TOPIC H: PHOTOSYNTHESIS

Learning Outcomes

Candidates should be able to:

Core Topic 6 – Cellular Physiology and Biochemistry

(a) With reference to the chloroplast structure, explain the light dependent reactions of photosynthesis (no biochemical details are needed but will include the outline of cyclic and non-cyclic light dependent reactions, and the transfer of energy for the subsequent manufacturing of carbohydrates from carbon dioxide).

(b) Outline the three phases of the Calvin cycle: (i) CO_2 uptake (ii) carbon reduction and (iii) ribulose bisphosphate (RuBP) regeneration and indicate the roles of ATP and NADP in the process.

(c) Discuss limiting factors in photosynthesis and carry out investigations on the effects of limiting factors, such as light intensity, CO_2 concentration and temperature, on the rate of photosynthesis.

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7. Limiting Factors in Photosynthesis
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   (c) Carbon Dioxide

1. Autotrophs: Producers for the Earth

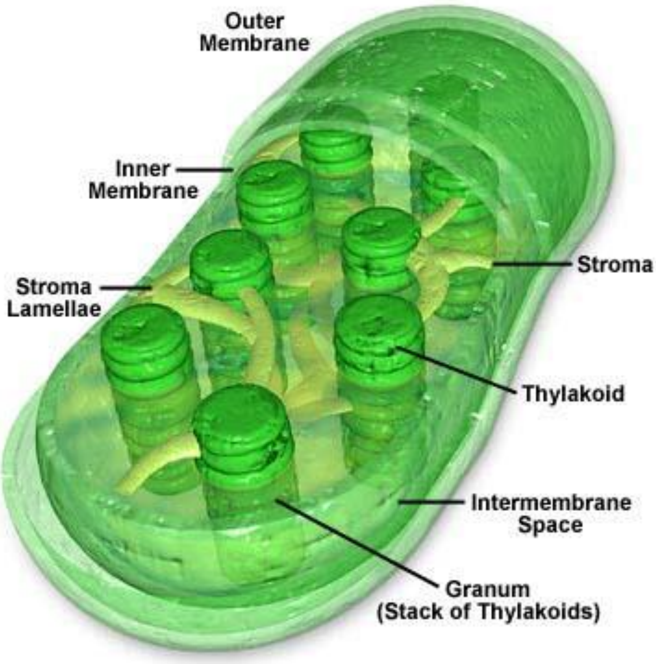
Photosynthesis nourishes almost all life on earth directly or indirectly. **Autotrophs** are organisms that sustain themselves without feeding on other organisms.

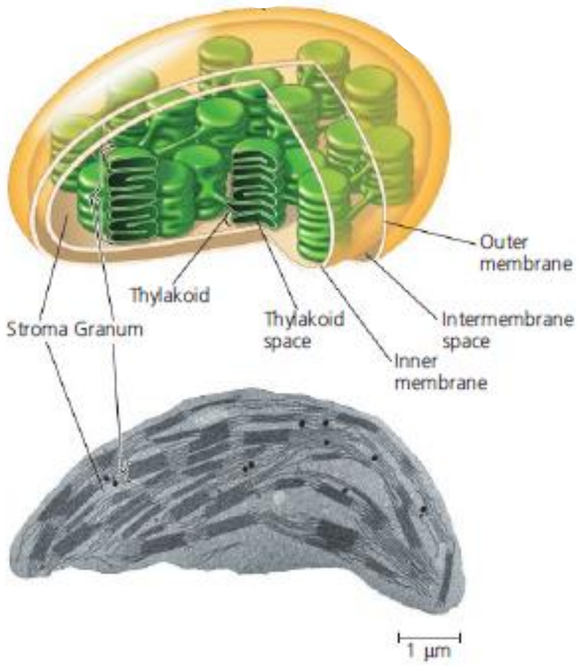
Autotrophs can produce their own organic (carbon-containing) compounds from carbon dioxide (an inorganic source of carbon) and other inorganic compounds obtained from the environment using the energy harnessed from the environment. As plants use light as a source of energy to synthesise these organic compounds, they are specifically known as **photoautotrophs**.

Heterotrophs feed on other organisms as they are unable to make their own organic compounds, unlike autotrophs.

Autotrophs undergo **photosynthesis** where **light energy** is used to **synthesize carbohydrates** from **carbon dioxide and water**. **Oxygen** is given out in the process as a by-product. The product formed is a sugar that is soon converted to starch for storage. There are two main stages in photosynthesis, namely the **light-dependent** and **light-independent** reactions / **Calvin cycle**.

2. Structure of the Chloroplast





The two major steps for photosynthesis take place in specific regions within the chloroplast:

1) Light-dependent reactions – **Thylakoids of the grana**
2) Light-independent reactions (the Calvin cycle) - **Stroma**

The **thylakoids** possess the following properties which allow the **light-dependent reactions** of photosynthesis to be carried out:

1) **Large surface area** of thylakoid membrane
   - Allows **photosystems and electron carriers** to be embedded.
   - Allows **stalked particles containing ATP synthase** to be embedded.

