Indira Gandhi
National Open University
School of Sciences

BBYCT-137
PLANT PHYSIOLOGY AND METABOLISM

Block

2

PHOTOSYNTHESIS AND TRANSLOCATION OF PHOTOSYNTHATES

Course Design Committee

Prof. G.C. Srivastava (Retd.)
Former Head,
Department of Physiology,
IARI, Pusa,
New Delhi-110012

Prof. Vijay Paul
Principal Scientist,
Division of Plant Physiology
IARI, Pusa,
New Delhi-110012

School of Sciences, IGNOU

Prof. M.S. Nathawat, Director, (Ex.)

Prof. Amrita Nigam

Prof. Jaswant Sokhi (Retd.)

Prof. Bano Saidullah (Retd.)

Prof. Neera Kapoor

Block Preparation Team

Prof. Amrita Nigam
SOS, IGNOU, New Delhi-110068

Dr. Eklavya Chauhan
Sr. Consultant,
SOS, IGNOU, New Delhi-110068

Editor

Prof. G.C. Srivastava (Retd.)
Former Head,
Department of Physiology,
IARI, Pusa,
New Delhi-110012

Course Coordinator: Prof. Amrita Nigam

Production

Mr. Hemant Kumar
SO(P), MPDD, IGNOU

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BLOCK 2 : PHOTOSYNTHESIS AND TRANSLOCATION OF PHOTOSYNTHATES

Block II deals with Photosynthesis and Translocation of food in phloem. Photosynthesis is the only process which can harvest solar energy and convert it into potential chemical energy, that is vital for life.

Unit 5 introduces you to the history and the foundations of the unique process of photosynthesis. It also makes you familiar with the physical principles and nature of light along with the structure and function of the photosynthetic apparatus. This unit also highlights the unique structural features of the photosynthetic pigments, especially Chl*a* which has the capacity to optimally utilize the photons from sun.

Unit 6 deals with the mechanism of light reaction wherein the solar energy is used to energize the photochemical reactions in the chloroplast grana to generate ATP and NADPH for the dark reaction via the membrane bound electron transfer chain. The light harvesting centers in the chloroplast grana thylakoids in fact, present a unique example of nature's solar energy trapping machinery with a high degree of efficiency. In this unit you will find the details of the Benson-Calvin cycle wherein the atmospheric CO_2 is fixed along with the participation ATP-NADPH (generated in the light reactions), for the synthesis of sugars. This unique set of reactions form the basis for the survival of the heterotrophs on this planet.This unit also highlights the characteristic features of the wonder enzyme *Rubisco,* which is capable of catalyzing both the carboxylation and oxygenation reactions.

A detailed discussion follows in **Unit 7** which deals with the reactions of C_2 cycle, which is an unavoidable phenomenon of the C_3 plants. Strategies to avoid this wasteful process have been described in the description of C_4 and CAM plants.Origin of these alternate photosynthetic pathways have been discussed in the light of the evolution of monocots and dicots.

Unit 8 is devoted exclusively to a discussion of systems that have been evolved by plants to translocate the food material made by photosynthesis via phloem to different parts of a plant. The modern views regarding the source to sink transition and phloem loading and unloading have been discussed.

Objectives

After studying the block, you will be able to :

- describe history of photosynthesis; understand the earlier experiments that led to the formulation of the modern equation of photosynthesis;
- discuss the various events in cyclic and non-cyclic photophosphorylation and the electron transport chain;
- understand the reactions of C_3, C_2 ,C_4 and CAM pathways; and
- discuss various hypotheses for the transport of photosynthates through phloem; and describe the process of phloem loading via the apoplast and symplast.

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UNIT 5

PHOTOSYNTHESIS

Structure

5.1 INTRODUCTION

In this Unit you will be studying the plant pigments including non-photosynthetic pigments and sunlight, which are required for photosynthesis—a process by which green plants and certain other organisms transform light energy into chemical energy in the form of sugars. This sugar can then be converted to other carbohydrates or other food materials like fats and proteins. The general importance of the process was recognized as long ago as 2000 years. The biblical saint, Isaiah, who lived between 700-600 B.C. said “All flesh is grass” recognizing that all food chains are finally traced to plants. Plants are also responsible for the fossil fuels such as petroleum, oil, and coal, which represent products of photosynthesis carried out millions of years ago in the carboniferous era. It is through this process that plants continuously purify air during daytime and thus allow animals to breathe.

The overall importance of this process is best expressed in the words of Eugene Rabinowitch, one of the great authors and researchers of

photosynthesis, who said "*Physiologically speaking all the animals on land and in the sea, including man are but a small brood of parasites living off the great body of the plant kingdom*", and "*if plants could express themselves, they would probably have the same low opinion of animals as we have of fleas and tapeworms – organisms that must lazily depend on others for survival*."

The photosynthetic products are utilized by humans and other animals to provide energy. He proceeded to state that "*without them no heart could beat, no amoeba could swim, no sensation could speed along a nerve, no thought could flash in the human brain*". Clearly, for all these activities we are dependent on plants.

It has been estimated that photosynthesis gives 200×10^9 tons of solid plant material per year which comes to about 70 to 80 tons of sugar equivalent per person! Clearly, photosynthesis represents the greatest chemical factory on earth. Unravelling the mechanism of the process has, therefore, been one of the most important tasks of plant biology.

The various sections and subsections are arranged in a chronological order. Beginning with experiments that led to the formulation of the basic equation, we have described the various photosynthetic pigments, especially the unique chlorophyll, and nature of the electromagnetic spectrum. You will also study about the unique properties of chlorophyll in terms of its absorption and action spectrum. Finally, we will briefly discuss the energy states of chlorophyll after its photoexcitation.

This unit is unique and interesting. Here, we have described in a story form a historical account of major experiments that led to the detailed knowledge of photosynthesis such as we have today. We have particularly emphasized how the various key concepts in photosynthesis were formulated.

Objectives

After studying this unit, you should be able to :

- outline the scientific developments that led to recognition of the necessary raw materials of photosynthesis and the important end products;
- list the essential and the accessory photosynthetic pigments and gain an idea of the structure and synthesis of chlorophyll;
- describe the nature of light and the electromagnetic spectrum and appreciate the unique properties of chlorophyll: absorption and action spectrum; and
- trace the photoexcitation of chlorophyll and its energy states.

Study Guide

You may find this unit a bit lengthy, but very interesting as you will study how the photosynthesis was discovered. It is important that you spend more time in studying it in continuity.

5.2 BASIC CONCEPTS-HISTORICAL BACKGROUND

5.2.1 Earlier Investigations

Although the very concept of photosynthesis is based on observations mentioned in the epic “Mahabharata” as early as 2600 BC that recognized the role of plants in harnessing the solar energy into food, the credit for crystallizing this concept goes to the Greek philosopher **Aristotle** (Father of Biology and Zoology). He proposed in 350 BC that plants, like animals, require food. This very assertion was confirmed only after almost 2000 years by **Joseph Priestley,** that it is not plants that require animals but in fact it is the animals who cannot live without plants. **Theophrastus**, a student of Aristotle called **Father of Botany**, was the first to indicate that plants obtained their food and nourishment through the roots. The Indian sage **Parasara** (ca. 100 BC) mentioned the role of plant pigments and their ability to make food.

Nicholas of Cusa proposed a very interesting experiment in 1450. He theorized that if we were to weigh a plant and grow it in a pot containing pre-weighed soil, irrigated with a weighed amount of water; then by comparing the initial and final weights of soil after a given period, would certainly demonstrate that the mass of plant is derived from water and not from soil. His speculation was simply based on the belief that it was only water which could make the plants grow. However, there was no experimental backing to this assumption.

This very idea that water is an important reactant came later also from the work of a Dutch physician, chemist, and alchemist **Jean Baptiste van Helmont** (Fig. 5.1a), who actually performed the experiments proposed by Nicholas of Cusa nearly two centuries earlier.

Results of his experiments were published after his death in 1648 by his son in **Ortus medicinae**. He grew a sapling of willow (*Salix*) tree initially weighing 5 lb (2.27kg) in a clay pot containing 200 lb (90.72kg) of soil. He watered the plants regularly with distilled water. After five years the tree was removed from the earthenware, and all the soil was brushed off its roots and put back in the pot. The tree now weighed 169 lb 3 oz (76.74kg) while there was not much loss of soil during this period. The weight of soil had decreased by only 2 oz (56.7g) (see Fig. 5.1b). He concluded that this 164.19 lb (74.39 kg) of wood, bark and roots were formed from **water alone**, which constituted the prime component of the plant body. These observations were recorded in his book entitled **Ortus medicinae** (On the Power of Medicine), published in 1648 in Amsterdam after his death. However, we know very well today that even though van Helmont was only partly correct and was not at all aware of the role of atmospheric gases or sunlight in plant growth, his pioneering experiments have contributed to advancement in our understanding of photosynthesis.

John Woodward (1699) was of the view that plants were not formed of water alone, but also grew well in muddy water, and perhaps some terrestrial matter got absorbed that also helped in plant growth. His experiments with mint plants in water (hydroponics) of different purity made him to conclude that soil was infact, responsible for increase in plant growth. This led to his “**Humus Theory**” that plants absorb all their nutritional requirements from soil humus.

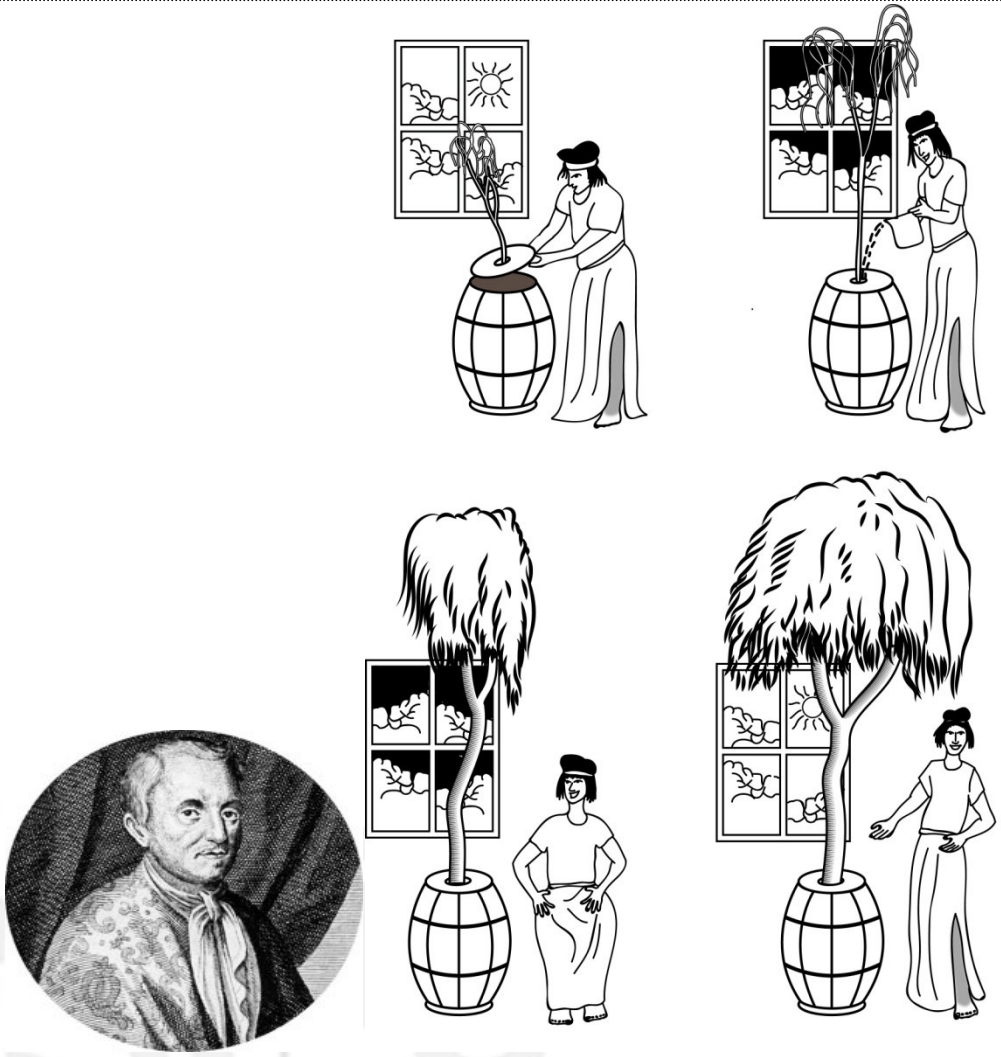


Fig. 5.1: a) Jean Baptiste van Helmont (12-1-1580 to 30-12-1644); b) The famous willow experiment by which van Helmont concluded that a plant grows from water alone.

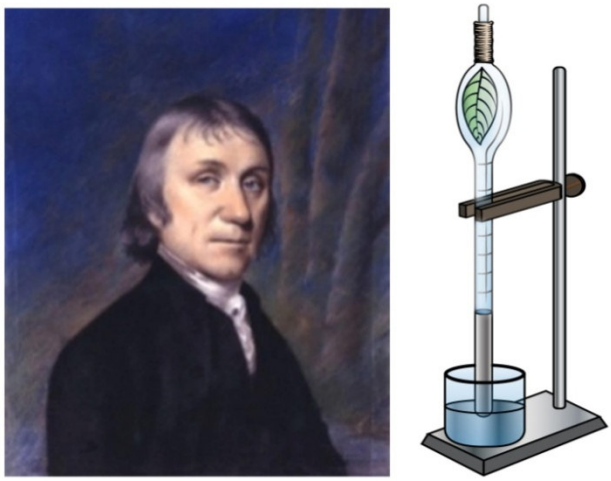
Fig. 5.2: Stephen Hales (17-9-1677 to 4-1-1761)

It was **Edme Mariotte** (1679) who was perhaps the first to propose that plants obtained part of their nourishment from the atmosphere. The English scientist **Stephan Hales** (Fig. 5.2), also called as Father of Plant Physiology, mentioned in 1727 in his book "**Vegetable Staticks**" that the leaves "very probably" derive nourishment from air and the process may involve light. His guess was ultimately proven correct by future researchers.

Interestingly, what the Swiss naturalist **Charles Bonnet** (1754) observed as emission of gas bubbles from leaves of an illuminated submerged hydrophyte , forms the most basic experiments on photosynthesis conducted in our school and undergraduate laboratories today, demonstrating the release of oxygen bubbles by *Hydrilla* sprigs.

The first convincing evidence of the participation of gases in the process of photosynthesis came in 1771 from a series of experiments by the English clergyman and chemist **Joseph Priestley** (Fig. 5.3 a). He and his contemporaries firmly believed that a noxious substance (called **phlogiston** by them) was released into the air because of burning of flame. He was intensely interested in the process by which bad air could be purified, or "**dephlogisticated**" into **dephlogiston**. At that time, chemists were obsessed with the idea of phlogiston, then considered a principle of flammability.

According to Priestley, plants dephlogisticated the foul air (see Fig. 5.3 b). Further, the pure air had properties like the gas which he had discovered and was released by focusing sunrays on the red oxide of mercury with the help of a huge lens, which was almost a foot in diameter. This very dephlogisticated air was later identified as oxygen.



(a) (b)

Fig. 5.3: a) Joseph Priestley (24-3-1733 to 6-2-1804); b) Classical experiments by Priestley who grew small twigs of mint in an inverted tube and piped air to a jar with live mouse. He proved that plants have the capacity to purify air.

Priestley's experiments excited the interest of a Dutch physiologist and chemist **Jan Ingenhousz (Ingen-Housz)** in Vienna (Fig. 5.4), Austria. He was a court physician to Empress Maria Theresa of Austria. In 1778, on a visit to England for a three-month vacation, he rented a villa and conducted some 500 experiments. He confirmed that not only mint, but even other plants purified air; but, more importantly, he found that the process will proceed only in the presence of sunlight and plants could purify air significantly even in a few hours. To quote from a book "**Experiments on Vegetables Discovering Their Great Power of Purifying the Common Air in Sunshine, and of Injuring at Night**" he said *" I was not long engaged in this enquiry before I saw a most important scene opened to my view: I observed, that plants not only have a faculty to correct bad air in six or ten days, by growing in it ,as the experiments of Dr. Priestley indicate, but that they perform this important office in a complete(sic) manner in a few hours; that this wonderful operation is by no means owing to the vegetation of plant, but to the influence of the sun upon the plant".* He also found that only the green parts of the plant purified air and not the non-green parts and that so long as the plants were green, the "*acrid, ill-scented, and even the most poisonous plants perform this office in common with the mildness and the most salutary".*

Fig. 5.4: Jan Ingenhousz (8-12-1730 to 7-9-1799)

In other words, **Ingenhousz** had recognized the participation of both chlorophyll and sunlight in his own way as he asserted that only green parts of plants are important for dephlogistation and that this process of purification is possible only in the presence of sunlight. Interestingly, he also reported that plants also generated "bad air" in darkness and even recommended the removal of plants from rooms at night to avoid poisoning or toxicity to the occupants.

Fig. 5.5: Antoine Lavoisier (26-8-1743 to 8-5-1794)

The true chemical nature of pure and impure air was not known to us and remained a mystery till the French chemist **Antoine Lavoisier** (Antoine-Laurent de Lavoisier) coined the term Oxygen in 1778. Lavoisier (Fig. 5.5) is hailed as the **Father of Modern Chemistry.** While discovering the **principle of combustion**, he identified the "pure" component of air as oxygen (O_2) and the "impure" air as carbon dioxide (CO_2).

Credit for the discovery of carbon dioxide, perhaps goes to the British physician and chemist **Joseph Black** (1756). Although he described this discovery as a component of air which he called **fixed air** in his work **"Experiments upon Magnesia Alba, Quicklime, and some Other Alcaline Substances"**. Fixed air was the same as **"gas sylvestre"** earlier reported by van Helmont.

Ingenhousz reinterpreted his "dephlogistation" results in the light of Lavoisier's work. He later put forth a hypothesis that plants split carbon dioxide in the presence of sunlight to use its carbon (C) for growth,and oxygen is given off as waste. As we would study in the next section, this model was a definite improvement over the earlier ideas of Priestley, but again was not entirely correct. This was therefore, also destined to get modified, and eventually it did happen almost 150 years later by the work of **C. B. van Niel.**

Fig. 5.6: Jean Senebier (6-5-1742 to 22-7-1809)

Another important advancement in this direction was made in1796 by **Jean Senebier** (Fig. 5.6), a Swiss pastor and botanist from Geneva. He established that CO_2 was essential for plant growth. He also demonstrated that plants absorb CO_2 and released dephlogisticated air (oxygen). Since a submerged green leaf did not produce oxygen when put in boiled distilled water (minus CO_2), oxygen must be coming from CO_2. Surprisingly, Ingenhousz also agreed with Senebier that CO_2 is taken up and decomposed by green leaves to produce oxygen, which is released into the atmosphere.

SAQ 1

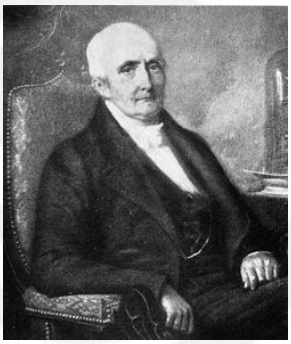
Match the experimental findings related to photosynthesis (given in column 1) with the names of scientists (given in column 2) who were responsible for the findings.

Column 1		Column 2	
a)	A sprig of mint can purify air injured by breathing of animals.	i)	Stephan Hales
b)	Plants are made of water alone	ii)	Antoine Lavoisier
c)	All kinds of plants purify bad air, but light is necessary for such purification.	iii)	Jean Senebier
d)	Soil is necessary for plant growth	iv)	Joseph Priestley
e)	Evolution of bubbles by *Hydrilla* sprigs	v)	Jan Ingenhousz
f)	CO_2 essential for plant growth.	vi)	Charles Bonnet
g)	Identified pure air as O_2 and impure air as CO_2.	vii)	van Helmont
h)	Author of the book " Vegetable Staticks"	viii)	J. Woodward

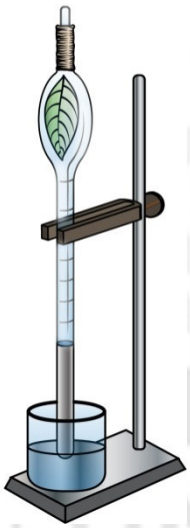
5.2.2 Development of Concept - Formulation of the Equation of Photosynthesis

By 1804, methods of quantitative measurements of gases were well established. In this year, another Genevian (but of French descent) **Nicholas Thēodore de Saussure** (Fig. 5.7a) published his *Recherches Chimiques sur la Vegetation* wherein he used an eudiometer (Fig 5.7b) and followed it by simple methods of gas analysis. His experiments on carbon assimilation confirmed the equivalence of release of O_2 to consumption of CO_2 during the process of photosynthesis. He observed that since the total weight of organic matter produced and oxygen evolved during this process in sunlight exceeded the weight of fixed air, i.e., CO_2 consumed, water must have some role of a raw material. So, attention was once again drawn to the essential role of/played by water; an aspect of photosynthesis which had been totally ignored after van Helmont. Work of de Saussure, therefore, confirmed the discoveries of Ingenhousz and Jean Senebier and by stressing on the essentiality of water, finally led to the following equation.

Carbon dioxide + water + light ⟶ organic material + oxygen



(a)



(b)

Fig. 5.7: a) Theodore de Saussure (14-10-1767 to 18-4-1845); b) This type of experimental set-up (an eudiometer) was employed by Nicolas-Théodorede Saussure to determine gas exchange during Photosynthesis. By putting a leaf or any other plant part in the bulb with a suitable support, the composition of air can be determined by use of an alkali water and pyrogallol at the end of the experiment.

Fig. 5.8: P. J. Pelletier (22-3-1788 to 19-7-1842)

In 1818, two French chemists **Pierre Joseph Pelletier** (Fig. 5.8) and **Joseph Bienaime Caventou** (Fig. 5.9) isolated a green pigment from leaves and named it **Chlorophyll** (=green leaf: Gr: *chloros* (yellow green) and *phyllon* (leaf). **Nehemiah Grew** (1682) is reported to have been the first to extract the leaf pigments (now called chlorophyll and carotenoids). Another Frenchman **René-Joachim-Henri Dutrochet** was the first to recognize in 1837 that chlorophyll was essential for plants to assimilate carbon dioxide. The German botanist **Hugo von Mohl** (Fig. 5.10), who is also credited for the discovery of protoplasm in plants, first described the chlorophyll granules or "*chlorophyllkörnern*" in detail. The term plastid, which was used to describe a chlorophyll granule was later replaced in 1883 by the term chloroplast.

Fig. 5.9: J.B. Caventou (30-6-1795 to 5-5-1877)

Fig. 5.10: Hugo von Mohl (8-4-1805 to 1-4-1872).

Fig. 5.11: J. Von Mayer (25-11-1814 to 20-3-1878)

Fig. 5.12: Jean Baptiste Boussingault (1-2-1801 to 11-5-1887)

Fig. 5.13: Julius von Sachs (14-11-1843 to 20-5-1909)

While in 1842, the German physician and physicist **Julius Robert Mayer** (Fig. 5.11) stated the Law of Conservation of Energy, i.e., energy is neither created nor destroyed. He made this claim in "*Organic Motion in its Relation to* Metabolism" published in 1845 and wrote *"Nature has put itself the problem how to catch in flight light streaming to the earth and to store the most elusive of all powers in rigid form. To achieve this aim, it has covered the crust of earth with organisms which in their life processes absorb the light of the sun and use this power to produce a continuously accumulating chemical difference ….These organisms are the plants; the plant kingdom forms a reservoir in which the fleeting sun rays are fixed and skillfully stored for future use; an economic provision to which the physical existence of mankind is inexorably bound. The plants take in one form of power, light; and produce another power: chemical difference".*

Meyer use the term "*power*" for energy and "*chemical difference*" for chemical energy. In fact, he elaborated the theme that photosynthesis mainly represented a process in which physical energy was conserved as chemical energy. In other words, plants produced organic matter as well as provided energy to sustain life. The process of photosynthesis could now be represented by the following equation:

$$CO_2 + H_2O + \text{light} \xrightarrow[\textit{Chloroplast}]{} \text{organic matter} + \text{chemical energy} + O_2\uparrow$$

A pioneer in agrochemistry, the French chemist **Jean Baptiste Boussingault** (Fig. 5.12) in1864 made quantitative measurements regarding the volumetric equivalence of uptake of CO_2 and production of O_2 in light.

According to him the value of assimilatory coefficient (O_2/ CO_2) was equal to 1. His work catalyzed the formulation of the equation for photosynthesis:

$$6\,CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6 + 6O_2\uparrow$$

The credit for conducting first physico-chemical studies on photosynthesis perhaps goes to the German physiologist **Julius von Sachs** (Fig. 5.13) in 1862-64 who demonstrated that starch grains are produced in leaves exposed to sunlight and these are the first visible products of this process. Starch accumulates in light but disappears in dark. Incidentally, starch is the most abundant photosynthetic product formed in chloroplasts.

Almost nine years after the formulation of the equation for photosynthesis, a Polish plant physiologist **Emil Godlewski Sr** (1873) discovered the correlation between the presence of CO_2 and starch formation in illuminated leaves. He modified further the apparatus used for the quantitative analysis of gaseous exchange.

Another very important development took place at the end of the last century. It was the elucidation of properties of chlorophyll in relation to light quality. **Theodor Wilhelm Engelmann** (Fig. 5.15c), a German botanist and physiologist demonstrated in 1882 that chloroplasts having chlorophyll were the sites of photosynthesis. The term chloroplast itself was established in 1883

as a better substitute for the earlier term ‘plastid’. We must remember that the chloroplasts had already been discovered earlier in 1837 by Hugo von Mohl who had called them “chlorophyllkörnern” or chlorophyll granules.

Three most ingenious experiments by Engelmann helped us to know as to which wavelengths of light are important in photosynthesis. By using spectroscopic techniques, he demonstrated that all colours and wavelengths of light were neither equally effectively absorbed by chlorophyll, nor induced an equal rate of photosynthesis.

Engelmann determined the **absorption spectrum** (A graph plotting light absorption by chlorophyll *vs* wavelength highlighting the best light absorbed) and **action spectrum** (A graph plotting the rate of photosynthesis vs wavelength).

Box 5.1: Clement A Timiryzeff

The Russian physiologist Clement A Timiryzeff (1874-75) (Fig. 5.14) was perhaps the first one to establish that red wavelength of the absorption spectrum of chlorophyll was most effective for photosynthesis. He also claimed that chlorophyll is an optical and photosensitizer of photosynthesis. He proposed that chlorophyll upon light absorption gets chemically transformed (what we know as oxidation today) and induces further reaction in photosynthesis. Surprisingly, his work on action and absorption spectrum of chlorophyll is less cited (see Govindjee & Krogmann, 2004).

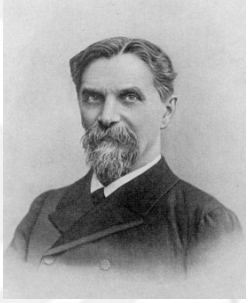
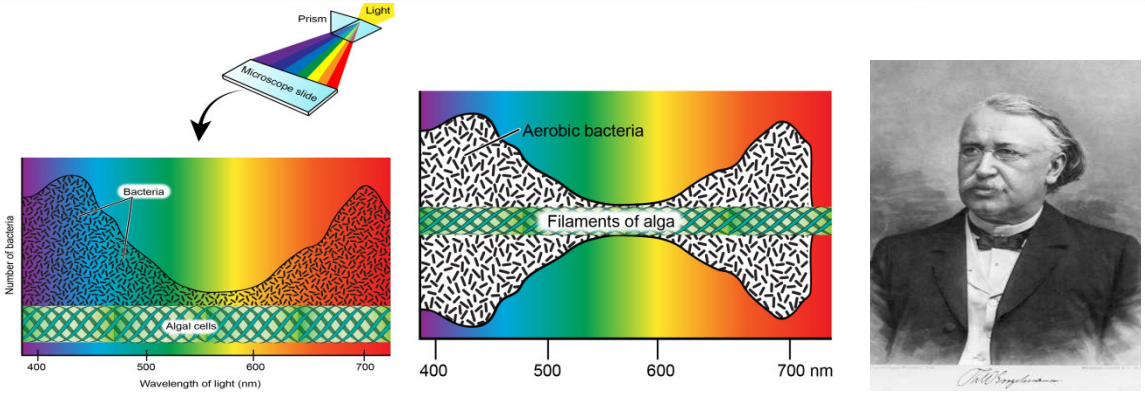


Fig. 5.14: Clement A Timiryzeff (3-6-1843 to 28-4-1920).

The third classical experiment by Engelmann was carried out with a filamentous green alga *Spirogyra*. He used a special type of microscope modified by Carl Zeiss Company. The microscope was fitted with a prism that could produce a spectrum on the microscopic slide. This mini-spectroscope could distinguish and measure different wavelengths of light. The algal filament was illuminated, and the visible spectrum readily observed /seen through the eyepiece by the viewer (Fig. 5.15 a). Thus, different sections of the algal filament were exposed to different wavelengths. To determine which wavelengths of light were effective in evolving oxygen he used a species of highly aerobic motile bacteria. A drop of bacterial suspension was introduced over the algal filament. He observed that the oxygen-dependent bacteria accumulated/congregated near those parts of algal filament that were illuminated with red and blue light (See Fig. 5.15 a, b). This experiment clearly demonstrated that red and blue wavelengths encouraged most oxygen production which attracted the **aerotactic** bacteria to clump/congregate in these portions of the algal filament.



(a) (b) (c)

Fig.5.15: a) Engelmann's experiments on the action spectrum measurements. A spectrum of light was projected onto the spiral chloroplasts of *Spirogyra* (a filamentous green alga); b) When introduced, the aerotactic or oxygen-seeking bacteria collected in blue and red regions where chlorophyll absorbs the most; c) T.W. Engelmann (2-10-1832 to 29-5-1897).

Engelmann subsequently found that the purple bacteria utilized the UV light for photosynthesis in a similar manner. Although Engelmann's work used sunlight as a source where all wavelengths are not emitted with the same intensity, this experiment proved a milestone in **photobiology**.

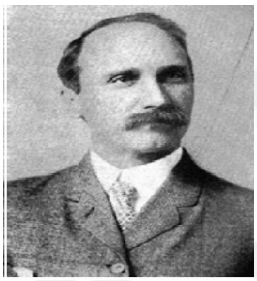


Fig. 5.16: Charles Barnes (1858-1910)

Surprisingly, the entire literature since Joseph Priestly described the process not by the term photosynthesis but by other names like "**assimilation of carbon**" of simply "**assimilation**". Credit for introducing the term photosynthesis goes to the American Botanists **Charles Barnes** (Fig. 5.16) and **Conway MacMillan**. Barnes proposed the terms **Photosyntax** and its alternate term **Photosynthesis** in 1893 to describe the biosynthetic process of light-dependent carbon dioxide reduction of organic matter in his paper '*On the food of green plants'*, Barnes argued that the term assimilation led to a lot of confusion with a process in animal physiology and thus defined '**photosyntax**' as the "**Synthesis of complex carbon compounds out of carbonic acid, in the presence of chlorophyll, under the action of light**". His work was discussed in a meeting of the Botanical Section of the American Association for the Advancement of Science in Wisconsin in 1893, where another American botanist Conway MacMillan preferred the term Photosynthesis. Although Barnes liked photosyntax more, but **photosynthesis** has since become the most accepted term.

SAQ 2

State whether the following statements are True (T) or False (F):

a) The Frenchman de Saussure elucidated the essentiality of water in photosynthesis [ ]

b) Chlorophyll was first extracted and named by Dutrochet. [ ]

c) The term "chlorophyllkörnen" was given by von Mohl to describe a chloroplast. [ ]

d) It was the work of Bossingault that led to the formulation of the equation. [ ]

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6 + 6O_2\uparrow$$

e) Godlewski was the first to demonstrate starch as the first visible product of photosynthesis. [ ]

f) Experiments by Engelmann proved the green wavelength was most effective for starch formation. [ ]

g) Most of the aerobic bacteria were concentrated near the algal filament exposed to blue and green region in the above experiment. [ ]

h) The term photosynthesis has a synonym in *Photosyntax* which was proposed by Barnes. [ ]

5.3 PHOTOSYNTHETIC PIGMENTS

The technique of chromatography (*Gr*: *chroma*=colour and *graphein*=to write) was invented by the Russian botanist **Mikhail SemenovichTswett** in 1903. He was the first to use this novel technique for separating the chlorophylls and carotenoids by passing their solutions through glass columns packed with calcium carbonate. He showed that Magnesium (Mg) was an essential part of chlorophyll and there was a definite structural relationship with the hemoglobin pigment present in blood. Although the chlorophyll of different plant species is the same, you will study in the later pages that it is in fact, a mixture of two different types.

The first detailed investigations on chlorophyll structure were reported by the German **Richard Willstätter** and the Swiss **A. Stoll**, which earned a Nobel Prize in Chemistry for Willstätter in 1915.

Pigment molecules which can process light into a utilizable form in a plant are called **photoreceptors.** A pigment is essentially made up of an integral protein component the **chromoprotein**. The specialized part of the chromoprotein responsible for absorbing light and thereby imparting color is the **chromophore.** The protein part of the chromophore is called **apoprotein.** Together, the chromophore and the chromoprotein make a complete molecule – the **holochrome.**

There are two types of photosynthetic pigments: **Essential** and **Accessory** pigments. A characteristic feature of all photosynthetic organisms is the presence of chlorophylls or bacteriochlorophylls.

5.3.1 Essential Pigments : Chlorophylls

A molecule looks like a spatula (Fig. 5.17a) and is composed of **two** parts, a **porphyrin 'head'** and a **phytol 'tail'**. The porphyrin head is in the form of a cyclic tetrapyrrole which is made up of **four** nitrogen-containing **pyrrole** rings (Fig. 5.17b) and one isopentenyl carbon ring (ICR).

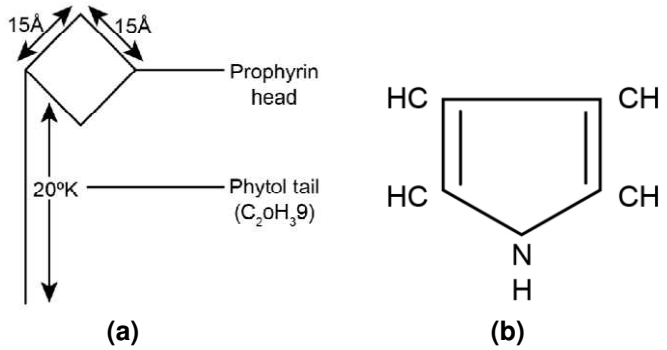


Fig. 5.17: a) A chlorophyll molecule; b) A pyrrole ring.

A magnesium ion (Mg^{2+}) constitutes the centre of the tetrapyrrole ring and is chelated to the four nitrogen atoms. The magnesium ion encased in a large central ring structure constitutes **chlorin.** Four nitrogen atoms from the chlorin surround and bind the magnesium atom. The pyrroles are numbered in a clockwise manner according to the conventional Fischer Numbering System (Fig. 5.18a) as per the recommendations of the IUPAC-IUB Joint Commission of Nomenclature (JCBN)). Two of the pyrroles are linked to magnesium by covalent bonds while the remaining two are linked via coordinate bonds.

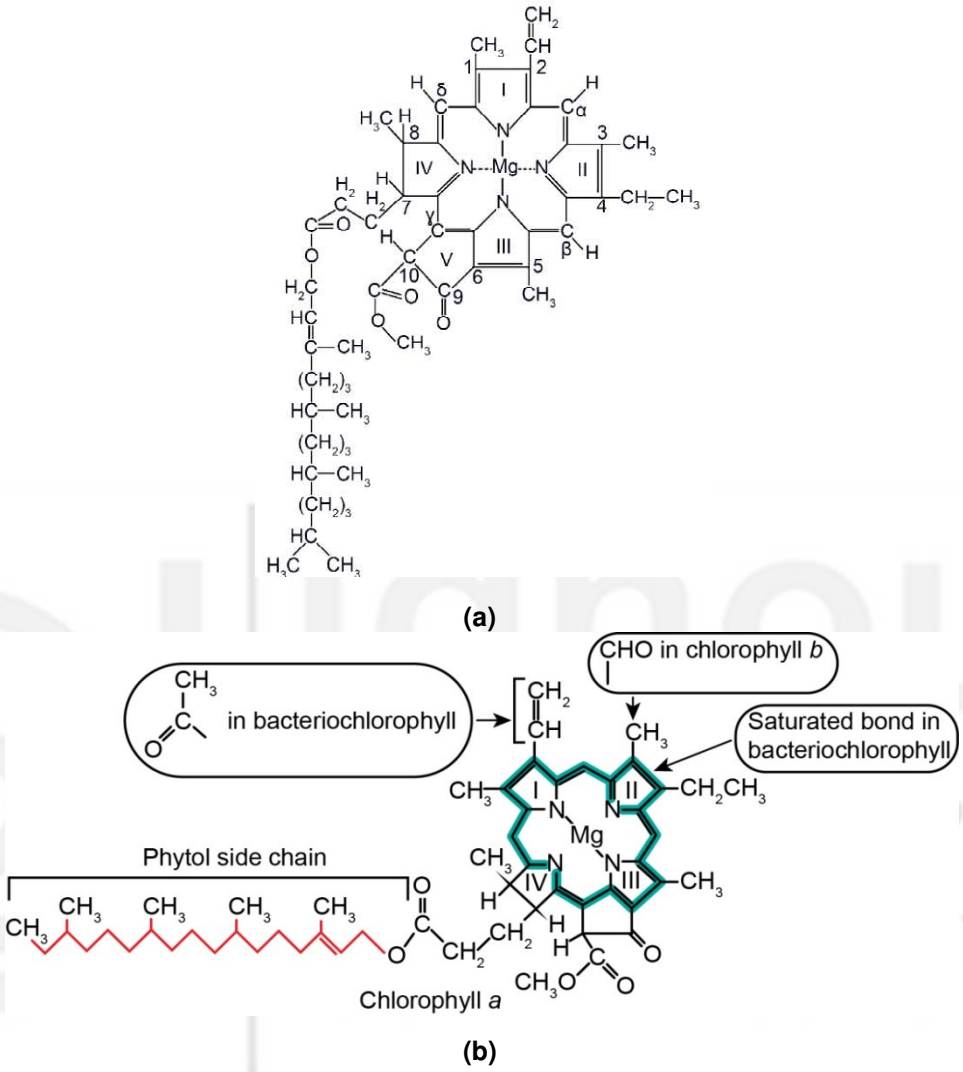


Fig. 5.18: a) Structure of chlorophyll$_a$ showing the porphyrin head and a phytol tail. The pyrroles are numbered in a clockwise manner; b) General structure of chlorophyll showing characteristic groups found Chl$_a$, Chl$_b$, and bacteriochlorophyll. The isopentenyl carbon ring is adjacent to the Porphyrin ring III (From Nelson & Cox).

A 20-carbon **phytol 'tail'** ($C_{20}H_{39}$) is attached (esterified) to the porphyrin ring IV of the 'head' at its 7th carbon position by a propionic acid residue (Fig. 5.18b). The angle between head and tail is 45^0. The head is a square of 15Å, and the tail is 20Å long and makes a typical 15 × 15 × 20 configuration (Fig. 5.17 b). The porphyrin head is hydrophilic and anchors the chlorophyll molecule in the chloroplast thylakoid membrane. The ring structure of the porphyrin head contains loosely bound electrons. This part of chlorophyll is functional in electronic transitions and oxidation-reduction (redox) reactions during photosynthesis. On the other hand, the phytol tail is lipid soluble and is derived from the 5-carbon **isoprene** (a precursor of carotenes, gibberellins, and steroids).

The complex structure of chlorophyll is structurally related to the porphyrin – like groups of cytochromes and hemoglobin. In chlorophyll, Mg^{2+} occupies the central position, while hemoglobin has Fe^{2+} positioned at its centre (Fig. 5.19). Another difference between the two is the presence of a non-pyrrole 5th ring in the porphyrin head of chlorophyll, which is absent in hemoglobin.

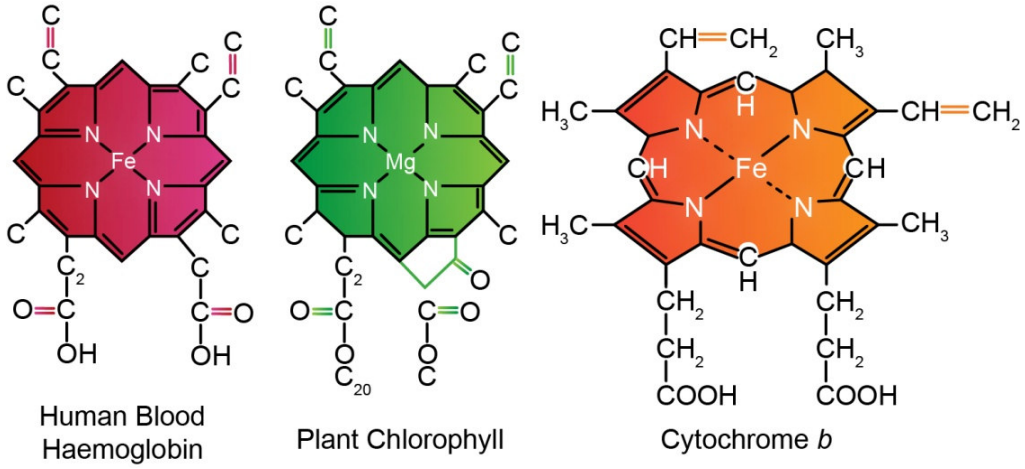


Fig. 5.20: Related structures of hemoglobin, chlorophyll, and cytochrome.

Both the chlorophylls, Chl$_a$ and Chl$_b$ are present in higher plants and the amount of the latter is on average almost double that of Chl$_a$. Both chlorophylls differ in their abilities to absorb light (absorption spectra), types of groups present in them, solubility properties as well as their molecular formula. Chl$_a$ has a methyl group (-CH_3) of II(B) pyrrole ring (Fig. 5.20). It appears green, absorbs light in the blue and red regions, is more soluble in organic solvents like ether. The molecular formula of Chl$_a$ is **$C_{55}H_{72}O_5N_4Mg$** and mol. wt. 893. Different types of Chl$_a$ have been identified. They have variable absorption maxima, viz., Chl$_{a670\text{-}673}$, Chl$_{a680\text{-}683}$, Chl$_{a695\text{-}700}$, P_{680} and P_{700}.

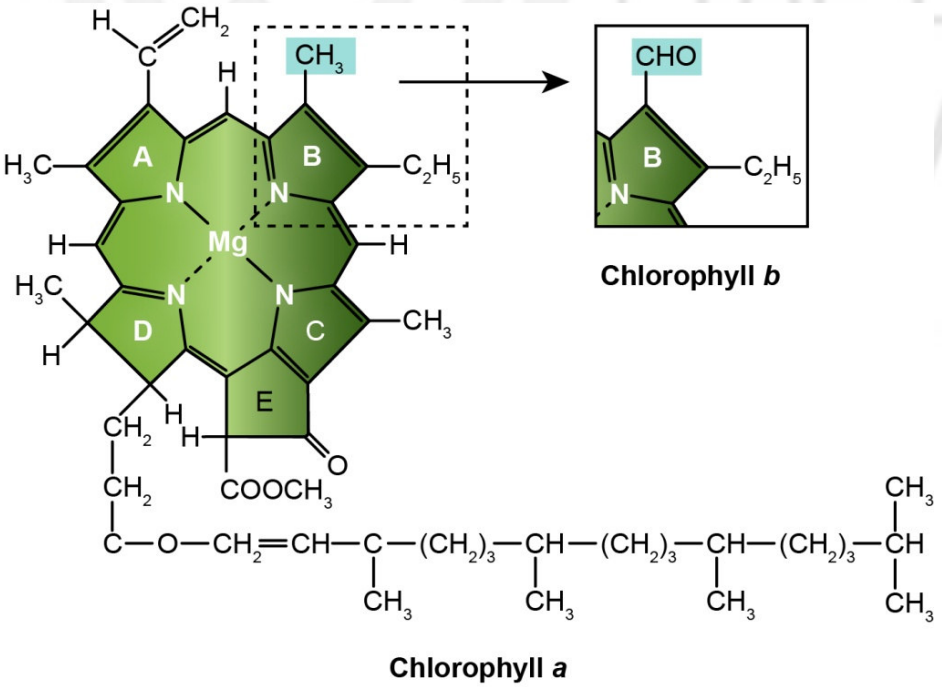


Fig. 5.20: Difference between Chl$_a$ and Chl$_b$. The methyl (-CH_3) group of chl$_a$ is replaced by aldehyde group (-CHO) in Chl$_b$ (From Taiz *et al*).

Chl$_b$ is structurally like Chl$_a$ except that the methyl group mentioned above is replaced by an aldehyde (-CHO) group (Figs. 5.18b, 5.20). This minor

alteration in structure results in its different properties. Chl_b appears yellowish and absorbs blue and red light but the absorption maxima are different. Two different Chl_b, viz., $Chl_{b\,640}$ and $Chl_{b\,650}$ are present. Chl_b is more soluble in methanol and ethanol. Chl_b has a molecular weight of 907 and molecular formula- $\mathbf{C_{55}H_{70}O_6N_4Mg}$. The distribution of other chlorophylls and their absorption properties have been summarized in the following Table 5.1.

Table 5.1 : Distribution of pigments and their absorptions peaks

Type of pigments	Distribution in Plant Kingdom	Absorption peaks in cells in nm	
Chl_a	All green plants (oxygenic photosynthetic organisms)	435	670-680
Chl_b	All green plants except diatoms brown, red and blue green algae	480	650
Chl_c	Diatoms and brown algae	---	645
Chl_d	Some red algae	---	740
Protochlorophyll	Etiolated plants	---	---
Bacterioviridin (*Chlorobium* chlorophyll)	Green Sulphur bacteria	---	750 or 760
Bacteriochlorophyll	Purple Sulphur bacteria		800, 850 and 890

Table 5.2: Distribution of Photosynthetic Pigments in Plant Kingdom

Pigment	Distribution in Plant Kingdom
1) Chlorophylls	
Chlorophyll-*a*	All photosynthesizing plants except bacteria.
Chlorophyll-*b*	Higher plants and green algae
Chlorophyll-*c*	Diatoms, dinoflagellates, and brown algae
Chlorophyll-*d*	In some red algae
Chlorophyll-*e*	In *Tribonema* and *Zoospores of Vaucheria*
Bacteriochlorophyll-*a*	Purple and green bacteria
Bacteriochlorophyll-*b*	In a strain of purple bacterium *Rhodopseudomonas*
Bacteriochlorophyll-*c*, *d* & *e* (*Chlorobium* chlorophyll or bacterioviridin)	Green bacteria
Bacteriochlorophyll-*g*	Heliobacteria
2) Carotenoids	
Carotenes	Mostly in algae and higher plants
Xanthophylls (Carotenols)	Mostly in algae and higher plants
3) Phycobilins	
Phycoerythrin	In blue-green and red algae
Phycocyanin	In blue-green and red algae
Allophycocyanin	In blue-green and red algae

Bacteriochlorophylls

Bacteriochlorophylls are the principal pigments present in bacteria engaged in anoxygenic photosynthesis. Discovered by **C.B. van Niel** (1932) these pigments absorb wavelengths of light in the ranges not absorbed by O^-_2 producing plants or cyanobacteria. Light wavelengths in the range of 705-1040nm (Far to Infra-Red) may be absorbed. Different groups of photosynthesizing bacteria contain various types of pigments viz., bacteriochlorophyll $_{a,b,c,cs,d,e,f,}$ and $_g$. Bacteriochlorophyll $_a$ is the most abundant of this group with a molecular formula **$C_{55}H_{74}O_6N_4Mg$** and a molecular wt. of 911. The structure of the porphyrin head differs from that of chlorophylls as the pyrrole ring is reduced with H. In addition, a vinyl group is replaced by an acetyl group (Fig.5.21). Bacteriochlorophyll shows absorption peaks at 358nm and 772nm. Different forms of bacteriochlorophylls occur in Green Sulfur bacteria, Heliobacteria and Purple bacteria.

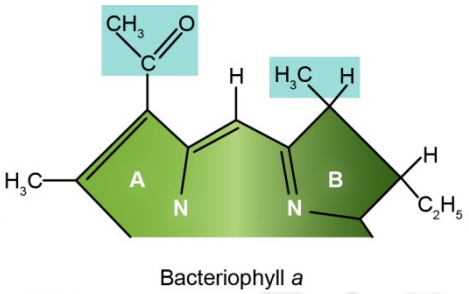


Fig. 5.21: Structure of bacteriochlorophyll. The pyrrole rings I and II are reduced with 2H. Also see Fig. 5.18b for comparison.

5.3.2 Accessory Pigments

Although chlorophyll $_a$ is an essential pigment, it cannot carry out the entire photosynthetic process alone. This is due to **two** main reasons:

1. Chlorophyll $_a$ absorbs light quanta only in two regions of the visible spectrum. If not harnessed, the other wavelengths will go waste.
2. Chlorophyll $_a$ is highly sensitive when present in a pure form and may get photo oxidized. The process of photo destruction is called **Solarization**.

Accessory pigments perform **two** important roles: **a**. Supportive and **b**. Protective.

a. They absorb photons/light quanta at wavelengths not covered by Chl$_a$ and transfer energy to the chlorophyll reaction centre, and

b. Protect the sensitive Chl$_a$ against photodynamic damage by quenching/detoxifying the reactive oxygen species.

1. Carotenoids

Carotenoids are accessory pigments present in all photosynthetic organisms. These pigments belong to the terpenoid group of compounds formed by isoprenoid pathway. They are highly unsaturated molecules made up of isoprene (C_5H_8) units. Each carotenoid molecule (C_{40}) is made up of a long chain which contains conjugated double bonds between carbon atoms. Six-carbon rings called “ionone” rings are present on both the ends of this open chain conjugated double bond system (Fig. 5.22 a).

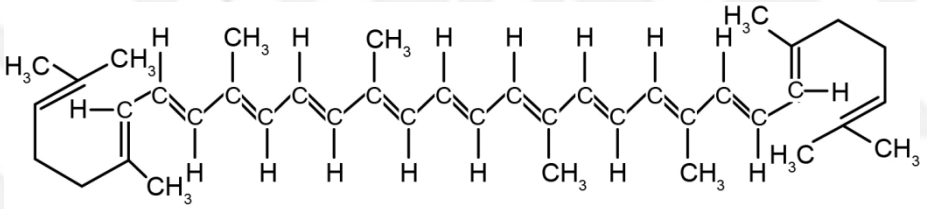
Carotenoids constitute a large group of fat soluble, orange, and yellow pigments which may also assume different colors like brown or red. The brilliant orange and yellow colors of autumn foliage is due to chlorophyll degeneration and unmasking of stable carotenoids at the time of leaf senescence.

Carotenoids absorb light in blue and green parts of the visible spectrum. They also exhibit fluorescence and emit orange and red light upon excitation.

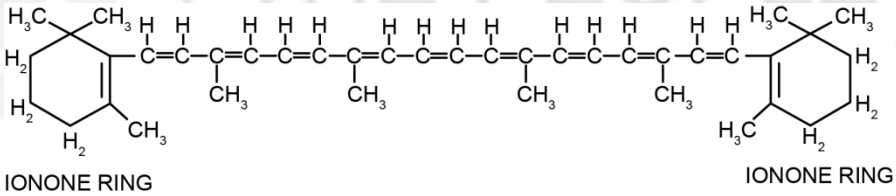
Carotenoids are of **two** types:

In animals, β-carotene is the precursor of Vitamin A (Retinol) - $C_{20}H_{30}O$.

a. **Carotenes** are orange or red-orange carotenoids with a general formula $C_{40}H_{56}$. Various types of carotenes differ only in the arrangements of molecules in space. **Lycopene** (red) is the basic carotene present in purple sulfur bacteria (Fig. 5.22 a). It also gives characteristic coloration to tomato fruits, flowers of *Calendula* and rose, carrots and red chillies. **Carotene** is another carotenoid which is an isomeric form of lycopene. β-carotene is the most abundant carotenoid and is present in the chloroplasts (Fig. 5.22 b). It absorbs maximum light in violet and blue-green ranges of the spectrum with maximum absorption at 450-460nm. β-carotene is one of the most efficient in preventing the photooxidation of chlorophyll.



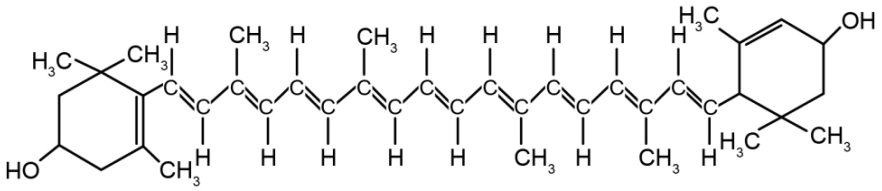
(a)



(b)

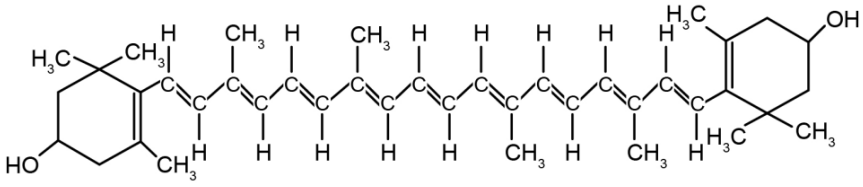
Fig. 5.22: a) Structure of Lycopene; b) Structure of β-carotene.

b. **Xanthophylls,** also called **carotenols**, are oxygen containing derivatives of carotenes. These are yellow to brownish pigments with a general formula $C_{40}H_{56}O_n$ .Oxygen may be present as hydroxyl, carboxyl, methoxyl or epoxide groups. Lutein/Luteol-- $C_{40}H_{56}O_2$ (Fig. 5.23 a) is the most common xanthophylls present in leaves of some flowers like dandelion and sunflower. Other common xanthophylls are isomers of lutein: zeaxanthin -- $C_{40}H_{56}O_2$ (Fig. 5.23 b), cryptoxanthin -- $C_{40}H_{56}O$, flavoxanthin -- $C_{40}H_{56}O_3$ and violoxanthin -- $C_{40}H_{56}O_4$ (Fig.5.23 c). Fucoxanthin -- $C_{40}H_{56}O_6$ gives characteristic color to brown algae. Absorption pattern of xanthophylls is like that in carotenes, but the xanthophylls are less soluble in carbon disulfide than the carotenes are. However, the xanthophylls are much more abundant in leaves.



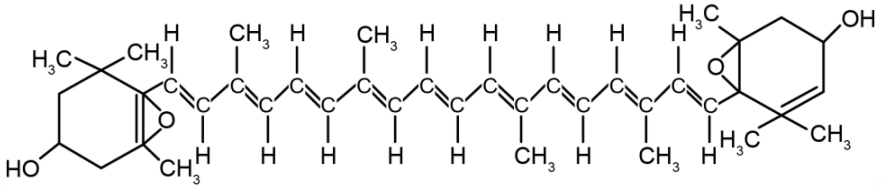
Lutein

(a)



Zeaxanthin

(b)



Violaxanthin

(c)

Fig. 5.23: Xanthophylls: a) Lutein; b) Zeaxanthin; and c) Violaxanthin.

2. Phycobilins

Phycobilins are photosynthetically active pigments present in prokaryotic cyanobacteria and eukaryotic red algae. Phycobilins are structurally related to bile pigments. Three phycobilins, viz., phycoerythrin (phycoerythrobilin), phycocyanin (phycocyanobilin) and allophycocyanin (allophycocyanobilin) are supplementary accessory light harvesting pigments. Phycobilins are structurally related to chlorophylls as they also have a tetrapyrrole organization.

Also, these pigments are called phycobiliproteins as they remain conjugated with a protein moiety. They are water soluble. The phycobiliproteins are organized in the form of macromolecular complexes called **phycobilisomes** in cyanobacteria and Rhodophyta (red algae). Phytochromobin is the only phycobilin reported from higher plants.

5.3.3 Non-Photosynthetic Pigments and Photoreceptors

There are many pigments which do not occur in the chloroplasts and do not have a direct role in photosynthesis. Yet they contribute significantly to protecting chlorophylls against excess light and UV-radiations. These non-photosynthetic pigments belong mostly to the secondary metabolite pool and occur in both lower and higher plants. Many fungi also possess these pigments. In higher plants, besides leaves, they are also present in many other plant parts like petals, fruits, stems and roots. Many of them play an important role in photomorphogenesis.

1. Flavonoids

As you have read that the carotenoids serve as accessory pigments in photosynthesis serving both supportive (antenna molecule) and protective roles. In addition, there are a variety of other coloured substances present in flowers and fruits which perform the role of protection from damaging UV rays and attracting insects for pollination.

