthophyll). The chlorophylls are green in color whereas carotene and xanthophylls are orange and yellow, respectively. Magnesium present in chlorophyll molecule is crucial to the capture of light energy.

All organisms actually contain a mixture of more than one kind of pigment, each serving a specific function. As the energy of light absorbed by carotenoids is rapidly transferred to chlorophylls, carotenoids are termed as **accessory pigments**.

9.2.3 ELECTRO MAGNETIC SPECTRUM:

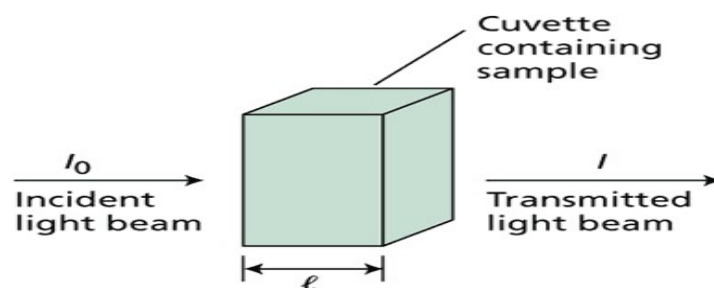


In the electromagnetic spectrum the wavelengths between 400-700nm is called as PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR).

9.2.4 PRINCIPLE OF LIGHT ABSORPTION BY PLANTS:

The depression of light intensity within the plant community is mainly due to interception of light by leaves. The absorption of radiant energy by a plant community would follow the Lambert-Beer's Law.

Beer's Law states that the absorption of light by a solution is proportional to the concentration of the solution and the distance through which the light travels



Here the absorbance is defined as $A = \log I_0 / I$ where I_0 is the intensity of light that is incident on the sample and I is the intensity of light that is transmitted by the sample.

Lambert expressed this relationship mathematically as

$$I = I_0 e^{-KX}$$

Where I_0 = Incident light intensity

I = Intensity of the light after passing through the solution

K = Absorption coefficient.

X = Distance or path length .

Davidson and Philip modified this equation for absorption of energy by a plant canopy. They substituted LAI for x (i.e the path length). The hypothesized equation for absorption of radiant energy by a plant canopy then becomes

$$I = I_0 e^{-KA}$$

$$(or) K = \log_e (I_0/I) / A$$

The proportion of incident light that is intercepted by the canopy does not depend on leaf area index alone, but also on the architecture of the canopy. In a crop the leaves are not isolated, but are arranged in a canopy. The way they are arranged will affect the proportion of incident light that is intercepted by the crop canopy as a whole. Thus, the extinction coefficient (K) is a measure of the light intercepting efficiency of the leaf area. Leaf characters of importance in this respect are: leaf angle, leaf area, continuity of leaf layers etc.

9.2.5 THE TWO PHOTO SYSTEMS:

9.2.5.1 Evidence for the existence of PSI and PSII. (Red drop & Emerson enhancement effect)

Experiments measuring the quantum yield (i.e. the number of O_2 molecules released for each Quanta absorbed) is approximately 0.1. The reciprocal of quantum yield is quantum requirement i.e. the number of quanta required for each O_2 molecule evolution. It is 10). If the quantum yield of photosynthesis is measured at different ranges of wave lengths most of the ranges are remarkably constant. However at the extreme red edge of the chlorophyll absorption (>680nm), the yield drops drastically. The phenomenon is known as **RED DROP**.

Conclusion: It is proposed that at this wave length only one photosystem remains in operation. As another photosystem is not able function at this wavelength quantum yield drops.

Another puzzling experimental result was the enhancement effect, discovered by Emerson. The rate of photosynthesis was measured separately with light of two different wave lengths and then the two beams were used simultaneously. When exposed to a wavelength more than 680 nm (far red region) a specific rate of photosynthesis was observed. Likewise when the exposure was given at wavelengths less than 680 nm some other effect was observed. When the system was exposed to the light of both wavelengths simultaneously, the effect on photosynthesis exceeded the sum of the two effects caused separately. This provided evidence that the two pigment systems worked in co-operation with each other and the increase in photosynthesis was due to synergism. This phenomenon is known as **Emerson Enhancement effect**. This experiment also explained that, there exist two photo systems (PS-I & PS-II)

Based on these two observations Hill and Fay Bendall (1960) proposed that light reactions of photosynthesis involve two photochemical events.