2) Thylakoid membrane is **impermeable to protons**
   - Allows **electrochemical proton gradient** to be set up between the thylakoid space and stroma.

The **stroma** is a dense fluid which **contains the enzymes and dissolved substrates** required for the **light-independent reactions** of photosynthesis, also known as the **Calvin cycle**.

Excess carbohydrate from photosynthesis is stored as **starch grains** in the chloroplasts. **Lipid droplets**, which are associated with the breakdown of membranes and lipids, also accumulate within the chloroplasts.

3. Photosynthetic Pigments

There are 2 types of photosynthetic pigments which are the **chlorophylls** and the **carotenoids.** These pigments are found in **photosystems (PS)** which are embedded on the thylakoid membranes. The role of the pigments is to **absorb light energy** and **convert it into chemical energy.**

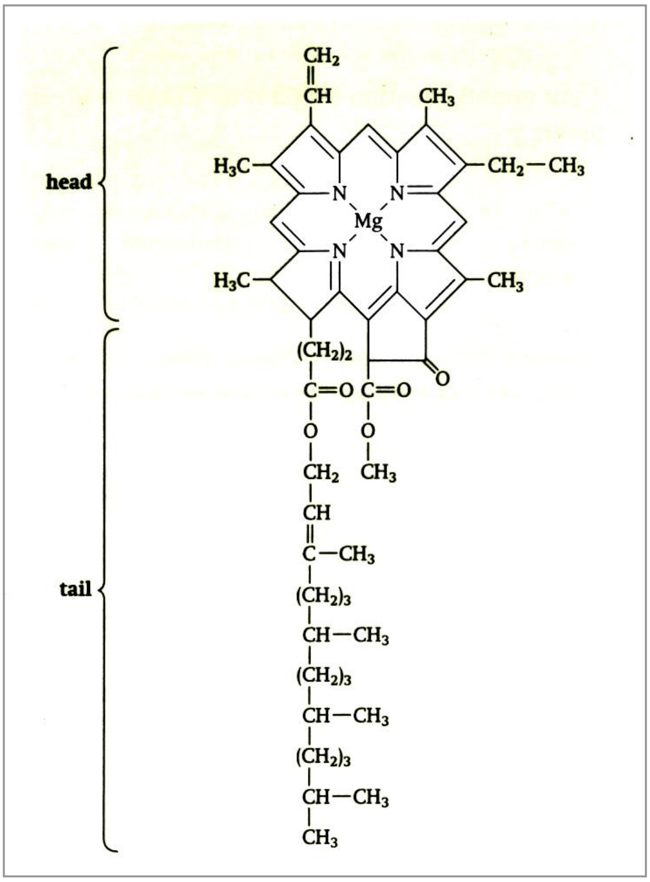
(a) Chlorophylls

There are 2 main types of chlorophyll – **chlorophyll a** and **chlorophyll b.** They absorb mainly **red** and **blue-violet light**, reflecting green light and therefore giving plants their characteristic green colour.

Their chemical structure consists of:

- a head made up of a porphyrin ring with a magnesium ion in the centre, and
- a long hydrocarbon tail which is joined to its head by an ester linkage.

Different chlorophylls have different side-chains on the head and these modify their absorption spectra.



Structure of Chlorophyll a

(b) Carotenoids

There are 2 main types of carotenoids – **carotenes** and **xanthophylls**. They are yellow, orange, red or brown pigments that **absorb strongly in the blue-violet light spectrum**. They are usually masked by the green chlorophylls.

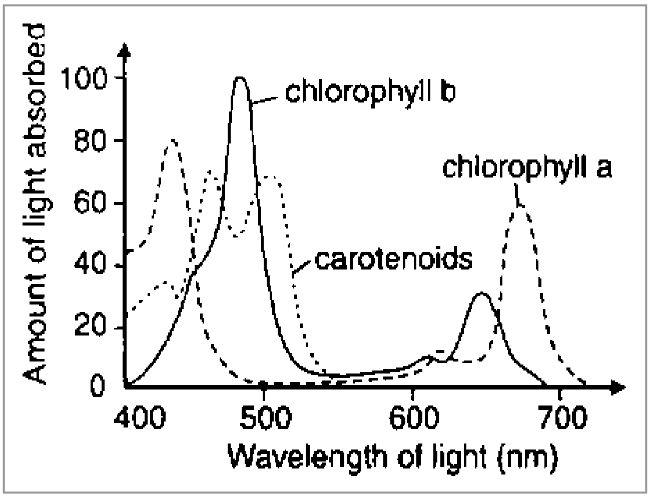
Carotenoids **protect chlorophylls from excess light and oxidation by oxygen produced during photosynthesis**.

4. Absorption and Action Spectra

a) **Absorption spectrum**
- A graph showing the **relative absorbance** of different wavelengths of light by a pigment.