Flavonoids belong to one of the largest classes of plant phenolics. It contains a 15-carbon skeleton consisting of two aromatic rings connected by a 3-carbon bridge (C6-C3-C6 composition).

Sometimes they may also occur in the chloroplasts and chromoplasts . Flavonoids serve as UV absorbing pigments. They absorb shorter wavelengths of the broad electromagnetic spectrum in the region of 280-320nm (UV), harmful to plants and thus allow an uninterrupted passage to photosynthetically active radiations. Thus, they protect plants against UV damage.

Flavonoids are classified based on the degree of oxidation of the three-carbon bridge. **Four** of the most important groups of flavonoids are: Anthocyanins, flavones, flavanols and isoflavones.

a) Anthocyanins

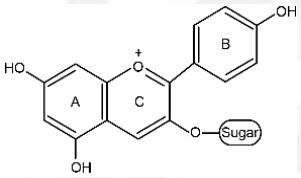


Fig. 5.24: Structure of anthocyanin.

These are the most widespread group of coloured flavonoids in plants. Anthocyanins occur as glucosides derived from the sugar-less part, the anthocyanidins. They get dissolved in cell sap of epidermal cells and impart bright colours like red, pink, purple and blue colours to flowers and fruits. Many different types of anthocyanins exist, and their characteristic colour depends on the number of hydroxyl and methoxy groups in its ring structure (see Fig. 5.24), and pH of the cell vacuole.

A high degree of colour variation by the anthocyanins combined with the presence of carotenoids, accounts for so many different colors and shades of flowers and fruits in nature. Anthocyanins and other flavonoids are also very important from the viewpoint of evolution. Presence of flavonoids has also been used as a vital basis for classification by taxonomists and evolutionary biologists.

b) Flavones and Flavanols

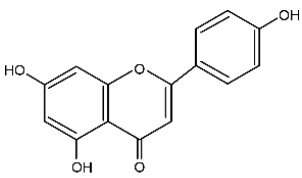


Fig.5.25: Structure of Flavones.

Flavones and flavanols are universally present in leaves of green plants. They also occur in petals of some flowers, imparting them with yellowish or ivory color. Their structure is like anthocyanins, except that their molecules have different central rings (Fig. 5.25).

Since these flavonoids absorb light at much shorter wavelengths than the anthocyanins, their coloration is not visible to human eyes. However, bees and other insects, who can see in the UV range of the spectrum, use them as nectar guides, indicating the location of pollen and nectar. There are special types of symmetric patterns or concentric rings that only bees can visualize as attractant cues, as in *Rudbeckia* sp. (Fig. 5.26 a, b).

(a) (b)

Fig. 5.26: a) The black-eyed Susan (*Rudbeckia* sp.) flower appears as yellow rays with brown central disc to humans; b) To bees, the inner portion of the rays appears "dark yellow" and the central disc "black", tips of the rays appear "light yellow". This is due to the distribution of UV-absorbing flavanols on the inner parts of the rays alone. This helps the bees to locate pollen and nectar (From Taiz *et al*).

In addition, the colourless flavones and flavanols located in the leaf epidermal cells absorb the dangerous UV-B radiations (280-320nm), and at the same time allow PAR (Photosynthetically Active Radiation) to pass through, thereby helping to protect the cells against UV-induced mutations.

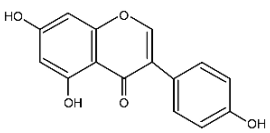


Fig. 5.27: Structure of Isoflavones.

Recently these two flavonoids have been found to play a regulatory role as modulators of polar auxin transport during plant development. Also, these flavones and flavanols, when secreted by the leguminous roots, contribute to the interaction between the roots and rhizobacteria during nitrogen-fixing symbiosis.

c) Isoflavonoids (Isoflavones)

Isoflavonoids differ from other flavonoids in their structure. These compounds have their aromatic ring B attached to carbon at 3rd position of central ring instead to the 2nd carbon position (Fig. 5.27).

Found mostly in legumes, the isoflavonoids exhibit a variety of very important biological functions. Roots of *Derris elliptica* yield an isoflavonoid-**Rotenone,** which is used as a rat poison and an insecticide. Many others show anti-estrogenic and anti-cancerous effects. Most important function of isoflavonoids is as **Phytoalexins** (antimicrobial compounds synthesized in plants in response to fungal or bacterial infection).

2. Betacyanins

Betacyanins were first extracted from common beet (*Beta vulgaris*). These pigments are deep red in color and along with the yellowish betaxanthins, belong to a larger group **betalains**. The betacyanins are aromatic indole derivatives synthesized from tyrosine and occur as glycosides. Interestingly, the betacyanins are present in the vacuoles as do the anthocyanins, but they are never present together. These pigments are common in Amaranth, *Bougainvillea* and in some cacti.

3. Phytochrome

Phytochrome is the most important pigment involved in a variety of photo morphogenetic responses. This protein-pigment photoreceptor can absorb red

and far-red light and blue light. It shows many responses of red/far-red reversibility in seed germination and floral induction.

4. Cryptochrome

Cryptochromes are yet another group of photoreceptors which show blue light-mediated responses. These are believed to be flavins and show many photomorphic responses together with phytochrome in lower and higher plants. They absorb light mainly in the violet/blue region of the spectrum (400-500nm). Some portion of UV-A region (320-400nm) is also absorbed.

5. Phototropins

Phototropins are flavoproteins localized in the plasma membrane. These pigments play an important role in protecting the photosynthesizing machinery from photoinhibition due to excessive light exposure.

You will read in detail about these blue light and UV receptors in the later chapters on Plant Responses to Light (Unit 15).

5.4 ROLE OF SUNLIGHT

You have read about all the photosynthetic pigments, their structure, and properties. Now we will study how sunlight is captured and triggers photosynthesis process.

5.4.1 Electromagnetic Spectrum

Earth receives enormous amount of sunshine which is the sole source of energy driving the process of photosynthesis. Processes of fusion and transmutation occurring on sun release tremendous amounts of heat and light radiations.

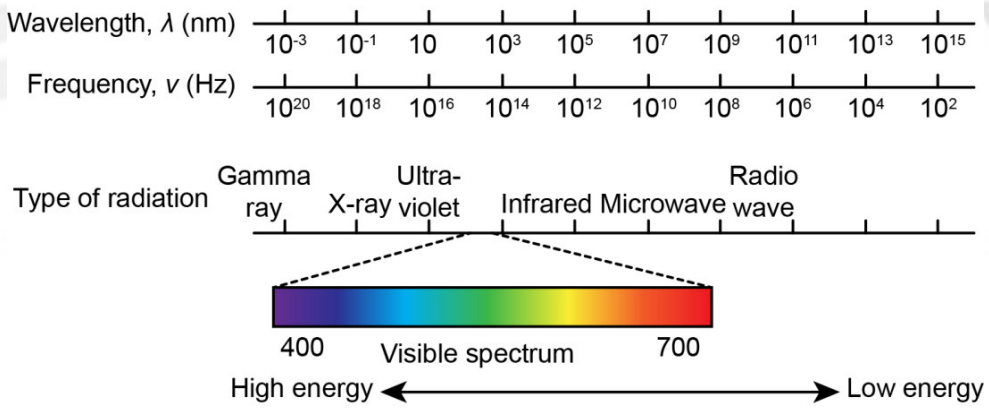


Fig. 5.28: Electromagnetic Spectrum. Wavelength (λ) and frequency (*ν*) are inversely related. Our eyes are sensitive to only a narrow range of wavelengths of radiation, the visible region, which extends from about 400 nm (violet) to about 700 nm (red). Short-wavelength (high-frequency) light has a high energy content; long-wavelength (low-frequency) light has a low energy content (After Taiz & Zeiger).

Of the total solar energy generated, about 40% (nearly 5×10^{20} kcal) reaches the earth surface. The remaining part is either dissipated (scattered in space) or absorbed by the atmosphere. Not the entire portion of light of different

wavelengths and energy continuously showering on earth are useful to plants for photosynthesis. Light is a particle, and a form of radiant energy. This particle is called **photon**. Light is that narrow band of energy within the electromagnetic spectrum of radiation emitted by sun that we can see. In other words, our eyes can perceive only a very limited/narrow range of frequencies out of this electromagnetic spectrum-the visible region or the visible spectrum which is the most vital region for photosynthesis (Fig. 5.28). In addition, the ultraviolet and infrared wavelengths are also important for photosynthesis in bacteria, though we cannot see them.

5.4.2 Absorption and Action Spectrum

Light and other forms of radiation are usually characterized by biologists in terms of wavelength. Thus, each photon is associated with a certain specific amount of energy called **quantum** (*pl*: quanta**)**.The energy carried by one mole of photons of different wavelengths is variable. To explain, the energy carried by photons of the blue light (274 kJ mol^{-1}) is much more than those in the red region (181 kJ mol^{-1}). Number of moles of photons of a particular wavelength needed to excite a mole of a particular pigment molecule are different.

Light needs to be first absorbed by a photosensitive pigment before any photobiological event can begin. Each pigment will absorb light quanta of a particular wavelength with variable intensity-which constitutes its **absorption spectrum.** In other words, absorption spectrum is an optical property of a solution. With the help of a spectrophotometer, the degree of absorption can be easily measured. This will be represented in the form of a graph depicting absorption as a function of wavelength. According to Hopkins & Hüner (2009) “More importantly, an absorption spectrum is like a fingerprint of a molecule. Every light absorbing molecule has a unique absorption spectrum that is often a key to its identification” (Table 5.3).

Table 5.3 : The Physical Nature of Light

The Physical Nature of Light Radiation of Principal Interest to Biologists.

Colour	Wavelength Range (nm)	Wavelength
Ultraviolet	100-400	
UV-C	100-280	471
UV-B	280-320	399
UV-A	320-400	332
Visible	400-740	
Violet	400-425	290
Blue	425-490	274
Green	490-550	230
Yellow	550-585	212
Orange	585-640	196
Red	640-700	181
Far-red	700-740	166
Infrared	*Longer than 740*	85

Out of the total incident light energy falling on leaves and other green parts, only a very small portion is absorbed by the photosynthetic pigments. A major portion of light is reflected and some of it is transmitted (Fig. 5.29). Photosynthetic pigments absorb light quanta only in the visible part of the electromagnetic spectrum (400-700nm) which constitute the **Photosynthetically Active Radiations** (**PAR**). However, many photosynthetic bacteria utilize the infra-red radiations /higher wavelengths for photosynthesis.

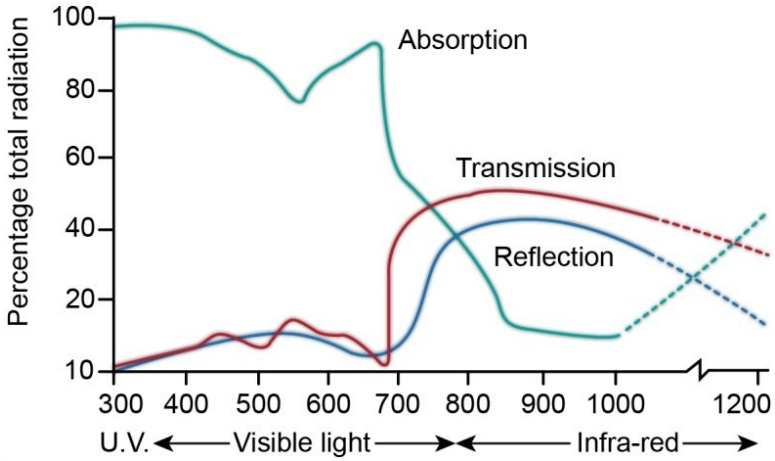


Fig. 5.29: Reflection, absorption, and transmission of light by green leaves.

Box 5.2 : Unique Matching of Chl$_a$ Spectra

Chlorophylls absorb light mainly in the violet-blue and red regions of the visible spectrum. A thick absorption band by chlorophylls in the violet blue regions is called **Soret band**. Absorption spectra of different chlorophylls show peaks at variable wavelengths (Fig. 5.30).

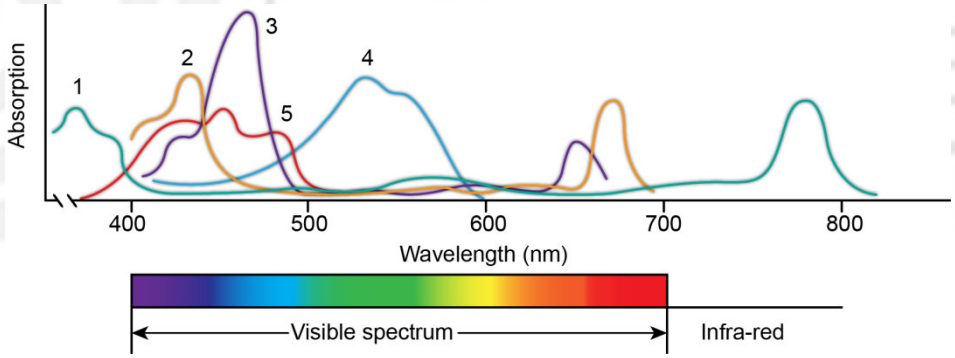


Fig. 5.30: Absorption spectra of photosynthetic pigments. (1.Bacteriochlorophyll; 2. Chl*a*; 3. Chl$_b$; 4. Phycoerythrins and 5. β-carotene).

Absorption spectra of Chl$_a$ and Chl$_b$ (*in vitro*) in acetone are shown in Fig. 5.31.

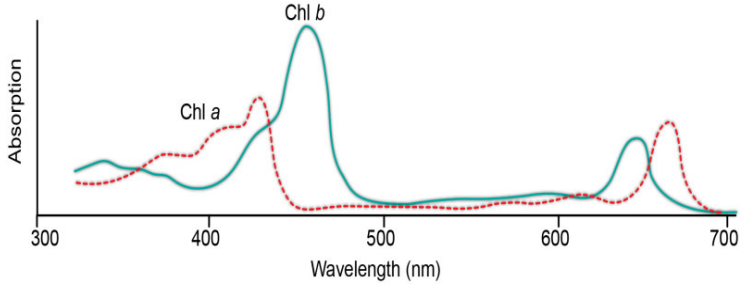


Fig. 5.31: Absorption spectra of Chl$_a$ and Chl$_b$ in acetone.

By way of explanation, it can be said that the **absorption spectrum** is an optical property of a solution. With the help of a spectroscope one can study the wavelengths absorbed by the plant extract or the solution such as the chloroplast pigments in acetone (see Fig.5.31). A modern spectrophotometer can now measure the degree to which these wavelengths are absorbed. An **action spectrum**, on the other hand tells us about the **relative activity** of a physiological process in different parts of the spectrum. The living cell must be illuminated with monochromatic light in different regions of the spectrum to obtain the action spectrum. Obviously, to associate a photoreceptor convincingly to a certain process of action, the two spectra must match. And they do in case of chlorophyll *a*. This makes the chlorophyll as the most efficient and universal chlorophyll (Fig.5.32).

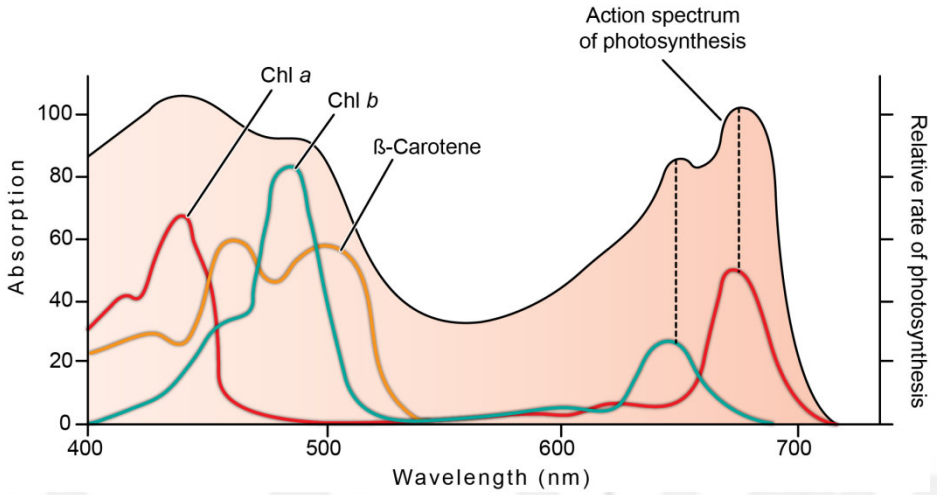


Fig.5.32: Action spectrum of, chl_a, chl_b, and β-carotene matches with the wavelengths at which light is absorbed by them. Photosynthesis takes place primarily at 680nm by chl_a at 650nm by, chl_b and at shorter wavelengths by Chlorophylls *a* and *b*, along with β-carotene (From Lodish *et. al*).

SAQ 3

a) Describe the experiment which led to the discovery of :

i) the role of chlorophyll in photosynthesis.

ii) the wavelength of visible spectrum that are effective in photosynthesis.

b) Differentiate between:

i) structure of Chl_a and Chl_b.

ii) absorption and action spectrum.

5.4.3 Absorption of Photons - Energy States of Chlorophyll

A molecule is said to exist in a normal, ground state when there is no magnetic effect, and it has its lowest energy status. Various transitions are possible after the absorption of light by chlorophyll. Absorption of light by chlorophyll is a very rapid process and requires only a femtosecond (fs=10^{-15} s). Absorption of

photons (h ϒ) or light quanta by the chlorophyll molecules results in excitation of chlorophyll (**photoexcitation**). In other words, the chlorophyll gets excited to a higher energy state, i.e., one of the two electrons is raised to a higher energy state. Although the **Stark Einstein Law** of photochemical equivalence state that only a single electron can be excited by one photon, the situation may be far more complex. As you have already read in the above discussion on the structure of photosynthetic pigments (Section 5.3) chlorophyll being a complex molecule has many electrons. Therefore, each of these electrons will absorb a light quantum (photon) of different wavelengths i.e., photons of a different energy level. Depending on the energy of the photon, the electron will be elevated (excited) to different energy levels (called **singlet states**). Since the energy of photon is inversely proportional to the wavelength, the excitation levels will vary, and exposure of white light will induce the chlorophyll to show different excited states at one given time. For example, absorption of blue light (58000 cal/Einstein) will excite chlorophyll to a much higher energy level than when it is exposed to red light (43000 cal/Einstein). 1 Einstein = 6.023×10^{23} photons).

With specific reference to the absorption of blue and red wavelengths, two main **excited states** or levels can be recognized. As mentioned above, exposure of chlorophyll to blue light (shorter wavelengths) excites it to a higher energy state called the **second excited singlet state**. This is highly unstable, and short lived with a half-life of 10^{-12} seconds. On the other hand, upon absorption of red light, chlorophyll is excited to a comparatively lower excitation level than that for the blue one. This level is called the **first excited singlet state.** This is also a short-lived stage having a half–life of 10^{-9}or 1 nanosecond). If these excited molecules do not get a chance to chemically interact with other molecules, they lose their extra energy to return to their ground state. There are **four** ways by which this excess energy is dissipated/lost: a) **Heat**; b) **Light,** c) **Inductive Resonance** (radiation less transfer), and d) **Photochemical reactions**. We shall now examine each of these possibilities:

a) **Heat:** The chlorophyll molecule decays to its lower first excited singlet state by losing its excitation energy as heat (Fig. 5.33). This is called **thermal deactivation**. This loss of energy results in a shifting of the emission spectra to the right, i.e., lower energy (See Box 5.3 Stokes Shift). There would be a further release of heat if the electron were to return to its ground state.

b) **Light:** Relaxation (quick drop) of electrons from the first excited singlet state may also result in the loss of excessive energy as reemission of photons. This production of light due to rapid decay of electrons in the excited state is called **fluorescence**. It is expressed by the equation $\upsilon = c/\lambda$ where c = speed of light ($3 \times 10^8 ms^{-1}$), ϒ = nu=number of wave crests, and ƛ = wavelength in nm (1 nm = 10^{-9}m). Light is emitted only when the electron relaxes from the first excited singlet state to the ground state and not from second to the first excited singlet state (Fig. 5.33). This is because transition of electron from second to the first excited singlet state is so rapid (10^{-14} to 10^{-13} s) that excess energy can only be released as heat and not as fluorescence.

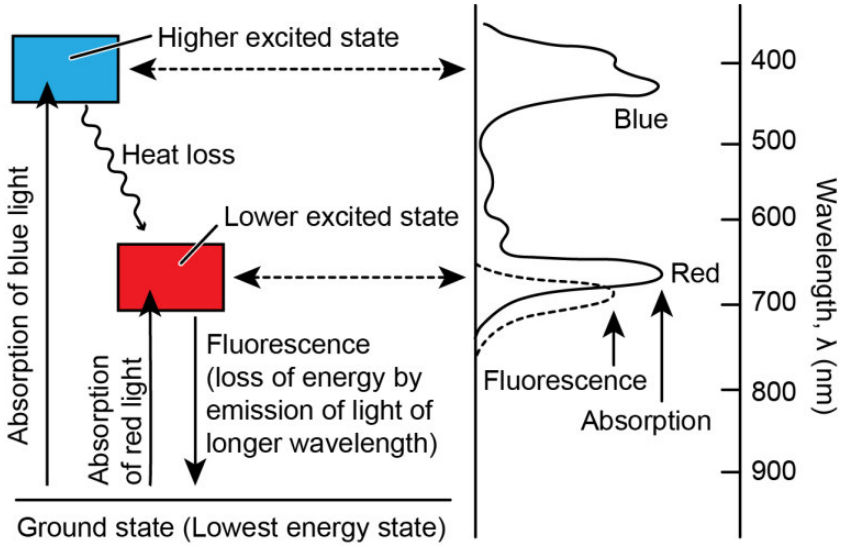


Fig. 5.33: Absorption of light by chlorophyll and its fate. An energy level diagram depicts various possible transitions upon absorption of light (After Taiz et al).

Box 5.3: Stokes Shift

Interestingly, fluorescence is shown only during the period of exposure to incident light. Since a portion of excitation energy gets converted to heat before the emission of photon during fluorescence, wavelength of fluoresced light is always of lower energy (longer wavelength) than that of light absorbed (Fig. 5.34). Therefore, when chlorophyll is excited either by blue light (450nm with 262kJ mol^{-1}) or red light (660nm with 181 kJ mol^{-1}), the fluorescence emission will always be on the right-hand side (long wavelength side) of red. Thus, a solution of chlorophyll when irradiated with blue light emits red fluorescence. The difference in wavelengths between absorption and emission spectra of the same electronic transition is called **Stokes Shift** (named after George G. Stokes).

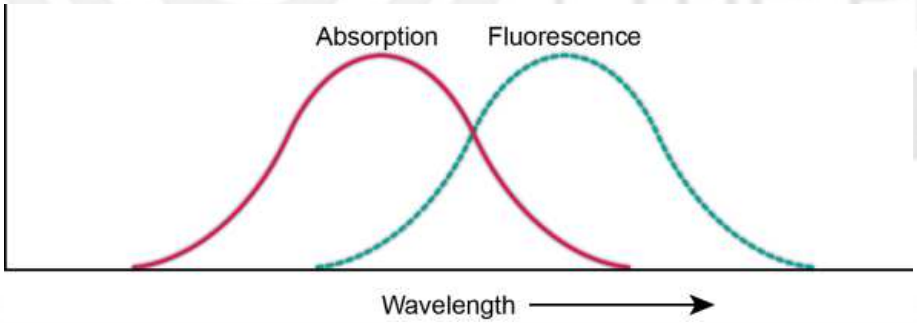


Fig. 5.34: The fluorescent light generally has a longer wavelength (less energy) than the absorbed light.

c) **Resonance Transfer:** The excited molecules may also transfer its excessive energy to another chlorophyll molecule by resonance transfer (radiation less transfer). In this case the absorption bands of the recipient and the fluorescent emission band of the donor molecule overlap with each other.

d) **Photochemical Reactions:** The excited molecule may return to another level or excited state called the **triplet** or **metastable triplet state**, which is long lived (10^{-3}s). Release of energy during this transition is called **phosphorescence**. Like fluorescence, the phosphorescing light will also

be of a higher wavelength than that of the incident light. But unlike fluorescence, the substances will emit phosphorescent light even after the incident light is cut off.

Since the metastable triplet state has a longer lifespan (10^{-3}s) and is much more stable, it may allow the occurrence of **photochemical reactions**, i.e., utilization of the excess energy in the excited triplet state of chl_a molecule to carry out the primary photochemical reaction by exciting the neighboring chromophore molecule. In this most vital reaction, chl_a is **photo oxidized** while the acceptor molecule gets **reduced**. The photochemical reaction is the fastest among the four alternatives and is also the most useful reaction to initiate photosynthesis. We shall study this aspect involving antenna molecules and the reaction centre in much greater details in the subsequent unit.

SAQ 4

Fill in the blanks in the following statements with appropriate words.

a) Light in the blue region of visible spectrum will excite the chlorophyll to a higher energy level than when excited by red light. This is because red light has …… energy than the blue light.

b) The second excited singlet state is short-lived and while relaxing to its lower excited singlet state, the electrons lose their excess energy as ……... .

c) Light emitted in fluorescence has a ……..…… wavelength than the excitation/incident light.

d) ……..….. results due to release of light energy when the electron returns from the excited first singlet state to the ground state.

e) Most useful utilization of excess energy is in the form of a ……..…… reaction.

5.5 SUMMARY

- Plants possess the unique ability to photosynthesize and convert light energy into chemical energy in the form of carbohydrates. In addition, the remarkable capacity to take up CO_2 from air and release O_2 makes photosynthesis the most crucial activity for supporting life on this planet. In addition to plants, algae and cyanobacteria also perform photosynthesis.

- Photosynthesis has a very interesting history where many plant physiologists, biochemists and naturalists have contributed towards our current understanding of this unique process. Only a very narrow band of PAR (Photosynthetically active radiation) is useful to plants. Selected wavelengths of PAR are absorbed by special pigments $Chl_{a,b}$, carotenoids and phycobilins, which act as photoreceptors . These

pigments belong to different classes of organic compounds and have specific absorption and action spectra. In plants the photosynthetic pigments are located on the thylakoid membranes of the chloroplasts in the form of specialized reaction centres (Photosystems I and II).

- Chl_a is the principal photosynthetic pigment which is excited by light. The excess energy gained by it, is partly lost as heat, or reemitted as light; some of this energy is also usefully harnessed to perform a photochemical reaction, which induces photolysis of water. The pigment chlorophyll, however, returns to its original, stable, ground state. Photolysis of water during the complex light reaction eventually helps in the generation of ATP and NADPH, which later provide the assimilatory power to drive the dark reaction of Calvin-Benson cycle to reduce CO_2 and synthesize carbohydrates.

5.6 TERMINAL QUESTIONS

1. Distinguish between PS I and PS II.
2. Answer the following questions:
   i) Who gave the term photosynthesis?
   ii) Trace the development of concept of photosynthesis by enumerating the contribution of various scientists.
   iii) Describe Engelmann's experiments on the role of light in photosynthesis.
   iv) With the help of suitable diagrams, explain the structure of chlorophyll.
3. Distinguish between Chl_a and Chl_b. Explain the absorption and action spectrum of chlorophyll. How is the bacteriochlorophyll different from chlorophyll?
4. What is the importance of accessory pigments? Give an account of carotenoids and carotenols.
5. Write a short note on non-photosynthetic photoreceptors.
6. Discuss the different ways by which the photoexcited electrons dissipate their excess energy.
7. Define the following:
   i) Photon
   ii) Absorption spectrum
   iii) Photosynthetically Active Radiation (PAR)
   iv) Fluorescence
   v) Phosphorescence
   vi) Resonance Transfer

5.7 ANSWERS

Self-Assessment Questions

1. a) Joseph Priestley

   b) Van Helmont

   c) Jan Ingenhousz

   d) J. Woodward

   e) Charles Bonnet

   f) Jean Senebier

   g) Antoine Lavoisier

   h) Stephan Hales

2. i) True; ii) False; iii) True; iv) True;

   v) False; vi) False; vii) False; viii) True.

3. a) i) Determination of relative photochemical efficiency of different wavelengths of visible spectrum. It closely matched with the absorption spectrum of chlorophyll.

      ii) Exposure of portion of algal filament to different wavelengths and measurement of photochemical efficiency (O_2 evolution was measured by differential accumulation of aerobic motile bacteria on the filament).

   b) i) Refer to Subsection 5.3.1

      ii) Refer to Subsection 5.4.2

4. i) less

   ii) heat

   iii) greater/longer

   iv) Phosphorescence

   v) Photochemical

Terminal Questions

1. Differences between PS I and PS II

PS I	PS II
1. It is located on the outer surface of thylakoid membrane towards stroma	1. It is located on the inner surface of the thylakoid membrane.

2. PSI is present in unstacked thylakoid membrane and causes light induced reduction of $NADP^+$.	2. It is predominantly present in stacked thylakoid membrane.
3. Here molecular oxygen is not evolved.	3. Molecular oxygen is evolved, and photolysis of water takes place.
4. It is involved in both cyclic and non-cyclic photophosphorylation.	4. It is involved in non-cyclic photophosphorylation.
5. Here strong reductant is produced which reduces $NADP^+$ to $NADPH + H^+$	5. PSII donates electrons to PSI when $NADP^+$ is reduced.
6. Reaction centre is made up of P_{700} a special chl *a* molecule.	6. Reaction centre is made up of P_{680}, a special type of chlorophyll a molecule.
7. Chl *a*/Chl *b* ratio is high (5)	7. Chl *a*/Chl *b* ratio is low (2-2.5)

2. i) Barnes
   ii) Refer to Sub Sections 5.2.1 and 5.2.2
   iii) Refer to Sub Section 5.2.2; Fig. 5.15
   iv) Refer to Section 5.3; Fig. 5.18
3. Refer to Section 5.3; Fig. 5.20,
   Refer to Section 5.4; Figs 5.34 and 5.35,
   Refer to Section 5.3; Fig.5.21.
4. Refer to Section 5.3
5. Refer to Section 5.3
6. Refer to Section 5.4
7. For i to vi Refer to Section 5.4.

Acnowledgements

Fig. 5.1 : https://www.britannica.com/biography/Jan-Baptista-van-Helmont

Fig. 5.2 : https://en.wikipedia.org/wiki/Stephen_Hales

Fig. 5.3 : .https://en.wikipedia.org/wiki/Joseph_Priestley

Fig. 5.4 : https://en.wikipedia.org/wiki/Jan_Ingenhousz

Fig. 5.5 : https://en.wikipedia.org/wiki/Antoine_Lavoisier

Fig. 5.6 : https://en.wikipedia.org/wiki/Jean_Senebier

Fig. 5.8 : https://en.wikipedia.org/wiki/Pierre_Joseph_Pelletier

Fig. 5.9 : www.daviddarling.info/encyclopedia/C/Caventou.html

Fig. 5.10 : https://en.wikipedia.org/wiki/Hugo_von_Mohl

Fig. 5.11 : https://en.wikipedia.org/wiki/Julius_von_Mayer

Fig. 5.12 : https://en.wikipedia.org/wiki/Jean-Baptiste_Boussingault

Fig. 5.13 : https://en.wikipedia.org/wiki/Julius_von_Sachs

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Fig. 5.15 : https://en.wikipedia.org/wiki/Theodor_Wilhelm_Engelmann

Fig. 5.16 : http://www.cropsreview.com/charles-barnes.html

Fig. 5.18 : https://img.tfd.com/mgh/ceb/thumb/Structure-of-chlorophyll-a-C55H72O5N4Mg.jpg

UNIT 6

PHOTOSYNTHESIS MECHANISM

Structure

6.1 INTRODUCTION

You have studied the historical background, photosynthetic pigments, and role of sunlight in photosynthesis in the preceding unit 5. In the present unit we are going to study the mechanism involved in photosynthesis. Photosynthesis is a complex process which is broadly divided into two distinct but related phases.

The light phase is directly dependent on absorption of photons and photoexcitation of chlorophyll, finally resulting in the photolysis of water. Electrons released during this process pass through a series of grana-membrane bound carriers, organized into two light-harvesting complexes (PS I and PS II), resulting in the generation of ATP and NADPH. ATP is generated via a specialized *CF_0-CF_1 ATP synthase* complex by creation of a proton-motive force.

The products of light reaction viz., ATP (high energy phosphate through photophosphorylation) and NADPH (reducing power) together constitute assimilatory power, which passes into the stroma for carbon-assimilation reactions (Calvin-Benson Cycle). This Dark reaction reduces CO_2 through the mediation of the bifunctional enzyme Rubisco. Reactions of the Calvin cycle occur in three stages: 1. CO_2-fixation, 2.Reduction of 3-phosphoglycerate (also called steps of glycolytic reversal), and 3. Regeneration of RuBP.

The cost of fixing $6CO_2$ to one hexose is 18 ATP and 12 NADPH, which are provided by the light reaction.

Four enzymes of Calvin cycle are activated indirectly by light.

Objectives

After studying this unit, you should be able to:

- list the evidences that lead first to the discovery of light and dark reactions and later to the two photochemical reactions;
- explain the Z-scheme of light reaction outlining the path of electrons in the electron transport chain from water to the final electron acceptor;
- Appreciate the structure of a chloroplast and its membranes and study the sites where PS I, PS II, and the components of the electron transport chain are located;
- compare cyclic and non-cyclic transfer of electrons;Outline the mechanism of photophosphorylation through chemiosmotic coupling along with the role of Q-cycle during generation of the proton gradient;
- appreciate the historical aspects associated with the discovery of Dark reactions;Outline the Benson-Calvin cycle and illustrate its connection with the energy capturing reactions in the thylakoid membranes; and
- critically analyse the energetics of C_3 cycle by calculating the number of ATP and NADPH molecules needed for CO_2 fixation.

6.2 EVIDENCE FOR LIGHT AND DARK REACTIONS

As discussed earlier in Unit 5, the process of photosynthesis was known in its bare outline at the beginning of the 20th century. Though the scientists did agree to the raw materials and products of photosynthesis, little was known regarding the source of molecular oxygen or sugars produced during this

process. With the prevailing methodology, it was not possible to confirm the origin of either products. Nor did it provide additional information about the intermediate steps. So, the entire phenomenon remained like a mysterious black box with little idea of the events that proceeded inside the box. Opening of this box meant the disruption of chloroplasts, and stoppage of photosynthesis.

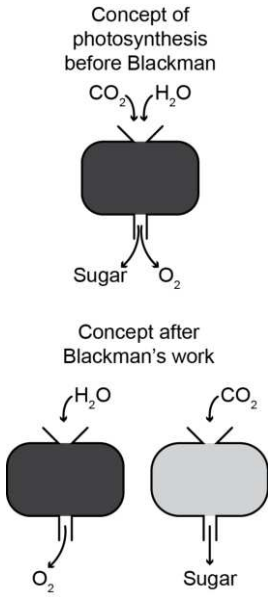


Fig. 6.1: Diagram to show evolution of concepts concerning the mechanisms of photosynthesis. The box above represents the concept at the end of the last century. After Blackman conducted the experiments to determine the Q_{10} of the photosynthetic reaction, it was concluded that the reactions in the "black magic-box" consisted at least two sub-sets of reactions, the light and dark reactions. The actual roles, that in the light box there is photolysis of water and in the dark box there is reduction of carbon dioxide was established much later.

6.2.1 Temperature Coefficient Experiments

This problem was finally tackled by an English plant physiologist F. F. Blackman who performed experiments considering the effect of temperature coefficient (Q_{10}).

Box 1: Q_{10} Temperature Coefficient.

For chemical and biochemical reactions, it is commonly observed that increase of temperature by 10°C, increases the rate of thermal reaction by two fold. The minimum energy which the reactants must possess for a reaction to proceed is called activation energy. The increase in temperature increases molecular collisions and so the proportion of molecules possessing activation energy for reaction to occur increases. Hence, the reaction rate increases.

The ratio of the rates of reaction at 1°C and (t + 10°C) is denoted by Q_{10} (**Temperature Coefficient)**

$$Q_{10} = \frac{\text{Reaction rate at (t+10)°C}}{\text{Reaction rate at 1°C}} = 2$$

(Q denotes Quotient and 10 stands for 10°C rise in temperature.)

For physical processes, the Q_{10} is in the range of 1.2 to 1.4, whereas enzymatic and physiological processes have Q_{10} ranging from 2 to 3.

The rate of photochemical reaction is not affected by temperature increases because the activation energy to substrate is supplied by photons of required energy and the energy for bond breaking process is also given by photons. Photochemical reactions have a Q_{10} of 1.

Q_{10} is a convenient indicator of the extent to which a reaction gets affected by temperature. Since the process of photosynthesis requires both light and chlorophyll, it is obvious that a photochemical reaction is involved. Blackman measured the Q_{10} of this reaction by increasing the temperature at low light intensity and found it to be 1 (unity). This indicated that this reaction was independent of temperature (in fact, all photochemical reactions are independent of temperature). He further demonstrated that if the plant is well illuminated and has an adequate supply of CO_2, the value of Q_{10} ranges between 2-2.5, thereby indicating the existence of some purely chemical (temperature-sensitive) reactions also in photosynthesis. So, existence of two distinct reactions meant that instead of the single magic box as mentioned above, there were two boxes (Fig 6.1).

1. One box contained the chlorophyll pigments whose function was controlled by light and was not temperature sensitive, and

2. The other box was the site for "dark" or chemical reaction.

Blackman's results can be easily duplicated in the laboratory by using a simple experimental set-up with the green submerged plants of *Hydrilla* or *Elodea*

(Fig.6.2). Oxygen bubbles are released from the cut portion of the stem when the plant is provided with dilute $NaHCO_3$ (source of CO_2) and enough light. The number of bubbles given out in a fixed interval of time at each of the several light intensities gives data which can be plotted as below (Fig. 6.3):

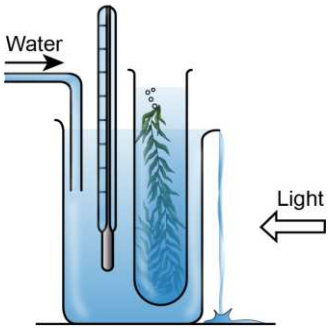


Fig.6.2: Experimental set up to demonstrate effect of light intensity on photosynthesis.

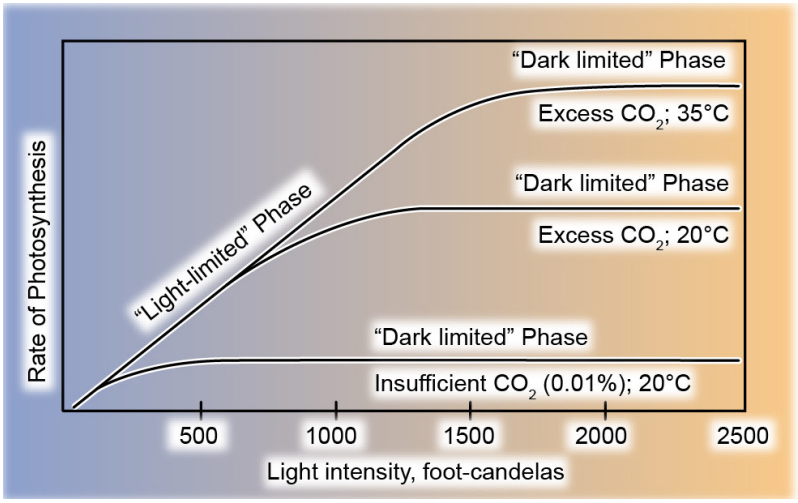


Fig. 6.3: Graph showing the effect of light intensity on rate of photosynthesis.

Since the rate of photosynthesis does not continue to increase indefinitely with increased light intensity, Blackman concluded that at least **two** distinct processes are operative: a) A light requiring phase, and b) a light – independent phase. The latter is called "dark" phase, although it also operates even in light.

Based on these experiments Blackman also proposed the "Law of Limiting Factors". This law states that the factor which is shortest in supply, or the slowest step, will limit the overall rate of the photosynthetic process. He observed that there was no effect by raising the temperature when light intensity was low and CO_2 concentration was high. However, under conditions of strong light and low (limiting) CO_2 concentrations, increase in temperature resulted in an increase in photosynthesis. This work led to the concept of light-limited and dark-limited photosynthesis. Later Warburg called this dark reaction as "Blackman reaction". You will perform these experiments in your practical.

6.2.2 Intermittent Light Experiments

The existence of light and dark reactions was further confirmed by the German Nobel laureate **Otto Warburg** (1919), and later by his American pupils, **Robert Emerson,** and **William Arnold**. They carried out experiments with alternating flashes of light and darkness given to unicellular green alga *Chlorella*. A mechanical device, consisting of an electric motor to which was attached a rotating disc with sectors cut in different sizes (see Fig.6.4) was set in the light path.

With this set-up they could obtain intermittent light and darkness. The mechanical device was later replaced by more sophisticated and accurate electronic flashes discharges. It was shown that if light and dark periods were separated by appropriate intervals (intermittent light flashes), the O_2 evolution per unit intermittent light was as high as 400% in comparison to the control, where the same amount of light was given continuously. This work further

suggested the presence of two phases and that since the dark reaction was slow, it limited the reaction under continuous light.

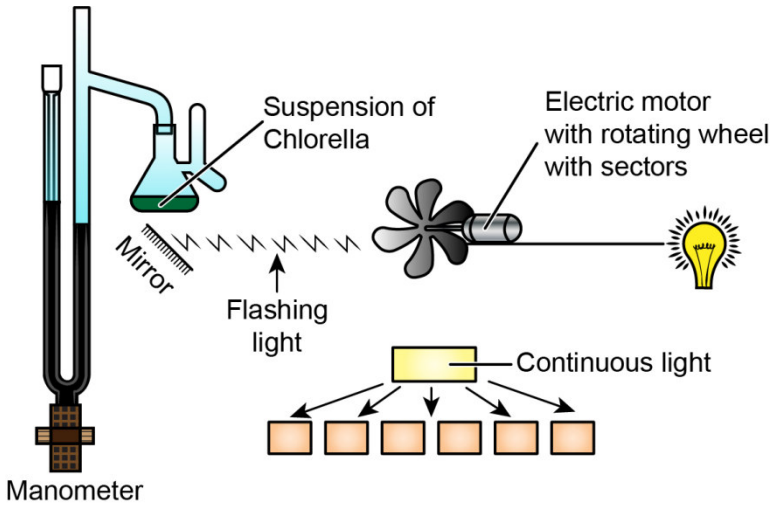


Fig. 6.4: The experimental set-up employed for the flashing light experiments.

The following equation summarizes the sequence of events based on Warburg's ideas:

Photochemical Reaction *Thermochemical Reaction*

A ⟶ B ⟶ C (Product)

Very rapid Q_{10}=1 Light insensitive Very slow; Q_{10}=>2.

Under continuous light B accumulates and becomes limiting and slows down the entire process while the intermittent light gives ample time for the conversion of B to C to occur, thereby allowing photosynthesis to proceed at an optimum rate. Warburg also calculated the minimum quantum requirement (minimum number of photons) for photosynthesis to be 3-4 per oxygen molecule evolved. You would study in the section of light reaction (6.3.1), this value (3-4 light quanta per oxygen) was an error by a factor of 2-3, as subsequent studies revealed this value to be between 8-10.

Later, Emerson & Arnold (1932) repeated these intermittent/Flashing light experiments (light= 10^{-3} s + Dark=0.055 s) and found that the dark phase was a temperature-sensitive reaction. They also showed that **only one** out of several hundreds of cooperating chlorophyll molecules is directly involved in photochemistry, i.e., in light mediated reactions.

This led to the concept of a "**photosynthetic unit**" In addition, several hundred pigment antenna molecules contributed their services to a single reaction centre chlorophyll. You will read more about the reaction centre along with the concept of "photo zyme" in Section 6.3.

6.2.3 Role of Light Reaction

It was the American microbiologist Cornelius B. Van Niel, who laid down the foundation of the modern concepts of photosynthesis by pointing out the role of light in this process. He performed experiments on the photosynthesis of green sulfur bacteria. These bacteria contain bacteriochlorophyll and survive on H_2S; and in the process released elemental sulfur. In nature these bacteria account or some very picturesque deposits of sulfur. Since this bacterium did

not evolve oxygen, it constituted **anoxygenic** photosynthesis, in contrast to **oxygenic** photosynthesis (O_2 releasing) that we knew till then. Van Niel believed that these types of bacteria did not require water and thus H_2S appears to be the hydrogen donor. According to him, the action of light was to cause the decomposition of H_2S into hydrogen and elemental sulfur.

$$nCO_2 + 2nH_2S + Light \longrightarrow (CH_2O)n + nH_2O + 2nS$$

which translates to

$$6CO_2 + 12H_2S \xrightarrow[Bacteriochlorophyll]{Light} C_6H_{12}O_6 + 6H_2O + 12S$$

The above equation indicated that the primary role of H_2S was that of a reductant to supply electrons. Adopting a comparative biochemical approach, he envisioned a parallel process to be occurring in case of photosynthesis in higher plants where water must serve as a reductant and the O_2 evolved in photosynthesis must come entirely from water rather than from both H_2O and CO_2, or from CO_2 alone. In other words, he pointed towards an analogy between the roles of H_2S and H_2O with those of S and O_2 and thus a general plan for both oxygenic and anoxygenic photosynthesis. This was a clear contradiction of the earlier ideas of Senebier that CO_2 was the source of O_2. If Van Niel's theory is correct, then the equation for photosynthesis would be written as:

$$6\ CO_2 + 12\ H_2O \xrightarrow[Chlorophyll]{Light} C_6H_{12}O_6 + 6\ H_2O + 6O_2\uparrow$$

Equally important, Van Niel also proposed on simple, purely thermodynamic considerations that the role of light would be to split H_2O (a process for which he coined the term "**photolysis**" of water.

This step would require maximum input of energy. The role of water or H_2S can be depicted by the following equation:

$$H_2S \longrightarrow 2H^+ + 2e^- + S$$

and

$$H_2O \longrightarrow 2H^+ + 2e^- + 1/2O_2\uparrow$$

6.2.4 Hill Reaction

Ideas of Van Niel found support by the classical experiments carried out by the British scientists **Robin Hill** and **R. Scarisbrick** in 1939, who demonstrated that even isolated chloroplasts and chloroplast fragments when illuminated and provided with suitable acceptors could release O_2 from water. They provided light to the isolated live chloroplasts from *Stellaria media* and found that acceptors like ferricyanide and ferrixylate got reduced from their ferric (Fe^{+++}) to ferrous (Fe^{++}) form (Fig 6.5). Later, other workers have shown that many other electron donors such as benzoquinone and 2, 6-dichlorophenol indophenols (DCPIP) can also be used as hydrogen acceptors (Fig. 6.6). In honor of its discoverer, the process of dye reduction by isolated illuminated plastids, leading to photolysis of water in the absence of CO_2 fixation is called **Hill Reaction** and the oxidants used are called Hill reagents or Hill oxidants. You will perform this experiment in your practical work.

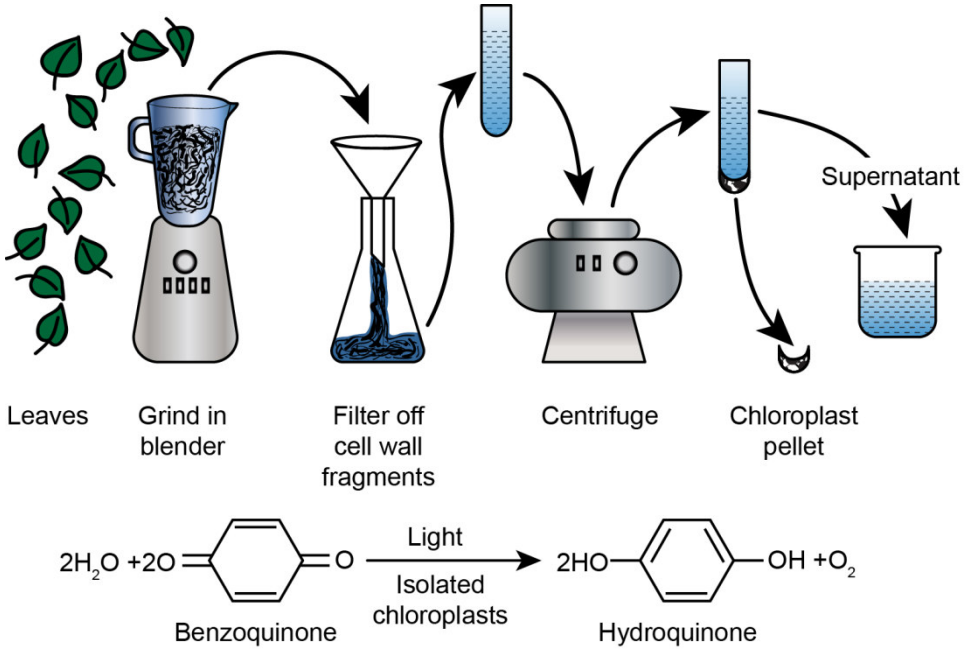


Fig. 6.5: A common procedure for the isolation of chloroplasts from leaves to demonstrate Hills reaction.

Hill adopted a clever strategy. He put some reduced hemoglobin in the chloroplast suspension which got readily oxidized by the O_2 released. He monitored the release of O_2 by a spectroscope.

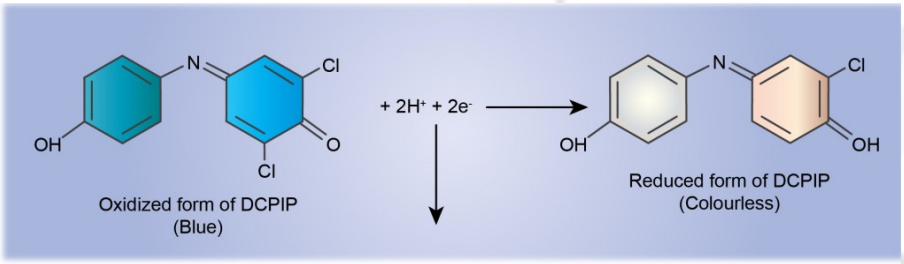


Fig 6.6: Demonstration of Hill reaction with DCPIP.

These experiments clearly indicated that some reactions of photosynthesis could take place in cell- free media by isolated chloroplasts. Also, the light dependent O_2 release in light reaction seems to be an independent process, as under these conditions neither CO_2 was consumed, nor any carbohydrate was produced. Only O_2 was evolved because of light-mediated /driven reduction of electron acceptors.

$$4Fe^{3+} + 2H_2O \longrightarrow 4Fe^{2+} + 4H^{+} + O_2 \uparrow$$

6.2.5 Confirmation of O_2 Source in Light Reaction

American chemists **Samuel Ruben** and **Martin Kamen** (1941) provided a much more convincing evidence that O_2 is indeed evolved from water. They used water containing O^{18}, the heavy, non- radioactive isotope of oxygen to study photosynthesis in the green alga *Chlorella*. They employed a mass spectrometer to detect O^{18}. The oxygen released was found to be labeled with O^{18} rather than from O^{16}. Subsequent work by **Alan Stemler** and **Richard Radmer** (1975) proved that the entire O_2 comes from water.

Since the investigations supported Van Niel's hypothesis, it also follows the analogy of his equation. Moreover, two H_2O molecules will be required as reactants to account for release of one O_2 molecule.

$$n CO_2 + 12 H_2O + Light \xrightarrow{chlorophyll} (CH_2O)n + 2H_2O + n\ O_2 \uparrow$$

The new correct representation of the overall reaction of photosynthesis is

$$6\ CO_2 + 12\ H_2O \xrightarrow[chlorophyll]{686 Kcal\ of\ radiant\ energy} C_6H_{12}O_6 + 6\ H_2O + 6\ O_2 \uparrow$$

$$6\ CO_2 + 12\ H_2O \longrightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_2 \uparrow$$

Carbohydrates (CH_2O) are synthesized from CO_2 and H_2O. Both carbon (red) and oxygen (yellow) in (CH_2O) comes from CO_2 while oxygen that is liberated (green) comes from H_2O.

However, light energy is not utilized directly to drive this reaction. Similarly, H_2O does not directly reduce CO_2 to carbohydrates.

Clearly, by the first half of the last century, the basic role of at least one of the two "boxes", the box in which electrons are produced from H_2O for reducing CO_2 was established without doubt (see Fig. 6.1).

6.2.6 Photophosphorylation and Production of Reducing Power (Photoreduction)

The role of the other box was clarified by the American biochemists **Wolf Vishniac** and **Severo Ochoa** (1951) along with **D.I.Arnon** who demonstrated independently that photolysis of water by illuminated chloroplasts led to the reduction of a vitamin B (niacin or nicotinamide) containing coenzyme **NADP** (Nicotinamide Adenine Dinucleotide Phosphate). Thus, NADP could also act as a Hill reagent.

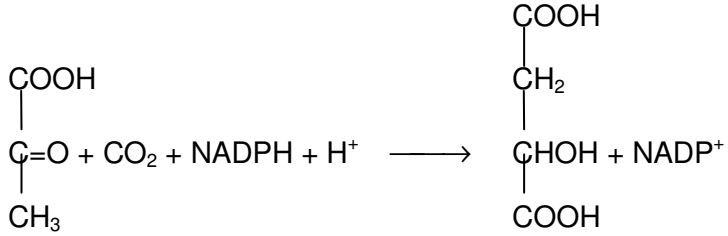
$$2NADP^+ + 2\ H_2O \xrightarrow[Chlorophyll]{Light} 2\ NADPH + 2H^+ + O_2 \uparrow$$

Since it was already known that the NADP in a reduced form i.e., NADPH could transfer electrons to a number of compounds in plant metabolism, like during the reductive carboxylation of pyruvate into malic acid, it was concluded that light induces photolysis which derives the electrons from water to reduce $NADP^+$ to NADPH, which is utilized for the dark reaction.

(i) $$H_2O \longrightarrow 2H^+ + 2e^- + \tfrac{1}{2}\ O_2$$

$$NADP^+ + 2H^+ \longrightarrow NADPH + H^+$$

(ii)



Pyruvic acid **malic acid** **Dark reaction**

or to put more generally

$$CO_2 + NADPH + H^+ \longrightarrow [CH_2O] + NADP^+$$

At nearly the same time, **D.I. Arnon,** working at the University of California, Berkeley made an outstanding discovery in 1954 (reviewed by Arnon, 1984). By using radioactive phosphate, labelled with P^{32}, he showed that isolated chloroplasts, when exposed to light, could synthesize ATP from ADP without oxygen. Till then, respiration was the only known source of ATP generation (oxidative phosphorylation). Since ATP was being synthesized from ADP and inorganic phosphate (Pi) by the chloroplasts only in light, Arnon suggested the term **Photosynthetic phosphorylation** or simply **photophosphorylation.**

$$\text{ADP} + \text{Pi} + \text{Light} \xrightarrow{\textit{Chloroplast}} \text{ATP} + \text{water}$$

The above-mentioned experiments led to three important conclusions for light reactions:

1. Occurrence of photosynthesis in the chloroplasts.
2. Reduction of $NADP^+$ and generation of ATP.
3. Utilization of NADPH and ATP in the subsequent reactions.

This discovery made good sense since photosynthesis is generally 10-20 times faster than respiration, the requirement for ATP for the dark reactions of photosynthesis (say, phosphorylation of ribulose to RuBP) cannot be met by the ATP supplied by respiration via the ordinary pathway of oxidative phosphorylation.

Interestingly, our summary equation does not mention ATP or NADPH. This is because these substances get used in the process of CO_2 reduction and sugar synthesis during the dark reaction and get regenerated as ADP and $NADP^+$ respectively. We shall formulate a complete equation for photosynthesis only after studying the dark reaction (Calvin Cycle) in the forthcoming section.

By this time, the spatial distribution of the primary and secondary processes occurring within the chloroplasts were known. These are represented in the form of a general scheme(Fig. 6.7) given by Arnon.