9.2.5.2 PHOTOSYSTEM I & II:

The reaction centre chlorophyll of **photo system I** absorbs maximally at 700 nm in its reduced state. Accordingly, it is named **P 700** (the P stands for Pigments), the reaction centre chlorophyll of **photo system II** absorbs maximum at 680 nm (**P 680**) so its reaction centre chlorophylls and associated electron transport proteins is located predominantly in the stacked regions of the grana lamellae. Whereas the photo system I, its associated antenna pigments, ATP synthase enzymes are found exclusively in the stroma lamella and at the edges of the grana lamellae. (At a wavelength greater than 680 nm PS-II can not operate. Therefore, quantum yield decreased beyond 680 nm. This is what red drop is.)

Photo system I contains **large amount of chlorophyll a**, a small amount of chlorophyll b and some B carotene. Photo system II also contains chlorophyll a, B carotene but a **large amount of chlorophyll b**.

9.2.6 LIGHT REACTIONS:

Almost all the chemical processes that make up the light reactions of photosynthesis are carried out by four protein complexes; photo system II (P 680), the cytochrome b-f complexes, photo system I (P 700) and the ATP synthase.

SEQUENCE OF LIGHT REACTIONS:

9.2.6.1 Water is oxidized to oxygen by Photo system II:

The chemical reaction by which water is oxidized is given by the following equation.



This equation indicates that four electrons are removed from two water molecules, generating an oxygen molecule and four hydrogen ions.

Robert Hill demonstrated that isolated chloroplasts evolved Oxygen when they were illuminated in the presence of suitable electron acceptor, such as ferricyanide. The ferricyanide is reduced to ferrocyanide by photolysis of water. This reaction is now called as **HILL REACTION** and it explains that water is used as a source of electrons for CO₂ fixation and Oxygen is evolved as a by product.

The protons produced by oxidation of water is released into the lumen of the thylakoid, not directly into the stromal compartment. These protons are eventually released from the lumen to the stroma through the process of ATP synthesis. In this way the electro chemical potential formed by the release of protons during oxidation of water contributes to ATP formation.

Water Oxidation System: Manganese (Mn) is an essential cofactor in the water oxidizing process. Analytical experiments indicate that four Mn ions are associated with oxygen evolving complex. Other experiments have shown that Cl and Ca ions are also essential for O₂ evolution.

9.2.6.2 ELECTRON TRANSPORT AND PHOSPHORYLATION:

The Z scheme (named because of its shape) illustrates electron transport and the production of NADPH and ATP in chloroplasts.

9.2.6.2.1 NON CYCLIC PHOTOPHOSPHORYLATION:

Non cyclic photophosphorylation involves integration of two photo systems (PS-I and PS-II). This is one of the means of ATP production in chloroplasts.

Non-Cyclic	:	Electrons follows a non cyclic track.
Photo	:	it is the light energy that drives electrons along this Track.
Phosphorylation	:	As electrons are driven along the track ADP is phosphorylated to yield ATP.

This can also be termed as non-cyclic electron transport to refer to the manner of electron flow during the process.

MECHANISM:

The primary flow of electrons within a given granum thylakoid may be initiated almost simultaneously for each photo system (PS I and PS II).

After excitation of P₇₀₀ the electrons are passed on to a chlorophyll molecule (A₀). The electrons are then passed to series of Iron-sulfur proteins (Fe-S) and finally to ferridoxin (Fd). The ferridoxin-NADP reductase serves to reduce NADP to NADPH which is used in the Calvin cycle to reduce CO₂.(Fig. 7)