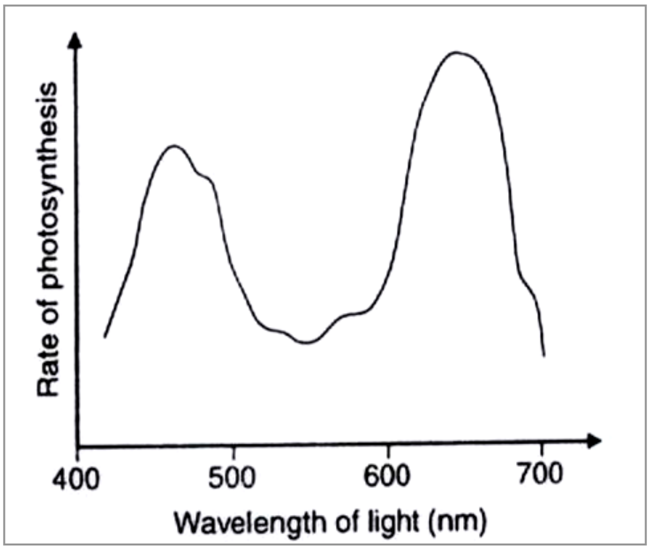
b) **Action spectrum**
- A graph showing the **effectiveness** of different wavelengths of light in stimulating photosynthesis.
- It is a record of the amount of photosynthesis occurring at each wavelength of light.

The close similarity/correlation between the **absorption spectrum** and **action spectrum** indicates that the photosynthetic pigments are responsible for absorption of light in photosynthesis.



Absorption Spectrum:

Amount of light absorbed by different pigments at different wavelengths of light

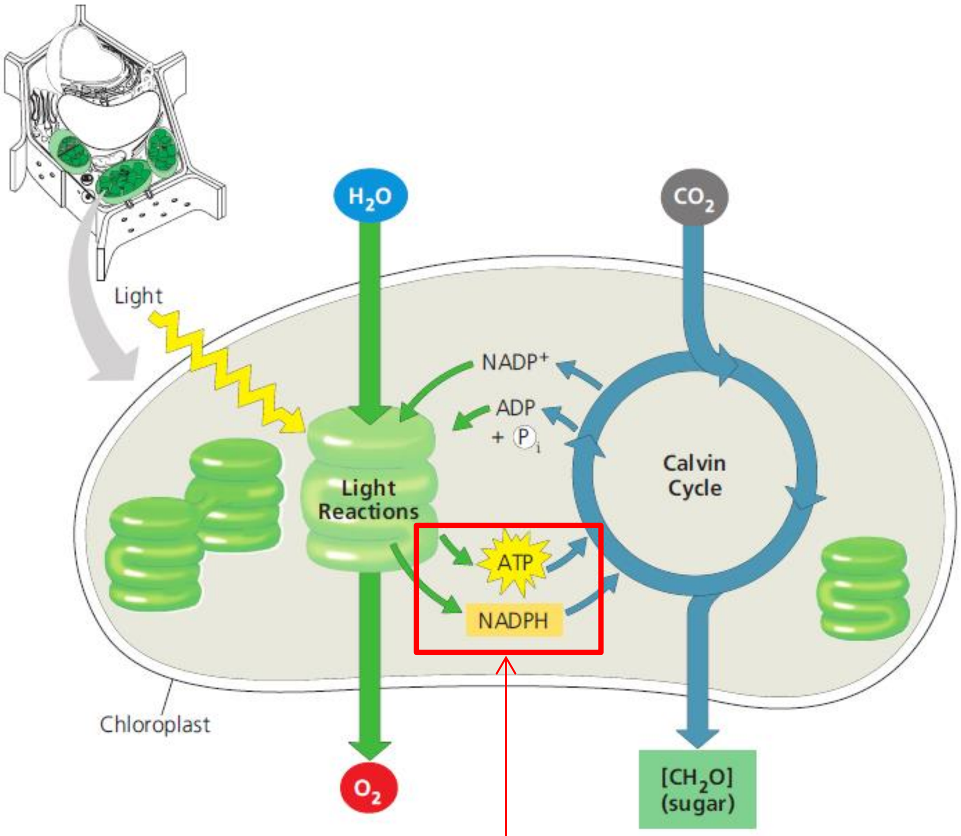


Action Spectrum:

Rate of photosynthesis at different wavelengths of light

5. Light-dependent Reactions

Light-dependent reactions occur in the **grana**. The objective of these reactions is to provide **ATP** and **reduced nicotinamide adenine dinucleotide phosphate (reduced NADP / NADPH + H^+)** for the light-independent reactions (Calvin cycle).



Light-dependent reactions provide the **ATP and reduced NADP** required to drive the Calvin cycle.