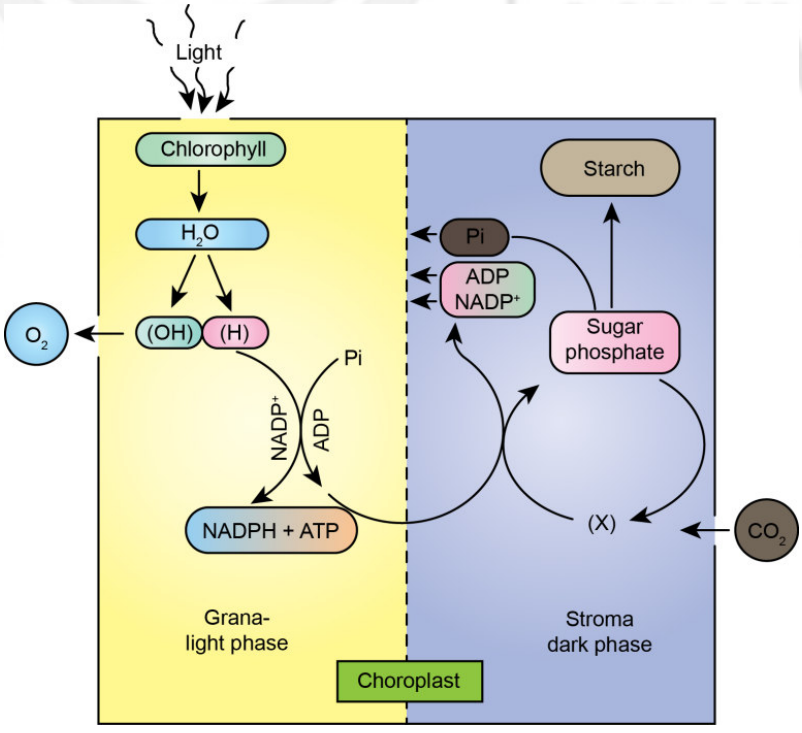


Fig. 6.7: A review of the photosynthetic process.

SAQ 1

a) i) Which of the experimental findings in photosynthesis gave the clue that the process consists of both light and dark reactions?

ii) What exactly is the role of the photochemical reaction?

b) Fill in the blanks in the following statements with appropriate words.

i) In photosynthesis O_2 is released because of ..... of ..... .

ii) Light splits ..... was shown by using an isotope of H_20 containing .....

iii) The action of light leads finally to reduction of ..... to ..... .

iv) Reduction of an electron acceptor and evolution of O_2 by isolated chloroplast on illumination is called .....

6.3 DISCOVERY OF TWO LIGHT REACTIONS

Let us now enquire into the detailed mechanism of both the light as well as the dark reactions. We begin with the light "box" and discuss the great conceptual advances in photosynthesis, i.e., the discovery that there are two light reactions (or boxes), and not one as was originally believed.

6.3.1 Quantum Requirements of Photosynthesis

In the earlier section (6.2.2) we discussed the flashing light experiments, which laid an emphasis on confirmation of the idea of the existence of light and dark reactions. But a second objective of doing these experiments was to determine the quantum requirement for photosynthesis to obtain an overall idea of the efficiency of the process and its mechanism. Since oxygen evolution is a measure of the photosynthetic process, the question that needed to be resolved experimentally was **"How many quanta of light are necessary for evolving one molecule of oxygen"?** This can be better understood through the concept of **Quantum efficiency,** which can be expressed in two ways-either as **quantum requirement** or as **quantum yield.**

Quantum yield ($q\varphi$) expresses the efficiency of a light mediated process when a photon is absorbed by a molecule. φ is equal to the ratio of the number of molecules reacted (M) to the number of photons absorbed (q). So $q\varphi = M/q$.

In other words**, φ=Number of photochemical products/Total number of quanta absorbed**. With reference to photosynthesis, it is measured as the amount of O_2 evolved or the amount of CO_2 taken up to the number of photons absorbed. On the other hand, quantum requirement (also called quantum number) or $1/q\varphi$ refers to the number of photons required to release one O_2 molecule or to reduce one CO_2 molecule.

With the development of the Atomic theory in the early part of the last century as also the new equation of photosynthesis (that a minimum of 4 electrons are needed to evolve a molecule of oxygen) and Einstein's Law of Photochemical Equivalence, the studies on quantum requirement of photosynthesis took an entirely new turn. According to the concept advanced by Einstein, in a photoelectric effect one hit by a photon leads to expulsion of only one electron at a time from the molecule. Thus, the overall **quantum requirement** (number of light quanta required for the evolution of one oxygen molecule) could serve as an indicator of the number of hits needed for an electron to be pulled up via chlorophyll from water to a higher energy state as in NADPH. On the other hand, **quantum yield** is the number of oxygen molecules released per photon of light.

As per the new equation of photosynthesis, four electrons need to be extracted from a H_2O molecule for the evolution of one oxygen molecule. This is because 4 H atoms are required to reduce one molecule of CO_2 by 2 molecules of H_2O. Also, the transfer of each H atom from water to CO_2 requires on quantum of light or photon.

$$4\ H_2O \longrightarrow 4H^+ + 4OH^-$$

$$4OH^- \longrightarrow 2H_2O + O_2 + 4e^-$$

$$4H^+ + CO_2 \longrightarrow (CH_2O) + O_2 + H_2O$$

You should remember that the quantum requirement must be in simple multiples of 4 (theoretical) and so one must choose a figure from amongst 4,8,12 and so on (Fig.6.8).

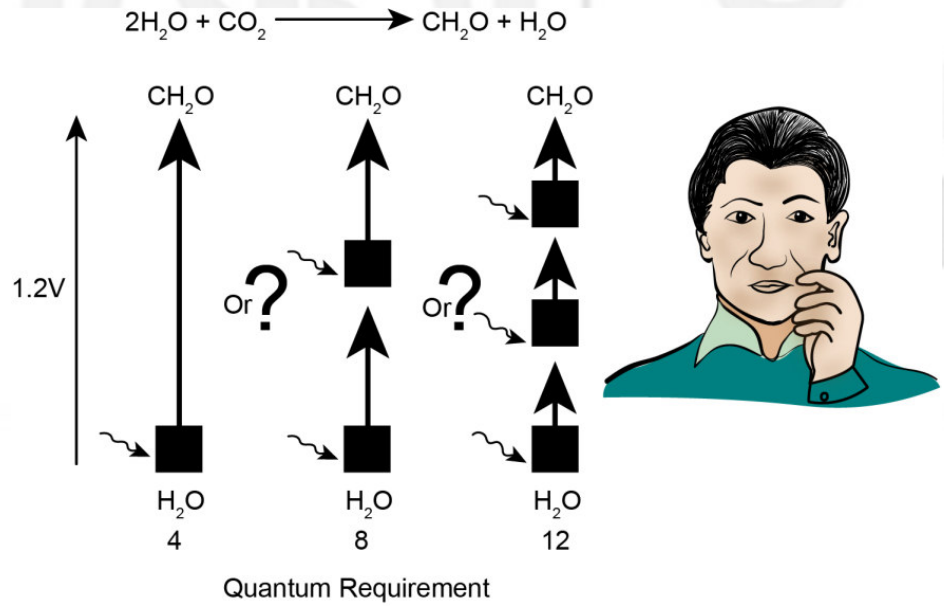


Fig 6.8: The diagram illustrates the significance of studies on quantum requirement to evolve oxygen during photosynthesis.

Although Warburg, who initiated these studies, found a quantum requirement of **four**. This would suggest an extraordinary efficiency of the photosynthetic process. Emerson and Arnold and later many other workers found that the quantum requirement was **8-10** meaning thereby that the overall photochemical process is a **two-step reaction** (since a quantum requirement of 4 indicates that to drive a single electron only one photochemical reaction is

involved).Each electron, therefore, needs to be excited twice. Thus, contrary to Warburg's conclusion, photosynthesis is not so efficient to convert the entire light energy into chemical energy and that there is a considerable loss of light energy during this process.

Box 6.3: Quantum Requirement for Photosynthesis

Let us analyze the above equation in detail. One molecule of glucose $(CH_2O)_6$ contains 686kcal (468.6 kJ) of energy. Therefore, 1/6 of glucose molecule represented in the above equation will possess 686/6=112kcal (approx.468.6 kJ). Since maximum photosynthesis occurs in red light (a red light photon contains about 40kcal of energy), it would mean that the efficiency with which plants can convert light energy into chemical energy would be 112/40x4=70%, which seems to be very high.

If we consider the quantum requirement for photosynthesis as 8-10, the quantum yield of O_2 production comes to 1/8= 0.125 or 1/10=0.1 in contrast to 0.25 proposed by Warburg.

6.3.2 Red Drop and Emerson Effect

Another hint that there are two photochemical reactions came from the laboratory of Robert Emerson, who found the second line of evidence while working on the absorption spectrum of photosynthesis in *Chlorella*. Emerson and Lewis (1943) measured the quantum yield of photosynthesis as a function of wavelength of light. It was observed that the value of *qp* remained constant through most of the red region of the spectrum (where maximum light is absorbed by Chl_a). It clearly means that any photon absorbed by chlorophyll was equally effective in driving photosynthesis in that range. However, these workers observed that there was a dramatic fall/drop in the values of *qp* at wavelengths greater than 680nm, i.e., the far- red region. Interestingly, some light was still being absorbed by the chlorophyll in this region. This phenomenon cannot be explained simply based on just a decrease in absorption by chlorophylls as *qp* or quantum yield is only a measure of photons that have been absorbed.

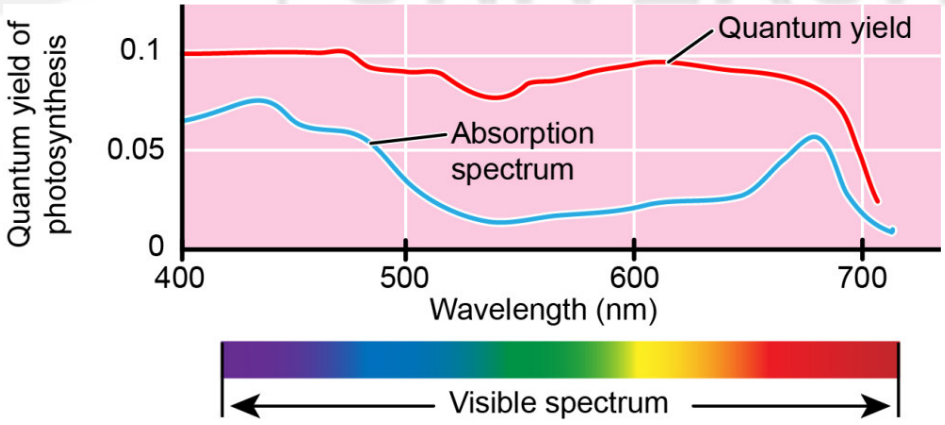


Fig. 6.9: Emerson "Red Drop" effect .Drastic fall in quantum yield at wavelengths greater than 680nm (Black curve). This indicates that far-red light alone is not sufficient to drive photosynthesis (From Taiz *et al*).

This pronounced decline in the quantum yield at wavelengths higher than 680nm was quite puzzling as it indicated this region to be less efficient for photosynthes is and was called **red drop**. This was highly perplexing since

Chl_a has been considered as the most important of all photosynthetic pigment molecules and to which in fact all energy is ultimately transferred from the other pigments.

In the subsequent experiments, Emerson and his colleagues demonstrated that the "red drop" caused by wavelengths greater than 680nm could be abolished/restored provided the two beams of light of different wavelengths- one red (650-680nm), and the other far red (700-720nm) were given simultaneously. The rate of photosynthesis under two simultaneous/superimposed light beams was two to three times more than the sum of rates obtained by the two light beams of individual wavelengths separately (Fig. 6.10)

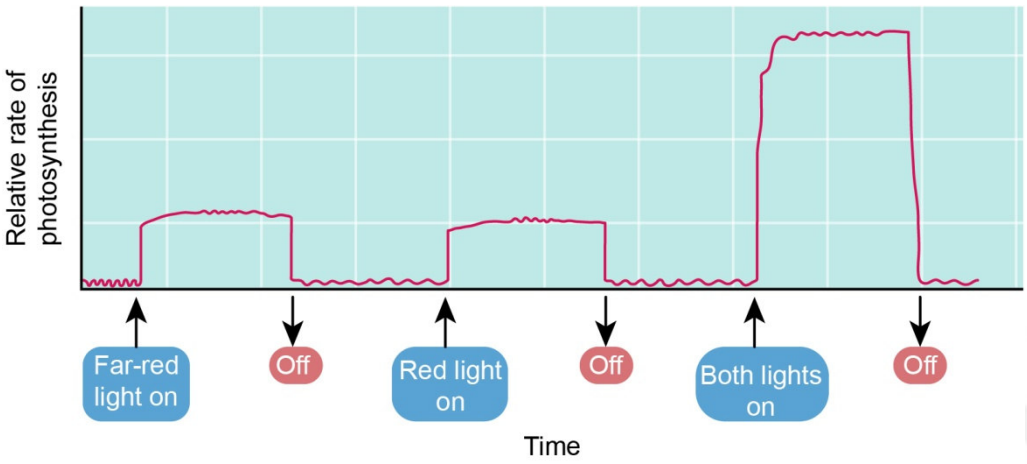


Fig. 6.10: **Emerson's Enhancement Effect. Greater photosynthetic rate upon exposure to red and far-red light together than the sum of the rates when given apart. Evidence of two photosystems working in tandem. (From Taiz *et al*).**

This increase in photosynthetic activity came to be known as **Emerson Enhancement Effect**.

Emerson effect provided a conclusive evidence that not one, but two photochemical reactions or events are involved in light reaction, each driven by a short-wavelength (<680nm) and long wavelength (>680nm) respectively. It was also established that to carry out the optimal process, both these systems must be driven simultaneously or in rapid succession. In addition, the experiments on saturation of chlorophyll by Emerson and Arnold (1932) gave some unexpected results.

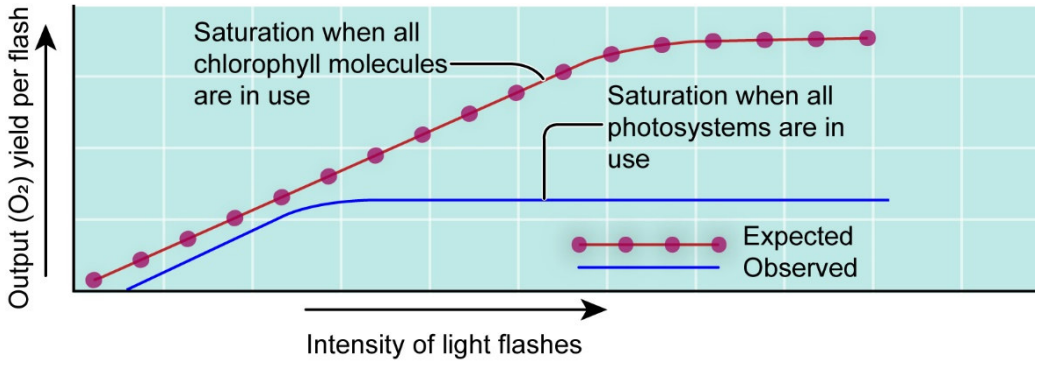


Fig. 6.11: Emerson and Arnold's experiment: After achieving photosynthetic saturation, no increase in output after further increase in light intensity.

Assuming that all the pigments of a plant were in use at the time of saturation, the oxygen yield in *Chlorella* upon exposure to brief flashes lasting a few milliseconds did not increase and saturation was achieved even with one oxygen molecule per 2500 chlorophyll molecules (see Fig. 6.11). Emerson and Arnold concluded that light is absorbed by a cluster of pigment molecules-both chlorophyll and the accessory pigments rather than independent pigment molecules. This gave birth to the concept of **photosystems.** Also, the reaction centre of the photosystem functions like an energy sink, which traps the excitation energy from the helper pigment molecules called antennae.

Box 6.4 : Quantasomes-- Photosynthetic units?

Earlier studies made by Emerson and Arnold suggested that about 2,500 chlorophyll molecules would be required to fix one molecule of CO_2 in photosynthesis. Since they function together as a unit, they were called as a photosynthetic unit. Later studies have revealed that 10 photons are required to produce a molecule of oxygen. So logically each individual photosynthetic unit must comprise 2500/10=250 chlorophyll molecules. Small granular structures have been observed by Park and Biggins (1961) by using freeze fracture studies combined with TEM, and later coined the term **Quantasomes**. The granules were present in the thylakoid membrane and were thought to depict the morphological expression of a photosynthetic unit. Each quantasome was measured to be 180x160Å with 250- 300 pigment molecules grouped together as a unit. Later studies have, however, cast a doubt as to whether these quantasomes represent photosynthetic units.

Recent investigations have shown that a light harvesting complex must be called photosynthetic unit, where only one member, chlorophyll*a*,is just one of the components, acting as reaction centre and transferring the electrons to an electron acceptor. The other participating accessory pigments act as antennae working to trap and transfer energy to the chlorophylls. Thus, the concept of Quantasome now appears to be unconvincing.

SAQ 2

a) List the evidences for the involvement of two light reactions instead of one in photosynthesis.

b) In the following statements fill in the blanks with appropriate words:

i) The photosensitive pigments transfer one electron at a time, while a molecule of $NADP^+$ requires ……..…… electrons for its reduction.

ii) Light energy captured by chlorophyll is used to produce a strong …….. from a weak one.

iii) Light energy absorbed by the antenna complex pigments is funneled to a single molecule of ……..…… .

iv) The expelled electron from Chl $_{P\ 680}$ travels through the electron transfer chain and reaches Chl $_{P\ 700.}$The final acceptor of electron is ……..….. .

6.4 PHOTOSYSTEMS I AND II

The twin discovery of red drop and Emerson's enhancement effect helped scientists to believe in the existence of two distinct photochemical processes or energy capturing reactions- one driven by shorter wavelengths and the other operative at wavelength greater than 680nm. Two different, yet connected pigment systems viz., Photosystem I and Photosystem II, each requiring different light energy and possessing different groups of pigments seem to be associated with these photochemical processes. These are called the **Photosystems** /**Pigment systems**. Biochemical studies have revealed that chl_a itself exists in many forms, each having their specific absorption maxima, e.g., $chl_{a\ 673,}$ $chl_{a\ 683\ and}$ $chl_{a\ 700}$ and a few more. These two photosystems have been biochemically isolated by density gradient centrifugation and have been shown to possess different types of chl_a molecules. The number of chl_b and carotenoid molecules is also variable.

Each reaction centre is surrounded by a light harvesting complex(LHC), which consists of antenna molecules in the form of chl_b and carotenoids, along with several associated proteins and metal and non-metal ions (see Fig. 6.12)

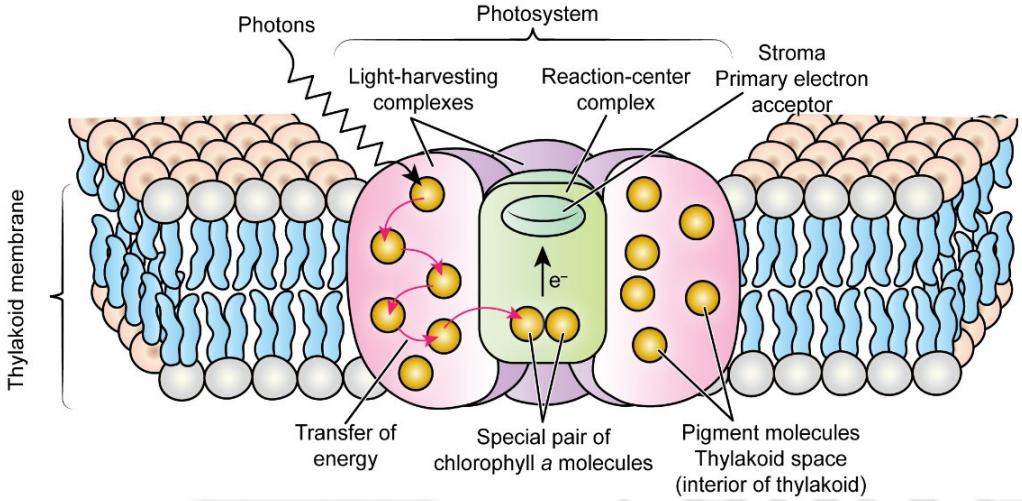


Fig. 6.12: Organization of a photosystem in the thylakoid membrane (From Taiz *et al*).

Photosystem I (PS I) is present in the lighter fraction. It operates in the form of a complex and contains a specially oriented molecule of chl_{a700} (called P_{700}) or the reaction centre (trap I) for this photosystem. It absorbs light quanta at 700nm. The reaction centre is surrounded by nearly 200 light harvesting chl_a molecules which operate at long wavelengths, i.e., $chl_{a\ 683}$, along with chl_b and carotenoids, electron carriers in the form of cytochrome *f*, plastocyanin, two cyt_{b563}, tyrosine residues, ferredoxin reducing substance (FRS) and several membrane bound Fe-S proteins. Photosystem I is richer in chl_a than Photosystem II while the amount of chl_b and carotenoids is lesser. PS I absorbs light at wavelengths greater than 680nm and operates at relatively low energy levels. However, it is a strong reductant and reduces $NADP^+$ to NADPH and H^+. PSI also produces a weak oxidant and is rich in Fe and Cu ions. This photosystem can operate independently. We will discuss the process of cyclic photophosphorylation later in this section.

Photosystem II (PSII) constitutes the heavier fraction with a larger proportion of chl_b and carotenoids than in PS I. Here the P_{680} (chl_a680) forms the reaction centre/trap II which is surrounded by the antenna molecules of $chl_{a\,673.}$ An electron donor (Z), a primary electron acceptor (Q), plastoquinone, Mn molecules and chloride ion are also present. The PS II is a strong oxidant and a weak reductant. It is responsible for the photolysis of water and evolution of molecular oxygen (see Fig. 6.13).

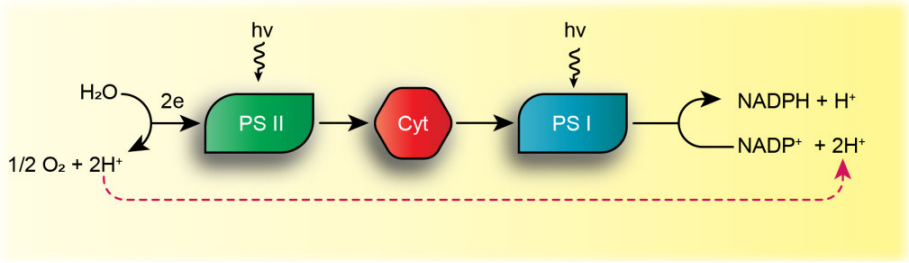


Fig.6.13 : A sequential arrangement of the multimolecular complexes of the electron transport chain take low-energy electrons from water and produce a strong reductant, NADPH +H^+ in the presence of sunlight. (From: Hopkins & Hüner).

Semi Genetic Autonomy

Mitochondria and chloroplasts are unique organelles in many ways. Both arise from pre-existing organelles and never *de novo*.

Both organelles possess circular DNA, show DNA-dependent RNA synthesis, 70S ribosomes, and protein synthesizing machinery. Yet, synthesis of many enzymes needed for the normal functioning and division of these organelles is controlled by the nuclear genes. This illustrates the semi-genetic autonomy of these organelles.

Both the photosystems are interconnected by an integral protein complex, the **cytochrome$_{b6\text{-}f}$complex** (Fig. 6.13) which is a large protein with many subunits. This complex also contains a **Rieske iron-sulfur protein (FeSR)** which is an electron carrier.

It is the interaction of weak oxidant of PSI and the weak reductant of PSII which is the driving force for ATP synthesis (photophosphorylation). Unlike PSI, the PSII always works in combination with PSI and not independently.

6.4.1 Organization of Photosynthetic apparatus in the Chloroplast

Photosynthesis is carried out in the chloroplasts which are specialized double membrane organelles in the green plants. The chloroplasts are internally separated into two distinct zones-**grana** and **stroma**. The grana consists of an extensively folded system of internal membranes (**thylakoids**) which are aggregated in the form of coin-like stacks (Fig. 6.14a-d). These stacks amplify the membrane surface area manifold, thereby increasing the light harvesting capability. Each such coin-like stack of thylakoids is called a **granum** (Fig. 6.14 c, d) and the stacked membranes as grana lamellae (Fig. 6.14 a, b). These lamellae are interconnected by other thylakoids which are not stacked and are in the form of exposed membranes. These are called **stroma lamellae** (Fig.6.14 b).The space inside the thylakoid of a grana is called **loculus**. The region outside the thylakoid constituted the **stroma**. The chloroplast envelope comprises two membranes which are a seat for many transport systems for metabolites.

Light reactions occur in the thylakoids, i.e., grana, while the carbon fixation (dark reaction) takes place in the stroma. In addition, the stroma is also a site for DNA, RNA and protein synthesizing machinery which can synthesize some of the proteins. Chloroplasts are semi-genetically autonomous organelles. As mentioned above, thylakoids are the sites for light reaction. The two photosystems or pigment systems, which are made up of the reaction centres, the antenna pigment –protein complexes, and many of the electron carrier proteins belong to the class of **integral proteins**. These proteins have a

characteristic orientation within the thylakoid membrane. One region of such protein projects into the stroma while the other one is oriented towards the lumen.

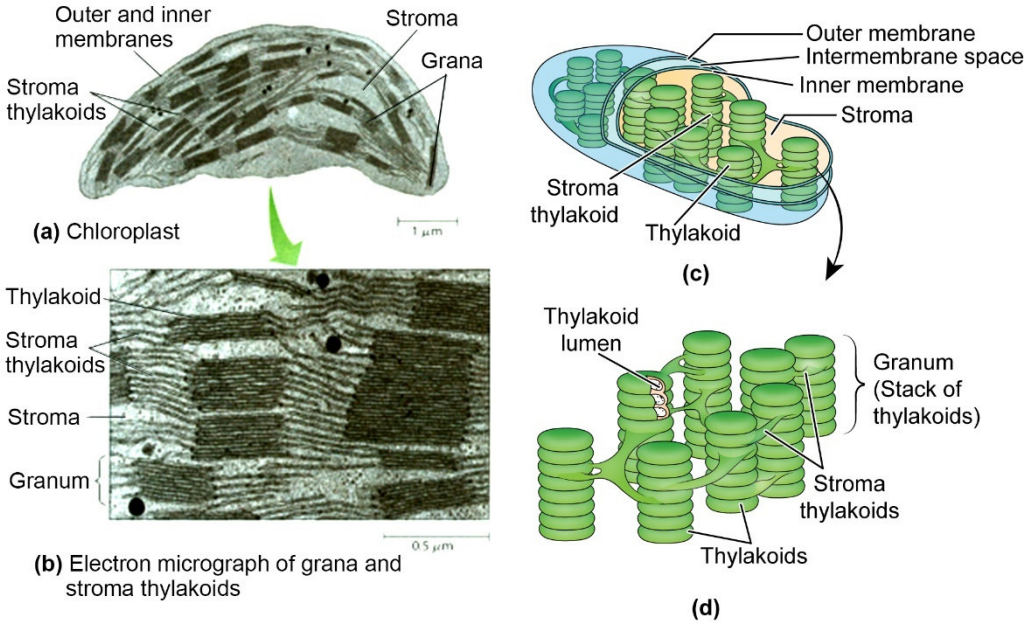


Fig. 6.14: Structural features of a chloroplast: a) chloroplast, b) EM graph of grana and stroma thylakoids; c) 3-D diagram of chloroplast; d) Grana and stroma lamellae in 3D (From Becker).

6.4.2 Photosynthetic Unit and Light Harvesting Complexes

Both the PSI and PSII reaction centres have been fractionated and crystallized to determine the precise 3-D location of the pigment molecules, using X-ray crystallography technique. Chlorophylls are associated with the accessory pigments and several specific proteins to form **pigment-protein complexes** (CP). The CPs have been electrophoretically separated and identified. A major portion of the chlorophyll molecules function as antennae which absorb the incoming photons. Since these protein-bound antennae are closely packed, they can optimize transfer of excitation energy to the adjacent pigment molecules via radiation less energy transfer/inductive resonance (Fig. 6.15). The proximity of all the antenna components ensure minimum waste of energy during its eventual arrival at the reaction centre-the site for photochemical oxidation-reduction reaction.

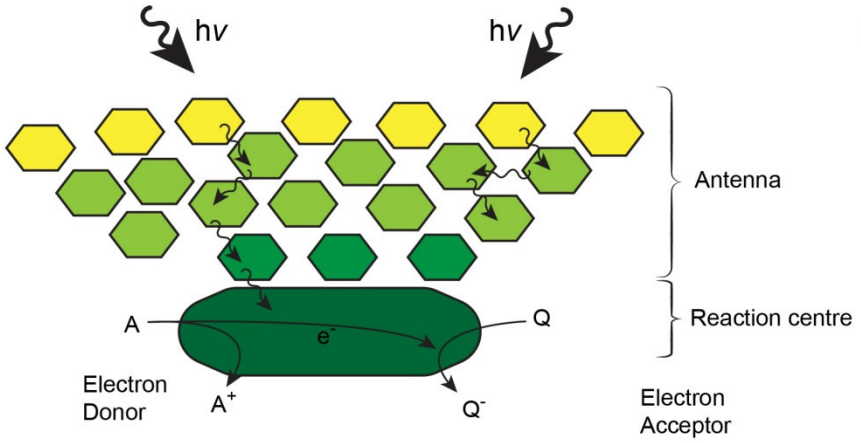


Fig. 6.15: A photosystem with chlorophyll antennae and the reaction centre. Transfer of excitation energy is by inductive resonance (From: Hopkins & Hüner).

Light harvesting complexes comprise pigment antennae along with their associated specific proteins. Complexes associated with PS I and PS II have been designated as Light Harvesting Complex (LHC I) and LHC II, respectively. LHC I contain four times more Chl_a content than that of Chl_b whereas the ratio of Chl_a to Chl_b is about 1.2 in LHC II. However, a major portion of Chl_b, about 50-60% Chl_a and xanthophylls reside in the LHC II.

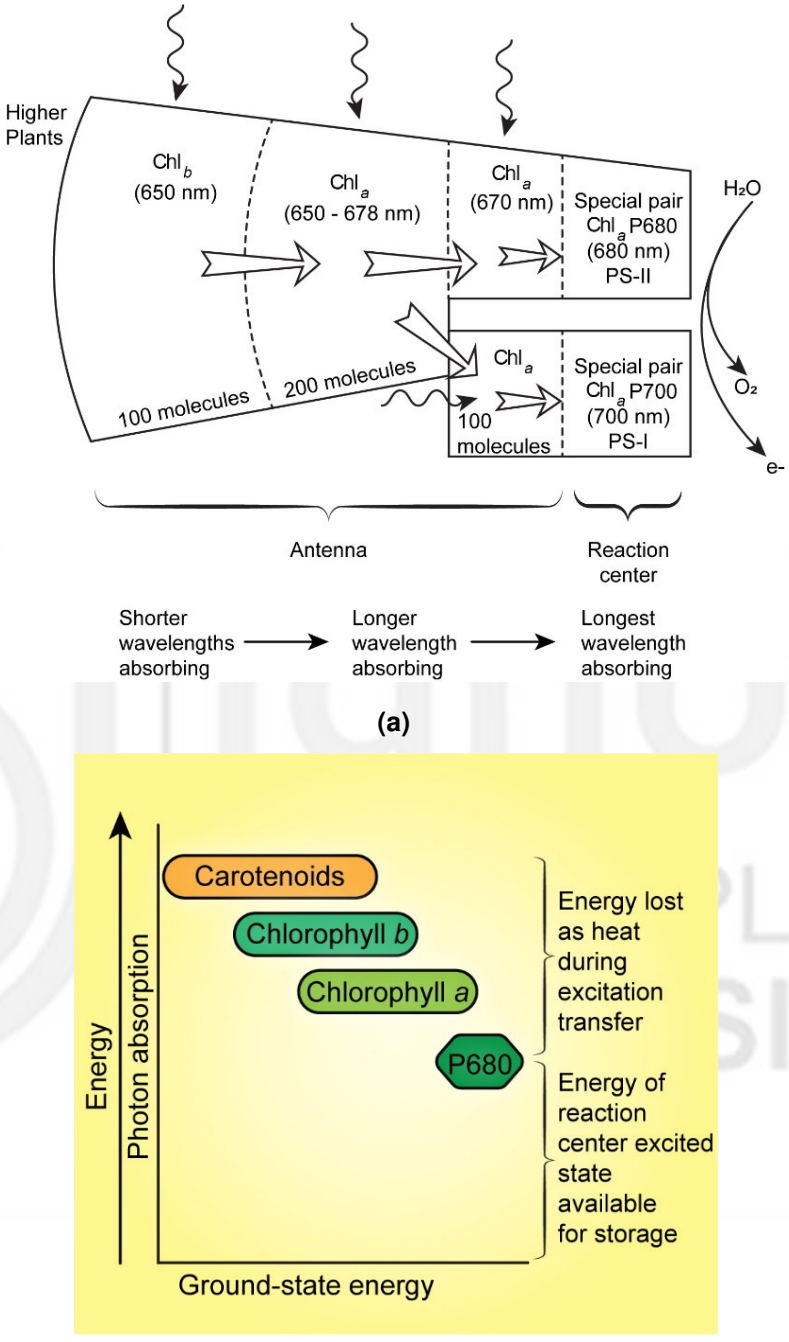


Fig. 6.16: a) Funnelling path of absorption and excitation transfer by light harveting antnnae to the reaction centres (From Lawlor); b) Loss of energy during excitation transfer(From Taiz *et al*).

The destination of the absorbed energy is the reaction centre, which comprises a Chl_a (trap) in a dimer form, along with its associated proteins and redox carriers. The reaction centre chlorophyll functions as an energy sink. This chlorophyll is unique in absorbing the lowest energy as it functions at the longest wavelengths. The reaction centre is the site where the conversion of light energy into chemical energy takes place. The reaction centre chlorophylls

of both the pigment systems I and II are designated according to their absorption maxima. Chlorophylls of PS I and PS II are called P_{700} (P=Pigment) and $P_{680,}$ respectively. Since these unique reaction centre chlorophylls absorb maximum light at 700 and 680nm respectively, they are accordingly called P_{700} and P_{680}. Presence of a single reaction centre associated with many light harvesting and core chlorophyll molecules has been well designed to provide maximum efficiency in terms of collection and utilization of light energy. It provides a funnel–like situation ensuring a continuous availability of excitation energy with an exceptionally high degree of efficiency (see Figs 6.16 a, b) so that only about 10% of the energy is lost during inductive resonance transfer.

These photsystems ,along with the cytochrome $b_6/_f$ complex are embedded in the thylakoid membranes.In addition, the CF_0-CF_1 coupling factor or ATP synthase is also present . These four complexes are represented in the form of large-sized integral proteins which span through the membrane. However, these proteins represent a **vectorial arrangement** i.e., their specific polypeptide regions are oriented either towards the stroma or the lumen.

Proteins of Complex I (LHC II—PS II) and Complex II (*Cyt $_{b/f}$*) are directed towards the lumen whereas Complex III (LHC-I--PS-I) along with Complex IV (CF_0-CF_1 coupling factor or *ATP synthase*) are directed towards the stroma.This specific orientation of the complex helps in conservation of energy through chemiosmosis as it ensures that the reduction of $NADP^+$ and the oxidation of water occur on opposite sides of the membrane. This is how a proton gradient is created in the desired direction i.e., from the lumen towards the stroma and both NADPH and ATP are produced on the stroma side. Proton gradient is created as a result of accumulation of H^+ ions in the lumen by the photolysis of H_2O by the LHC II. In addition, the *Cyt b_6/f* pump also contributes H^+. These protons enter through the proton channels of the CF_0-CF_1 coupling factor (*ATPase*) from the lumen side to the stromal side to chemo-osmotically generate ATP (see Fig. 6.17).

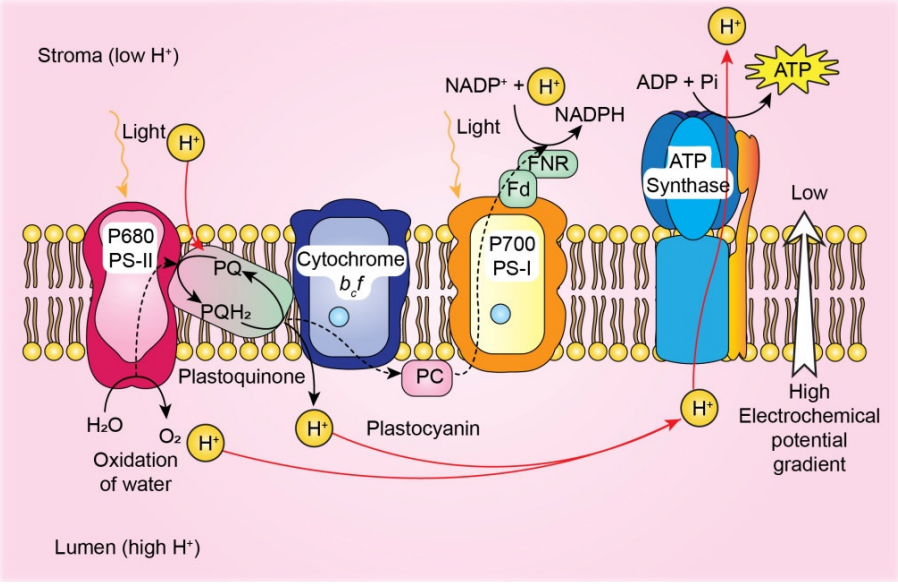


Fig. 6.17: The vectorial arrangement of four protein complexes on the thylakoid membrane showing electron and proton transport finally leading to the generation of ATP. Water is photolyzed in the lumen while NADPH and ATP are formed on the stromal side (From Taiz *et al*).

Another interesting feature of the thylakoid membranes of chloroplasts is the spatial separation/segregation in terms of distribution of the major protein complexes. This is called **lateral heterogeneity**. The two LHCs are not positioned on the membrane as depicted as static super complexes in the above figures. The PS I/LHC I complex and CF_0-CF_1*ATPase* occur exclusively in the **non-appressed regions** (where membranes are not paired to make grana) of the thylakoid. Therefore, these two will be located on the stroma lamellae and at the edges/margins of the grana lamellae stacks , as well as regions not forming the grana. On the other hand, the entire PS II/LHC II along with their associated electron transport proteins are located in the **appressed regions** of the grana thylakoids/lamellae (Fig . 6.18 a, b).

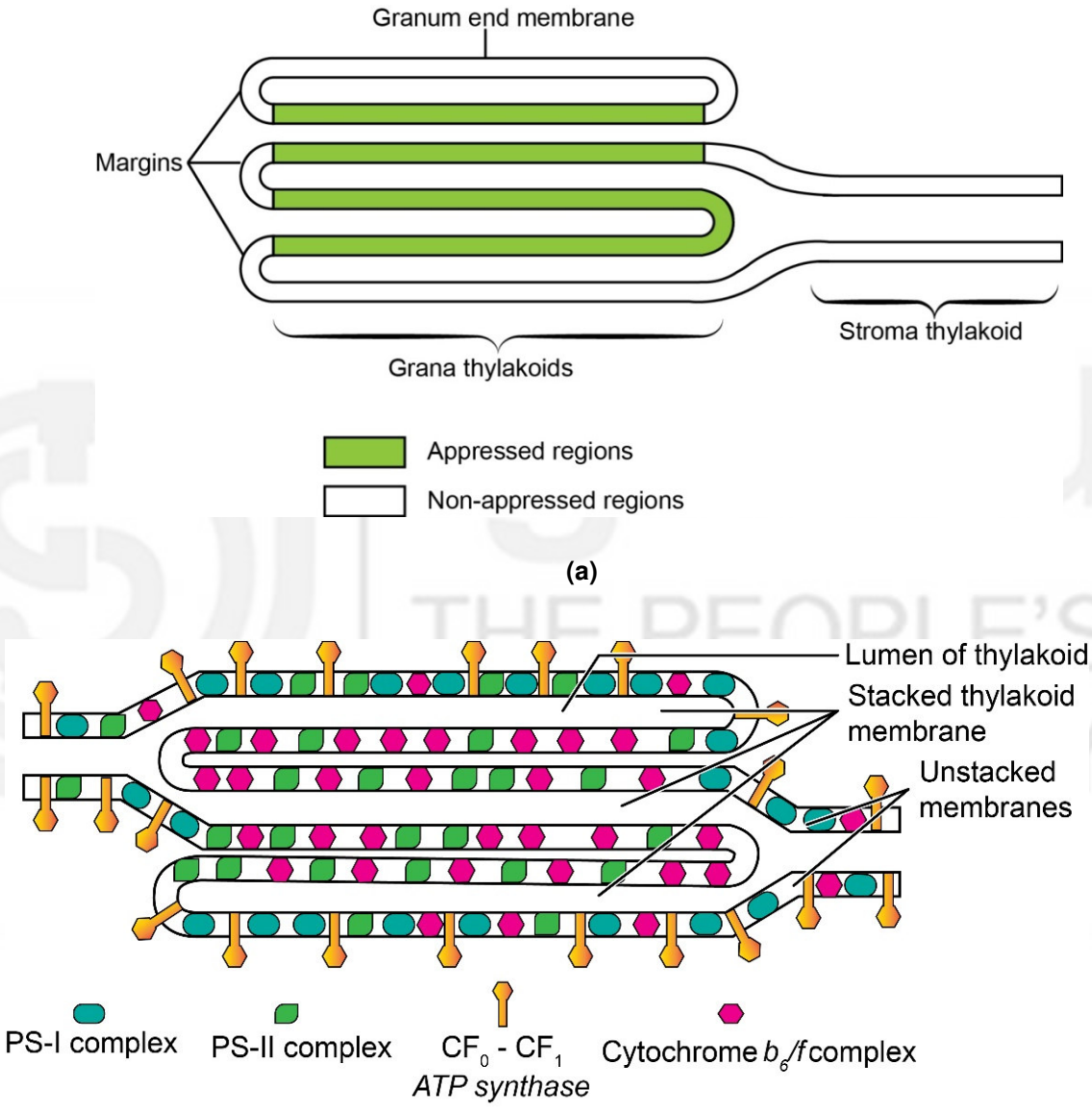


Fig. 6.18: Thylakoid membranes showing lateral homogeity in PSI and PSII distribution. a) Appressed membranes occur in the interior of grana stacks and are not exposed to the stroma; the grana margins, grana end membranes and stroma thylakoids represent the non-appressed zones; b) PSI and *ATP synthase* units are located in non-appressed (unstacked) areas while PSII complexes are located exclusively in the appressed regions. Other components like Cyt b_6/f complex, PQ and PC are uniformly distributed.

The third complex viz., Complex II or *Cyt b_6/f* complex is uniformly distributed in both appressed as well as non-appressed regions. The spatial segregation between the two photoevents suggests that one or more electron carriers in the form of **mobile carriers** will be required to deliver electrons from LHC II to LHC I, and these carriers must also be able to diffuse from the grana to the stroma and at the same time not be strictly an integral/permanent part of any of the above mentioned complexes. Three such mobile carriers that have been identified are **plastoquinone** (PQ), **plastocyanin** (PC) and **ferrodoxin.**

Plastoquinone is a hydrophobhoic molecule and diffuses within the whole diameter of the lipid matrix of the thyloakoid membrane with an incredible speed. It can traverse the said distance in less than a millisecond. Due to the exceptionally high lateral mobility (differential coefficient of 10^6 cm $^{-2}$ s^{-1}), PQ carries electrons between PS II and the cytochrome complex.

Plastocyanin is a peripheral (extrinsic) copper-protein complex which diffuses along the lumenal surface/side of the membrane. It carries electrons between the cytochrome complex and PS I/ LHC I. Ferrodoxin is again a low molecular weight iron-sulfur protein which operates on the stroma side of the thylakoid membrane. After receiving electrons from PS I , and with the assistance of Fd-NADP reductase, it reduces $NADP^+$ to NADPH.

Although, not fully understood, this large spatial segregation between the two photosystems is thought to improve the efficiency distribution between them. Interestingly, the water splitting photosystem is predominant, and the ratio of PS II to PS I in a chloroplast is usually 1.5:1. The situation is reverse in cyanobacteria where the photosynthetic lamellae possess a higher proportion of PS I than PS II. However , a change in light conditions may induce variations in this ratio.

Moreover, the stacking of the thylakoid membranes in the form of a grana is not as static as it appears from the electron micrographs. Actually the stacking pattern is quite dynamic and depends upon the concentration of K^+ and Mg^+ ions. Oxygenic photosynthetic bacteria possess only a single photocentre.

Excitation of Reaction Centre by Exciton Transfer

We have already discussed about the two photosystems working in tandem (operating in series) and the components participating in the transfer of electrons from the antenna to the reaction centre. Let us now briefly review the mechanism of electron transfer beginning from the photoexcitation of chlorophyll till the final reduction of $NADP^+$ and the chemiosmotic mechanism for ATP synthesis.

Upon photoexcitation, the antenna molecules transfer their energy to the adjacent chlorophyll molecules. In this process the donor chllorophyll comes back to its ground state while the recipient becomes excited. This process of energy transfer is called **exciton transfer**. This process continues and extends to the subsequent neighbours, till it finally excites the chlorophyll molecule at the reaction centre (trap). As a result of this energization, an electron from this reaction centre chlorophyll gets promoted to a higher – energy orbital. Since the electron here is loosely bound to the chlorophyll, it

can be easily lost, provided there is an electron acceptor positioned nearby. Loss of an electron from this reaction centre chlorophyll leaves it in an oxidized state, i.e., with a positive charge.

In the process, the electron acceptor itself becomes negatively charged. At the same time, the vacancy on an electron created in the reaction centre chlorophyll needs to be filled. The entire process would be a sheer waste if the same acceptor were to donate back the electron to the reaction centre chlorophyll. In that case, the entire extra energy would be converted into heat, which is undesirable. Instead, the lost electron is replaced by another electron from a neighbouring electron donor molecule which now becomes positively charged.

The oxidized reaction centre of the previous electron donor is now re-reduced by a secondary donor, which itself gets reduced by a tertiary donor. This sequence goes on down the electron transport chain, the components of which are arranged in a decreasng /descending manner with respect to decreasing negative redox potential (or increasing positive redox potential.

Thus, the photoexcitation of chlorophyll at the reaction centre results in electrical charge separation by which the positive and negative charges are segregated by a very rapid series of secondary chemical reactions.This initiates the subsequent oxidation-reduction reactions.

It has been calculated that the secondary reaction separates the charges very rapidly, say in nearly 20 picoseconds (1 picosecond ps= 10^{-12} s) and involves a gradual loss of energy.Thus, these reactions are irreversible.The generalized scheme for electron transfer and charge separation at the reaction centre has been depicted in Fig. 6.19.

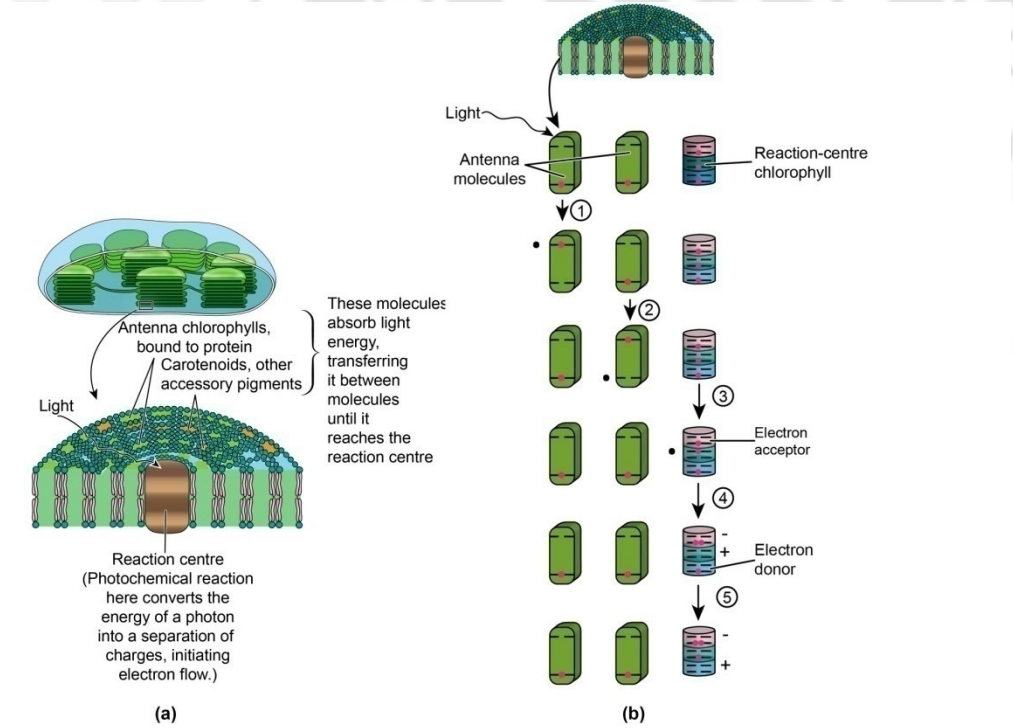


Fig. 6.19: a) A generalized scheme depicts the conversion of energy of an absorbed photon into separation of charges at the reaction centre; b) An enlarged view showing charge separation (From Nelson & Cox).

6.4.3 Non-Cyclic and Cyclic Photophosphorylation - Z- Scheme

You have already studied in the previous section that photosynthesis essentially involves a photochemical reduction of CO_2.Role of light reaction is to provide adequate energy in order to generate strong reducing equivalents like reduced $NADP^+$ ($NADPH + H^+$) by splitting water along with additional energy in the form of ATP. Both these events are accomplished with the help of the photosynthetic electron transport chain.

Our current understanding of the two photosystems working in tandem is a modern version of the **"Z" scheme** or zig-zag scheme originally proposed by Robin Hill (see Fig. 6.22). In both the photosystems, the electron expelled from the chlorophyll after its photoexcitation travels through several electron carriers and has two options. The electrons are either **cycled back** or **get consumed** in reducing $NADP^+$ to $NADPH + H^+$. At certain places during its transport, the extra energy carried by this electron is utilized/used in the formation of ATP. Formation of ATP form ADP and *Pi* in photosynthesis is called **photophosphorylation**.

Here all the reactions are arranged vertically at their midpoint redox potentials (i.e., against the redox scale) to illustrate as to how an electron from a lower energy level (as in water) is being raised to a high energy state (as in NADPH). The two photosystems are connected by a "**dark bridge**", in which, for a short distance, electrons move from one carrier to another without any need of light. This is made possible because in each photochemical act, the electron is excited to a level slightly more than required for the next photosystem to be excited. It is believed that the downhill journey of electrons provides a mechanism of synthesis of extra ATP molecules. Over the years a lot many new carriers and the intermediates have been discovered, which has given us a much-detailed understanding/insight of the zig-zag journey (in the shape of the letter **Z** of electrons and their carriers positioned between PS II and PS I. Also, the four participating major protein complexes show a distinct vectorial arrangement on the thylakoid membrane (described in the earlier section 6.4.2).The overall process carried out by these complexes involves:

a) Oxidation of water into O_2 by the PS II and the release of protons into the thylakoid lumen.

b) Oxidation of plastohydroquinone (PQH_2) (which were earlier reduced by PS II), by cytochrome $_{b6/f}$ and their subsequent delivery of electrons to PS I. The oxidation of PQH_2 is coupled to proton transfer from stroma into the lumen. Excess of protons generates a proton motive force.

c) Reduction of $NADP^+$ to $NADPH + H^+$by PS I in the stroma involving the action of ferredoxin (*fd*) and the flavoprotein *ferredoxin-NADP reductase* (*FNR*).

d) Diffusion of protons through proton channels from the lumen into the stroma prompting ATP production by *ATP synthase*.

Stark-Einstein Law

German-born physicists Johannes Stark and Albert Einstein, formulated the Law of Photochemical equivalence to explain the reactions induced by light. According to this Law, for every quantum of radiation (a unit of electromagnetic radiation) absorbed, one molecule of the substance reacts.

1 quantum = $h \times \nu$

h= Planck's Constant

ν =Frequency of radiation (Greek letter nu).

Since we express the quatitative measure of substances in terms of gram moles (one gram mole comprises $6.022140857 \times 10^{23}$ molecules, i.e., the Avogadro's number). Thus, the Stark-Einstein Law can be restated as : for every mole of a substance that reacts, $6.022140857 \times 10^{23}$ quanta of light are absorbed.

Photolysis of water and PS II

Electron transport begins when the excitation energy reaches the PS II reaction centre trap, P_{680} (Fig. 6.20). The photoexcited form of P_{680} is designated as P_{680^*} (* =excited). Since PS II is a strong oxidant, it ensures the rapid compensation/replacement of electron lost by the photo oxidized P_{680}. This is accomplished by photolysis (splitting) of water and extracting electrons from it. PS II is associated with enzymes associated with water splitting complex, which oxidizes water by the following reaction.

Box 6.5: Oxygen-Evolving Complex

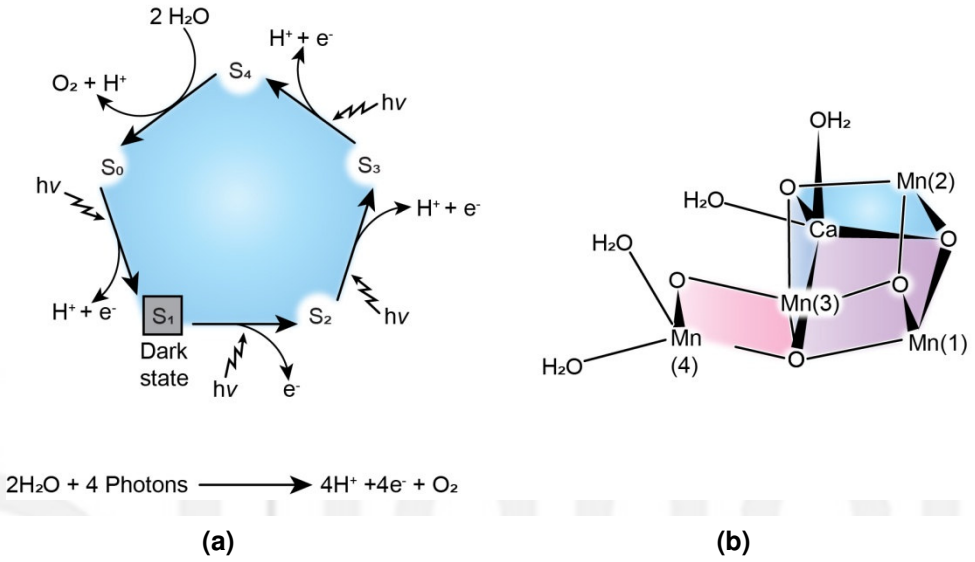


Fig. 6.20: a) The oxygen evolving complex; b) Structure of water evolving complex.

A small complex of proteins called the **Oxygen-Evolving Complex** (OEC), located on the lumen side of the thylakoid membrane, is associated with four Mn and Cl ions.OEC is responsible for photooxidative splitting of water and evolution of molecular oxygen.

$$4H_2O \longrightarrow 4H^+ + 4(OH^-)$$

$$4(OH^-) \longrightarrow 2\,H_2O + O_2 + 4e^-$$

$$2\,H_2O \longrightarrow 4H^+ + O_2 + 4e^-$$

Liberation of one O_2 from $2H_2O$ involves release of four protons. Thus, PS II must utilize the energy of four protons for the evolution of one molecule of O_2. Photooxidation of P_{680^*} results in the *charge separation* yielding P_{680}^+ and pheophytin $Pheo^-$. In other words, P_{680^*} rapidly transfers its electron within picoseconds to Pheo and becomes P_{680}^+. Pheophytin is a form of Chl_a where two hydrogens replace the Mg core, and after receiving an electron becomes $Pheo^-$ (Fig. 6.21).

Stark-Einstein Law states that energy of one photon is responsible for the release of one electron. So, this step of charge separation between P_{680}^+ and $Pheo^-$ is the actual **photochemical change** where light energy is converted into chemical energy-light energy being stored in the form of redox potential energy.

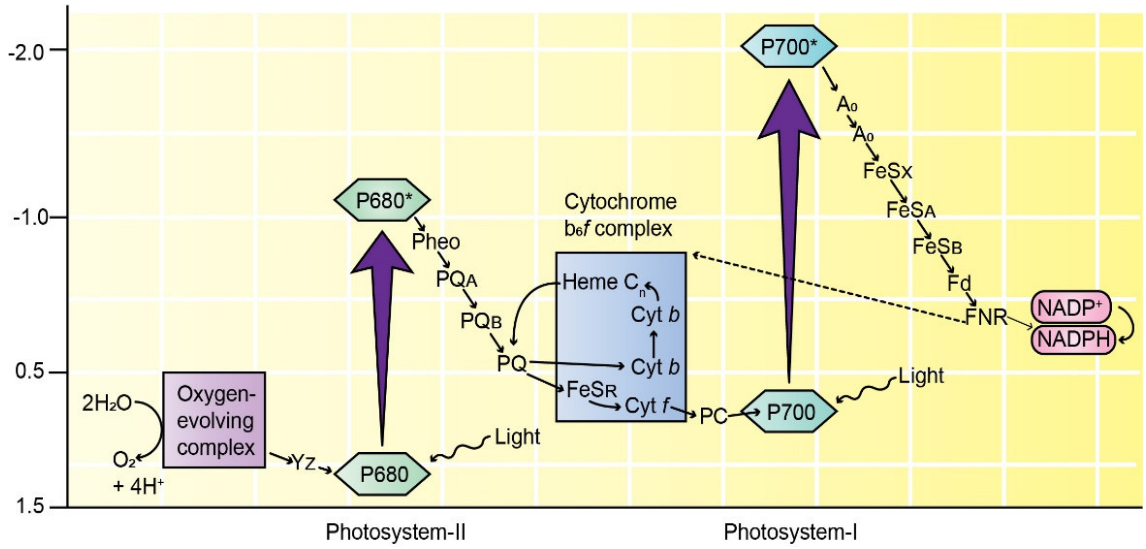


Fig. 6.21: Detailed Z-scheme for O_2-producing photosynthetic organisms (From Taiz *et al*).