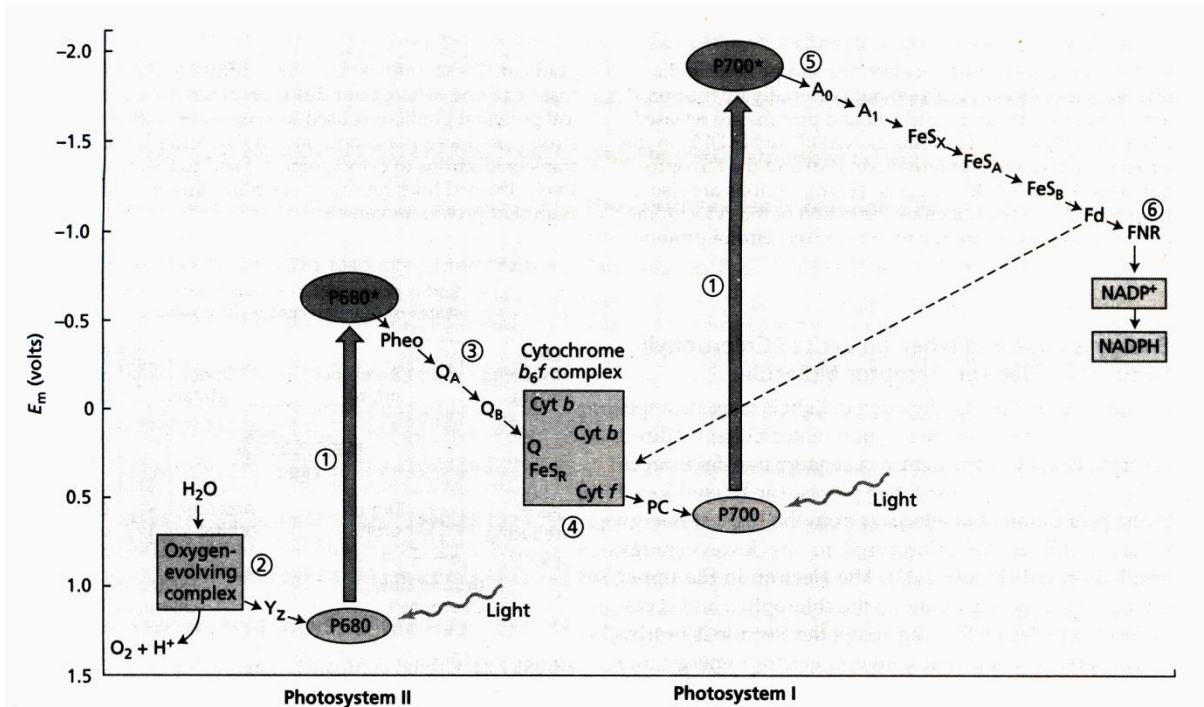


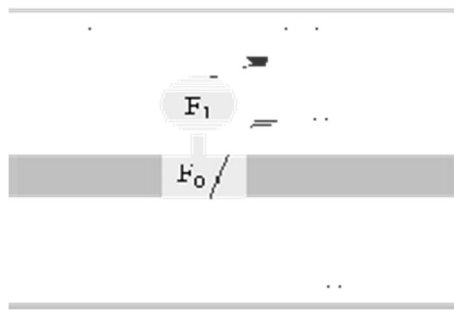
FIGURE Detailed Z scheme for O₂-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, that has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the

acceptors Q_A and Q_B, which are plastoquinones. (4) The cytochrome *b₆f* complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces P700* (oxidized P700). (5) The acceptor of electrons from P700* (A₀) is thought to be a chlorophyll, and the next acceptor (A₁) is a quinone. A series of membrane-bound iron-sulfur proteins (FeS_X, FeS_A, and FeS_B) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin-NADP reductase (FNR) reduces NADP⁺ to NADPH, which is used in the Calvin cycle to reduce CO₂ (see Chapter 8). The dashed line indicates cyclic electron flow around PSI. (After Blankenship and Prince 1985.)

*Figure.7 is from reference 6

The transfer of electrons to NADP creates deficit (commonly referred to as a hole) in photo system I. However this deficit is made up by the excitation of P₆₈₀ of photo system II. The excited P₆₈₀ of PS II transfers electrons to pheophytin, plastoquinones, and cyt b₆-f complex. Cytochrome b₆-f complex transfers electrons to plastocyanin (PC), which in turn reduces P₇₀₀* (excited P700). The hole created in photo system II is filled by-electrons that are derived from the oxidation of water.