As the energy for the synthesis of ATP comes from light, it is called **photophosphorylation**. There are 2 types of photophosphorylation – **cyclic** and **non-cyclic.**

Light-dependent reactions (photophosphorylation)

- **Cyclic** photophosphorylation
- **Non-cyclic** photophosphorylation

(a) Photosynthetic Units

There are 2 types of photosynthetic units: **photosystem I (PSI)** and **photosystem II (PSII).**

Each photosystem comprises:

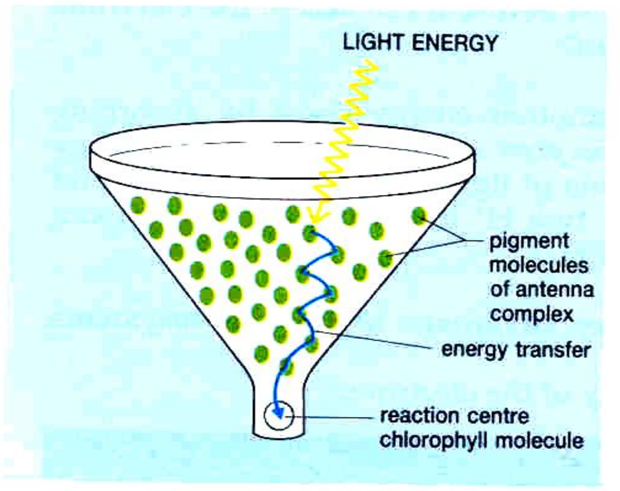
i. **Light-harvesting complexes**
- Made up of **accessory pigments** (chlorophylls and carotenoids).
- Accessory pigments **funnel the energy absorbed from light** to the reaction centre in either PSI or PSII.
- The light-harvesting complexes surround the reaction centre.

ii. A **reaction centre**
- A protein complex that includes **two special chlorophyll a molecules (primary pigments)** and a molecule called the **primary electron acceptor**.
- Two different forms of special chlorophyll a, **P680** and **P700** *(P stands for primary pigment of chlorophyll a while 680 and 700 are their absorption peaks' wavelength in nm).*
- The two special chlorophyll a molecules **each emit one electron** and the two electrons are accepted by the primary electron acceptor.

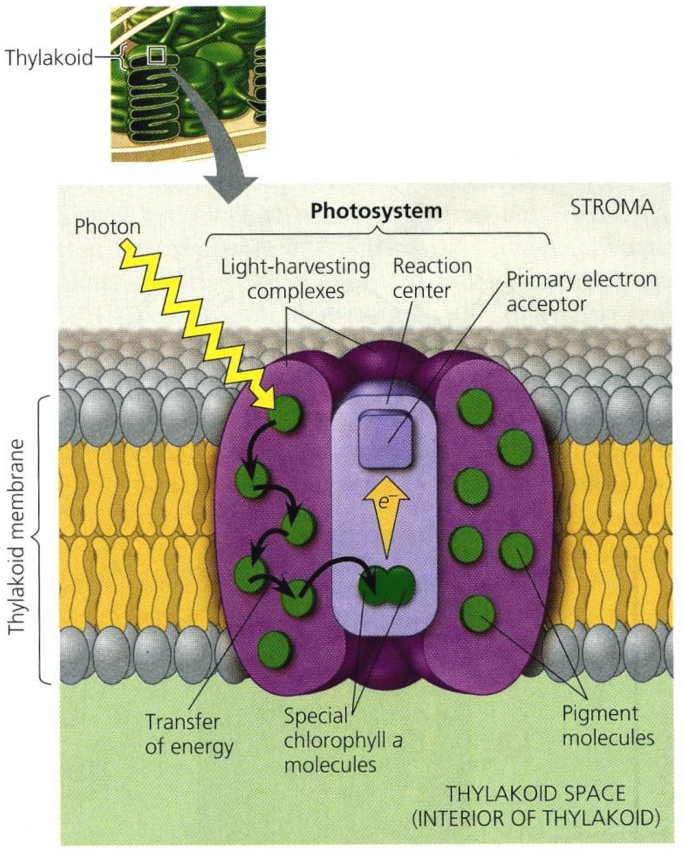
(b) Excitation of Primary Pigments by Light (Absorption of Light Energy)

Primary pigments (i.e. special chlorophyll a in the reaction centres) are molecules that absorb energy from accessory pigments. When the energy levels of the electrons found in the primary pigments are boosted, they reach an **excited state**. The excited pigments will then **emit their electrons**, leaving **positive 'holes'** in their molecules.

$$\textbf{chlorophyll} \xrightarrow{\textbf{light}} \textbf{chlorophyll}^{+} + e^{-}$$