Two membrane bounded proteins **D_1** and **D_2** prevent the wasteful charge recombination between P_{680}^+ and $Pheo^-$, by rapidly passing the electrons to the quinone electron acceptors **Q_A** forming **$P_{680}^+ Pheo\ Q_A^-$** . Later two electrons are transferred to **Q_B** forming **Q_B^{2-}** which finally receives two protons from the stroma side of the medium to form a fully reduced plastohydroquinone (**PQH_2**).

After its release from PS II, the plastoquinone exhibits a lateral diffusion through the membranes to finally reach the **Cytochrome *b_{6f}* complex**. This complex is represented by a large multisubunit protein containing many prosthetic groups. This membrane spanning redox complex has the following constituents:

a) Cytochrome $_{b6}$ (***Cyt_{b6}***), a b-type haem,

b) Cytochrome $_f$ (***Cyt_f***), a c-type haem, and

c) A redox component Rieske iron-sulfur protein (**FeS_R**).

Both the Rieske FeS protein and Cyt $_f$ are located on the luminal face of the thylakoid membrane. The movement of electrons and protons through the Cyt $_{b6/f}$ complex has been convincingly explained by the **Q Cycle** (see Fig.6.23) which highlights some differences between the cyclic and non-cyclic (linear type) electron and proton transfer.

In the non-cyclic/linear chain (Figs. 6.21, 6.22 a), the PQH_2 produced by the action of PS II first gets oxidized near the luminal side of the complex. Thereafter, it transfers its two electrons to the Rieske iron-sulfur protein (**FeS_R**) and one of the b-type cytochromes. Two protons are simultaneously expelled into the lumen. Electrons from the FeS_R get transferred to Cyt $_f$, and finally to Plastocyanin, which eventually reduces P_{700} of PS I. An electron is transferred from the reduced *b* type cytochrome to the other *b*-type cytochrome, and ultimately reduces PQ to plastosemiquinone (PQ^-).

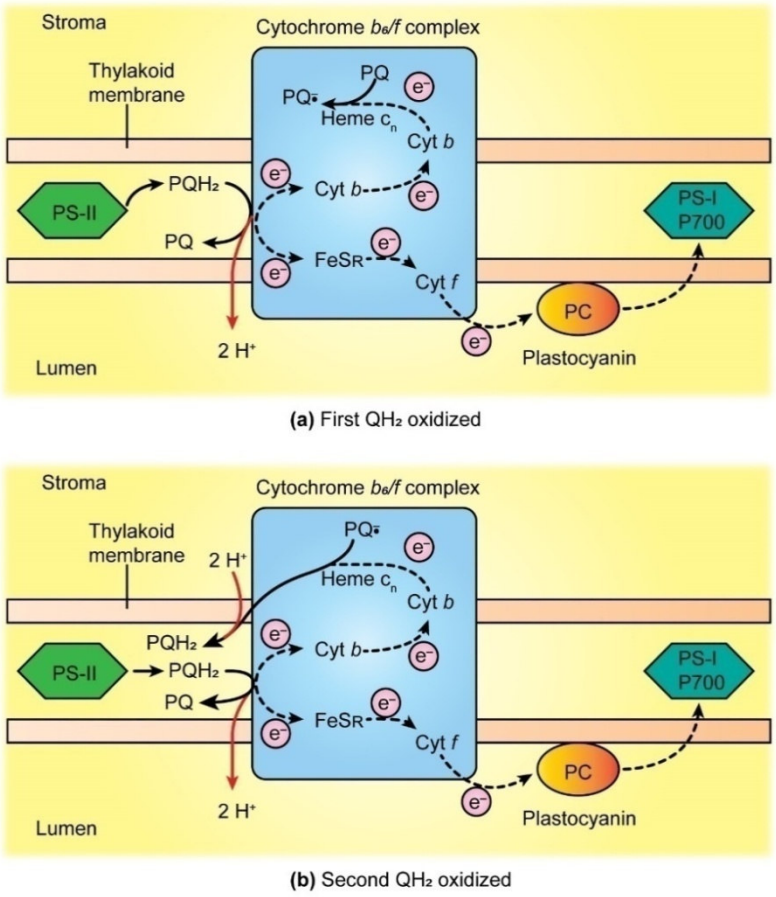


Fig. 6.22: a) non-cyclic/linear chain process of transfer of electrons/proton transfer in the cytochrome b_6f complex; b) The cyclic transfer of electrons (From Taiz *et al*).

The electron flow connecting the two photosystems along with unequal H^+ concentration on the two faces/sides creates an electrochemical potential across the membrane which energizes the machinery to synthesize ATP by chemiosmosis (Fig. 6.23).

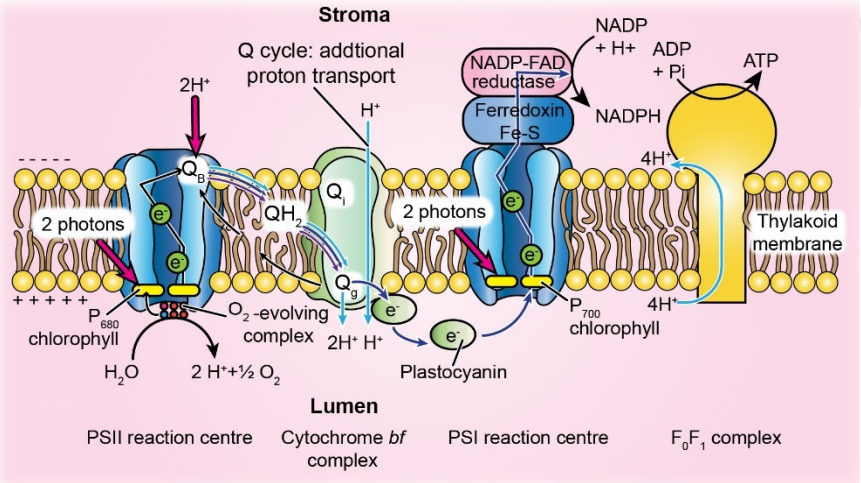


Fig. 6.23: Linear/noncyclic flow of electrons requiring the participation of both PS I and PS II. Electrons removed from H_2O are transferred to P_{680}*. The cytochrome$_{bf}$ complex accepts electrons from QH_2 and releases two protons into the lumen. Operation of the Q cycle translocates additional protons across the membrane to the lumen, resulting in the generation of a proton-motive force. Electrons released from the light excited P_{700} molecules move to the stromal surface via a series of carriers to $NADP^+$ to form NADPH.The proton-motive force powers the generation of ATP at the F_0F_1 complex (After Lodish *et. al*).

In the cyclic type of transfer (Fig 6.22b), the PQH_2 is first oxidized releasing $2H^+$ and $2e^-$. One of the two electrons passes to the Rieske iron-sulfur protein(**FeS_R**), thereafter to the haem of Cyt $_f$, and finally being picked up by the copper binding protein **Plastocyanin,** which reduces P_{700} of PS I. The second electron passes through two *b*-type cytochromes finally reducing plastosemiquinone (Q^-.) to plastohydroquinone (QH_2). During this process, two protons are taken up from the stroma per one electron delivered to P_{700}. Thus, for every 2 electrons delivered, 4 protons are transported across the membrane which get accumulated in the lumen.

Located at different sites of the thylakoid, the two photosystems require a **mobile element** which can deliver the electrons from PS II to PS I. Since the membrane spanning **Cyt $_{b6/f}$ complex** is too large to do this job, it is the **plastocyanin(PC)** and **plastoquinone (PQ)** which serve as electron delivery agents(carriers). PC is a small, water soluble, copper containing peripheral protein that can diffuse freely along the luminal surface of the thylakoid membrane and transfers electrons from Cyt $_{b6/f}$ to P_{700} .

The PS I reaction centre also occurs in the form of a large multi –subunit complex comprising 100 chlorophylls as its integral part in its core antenna, which along with P_{700} are bound to the proteins **$P_{sa}A$** and **$P_{sa}B$** (Fig. 6.24). In addition, there are electron transfer cofactors. All these electron acceptors are strong reducing agents. The electron carriers in this complex include a chlorophyll molecule, along with another quinone, **phylloquinone** (Vit K_1).

Quantum requirement for oxygen evolved : How many photons are required to evolve one O_2 molecule? We have seen that two excitations (one each at PS II and PS I) are required to evolve one O_2 molecule from H_2O to $NADP^+$. Since photolysis of water and evolution of one O_2 molecule at PS II by $P680^+$ generates 4 electrons, their transport from PS II to PS I would require 4 x2 = 8 photons. Thus, the minimum theoretical QR for O_2 evolution is 8 photons/O_2 molecule. Likewise, the theoretical quantum yield (QY) (=1/QR) would be 1/8 or 0.125 mol of oxygen evolved per photon absorbed.

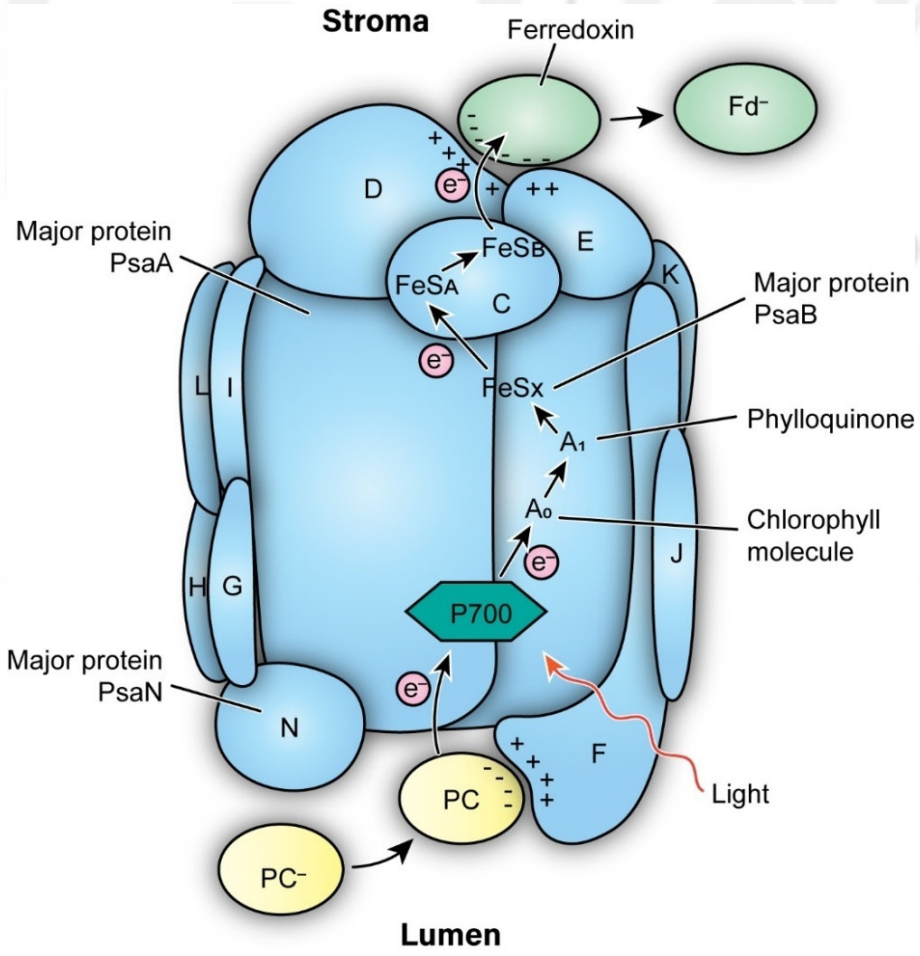


Fig. 6.24: Structural model of the PS I reaction centre with two major core proteins PsaA and PsaB along with minor proteins (labelled as C to N). Electrons are transferred from PC to the soluble iron-sulfur protein ferredoxin via P_{700}, phylloquinone, and many Fe-S centres (From Taiz *et al*).

At the same time, light driven charge separation also occurs at the PS I where the excitation energy is transported to the reaction centre P_{700}. The redox potential of P_{700} changes dramatically from +0.4eV to about -0.6eV, giving rise to the excited form P_{700^*}. This photoexcited form now gets photo oxidized to P_{700}^+ (i.e., there is an electron deficiency or 'hole' in the P_{700} molecule) by a chlorophyll acceptor A_o. The electron then passes through a quinone A_1, and subsequently through a series of membrane-bounded Fe-S proteins (viz., FeS_X, FeS_A and FeS_B), and finally on the stroma side of the membrane to ferredoxin (Fd). Fd is a Fe-S protein soluble in the stroma and reduces $NADP^+$ with the help of a membrane-associated enzyme *ferredoxin-NADP reductase* to yield NADPH + H^+, thus completing non-cyclic electron transport. The electron-deficiency in P_{700}^+ ('hole') is compensated/satisfied by withdrawing an electron from reduced PC, the initial source of which was the oxidation of water at PS II. Thus, the electrons pass continuously between water and $NADP^+$ through the coordinated functioning of the two photosystems and the cytochrome complex (Fig. 6.24).

Cyclic Photophosphorylation

Under certain special conditions, cyclic flow of electrons occurs, which does not conform to the Z-scheme. If PS II is absent and there is low availability of $NADP^+$ in the medium, or even if monochromatic light >680nm is provided under experimental conditions-only PS I operates. Under such conditions water is not photolyzed and therefore, no O_2 is evolved (anoxygenic). The electrons flow from the photoexcited P_{700} via A_0 and A_1, quinones, the iron sulfur proteins FeS_x, FeS_A and FeS_B, ferredoxin, $Cyt_{b6/f}$ complex, PC, and back to P_{700}.

$$2Chl + 2hv \longrightarrow 2Chl^*$$

$$2Chl^* \longrightarrow 2Chl^+ + 2e^- \text{ (electrons)}$$

$$2C + 2e^- \longrightarrow 2X^-$$

$$2X^- + 2Fe^{3+} Cyt_1 \longrightarrow 2X + Fe^{2+} Cyt_1$$

$$2Fe^{2+} Cyt_1 + ADP + Pi + 2Fe^{3+} Cyt2 \longrightarrow 2Fe^{3+} Cyt_1 + 2Fe^{2+} Cyt_2 + ATP$$

$$2Fe^{2} Cyt_2 + 2Chl + \longrightarrow 2Fe^{3+} Cyt_2 + 2Chl$$

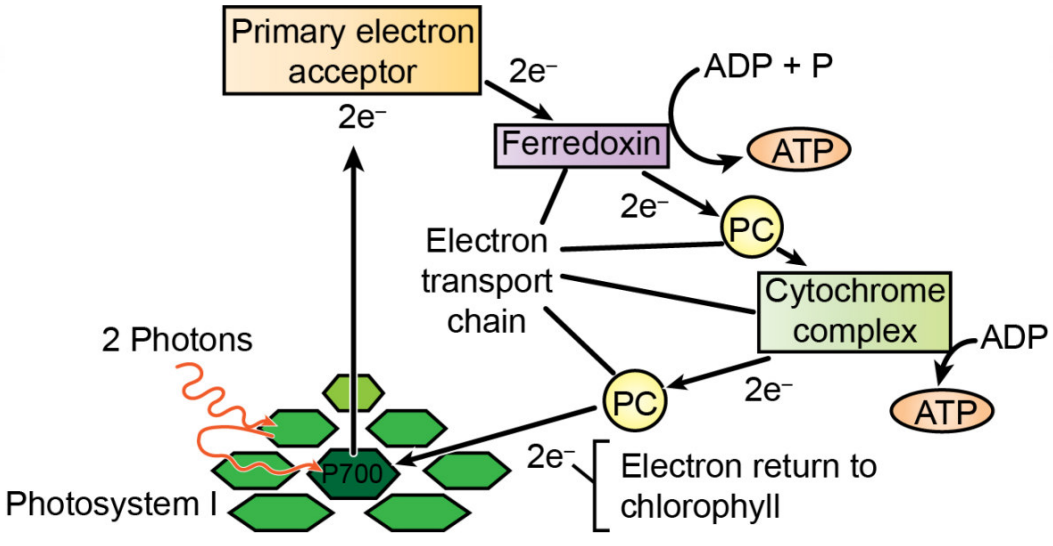


Fig. 6.25: Reactions occurring in cyclic photophosphorylation.

This process also involves proton pumping into the lumen along the pH gradient, which is used for ATP synthesis --steps between *fd* and Cyt$_{b6}$ and/or between Cyt $_{b6}$ and Cyt $_{f}$ (Fig. 6.25). Although the molecular mechanism of cyclic electron flow which generates only ATP, but no NADPH is not very well understood, it certainly accounts for retardation in CO_2 uptake (i.e., red drop). On the contrary, running of both PS I and PS II would result in photosynthetic rate enhancement or Emerson effect. Although cyclic electron chain may not be a part of normal photosynthesis, it is believed to serve as a source of ATP for other biosynthetic processes like the synthesis of nucleic acids, proteins, and pigments. Cyclic flow of electrons /cyclic photophosphorylation is not inhibited by DCMU (a known inhibitor of non-cyclic transport).We shall study more about these inhibitors at the end of this unit.

Proton Transport and Photophosphorylation

Light driven ATP synthesis is called **photophosphorylation.** ATP is required not only to fix atmospheric CO_2 in dark reaction but also to energize synthesis of amino acids, starch, and fatty acids. In addition, the active transport of proteins and other metabolites across the chloroplast envelope also requires ATP. Electron transport where both the photosystems are operative and water continuously supplies electrons to help in the formation of NADPH is called **linear or non-cyclic**, and the ATP is generated during this process which is termed **non-cyclic photophosphorylation**. Although both the PS I and PS II are located on different regions of the thylakoid, yet they are linked by the Z-scheme. On the other hand, ATP synthesis during cyclic electron transfer where PS I transfer electrons independently of PS II is called **cyclic photophosphorylation**.

Box 6.6 : Pseudocyclic photophosphorylation.

A third mechanism by which ATP can be generated is the "oxygen –dependent FMN catalyzed photophosphorylation" or **pseudocyclic photophosphorylation** (Arnon et al 1954)**.**This process takes place even in the absence of CO_2 and $NADP^+$. Flavin Mononucleotide (FMN) is an auto oxidizable hydrogen acceptor which breaks water molecule in the presence of chlorophyll and light to produce $FMNH_2$ and oxygen. ATP can be generated from ADP and inorganic phosphate simultaneously. During this process FMN is reduced and oxygen is evolved. $FMNH_2$ is oxidized back by oxygen to yield FMN and water (Fig. 6.26).

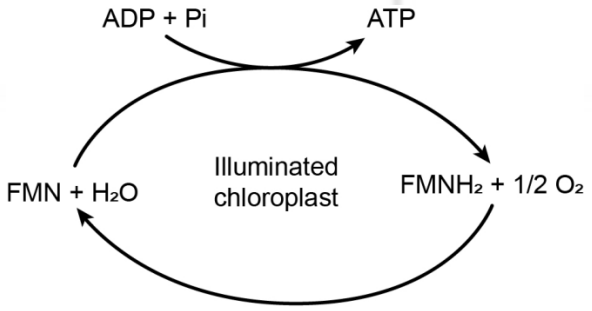


Fig. 6.26: Pseudocyclic Photophosphorylation (oxygen-dependent FMN-catalyzed photophosphorylation.

Non-cyclic photophosphorylation generates both ATP and $NADPH^+ + H^+$. (**reducing power**) which together constitute the aim of light reaction – **Assimilatory Power**. Assimilatory power is used in reducing CO_2 to carbohydrates in the Calvin cycle. On the other hand, the cyclic photophosphorylation generates only ATP but no reducing power. It is

believed that the metabolic activities in the chloroplast are regulated/maintained by the ratio of ATP/ $NADPH^+$, which in turn, depends upon the switchover between non-cyclic and cyclic photophosphorylation.

To summarize, for every oxygen molecule evolved in light reaction, 3 molecules of ATP and 2 molecules of NADPH + H^+ are produced. Since a total of 6 molecules of O_2 are evolved in C_3 plants, a total of 18 ATP and 12 molecules of NADPH + H^+ formed as depicted in the equation given below:

There are two main mechanisms by which protons are accumulated in the lumen for ATP production.

a) Oxidation of water deposits **2** protons into the lumen per H_2O molecule oxidized, and

b) A PQ-cytochrome proton pump. The movement of protons across the membrane is best explained by the **Q-Cycle**,originally proposed by P.Mitchell, which suggests that for each pair of electrons passing from PQ through the Rieske Fe-S centre and from Cyt_f to PC, **four** protons are translocated from the stroma into the thylakoid lumen. In accordance with this hypothesis, each pair of electrons will contribute **six** protons in all to the gradient (**4** from Q Cycle and **2** from oxidation of water) while passing through non-cyclic electron transport (i.e., from H_2O to $NADP^+$). Likewise, the number of protons transferred per pair of electrons in cyclic electron transport is 4.

Summary of Light Reaction :

$$24H_2O \longrightarrow 24OH^- + 24H^+$$

$$24OH^- \longrightarrow 24e^- + 24OH$$

$$24OH \longrightarrow 12H_2O + 6O_2$$

$$18\ ADP + 18Pi \longrightarrow 18\ ATP$$

$$12NADP + 24e^- + 24H^+ \longrightarrow 12\ NADPH + 12\ H^+$$

$$24H_2O + 18ADP + 18Pi + 12NADP \longrightarrow 12H_2O + 18ATP + 12NADPH + 12H^+$$

SAQ 3

Fill in the blanks with appropriate terms/words:

a) ……. forms the reaction centre for Photosystem I.

b) PS II is a strong …… while PS I is a strong …….. .

c) $Mn^{2+}$and ……..…… ions are essential forphotolysis of water at photosystem …… .

d) Both the photosystems PS I and PS II are interconnected by an integral protein complex called …… .

e) Energy is transferred from the antenna molecules to the reaction centre by the process of …… .

6.5 CHEMIOSMOTIC COUPLING AND ATP GENERATION

Capture of light energy results in the formation of ATP and NADPH. Light-dependent ATP synthesis is called photophosphorylation.Discovered by Arnon et al. in the 1950s, this process requires electron flow. ATP generation has been best explained by the **Chemiosmotic coupling mechanism**—proposed by Peter Mitchell in the 1960s.

This Chemiosmotically coupled ATP synthesis mechanism seems to operate in the chloroplasts, mitochondria and also in bacteria.There are differences in ion concentration and in eleterical potential across membranes which provides free energy that can be utilized by the cell. Such a source of energy can also be provided by the unequal chemical potential on both sides of the membrane. Since the asymmetric nature of the thylakoid membrane allows free movement of protons only in one direction, i.e., from the stroma into the lumen and **not vice-versa,** more H^+ keep on accumulating in the lumen.

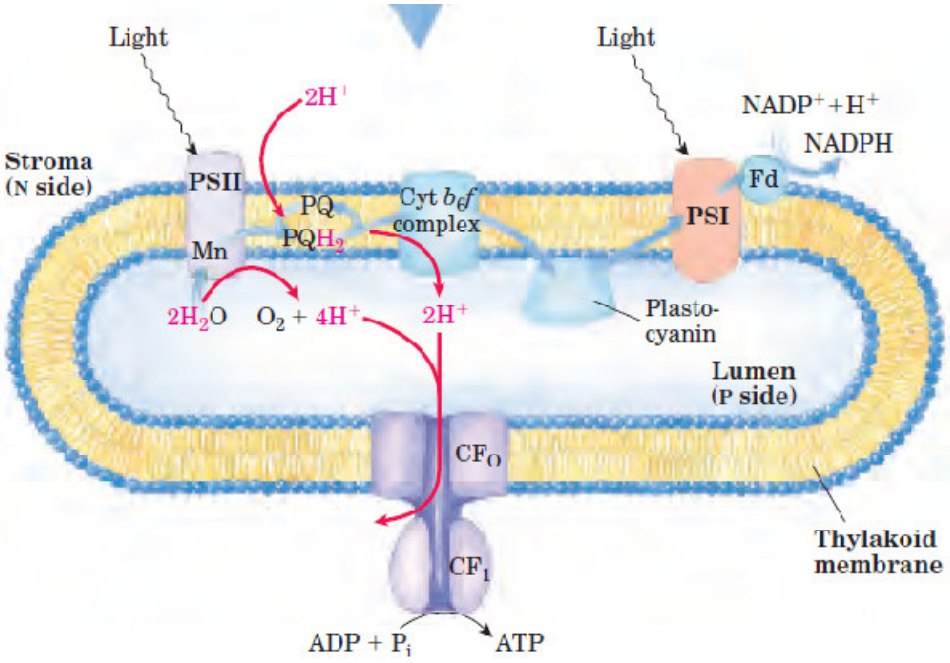
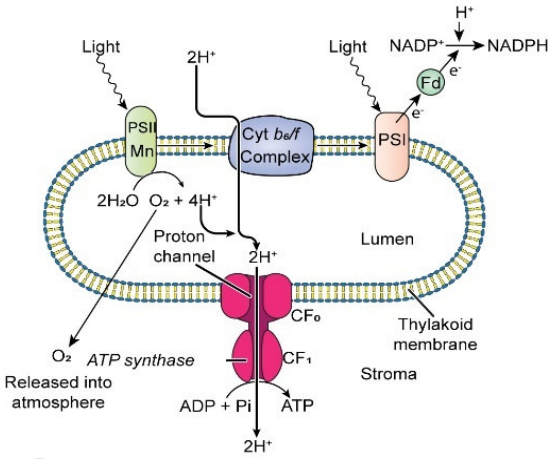


Fig. 6.27: Proton and electron flow in the thylakoids. Whereas the electrons move from water through PS II, chain of intermediate carriers, PS I, to $NADP^+$ (depicted by blue arrows), the protons (depicted by red arrows) get continuously pumped into the thylakoid lumen by carriers linking the two photosystems. The protons re-enter the stroma through proton channels of F_0 (CF_0) subunit along a proton gradient and the F_1 subunit of ATP synthase catalysis ATP synthesis (From Nelson& Cox).

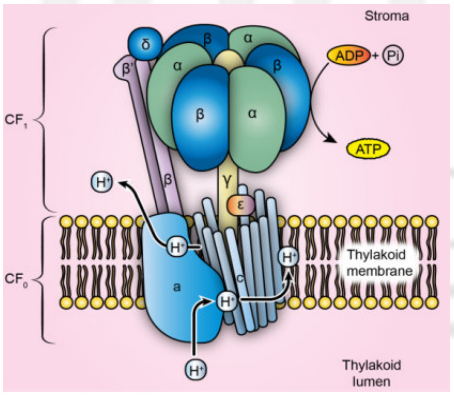
As a result, the lumen becomes much more acidic (higher H^+) than the stroma, which is more alkaline. This creates a proton (H^+) gradient across the thylakoid membrane (Fig.6.27).The total energy available for ATP synthesis was termed **proton motive force** by P. Mitchell. The proton gradient operates across the thylakoid membrane from the lumen (inside) to the stroma (outside) in a chloroplast. In contrast, the proton gradient in mitochondria occurs in an opposite direction i.e., from the inter mitochondrial space (outside) to the matrix (inside) as seen as discussed in Unit 10.

Box 6.7 : ATP Synthesis.

ATP synthesis takes place with the help of *ATP synthase* (also called $CF_o–CF_1$). This chloroplast $CF_o–CF_1$ *synthase* is a multi-subunit complex. As described earlier, the *ATP synthase* complexes are preferentially localized in the stroma lamellae in the non-appressed regions of the thylakoids. The hydrophobic part CF_o is embedded in the membrane while the other portion CF_1 projects out into the stroma (Fig. 6.28a). Differences in H^+ concentrations of 1,000 to 2000 fold may exist across the thylakoid membrane. This corresponds to a pH gradient (delta pH) of 3-4 units. The proton motive force urges the protons to move towards the stroma along a proton gradient through **proton channels** located in the CF_o, and pass through the catalytic sites of CF_1 where energy is released for the synthesis of ATP.



(a)



(b)

Fig. 6.28: a) Proton gradient and ATP formation on the stromal face of the thylakoids; b) A model of chloroplast F_1F_o*ATP synthase* enzyme showing multiunits and path of protons. The protons are transported by rotation of the c polypeptide and ejected on the stroma side (from Taiz *et al*).

Most of the portion of CF_o is thought to rotate like a motor during the proton transport. Each rotation of this enzyme generates three ATP molecules (see Fig. 6.28 b).

Herbicides: Inhibitors of Photosynthetic Electron Transport

Different classes of herbicides act by either blocking the biosynthesis of vital metabolites like lipids,carotenoids, and amino acids, or by interfering in the cell division processes. Some herbicides are very effective killers as they block the electron flow during photosynthesis. For example, a urea-derivative **DCMU** (dichlorophenyl-dimethylurea)--also called diuron, **CMU** (chlorophenyl-dimethylurea), **atrazine** (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine), and **paraquat** (methyl viologen) are some of the widely used herbicides.

DCMU is an inhibitor of non-cyclic photophosphorylation as it blocks the flow/transfer of electrons from the PS II to PS I. It competes with the binding site of PQ, which is normally occupied by PQ_B. As a result of this interference with the binding of PQ to its assigned site, the electron flow from the PQ_A to PQ_B is blocked (Fig.6.29). The triazine herbicides (**atrazine**, **triazine** and **simazine**) also have a similar mode of action. **Paraquat** belongs to the category of bipyridinium viologen dyes. It blocks the flow of electrons to $NADP^+$ at PS I by accepting itself the electrons from the early acceptors of PS I thereby preventing them to reach $NADP^+$. Hence NADPH formation is blocked (Fig. 6.29). In addition, paraquat also produces superoxide radicals causing additional toxicity/damage to plants by inactivating chlorophyll and lipid peroxidation of chloroplast membranes.

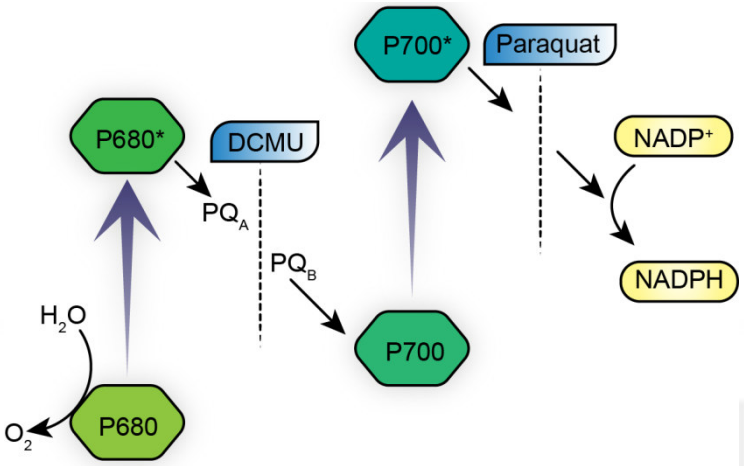


Fig 6.29: Mechanism of action of DCMU and Paraquat: DCMU competes for the binding site of plastoquinone and blocks electron flow at the PQ acceptors of PS II. Paraquat accepts electrons from early acceptors of PS I thus, preventing them to reach $NADP^+$ (From Taiz *et al*).

SAQ 4

Match the components of ATP generation in Column 1 with those in column 2.

	Column 1		Column 2
a)	DCMU	i)	Prevents electrons to reach $NADP^+$
b)	Proton-motive force	ii)	Rotary motion
c)	Photosynthetic units	iii)	Peter Mitchell
d)	Paraquat	iv)	Electron flow blockadeat PQ
e)	CF_0	v)	Quantasomes

6.6 THE DARK REACTION – PHOTOSYNTHETIC CARBON REDUCTION (C_3) CYCLE

You have studied in the above sections as to how do the photosynthetic organisms, both prokaryotes and eukaryotes generate ATP by light-dependent reactions to provide the complete assimilatory power for the next important step (phase), i.e., **CO_2 fixation** (also called carbon fixation/CO_2 assimilation) (Fig 6.30). The incorporation of CO_2 into carbohydrates takes place in the

stroma of the chloroplast where all the necessary enzymes are present. Since the set of reactions of carbon fixation are temperature sensitive and independent of light, they are popularly called as **Dark Reaction** (It is worth remembering that they were also called Blackman Reactions).

6.6.1 Calvin – Benson Cycle

The process of CO_2 fixation was elucidated by Melvin Calvin, Andrew Benson and James A. Bassham at the University of California, Berkley. Prof. Calvin was awarded the Nobel Prize in 1961 for unraveling the minute details of the sequence of reactions of what is now **Calvin- Benson Cycle** or simply as **Calvin Cycle.** It is also called **Photosynthetic Carbon Reduction Cycle (PCR Cycle).**

Calvin's success can also be attributed to the availability of long-lived radioisotope of carbon ^{14}C (half- life of nearly 5720 years) by **Ruben** and **Kamen**. In his Nobel address in 1961, Calvin himself acknowledged that "…. the discovery of the long-lived isotope of carbon, ^{14}C, by Samuel Ruben and Martin Kamen in 1940 provided the ideal tool for the tracing of the route along which carbon dioxide travels on its way to carbohydrates".

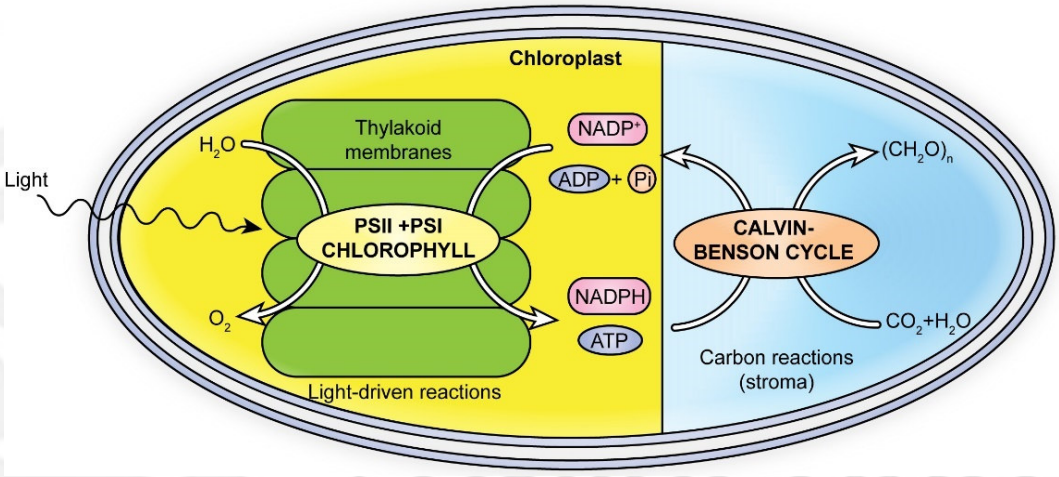


Fig. 6.30: A generalized scheme of reactions occurring in the chloroplast. Products of the light reaction (ATP and NADPH) are utilized in the Calvin-Benson (C_3 Cycle) where CO_2 is fixed, and sugars are formed (After Taiz*et al*).

6.6.2 Classical Experiments of Calvin

A suspension of *Chlorella* was fed with radioactive sodium bicarbonate ($NaH^{14}CO_3$), which results in the formation of $^{14}CO_2$. The suspension was placed in a glass container that looked like a "Lollipop" and then the contents were extracted in hot alcohol after brief period of illumination and concentrated by evaporation. Its constituents were then separated by chromatography, and those that were radioactive identified by the technique of radioautography. Once a certain spot was identified on the autoradiogram, one could go back to the original chromatogram, cut it out, elute the compound and determine its structure and investigate its properties (see Fig. 6.31).

Being a chemist, Calvin went a step further and employed a technique of "**molecular dissection**", whereby the terminal carbon atom in a radioactive intermediate was oxidized to carbon dioxide which was trapped as $BaCO_3$ by

passing it through a barium hydroxide solution. The radioactivity in the carbon atom was determined by the Geiger-Müller counter. Calvin could thus not only tell whether a particular carbon atom was radioactive, but also to what extent in each sample. It was found that the first compound to become radioactive, already within a few seconds of photosynthesis, was the three-carbon compound ***3-phosphogycerate* (*3-PGA*)**. Studies made by Calvin on *Scenedesmus* also confirmed this. Later, **Rosenberg & Gaffron** (1950) found similar results in the green tissues of higher plants like soybean, Geranium, and barley leaves.

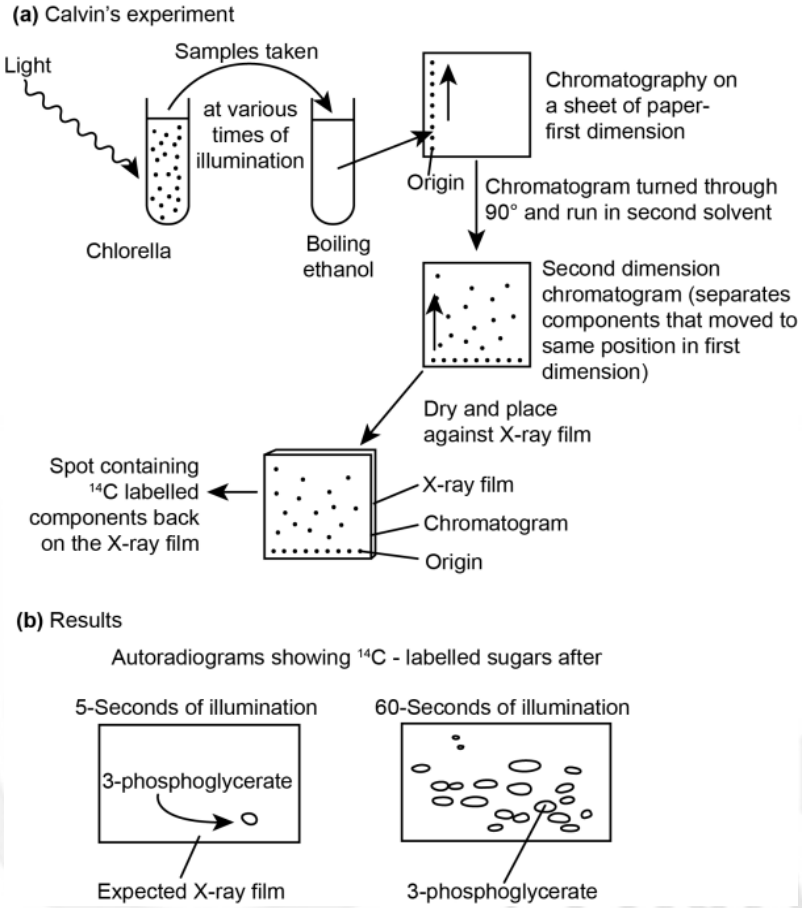


Fig. 6.31: Calvin's experiment using $^{14}CO_2$ to demonstrate that 3-phosphoglycerate was the first product of carbon fixation (From Smith & Wood).

Since only the terminal carbon atom was radioactive, it was clear that CO_2 was added to a pre-existing acceptor molecule. Initially, thought to be a 2C molecule, later the acceptor was identified as a 5-C sugar ***Ribulose bisphosphate* (*RuBP*)**. The RuBP is made up of an active two-carbon fragment which accepts the CO_2 molecule to form 3-PGA. Later the corresponding enzyme catalyzing the addition of CO_2 to *RuBP* viz*.,* ***Ribulose 1,5-bisphosphate carboxylase oxygenase* (*Rubisco*)** was also discovered in the 1940s by Calvin. Upon allowing photosynthesis to take place for longer periods, ^{14}C labeling increased in other ester phosphates with 3C, 5C, and later 6C, thereby suggesting that conversion of 3-PGA to carbohydrates was taking place by steps that were something like the reversal of sequence of reactions of glycolysis of respiration. During this process, the primary acceptor RuBP was being continuously regenerated. The Calvin-Benson cycle proceeds in **three** highly coordinated stages within the chloroplast stroma (Fig 6.32).

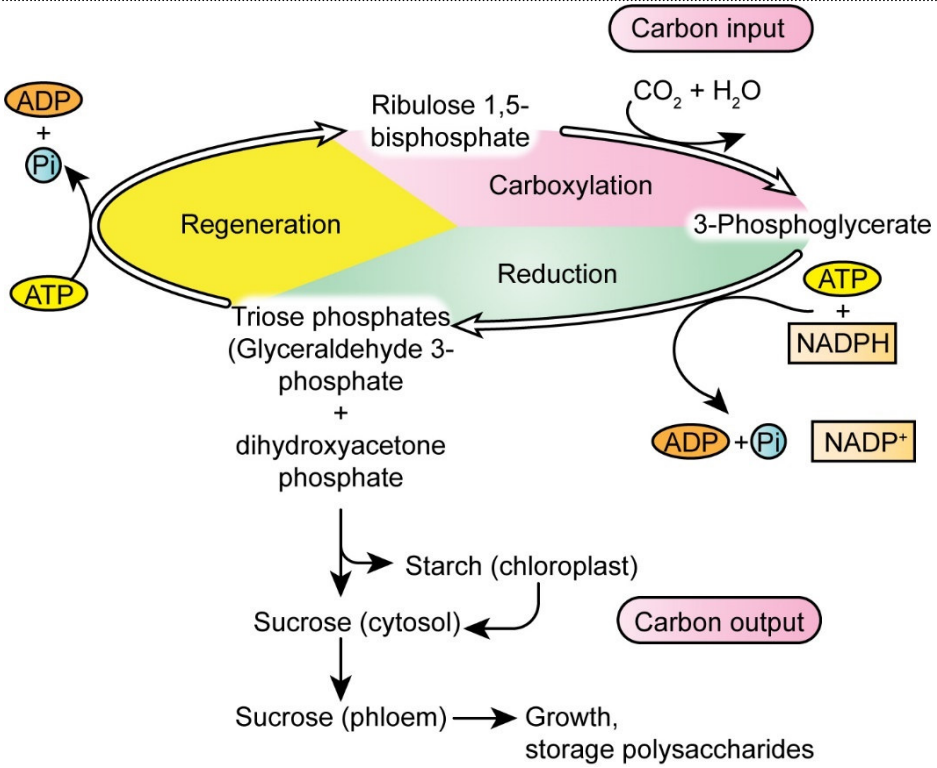


Fig. 6.32: Three stages of Calvin-Benson Cycle: 1. Carboxylation, 2. Reduction, and 3. Regeneration (From Taiz & Zeiger).

1. **Carboxylation** of the CO_2 acceptor molecule where the 5-carbon acceptor, RuBP reacts with CO_2 to form two molecules of a 3-carbon intermediate compound (3-PGA).

2. **Reduction** of 3-PGA to triose phosphates (3-carbon carbohydrates) by using the photosynthetically generated ATP and NADPH (steps of **Glycolytic Reversal**), and

3. **Regeneration of CO_2 acceptor.** A series of ten enzyme catalyzed reactions including one which is ATP requiring, regenerate RuBP. At steady state, the input of CO_2 equals the output of triose phosphates.

Now let us know about these steps in details.

STEP I Carboxylation

This step involves the addition of six molecules of CO_2 to 6 molecules of the primary acceptor, a 5- carbon sugar, **ribulose 1,5-bisphosphate (RuBP)** to yield 12 molecules of the first stable product **3-phosphoglycerate (3-PGA),** which is a 3 carbon compound.

This reaction is catalyzed by the chloroplast enzyme ***ribulose 1,5-bisphosphate carboxylase oxygenase***, popularly called ***Rubisco*** (also written as ***RuBisCo, rubisco, RuBPCase, or RuBPco***). It is a bifunctional enzyme which has also the capability to function as an *oxygenase*. RuBP has an active 2-carbon site. The immediate product of carboxylation is an unstable 6-C intermediate 2-carboxy-3-ketoarabinitol 1,5-bisphosphate. It is formed by the elimination of H^+ from the carbon 3 on RuBP. Addition of CO_2 converts this unstable **enediol** to a stable 2-carboxy-3-ketoarabinitol 1,5-bisphosphate, the hydration of which yields two molecules of 3-PGA (Fig. 6.33). Since the first visible product is a 3C compound, plants showing this pattern are called C_3 plants.

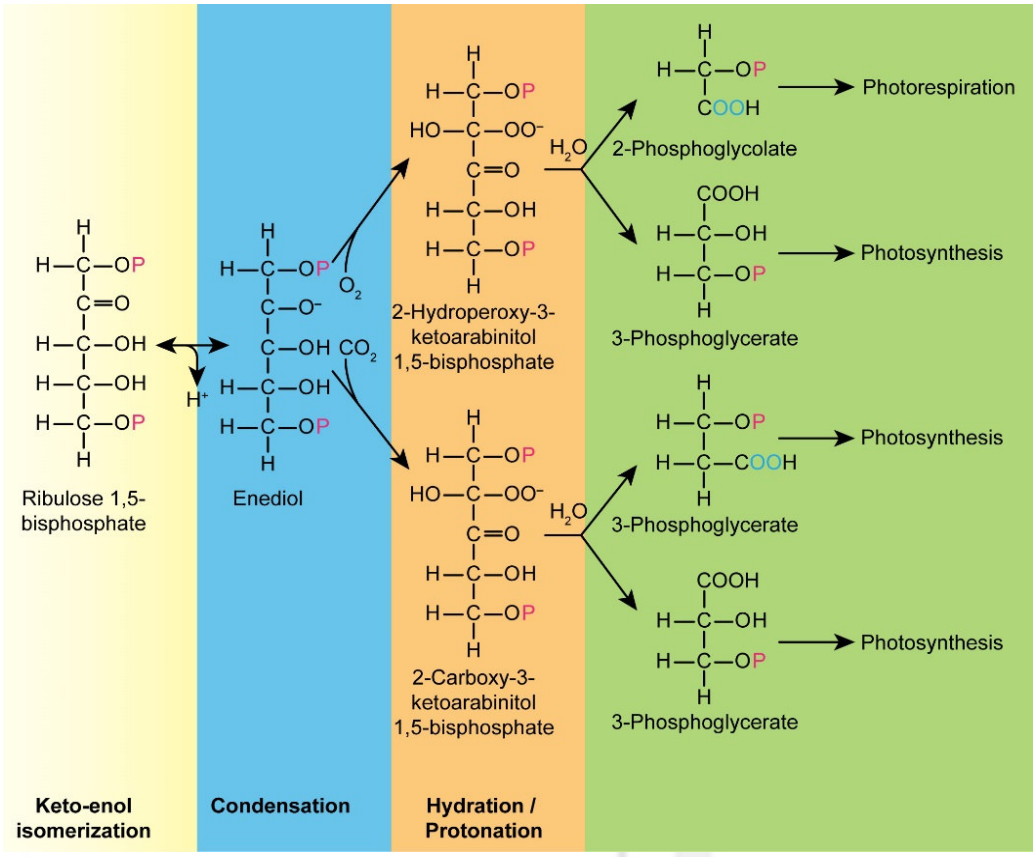


Fig. 6.33: Carboxylation and oxygenation of ribulose 1,5-bisphosphate catalysed by *Rubisco* (After Taiz et al).

Interestingly, the carboxylation step of Calvin cycle is an energetically favorable one with a ΔG of -35kJ mol^{-1} and is the only step which does not need an input of energy from the light reaction. The remaining two steps do require ATP.

Step II Reduction (Glycolytic Reversal Steps)

The 3-PGA formed in Step I is converted into glyceraldehyde 3-phosphate in **two** steps. These steps are essentially **reverse** of the corresponding steps in glycolysis—hence called steps of **glycolytic reversal**. However, NADPH is used here rather than NADH, which operates in glycolysis.

12 molecules of 3-PGA are first phosphorylated by 12 molecules of ATP (formed in the light reaction) yielding 12 molecules of a mixed anhydride **1, 3-bisphosphoglycerate (1, 3-BPGA)**. This reaction is catalyzed by *3-phosphoglycerate kinase* and requires Mg^{2+}.

Next, the actual reduction takes place when the light-reaction generated 12 molecules of NADPH reduce 12 molecules of 1,3-BPGA to **12 glyceraldehyde 3-phosphate** (3C). This reaction is catalyzed by the enzyme *NADP-glyceraldehyde-3-phosphate dehydrogenase* and requires Mn^{2+} ions. 12 molecules of Pi in the form of H_3PO_4 are released during the process. Interestingly, this enzyme is activated by light. Most of this triose phosphate is used to regenerate the primary acceptor RuBP. However, a small fraction of the “extra” fixed carbon in glyceraldehyde 3-phosphate may be used immediately as the source of energy by leaving the chloroplast via a triose phosphate antiporter into the cytosol where it undergoes glycolysis or gets converted into sucrose.

Step III Regeneration of RuBP (Reductive Pentose Phosphate Pathway)

For the continuous CO_2 assimilation, and to prevent depletion of intermediates in Calvin-Benson Cycle, a constant regeneration of RuBP is a must. The regeneration of this primary acceptor is made possible by a series of complex reactions involving reshuffling of carbons, recycling, and transformation with 3-,4-,5-,6-,and 7-carbon sugars as intermediates. Out of the 12 molecules of glyceraldehyde 3-phosphate (12 × 3C= 36 carbons), only two molecules (2 × 3C = 6 carbons) are utilized in the synthesis of fructose 6-phosphate/fructose/sucrose/starch. The remaining ten molecules (10 × 3C = 30 carbons) are recycled to generate back 6 molecules of RuBP (6 × 5C = 30C). Thus, there is a net assimilation of only six carbons. Or so to say that one turn of Calvin cycle fixes only one carbon of the sugars (Fig. 6.34).

Glyceraldehyde 3-phosphate shows four types of reactions. Out of 12 molecules of glyceraldehyde 3-phosphate (12 × 3C= 36 carbons), five molecules isomerize to give **5 dihydroxyacetone phosphate (DHAP)** by the enzyme *triose phosphate isomerase* .Three of these DHAP molecules (3 × 3C = 9C) condense with 3 molecules of glyceraldehyde 3-phosphate (3 × 3C = 9C) to yield 3 molecules of **fructose 1,6-bisphosphate**(3 × 6C=18C). This reaction is catalyzed by the enzyme *trans aldolase*. One of these three molecules gets dephosphorylated by the enzyme *fructose 1,6-bisphosphatase* to form **fructose 6-phosphate** (6C), which is released as the final product of photosynthesis.

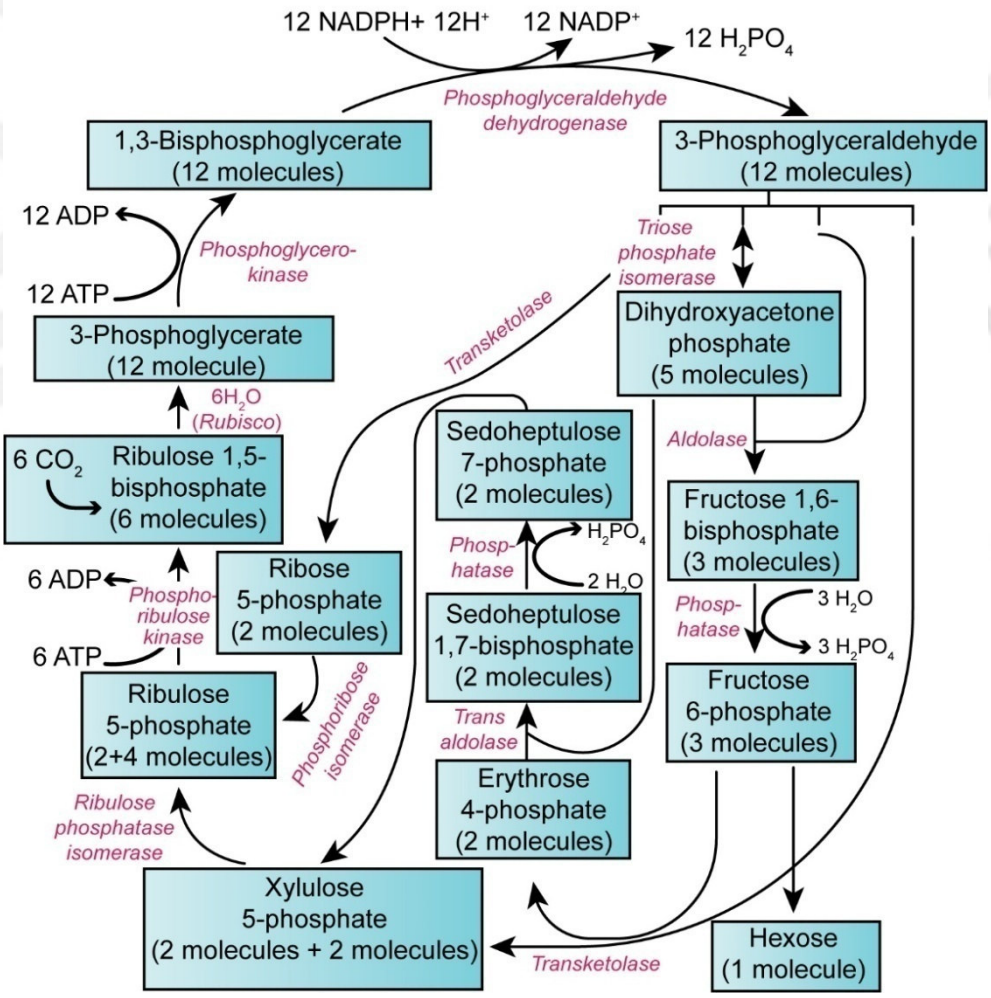


Fig. 6.34: A schematic diagram of the path of carbon in the Calvin-Benson Cycle - Dark reaction (From Verma).

The remaining two molecules of fructose 6-phosphate (2 × 6C = 12 carbons) combine with 2 molecules of glyceraldehyde 3-phosphate (2 × 3C = 6C) and produce two molecules each of a four-carbon compound **erythrose 4-phosphate** (2 × 4C=8 carbons) and **xylulose 5-phosphate** (2 × 5C=10C). The enzyme *transketolase* catalyzing this reaction, requires thiamine pyrophosphate (TPP) and Mg^{2+} as cofactors.The erythrose 4-phosphate (2 × 4C=8 carbons) reacts with another 2 molecules of DHAP (2 × 3C = 6C) to form 2 molecules of a 7-carbon **sedoheptulose 1, 7-bisphosphate** (2 × 7C = 14 carbons). This bisphosphate now loses its P at the carbon 1 to become **sedoheptulose 7-phosphate** (2 × 7C = 14 carbons).The remaining two molecules of glyceraldehyde 3-phosphate (2 × 3C=6C) react with two molecules of sedoheptulose 7-phosphate(2 × 7C=14Carbons) to form yet another set of compounds, viz., two molecules each of **ribose 5-phosphate** (2 × 5C=10 carbons) and **xylulose 5-phosphate** (2 × 5C=10 carbons) Now, a total of 4 molecules of xylulose 5-phosphate(4 × 5C=20 carbons) epimerize to yield 4 molecules of **ribulose 5-phosphate** (4 × 5C=20 carbons). Another two molecules of **ribulose 5-phosphate** (2 × 5C=10 carbons)are formed by isomerization of the remaining two molecules of **ribose 5-phosphate** (2 × 5C = 10 carbons).Thus, a total of 6 molecules of ribulose 5-phosphate (6 × 5C = 30 carbons) ultimately get phosphorylated by ATP from light reaction to form six molecules of the primary acceptor **ribulose 1,5-bisphosphate** (6 × 5C = 30 carbons). A summary of reactions along with their enzymes is given in Table 6.1.

Table 6.1: A summary of various reactions and enzymes participating in the Calvin-Benson cycle.