In addition to the energy stored as redox equivalents (NADPH) by the light reactions a portion of the photons energy is utilized for the synthesis of ATP during the transfer of electrons between plastoquinone and cyt b₆-f complex. This phenomenon of synthesis of ATP in light reactions of photosynthesis is known as **photophosphorylation**. It is now widely accepted that photophosphorylation works via the **chemi-osmotic mechanism** first proposed in 1960 by **Peter Mitchell**.



The basic principle of chemi-osmosis is that in concentration differences and electrical potential differences across the membranes are a source of free energy that can be utilized by the cell for the synthesis of ATP. In the light reactions electron flow is coupled to proton translocation, creating transmembrane proton motive force (pmf). The energy in the proton motive force is then used for synthesis of ATP by the enzyme called ATP synthase.

This synthesis of ATP coupled to a linear flow of electrons from water to NADP is called as non cyclic photophosphorylation. The precise number of ATP molecules formed in non-cyclic photophorylation is unclear although it is generally thought **two** per O₂ evolved.

9.2.6.2.2 CYCLIC PHOTOPHOSPHORYLATION:

The cyclic photophosphorylation operates when chloroplasts are illuminated with wave lengths of light greater than 680nm. Under these circumstances only photo system I is activated and electrons are not removed from H₂O. When the flow of electrons from H₂O is stopped, non cyclic assimilation retarded, oxidized NADP is no longer available as an electron acceptor. Activation of photo system I by wave lengths of light greater than 680 nm causes electron to flow from P₇₀₀ to chlorophyll molecule and Ferridoxin. Then the electrons instead of pass on to NADP return back to P₇₀₀ via cyt b₆-f complex, plastoquinone and plastocyanin.

Cyclic transport system is likely to result in the synthesis of ATP at two locations. One is between Fe-s protein and cyt-b₆ complex and another between cyt-b₆ and cytochrome f.

Significance

Evidence for the operation of cyclic electron transport in C₃ plants *in vivo* is limited but it has been demonstrated under physiological conditions *in vivo* in C₄ plants where there is an additional ATP requirement in their carbon fixation pathway. It may also play an important role in the synthesis of ATP required for protein synthesis during PS II repair following photo inhibition.

9.2.6.2.3 PSEUDO CYCLIC PHOSPHORYLATION:

Another source of generation of ATP is that electrons might be transferred from ferridoxin back to oxygen reducing it to water. It is possible that this process might also involve an electron transport chain and produce ATP. Here the electron that is cycled back to reduce molecular oxygen to water is not the same that is released from the water. Hence it is called as pseudo cyclic phosphorylation.

9.2.7 CARBON DIOXIDE FIXATION /DARK REACTIONS OF PHOTOSYNTHESIS:

During the light reactions of photosynthesis, the photochemical oxidation of water to molecular oxygen is coupled to the generation of reduced pyridine nucleotide (NADPH) and ATP. The reactions associated with the reduction of CO₂ to carbohydrate are coupled to the consumption of NADPH and ATP. These reactions are referred to as the Dark reactions of the photosynthesis.

9.2.7.1 The C₃ Cycle (C₃ Photosynthetic Carbon Reduction Cycle)

The PCR cycle is sometimes referred to as the Calvin cycle in honor of its discoverer, the American biochemist Melvin Calvin, other pathways associated with the photosynthetic fixation of CO₂, such as the C₄ photosynthetic carbon assimilation (PCA) cycle and the C₂ photo respiratory carbon oxidation cycle (PCO), are either auxiliary to or dependent on the basis PCR cycle.

In the C₃, PCR cycle, carbon dioxide from the atmosphere and water are enzymatically combined with a five-carbon acceptor molecule to generate two molecules of a three carbon intermediate. These intermediates are reduced to carbohydrate using the photo chemically generated ATP and NADPH in the light reactions. The cycle is completed by the generation of five-carbon acceptor.(Fig.8)

The C₃ PCR cycle proceeds in three stages:

1. **Carboxylation** of the CO₂ acceptor, ribulose 1,5 – bisphosphate, to form 2 molecules of 3. Phosphoglycerate, the first stable intermediate of the PCR cycle.
2. **Reduction** of this carboxylic acid to a carbohydrate in the form glyceraldehydes 3- phosphate.
3. **Regeneration** of CO₂ acceptor, ribulose 1.5-biophosphate from glyceraldehydes 3-phosphate.