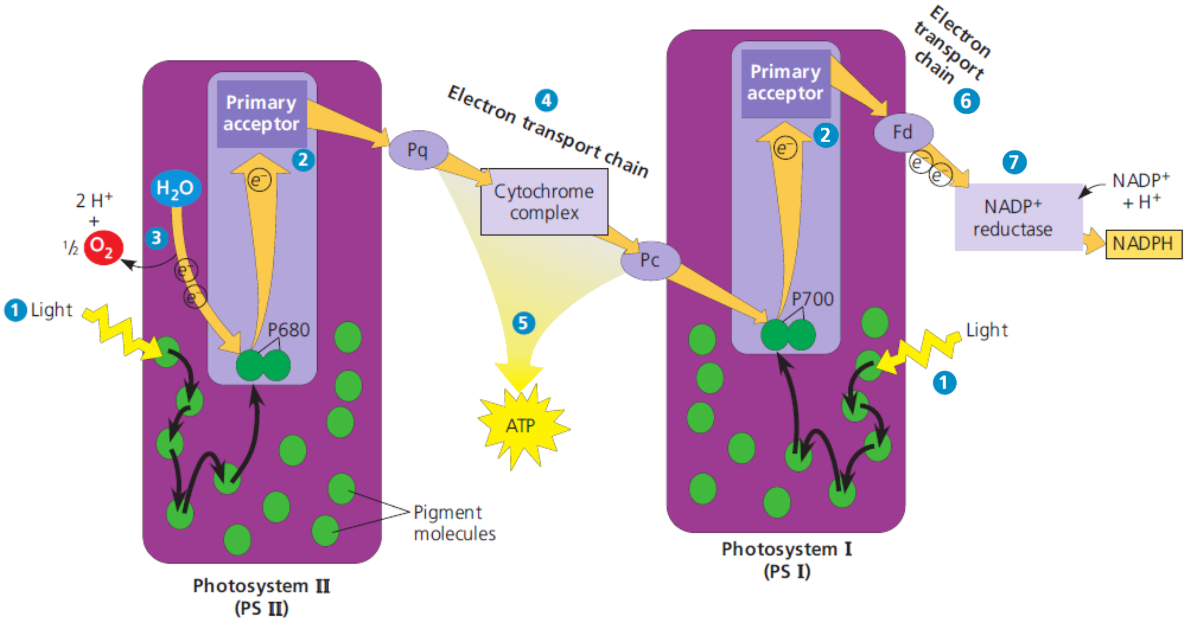


Each antenna complex in the thylakoid membrane consists of several hundred chlorophyll and accessory pigment molecules which funnel the energy they absorb from sunlight to a single reaction centre chlorophyll.



How a photosystem harvests light. When a photon strikes a pigment molecule in a light-harvesting complex, the energy is passed from molecule to molecule until it reaches the reaction center. At the reaction center, an excited electron from one of the two special chlorophyll *a* molecules is captured by the primary electron acceptor.

(c) <u>Non-cyclic Photophosphorylation</u>



Step 1:

- **<u>Light</u>** of particular wavelengths strikes an **<u>accessory pigment molecule</u>** in the **<u>light harvesting complex</u>** of **<u>PSII and PSI</u>**.
- This **<u>energy</u>** is relayed to **<u>neighbouring accessory pigment molecules</u>** until it **<u>accumulates</u>** and reaches one of the two **<u>P680 chlorophyll a</u>** molecules in the **<u>reaction centre</u>** of **<u>PSII</u>**.
- The same occurs for the **<u>P700 chlorophyll a</u>** molecules in the **<u>reaction centre</u>** of **<u>PSI</u>**.

Step 2:

- This **<u>excites one of the P680 electrons and one of the P700 electrons</u>** to a **<u>higher energy state</u>**, which subsequently gets emitted and captured by the **<u>primary electron acceptor</u>** within each PS.
- A **<u>positive 'hole'</u>** is left behind in each P680 and P700 chlorophyll a molecule in PSII and PSI respectively.

Step 3 (occurs concurrently with Step 4):

- **<u>Photolysis of water</u>** occurs when **<u>an enzyme catalyses the splitting of a water molecule into protons</u>**, **<u>electrons</u>** and **<u>molecular oxygen</u>**.