S. No	Reactions	Enzyme
1.	6 Ribulose-1,5-bisphosphate + $6CO_2$ + $6H_2O$ → 12 (3-phosphoglycerate) + $12H^+$	*Ribulose-1,5-bisphosphate carboxylase/ oxygenase*
2.	12 (3-phosphoglycerate) + 12 ATP → 12 (1,3 – bisphosphoglycerate) + 12 ADP	*3-Phosphoglycerate kinase*
3.	12 (1,3 – bisphosphoglycerate) + 12 NADPH +12 H^+ →12 glyceraldehyde-3-phosphate (3-phosphoglyceraldehyde) + 12 $NADP^+$ + 12 Pi	*NADP glyceraldehhyde-3-phosphate dehydrogenase*
4.	5 Glyceraldehyde-3-phosphate → 5 dihydroxyacetone-3-phosphate	*Triose phosphate isomerase*
5.	3 Glyceraldehyde- 3-phosphate + 3 dihydroxyacetone-3-phosphate → 3 fructose-1, 6-bisphosphate	*Aldolase*
6.	3 Fructose-1, 6-bisphosphate + $3H_2O$→ 3 fructose 6-phosphate + $3P_1$	*Fructose-1, 6-bisphosphatase*
7.	2 Fructose-6-phosphate + 2 glyceraldehyde-3-phosphate → 2 erythrose-4-phosphate + 2 xylulose 5-phosphate	*Transketolase*
8.	2 Erythrose-4-phosphate + 2 dihydroxyacetone-3-phosphate → 2 sedoheptulose-1 7-bisphosphate	*Aldolase*

9.	2 Sedoheptulose-1, 7-bisphosphate + $2H_2O$ → 2 sedoheptulose 7-phosphae +$2P_1$	*Sedoheptulose-1-7, bisphosphatase*
10.	2 Sedoheptulose-7 – phosphate + 2 glyceraldehyde 3- phosphate →2 ribose-5-phosphate + 2 xylulose-2-phosphate	*Transketolase*
11.	4 Xylulose-5-phosphate → 4 ribulose-5-phosphate	*Ribulose-5-phosphate epimerase*
12.	2 Ribose-5-phosphate → 2 ribulose-5-phosphate	*Ribose-5-phosphate isomerase*
13.	6 Ribulose 5-phosphate + 6 ATP → 6 Ribulose-1, 5-bisphosphate + 6ADP + $6H^+$	*Ribulose-5-phosphate kinase*

Net: $6CO_2 + 11H_2O + 12NADPH + 18ATP \rightarrow$ Fructose-6-phosphate + $12NADP^+ + 6H^+ + 18ADP + 17Pi$

You must appreciate that the original number of molecules of the acceptor are regenerated to maintain a steady state of carbon reduction.

It is, therefore, clear that **six turns** of Calvin cycle (6 × {1C+5C} = 36 carbons) will fix carbons equivalent to one additional hexose sugar (6C) as a net product, and at the same time would also regenerate 6 molecules of RuBP (6 × 5C=30 carbons)

$6CO_2$ + 12 NADPH +$12H^+$+ 18 ATP $\longrightarrow$ $C_6H_{12}O_6$ + 12 $NADP^+$ +$6H_2O$ + 18ADP +18Pi

There is a net gain of 6 molecules of water in the Calvin cycle. 12 molecules of water are generated in the reduction step of 3-PGA to glyceraldehyde 3-phosphate, out of which 6 molecules get consumed by RuBP while accepting CO_2.

12PGA + 12 NADPH +$12H^+$+ 12 ATP $\longrightarrow$ 12Gl 3-Ph + 12 $NADP^+$ + $12H_2O$ + 12ADP + 12Pi

Box 6.8 : Energetics of Calvin cycle.

If you carefully calculate the requirements for input of Calvin cycle, you will find that for the uptake of 6 molecules of CO_2 (involving six turns of the PCR cycle), a total of 12 molecules of NADPH and 18 ATP are utilized. This would mean that 2 molecules of NADPH and 3ATP molecules are required for the reduction of each CO_2 molecule. The ratio of ATP/$NADP^+$ for this cycle is 3/2=1.5. Since each $NADP^+$ stores 2 electrons, 4 electrons will be required to fix each CO_2 molecule. A total energy input of 529 kJ mol^{-1} of CO_2 is required for this purpose. On the other hand, oxidation of one molecule of a hexose sugar yields just 469 kJ mol^{-1} of CO_2. Thus, the **energy storage efficiency** =469/529=0.88=88%. If we were also to include the energy consumed as 3ATP per CO_2 (i.e., 3 × 31.4 kJ mol^{-1}=282kJ mol^{-1}) which is needed to regenerate RuBP, the percentage for energy storage efficiency would go down to 469/811= approx. 58% (529+282= 811).

6.6.3 *Rubisco*-Structure and some Modern Concepts

The enzyme *Ribulose 1, 5-biphosphate carboxylase oxygenase* (***Rubisco*/*RuBisCO*/*RuBPCase*/*RuBPco***) is a copper containing large protein with a molecular wt. of 560,000 Daltons.It is located in the chloroplast stroma. The enzyme was discovered by Melvin Calvin at Berkley in the 1940s. It was previously called *carboxydismutase* since it can catalyze both a carboxylase

reaction as well as dismutation (an intramolecular oxidation-reduction reaction. The enzyme exists in all photosynthetic organisms and comprises up to 50% of the soluble protein (6mg per mg chlorophyll) in leaves of higher plants. It is rightly considered as the most abundant protein present on the earth. It is estimated that 4 x 10^{13} g of the enzyme is made every second. This single complex protein can be distinguished from the remaining leaf proteins by ultracentrifugation and is called **Fraction I protein**. This high concentration of Rubisco indicates a significant role for the plant. Large amounts/ sheer abundance of this enzyme is needed to compensate for its extremely slow activity. It has a turnover of only 3-4 molecules of CO_2 per second.

6.6.4 Regulation of Calvin – Benson Cycle

Earlier it was thought that the dark phase of photosynthesis did not require much regulation and the activity of *Rubisco* was a rate limiting one. Recent studies, however, indicate otherwise. You should be aware that photosynthesis does not operate in isolation and therefore, like all metabolic pathways, is also subject to various controls like light and enzyme activities.

Autocatalytic regeneration of RuBP

Normally, the newly fixed carbon is channelized by the Photosynthetic Carbon Reduction Cycle (PCR cycle) to form sugars. However, when necessary, this carbon can also be used by the PCR cycle to increase the levels of CO_2 and RuBP through catalytic regeneration of RuBP.

Since there is no photosynthesis at night, the concentration of RuBP falls substantially. The availability of this CO_2 acceptor will be extremely low upon exposure of light. This would limit the rate of photosynthesis. So, the extra carbon for this purpose could be taken back from starch. Also, to support rapid photosynthesis, the Calvin cycle can provide an extra supply of the acceptor RuBP by autocatalytically generating it, instead of channelizing it towards the synthesis of sugars Fig. 6.35).

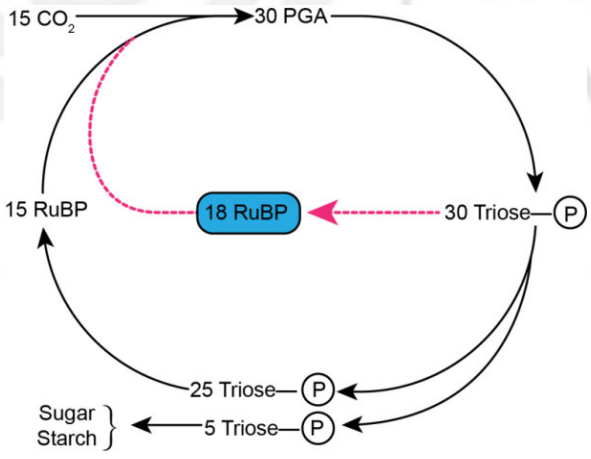


Fig. 6.35: Autocatalytic properties of Calvin-Benson cycle. To increase the rate of photosynthesis, the amount of receptor molecules is increased by retaining carbon within the Calvin cycle (dashed arrows)(From Hopkins & Hüner).

This ensures rapid supply of RuBP for rapid photosynthesis. The time required to build up the necessary/requisite levels of intermediates of the PCR cycle during transition from dark to light is called **Photosynthetic induction time**. It is only after this time that carbon is channelized further for the synthesis of sugars and starch.

SAQ 5

Give one- word answer:

a) The first compound in which the radioactivity appeared was ……. .

b) The CO_2 acceptor molecule in Calvin Cycle is …….. .

c) The final product of CO_2 fixation is …….. .

d) The number of ATP molecules required for the fixation of six molecules of CO_2 is …….. .

e) The number of ATP molecules used to produce a molecule of glucose is ……..…….. .

f) The number of NADPH used for fixation of 6 CO_2 is …….. .

g) The number of molecules of ATP required for the regeneration of one RuBP is ……..…….. .

6.7 SUMMARY

- The pioneering experiments suggested the presence of two phases-the light and the dark reactions. The foremost step of light reaction is the photoexcitation of chlorophyll by the photons, which ultimately leads to the photolysis of water molecule.
- The transfer of electrons from H_2O to NADPH is mediated by two separate photochemical reactions through two sets of pigment molecules organized in the form of light harvesting complexes, representing the PS I and PS II.
- To drive the dark reactions of photosynthesis, apart from NADPH, ATP is also necessary. Since photosynthesis proceeds at a much faster rate than respiration, both photosystems in green plants function together to synthesize and transport ATP by a process known as photophosphorylation, a process which is independent of oxidative phosphorylation carried out in mitochondria.
- Complete assimilatory power can be provided only by non-cyclic photophosphorylation where both the photosystems PS I and PS II are operative and ATP and NADPH are generated. These two products will help to drive an endergonic pathway involving the fixation of atmospheric carbon dioxide through Calvin cycle in the stroma. Calvin cycle has three phases: i) pick up of CO_2 by the primary acceptor RuBP(5C), catalyzed by the unique enzyme *Rubisco*. This results in the formation of a stable 3C compound—3-phosphoglycerate, ii) reduction of 3-phosphoglycerate to triose phosphates like glyceraldehyde 3-phosphate (3C) using light reaction generated ATP and NADPH, and iii) Regeneration of the CO_2 acceptor RuBP.

- *Rubisco* activity is regulated indirectly by light and involves a series of complex interactions between Mg^{2+} fluxes across the thylakoid, chloroplast pH alterations, CO_2 activation, and an activating protein *Rubisco activase*. Four other enzymes of the PCR cycle are also regulated by light via the participation of the electron carrier ferredoxin, and an iron-sulfur protein thioredoxin.

6.8 TERMINAL QUESTIONS

1. The photochemical reactions in grana capture light energy and convert it into chemical energy as ATP and NADPH. Why is carbon fixation necessary?

2. Give the raw materials and end-products of the following reactions of photosynthesis.

Photosynthesis Reactions	Raw Material	End-Product
i) PSI		
ii) PS I followed by electron transport		
iii) PS II		
iv) PS II followed by electron transport		
v) Cyclic photophosphorylation		
vi) Carbon fixation		

3. Differentiate between:

   i) PS I and PS II

   ii) Cyclic and non-cyclic electron transfer

   iii) Photophosphorylation and Oxidative phosphorylation

4. Write the summary equation for light reaction.

6.9 ANSWERS

Self-Assessment Questions

1. a) The value of Q_{10} was more than 1;

   b) Splitting of water, formation of ATP and reducing power.

   c) i) Photolysis, H_2O ii) H_2O, O^{18}

      iii) $NADP^+$, NADPH iv) Hill reaction

2. a) i) Quantum requirement (number of light quanta required for each molecule of O_2 produced was 8 instead of 4.

   ii) The quantum yield drops at higher wavelengths (red region) but it is abolished when a beam of shorter wavelength was simultaneously given.

   b) i) two

   ii) electron donor

   iii) reaction centre chl $_a$

   iv) $NADP^+$

3. a) P_{700}

   b) reductant, oxidant

   c) Cl^-, II

   d) cytochrome $_{b6/f}$ complex

   e) resonance transfer

4. a) Electron flow blockade at PQ

   b) Peter Mitchell

   c) Quantasomes

   d) Prevents electrons to reach $NADP^+$

   e) Rotary motion

5. a) PGA

   b) RuBP

   c) Glucose

   d) 18

   e) 12

   f) 12

   g) one

Terminal Questions

1. Firstly, the storage of energy in the form of carbohydrates is much more convenient and lot more energy can be stored in this form. Secondly, carbon skeleton of carbohydrates is needed for various biosynthesis.

2.

Photochemical Reactions	Raw Material	End Product
i) Excitation of PS I	hv + light harvesting pigment complexes + P_{700}	Excited electrons

ii)	PSI followed by electron transport	hv + (light harvesting pigment complexes + P_{700}) + $NADP^+$	NADPH
iii)	Excitation of PS II	hv + light harvesting pigment complexes, P_{680}	Electrons
iv)	Excitation of PS II followed by electron transport	hv + light harvesting pigment complexes + P_{680} + ADP + Pi	ATP + electrons
v)	cyclic photophosphorylation	H^+ reservoir, ADP + Pi	ATP
vi)	Carbon fixation	RuBP + CO_2 + ATP + NADPH	Sugars + ADP + Pi + $NADP^+$

3. i)

	PSI		PSII
1.	It is located on the outer surface of thylakoid membrane towards stroma.	1.	It is located on the inner surface of the thylakoid membrane.
2.	PSI is present in unstacked thylakoid membrane and causes light induced reduction of $NADP^+$.	2.	It is predominantly present in stacked thylakoid membrane.
3.	Molecular oxygen is not evolved.	3.	Photolysis of water takes place and molecular oxygen is evolved.
4.	Involved in both cyclic and non-cyclic photophosphorylation.	4.	Participates only in non-cyclic photophosphorylation.
5.	Here strong reductant is produced which reduces $NADP^+$ to NADPH + H^+	5.	PSII donates electrons to PSI when $NADP^+$ is reduced.
6.	Reaction centre is made up of P_{700} a special chlorophyll a molecule.	6.	Reaction centre is made up of P_{680}, a special type of chlorophyll a molecule.
7.	Chl_a/Chl_b ratio is high (5)	8.	Chl_a/Chl_b ratio is low (2-2.5)

ii)

	Cyclic Electron Transport System		**Non-cyclic ElectronTransport system**
1.	Disributed in some bacteria.	1.	Occurs in cyanobacteria, higher plants and algae.

2. Only PSI operates	2. Both PSI and PS II operate.
3. Electron ejected by chlorophyll molecule returns to same chlorophyll. $NADP^+$ is not involved.	3. Electron ejected by PS II is finally accepted by $NADP^+$ via PS I. Thus, electron ejected by PS II does not return to same chlorophyll.
4. $NADPH + H^+$ is not produced. Only ATP is produced (by the coupling factor)	4. $NADPH + H^+$ is produced along with ATP (by the coupling factor).Complete assimilatory power formed
5. Photolysis of water does not occur. So no evolution of oxygen.	5. Photolysis of water evolves oxygen.
6. The flow of elecrons is as follows : Chl $P_{700} \rightarrow A_0 \rightarrow A_2 \rightarrow$ Fd $\rightarrow$ PQ $\rightarrow$ Cyt $_{b6/f}$ Complex $\rightarrow$ PC (P_{700}).	6. The flow of electrons is as follows : Chl P 680 $\rightarrow$ Pheo $\rightarrow$ $Q_A \rightarrow Q_B$ PQ $\rightarrow$ Cyt $b_6/f \rightarrow$ PC $\rightarrow$ chl $\rightarrow$ P700 A_0 Fd $NADP^+$.
7. Protons release at only one step ($PQ\text{-}PQH_2$).	7. Protons released at two steps (between water and PS II and PS I ($PQ - PQH_2$).
8. System is not sensitive to either antimycin or dichlorophenyl dimethyl urea (DCMU).	8. DCMU inhibits flow of electrons from water to $NADP^+$.
9. No external electron donor required.	9. Requires an external electron donor like H_2O or H_2S.

iii)

Photophosphorylation	**Oxidative phosphorylation**
1. Takes place in chloroplast during light reaction.	1. Operates in mitochondria during respiration.
2. 18 molecules of ATP are consumed in formation of one glucose molecule.	2. 38 or 36 molecules of ATP are produced by oxidation of one molecule of glucose.
3. Oxygen is not used except in pseudo cyclic photophosphorylation.	3. Oxygen is used in the process. Final electron acceptor is oxygen in aerobic respiration.
4. All green cells on illumination show photophosphorylation	4. All living aerobic cells perform oxidative phosphorylation.
5. Photophosphorylation produces reduced coenzymes.	5. It oxidizes reduced coenzymes. i.e. $NADH + H^+$, and $FADH_2$

6. Energy stored in ATP is obtained from sun.	6. Energy stored in ATP is obtained by oxidation of organic compounds.
7. Splitting of water occurs in this process.	7. Synthesis of metabolic water (i.e. new water) occurs in this process.
8. Flow of electrons from water to NADP+ (i.e. from+ 0.82 volt to --0.6 volt) against electrochemical gradient occurs at the cost of solar energy.	8. Flow of electrons from reduced coenzymes to oxygen occurs as per oxidation reduction potential.
9. Photophosphorylation takes place in thylakoid membrane of chloroplast.	9. Oxidative phosphorylation takes place in inner membrane of mitochondria.
10. Four protons are transported.	10. Six protons are transported.

4. Summary equation for light reaction.

$$24H_2O \longrightarrow 24OH^- + 24H^+$$

$$24OH^- \longrightarrow 24e^- + 24OH$$

$$24OH \longrightarrow 12H_2O + 6O_2$$

$$18\ ADP + 18Pi \longrightarrow 18\ ATP$$

$$12NADP + 24e^- + 24H^+ \longrightarrow 12\ NADPH + 12\ H^+$$

$$24H_2O + 18ADP + 18Pi + 12NADP \longrightarrow 12H_2O + 18ATP + 12NADPH + 12H$$

Annexure 1 : *Rubisco*

Rubisco (**EC 4.1.1. 39**) in higher plants is in the form of a hexadecameric complex consisting of two dissimilar types of subunits, but in equal number. There are eight large catalytic subunits (called LSU) with a molecular weight of 55kDa and eight small regulatory subunits (called SSU) with a molecular weight of 15 kDa. There is one catalytic site on each large subunit, i.e., eight catalytic sites in all per holoenzyme (Fig 1 a).

The large catalytic subunits are synthesized within the chloroplast and genes coding for them are located on the chloroplast genome or **plastome**. In contrast, the smaller regulatory subunits are coded by a gene located in the nucleus. The formation of a fully functional *Rubisco* complex involves many steps. After the large subunits have been synthesized in the chloroplast stroma and the smaller subunits have been imported from the cytosol, the two get assembled into a complex with the help of a binding protein (BP). The binding protein is itself synthesized in the cytosol and gets transported into the chloroplast stroma. The assembly of holoenzyme requires ATP and Mg ions (Fig. 1 b).

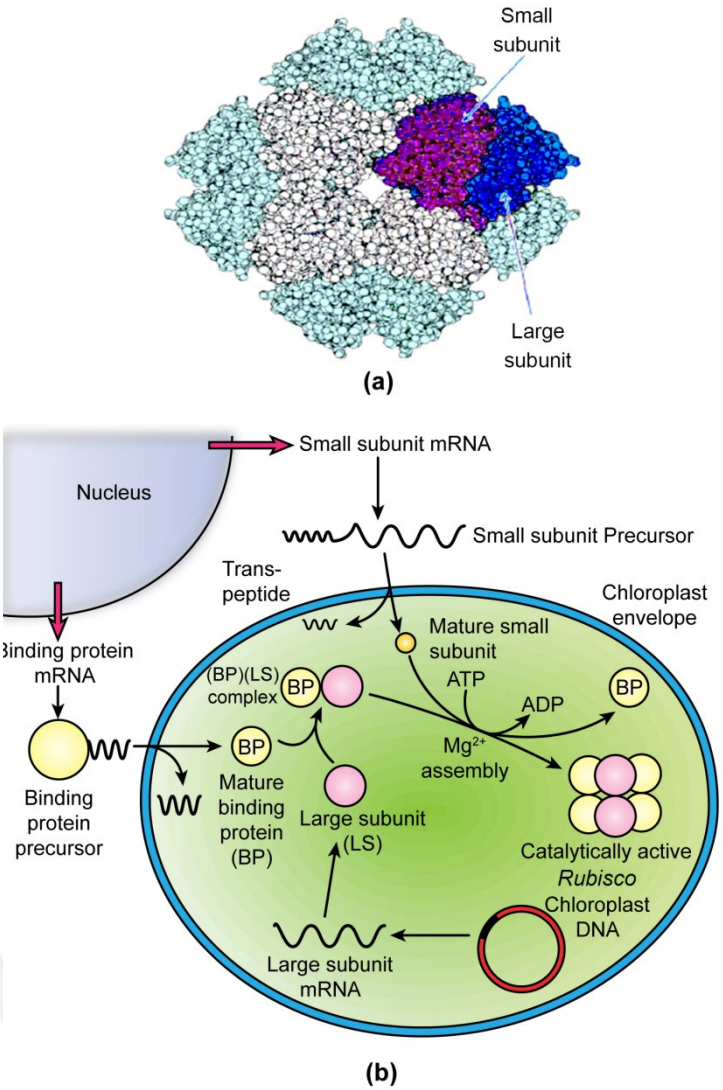


Fig. 1: a) Structure of the *Rubisco*. The enzyme has eight large subunits (one depicted in red and the others in yellow, and eight small subunits (one shown in blue and others in white). The active sites are located within the larger subunits (From Stryer); b) *Rubisco*: Synthesis and assembly. Small *carboxylase* subunits are synthesized in the cytosol while the large subunits are made within the chloroplast stroma. The catalytically active enzyme is assembled in the stroma (From Smith and Wood).

Rubisco shows a high affinity for its substrate RuBP and catalyzes two principal reactions: The active site of *Rubisco* can accept both CO_2 and O_2 as substrate (Fig. 2). Interestingly, O_2 has a higher affinity for the active site and thus, binds at a much higher rate than CO_2. Perhaps this is the reason why CO_2 needs to be constantly shuttled into the chloroplast from the outside to ensure its maximized exposure to *Rubisco* to run the Calvin cycle. The bifunctional enzyme *Rubisco* performs conformational changes before catalyzing carboxylation or oxygenation.

The low affinity of the enzyme for CO_2/O_2 is partly counter balanced by the high quantity of the enzyme. The *carboxylase/oxygenase* property of the enzyme is regulated by CO_2/O_2 ratio of the atmosphere. If the ratio is high, *carboxylase* activity is favored, while more *oxygenase* activity takes place at low ratio (Fig. 2). The *oxygenase* activity of this enzyme leads to photorespiration where the two-carbon molecule phosphoglycoate is metabolized by a set of reactions by a C_2 cycle, the details of which you will study in the next unit (Unit 7).

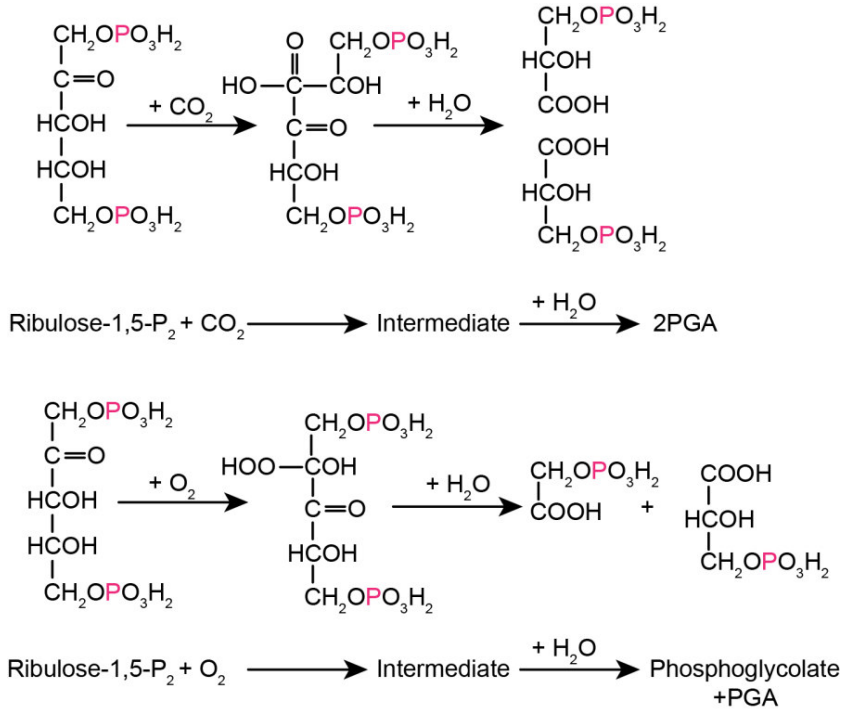


Fig. 2: Comparison between the action of *RuBP carboxylase* and *RuBP oxygenase.*

Rubisco is also unique in the sense that it is among very few enzymes which is autocatalytic. It regenerates one of its own substrates while having a synthetic capacity at the same time.

Various attempts have been made with limited success to genetically modify *Rubisco* by either eliminating its *oxygenase* activity, or by engineering it to fix atmospheric carbon at a much faster rate by introducing the gene for *Rubisco* from photosynthetic bacteria. This is because the *Rubisco* from photosynthetic bacteria is simpler in organization that its counterpart in the higher plants.

Annexure 2 : Do Dark Reactions really occur in Dark?

The PCR cycle has been traditionally called the Dark reaction, as it has long been believed that the reactions occurring within are thermochemical and **do not** require a direct participation of light.

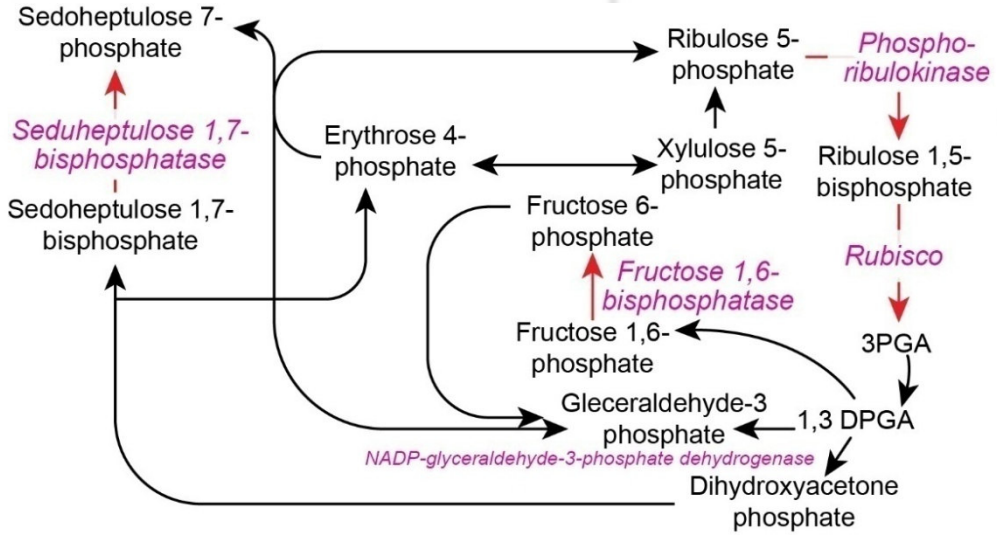


Fig. 1: Regulation of Calvin cycle by four major enzymes (in pink) which are themselves activated by metabolic changes initiated by the light reaction (From Peter Scott).

However, it is now known that the activity of many of the enzymes of Calvin cycle is indirectly affected by light reactions of photosynthesis. Some key steps/enzymes in the Calvin cycle do not operate in dark yet seem to control/regulate the activity of this cycle (Fig. 1).These enzymes get activated by metabolic changes which are initiated by the light reaction. These are *Rubisco, Fructose 1,6-biphosphatase, Sedoheptulose 1,7-biphosphatase,* and *Phosphoribulokinase* and *NADP-glyceraldehyde-3-phosphate dehydrogenase*.

Upon illumination, the following four sequence of events take place:

1. Light reaction results in the generation of H^+. There is a net movement of protons into the thylakoid lumen. A proton gradient equivalent to 3.0 pH units is generated. As a result, the pH of the stroma, which was originally around 5.0 in dark, increases to pH 8.0 in light. This activates the above-mentioned enzymes.

2. To compensate the charge imbalance across the membrane, Mg^{2+} ions move out of the lumen into the stroma (opposite to the direction of the proton flux). High Mg^{2+} concentration in the stroma also stimulates *Rubisco* and other enzymes (Fig.2 a).

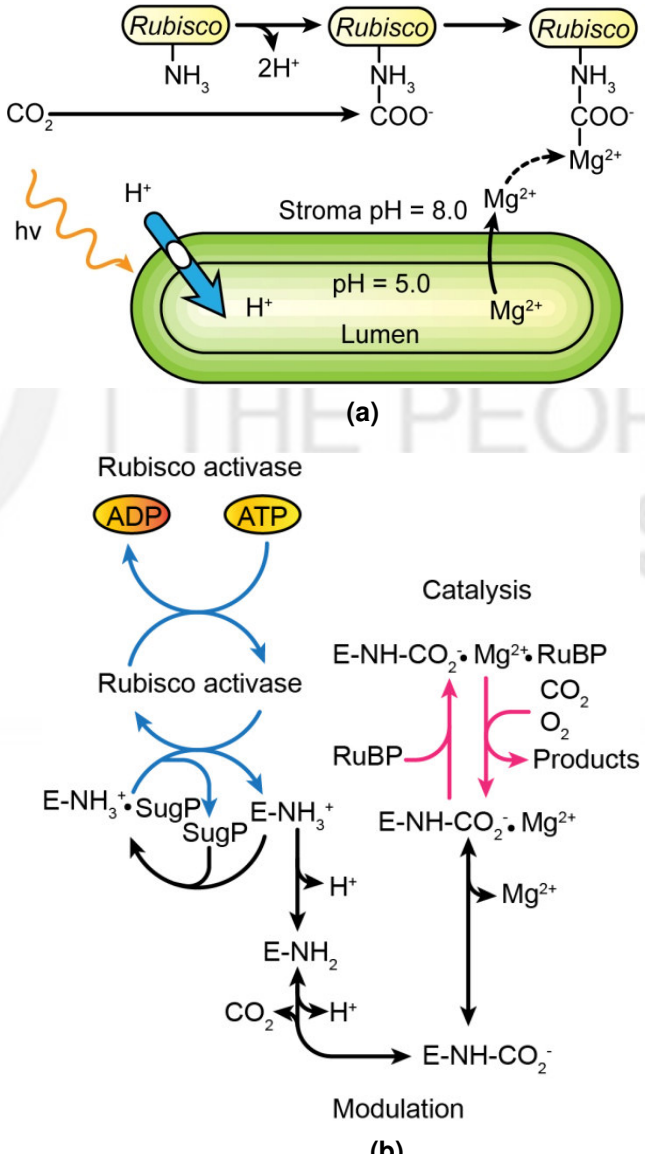


Fig. 2: a) Stimulation of *Rubisco* by increase in pH and Mg^{2+} concentration) (From Hopkins & Hüner); b) Role of CO_2 both as a substrate and an activator in *Rubisco*-catalyzed reactions (From Taiz & Zeiger).

Recent studies made by Lorimer et al. (cited in Taiz; Hopkins & Hüner) using isolated *Rubisco in vitro* have demonstrated clearly that CO_2 is not just a substrate but also a modulator for the slow acting *Rubisco*. All three factors, viz., CO_2, Mg^{2+}, and pH contribute towards *Rubisco* activation. Upon increase in pH in the lumen, CO_2 first reacts with the amino group of a specific lysine in the active site of *Rubisco*, resulting in the formation of a **carbamate.** Increased pH favors the release of 2 protons during the carbamate formation. At the same time, increase in Mg^{2+} concentration helps the coordination of Mg^{2+} to carbamate forming a carbamate- Mg^{2+} complex, which is the active form of *Rubisco*. CO_2 binds to this activated enzyme to release 2 molecules of 3-phosphoglycerate.

Rubisco first interacts with a special type of protein ***Rubisco activase*.** This reaction requires ATP (Fig.2 b). The *activase* induces certain structural changes in Rubisco so that its tightly bound sugar phosphate –like molecules are released, making the enzyme ready for activation via carbamation and metal binding.

3. In addition to *Rubisco*, four other enzymes of the Calvin cycle are controlled by light via the **ferredoxin-thioredoxin system**. This redox system comprises ferredoxin-thioredoxin reductase enzyme and thioredoxin. Here, a product of the photosynthetic electron transport chain viz., reduced ferredoxin transforms the regulatory protein thioredoxin to its reduced form, which in turn converts the target enzyme into the reduced state, thereby enhancing its catalytic activity (Fig.3 a, b). Increase in ATP levels in the stroma due to the above events stimulates further the *Rubisco* activity.

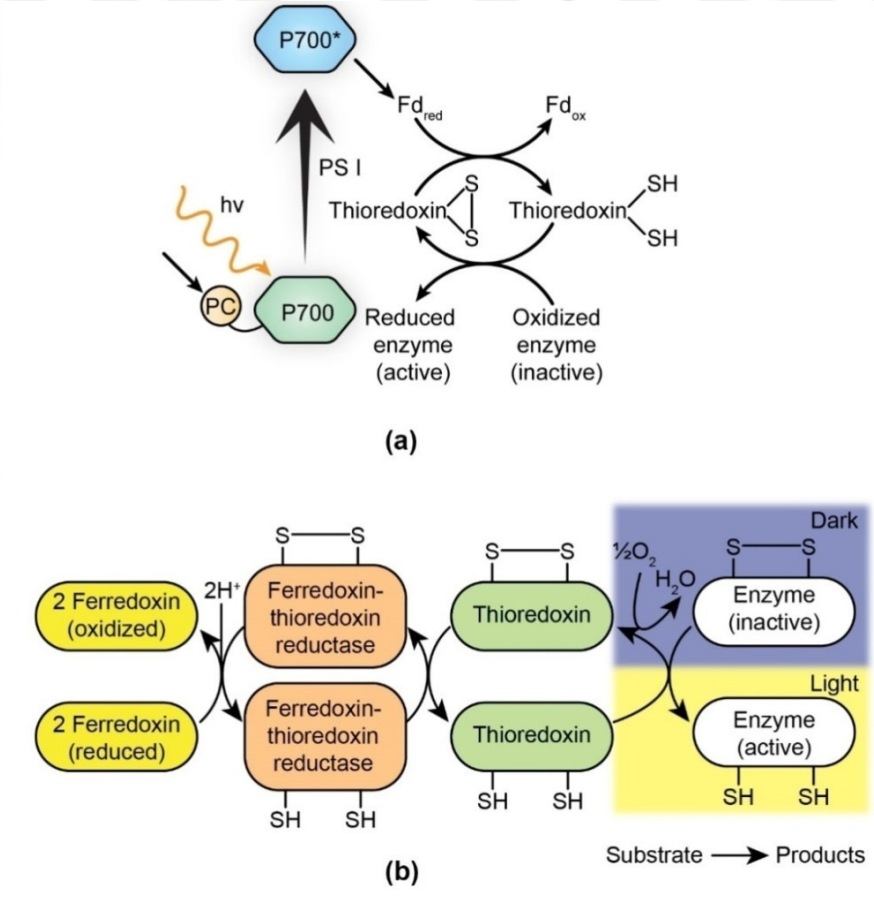


Fig. 3: a) Light-driven enzyme activation by Ferredoxin-thioredoxin system. (From Hopkins & Hüner); b) A similar representation of the ferredoxin-thioredoxin system (From Taiz *et al*).

A mechanism reverse to reduction (activation) pathway operates in dark which involves deactivation of target enzymes. *Rubisco* activity is 'switched off' in dark by an inhibitor bound to this enzyme. It is a C6 compound, 2-carboxy-D-arabinitol 1-phosphate, similar in structure to the transition unstable compound formed during CO_2 fixation by RuBP. The inhibitor depletes upon illumination thereby activating *Rubisco* (Fig.4).

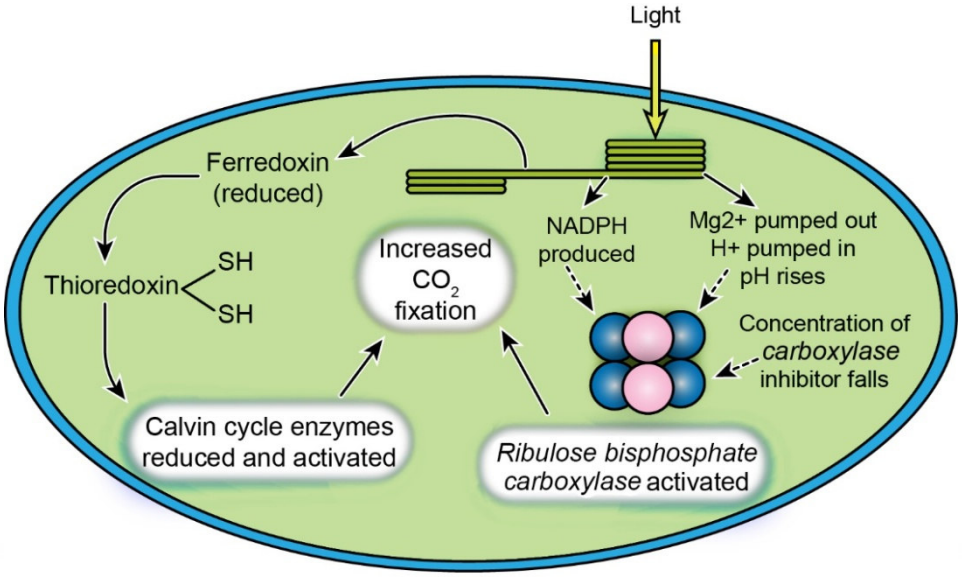


Fig. 4: Regulation of CO_2 fixation by light. Light stimulates NADPH; Mg^{2+} efflux and H^+ influx and increases pH; this results in lowering in concentration of RuBP carboxylase concentration which stimulates higher CO_2 fixation. Light activates CO_2 fixation by stimulating the ferredoxin-thioredoxin system (from Smith and Wood).

UNIT 7

C_2, C_4 AND CAM PLANTS

Structure

7.1 INTRODUCTION

This unit gives you a comparative account of the alternative mechanisms operative in plants, in addition to the C_3 or Calvin-Benson cycle. Details of an exceptional biochemical pathway-Photorespiration (C_2 pathway), which involves light dependent uptake of molecular oxygen and release of CO_2 without release of energy. The CO_2 – concentrating mechanisms of C_4 plants have been described in detail. The strategies developed by CAM plants to photosynthesize under desert conditions are also highlighted. We have also described bacterial and chemosynthesis, where the main differences with green plant photosynthesis have been highlighted. The external and internal factors affecting the photosynthetic process have been discussed in a holistic manner with reference to productivity. Finally, we have briefly discussed the efforts made in the field of modern biotechnology to improve the photosynthetic efficiency, and some recent success stories which present a promising scenario for increasing the productivity to feed the ever-increasing human population.

Objectives

After studying this unit, you should be able to:

- discuss the observations that led to the discovery of photorespiration;
- appreciate the need of photorespiration in plants; know the light and the dark side of photorespiration;
- describe Calvin cycle and calculate the number of ATP and NADPH molecules required for the fixation of one molecule of CO_2;
- relate the importance of Kranz anatomy with C_4 cycle, and give reasons for the higher photosynthetic efficiency of C_4 plants;
- know the mechanism and ecological significance of CAM pathway; and
- describe the effect of various environmental factors on photosynthesis; and gain an idea of the prospects of increasing photosynthetic efficiency through modern techniques of biotechnology.

7.2 THE C_2-OXIDATIVE PHOTOSYNTHETIC CARBON CYCLE (PHOTORESPIRATION)

So far you have learnt that O_2 is released and CO_2 is used during photosynthesis. Even though O_2 is a by-product, its increased concentration may result in lowering the rate of photosynthesis. Long ago, Otto Warburg in 1920s noted that with increasing O_2 concentration, photosynthesis in *Chlorella* was inhibited. This came to be known as "**Warburg's Effect**". This type of O_2-induced photosynthetic inhibition was later confirmed in many higher plants, and seems to operate in C_3 plants like soybean, tomato, wheat, and oats. However, the process is not operative in C_4 plants like maize, sorghum, and sugarcane. Greater inhibition of photosynthesis by O_2 was observed at a time when light levels are saturating, and CO_2 levels are low (Fig. 7.1)

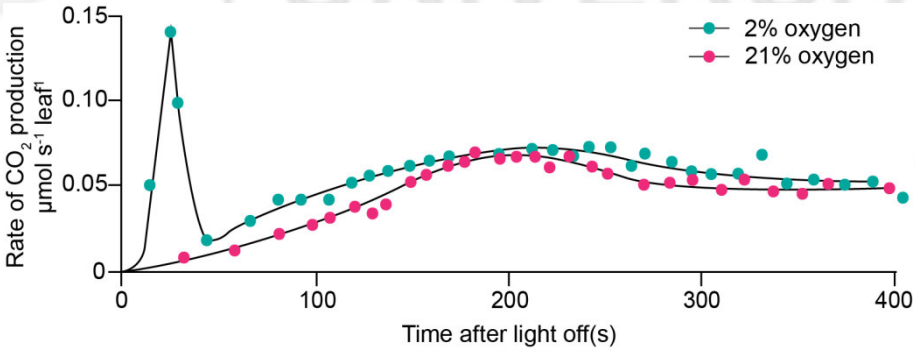


Fig. 7.1: A post-illumination burst of CO_2 is shown by photosynthesizing leaves, which varies in strength according to ambient O_2 concentration.

Even the normal ambient concentrations of O_2 (21%) is sufficient to slow down the rate of photosynthesis. There was a decline in the rate of photosynthesis when the concentration of O_2 was doubled from its normal value of 21%. Interestingly, when the O_2 concentration was reduced to 2%, rate of photosynthesis doubled. These studies suggested a possible competition between CO_2 and O_2 during photosynthesis. It was widely believed that this

photosynthesis decline may be either due to the role of O_2 in inhibiting NADPH generation or the re-oxidation of primary photochemical product/s by O_2.. Thus, the existence of photorespiration has been suspected for long.

However, definitive evidence came in 1960s when botanists used O^{18} which allowed movement of O_2 uptake even upon exposure of a plant to constant illumination. When the consumption of O^{18} was monitored by mass spectrometry in the surrounding atmosphere, it was discovered that the rate of uptake of O^{18} became significantly higher after illumination.

Decker (1955), Krotkov (1963), and others demonstrated that when the CO_2 output of plants was monitored continuously in light and then upon transfer to darkness, there was an immediate "burst" in the net CO_2 output in certain plants. Curiously, this post illumination burst became more intense if the O_2 concentration in the atmosphere was higher (Fig.7.1). This "burst" of CO_2 was also found to be directly proportional to the CO_2 concentration. Krotkov (1963) suggested that a different type of respiratory process-**photorespiration** replaces light suppressed dark respiration. Both these types of respirations differ in their sensitivity to O_2, temperature as well as the metabolic inhibitors.

We have been traditionally treating photosynthesis and respiration as two independent processes. Under saturating light conditions, a certain CO_2 concentration at which the uptake of CO_2 for photosynthesis just balances (is equal to) the evolved CO_2 by respiration. This is called **carbon dioxide compensation point.** There exists a great variation among species in the value of the CO_2 concentration at the CO_2 compensation point. This also depends on temperature.

Photorespiration is known to occur in those plants with relatively high CO_2 compensation point (50ppm or more) like the C_3 plants (e.g., wheat, oats, tomato, legumes).On the other hand the C_4 plants like sugarcane, maize and sorghum lack or exhibit negligible photorespiration since they have very low CO_2 compensation point (1 to 5ppm).So these plants can utilize the CO_2 from the atmosphere under any O_2 concentration

7.2.1 Photorespiratory Pathway

The detailed mechanism whereby CO_2 is evolved has been investigated by many American biochemists. The coordinated, complex set of reactions are not confined to the chloroplasts alone. Two more subcellular organelles: the peroxisomes and the mitochondria also participate in this pathway. Photorespiration (also called C_2 cycle /glycollate metabolism /C_2 oxidative photosynthetic carbon cycle) also integrates the Calvin cycle and nitrogen metabolism and is compartmentalized in these three organelles — a unique phenomenon. Remarkably, the transmission electron micrographs do show these organelles closely appressed to each other indicating that there is indeed some important functional relationship between them.

The initial reaction involving oxidation of RuBP by the oxygenase catalytic activity of *Rubisco* occurs in the chloroplast. Oxygenation of RuBP (**5C**) produces one molecule each of 3-phosphoglycerate (**3C**—which goes into the Calvin-Benson cycle), and a 2-carbon compound, 2-phosphoglycolate. (Fig.7.2). Both these reactions (carboxylation and oxygenation) take place within the same active site.

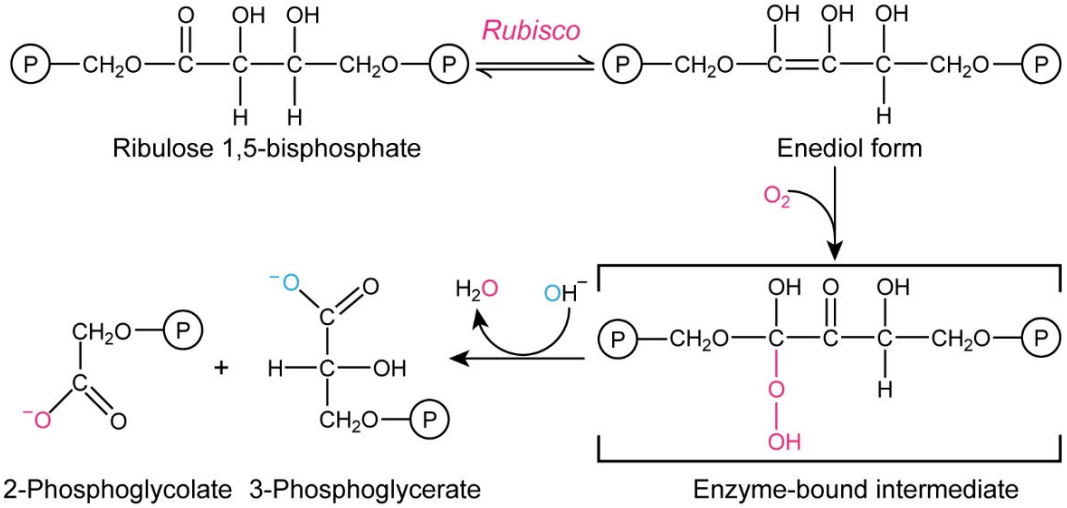


Fig. 7.2: Fate of *Rubisco* with reference to O_2 and CO_2. In the presence of O2, *Rubisco* acts as an *oxygenase*, incorporating into RuBP to form an unstable intermediate 2-phosphoglycolate.

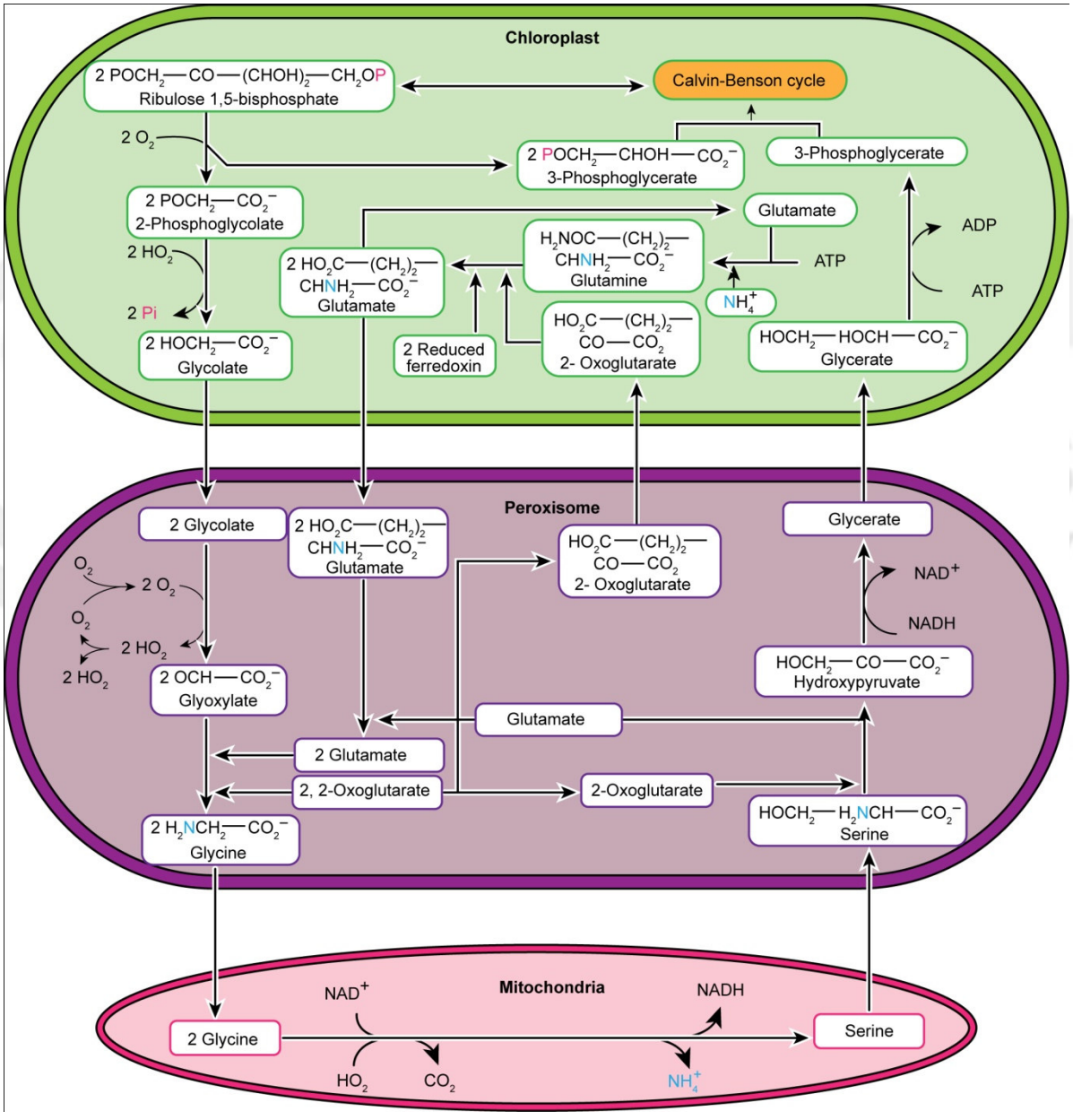


Fig.7.3: A diagrammatic representation of the C_2 cycle highlights cooperation between three organelle compartments. Note that O_2 is incorporated at two places (From Taiz *et al*).

The metabolically useless 2-phosphoglycolate gets rapidly hydrolyzed to glycolate, which then moves out of the chloroplast via a transporter protein in the chloroplast inner envelope membrane and diffuses into the peroxisomes (Fig. 7.3). The glycolate gets oxidized to glyoxylate, and H_2O_2 is produced in this reaction. H_2O_2 gets scavenged by the enzyme *catalase*. The glyoxylate undergoes transamination with glutamate, giving rise to the amino acid glycine (2C). This amino acid now exits the peroxisome and enters the mitochondrial matrix, where two molecules of glycine are acted upon by a multienzyme complex and NAD^+ to produce one molecule each of another amino acid serine (3C) and NH_4^+. In addition, one molecule of CO_2 is also released. Serine (3C) reenters the peroxisome where it undergoes deamination to form hydroxy pyruvate (3C), that subsequently gets reduced to glycerate (3C) by a NADH-mediated reduction. Finally, glycerate reenters the chloroplast stroma via the same transporter protein and gets phosphorylated by spending ATP to yield 3-phosphoglycerate. Complete set of reactions of the C_2 cycle are illustrated in Fig. 7.3.

Operating simultaneously are a set of reactions which help in the assimilation of ammonium ions produced during this C_2 cycle. NH_4^+ generated by the oxidation of glycine diffuses into the chloroplast and converts into glutamine by the expenditure of ATP. Subsequently, the glutamine reacts with the 2-oxoglutarate coming from the peroxisome to yield two molecules of glutamate. Reactions of the C_2 cycle along with the participating enzymes have been depicted in the Table 7.1.

Table 7.1: Summary of the Reactions of the C_2 oxidative photosynthetic carbon cycle (after Taiz & Zeiger)

S. No	Reactions*	Enzyme
1.	**2** Ribulose 1,5-bisphosphate + $2O_2 \rightarrow$ **2** 2-phosphoglycolate + **2** 3-phosphoglycerate	*Rubisco*
2.	**2** 2-phosphoglycolate + $2H_2O \rightarrow$ 2 glycolate + 2 Pi	*Phosphoglycolate phosphatase*
3.	2 Glycolate + $2O_2 \rightarrow$ 2 glyoxylate + $2H_2O_2$	*Glycolate oxidase*
4.	2 $H_2O_2 \rightarrow 2H_2O + O_2$	*Catalase*
5.	2 Glyoxylate + 2 glutamate $\rightarrow$ 2 glycine + 2-oxoglutarate	*Glyoxylate : Glutamate aminotransferase*
6.	Glycine + NAD^+ + [GDC] $\rightarrow$ CO_2 + NH_4^+- + NADH + methylene-[GDC]	*Glycine decarboxylase complex (GDC)*
7.	Methylene -[GDC]+ glycine + $H_2O \rightarrow$ serine + [GDC]	*Serine hydroxymethyl transferase*
8.	Serine + 2-oxoglutarate $\rightarrow$ hydroxypyruvate + glutamate	*Serine : aminotransferase*
9.	Hydroxypyruvate + NADH + $H^+ \rightarrow NAD^+$ + glycerate	*Hydroxy pyruvate reductase*
10.	Glycerate + ATP $\rightarrow$ 3-phosphoglycerate + ADP	*Glycerate kinase*
11.	Glutamate + NH_4^+ + ATP $\rightarrow$ glutamine + ADP + Pi	*Glutamine synthetase*
12.	2-Oxoglutarate + glutamine + $2Fd_{red}$ + 2 $H^+ \rightarrow$ 2 glutamate + $2Fd_{oxid}$	*Ferredoxin-dependent glutamate synthase (GOGAT)*

Reactions 1, 2, 10, 11 and 12 occur in the chloroplast; Reactions 3,4,5, 8 and 9 take place in the peroxisome; Reactions 6 and 7 occur in the mitochondrion. A careful appraisal of the net reaction of the C_2 oxidative photosynthetic cycle highlights some important points (Table: 7.2).

2RuBP +**3**O_2+ **2**Fd $_{red}$ +**2**ATP $\longrightarrow$ **3** 3-PGA+ CO_2+ **2**Fd $_{ox}$ +**2**ADP +**2**Pi

---

Table 7.2 Net reactions of the C_2 oxidative photosynthetic carbon cycle

2 Ribulose 1,5-bisphosphate + **3**O_2 + glutamate

↓ **(reaction 1 to 9)**

Glycerate + **2** 3-phosphoglycerate + NH_4+ + CO_2 + **2**P_i + 2-oxoglutarate

Two reactions in the chloroplasts restore the molecule of glutamate :

2-Oxoglutarate + NH_4^+ + [(2Fd_{red} + 2 H^+), ATP]

(reactions 11 and 12)

↓

Glutamate + H_2O + [(2Fd_{oxid}), ADP + Pi]

and the molecule of 3-phosphoglycerate :

Glycerate + ATP

↓ **(reaction 10)**

3-Phosphoglycerate + ADP

Hence, the consumption of three molecules of atmospheric oxygen in the C_2 oxidative photosynthetic carbon cycle (two in the *oxygenase* activity of *Rubisco* and one in peroxisomal oxidations) take place resulting in:

- the release of one molecule of CO_2 and
- the consumption of two molecules of ATP and two molecules of reducing equivalents (2 Fd_{red}+ 2H^+) for
- incorporating three-carbon skeletons back into the Calvin-Benson Cycle, and
- restoring glutamate from NH_4^+ and α-ketoglutarate.

Thus, the pathway serves to recycle three carbon atoms (ending up as PGA) out of the 4 carbon atoms, i.e., 2 molecules of glycolate. One of them is lost as CO_2. Also, of the two –NH_2 groups donated in the transamination reaction, one is given up as NH_3. These additional inputs of ATP and NADPH would obviously result in the photosynthesis – associated energy cost as well as raising the photon requirement (from 8 to 14 for assimilation of one molecule of CO_2.

Three factors, viz., the kinetic properties of *Rubisco*, temperature, and the relative concentrations of CO_2 and O_2 determine the balance between C_3 and C_2 cycles. For example, the efficiency of photosynthetic assimilation will be adversely limited by changes in the photorespiratory rates. As the temperature increases, there is a progressive shift from C_3 to the C_2 cycle.

So, we can now define photorespiration as "**Light-stimulated oxidation of photosynthetic intermediates to CO_2 with expenditure of energy**".