The Carboxylation of Ribulose Bisphosphate:

CO₂ enters the PCR cycle by reacting with ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglycerate, a reaction that is catalyzed by the chloroplast enzyme ribulose bisphosphate carboxylase/oxygenase, referred to by the acronym **RUBISCO**.

The reduction step of the C₃ PCR cycle:

In this stage, the 3-phosphoglycerate formed as a result of the carboxylation of RuBP (Ribulose 1,5-bisphosphate) is first phosphorylated to 1,3-bis phosphoglycerate by the ATP generated in the light reactions and is then reduced to glyceraldehyde 3-phosphate, using the NADPH generated by the light reactions. The chloroplast enzyme NADP-glyceraldehyde 3-phosphate dehydrogenase catalyzes this step.

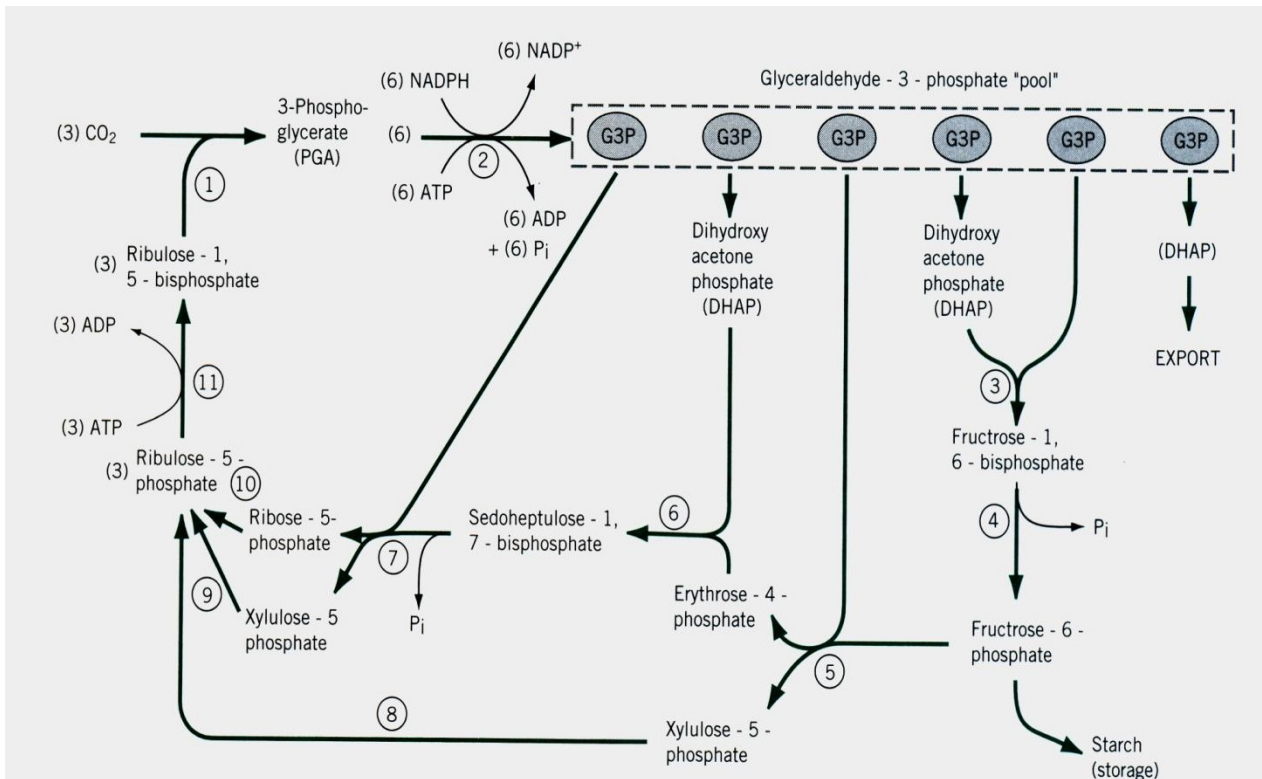


FIGURE 10.3 The photosynthetic carbon reduction (PCR) cycle. Numbers in brackets indicate stoichiometry. Enzymes, indicated by circled numbers are: (1) ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco); (2) 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase; (3) aldolase; (4) fructose-1,6-bisphosphatase; (5) transketolase; (6) aldolase; (7) sedoheptulose-1,7-bisphosphatase; (8, 9) ribulose-5-phosphate epimerase; (10) ribose-5-phosphate isomerase; (11) ribulose-5-phosphate kinase.