$$H_2O \xrightarrow{\text{enzyme}} 2H^+ + 2e^- + \tfrac{1}{2}O_2$$

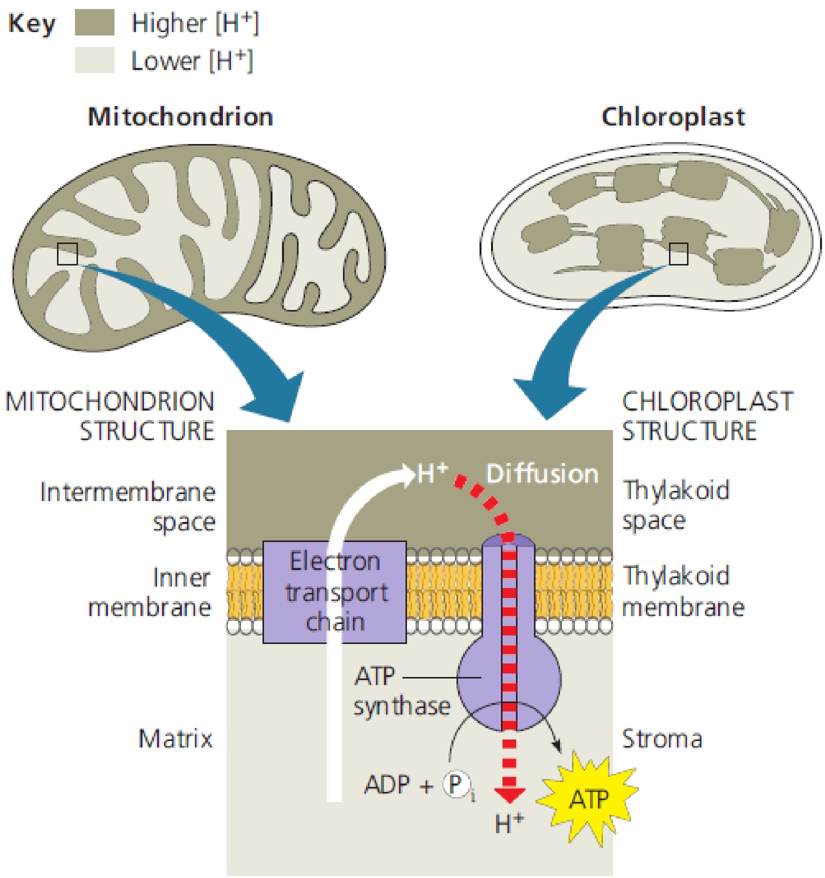
- Electrons from the photolysis of water are used to fill up **<u>positive 'holes'</u>** in the **<u>reaction centre of PSII</u>** to return **<u>$P680^+$ to ground state</u>**.

Step 4 (occurs concurrently with Step 3):

- The **<u>photoexcited electron</u>** (that was emitted by P680 in PSII previously) passes from the **<u>primary electron acceptor of PSII to $P700^+$ in PSI, to fill the positive 'hole' in $P700^+$.</u>**
- This occurs via an **<u>electron transport chain</u>** made up of **<u>electron carriers, each with an energy level lower than the one preceding it</u>**.

Step 5:

- **Energy** from the **electron transfer** down the chain of electron carriers is used to **actively pump protons** from **the stroma into the thylakoid space**.
- This **generates an electrochemical proton gradient** for the **synthesis of ATP**.
- **Protons** diffuse through the **stalked particle** containing **ATP synthase** which catalyzes the **synthesis of ATP from ADP and P_i**.
- This process by which protons (H^+) diffuse through a stalked particle for the synthesis of ATP is known as **chemiosmosis**.



Comparison of chemiosmosis in mitochondria and chloroplasts. In both kinds of organelles, electron transport chains pump protons (H^+) across a membrane from a region of low H^+ concentration (light gray in this diagram) to one of high H^+ concentration (dark gray). The protons then diffuse back across the membrane through ATP synthase, driving the synthesis of ATP.

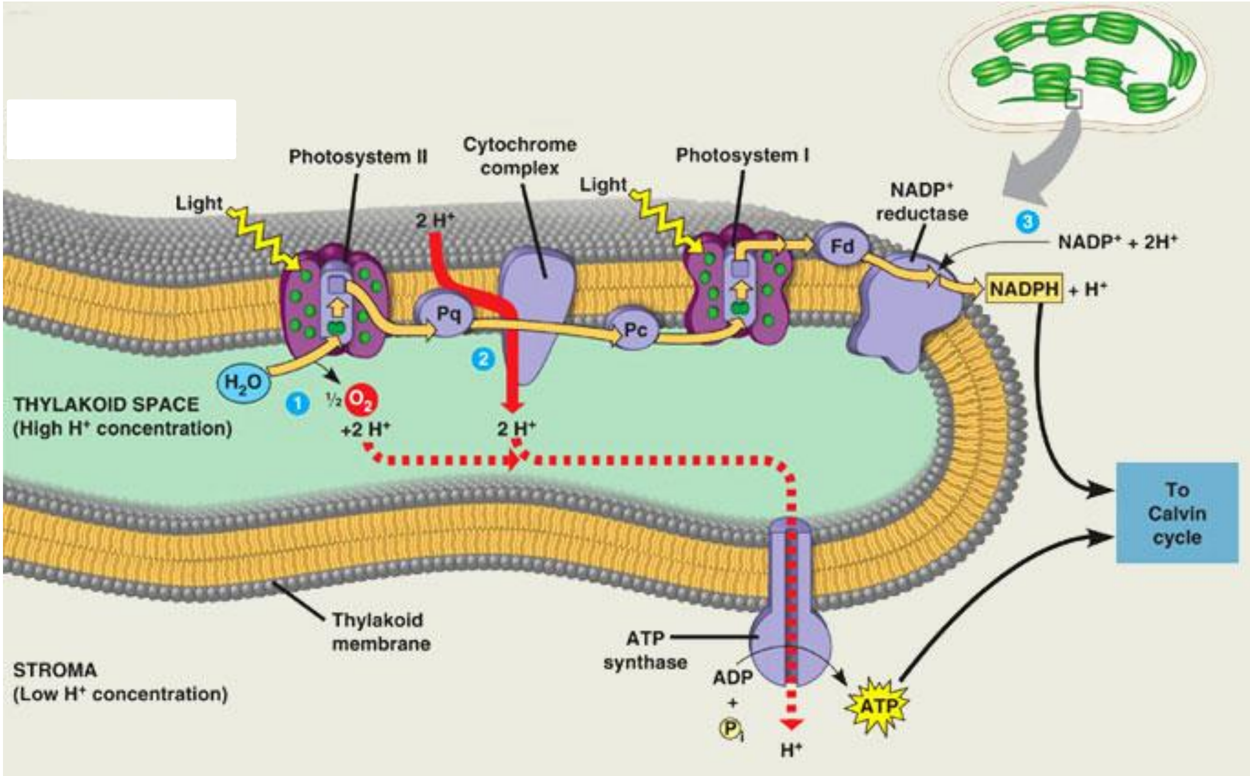
Step 6:

- **Electrons and protons** are **passed down a second electron transport chain from the primary electron acceptor of PSI to** the protein **ferredoxin** (the last electron carrier).

Step 7:

- The enzyme **NADP reductase** catalyses the **transfer of electrons from ferredoxin to oxidised NADP (the final electron and proton acceptor)** to form **reduced NADP**.

$$NADP^+ + 2e^- + 2H^+ \xrightarrow{\text{NADP reductase}} NADPH + H^+$$



The above diagram shows a current model for the organization of the thylakoid membrane. As electrons pass from carrier to carrier in redox reactions, protons removed from the stroma are pumped into the thylakoid space storing energy as a proton-motive force (electrochemical proton gradient).

The electrochemical proton gradient is established across the thylakoid membrane:

(a) Higher concentration of protons in thylakoid space
   1. Water undergoes photolysis in the thylakoid space, generating protons in the process.
   2. As plastoquinone (Pq), a mobile carrier, transfers electrons to the cytochrome complex, protons are translocated across the membrane into the thylakoid space.

(b) Lower concentration of protons in stroma
   3. Protons are removed from the stroma when they are taken up by oxidised NADP.

The diffusion of H^+ from the thylakoid space back to the stroma (down the electrochemical proton gradient) powers the ATP synthase. These light-driven reactions store chemical energy in NADPH + H^+ and ATP, which shuttle the energy to the sugar-producing Calvin cycle.

(d) <u>Cyclic Photophosphorylation</u>

In cyclic photophosphorylation, the electrons follow a different route.

PSI is now **<u>both a donor and acceptor of electrons</u>**. The excited electrons in the primary electron acceptor of **<u>PSI</u>** pass to **<u>ferredoxin</u>** and back to the **<u>cytochrome complex in the electron transport chain</u>**. The electrons eventually return to the **<u>PSI reaction centre</u>**.

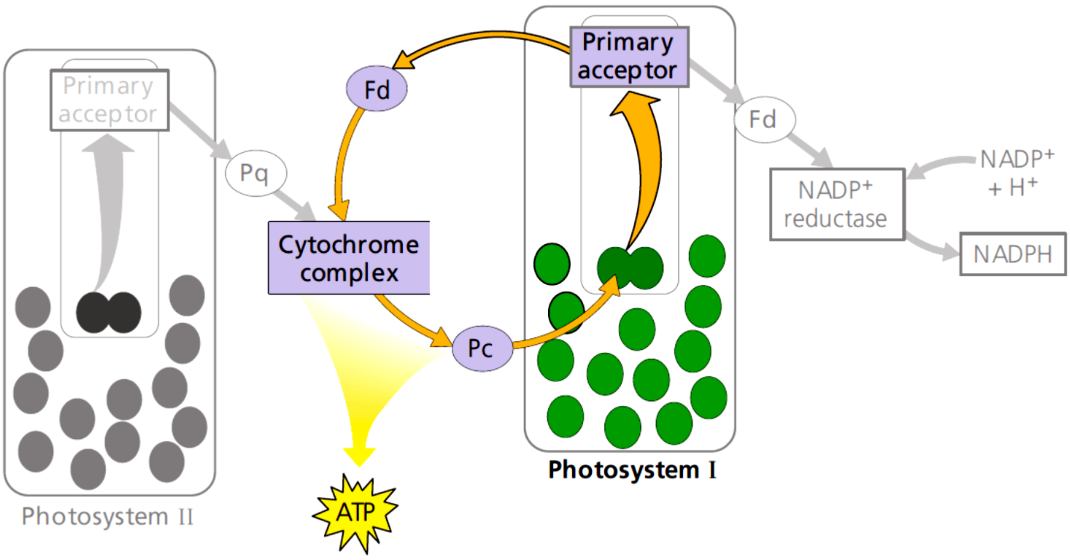
Cyclic photophosphorylation **<u>does not involve photolysis of water</u>**.