Box. 7.1: Inability of *Rubisco* to distinguish between O_2 and CO_2

You may be aware that although the reaction with oxygen is harmful to the plant, why in the first place did the evolution of *Rubisco* produce an active site unable to discriminate between CO_2 and O_2? A part of the answer perhaps lies in the fact that *Rubisco* evolved more than 2.5 billion years ago, probably from sulfur metabolism enzymes. During that time, the O_2 concentration was too low and thus there was no selective pressure for this enzyme to choose between CO_2 and O_2. In fact, it was the advent of oxygenic photosynthesis which perhaps brought about a drastic change in the atmospheric gaseous composition. You should also keep in mind that the modern atmosphere contains about 20% O_2, while the CO_2 concentration is a meagre 0.04%. Even though *Rubisco* has a much lower K_m value (higher affinity) for CO_2 (9μM), as against 350μM for oxygen, it is the extremely high concentrations of O_2 which encourage O_2 fixation by *Rubisco*. This obviously results in a wastage of energy. In addition, an increase in temperature also favors oxygenation. The relative solubilities of CO_2 and O_2 at higher temperatures are also different. The ratio of O_2 to CO_2 increases at high temperature. Interestingly, increase in temperature also lowers the affinity of *Rubisco* for CO_2. As more and more CO_2 gets assimilated, the ratio of O_2 to CO_2 increases in the leaf air spaces. This further favours the wasteful *oxygenase* reaction. Thus, higher O_2 concentrations inhibit photosynthesis. Production of 2-phosphoglycolate is a disadvantage since, apart from loss of a potential carbon atom that could have been otherwise fixed, the state of oxidation becomes higher than had been existing before because of the oxygenation reaction. Eventually, one of the two carbons constituting the 2-phosphoglycolate is respired away as CO_2. Thus, instead of the fixation of CO_2, plants under conditions of high light intensity and high temperature evolve O_2. Yet no energy is released in photorespiration. During this process 25% of *oxygenase* reaction takes place while the pathway helps to recover almost 75% of the carbon by the conversion of *oxygenase*-synthesized 2-phosphoglycolate back to 3-phosphoglycerate. It is estimated that if *oxygenase* activity, and photorespiration could be avoided, plants would have fixed 30% more carbon.

7.2.2 Significance of Photorespiration

At first sight, the loss of CO_2 and NH_3 does appear as a wasteful process. There may be upto 50% loss of total carbon fixed through the C_3 cycle along with a consumption of ATP and reduced coenzyme. No energy is released in the process and the productivity is lowered.

A very logical question, therefore, comes to our mind. Why must a plant undertake such an inefficient and wasteful process? If one looks at the whole rationale of the process, we can find out some positives within this otherwise costly and wasteful process.

1. Since a C_3 plant cannot escape the *oxygenase* function of *Rubisco* in nature, and that by losing one carbon atom (and another of nitrogen), the three other carbon atoms are at least conserved, and in the process, one molecule of phosphoglycerate is formed which is ready to be reduced to phosphoglyceraldehyde and enter the Calvin cycle, or be converted directly into sugar. The C_2 cycle therefore, salvages/recovers 75% of the carbon which would have been otherwise lost as glycolate. This salvaging/recovery role of C_2 cycle perhaps justifies its evolutionary importance.

2. Recent experimental studies support the idea of C_2 cycle as a type of "**safety valve**" under stressful situations with high light and limited CO_2 supply. Sufficient O_2 present currently perhaps helps in protecting the plant from photooxidative damage, and it keeps the electron transport chain operative. Photorespiration thus acts as an electron sink for the reactive oxygen species formed during light reaction.

3. Phosphogluconate inhibits some of the key enzymes of Calvin cycle like *triose-phosphate isomerase* (active in regeneration of RuBP), and *phosphofructokinase* (for replenishment of Calvin cycle intermediates). In addition, via this cycle, glyoxylate (inhibitor of *Rubisco*) is not allowed to accumulate.

4. The amino acids glycine and serine are synthesized during this process, which contribute to several other metabolic pathways.

5. H_2O_2 is now considered to be an important signaling molecule in conditions of abiotic and biotic stress. A major source of H_2O_2 is the C_2 cycle itself.

Viewed this way, photorespiration is not entirely a counter productive and a detrimental/harmful process but may even be beneficial to plants under unfavorable conditions. One should, therefore, look upon this process as a "**necessary evil**", vital for the survival of C_3 plants.

Finally, the term photorespiration itself is misleading as it is essentially not a mitochondrial process. Also, it does not generate ATP. Infact, it results in a net loss of carbon. This is more of a salvage pathway involving oxygenase activity.

Photorespiration and Dark respiration both are oxidative processes, they intake O_2 and yield CO_2. But they differ in the following main features (Table 7.3):

Table 7.3: Difference between Photorespiration and Dark respiration

Photorespiration	Dark Respiration
1. Substrate for photorespiration is glycolic acid	1. Substrate for dark respiration is carbohydrate, protein, fat, organic acid.
2. It occurs in green cells only.	2. It occurs in all living cells.
3. It occurs only in presence of light.	3. It occurs in light as well as in dark.
4. It depends upon CO_2/O_2 concentration of atmosphere High O_2 concentration (21%) and less CO_2 concentration (less than 330 ppm) favors photorespiration.	4. It does not depend upon CO_2/O_2 concentration of atmosphere.
5. Substrate is recently assimilated.	5. Substrate is not recently assimilated.
6. It involves peroxisomes, chloroplasts, and mitochondria.	6. It involves cytoplasm and mitochondria.
7. Hydrogen peroxide is formed.	7. Hydrogen peroxide is not formed.
8. Neither reduced coenzymes ($NADH/FADH_2$) nor ATP are produced; hence it is a wasteful process.	8. Reduced coenzymes and ATP are produced for cellular maintenance, synthesis of other products.
9. $NADH_2$ is oxidized to NAD^+	9. NAD^+ is reduced to NADH.

10. It increases with the availability of oxygen.	10. It is not changed by change in oxygen concentration.
11. It involves oxidation both by transfer of electrons to O_2 and incorporation of an oxygen atom derived from molecular O_2.	11. Terminal oxidation involves transfer of electrons to O_2 and the formation of water.
12. Photorespiration is not essential for survival of organism.	12. It is essential for survival of organism.
13. It consumes O_2 at 3 places and release CO_2 at only one place.	13. O_2 is consumed only in terminal oxidation while CO_2 is released at several places.
14. Transamination reaction is involved. One molecule of NH_3 is released per molecule of CO_2 released.	14. No such reaction occurs.

SAQ 1

a) State whether the following statements are true or false.

i) C_3 pathway of photosynthesis is sensitive to concentration of atmospheric oxygen.

ii) Photorespiration gets completed by participation of two organelles.

iii) C_2 pathway integrates with both, nitrogen metabolism and the Calvin cycle.

iv) Catalase enzyme removes H_2O_2 in the peroxisome by converting H_2O_2 into water and oxygen.

v) Dephosphorylation of 2-PGA results in the formation of glyoxylate by the action of phosphoglycolate phosphatase.

vi) Ammonia is produced in the mitochondrion by glycine dephosphorylation during the C_2 cycle.

b) Fill in the blanks.

i) The relative carboxylase and oxygenase activities of *Rubisco* are a function of ……..of the enzyme.

ii) Photorespiratory pathway operates across three sub-cellular organelles: the …… , the …….. and the…… .

iii) Reassimilation of ammonia takes place in the …….. via the …….. pathway.

iv) Photorespiration …….. the net energy cost associated with carbon dioxide fixation.

v) In dry conditions, the oxygenase activity of *Rubisco* can …….. significantly.

vi) ……. oxidation reactions occur during photorespiration.

7.3 C_4 CYCLE – INORGANIC CARBON CONCENTRATING MECHANISM

In the previous unit you have read that plants where RuBP combines with CO_2 to form 3-PGA are the C_3 plants. These plants also show the wasteful process of photorespiration thereby losing the valuable carbon to the atmosphere. Here the *Rubisco* performs the function of oxygenase as well as the carboxylase.

For a long time, Calvin cycle was thought to be the only photosynthetic pathway operative in higher plants. As early as in 1965, **Kortschack, Hartt and Burr** demonstrated Calvin cycle in sugarcane and *Zea mays* and observed that certain 4C compounds/acids like oxaloacetate and malate were the early products of photosynthesis which did not appear after some time. This was a clear indication that these monocots were perhaps first fixing CO_2 temporarily into a 4-carbon compound, in addition to the ongoing Calvin cycle. The mechanism underlying these interesting observations was worked out in details by **Marshall Hatch** and **Rodger Slack** in 1966, who, by using $^{14}CO_2$ established that oxaloacetate was the first product while malate and aspartate were the first stable 4C compounds in the photosynthesis of these monocot plants. These workers also found out that malate subsequently converts into 3-phosphoglycerate. Thus, another CO_2 –fixation pathway was established and was called **Hatch and Slack Pathway** or **Hatch, Slack and Kortschack Pathway (HSK**)/or simply **C_4** pathway. Since the first products are 4C-dicarboxylic acids, the pathway is also called **C_4 –Dicarboxylic Acid Pathway.**

Land plants have evolved a major carbon-concentrating strategy through C_4 photosynthesis, which on one hand compensates for limitations of low levels of CO_2 in the atmosphere, and minimizes *oxygenase* activity of *Rubisco* on the other, so that photorespiration-associated energy losses are minimized.

This unique metabolic pathway takes place in two distinct cell types which exhibit spatial isolation, i.e., they are separated by their own membranes.

A special type of leaf anatomy characterizes the C_4 plants. A central bundle sheath region consisting of a ring of large, chlorophyll containing cells, are surrounded by more loosely arranged spongy mesophyll cells giving the appearance of a ring or wreath.

Box 7.2: Origin of C_4 Pathway

C_4 cycle is known to operate in over 1500 tropical and subtropical species belonging to 19 different angiosperm families. Interestingly, many of them are represented by both C_3 as well as C_4 representatives, indicating their polyphyletic origin. This also suggests that the C_4 pathway is of much recent origin and has evolved in different taxa at different times. The common environmental factors driving their origin are perhaps tropical and xeric habitats. Members of monocot families like Poaceae (maize, sugarcane, *Sorghum*, *Panicum*, *Pennisetum* and other tropical grasses), and Cyperaceae (sage family) along with some dicot families like Chenopodiaceae, Amaranthaceae , Asteraceae, Euphorbiaceae, Portulacaceae, and Nyctaginaceae . Wheat and rice are notable exceptions. They are primarily grass species but grow in temperate climates, and therefore, exhibit C_3 cycle. A very interesting example of the influence of environmental conditions on photosynthesis mode is seen in *Atriplex*.

C_4 plants show anatomical similarities in their leaves. This is markedly different from the internal structure of a C_3 plant. As you have read in the Unit 5 of BBYCT-135 Course on Plant Anatomy, a typical dicot leaf has two distinct tissues - tightly packed palisade cells and loosely arranged mesophyll cells with large air spaces. In sharp contrast, leaves of C_4 plants show much closely packed vascular bundles with smaller air spaces. Each vascular bundle is surrounded by a sheath of large, tightly packed, thick walled parenchymatous cells with large number of chloroplasts. These cells constitute the **bundle sheath**. These tube-shaped tissues are in turn surrounded by loosely packed, helically arranged spongy **mesophyll cells** with smaller chloroplasts. This characteristic wreath like configuration of the bundle-sheath cells was first observed and described by the German anatomist G. Haberlandt in as early as 1884, named it as **Kranz type anatomy** (Kranz in German means a wreath). Haberlandt had even suggested that this special type of anatomy was indicative of some division of labor between the mesophyll and bundle-sheath cells.

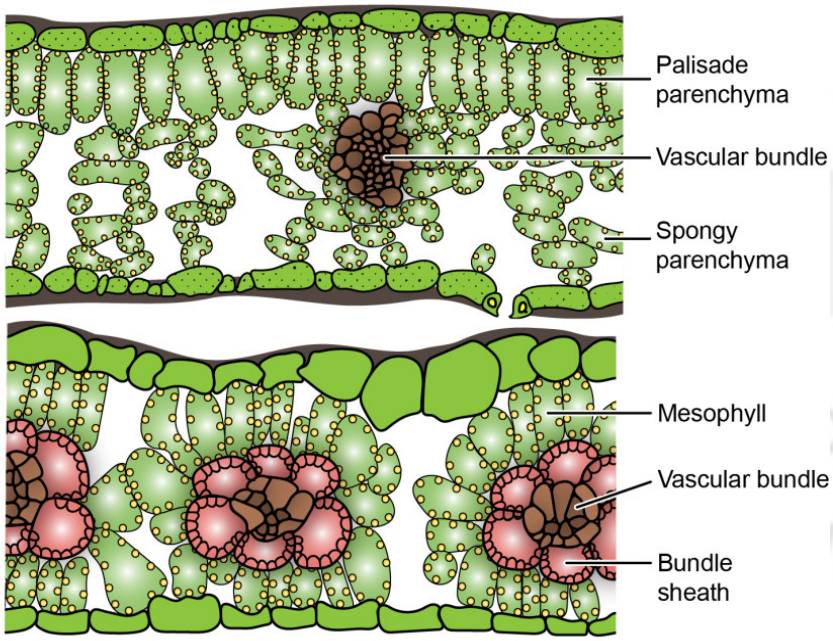


Fig. 7.4: Comparison between the leaf anatomy of a C_3 (upper) and a C_4 plant (below). In the C_4 leaf the assimilatory parenchyma is organized concentrically around the vascular tissue. The bundle sheath cells are large with big chloroplasts. They are surrounded by loosely packed helically arranged mesophyll cells giving the appearance of a wreath or "Kranz".

The typical dark-green veins in a C_4 plant represent the vascular bundles along with their bundle sheath. Another characteristic feature of C_4 leaf anatomy is the presence of **dimorphic plastids**. Chloroplasts in cells of the bundle sheath are much larger but **agranal** (lacking granal stacks), more concentrically arranged with unstacked thylakoid membranes. However, these plastids have abundant starch (Fig. 7.5). On the other hand, the randomly arranged, small sized chloroplasts of the mesophyll are **granal** (with well-developed grana) but starch free. In addition, a reticulum of membranes called **peripheral reticulum** is localized inner to the chloroplast envelope.

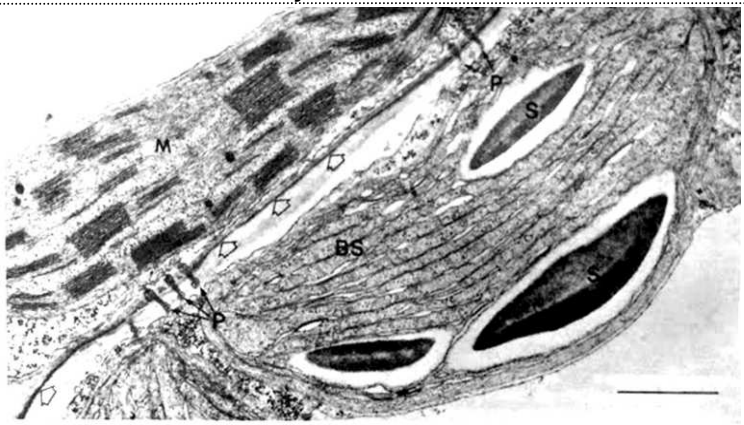


Fig. 7.5: Electron micrograph of maize leaf (C_4) showing chloroplast dimorphism. Plasmodesmata (P) connect the two cells. The bundle sheath chloroplasts (BS) are agranal and contain starch (S). Mesophyll chloroplasts (M) are granal but starch free (From Mohr & Schopfer).

This typical anatomical adaptation ensures a continuity of metabolites between two types of tissues. In some plant species, plasmodesmata have been seen to connect cells of the bundle sheath and mesophyll. This anatomical arrangement also ensures very efficient CO_2 trapping from throughout the cytosol (cytoplasm outside the organelles), a short diffusion path of the fixed CO_2 by the transfer of C_4 and C_3 acids and an equally efficient system of sugar export from the bundle sheath to the vascular tissue. The enzyme *PEP carboxylase* abounds in the outer mesophyll cells, whereas *RuBP carboxylase* is restricted to the bundle sheath cells. The overall strategy is to trap maximum atmospheric CO_2 and to transport it to the bundle sheath cells to enhance the Calvin cycle. The plasmodesmata enable the diffusive flux of metabolites between mesophyll and bundle sheath cells.

The C_4 pathway takes place simultaneously in mesophyll and bundle sheath cells. Both the cells have their cell walls and cell membranes. Here, the initial CO_2 fixation and the *Rubisco* activity are **spatially separated** into these two morphologically distinct types. The C_4 pathway can be described in the form of **five** distinct stages:

1. The initial substrate for C_4 pathway is HCO_3^- and not CO_2.Carbon dioxide first dissolves in water in the mesophyll cytoplasm and gets ionized into HCO_3^- by the enzyme *carbonic anhydrase*. Using this HCO_3^- as a substrate, carboxylation of the C_3 compound phosphoenol pyruvate (PEP) in the mesophyll cells is catalyzed by the enzyme *phosphoenolpyruvate carboxylase* (*PEPcase*).This enzyme shows a very high affinity for HCO_3^- and can thus, fix CO_2 many times more than *Rubisco.* Moreover, *PEPcase* does not have an *oxygenase* function, so no competition exists between CO_2 and O_2 unlike *Rubisco*. *PEPcase* fixes and concentrates CO_2 in the form of malate. This would help *Rubisco* to act to its maximum efficiency. Also, the enzyme *oxygenase* activity will be suppressed. The reaction product, oxaloacetate (4C) is moderately stable and gets subsequently reduced to malate (4C) by the expenditure of NADPH or gets deaminated (transaminated) with glutamate to yield aspartate (4C).

2. Both malate and aspartate are stable and are transported into the adjacent bundle-sheath cells through plasmodesmata connections.

3. The 4-carbon acid (malate) is oxidized and decarboxylated to yield pyruvate(3C) and CO_2. $NADP^+$ is reduced by either *$NADP^+$ malic enzyme* or by *PEP carboxykinase*. Labelling experiments have confirmed that the CO_2 molecule released in the bundle sheath cells is the same which was fixed into OAA in the mesophyll cells. This CO_2 is now fixed (for the second time) by *Rubisco* and the reaction is the same as described in the C_3 cycle which CO_2 gets incorporated into C-1 of the 3-PGA.

4. The pyruvate (3C) so formed after the decarboxylation of malate and the second CO_2 fixation is transferred back in the mesophyll cells.

5. Back in the mesophyll cells, pyruvate gets converted into PEP by a unique reaction which involves the simultaneous phosphorylation of two different molecules by one molecule of ATP: Pyruvate to PEP and phosphate to pyrophosphate. The pyrophosphate is subsequently hydrolyzed to phosphate and an additional molecule of ATP is required to transform AMP to ADP. Thus, two high energy phosphate groups of ATPs are utilized in the regeneration of PEP, and to keep the reaction going. The general outline of the C_4 mechanism is illustrated in Fig.7.6 a, b.

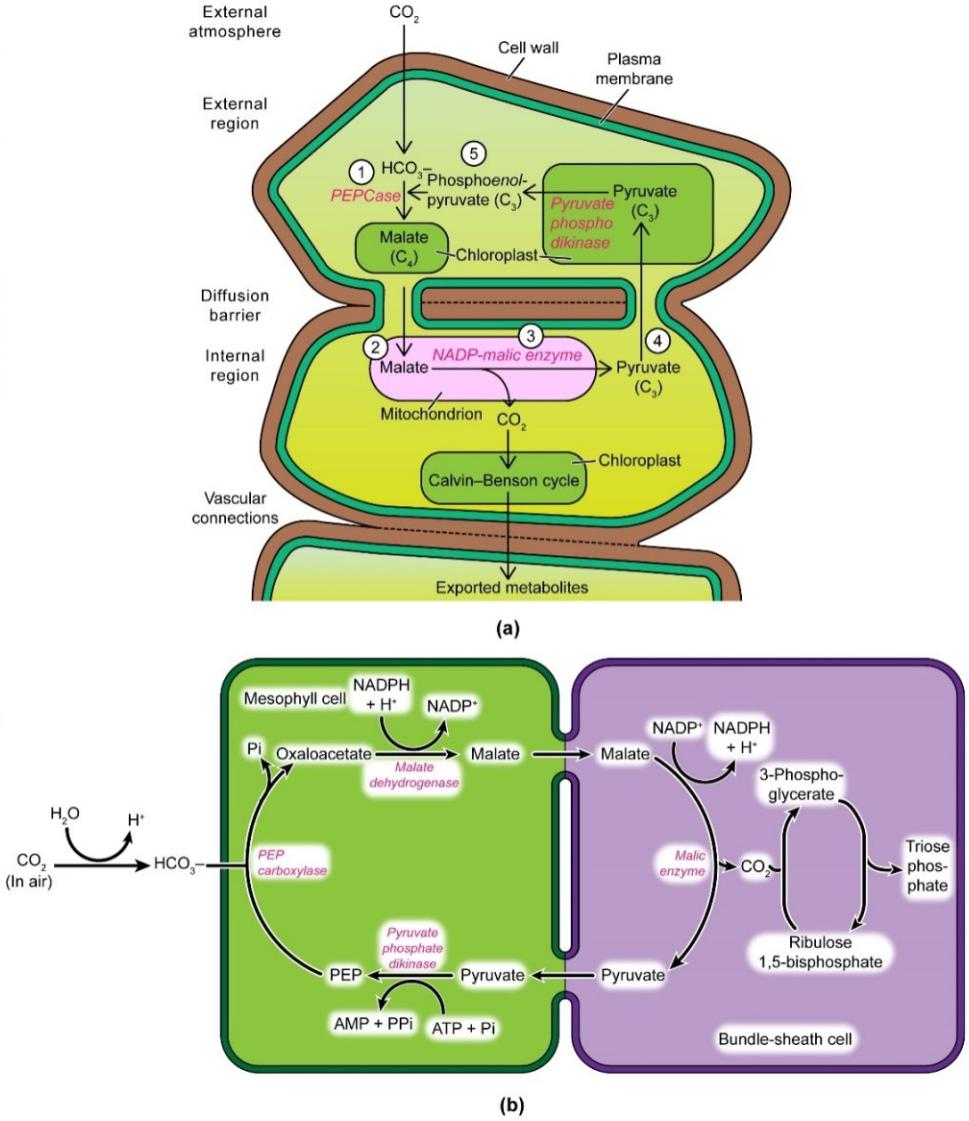


Fig. 7.6: a) General outline of C_4 cycle depicting FIVE successive stages in two different compartments (Modified from Taiz *et al*). b) Another outline of C_4 cycle shows a spatial isolation of the two cycles and chemical transformation of CO_2.

In Mesophyll Cells

PEP (3C) + H_2O + NADPH + CO_2 → Oxaloacetate (4C)

Oxaloacetate (4C) → Malate(4C) In Bundle Sheath Cells

Malate(4C) + NADP → Pyruvate(3C) + NADPH+ CO_2

Pyruvate(3C) + ATP → PEP(3C) + AMP + $H_2P_2O_7^{2-}$

$P_2O_7^{4-}$ + H_2O → $HOPO_3^{2-}$ (in Mesophyll)

2AMP + 2ATP → 2 ADP

Net CO_2 (mesophyll) + 2ATP +2 H_2O → CO_2 (bundle sheath) +2ADP+2H+2 $HOPO_3^{2+}$

The fixation of 6 molecules of CO_2 through C_4 pathway requires 6 × 2 = 12ATP (in C_4 cycle) and the usual 18 ATP in PCR cycle. Total ATP requirement = 12 + 18 = 30 ATP per 1 molecule of glucose synthesized.

Various enzymes involved in the C_4 plants along with the enzymes are given below in Table 7.4:

Table 7.4: Summary of the reactions and the enzymes involved in C_4 cycle

ENZYME	REACTION
1. *PEPCase*	Phosphoenolpyruvate + HCO_3→ oxaloacetate + Pi
2. *NADP-malate dehydrogenase*	Oxaloacetate + NADPH + H^+→ malate + $NADP^+$
3. *Aspartate aminotransferase*	Oxaloacetate + glutamate → aspartate + α-ketogutarate
Decarboxylating enzymes	
4. a. *NADP-malic enzyme*	Malate + $NADP^+$→ pyruvate + CO_2 + NADPH + H^+
b. *NAD-malic enzyme*	Malate + NAD^+→ Pyruvate + CO_2 + NADH + H^+
5. *Phosphoenolpyruvate carboxykinase*	Oxaloacetate + ATP → phosphoenolpyruvate + CO_2 + ADP
6. *Alanine aminotransferase*	Pyruvate + glutamate → alanine + 2-oxoglutarate
7. *Pyruvate-phosphate dikinase*	Pyruvate + P_i + ATP → phosphoenolpyruvate + AMP + PPi
8. *Adenylate kinase*	AMP + ATP→ 2ADP
9. *Pyrophosphatase*	PP_i+ H_2O → 2 Pi
Note : P_i and PP_i indicate inorganic phosphate and pyrophosphate, respectively.	

Despite the novelty of the C_4 cycle, you should be clear about this pathway that it is not totally independent of the Calvin cycle, and thus, must be viewed as an **adjunct** to the C_3 pathway. The C_4 cycle serves as a CO_2-concentrating

mechanism for the bundle sheath cells where a high concentration of CO_2 favors the carboxylase activity of *Rubisco*, thereby suppressing photorespiration. These triose phosphates are ultimately synthesized by the PCR cycle.

To conclude, two main features of C_4 plants help to overcome the harmful effects of high temperature and high light intensity, where they grow:

1. High photosynthesis rates under warm climates due to high affinity of *PEPcase* for its substrate HCO_3^-. Stomatal opening is reduced at high temperature which reduces water loss and makes these plants well adapted for drought and desiccation conditions that characterize the typical deserts.

2. High concentration of CO_2 in bundle sheath cells suppress *oxygenase* activity thereby minimizing the harmful C_2 Cycle.

Major differences between the C_3 and C_4 plants are summarized in Table:7.5.

Table 7.5: Differences between C_3 and C_4 plants

C_3 Plants	C_4 Plants
1. It occurs in most green plants except some tropical monocots and a few dicots.	1. It occurs in tropical monocots and a few dicots.
2. All the green cells of the leaf have Calvin cycle.	2. Plants have Hatch-Slack cycle in the mesophyll and Calvin cycle in the cells of the bundle-sheath.
3. All green cells of the plant possess only one CO_2 acceptor, the Ribulose 1, 5 bisphosphate.	3. There are two CO_2 acceptors in leaf cells. phosphoenolpyruvate (PEP) in the mesophyll and RuBP in the cells of the bundle-sheath.
4. A 3-carbon compound. Phosphoglyceric acid is the first stable product of photosynthesis	4. A 4-carbon compound oxaloacetic acid is the first product . The stable products are malic acid and aspartic acid.
5. Photorespiration occurs which reduces the photosynthetic yield.	5. Photorespiration do not occur and, therefore, the efficiency of photosynthesis is further increased.
6. Calvin cycle occurs in all the green cells because Ribulose bisphosphate and the enzymes of the Calvin cycle are present within them.	6. Ribulose bisphosphate and the enzymes of the Calvin cycle are present only in the bundle sheath cells and the mesophyll cells contain the enzymes of the Hatch-Slack cycle.
7. In C_3 plants the optimum temperature ranges between 10-25℃	7. In the C_4 plants the optimum temperature ranges between 30-45℃.
8. In C_3 plants the maximum light intensity for photosynthesis is 1000-1200-foot candles and it is the saturation intensity.	8. In C_4 plants photosynthesis occur at a much higher light intensity, and even full sunlight is not the saturation intensity.

9.	Calvin cycle can reduce the CO_2 concentration around the plants to only 50 ppm due to lesser affinity of RuBP for CO_2.	9.	Hatch-Slack cycle can reduce the CO_2 concentration to even less than 10 ppm due to stronger affinity of PEP for CO_2.
10.	C_3 plants are less efficient in photosynthesis. The net rate of the process is only15.35 mg CO_2 per dm^2 of leaf area per hour.	10.	C_4 plants are more efficient in photosynthesis. The net rate of the process is 40-80 mg of CO_2 per dm^2 of the leaf area per hour.
11.	In C3 plants Oxygen has an inhibitory effect on photosynthesis	11.	In C4 plants no inhibition by Oxygen occurs in this process.
12.	The value of compensation point is higher.	12.	The value of compensation point is lower.
13.	C_3 plants do not have "Kranz anatomy.	13.	Kranz anatomy is found in these plants.
14.	All the green cells of leaf possess same type of chloroplast with well-defined grana and both photosystems I and II.	14.	Chloroplasts are dimorphic. Mesophyll cells have granal chloroplast whereas the bundle sheath cells have very large chloroplast with starch grains. Chloroplasts lack photosystem II and depends on the chloroplast mesophyll for the supply of NADPH.

Box 7.3: Kranz Anatomy and the Environment

One cannot fail to appreciate also another advantage to the plants by virtue of the Kranz anatomy. Oxygen tension is likely to be high in the outer areas and low in the innermost parts of the leaf, thus, diminishing oxygenase activity of *Rubisco*. However, if there is some unavoidable photorespiration in the inner bundle sheath cells, the CO_2 so formed can be trapped again by *PEPcarboxylase*, as it tries to escape. No wonder, then, the C_4 plants are highly efficient photosynthesizers. Grasses are widely considered to be one of the most photosynthetically efficient species colonising the earth and this may be due to the C_4 pathway they possess.Many of our important crops like corn, besides sugarcane mentioned above, are also C_4 plants. On the other hand, the dicots are by and large C_3 plants. However, there are some notable exceptions among dicots, for example, sugarbeet and members belonging to the family Chenopodiaceae and Portulacaceae. It is of interest that these plants also have the "Kranz" anatomy – much like monocots – instead of a palisade layer and spongy parenchyma typical of dicotyledonous leaves. Overall, C_4 pathway is an adaptation to enable plants to survive better under conditions of higher temperatures and poor water supply – it is of interest that if one studies the distribution of $C_3$and C_4 species geographically, the former dominate in the temperature regions, but as one approaches the equator, the C_4 species dominate.

Kranz anatomy is also seen in the C_4 dicot *Flaveria australasia* (Asteraceae). It looks as if spatial isolation of C_4 and PCR cycles; along with Kranz anatomy have been traditionally thought to be the key prerequisites for plants showing Hatch and Slack cycle.

However, there are some interesting exceptions where C_4 carbon fixation takes place without Kranz anatomy. For example, *Bienertia sinuspersici* (Amaranthaceae), and

species like *Suaeda aralocaspica* (=*Borszczowia aralocaspica*), *Bienertia cycloptera* and *B. kavirense*, growing in hot, dry, and highly saline environments of Deserts in Central Asia and Persian Gulf, are known to conduct single celled C_4 photosynthesis within individual chlorenchyma cells. The chloroplast itself is partitioned into two distinct cellular compartments-a central compartment (CCC) and the peripheral chloroplast compartment (PCC).

Some diatoms (unicellular marine protists) and certain unicellular algae can also perform C_4 photosynthesis within a single cell. The plasma membrane –bounded HCO_3^- pumps accept HCO_3^- in the cytoplasm from where it is transported to *Rubisco*-rich **carboxysomes.** The carboxysomes are specialized protein-rich micro-compartments which contain enzymes for photosynthesis. Carbon dioxide is concentrated with the help of carboxysomal *carbonic hydrase* which then supplies it to *Rubisco*.

7.3.1 Energy Costs for C_4 Mechanism

The C_4 crop plants are much more productive than their C_3 counterparts as the rate of their CO_2 fixation is almost 2-3 times more. However, this enhanced productivity requires an additional input of energy. As is clear from the above equation, 2 high energy phosphate groups of ATPs are utilized for regeneration of 1 mol of PEP (acceptor of CO_2). So, enrichment of each CO_2 molecule (building up of CO_2 concentration in the bundle sheath cells) in the C_4 pathway will spend 2 additional ATPs. This is exclusive of the 3 ATP and 2 NADPH required for fixation of one CO_2 molecule in the PCR cycle. Thus, the net energy requirement for assimilation of CO_2 by the Hatch and Slack plants is 5 ATP and 2 NADPH. You should realize that despite this costly CO_2 fixation mode, the C_4 plants are more productive than C_3 plants as there is practically no photorespiratory loss at higher temperatures. Since this gain more than compensates for the additional energy expense in C_4 plants, the net requirement of ATP and NADPH per CO_2 mol fixed (photosynthesis-photorespiration) is much lower in C_4 plants than in C_3 plants.

Variations among C_4 cycle : Within the general pattern of C_4 cycle described earlier the release of CO_2 in the bundle sheath cells occurs in three different ways. Although the primary carboxylation reaction occurring in the mesophyll cell cytosol is the same for all the three cell types, viz, $NaDP^+$ - Me (*Malic Enzyme*) type , NaD^+ - Me (*Malic Enzyme*) type, and PCK (*PEP-Carboxylase*) type. They are distinguished on the basic of the nature and intracellular location of the decarboxylation catalyzing enzyme (CO_2 concentrating enzyme) in the bundle sheath.

SAQ 2

a) Complete the following statements with appropriate words/terms.

i) CO_2 is accepted by a 3 - carbon compound ……. in C_4 plants.

ii) An interesting anatomical characteristic of Hatch and Slack plants is called ……anatomy, where vascular bundles are surrounded by …… rings of cells.

iii) Dimorphic plastids in C_4 plants show that …….. enzymes are absent in plastids of mesophyll cells, while…… is absent from those of bundle-sheath cells.

iv) Sugars are produced in …….of C_4 plants.

v) In C_4 plants, a total of ….. ATP molecules are required to fix 6 molecules of CO_2.

b) Which among the following statements are true? Write T for true and F for false in given boxes.

i) Wheat and rice are the major cereal C_4 crops. [ ]

ii) No photorespiration occurs in C_4 plants. [ ]

iii) RuBP has a higher affinity for CO_2thanPEP. [ ]

iv) In NAD^+-Me type, malate is the C_4 molecule that is transported from mesophyll to the bundle-sheath cells. [ ]

v) In C_4 cycle, a total of 2 carbons are needed for CO_2 enrichment per 1CO_2 molecule. [ ]

7.4 CRASSULACEAN ACID METABOLISM (CAM)

Dry and hot habitats offer very stressful conditions for photosynthesis. Yet some categories of plants have successfully adapted themselves to conditions of severe water shortage. Many cacti, succulents, some epiphytes growing in the tropical rain forests along with many orchids have developed a unique strategy to photosynthesize under desert conditions.

Such plants exhibit a specialized pattern of inorganic carbon concentrating mechanism of photosynthesis, viz., **Crassulacean Acid Metabolism (CAM)**. It is so named as it was first discovered in *Bryophyllum calycinum* , a member of the family Crassulaceae. This unique pattern of photosynthesis has been reported in plants belonging to 23 different angiosperm families, many of which include commercially important plants like pineapple, cacti, and aloes. Some of the families with CAM plants are Bromeliaceae,Cactaceae, Euphorbiaceae, Vitaceae, Orchidaceae, Cyperaceae and Agavaceae and many more. In addition, CAM plants are also represented in Gymnosperm families Zamiaceae and Welwitschiaceae (Gnetales) and the pteridophytes of the family Isoetaceae, Polypodiaceae and Vittariaceae.

Although not as efficient as the C_3 or C_4 plants, the CAM mode of carbon fixation is well adapted to survive under conditions of extreme water deficiency. The plants are well adapted to avoid transpiration and photorespiration on account of their **scotoactive** stomatal behavior, which means that in these plants, the stomata remain open during night, and closed during daytime. This helps them in maintaining a high concentration of CO_2 in leaves during daytime.CAM plants exhibit a combination of C_3 and C_4 cycles which occur both spatially (in different parts of the same cell) and temporally, i.e., C_4 cycle at night and C_3 cycle during day.

Incidentally, the French scientist de Saussure observed as early as in 1804 that *Opuntia* (cactus), when illuminated produced O_2 even in the absence of CO_2 !! An Englishman, Benjamin Heyne, while staying in India in 1812-13 made some very interesting observations. He found that the leaves of the ornamental plant *Bryophyllum calycinum* growing in his garden had a normal taste of herbs in the afternoon, whereas if chewed in the morning, the taste was very acidic.

In CAM plants parenchyma cells store acids and water. Transpiration and CO_2 uptake occurs only at night time when the stomates open. There is a reciprocal diurnal fluctuation of malic acid and stored glucans. Since CO_2 capture and *Rubisco* action are temporally separated, i.e., the two events are separated over time. This is in sharp contrast to the situation found in C_4 plants, where these two processes are spatially isolated.

CO_2 fixation at night is made possible by the acceptor PEP (3C) and the reaction is catalyzed by the enzyme *PEP carboxylase*. This nocturnal CO_2 fixation or "dark carbon fixation" follows the same pattern as in C_4 plants. In addition, CO_2 is also obtained endogenously from Krebs cycle.Despite enhanced CO_2 availability, a high concentration of CO_2 cannot be attained in a plant at night as it is rapidly utilized for malate synthesis by the enzyme *PEP carboxylase*. Moreover, this enzyme operates at low CO_2 concentration. The 3C acceptor PEP is generated by either degradation of starch or by glycolytic breakdown of stored carbohydrates like glucans. The immediate product OAA (4C) formed is quickly reduced to malate by *NAD-dependent malate dehydrogenase* enzyme in the cytosol. Malate (4C) is pumped at the expense of energy into the vacuoles where it remains for rest of the night. This makes the vacuolar sap highly acidic. Since CO_2 uptake takes place mainly during night, accumulation of organic acids in the large vacuole leads to a high degree of acidification of the cell sap at night. Thus, the malate level is very high before sunrise while the carbohydrate level is the lowest as it has been used in malate synthesis. These acids will gradually get decarboxylated during daytime when carbohydrates are formed in photosynthesis. CO_2 so produced is fixed by the Calvin cycle.

Since most of malic acid is in an undissociated form, it does not exert much osmotic pressure than when in its ionized form, thereby allowing more accumulation possible in unusually large vacuoles whose contents become highly acidic (approx. pH 3.0) during the night. ATP required for CAM metabolism is generated by the oxidative phosphorylation in the mitochondria.

During day time, the stored malate is retrieved from the vacuole, transported to the chloroplast and decarboxylated by mechanisms which you have already studied in the preceding sections for C_4 plants, viz., *NADP malic enzyme*, Mitochondrial *NAD enzyme* or mitochondrial *phosphoenolpyruvate carboxykinase* to give pyruvate, $NADP^+$/NADPH and CO_2. The evolved CO_2 is now made available to the chloroplast or Benson-Calvin cycle. On the other hand, the corresponding 3-carbon acids are converted to sucrose or starch. Events occurring in CAM plants are depicted in Fig. 7.7.

Although CAM plants do show some similarities with the C_4 plants, there are significant differences too. Whereas a special type of anatomy is required to bring about spatial separation between C_4 carboxylation and PCR cycle, both the processes do occur simultaneously in CAM plants in the same cell except that they are separated in time. Also, in CAM plants, the PEP is derived from stored carbohydrates and in turn could also act as a substrate for their resynthesis.

From the point of view of evolution, it is believed the occurrence of CAM cycle in primitive ferns and *Welwitschia* itself indicates that this mode of photosynthesis perhaps evolved earlier than the C_4 mode.

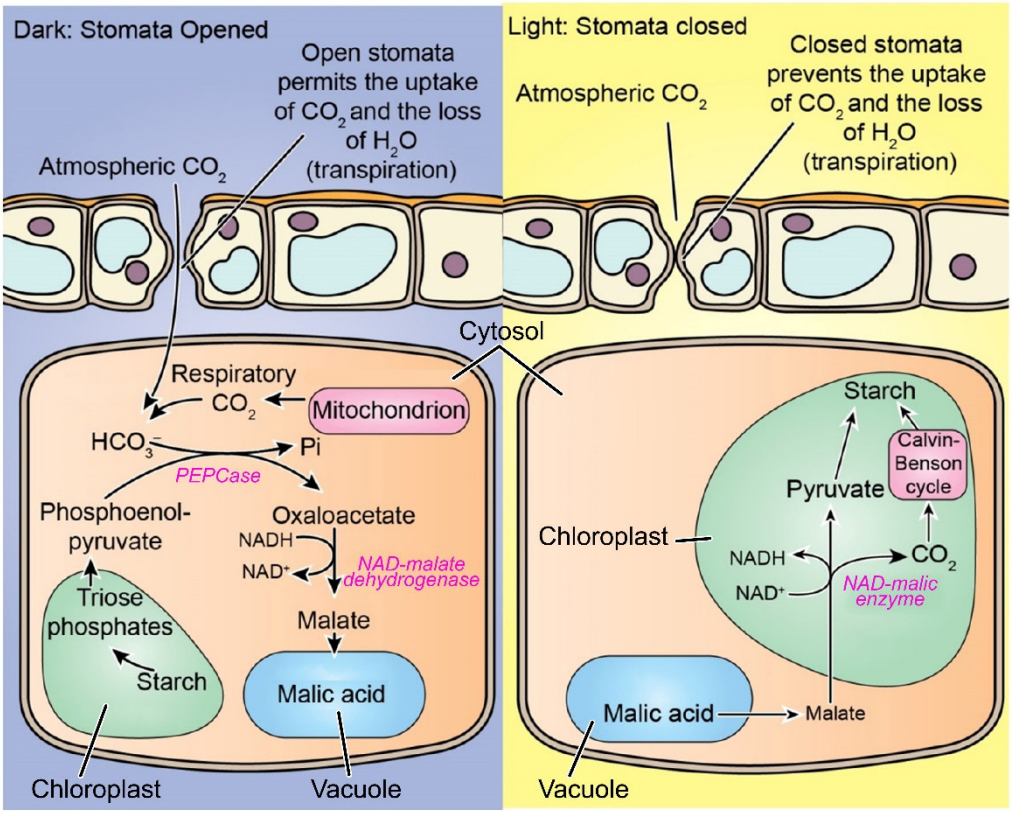


Fig. 7.7: CAM metabolism highlighting temporal separation of CO_2 uptake and Calvin-Benson Cycle (From Taiz *et al*).

7.4.1 Significance of CAM Plants

CAM plants are also unique as they can grow in exceptionally dry habitats where both C_3 and C_4 plants fail to survive. Ability to close stomata during day along with the capacity to retain and reassimilate the respired CO_2 helps to prevent loss of vital carbon during severe drought conditions.

Some CAM plants also exhibit a facultative approach towards photosynthesis modes. These plants can shift their photosynthetic mechanism in response to variations in environmental conditions.The ice plant *Mesembryanthemum crystallinum* (Aizoaceae), *Clusia* and *Agave* are typical CAM plants, but can switch over to C_3mode under conditions of abundant water supply. On the other hand, a member of Bromeliaceae, *Tillandsia usneoides*is is not a succulent, yet it photosynthesizes by the CAM mode. Although CAM plants are photosynthetically less efficient than the C_4 plants, they are best adapted to xeric environments.

SAQ 3

a) Fill in the blanks:

i) CAM plants store …….. in vacuoles at night.

ii) These plants conserve water by ……stomata during day time and keeping them……during night.

iii) Two types of cycles viz, …… and…… operate in CAM plants.

iv) Desert succulent plants exhibiting CAM cycle have ……. photosynthetic rate.

b) Which among the following statements are true? Write T for true and F for false in given boxes.

i) CAM plants have few, scattered photoactive stomata. [ ]

ii) Photorespiration in present in CAM plants. [ ]

iii) CAM plants exhibit chloroplast dimorphism like that in C_4 plants. [ ]

iv) There is a diurnal acid cycle in CAM plants involving acidification and deacidification. [ ]

7.5 BACTERIAL PHOTOSYNTHESIS AND CHEMOSYNTHESIS

Many bacteria also perform photosynthesis. Since they use inorganic materials, they are called **photolithotrophs** or simply photosynthetic bacteria. One of the notable features of bacterial photosynthesis is that these photoautotrophs are **anoxygenic** in nature, i.e., compounds other than water are donors of electrons; thus, oxygen is not released. As you have already read in Unit 5 on discovery of photosynthesis and early concepts, it was C.B. van Niel, who first characterized photosynthesis is purple sulfur bacteria and his work helped to established H_2O as a source of O_2. In addition, bacterial photosynthesis utilizes light energy in infra-red region of the spectrum – which appear dark to human eyes. Only one photosystem operates in bacteria. There are **three** major groups of photosynthetic bacteria.

1. **Green sulfur bacteria -** These are nitrogen fixing prokaryotes containing bacteriochlorophyll, mainly bacterioviridin (*chlorobium* chlorophyll) and carotenoids. They absorb light quanta at an optimum of 650-660 nm and H_2S is the electron donor.

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

e.g., members of Chromatiaceae, viz., *Chlorobium*, *Chlorobacterium* and *Chloropseudomonas.*

2. **Purple Sulfur Bacteria**- These are two types

a) Purple sulfur bacteria : These bacteria contain bacteriochlorophyll which absorbs invisible infra-red wavelength in the regions to 800-890 nm. Different sulfur compounds and molecular hydrogen are the hydrogen donors.

$$6CO_2 + 15\ H_2O + 3Na_2S_2O_3 \xrightarrow{\text{light}} C_6H_{12}O_6 + 6H_2O + 6NaHSO_4 + \text{Energy}$$

e.g., *Chromatium.*

b) Purple non-sulfur bacteria : They have bacterial chlorophyll but unlike the purple sulfur bacteria, they utilize non-sulfur compounds, like alcohols and organic acids as the electron donors.

$$6CO_2 + 12CH_3CHOH.CH_3 \longrightarrow C_6H_{12}O_6+12CH_3CO.CH_3+6H_2O$$

e.g., Members of Rhodospirillaceae viz., *Rhodospirillum, Rhodopseudomonas*

3. **Heliobacteria -** Heliobacteria belong to the primitive archaebacteria and do not have membrane bound chlorosomes on chromatophores as seen in other photosynthetic bacteria. They contain plasma membrane-bounded bacteriochlorophyll $_g$ which acts as the reaction center and absorbs light in 788, 576 and 370 nm., e.g., *Halobacterium*.

Mechanism of Bacterial Photosynthesis

Since only one pigment system (P890) in found in bacteria, only one photochemical act is possible. In such a situation, Emerson's enhancement effect is absent. Bacterial photosynthesis is characterized by reduction of NAD^+ to $NADH+H^+$ instead of the usual $NADP^+ \longrightarrow NADPH + H^+$ in green plant photosynthesis. Major differences between green plant and bacterial photosynthesis are summarized in Table 7.6 :

Table 7.6: A comparison between Bacterial and Green plant photosynthesis.

Bacterial Photosynthesis	Green Plant Photosynthesis
1. Photosynthetic apparatus is chromatophore.	1. Photosynthetic apparatus is chloroplast.
2. Photosynthetic pigments are bacteriochlorophyll, bacterioviridin, and open chain aliphatic carotenoids.	2. Photosynthetic pigments are chlorophylls, carotenoids and phycobilins.
3. Reaction center is P_{890}, which absorbs > 700 nm wavelength of light.	3. Reaction center is P_{700} and P_{680} which absorbs <700 nm wavelength of light.
4. Two photosystems are connected in parallel.	4. Two photosystems are connected in series.
5. O_2 is not evolved as there is no photolysis of water.	5. O_2 is evolved as there is photolysis of water.
6. Emerson effect is not seen	6. Emerson effect is seen.
7. Reductant used is NAD^+	7. Reductant used is $NADP^+$
8. Cyclic electron transport is more common	8. Non-cyclic electron transport is more common.
9. Cytochromes involved here in electron transport are of "c" type.	9. Cytochromes involved here in electron transport are of "b" type.
10. Pigment carotenoids are open chain aliphatic molecules.	10. Carotenoids contain bicyclic α and β carotene.

7.5.1 Chemosynthesis

Autotrophic non-green aerobic bacteria utilize chemical energy in place of solar energy to synthesize food. In this case CO_2 assimilation takes place by energy derived from oxidation of certain inorganic compounds. This process is called **Chemosynthesis**. Chemosynthetic bacterial include phylogenetically diverse taxa including methanogenic archaea, sulfur oxidizing proteobacteria, and iron-oxidizing bacteria. Thus, chemosynthesis can be defined as the biological conversion of one or more carbon-containing molecules (usually CO_2 or methane) as a source of energy replacing sunlight/in place of sunlight. Many of these microorganisms live in dark regions of the earth like deep oceans, hydrothermal vents, cold seeps, isolated cave water. Chemosynthesis is believed to be perhaps the first type of metabolism that evolved on earth, much before photosynthesis and cellular respiration.

Oxidation of different inorganic substrates like nitrogen, sulfur iron compounds as well as carbon, phosphorus and hydrogen by the chemosynthetic bacteria is an exergonic reaction, i.e., it results in release of ATP. The reactions carried out by nitrifying bacteria are given below:

Nitrifying bacteria: These bacteria convert ammonium ions to nitrates

These bacteria convert ammonia in soil into nitrates (NO_3^-) by a two step process. During the first step called **nitritation**, *Nitrosomonas* converts NH_3 into NO_2 (Nitrogen dioxide).

$2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O$

Nitration forms the second step of nitrification, where nitrogen dioxide is converted to nitrates

This conversion is carried out by *Nitrobacter*

$2H^+ + 2e^- + ½O_2 \rightarrow H_2O$

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

$2NO_2^- + O_2 \rightarrow 2\ NO_3^-$

Box 7.4: Submerged Aquatic Macrophytes (SAM)

Some vascular plants carry out their entire life cycle under water. These are called Submerged Aquatic Macrophytes (SAM) e.g., pondweeds and sea grasses. Availability of CO_2 exerts some evolutionary selective pressure on such plants.

The availability of CO_2 varies considerably, according to pH. Both inland and marine waters are alkaline and characterized by low levels of dissolved CO_2. In more productive inland lakes, low pH results in an increase in dissolved CO_2 content whereas HCO^-_3 ions are more abundant at high pH. SAM plants are thought to have originated from C_3 progenitors. Although the adaptive significance of the so called "CO_2 pumps" for HCO_3 in SAM plants is not fully understood, considerable CO_2 concentrations are achieved in leaves which suppresses photorespiration.

SAQ 4

a) Complete the following statements with appropriate words/terms.

   i) Photosynthetic bacteria use electrons from compounds other than …… .

   ii) Green sulfur bacteria have chromatophores containing the pigment …… .

iii) Heliobacteria are primitive …….. where the membrane-bounded chromatophores are ……..

iv) In bacteria, the coenzyme ……. is reduced to…….. .

v) Chemosynthetic bacteria utilize……..energy to synthesize food.

b) Which among the following statements are true? Write T for true and F for false in boxes.

i) Many of the chemosynthetic bacteria utilize sunlight for CO_2 assimilation. [ ]

ii) *Chromatium* is a purple non-sulfur bacterium. [ ]

iii) Both cyclic and non-cyclic electron transport operates in photosynthetic bacteria. [ ]

iv) Wave lengths greater than 700nm are utilized by bacteria. [ ]

v) Chemosynthesis is carried out by cyanobacteria. [ ]

7.6 FACTORS AFFECTING PHOTOSYNTHESIS

The rate of photosynthesis is affected by several factors which are both **external** and **internal**. These factors interact with each other and the overall rate reflects the ability of a plant to respond to different environmental conditions. The external factors include light, CO_2, O_2, temperature, water, mineral nutrients, and pollutants. The internal factors affecting photosynthesis are chlorophyll content, leaf age, hydration of the protoplasm, accumulation of photosynthetic end products, and hormones.

7.6.1 The Principle of Limiting Factors

Earlier physiologists believed that the intensity of factors affecting photosynthesis could be measured in terms of three cardinal points, viz., minimum, optimum, and maximum. Optimum point is that value where a physiological process proceeds at its highest velocity. Liebig was the first to appreciate that the smallest factor in soil was perhaps important in affecting the overall rate of productivity and proposed this idea as "**Law of Minimum**".

Blackman (1905) elaborated upon Liebig's ideas and gave the **Law of Limiting Factors**. The law states that "when a process is conditioned as to its rapidity by a number of separate factors, the rate of this process is limited by the pace of the slowest factor". This slowest factor has been called the **Limiting Factor**. A limiting factor does not mean one which is required in small amounts, but the one which is present in amounts lesser than its requirement. This law is applicable to all processes including photosynthesis, which are affected by many factors. A limiting factor is deficient to an extent that increase in its concentration results in an increase in the rate of the process keeps on doing so till some other factor becomes limiting.

Current thinking is that the limiting effect of factors may be a 'relative' one rather than an absolute one. Therefore, it would be more appropriate to call it is "Law of relatively limiting factors". Thus, to obtain favorable results for

photosynthesis, increase in any one factor may not do the job, as many factors need to be increased proportionately.

7.6.2 External Environmental Factors

There are several important factors that affect photosynthesis. We will discuss them one by one in this section.

Light Intensity : Light is a limiting factor for photosynthesis. The light intensity has a favorable effect on the rate of photosynthesis. However, optimum light intensity required is different for sun-loving (**Heliophytes**) and the shade-loving plants (**Sciophytes**). The optimum light intensity is also termed as the **Saturation effect**. Decrease in light intensity results in a decline in the rate of photosynthesis. A stage is arrived at where no CO_2 absorption from the atmosphere takes place. Also, no O_2 is either evolved or absorbed. The light intensity at which there is no apparent gaseous exchange between the plant and the environment is called **Light Compensation Point**. In other words, it can also be defined as the amount of light reaching a photosynthesizing leaf at which photosynthetic CO_2 uptake exactly balances respiratory CO_2 release.

The value of light compensation point is much lower in sciophytes (<100-foot candles) than for the heliophytes (100-400-foot candles). By increasing the light intensity beyond the light compensation point, there is also rise in temperature, which results in photorespiration in C_3 plants. At this time, another point is reached i.e., **CO_2 compensation point**, where no CO_2 efflux from the plant takes place as the process of photosynthesis and photorespiration balance each other. Or to put is simply, the CO_2 compensation point is that CO_2 concentration at which the rate of respiration balances the rate of photosynthesis. Exposure to light intensity above saturation results in photo-oxidation and photoinhibition of the photosynthesizing machinery called **Solarization**.

Light Quality : As you have already learnt in Unit 5, photosynthesis in higher plants occurs within the limits of PAR (400-700nm).Minimum photosynthesis take place in green light.

Although red light is the most effective for photosynthesis, followed by blue light, the shade-loving plants may show some variation in their action spectra.

Light Duration : Light duration does not affect the rate of photosynthesis. However, the total photosynthesis depends on the time for which light is available. However, rate of photosynthesis is higher when exposed to intermittent light than in continuous light.

Carbon Dioxide : The CO_2 concentration in the atmosphere is about 0.04% (400 ppm). An increase in CO_2 concentration will result in a higher rate of photosynthesis for C_3 plants as their saturation is much higher than the C_4 plants and therefore, the current CO_2 concentration is limiting for C_3 plants. Photorespiration also gets suppressed at higher CO_2 concentrations. A prolonged exposure to higher levels of CO_2 would result in a decline in photosynthesis rate as the stomata close, thereby inhibiting gaseous exchange.

Temperature : Plant species show a high degree of variation with respect to the temperature requirements for optimum photosynthesis. While some conifers can photosynthesize at an unbelievably low temperature of -35^{0}C, the

other extreme is some species of thermophilic algae thriving in hot springs at 75^0C. However, the optimum temperature for photosynthesis for C_3 plants is 20-25^0C. C_4 plants generally survive at comparatively higher temperatures (35-45^0C). Desert plants/CAM plants are adapted to survive even at higher temperatures. In general, the tropical plants have a higher temperature optimum than the plants growing in temperate areas. Higher temperatures do not affect photochemical reactions but affect enzyme-mediated reactions like those of the Dark reaction. The overall Q_{10} for photosynthesis is 2. Thus, temperature is limiting factor.

Water : Water is an essential raw material for photosynthesis and is directly involved through photolysis in providing electrons and protons for the reduction of $NADP^+$. Indirectly, water is involved in the stomatal movements, besides hydrating the protoplasm. Under normal circumstances, water may not be a limiting factor. However, under conditions of water stress, there is a drastic decline in rate of photosynthesis due to reduction in leaf water potential.

Oxygen : Oxygen is not a limiting but an inhibitory factor. A decline in the ambient O_2 concentration increases photosynthesis whereas increase in O_2 concentration inhibits photosynthesis by encouraging the oxygenase activity of Rubisco, thereby increasing photorespiration. O_2 concentration beyond 2.5% becomes inhibitory, a phenomenon first observed by Warburg (1920) and now called Warburg's Effect. Since CAM plants exhibit photorespiration, they also respond to increased oxygen levels. However,C_4 plants are not sensitive to oxygen.

Mineral Nutrients : Many mineral elements, especially those which are either constituents of chlorophyll, used as cofactors for the enzymes or the constituents of the electron carriers are very crucial. Mg (a constituent of chlorophyll), Fe and Cu (for synthesis of chlorophyll and part of cytochromes and plastocyanin), Mn, Cl and Ca (for photolysis of water), P (for ATP synthesis), Zn(cofactor for carbonic anhydrase) affect the process directly. Rate of photosynthesis is directly affected by nitrogen supply as nitrogen constitutes the basic pyrrole of chlorophyll and is a part of all enzymes.

Air Pollutants : Air pollutants like smoke, dust, flyash, hydrogen fluoride, sulfur dioxide, carbon monoxide, nitrogen oxide, ozone have an adverse effect on photosynthesis. After dissolving in the cell sap, these pollutants make it highly acidic, thereby inhibiting various pH specific enzymes, and even stomatal closure. Gases like SO_2, NO_2 and ozone are strong oxidizing agents, which damage the permeability of membranes, induce free-radical formation, degrade chlorophyll and proteins.

7.6.3 Internal Factors

In addition to the external factors, there are several internal factors which affect photosynthesis in plants.