*Figure.8 is from reference 5

Regeneration of Ribulose 1, 5 -Bisphosphate:

One molecule of glyceraldehyde 3-phosphate is converted to dihydroxy acetone 3-phosphate (DHAP). The DHAP then undergoes aldol condensation with a molecule of glyceraldehydes 3-phosphate to form fructose 1,6-bis phosphate. This product is hydrolyzed to fructose 6 phosphate. This fructose 6 phosphate combines with third molecule of glyceraldehyde 3-phosphate to give erythrose 4-phosphate and xylulose 5-phosphate. This reaction is catalyzed by transketolase.

Erythrose 4-phosphate then combines with DHAP to yield a seven carbon sugar, sedoheptulose 1,7-bisphosphate which is further hydrolyzed to give sedoheptulose 7-phosphate. Sedoheptulose 7-phosphate donates a two carbon unit to the fifth molecule of glyceraldehyde 3-phosphate and produce ribose 5-phosphate

and xylulose 5-phosphate as its products. Two molecules of xylulose 5-phosphate are epimerized to give ribulose 5-phosphate. The third molecule of ribulose 5-phosphate is formed by the isomerization of ribose 5-phosphate. Finally, ribulose 5-phosphate is phosphorylated with ATP. Thus generating CO₂ acceptor ribulose 1,5-bisphosphate.

Energy requirement: In order to synthesize the equivalent of 1 molecule of hexose sugar, 6 molecules of CO₂ are fixed at the expense of 18 ATP and 12 NADPH. In other words, the PCR cycle consumes 2 molecules of NADPH and 3 molecules of ATP for every CO₂ fixed.

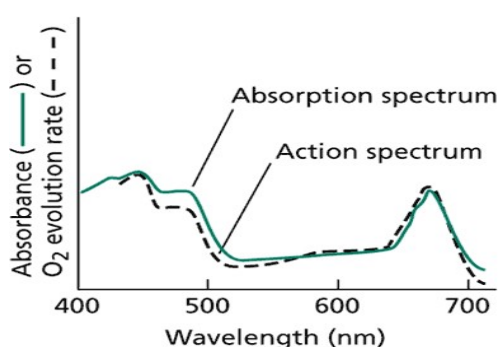


ANNEXURE

Apart from stroma lamellae ribosomes serving as sites for the protein synthesis are also found scattered in this region. Lipid, protein and nucleic acid metabolisms are also found in this region.

Chlorophyll molecule is a typical porphyrin derivative possessing a cyclic tetrapyrrolic structure in which one pyrrole ring is partially reduced. The tetrapyrrolic nucleus contains a **non-ionic magnesium** atom held by two covalent and two coordinate bonds in the centre of the molecule. Magnesium is crucial to the capture of light energy. The presence of methyl group (CH₃) at position 3 in the second pyrrole ring makes chlorophyll-a and when it is replaced by formyl group (-CHO) it forms chlorophyll-b.

		Chemical formula	Absorption peaks
1	Cholorophyll-a	C ₅₅ H ₇₂ O ₅ N ₄ Mg	662, 430nm
2	Cholorophyll-b	C ₅₅ H ₇₀ O ₆ N ₄ Mg	642, 453nm



An action spectrum is measured by plotting a response to light such as oxygen evolution, as a function of wavelength. If the pigments used to obtain the absorption spectrum are the same as those that cause the response, the absorption and action spectra will match. In the example shown here, the action spectrum for oxygen evolution matches the absorption spectrum of intact chloroplasts quite well, indicating that light absorption by the chlorophylls mediates oxygen evolution.

*For further information see reference.6