Chlorophyll Content : The chlorophyll content affects the rate of photosynthesis indirectly. Normally, the chlorophyll content is always many times more than the optimum. Non-green or variegated leaves do not show photosynthesis. Heliophytes have lesser chlorophyll content in their leaves than the sciophytes.

Age of Leaves : Rate of photosynthesis is higher in mature leaves than in the younger ones. This is probably due to the higher chlorophyll content. However, the rate of photosynthesis begins to decline as the leaf ages. There is a decline in chlorophyll content as well as more catabolic reactions set in.

Hydration of the Protoplasm : It is essential to have the protoplasm in a hydrated state for efficient photosynthesis. It is believed that the protoplasmic factors are more active in hydrated protoplasm.

Accumulation of Photosynthetic End-Products : When the rate of utilization and translocation of food decreases, it would lead to accumulation of the photosynthetic products resulting in "feedback inhibition", and hence, a decline in photosynthetic rate.

SAQ 5

Match the features/terms in column 1 with those in column 2.

	Column 1		Column 2
i)	Liebig	a.	Inhibitory effect of O_2
ii)	Sciophytes	b.	Light compensation point
iii)	Optimum light intensity	c.	Limiting factors
iv)	No apparent exchange of gases	d.	400-700 nm
v)	Warburg's effect	e.	Saturation point
vi)	Solarization	f.	Shade loving
vii)	Blackman	g.	Law of minimum
viii)	PAR(Photosynthetically Active Radiation)	h.	Photo oxidation

7.7 PHOTOSYNTHESIS, PRODUCTIVITY AND HUMAN WELFARE

Finally, let us examine the relevance of studies made on the mechanism of photosynthesis to agriculture and productivity. We shall first take up the question: What is the theoretical maximum efficiency in photosynthesis? Then we shall dwell into the status of actual efficiency prevailing in the field and in nature at large. And if the efficiency is low, we shall look for the reasons of the same and examine the modern strategies which are expected to improve photosynthetic efficiency.

As you learnt in Unit 5, Warburg found a quantum requirement for every CO_2 molecule reduced (or O_2 evolved). It has now been established that the true quantum requirement for photosynthesis is 8 light quanta. This changes our outlook on photosynthetic efficiency. Since $8 \times 6 = 48$ light quanta are needed

to fix 6CO_2 molecules and 1 light quantum of red light contains 40.8 Kcal of energy, the total energy needed will be 48 × 40.8 = 1958.4Kcal.The efficiency of photosynthesis is 686/1958.4 × 100 = 35.02%.

Nonetheless, in sharp contrast to the above figure of 35% under natural conditions, the actual efficiency is rarely more than 1-2% of the total receipt of energy. There are several reasons for the inability of plants to tap free energy from sunlight. Firstly, nearly 20% of even the PAR is wasted. Then, if we consider the total plant productivity, e.g., of a crop through a whole year, one can immediately observe many other wastages. For example, many a times a piece of fertile land can be left lying barren without any plant cover, simply because most crops are seasonal, and it is rather a short span of time when a plant has a fully developed leaf surface. Moreover, not every part is useful or edible in a crop plant. Thus, when calculated on a seasonal or yearly basis, the efficiency of photosynthesis is extremely low. However, in some of the most advanced agricultural systems in the developed countries using best conditions of irrigation, optimum use of fertilizers, the efficiency may reach 12%. Still 1/3 of theoretical efficiency is achievable.

Now the question arises as to how we can increase productivity via photosynthesis, since the world productivity is much lower than attainable theoretically and that photosynthesis itself is an inefficient process in terms of conversion of light energy into biomass. Therefore, one of the pressing needs of agriculture is to somehow manipulate light energy assimilation which could be translated in the carbon gain.

Exposure to a higher CO_2 concentration for increasing C_3 photosynthesis is one of the options. However, it may not be a good idea to allow increase in ambient CO_2 concentrations, which have already become a matter of alarming concern. Even under experimental conditions, short-term exposure to high CO_2 may enhance photosynthetic rate, but a prolonged exposure is shown to reduce RuBP regeneration and *Rubisco* inactivation in many species. Similarly, manipulation of temperature, water and nutritional status could bring about some increase in productivity.

7.7.1 Agricultural Biotechnology

Plant breeding techniques have helped tremendously in increasing productivity in modern agriculture. The green revolution stands by itself as a classical example. But the important question is whether there is enough scope for increasing yields by these conventional methods alone? In view of ever-increasing concerns regarding food shortages and increasing human population, the UN (United Nations) and FAO (Food and Agricultural Organization) estimates predict that the food production will need to nearly double by 2050. Will the conventional methods alone be able to meet this challenge?

Undoubtedly, the major goal will be improving the photosynthetic efficiency of this multi-step process by modern techniques of molecular biology and biotechnology, so that plants could be genetically manipulated for increased carbon gain, growth potential, and nutritional value. In addition, developing sustainable and climate resilient cropping systems with decreased water and nitrogen requirements will also contribute towards achieving this goal.

Agricultural biotechnologists are following a multi aspect strategy to manipulate the chloroplast genome to achieve better photosynthetic efficiency. Some experiments have even shown promising results which has sparked very strong possibilities of "turbocharging" photosynthesis to feed the world. Some aspects and targets are briefly mentioned below:

i) To modify the catalytic activity of Rubisco to increase its preference specifically to CO_2 rather than O_2. This would slow down photorespiration and increase carboxylation.

ii) To enhance light tolerance and minimize photodamage by triggering the protective non-photochemical fluorescence quenching (NPQ) system of the chloroplasts.

iii) Modification of thioredoxin (Trx) composition of plants to enhance photosynthetic efficiency. Thioredoxins are small proteins in plastids which have the capability to sense and transmit information regarding the redox state and act as coordinators of the enzymes involved in Calvin-Benson cycle.

iv) Strengthening the intrinsic photoprotective mechanisms operative at the reaction centers that prevent the formation of reactive oxygen species (ROS) and free radicals under fluctuating environmental conditions.

v) Enhancing photosynthesis in microbes, especially cyanobacteria by altering their PS II electron transport capacity.

vi) Scientists of the International project "Realizing Increased Photosynthetic Efficiency (RIPE) have engineered a model crop of tobacco to overexpress a native H-protein which reduces photorespiration at elevated temperatures, thereby increasing production by 27-47%.

vii) Other scientists are trying to tackle the problem of photosynthetic efficiency by introducing C_4 metabolism into C_3 plants, to improve their CO_2-fixation and water use efficiency, much like those with CO_2-concentrating mechanisms (CCMs), although it has not been successful so far.

viii) Introduction and expression of certain specific genes from cyanobacteria (FBP/*SBPase* and *ictB*) into tobacco chloroplasts for enhanced photosynthesis.

ix) Introduction of key C_4 genes into rice to engineer a C_4 version of rice at the International Rice Research Institute have shown promising results. Through new genome-editing methods it may soon become a reality that rice plants may acquire an internal organization closer to Kranz anatomy and result in a dramatic increase in crop production.

x) Very recently a wide range of cyanobacteria have been discovered growing near beach rock in Australia and Yellowstone which photosynthesize at near infra-red light. This discovery is unique as most of the life on earth uses visible red light in the process of photosynthesis. Another form of chlorophyll viz., Chl_f takes over the

role of absorption in near infra-red light. Discovery of this type of photosynthesis 'beyond red light' is very important for scientists who are trying to engineer crops for more efficient photosynthesis in a wider range of light regimes.

Modern genetic tools hold great promise to boost crop yields to feed billions of more people on earth. And finally, the excitement over a human–made artificial photosynthesis is not without reason as it is no more fiction. There are reports of initial success in creating a "synthetic leaf" that can collect solar energy and converting it to liquid fuel by applying photosynthetic reactions.

SAQ 6

Fill in the blanks by suitable terms/words:

i) The theoretical efficiency of photosynthesis is about……., which is much ……. than its actual efficiency.

ii) In developed nations with advance agricultural systems, the efficiency has been enhanced to certain level by using best conditions of ……and optimum use of …….

iii) Exposure to a high concentration of…….. could increase photosynthesis in …….plants. However, long term exposure could be…….. .

iv) Agricultural biotechnology holds great promise in improving the efficiency of photosynthesis by introducing genes from …….. plants into …….plants to improve their CO_2-fixation capacity.

v) Genetic manipulation of *Rubisco* will result in a decrease in its …….activity, thereby decreasing …….. and increasing the fixation of ……...

Box 7.5: Amazing *Elysia chlorotica* - the "solar-powered sea slug"

Elysia chlorotica also known as 'solar powered sea slug' is an invertebrate belonging to phylum Mollusca which has chloroplast in its gut lining. The sea slug is unusual in that it survives on green algae by digesting its nutrients and absorbing its chloroplasts. The chloroplast remains in the animal's digestive tract and photosynthesizes for the animal there making it solar powered. It was a challenge for researchers how chloroplast from algae could function properly in the gut of the animal, as chloroplast requires proteins from the chloroplast and the nucleus, as well and *Elysia chlorotica* being an animal, would be lacking proteins obtained from the plant's nucleus.

Research provided evidence through DNA gel electrophoresis that slug had the DNA required for photosynthesis. During evolution, gene transfer has occurred not only in bacteria but in eukaryotes as well since the gene present in the gut of the animal provides evidence for transfer of DNA fragments from algae to eukaryotes.

7.8 SUMMARY

- The enzyme *RuBP carboxylase*, is totally unable to discriminate CO_2 from O_2, leading to an oxygenase activity and photorespiration. Often, therefore, instead of two molecules of PGA only one molecule of PGA is formed - the other product being a molecule of 2-carbon glycolic acid. Two molecules of glycolic acid can be recycled to yield another molecule of phosphoglyceric acid with the loss of a molecule of CO_2. However, because of the more efficient process of CO_2 fixation in C_4 plants, any CO_2 released in light is wholly recaptured by PEP-carboxylase. Hence, they do not show photorespiration. One of the goals researchers have is to convert C_3 plants into C_4 plants by gene manipulation and eliminate photorespiration to conserve fixed carbon lost during the process.
- The C_4 plants are a special group of plants (mainly represented by the grasses) where the primary acceptor of carbon dioxide is a 3-carbon compound, phosphoenol pyruvate, and the first product is a 4-carbon compound, oxaloacetic acid, which is reduced to malic acid.
- The enzyme which fixes CO_2 in these plants is called *PEP-carboxylase*. Since this enzyme has higher affinity of CO_2 (as compared with *RuBPcarboxylase*), the C_4 plants carry out photosynthesis more efficiently.
- The4-carbon acids cannot, however, be converted to carbohydrates directly. Instead, the fixed CO_2 reenters the Calvin cycle by a reaction which is still not understood properly.
- But it is well established that in a typical monocot leaf, the outer cells have *PEP carboxylase*; on the other hand, the bundle sheath cells -more centrally located - have *RuBP carboxylase*. The organic acids are believed to be transported to bundle sheath cells when the CO_2 is released to be refixed by the C_3 cycle.
- The CAM plants (a group to which cacti and many other succulents belong) essentially constitute a variant group of C_4 plants in which stomates open at night to allow CO_2 fixation by *PEP-carboxylase* whereas the process of reentry of CO_2 into Calvin cycle and reduction of PGA to phosphoglyceraldehyde by NADPH takes place during the day.
- The photosynthetic units - originally proposed on purely theoretical grounds -can be seen in electron micrographs.
- In bacteria even a detailed model of the reaction center of the photosynthetic machinery has become recently available. It is expected that soon, a detailed model will become available also of the organization of the photosynthetic machinery in higher plants.
- The area of molecular biology of chloroplast is also developing at a rapid rate and not only proteins, but each gene related to photosynthesis is being identified.
- Simultaneously, genetic engineering techniques to transform the photosynthetic machinery in chloroplasts are also being developed and it is possible that photosynthesis can be made more efficient in future in selected economically useful plants.

7.9 TERMINAL QUESTIONS

1. The photochemical reactions in grana capture light energy and convert it into chemical energy as ATP and NADPH. What is the necessity of carbon fixation?

2. Give the raw materials and end-products of the following reaction of photosynthesis.

Photosynthesis Reaction	Raw Material	End-Product
i) PS I ii) PS I followed by electron transport iii) PS II iv) PS II followed by electron transport v) Cyclic phosphorylation vi) Carbon fixation		

3. Compare photorespiration and mitochondrial respiration.

   (**Hint :** Compare with respect to substrate, enzyme, waste products, gain of energy and loss of carbon).

4. Distinguish between :

   i) C_4 and CAM plants.

   ii) Oxygenase and Carboxylase activity of *Rubisco*.

   iii) CO_2 compensation point and light compensation point.

5. Write short notes on :

   i) Warburg effect

   ii) Kranz Anatomy

   iii) Variants of HSK pathway

   iv) Law of Limiting Factors

   v) Solarization

6. List the important differences between bacterial photosynthesis and green plant photosynthesis.

7. Explain the ecological significance of photorespiration. Is it a necessary evil?

8. Discuss the role of external environmental factors on photosynthesis.

9. How is chemosynthesis different from photosynthesis in green plants?

10. Discuss the various strategies which are employed to increase the photosynthetic efficiency of crop plants.

11. Can true photosynthesis operate beyond 700 nm? Comment

12. How are the agricultural biotechnologists trying to manipulate the chloroplast genome to 'turbocharge' photosynthesis to meet global food demands?

7.10 ANSWERS

Self-Assessment Questions

1. a) i) True; ii) False; iii) True; iv) True; v) False; vi) False

   b) i) kinetic properties

      ii) chloroplast, peroxisome, and mitochondrion

      iii) chloroplast, *glutamine-synthetase-glutamate α-ketoglutarate aminotransferase* (GS-GOGAT)

      iv) increases

      v) increase

      vi) Two

2. a) i) Phosphoenol pyruvate

      ii) Kranz, two

      iii) Calvin cycle, PS II

      iv) Bundle sheath cells

      v) 30

   b) i) False; ii) True; iii) False; iv) False; v) True

3. a) i) malic acid

      ii) closing, open

      iii) C_3, C_4

      iv) slowest

   b) i) False; ii) True; iii) False; iv) True

4. a) i) water

      ii) bacterioviridin

      iii) archaebacteria, absent

      iv) NAD+; $NADH+H^+$;

      v) chemical

   b) i) False; ii) False; iii) True; iv) True; v) False.

5. i) Law of minimum
   ii) Shade loving
   iii) Saturation point
   iv) Light compensation point
   v) Inhibitory effect of O_2
   vi) Photo oxidation
   vii) Limiting factors
   viii) 400-700 nm
6. i) 35%; higher
   ii) irrigation; fertilizers
   iii) CO_2; C_3, harmful
   iv) C_4; C_3
   v) oxygenase; photorespiration; carbon

Terminal Questions

1. Firstly, the storage of energy in the form of carbohydrates is much more convenient and lot more energy can be stored in this form. Secondly, carbon skeleton of carbohydrates is needed for various biosynthesis.

2.

Photosynthesis Reaction	Raw Material	End-Product
i) PS I	hν + light harvesting pigment complexes + $P_{700.}$	Excited electrons
ii) PS I followed by electron transport	hν + (light harvesting pigment complexes + P_{700}) + NADP	NADPH
iii) PS II	hν + light harvesting pigment complexes + P_{680}	Electrons
iv) PS II followed by electron transport	hν + light harvesting pigment complexes + P_{680} + ADP + Pi	ATP+ electrons
v) Cyclic phosphorylation	H^+ reservoir, ADP + Pi	ATP
vi) Carbon fixation	RuBP + CO2 + ATP + NADPH	Sugars + ADP + Pi + $NADP^+$

3.

Item	Respiration	Photorespiration
Substrate	Carbohydrates, fats, proteins, or their monomeric units + O_2	RuBP
Enzymes	Various enzymes of glycolysis. TCA cycle, electron transfer chain	*RuBP carboxylase/ oxygenase*
Loss	Loss of carbon	Loss of carbon
Gain	Energy 36 ATP/glucose	No ATP
Waste	CO_2, H_2O	CO_2, NH_3

4. i) Refer to Sections 7.3 and 7.4.
   ii) Refer to Section 7.2.
   iii) Refer to Subsection 7.6.2.
5. i) Refer to Section 7.2.
   ii) Refer to Section 7.3.
   iii) Refer to Subsection 7.3.2.
   iv) Refer to Section 7.6.
   v) Refer to Section 7.6.
6. Refer to Section 7.5.
7. Refer to Subsection 7.2.2.
8. Refer to Subsection 7.6.2.
9. Refer to Section 7.5.
10. Refer to Section 7.7.
11. Refer to Section 7.7.
12. Refer to Section 7.7.

UNIT 8

TRANSLOCATION IN PHLOEM

Structure

8.1 INTRODUCTION

In the previous units you have studied that one of the necessities of plants is water. It is taken up by the roots. Another purpose served by the roots is to absorb water soluble mineral nutrients from the soil. Mineral nutrients move together with water in long distance pathways provided by the vascular system to reach the entire plant. Roots cannot feed themselves in the darkness of soil. Several other tissues in the plant body also lack photosynthetic apparatus completely or possess it partly in the sense that they cannot manufacture enough food required to support their life processes. Leaves on the other hand

rely on roots for water and mineral nutrients but manufacture more food than they actually need. Hence, leaves serve as the source of food for other tissues which may store excess of it so that it can be used by plant for the perpetuation and spread of species.

Phloem tissue evolved as a channel to transport these photosynthates downwards to various parts of the body. Now, how is such a complementary division of labour in tissues particularly between roots and leaves made possible? And how are the organic molecules made by photosynthesis and other biosynthesis distributed in various parts of plants? It is believed that during the evolution of early land plants, their upright growth made possible the differentiation of tissue into photosynthetic and non-photosynthetic types.

Objectives

After studying this unit, you should be able to :

- describe transport network for the translocation of food material and appreciate the importance of translocation;
- explain the concept of source and sink relationship with respect to translocation;
- describe the structural and functional organization of phloem, particularly near the region of loading and unloading in sieve tubes;
- describe various experiments performed to study phloem transport; and
- explain the mechanism of phloem loading and unloading explain the pressure-flow model for translocation.

8.2 WHY IS TRANSPORT NECESSARY?

In higher plants, all cells do not have the capacity to photosynthesize, i.e., manufacture food. In such a situation, the photosynthetic parts, i.e., leaves conserve energy in the form of carbon compounds or *photo assimilates* and provide energy to the non-photosynthesizing parts like roots, tubers, rhizomes or bulbs etc. The **photo assimilate** first accumulates in the leaf either as sucrose or starch by a process called **starch allocation.**

The synthesized food must be transported from its manufacturing centre (*source*) to the non-green portions of a plant (**sink**) where it is utilized or stored or to be metabolized later. Storage tissue like fruits and seeds germinate and regenerate the plant in the appropriate season. The distance between the source and sink may vary from a few millimeters to more than 100 meters. The long-distance transport of photo assimilates is called **translocation**.

The extensive transport system carries the photo assimilates along with products of nitrogen metabolism over long distances. It is obvious that diffusion alone cannot achieve translocation over long distances. Thus, there must be a convective flow in the specialized vascular system to supply the photosynthates to all needy tissue. The transport system must be as extensive and ramified as the arterial and venous network in an animal body. However,

plants lack such a specialized pump as heart meant for blood circulation. The function of translocation occurs in vascular plants by phloem. It ensures efficient distribution of photosynthetic energy and carbon between organs of a plant, a process called **carbon partitioning**.

Downward and Upward Translocation

The translocation of food in plants takes place in downward, upward, and lateral directions.

a) **Downward translocation:** The entire organic food synthesized by the leaves is not retained by them, and most of it is translocated in the downward direction into roots and other underground portions of the plant, to be utilized for their nutrition and maintenance. The excess unutilized food is converted into insoluble form and stored in tubers, roots, rhizomes, and bulbs. Downward translocation is the most common mode of transport.

b) **Upward translocation:** Upward translocation takes place in situations that demand a supply of assimilates. For example, stem apices lie above the foliage and need a constant availability of foodfor growth. Also, after the autumn leaf fall in deciduous plants, formation of new foliage and renewal of cambial activity in spring also needs an upward food supply. In addition, upward translocation of food is needed for the formation of new buds, formation of new stems and leaves from underground storage organs, germination of seeds as well as development of flowers,seeds, or fruits.

c) **Lateral translocation:** This type of translocation takes place in certain parts of stem and root. This is performed mainly by medullary rays. When the source of manufacture of food and the sink or the organ of utilization lies on opposite sides or migration of photosynthates from a foliated branch to phloem of a defoliated one are examples of this type of translocation. Radial translocation of solutes also takes place from the cells of the pith to cortex.

SAQ 1

Complete the following statements:

a) The two elaborate transport networks …….. and ……. run parallel to each other in plants. .

b) Phloem translocates food from ……. to …… .

c) …….. are arranged in a linear array running vertically through the length of phloem.

d) Plants need extensive and ramified transport system to carry the products of …….. and ……. metabolism to their various parts.

e) After autumn leaf fall, the food supply is needed through …… translocation to the new foliage.

8.3 PATHWAYS OF TRANSLOCATION

Various parts of a plant are linked by an elaborate plumbing network for transport of food which is formed by phloem. This network runs parallel to another major transport system formed by xylem, which is responsible for the uptake of water and mineral nutrients from roots and their distribution throughout the plants. These two long-distance transport pathways phloem is generally located on the outer side of the primary and secondary vascular tissue. The primary tissue are surrounded by the compactly arranged sclerenchymatous bundle-sheath cells, whereas only some layers (most recent-innermost) are functional in case of old-stems showing secondary growth and annual rings. Sieve tubes are the cells of the phloem which are joined into long interconnecting pipelines and are living at maturity whereas xylem is not.

The term **phloem** was introduced by Nägeli in 1858 and is derived from the Greek word *phlois*="bark". It is infact, the innermost layer of the bark. It is generally located on the outerside of the primary and secondary vascular tissue. The primary tissues are surrounded by the compactly arranged sclerenchymatous bundle-sheath cells, whereas in plants with secondary growth, only some layers (most recently formed the innermost) are functional and constitute the inner bark. The location of phloem with respect to other tissues can be easily examined by a single transverse section.

It is believed that phloem evolved in early land plants as a means of downward transport of photosynthates (sugars and other metabolic products of photosynthesis)) from the aerial food producing parts to the lower non-photosynthetic portion of a plant.

We know that phloem is a complex tissue comprising 4 types of cells which you have studied in Unit 1 of BBYCT-135:

i) Conducting cells or **sieve elements** (sieve tube is a general term denoting both the highly differentiated sieve tube elements of the angiosperms as well as -- specialized sieve cells in gymnosperms (e.g., conifers) and ferns.

ii) Companion cells (closely associated with sieve cells in flowering plants whereas in non-flowering representatives, it is the albuminous cells that are associated with sieve elements.

iii) Phloem parenchyma and,

iv) Phloem fibers.

8.3.1 Sieve Elements and P-Proteins

Sieve elements form a continuous system throughout the length of the plant through its elongated cells which are connected end-to-end. These cells resemble hollow pipelines (cellular channels) through which the metabolites flow. These are specialized **sieve plates** present at the end walls separating two adjacent sieve tube elements. These plates are perforated structures which interconnect the sieve elements (tubes). The pore area may range from 1µm to 15µm. The pores are like open channels for transport of substances.

Sieve tube elements are unique as they lose their nucleus at maturity. In addition, the vacuolar membrane or tonoplast is also lost during differentiation. Also absent from the mature cells are Golgi bodies, ribosomes, microtubules, and microfilaments (Fig.8.1 a). A detailed study of serial ultrath in sections viewed under the transmission electron microscope reveals that smooth ER, plastids, and modified mitochondria are present. The cell lumen of the sieve element appears open except for transcellular strands that merge with neighboring sieve cells at the sieve pore plate via plasmodesmata. The neighboring sieve tube elements have continuous plasma membranes. The continuity of plasma membrane and cytoplasm makes the sieve tubes a longitudinally extending symplast. In sharp contrast, cells of the xylem are dead at maturity, with lignified secondary walls. Do you remember as to what the xylem vessels form, symplast or apoplast?

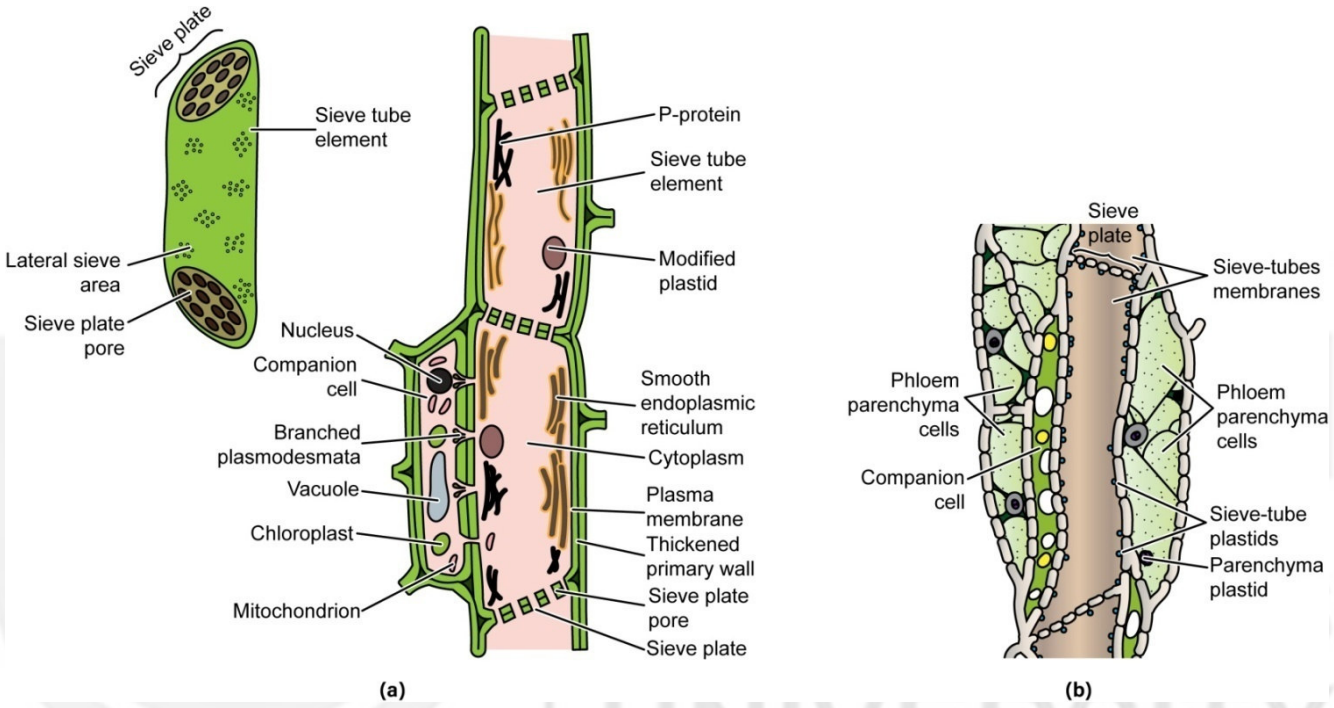


Fig. 8.1: a) Diagrammatic representation of mature sieve elements (From Taiz et al); b) The sieve plates and sieve pores in longitudinal section reveal sieve plates (From Hopkins & Hüner).

TEM pictures reveal that the sieve plate pores are callose-lined. A slimy proteinaceous component occurs in the cytoplasm of sieve elements called **P-protein** (phloem protein). This has been referred to as **slime** in traditional literature. P-protein occurs in all dicots and many monocots. However, it is absent in gymnosperms. P-proteins can be of different shapes; granular, crystalline or fibrillar. In young and immature cells, P-proteins assume the shape of discreet P-protein bodies and disperse into fibrillar forms during sieve cell maturation. The two major proteins forming P-proteins have been characterized at the molecular level in certain plants and are believed to be synthesized in the companion cells.

When plants are cut or injured, P-proteins flow to the sieve plate, there by plugging or blocking the sieve plate pores. In the absence of such a protective sealing mechanism, the metabolites would continue to flow out of the wounded part resulting in eventual death of the plant. Thus, P-protein apparently plays a role like the blood protein fibrin, in whose absence, a person would bleed to death.

Grazing by herbivores can cause serious damage by tearing and ripping the leaves apart easing the leaf sap pressure in phloem, which has been estimated to be nearly 100 times that in the aorta of the human heart. However, the damage is contained immediately to prevent any large drop in pressure by the activity of P-protein which rapidly coil up and help in the blockage of sieve plates. Subsequently, the callose deposition completely blocks the endplates. Interestingly, any repair of this damaged sieve element is possible only when new phloem cell are produced by cell division.

Callose is produced in the sieve pores as long-term solution to sieve damage. This is called as the **wound callose** as it helps in sealing off the damaged sieve elements. The callose gets gradually removed/dissolved from these sites by callose-hydrolyzing enzymes as the sieve cells recover from damage. Many plants that are resistant to sap-feeding insects possess special genes for callose deposition to prevent penetration by insect mouth parts or by plugging off the pores after insect penetrations.

8.3.2 Companion Cells

Each sieve element is compulsorily associated with one or more neighboring companion cells. The two arise from a common/same mother cell and are connected by plasmodesmata. They are true companions, in the sense that companion cells live as long as the sieve cells live and enjoy a functional relationship. Companion cells possess dense cytoplasm with organelles, especially large number of mitochondria (Fig.8.1b),which is indicative of its high metabolic status. These cells are known to synthesize proteins, P-proteins, and ATP which are supplied to the sieve elements through plasmodesmatal connections.

There are three types of companion cells that occur at the phloem loading (near the sieve tubes of leaves) and unloading sites (near the sieve tubes into any sink) and at different anatomical situations. These are :

i) **Ordinary companion cells** – for movement of sugars from mesophyll to the minor veins. They have many chloroplasts with well-developed thylakoids. The cells walls have a smooth inner surface. Plasmodesmata are few.

ii) **Transfer cells** – cells similar to ordinary companion cells, but with extensive cell wall invaginations (ingrowths), especially on the face away from the sieve element. Wall ingrowths greatly amplify the membrane surface area for increased transfer of metabolites from the source to the sink (Fig 8.2).

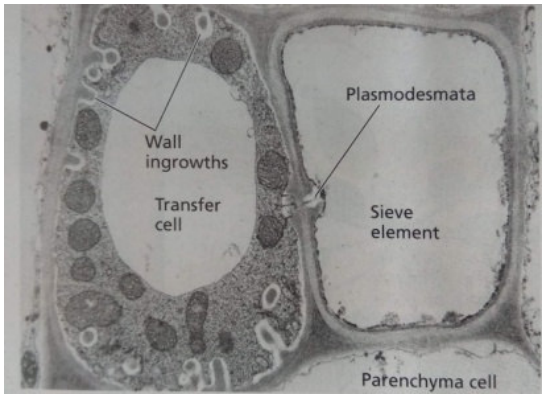


Fig. 8.2: Electron Micrograph of transfer cells with extensive wall ingrowths (From Brentwood & Cronshaw, 1978 in Taiz *et al*).

iii) **Intermediary cells** – have a large number of plasmodesmatal connections – making them particularly suitable for symplastic transport (Fig. 8.3). These cells are characterized by numerous small vacuoles, poorly differentiated thylakoids, and absence of starch grains in the chloroplasts (Fig. 8.3).

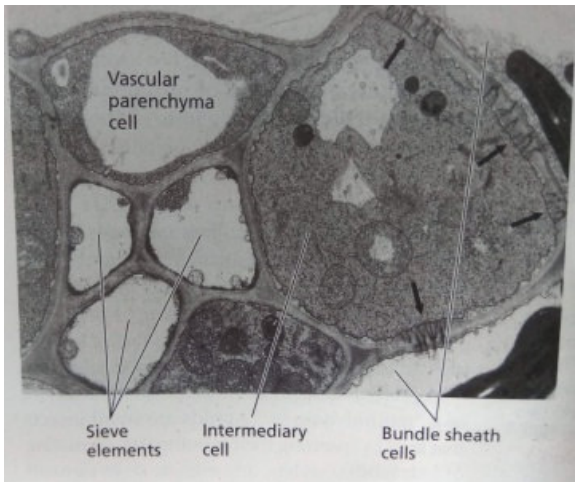


Fig. 8.3: Transmission electron micrograph of phloem showing intermediary cells with plasmodesmatal connections (From Turgeon, et al 1993 in Taiz *et al*).

Plants generally transfer the photosynthates from mesophyll cells to sieve elements apoplastically by companion cells and transfer cells. Symplastic transport of photosynthates takes place by the intermediary cells.

In addition to the translocation – mediating elements, phloem also contains cells which provide mechanical support. These are called **supportive cells.** Such cells are characterized by a thick, rigid secondary cells wall and are dead at maturity. These include **fibres** and **sclereids**.

8.3.3 The Source and Sink Relationship

The process of translocation of sap in the phloem does not necessarily/ always exclusively follow any one direction, i.e., downwards,or upwards. Also, this process is not governed by gravity. The photosynthates move from tissues, where they are produced (**source**) to those regions where they are needed for either metabolism or storage (**sink**). In other words, a source is an organ or tissue which produces more photosynthates than is required for its own growth and metabolism, and thus, exports the excess assimilates. A sink, on the other hand, can be considered as a net importer of photosynthates where it is utilized. In most plants, actively photosynthesizing tissues like mature leaves and green stems are the main sources, whereas roots, stems,and fruits usually serve as sinks. This source-to-sink translocation pattern has been experimentally proved by the classical girdling and the more recent radioactive labelling techniques.

A tissue organ with photosynthates may not necessarily always act as a source. A storage organ which is non-green, may also act as a sink. For example, the biennial wild beet (*Beta maritima*) root functions as a sink during the first year of its growth. During this period, it receives sugars from the leaves (source) and accumulates them. However, in the subsequent second growing season, this very root, which was earlier a sink, becomes a source itself, and contributes to remobilization of sugars to produce new shoots.

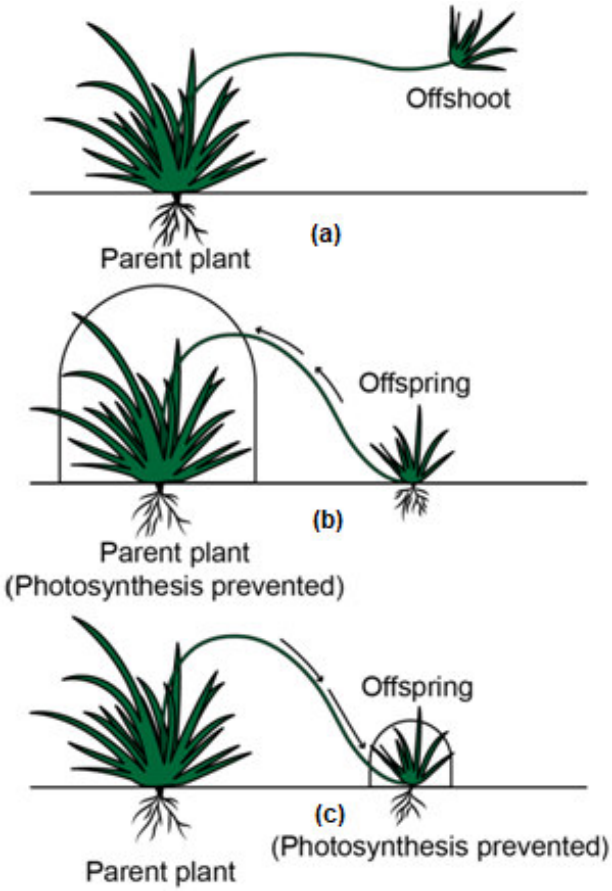


Fig.8.4: a) *Saxifraga*plant with an offshoot; b) Supply of food by the offspring; c) Supply of food by the parent plant.

In other words, the sink and sources may vary at any given point of time. An experiment on the plant *Saxifraga* (Fig. 8.4) illustrates this point. This plant spreads out by giving out long offshoots with a bunch of leaves and potential root system at the end. If the offshoots come in contact with moist soil, they develop into a self-sufficient shoot-root system. Till an independent system develops, the entire supply of water and minerals to the juvenile bunch of leaves is provided by the roots. The phloem transport system initially provides the buds of distant shoot with the necessary nutrition. When fully grown, the new cluster of leaves themselves become excess producers of photosynthates and contribute their output to the parent plant. If we shutoff the light falling on the parent plant or the off shoot (Fig. 8.4 b and c) over a long/substantial period, so that either of them becomes incapable of photosynthesis, which way will the phloem translocate the food? We are sure that you have already arrived at the correct answer. It is observed that translocation takes places/occurs from the system in which photosynthesis takes place to the area/tissue in which it is prevented. Thus, the two systems can act as a sink at one time and source at another. In the above experiment, the direction of phloem transport is therefore, dependent on the relative proportion of photosynthates in the two systems of leaves.

Similar situations among typical sinks also exist. In tropics, when the new leaves in deciduous plants emerge in the spring on a denuded (leafless) tree, they need the supply of nutrients. What could be the possible source of nutrients in the absence of pre-existing mature leaves? Naturally, the nutrients

would come from the stored stock of metabolites in the sink tissues. In spring, both the xylem and the phloem receive metabolites from all over the plant body and deliver them to the buds.

As mentioned earlier, the source-to-sink movement of photosynthates occurs as a general pattern. However, there are some specific pathways which modify the translocation patterns where all sinks of a plant may not be supplied by all their sources but are characterized by a preferential source. However, there are some generalized patterns governing these translocation pathways :

a) Proximity of source to sink. The upper mature leaves provide photo assimilates to the growing short tip and immature leaves on account of their proximity. The lower leaves are the source for roots. However, the intermediate leaves are capable of both upward and downward translocation.

b) Most preferred routes of translocation are those where the sources are linked to sinks by direct vascular connections.

c) Different tissues/organs may become sources/sinks, depending upon the developmental stage of the plant, i.e., organs may undergo a conversion from sink to source at one time or another. Whereas the root and shoot apices act as major sinks during vegetative growth, fruits assume the role of a sink in the reproductive phase/stage.

d) In many cases, wounding of the tissue may interfere in translocation. This necessitates an alteration of translocation pattern.

8.3.4 Sampling of Phloem Sap

Now we must ask a very logical question. What is the chemical composition of the phloem exudates? In what chemical form is the photosynthate translocated? One way to collect the phloem exudates is by making an incision into the bark and inserting microcapillary tubes. This procedure has some drawbacks as the injured portion induced plug formation. In addition, a true picture of chemical composition may not emerge as injury also results in a sudden high turgor pressure release which changes the chemical composition of cell cytoplasm by disrupting the organelles in sieve cells.

In some plants, phloem sap exudes from the wounded areas of the sieve elements. Such samples may be contaminated by the contents of the injured/damaged surrounding cells. Aphid stylets have been exploited as the most reliable approaches for analyzing the phloem sap.

Four very fine sharp stylets of each aphid puncture the tissues and get inserted into the phloem (Fig. 8.5 a-c). There is a small drop in pressure in the sieve tubes, which is not sufficient to trigger P-protein coiling. The flow in the phloem continues and the minor drop in pressure creates a false sink in the plants the system transports sap to the insect whose stylets function as a “natural syringe”. The proboscis of aphids can be cut off from the insect and the phloem sap continues to exude out of the stylet.

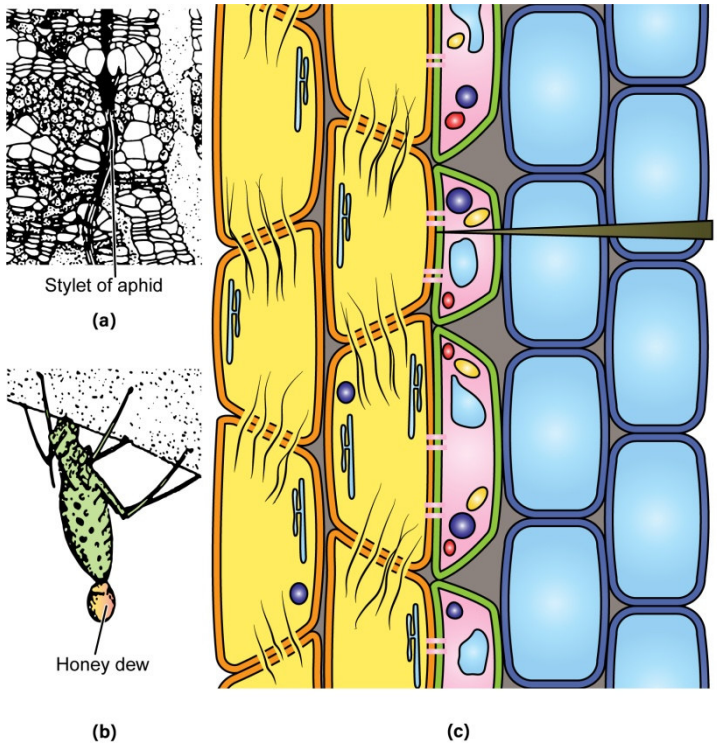


Fig. 8.5: a) An aphid penetrating the bark with its stylet; b) Anal end of aphid showing honey-dew droplet; c) Aphid stylet penetrating the sieve tube element (from Scott).

Chemical analysis of the relatively uncontaminated sap/exudates has revealed that water is the most abundant component of the phloem sap. The phloem exudates contain carbohydrates and amino acids. Among carbohydrates, sucrose in the most commonly translocated photoassimilate sugar. It is believed that the non-reducing sugar sucrose is less reactive than its reducing counterparts like glucose and fructose and is thus favored for translocation. In some species, however, many mobile carbohydrates may get combined with sucrose and then get translocated (Fig.8.6). For instance, sugars can be translocated as raffinose (sucrose + 1 molecule of galactose), stachyose (sucrose + 2 molecules of galactose), and verbascose (sucrose + 3 molecules of galactose).

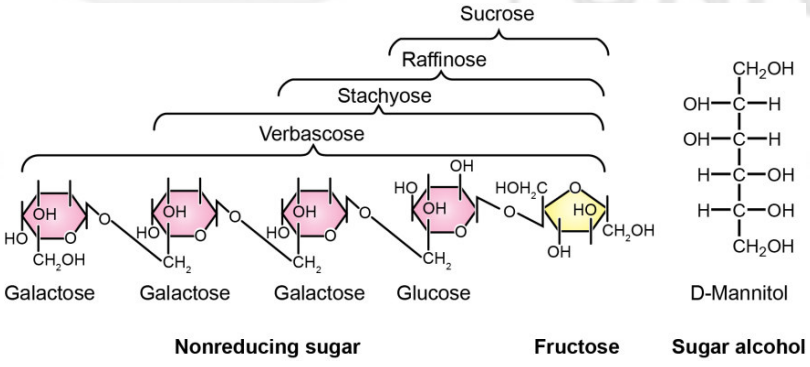


Fig.8.6: Compounds usually translocated in the phloem (From Taiz *et al*). All sugars in the raffinose series are non-reducing (From Taiz et al).

Also, the sugar alcohols mannitol and sorbitol are the translocatable photosynthates in certain families (Oleaceae and Rosaceae). In addition to sugars, nitrogen is translocated in the phloem in the form of amino acids like glutamate and aspartate and their respective amides glutamine and asparagine. The concentration of amino acids and proteins is, however, too low as compared to sugars. Proteins include P-protein (PP_1 and PP_2), along

with a wide variety of enzymes including *protein kinases*, thioredoxin, ubiquitin and chaperones. Recent studies indicate that over 100 proteins have been detected in the sieve elements which are involved in protein synthesis, along with a few ribosomal proteins. The inorganic ions in phloem include mobile elements like chloride, magnesium, phosphate, and potassium. On the other hand, relatively immobile elements the calcium, iron, nitrate, and sulfur are not transported.

Some endogenous plant hormones like auxins, gibberellins, cytokinins and abscisic acid along with nucleotide phosphates have also been identified in the phloem exudate. The chemical composition of phloem exudate from phloem cuts in castor bean has been depicted in the following table (Table 8.1):

Table 8.1: Composition of phloem sap collected as an exudate from *Ricinus communis*.

Component	Concentration (mg/mL)
Sugars	80-106
Amino acids	5
Potassium	2–4
Organic acids	2–3
Protein	1–2
Chloride	0.3–0.7
Phosphate	0.3–0.6
Magnesium	0.1

SAQ 2

a) Match the items given in column A with those listed in column B.

Column A		Column B	
i)	Sieve plate pores	a)	Cellular channels running throughout the plant
ii)	P-proteins	b)	Cellulose pipelines running throughout the plant
iii)	Stylet	c)	Transfer cells
iv)	Wall invaginations	d)	Callose
v)	Sieve tubes	e)	Phloem exudates
vi)	Xylem vessels	f)	Prevent the flow of phloem sap from injured cells

b) Which of the following statements are true? Write T for true and F for false in the given boxes.

i) Sieve tube elements are smaller in radius than xylem vessels and tracheids. [ ]

ii) Companion cells have numerous mitochondria to carry high metabolic activity. [ ]

iii) Transfer cells are located at the site of heavy transport of organic solutes and metabolites. [ ]

iv) Sugars are transported via both apoplastic and symplastic routes. [ ]

v) Transfer cells translocate metabolites to mesophyll cells and load them into the sieve tubes. [ ]

vi) Reducing sugars are not the principal translocable photo assimilates. [ ]

vii) The sources and sinks are always fixed. [ ]

8.4 EXPERIMENTS ON TRANSLOCATION OF ORGANIC SUBSTANCES (EVIDENCE)

To begin with, it was necessary to establish the basic fact that the metabolites flow from the source to the sink through phloem. Some of the earliest experiments performed in this regard were made in the seventeenth century by M. Malpighi. He removed the ring of bark (having phloem) from the inner wood portion (containing xylem) from stems. The exposed portion was sealed with molten wax. This technique which separates the two tissue at the vascular cambium is called **Girdling or Ringing**. After a few weeks, the bark on the upper side showed swelling in the region immediately above the girdle (Fig. 8.7) while the lower side retained the initial diameter, thereby suggesting that the food photosynthate moved via phloem and accumulated in the upper part.

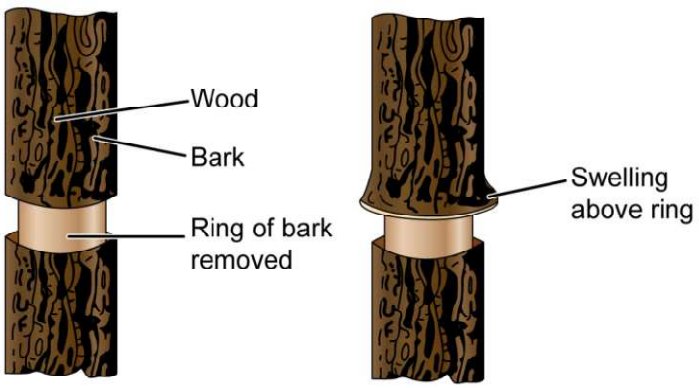


Fig. 8.7: Ringing (Girdling) of a tree. a) A ring of bark is removed from the tree, stripping the bark away, down to the xylem; b) Swelling in the bark above the ring.

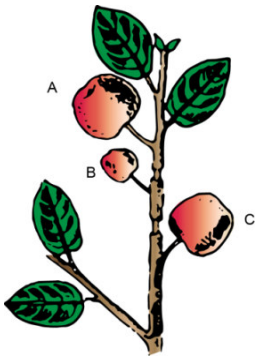


Fig. 8.8: Girdling at two positions, above and below the apple B (After Münch).

In the beginning of nineteenth century, Münch cut away two such rings in an apple tree (Fig. 8.8). The apple B with girdles both below and above it stopped growing, while the other two apples A and C grew perfectly well because they could draw their metabolites from above and below respectively.

Girdling operations are carried out routinely in fruit trees to make more food available and hence larger fruits. The intact cambium helps in healing of incisions. Interestingly girdling/ringing cannot be carried out in monocots. Can you suggest a reason for this?

The classical girdling experiments have been repeated and modified over the years by using both surgical and non-surgical as well as by alternate means to hamper/interfere with the activity of phloem. All experiments do support the idea that photo assimilates are translocated by the phloem.

8.4.1 Analysis of Phloem Exudates

A more direct evidence of the role of phloem in translocation of photo assimilates comes from a chemical analysis of the phloem exudates. There are operational problems in this approach as the living cells of phloem respond very quickly to injury. In addition, the exudates have many sources of contamination as other parenchyma cells also participate in translocation process.

Nature has provided a simple, neat technique to circumvent the problem of obtaining the uncontaminated contents from the sieve tubes. Aphids, phloem sap sucking insects draw their nutrition from phloem by inserting their stylets right into the sieve element as already described in section 8.3 (see Fig 8.5).

The turgor pressure of the sieve tube is sufficiently high so that the sap simply flows into the aphid's alimentary canal. A honey-dew drop can be seen at the anal end of a well-fed aphid. If the insect is cut off from the plant just before the point of entry of stylets, with the stylets still inserted into the bark, phloem sap keeps exuding from the cut end. The exudate called honey drop provides information on the content of the phloem sap. The location of the sharp tip of the stylets is determined by microscopic observations which show that the stylets penetrate single sieve elements like a micropipette. This simple technique provides valuable information on the transported material and the rate of transport under different conditions (temperature, soil, water content etc.) in a fully functioning intact plant.

8.4.2 Use of Radioactive Compounds

At the end of World War II when radioisotopes were made available as markers, experiments were conducted to trace the pathway of photosynthesis using radioactive $^{14}CO_2$. A single leaf of sugar beet (*Beta vulgaris*) was allowed to photosynthesize in a closed chamber containing $^{14}CO_2$ as carbon source. After 10 minutes, the petiole of this leaf was dipped in liquid nitrogen to immobilize the radiolabeled photosynthates. Cross-sections of frozen petiole were placed and exposed on an X-ray film and the resulting radioautograph revealed that the radioactive photo assimilate was localized exclusively in the phloem (Fig. 8.9).

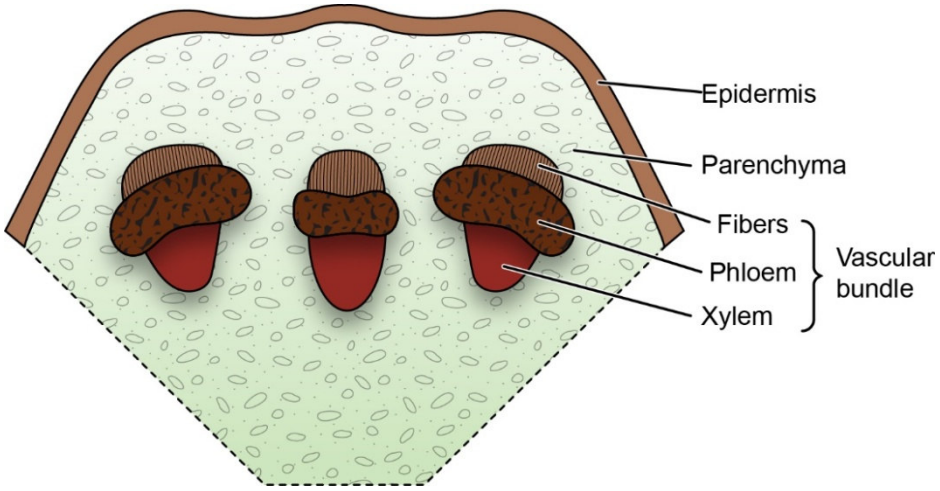


Fig. 8.9: Location of radioactivity (black areas) in the phloem of sugarbeet petioles after 10 minutes of photosynthesis in an atmosphere containing $^{14}CO_2$ (From Hopkins & Hüner).

In alternate experiments, the stem was cut into several segments a few hours after setting up the experiment and radioactive count of ^{14}C in various stem segments was calculated by the Geiger counter (Fig. 8.10).

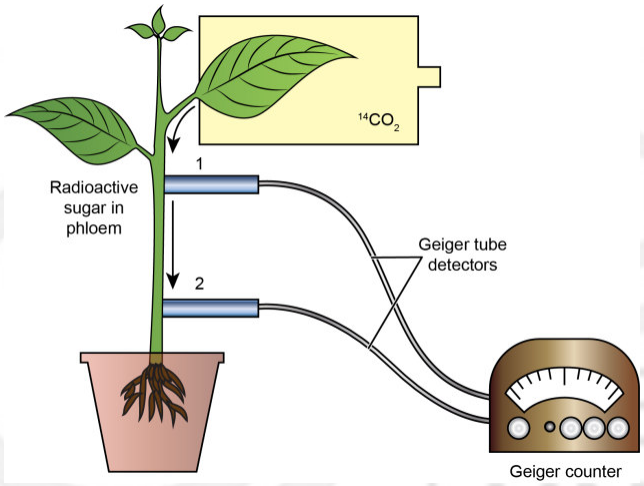


Fig. 8.10: Experimental design for measuring the velocity of phloem sap movement. A leaf is sealed in a closed chamber containing radioactive carbon dioxide ($^{14}CO_2$).

The leaf is illuminated, and the radioactive carbon dioxide is incorporated into sugar via photosynthesis. The translocation of the radioactive sugar can be detected by a Geiger tube positioned against the stem. The time required for sugar to move a known distance from Geiger tube 1 to Geiger tube 2 can be used to calculate the velocity of phloem sap movement. It was observed thatonly those parts of plant contained the ^{14}C photoassimilate that were in continuation with the leaf receiving $^{14}CO_2$.

SAQ 3

Select the correct alternate word(s) given in parenthesis for the statements listed below:

a) The technique of (photography/autoradiography) is used to point the location of radioactive carbon in the cell organelles.

b) In a girdled plant, it is the (root/stem) that dies first.

c) Infection of sap-sucking aphids yields (contaminated/uncontaminated) sap for chemical analysis.

d) Phloem sap is rich in (amino acids/nucleic acids)

e) ($^{14}CO_2$/$^{15}NO_2$.) is used in experiments to trace the path of photosynthates through the phloem.

f) The technique of girdling is (suitable/unsuitable) for monocots.

8.5 PHLOEM LOADING AND UNLOADING

To understand the transfer of materials from the food manufacturing leaf mesophyll cells to the sieve tubes, we must first examine the anatomy of a minor leaf vein shown in Fig. 8.11.

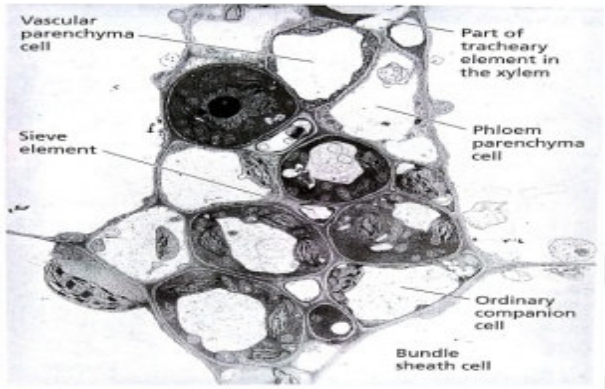


Fig.8.11: EM graph of the small vein in a source leaf of sugar beet showing short-distance transport movements from the mesophyll to the sieve tube elements (From Evert and Mierzawa 1985 in Taiz *et al*).

We can observe a xylem vessel (X) and two sieve elements (SE) which are relatively smaller than other cells and without cytoplasm, the modified companion cells, or transfer cells (TC) with dense cytoplasm and phloem parenchyma (PP) which is less dense and vacuolated. The transfer cells are associated with bundle sheath and mesophyll cells. How are the photosynthates translocated from the manufacturing units – mesophyll cells into the sieve elements, which now act as the source end? The translocation of photo assimilates from the mesophyll chloroplasts to the sieve elements is called **Phloem Loading**. Although, the path from the mesophyll (source) to the sieve element (sink) is only three to four cells long, the process of phloem loading involves four steps:

1. During daytime, photosynthesis in the mesophyll chloroplasts produces triose phosphates which are transported to the cytosol where they get converted into sucrose.

2. A short-distance transport of sucrose takes place from the mesophyll cytosol to the vicinity of sieve element in the minor veins, traversing just 3-4 cells i.e., only a few cell diameters.

3. The actual act of phloem loading involves the transportation of sucrose into the **sieve element-companion cell complex (SE-CC**). The two components of this complex i.e., sieve elements and the companion cells work as a single functional unit.

4. Sucrose and other solutes are now translocated through the vascular system away from the leaf source to the sink, through a process called **export.** This vascular system-mediated export of sugars is called **Long-Distance Transport.**

8.5.1 Loading via Apoplast and Symplast

The initial short distance transport of photosynthate from the mesophyll cells to the phloem can take place through apoplastic or symplastic pathway. Although the sugars move entirely through the symplastic (through cytoplasm via plasmodesmatal connections) pathway (Fig 8.12a), or at some point might enter the apoplast (space between cell wall and the plasma membrane) before finally entering the phloem. The two types of transport may occur in different species.

In apoplastic loading, the sugars initially get transported through the symplasm till the bundle-sheath cells after which they enter the apoplast quite near the sc-cc complex just prior to loading (Fig. 8.12 b).

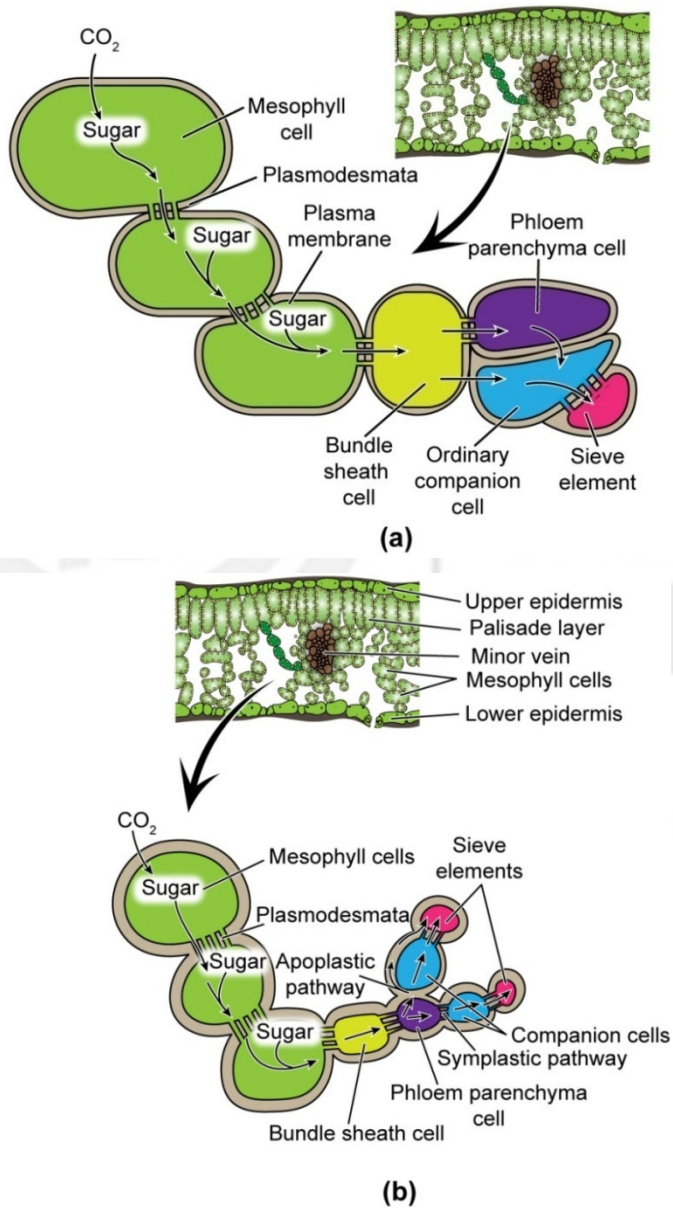


Fig.8.12: Schematic diagram of phloem loading. a) Plasmodesmata-mediated symplastic pathway; b) Partly apoplastic pathway; the sugars enter the apoplast just prior to loading into the companion cells (Modified from Taiz *et al*).

BOX 8.1 : Driving force for long distance transport.

The process of loading at source and unloading at sink provides the driving force for long-distance transport and is, thus, of considerable basic, as well as agricultural importance. A thorough understanding of these mechanisms should provide the basis of technology aimed at enhancing crop productivity by increasing the accumulation of photosynthates by edible sink tissues, such as cereal crops.

The sugars from apoplast are loaded into the SC-CC complex by an active ATP-dependent mechanism that involves a plasma membrane bound sucrose – H^+- Symporter (Fig. 8.13). Symplastic phloem-loading process operates against a concentration gradient and involves active transport. Energy for uptake is provided by the plasma membrane ATPase proton pump. A sugar - H^+ cotransport (Refer glossary) operates where the ATPase pumps protons out of the cell into the apoplast. A high proton concentration in the apoplast generates a proton gradient whose energy is used to drive the influx/transport of sucrose into the symplast of Se-Cc complex through a sucrose –H^+ symporter (Fig. 8.13). Experimental evidence indicates that sugar uptake is accompanied by increase in pH (i.e., depletion of protons).

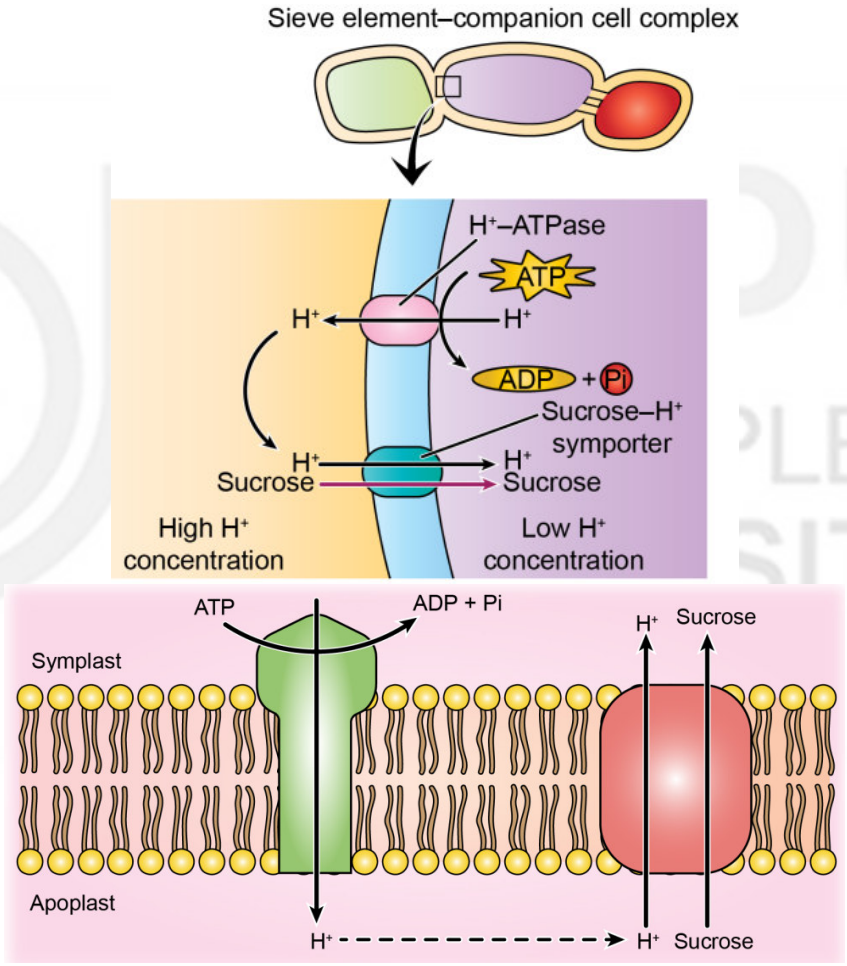


Fig. 8.13: Apoplastic sieve-element loading through ATP-dependent sucrose transport via a sucrose-H^+ symporter(Modified from Taiz *et al* and Hopkins and Hüner).

Several sucrose –H^+ symporters have been localized and identified in the phloem. The process of sucrose loading in the apoplastic pathway may also be regulated by some other factors like sieve element turgor pressure, sucrose concentration in the apoplast, potassium ion presence, and the availability of the numbers of symporter protein molecules.

Some species have evolved a symplastic mechanism for phloem loading. In contrast to the apoplastic loading process, where only sucrose is transported and only few plasmodesmata connect to the minor vein phloem, the symplastic pathway involves the participation of special cells present near the phloem called **intermediary cells**. These cells have many plasmodesmatal connections with the photosynthetic mesophyll cells, which make a symplastic continuum between the sieve element-companion cell (SE-CC) complex and the surrounding cells (Fig. 8.13).

Such intermediary cells exist in *Coleus* (now included in the genus *Plectranthus* – family Lamiaceae), and some cucurbits like pumpkin and squash (*Cucurbita pepo*), and melon (*Cucumis melo*).

8.5.2 Polymer Trapping Model for Symplastic Loading

It has been observed that in some species the chemical composition of sap in the sieve element is somewhat different from that of the surrounding phloem tissue. It is logical that the symporters in the apoplast mediate a selective uptake of sucrose. In sharp contrast, it is difficult to explain the selective symplastic loading through plasmodesmata. Moreover, the sieve elements and companion cells have a higher osmotic content than the mesophyll, which should favour diffusion in the reverse direction. The accumulation of sugars does take place selectively against a concentration gradient.

A **polymer trapping model** has been proposed to explain the symplastic phloem loading of sugars (Fig. 8.14).

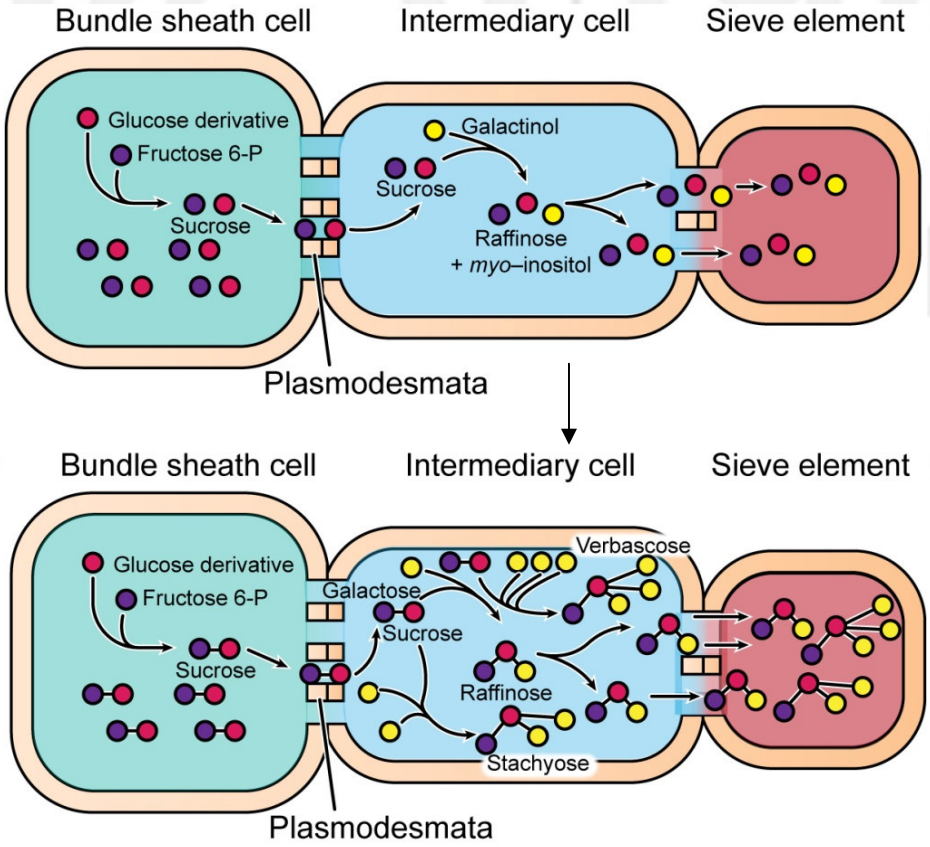


Fig. 8.14: Polymer trapping model of phloem loading (After Taiz& Zeiger).

Investigations on cucurbits shows that the sucrose synthesized in the mesophyll cells diffuses from the source to the bundle sheath cells through

plasmodesmetal connections finally reaches into the companion cells via the intermediary cells. In these cells, sucrose is converted into a series of oligosaccharides, viz., raffinose (1 mol. sucrose + 1 ml galactose), stachyose (1 ml. of sucrose + 2 molecules of galactose), or verbascose. These results in an increase in the size of carbohydrate molecule, and these polymers cannot get back into the bundle sheath cells and are thus trapped, but at the same time can diffuse readily into the sieve cells due to a larger concentration in the intermediary cells. This generates a low concentration of sucrose in the companion cells thereby driving the net movement of sucrose into the phloem by diffusion.

Since sucrose gets continuously utilized in the intermediary cells, a concentration gradient is maintained so that there is a flow of sucrose from the mesophyll. The polymer-trapping model envisages that the plasmodesmatal connections between intermediary cells and sieve elements must be wider to allow the passage of the large sized polymers,viz.,raffinose and stachyose.

Passive Symplastic Phloem Loading

A new type of mechanism of phloem loading has been observed in some tree species. Although abundant plasmodesmata exist between the SE-CC complex and the surrounding cells, the intermediary cells are absent. Also, sugars are not loaded in the form of polymers like raffinose or stachyose but as sucrose only. In contrast to both the apoplastic and symplastic polymer trapping modes, this mechanism suggests that the driving force for long-distance transport is generated in the mesophyll. The overall concentration of transport sugars in source leaves is high, which is responsible for maintaining a concentration gradient towards the sieve elements.

To summarize, the different types of phloem loading pathways depend upon their plant type, growth habit, anatomical characteristics, types of sugars transported, origin of driving force and more. The broad patterns of phloem loading have been summarized in Table 8.2:

Table 8.2: Different patterns found in apoplastic and symplastic loading (From Taiz & Zeiger).

Patterns in apoplastic and symplastic loading			
Feature	**Apoplastic loading**	**Symplastic polymer trapping**	**Passive symplastic loading**
Transport sugar	Sucrose	Raffinose and stachyose in addition to sucrose	Sucrose
Characteristic Companion cells	Ordinary companion cells or transfer cells	Intermediary cells	Ordinary companion cells
Number and conductivity of plasmodesmata connecting the SE-CC complex to surroundings cells	Low	High	High

Dependance on active carriers in SE-CC complex	Transporter driven	Independent of transporters	Independent of transporters
Overall concentration of transport sugars in source leaves	Low	Low	High
Cell type in which driving force for long-distance transport is generated	Sieve element companion cell complex	Intermediary cells	Mesophyll
Growth habit	Mainly herbaceous	Herbs and woody species	Mainly trees

8.5.3 Sink to Source Transition and Phloem Unloading

After reaching its target sink, the photosynthate is unloaded to the final sink tissues like storage tissue (roots and stems), developing vegetative organs (root tips, shoot tips and young leaves) or reproductive structures (fruits and seeds). The import of sugars from sieve elements to this sink tissue is called **phloem unloading**. At first glance, the process of unloading appears to be reverse of the loading process. However, there are some differences. In general, the process involves three broad steps :

a) Phloem unloading involves the exit of sugars from the sieve elements of the sink tissues.

b) Post-sieve element transport involves movement of these sugars to the cells in the sink via a short distance transport.

c) Storage and metabolism is the final step where the sugars get metabolized or are simply stored in the sink tissue.

Phloem unloading may take place by symplastic or apoplastic routes, depending upon the nature of the sink tissue. In young dicot leaves, like those of beet root and tobacco, the phloem unloading, and short distance transport are entirely symplastic (Fig 8.15 a, b).

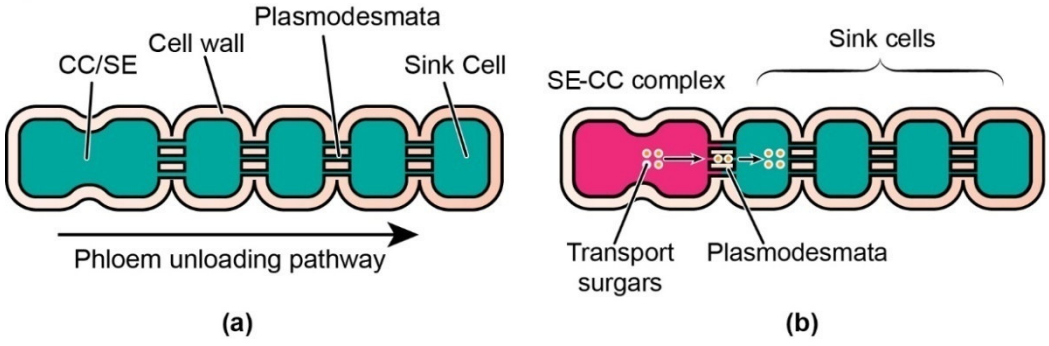


Fig. 8.15: a) Symplastic phloem unloading and short-distance transport; b) Symplastic pathway.

On the other hand, storage organs like fruit and seeds may show an apoplastic step in addition to the symplastic one. This is because there are no

plasmodesmatal connections between the maternal and the embryonal tissues. The apoplastic step can take place from the CC-CE to the adjoining cell, followed by the symplastic connections (Fig. 8.16) or occurring later in the pathway (Fig. 8.17).

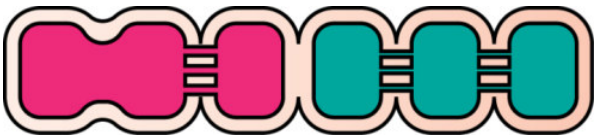


Fig. 8.16: Apoplastic transport from CC-CE to the adjoining cell.

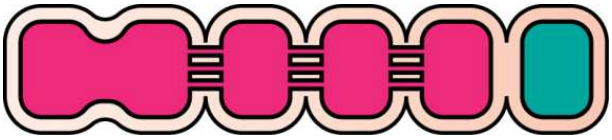


Fig. 8.17: Symplastic transport in the farther cells.

The three possible routes of phloem unloading are summarized in Fig. 8.18.

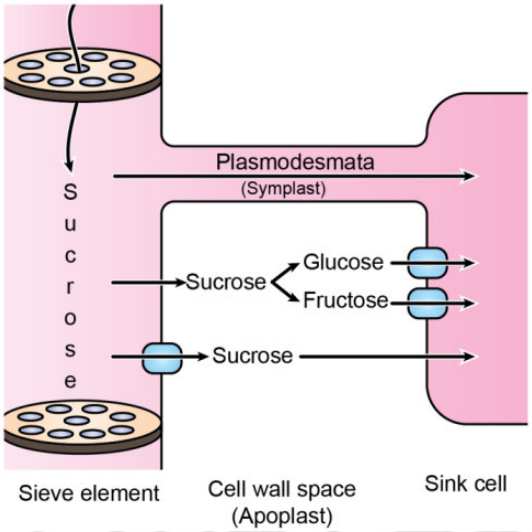


Fig. 8.18: Three possible routes for sugar unloading. A favorable diffusion gradient is maintained after the entry of sugars into the sink cells (From Hopkins& Hüner).

In apoplastic transport, the photoassimilate sugar either remains same or can even get partially changed i.e., gets hydrolyzed into glucose and fructose in the apoplast by the enzyme invertase.

$$\text{Sucrose} + H_2O \xrightarrow{\textit{Invertase}} \text{glucose} + \text{fructose}$$

Is there an individual sieve cell leading from a specific photosynthetic cell to every non-photosynthetic organ? Perhaps not. In fact, there are breaks between the sieve tubes at intervals. There is a constant leakage of sucrose from the phloem that helps the translocated carbohydrate to get efficiently transported to wherever it is required. This pathway is seen to operate in seeds of *Zea mays* (maize), *Pennisetum* sp (pearl millet) and *Sorghum* (jowar). If there is not demand for sucrose at the leakage point, it is taken up again by companion cells, and then reloaded.

In case of high demand for carbohydrates (e.g., in growing meristems of an expanding leaf), sucrose gets consumed by getting reloaded into the phloem. Metabolization by the sink permits continued diffusion from the phloem tissue.

Whereas symplastic import into sink tissues is a passive process and does not require metabolic energy, the apoplastic import is an energy-dependent process as sugars must cross two membranes – plasma membrane of the sugar-releasing cell and plasma membrane of the sink cell. Also, if the sugars are being transported into the vacuoles of the sink, they must traverse the tonoplast. There are energy-dependent transporters which facilitate sucrose unloading.

It has been observed that the transition of a leaf from becoming sink to source is a gradual process. For example, leaves of bean and tomato are to begin with, essentially sink organs. Gradually, as the leaf expands, say to about 25%, a gradual transition occurs, which gets completed by the time leaf expansion reaches 50%. In such cases the phloem gets loaded from the apoplast. Gradually the leaf assumes the role of sugar exporter. In compound leaves, the basal leaflets mature early, and are responsible for exporting photo assimilates to the later developing distal leaflets. Since transition from sink to source amounts to reversal in transport direction, it is also accompanied by several functional and anatomical changes.

SAQ 4

In the following statements fill in the blank with appropriate words :

a) Phloem loading involves the translocation of photosynthates from chloroplasts to …… …… .

b) ……. ……. are specialized and modified companion cells with wall invaginations to increase the ……..for greater transport.

c) Apoplastic transport of photo assimilates into the SC-CC complex requires ……. and involves a membrane bound …… symporter.

d) In symplastic phloem loading polymer trapping occurs in intermediary cells where …….. is converted to raffinose or …… .

8.6 MECHANISM OF PHLOEM TRANSPORT

The efficiency and magnitude of translocation of food material is evident from the annual yields of various crops and fruits. It is also apparent that long distance transport of photo assimilates through the phloem are translocated in a different manner as does the transport through xylem. Moreover, the measured velocities of photoassimilate transport are very high (90-150cm h^{-1}), which is many times greater than diffusion. Two parameters, i.e., **velocity** (linear distance travelled per unit time) and **mass transfer rate** (quantity of material passing through the given cross section of phloem/sieve elements per unit time. Since diffusion is too slow a process (1m/32years) to account for high velocities of translocation (90-250cm h^{-1}) and sieve element mass transfer rate (1-15g$h^{-1}cm^{-2}$), the question is, what is the mechanism of translocation? Before we study the different theories proposed to explain the mechanism of translocation, you must be clear that any proposed mechanism must account for some of these important factors, viz., a) structure and nature

of the living sieve elements, b) role of sieve plates and p-proteins, c) rapid rates of translocation over long distances, d) simultaneous multidirectional translocation, e) phloem loading, and f) phloem unloading. Several theories have been proposed to explain the mechanism, but most of them stand rejected on account of theoretical and experimental grounds. The most credible and generally accepted model to explain the phloem translocation is the **Pressure Flow Model**. Incidentally, this is one of the earliest proposed models and has been modified by many investigators. A detailed description of the model follows:

8.6.1 Münch Mass Flow (Pressure Flow) Model

This model was initially proposed by Ernst Münch in 1930 in a very simplistic manner. According to this model, osmotically generated pressure gradient ($\Delta\Psi p$) between the source and the sink is the main driving force for flow of photo assimilates in the sieve elements. This pressure gradient is established by phloem loading at the source and phloem unloading at the sink.

The process of translocation of photo assimilates

Energy driven loading of sugars into the SC-CC complex at the source constitutes the first step in the process of photoassimilate translocation. This process takes place in the minor veins of a leaf. As the concentration of solute increases in the sieve elements, a low (negative) solute potential ($\Delta\Psi s$) is generated which in turn results in a steep drop in water potential ($\Delta\Psi w$). This causes an osmotic uptake of water by the sieve elements from the xylem. A high turgor (Ψp) or hydrostatic pressure results. There is bulk flow of water and solute from the source to the sink. Phloem unloading at the sink end (e g. a storage cell/root) results in a decrease in sugar concentration in the sieve elements. Consequently, a high solute potential (more positive), along with a rise in water potential makes water leave the phloem to enter back into xylem. As water flows out, the turgor pressure is lowered.

Sieve plates present in the sieve-elements resist the moving phloem sap, to maintain a considerable pressure gradient in the sieve elements between the source and the sink. Thus, water will continue to move in at the source and out of the sink carrying the photoassimilate passively along as long as the pressure gradient is maintained (Fig. 8.19).

The pressure flow model indicates clearly that the movement of phloem sap is due to mass flow and not by osmosis, and no membranes are crossed. Thus, the flow of sugars is against the water potential gradient, but not against the law of thermodynamics. Being driven by pressure gradient, the mass flow of solutes is contrary to the water-potential gradient- mediated osmosis. Solute potential does not seem to play a role here as the solutes and water move with same rate. The solute translocation seems to be a passive process, without any direct participation of ATP. However, active membrane transport is one of the three different mechanisms which are together responsible for enhancing sugar concentration in the sieve elements of the source i.e., loading and unloading of sieve elements – the other being photosynthesis in the mesophyll and photo assimilation of transport sugars in intermediary cells. The process of pressure flow has been pictorially depicted in Fig.8.19.

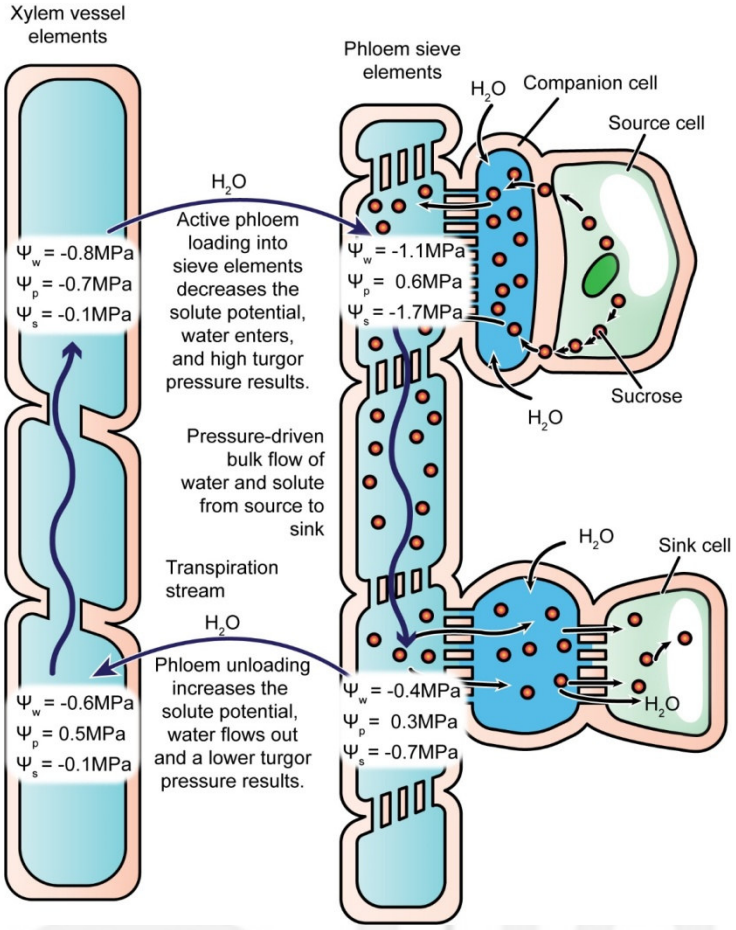


Fig. 8.19: The Pressure Flow Model of Translocation (From Taiz & Zeiger).

Box 8.2: Components of Water Potential

Water potential (-*Ψ*/*Ψw*) is the potential energy of water per unit volume.

$$\Psi w = \Psi s + \Psi p; \quad \Psi p = \Psi w - \Psi s$$

Ψs (-) = solute potential = water moves from a dilute solution towards a concentrated solution (with a negative sign).

Ψp (+) = pressure potential = water moves from high pressure towards low pressure (like squeezing a water filled balloon or a water pump)-(with a positive sign).

Water potential other than that of pure water is negative. So lower potential means a more negative value, and higher potential a less negative value.

If water potential of the source (the region supplying the water) is higher than the water potential of the sink (the receiving region), there is a spontaneous transfer of water from source to sink.

(-*Ψ* source > -*Ψ* sink)

Accumulation of sugars in the sieve elements results in a low (more negative) solute potential. The water potential (∆*Ψw*) also drops, and this allows water to enter the sieve elements and results in an increase in turgor pressure (*Ψp*).

Box 8.3 : Experiment to demonstrate mass flow

We can easily demonstrate the principle of mass flow in the laboratory with the help of a simple experiment (Fig. 8.20). Two chambers with a semi permeable membrane (e.g., dialysis tubing) can be set in side-arm flasks. These will act as simple osmometers. Both the osmometers are connected by a glass tube P (phloem) while the water in the two flasks is continuous via another glass/plastic tubing T (xylem). Osmometer **A** an act as a **source** as it initially contains a concentrated sucrose solution and a dye. Osmometer **B** acts as a **sink** and contains only water. After some

time, water moves by endosmosis through tubing T from B to A, as a hydrostatic pressure is generated by A. As a result, there is en mass flow of sucrose and dye from A to B. The flow will cease only when the sucrose concentration in both the osmometers becomes equal. In an intact plant, of course the solute is being removed/withdrawn from the sink (B) and added at the source (A) to maintain a continuous flow.

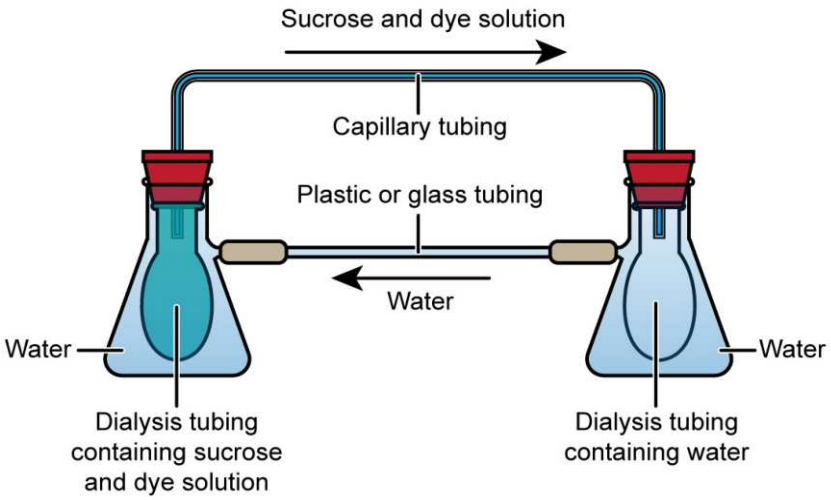


Fig. 8.20: Experimental set-up to demonstrate mass flow (From Hopkins & Hüner).

Most of the predictions of mass flow model have been confirmed by later scientists.

1. Modern Confocal Laser Scanning Microscopy (CLSM) of living sieve elements has confirmed that the sieve plate pores are opened to allow unobstructed movement.

2. Bidirectional movement of photoassimilates by a single sieve element is absent. However, a bidirectional movement in possible in different sieve elements.

3. Translocation of solutes as such does not require much expenditure of energy. However, the processes of phloem loading, and unloading do spend ATP.

4. There is in fact sufficient pressure gradient between the source and sink to drive translocation in the phloem. Values of 0.41 MPa have been recorded in many plants which is enough for driving the flow of solutes.

Thus, even in the absence of any direct evidence for the pressure-flow hypothesis, the mass flow model is by far the most acceptable one.

8.6.2 Electro-osmotic Flow Hypothesis

This theory was proposed by Fensom (1957) and Spanner (1958). In electroosmosis the ions flow across a membrane in response to electrical gradient. Ions pull along water and other contents because of solvent drag. In this hypothesis it is visualized that the sap flows in the lumen of the sieve element and electroosmosis occurs across the sieve plate. The basic idea of this model is represented in Fig. 8.21.

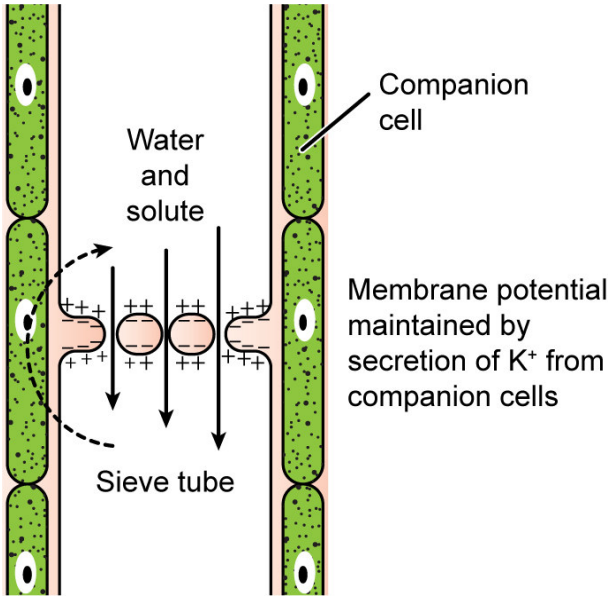


Fig. 8.21: Diagrammatic representation of electro-osmotic flow model. Active uptake of K^+ by the companion cell from its associates sieve tube on the left and the secretion of K^+ into the sieve tube at the right side by its companion cello generate both a potential difference and a K^+ concentration gradient. This causes a flow of K^+ ions and other solutes from right hand side to the left (After Spanner).

The pores of sieve are negatively charged, and many positive ions are associated with them. The companion cells of consecutive sieve elements are engaged in K^+ion uptake and release. This generates a K^+ gradient in the direction of phloem sap flux; the fluxes of other solutes are coupled to K^+ flux and move along with the electroosmotic flow from one sieve tube to the next.

The model has several advantages over the Münch model. The presence of P-proteins, and occlusion (complete obstruction or blocking) of sieve plate pores by filaments bearing negative fixed charges are in favor of this model. While these filaments would greatly prevent the flow of phloem sap under a pressure gradient, they would make electroosmotic flow more efficient. However, there are some negative aspects of the modeltoo:

a) The apparent irrelevance of the source-sink long distance relationship.

b) Extremely large expenditure of energy (ATP) for K^+ ion uptake and release by the companion cells and

c) Contradiction of the model in respect of anionic fluxes which would be excluded by electroosmosis across channels bearing negative fixed charges. The model remains an interesting candidate for the mechanism of translocation in phloem, even though no experimental evidence has proved its basic locations.

Although a potential difference of 4-48 mV has been observed by subsequent experiments, which is adequate for an electro-osmotic flow of solutes at an optimal rate, many answers are still awaited.

8.6.3 Protoosmotic Model

This model was proposed by M.M. Amin (1982) and is based on the fact that there exists a metabolically generated pH imbalance between the sinks and

the sources. As a rule, all cells which use exogenous metabolites and whose metabolism is based on energy derived from respiration generate excess H^+, whereas cells in which photosynthesis and nitrite reduction processes exceed their respiratory activity require uptake of H^+ to maintain the cytoplasm at neutral pH. Thus H^+ flux from sinks (roots) to sources (leaves), is needed (Fig. 8.22).

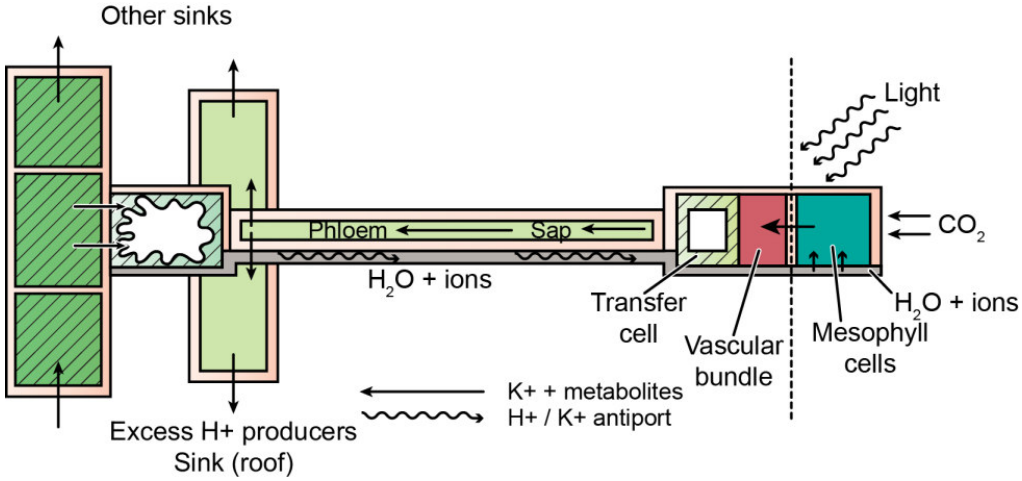


Fig.8.22: Proton flux down its gradient from sinks to sources causes K^+-counter flux in the phloem which is thought to drive metabolites to sinks.

Being a downhill process, the imbalance in the pH of sources (alkaline) and sinks (acidic), provides a source of energy which can be used for phloem transport. The model conceives a long-distance translocation of H^+/K^+ antiport process, well known for plant cell membranes. This flux of H^+ from sinks to sources is charge compensated by K^+ flux. The K^+ flux is electro osmotic in nature which carries other solutes with it (hence named proto-osmosis). As against the case of electroosmotic model discussed above, sink-source relationship in exchanging materials as per their metabolic activities is fully incorporated in this model. However, no experimental evidence has been advanced yet either to substantiate or refute this model.

Interestingly, this model is applicable to radial transport between phloem and xylem. Xylary sap is always acidic while phloem sap is invariably alkaline and rich in K^+. Flux of H^+ from xylem to phloem would in this case bring K^+ and water into xylem by proto-osmosis. This would enrich phloem sap (i.e., enhance the concentration of sugars) as the sap moves towards a sink, e.g., fruit. Tracer experiments have, indeed, shown the movement of water (tritium) and K^+ from phloem to xylem in a cyclic form: phloem of stem → xylem → leaf → phloem.

8.6.4 Factors Affecting the Rate of Translocation of Photosynthates

Various factors, both external and internal, affect the rate of translocation.

a) **Light:** More translocation of photo assimilates takes place in dark than during daytime.

b) **Temperature:** Movement of sugars is faster towards the root when temperature is high.

c) **Metabolic status:** Tissues with active cell division are more efficient sinks than winter buds where metabolism is very low.

d) **Minerals:** Phosphorus and boron have been shown to enhance the rate of translocation.

e) **Respiratory inhibitors**: Metabolic inhibitors reduce the rate of translocation as ATP is needed for phloem loading and unloading.

f) **Phytohormones:** Translocation rate increases auxins and gibberellins due to large food requirements in shoot and root apices.

SAQ 5

Give one word for each of the statements listed below:

a) Uptake of organic solutes by sieve elements from adjacent parenchyma cells, companion cells or transfer cells.

b) The movement of ions across a membrane in response to electrical gradient that pulls along water and other contents because of solvent drag.

c) The flow of solute from source to sink due to pressure gradient.

d) The flux of proton down its gradient from sink to source and counter flux of K+ from source to sink to drive metabolites to sink.

8.7 SUMMARY

In this unit you have studied that:

- Plants need extensive plumbing network to transport organic material, primarily the products of photosynthesis from the sites where they are synthesized to the sites where they are consumed or stored.
- This transport is carried out through a network of phloem tissue that extends from roots through the stem to the very tip of each leaf.
- Translocation in the phloem is defined as the movement of photosynthates from the source to the sink. The photo assimilates include sugars, organic molecules like amino acids, organic acids, and proteins. In addition, inorganic solutes in the form of ions are also transported.
- The sites or the parts of plant from where the organic materials are transported are called the **sources** and the sites or parts of the plant that receive the material from the source are called the **sinks**. Depending upon the need of the plant, a onetime source can also become a sink.

- Translocation of photosynthates takes place in the sieve elements of phloem. Experimental studies involving girdling and autoradiography support translocation of organic materials through sieve elements.
- Companion cells have a special structure and location. They support translocation by transporting photosynthates from the mature leaves to the sieve elements.
- Transfer cells are modified companion cells. Their wall invaginations and plasma membrane and numerous plasmodesmata increase the surface area for transport of materials.
- Sucrose in the most translocated sugar. There are other non-reducing sugars like raffinose, stachyose and verbascose which are translocated through specialized companion cells. In addition, however, amino acids and proteins and inorganic ions are also transported.
- Transport of sugars and other substances into sieve cells at the source is called **phloem loading**. Likewise, **phloem unloading** is the transport of sugars out of the sieve elements of the sink cells. Both symplastic and apoplastic processes occur solely or in combination in phloem loading and unloading in different plants.
- The mechanism of translocation can be most satisfactorily explained by the Pressure-flow model where the photosynthates show bulk flow through the sieve cells in response to an osmotically generated pressure gradient.
- Another model viz., electroosmotic model visualizes the flow of material due to electrical gradient created by uptake and release of K^+ ions by consecutive sieve elements. The protoplasmic model proposes a continuous flux of H^+ ions from sink to source compensated by K^+ flux from source to sink. To counter flux of K^+ is thought to drive metabolites to sink. Both these models lack strong backing of experimental evidence.

8.8 TERMINAL QUESTIONS

1. Compare vessels and tracheids of xylem with sieve tube members of phloem.
2. Why is the phenomenon of translocation of food in sieve elements a difficult process to study?
3. Phloem sap can be collected for the analysis by making incision in the bark. However, the collection of sap by aphid method is superior. Why?
4. Why cyclosis or diffusion cannot account for the transport of organic solutes in a plant?
5. Explain the polymer trapping method of phloem loading.

8.9 ANSWERS

Self-Assessment Questions

1. a) xylem, phloem
   b) source, sink
   c) Sieve tube elements
   d) photosynthesis, nitrogen
   e) source, sink
2. a) i) Callose
      ii) Prevent the flow of phloem sap from injured cells
      iii) Phloem exudates
      iv) Transfer cells
      v) Cellular channels running throughout the plant
      vi) Cellulose pipelines running throughout the plant
   b) i) False; ii) True; iii) True; iv) False;
      v) False; vi) True; vii) False
3. a) autoradiography
   b) root
   c) uncontaminated;
   d) amino acids
   e) $^{14}CO_2$
   f) unsuitable
4. a) sieve cells/elements
   b) transfer cells, surface area
   c) ATP/energy, sucrose-H^+
   d) sucrose, stachyose
5. a) loading
   b) electroosmosis
   c) Munch pressure flow hypothesis
   d) protoosmotic hypothesis

Terminal Questions

1. Xylem vessels are dead cellulose pipes running through the plant. They form part of apoplastic route whereas the sieve-tubes of phloem are living cytoplasmic channels forming symplasm. Water and mineral ions are transported through xylem vessels and trachieds, whereas photo assimilates and organic solutes including hormones are translocated through sieve-tubes of phloem.

2. Transport in phloem is difficult to study because the cells involved are very delicate and set easily damaged. When phloem cells are injured, beaded chains of G-protein filaments are formed. Besides, a slimy plug of callose develops in each plate pore.

3. Collection of phloem sap does not provide selective sampling of the sieve tube contents, since the phloem contains numerous other cells. Whereas aphid method provides only uncontaminated sieve tube sap because the stylets are inserted in a single sieve tube element.

4. The observed rates of phloem transport are much higher than the rates that can possibly be achieved by cyclosis or diffusion.

5. This model explains the symplastic loading of sugars. Sucrose from the mesophyll cells diffuses into the intermediary cells through plasmodeslmatal connections. Sucrose and galactose synthesize different polymers viz., raffinose and stachyose, which diffuse symplastically into the sieve elements through wider plasmodesmata.

Acnowledgements

Fig. 8.1 : http://plantphys.info/plant_physiology/translocation.shtml

Table 8.1 : http://plantphys.info/plant_physiology/translocation.shtml

GLOSSARY

Absorption spectrum : A graph showing the amount of light energy absorbed by a substance against the wavelength of light.

Accessory pigment : Pigments that initially absorb light energy and transfer it to chlorophylls for photosynthesis.

Action spectrum : A graph that represents amount of oxygen evolved/carbon dioxide absorbed against the wavelength of light absorbed.

Allocation : The control of the diversion of photosynthates into various metabolic pathways in source tissue.

Antennae pigment protein complex : A group of pigments and associated proteins that absorb light and transfer it the reaction centre complex.

Antioxidants : A chemical compound or substance which reduces damage by inhibiting oxidation.

ATP synthase : Enzyme that produces ATP from ADP and inorganic phosphate (Pi) present on the surface of thylakoids (Also called *ATPase/CF_0-CF_1*).

Apoplastic pathway : The pathway that proceeds along apoplast i.e., the space between cell wall and plasma membrane.

Bacteriochlorophyll : A type of chlorophyll, occurring in forms *a* and *b*, found in photosynthetic bacteria.

Bark : All tissue outside the vascular cambium; in old trees, divided into inner living bark (functional secondary phloem) and dead outer bark.

Bundle sheath : One or more layers of cells surrounding a vascular bundle.

Callose : A complex carbohydrate (1,3-linked glucan) associated with the pores of sieve tube members, pollen grains, pollen tubes, and primary walls of many living cells.

C_2 photosynthetic pathway : A process in plant metabolism where light-dependent uptake of molecular oxygen (O_2) takes place along with release of carbon dioxide (CO_2) from organic compounds (Syn:Photorespiration).

Calvin cycle	:	(*Syn*: C_3/reductive pentose phosphate/Photosynthetic carbon reduction cycle). This pathway causes the reduction of CO_2 to carbohydrates and consists of three phases. In first phase CO_2 is fixed by combining with ribulose-1,5-bisphosphate in presence of *Rubisco*. The second phase consists of reduction of 3-phosphoglycerate to triose phosphate.The third phase consists of formation of hexose sugar and regeneration of ribulose-1,5-bisphosphate through various enzyme-mediated reactions.
CAM Plants	:	Plants showing the presence of Crassulacean acid metabolism, where CO_2 is fixed at night and stored as malate (a four-carbon molecule) in the vacuoles. During the daytime it is transported out of the vacuoles and decarboxylated. The CO_2 released by malate is assimilated by the Calvin cycle in the stroma of chloroplasts. The stomates open during night and close during the daytime.
Carboxylation	:	A chemical reaction in which a carboxylic acid group is added to a substrate.
Carotenoid	:	A group of fat-soluble, yellow, orange, red, or purple pigments widely distributed among plants. They are subclassified into two groups, carotenes and xanthophylls. Function to assist the chlorophylls.
Chlorophyll	:	Light absorbing green pigment essential for photosynthesis.
Chlorosis	:	Decreased chlorophyll content due to its loss or reduced production. Can be physiological or pathological.
Chromophore	:	A chemical group capable of absorbing selective light. Helps in determining the color of organic compounds.
CO_2 fertilization effect	:	Increase in CO_2 levels leading to enhanced CO_2 photosynthetic fixation and increased plant cover in warm and arid environments.
CO_2 compensation point	:	The concentration of CO_2 at which the rate of photosynthesis and respiration are equal.
Companion cell	:	The cells closely associated with sieve tube elements through plasmodesmatal connections and supporting the function of photosynthate translocation.

Compensation point : It is the light intensity on the light curve at which the amount of carbon dioxide released in respiration equals the amount used in photosynthesis and the amount of oxygen used in respiration equals the amount released in photosynthesis. In other words, rate of photosynthesis exactly matches the rate of respiration.

Cotransport : The simultaneous transport of two solutes/ions by the carrier. Usually one solute moves down along its chemical potential gradient, while the other moves against it.

Cyclic photophosphorylation : Formation of ATP by the cyclic electron transport where the excited electrons from PSI are cycled back to it through Cytochrome b_6, cytochrome $_f$ and plastoquinone. Operates at wavelengths higher than 680nm (photosystem I). The photosystem II does not operate.

Deamination : Removal of an amine group from organic molecule.

Electromagnetic spectrum : The range of all possible frequencies of electromagnetic radiation according to energy, constituting the electromagnetic spectrum.

Emerson effect : The experimental observations made by Robert Emerson in 1957 that photosynthetic efficiency is enhanced in the presence of long wavelength red light when that light is supplemented with shorter wavelength than red light. It is the synergistic effect of red and far-red light on the rate of photosynthesis, as compared with the sum of the rates when the two different wavelengths are given separately.

Export : The translocation of transport sugars away from the source tissues such as mature leaves.

Fluorescence : Emission of extra energy by an excited molecule in the form of light of a greater wavelength than the wavelength of the absorbed light.

Frequency : Waves that pass a fixed place per unit time.

Glycolate metabolism : (C_2 cycle) Biochemical pathway occurring during photorespiration in plants that involves chloroplasts, mitochondria, and peroxisomes. Ribulose-1,5-bisphosphate combines in the presence of *Rubisco* to form 2-phosphoglycolate and 3-phosphoglycerate. 2-Phosphoglycolate is later converted to glycolate which is transported to peroxisomes and mitochondria where it loses NH_4^+ and CO_2 and is eventually converted to 3-phosphoglycerate, without the reduction of coenzymes or generation of ATP.

Glycoside : Chemical compounds composed of two parts - a sugar and another compound linked by a glycosidic bond. Formed by the replacement of a hydroxyl group in the sugar molecule. They yield one or more sugars upon hydrolysis.

Grana lamellae : Stacked thylakoid membranes present in the matrix of chloroplasts. Each stack is a granum. The membranes without stacking are the stroma lamellae.

Hatch-Slack pathway : (C_4 cycle) Biochemical pathway which results in fixation and reduction of CO_2 in two stages; occurs in two different cells, mesophyll cells and bundle sheath cells. CO_2 is fixed to form oxaloacetate which equilibrates to form malate or aspartate. Malate or aspartate is transported to bundle sheath cell where malate or aspartate is decarboxylated to form pyruvate. The CO_2 released in the bundle sheath cells is fixed by RuBP through Calvin cycle which results in the formation of carbohydrates.

Import : Transport of sugars into sink organs such as young leaves, roots, tubers, etc.

Kranz anatomy : The wreath like arrangement of bundle sheath cells and mesophyll cells around the vascular bundle. It is present mainly in the C_4 plants.

Light channeling : The propagation of light through the vacuoles of palisade cells and air spaces in between mesophyll cells.

Light compensation point : Intensity of light at which rate of photosynthesis (CO_2 uptake) is equal to respiration (CO_2 release).

Light scattering : Randomization of the movement of light due to reflection and refraction caused by air spaces and water inside the leaf of a plant.

Light harvesting complex : Complexes associated with the accessory pigment protein complex, aiding in the absorption of light. They are associated with pigment systems I and II and transfer the light energy to the reaction centre.

Lignin : Highly branched phenolic complex polymer, made up of coniferyl, sinapyl, or *p*-coumaryl alcohols; becomes associated with cellulose in primary and secondary cell walls, especially in secondary xylem, and gives strength to the cell wall.

Mass spectrometer : An apparatus for obtaining the mass spectrum of a beam of ions by means of suitably disposed magnetic and electric fields.

Net photosynthesis : Photosynthetic carbon fixation minus the carbon released as carbon released as carbon dioxide by processes such as respiration/photorespiration.

Non- cyclic photophosphorylation : Formation of ATP by non-cyclic electron transport where the electrons flow from H_2O to $NADPH^+$ through Photosystem II and Photosystem I.

Oxygenation : Addition of oxygen to any system/molecule.

P_{680} : A special molecule of chlorophyll $_a$ of photosystem II reaction centre that accepts energy from the light-harvesting pigments of photosystem I and transfers it by loss of a high-energy electron to another electron acceptor.

P_{700} : A special molecule of chlorophyll $_a$ of Photosystem I that accepts energy from the light- harvesting pigments of photosystem I and transfers it by loss of a high-energy electron to another electron acceptor.

P-protein : It is phloem protein found in all dicots and many monocots but absent in gymnosperms. Prevent leakage by sealing damaged sieve element pores. Earlier called “slime”.

Palisade cells : Elongated photosynthetic cells present below the epidermis of a leaf.

Partitioning : The differential distribution of photosynthates among various sink organs in a plant.

Phenylpropanoids : A family of organic compounds found throughout the plant kingdom, are synthesized from the amino acid phenylalanine. They are an important component of a number of structural polymers and provide many benefits to the plants which include, protection from ultraviolet light and pathogens

Phloem : Tissue that translocates the products of photosynthesis from mature leaves to areas of growth and storage.

Phloem loading : The movement of photosynthate from the mesophyll chloroplasts to the sieve elements of mature leaves.

Phloem unloading : The movement of photosynthate from the sieve cells into the sink tissue.

Phosphorescence : Release of extra energy in the form of light slowly in phased manner where the electrons re-release the energy at a slower rate than it was absorbed.

Photoinhibition : The inhibition of photosynthetic capacity caused by excess light-induced protein damage.

Photophosphorylation : It refers to the process of transfer of a phosphate group into ADP to synthesize energy rich ATP molecule using the energy of sunlight during photosynthesis.

Photosystem : One of two interacting energy-collecting and energy-transferring system that operate in chloroplasts.

Phycocyanin : Any of several blue, water-soluble protein pigments present in most blue-green algae.

Phycoerythrin : Any of several water-insoluble red protein pigments present in most blue-green algae and all red algae..

Photorespiration : Uptake of O_2 and release of CO_2 by plants when exposed to light. Photorespiration occurs due to oxygenase activity of *Rubisco* where molecular oxygen serves as a substrate and result in the formation of 2-phosphoglycolate. 2-phosphoglycolate enters the glycolate cycle/C2 pathway resulting in release of $CO_2$and NH_4^+.

Photosynthate : Any substance that gets synthesized during the process of photosynthesis.

Photosynthetically active radiation : Region of electromagnetic spectrum (400nm-700nm) absorbed by photosynthetic pigments.

Photosynthetic unit : Number of pigment molecules required for the evolution of a molecule of oxygen.

Photosystem : A functional unit of chloroplast that absorbs light energy for driving electron transport and causes the formation of proton gradient for synthesis of ATP. There are two types of energy collecting and energy transferring photosystem reaction centres (Photosystem I and Photosystem II) Photosystem I absorbs far red light and result in the oxidation of plastocyanin and reduction of ferredoxin. Photosystem II absorbs red light, oxidizes water, and reduces plastoquinone.

Plastocyanin : Water soluble copper containing protein associated with Cytochrome $_{b6f}$ complex and photosystem I.

Q_{10} (Temperature coefficient) : The ratio of rate of a chemical reaction occurring at a particular temperature and when occurring at similar conditions but a temperature lower by 10ºC.

Quantum : A discrete unit of electromagnetic energy. An entity having particle like properties. With reference to light, the amount of energy associated with one particle like unit or photon.

Quantum yield : Number of O_2 molecules released or CO_2 molecules fixed per photon of light in photosynthesis.

Quantasomes : Granular structures observed on the surface the thylakoids which may be the morphological expression of photosynthetic unit containing 200-300 chlorophyll pigments.

Reaction Centre : Special chlorophyll $_a$ molecules associated with proteins and redox carriers involved in the light reactions of photosynthesis.

Reactive oxygen species (ROS) : Highly reactive ions or free radicals that contain oxygen molecule(s).

Reassimilation : A second or subsequent assimilation that is absorption of nutrients into the system.

Redox potential/(Oxidation reduction potential) : Potential required in a cell to cause reduction at cathode and oxidation at anode. Redox system with a high positive or less negative redox potential value acts as an electron acceptor and a system with a high negative redox potential value acts as an electron donor.

Rubisco	:	*Ribulose bisphosphate carboxylase/oxygenase* enzyme in the chloroplast. Bifunctional enzyme; carboxylase in C_3 and C_4 pathways, oxygenase in photorespiration; Most abundant protein present on earth.
RuBP (Ribulose bisphosphate)	**:**	A 5- carbon bisphosphate pentose which acts as an acceptor of CO_2 in Calvin cycle or O_2 in photorespiration.
Sieve area	**:**	The pores present on sieve elements that interconnect the sieve tube elements.
Sieve plate	**:**	A region of the cell wall of a sieve tube member where pores are concentrated.
Sieve tube	**:**	A column of sieve tube members that functions in the transport of organic solutes in the phloem of angiosperms.
Sieve tube member	**:**	An elongated cell with pores on its end walls.
Sink	**:**	The non-photosynthetic organs of the plants that do not produce enough photosynthates to support their own growth and storage requirements.
Sink activity	**:**	The rate of uptake of photosynthate per unit weight of sink tissue
Sink capacity	**:**	A natural or artificial reservoir that accumulates and stores some chemical compound for an indefinite period.
Sink size	**:**	The total weight of sink tissue
Sink strength	**:**	The ability of a sink to mobilize photosynthate towards itself
Sieve element	**:**	Sieve element is a term that includes highly differentiated sieve tube elements in angiosperms and relatively unspecialized sieve cells of gymnosperms.
Source	**:**	The plant organs which can produce photosynthate more than their own needs.
Steady state	**:**	The state at which concentration of intermediates in a reaction are constant
Stomatal apparatus	**:**	A pair of guard cells and associated subsidiary cells involved in the opening and closing of the pore between the guard cells.

Stroma : The fluid substance within an organelle, such as a plastid. In fungi, a large mass of somatic (vegetative) hyphal tissue.

Symplastic pathway : The pathway that proceeds along symplast i.e., cytoplasm through plasmodesmatal connections between the cells.

Thylakoids : Several flattened sacs, present within the chloroplasts, arranged in stacks or grana. These are bounded by pigmented membranes and are the sites of the photochemical reactions of photosynthesis.

Tracheid : An elongated, empty cell of the xylem without perforated walls that is active in longitudinal transport of water and mineral nutrients in vascular plants.

Transfer cell : A parenchyma cell modified with internal extensions of the cell wall that greatly increase the surface of the plasma membrane.

Vessel : A long, hollow series of vessel members connected to each other end-to-end in the xylem that functions in longitudinal transport of water and mineral nutrients in angiosperms and some ferns.

Warburg's effect : Inhibition of photosynthesis due to oxygen.

Wavelength : Distance measured over which similar wave phase repeats itself. It characterizes light energy, and in visible spectrum it refers to a color.

Z scheme of photosynthesis : Arrangement of photosystem I, II, electron transport system and other electron carriers in a zig-zag manner according to their redox potential.

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