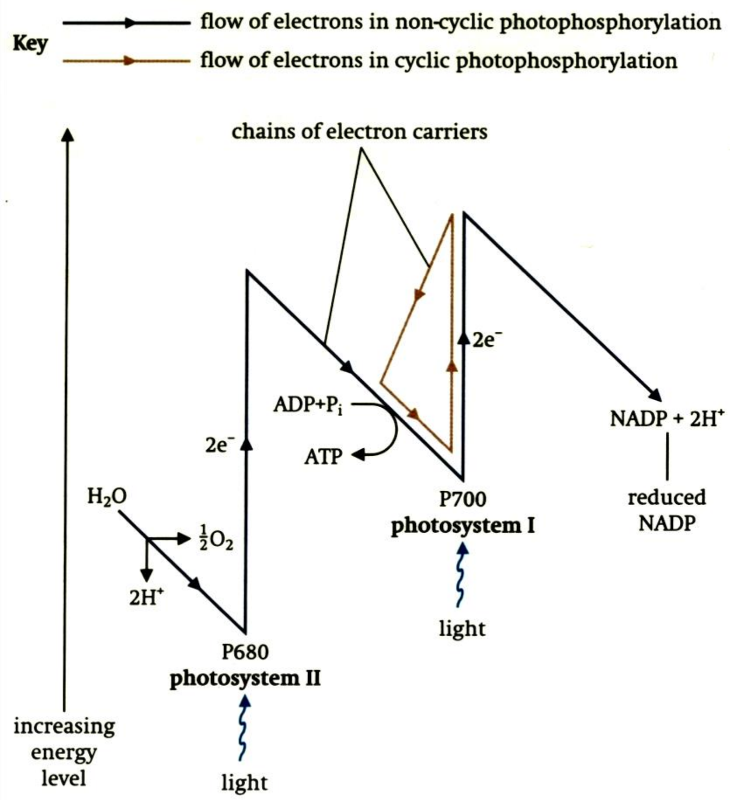
The energy released during the cycle of electrons down the chain of electron carriers allows **<u>protons to be actively pumped from the stroma into the thylakoid space</u>**, **<u>generating an electrochemical proton gradient across the thylakoid membrane</u>**, just like in non-cyclic photophosphorylation.

The electrochemical proton gradient allows for the synthesis of ATP by the stalked particles embedded on the thylakoid membrane.

<u>Cyclic photophosphorylation</u> yields **<u>only ATP</u>** whereas **<u>non-cyclic photophosphorylation yields O_2, ATP and reduced NADP</u>**.

- Light-dependent reactions (photophosphorylation)
  - **<u>Cyclic</u>** photophosphorylation
    - ATP
  - **<u>Non-cyclic</u>** photophosphorylation
    - ATP
    - O_2
    - Reduced NADP





Light-independent Reactions (Calvin Cycle)

The light-independent reaction / Calvin cycle occurs in the **stroma** of the choloroplast. There are 3 main stages:

(a) **CO_2 fixation** (CO_2 uptake)

$$RuBP + CO_2 + H_2O \xrightarrow{\text{RuBP carboxylase-oxygenase (Rubisco)}} \text{unstable 6C intermediate} \longrightarrow 2\ GP$$

RuBP = ribulose bisphosphate, 5C sugar

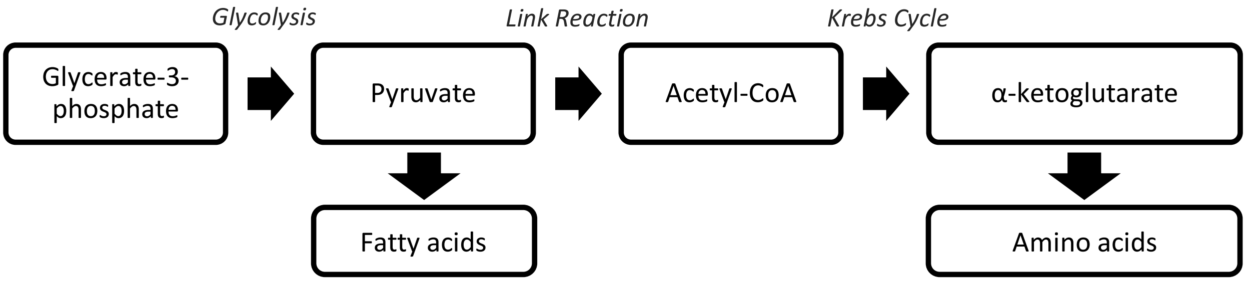
GP = glycerate-3-phosphate previously known as Phosphoglyceric acid (PGA)

RuBP carboxylase-oxygenase (Rubisco) is present in large amounts in the stroma of the chloroplast.

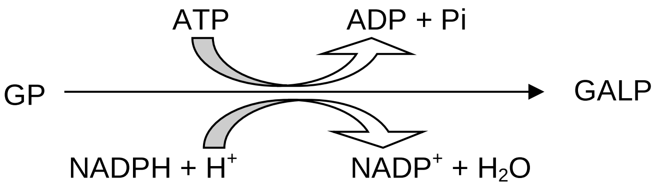
It catalyses the **fixation of CO_2** by a 5C sugar known as **ribulose bisphosphate (RuBP)**, which gives an **unstable 6C intermediate** that **immediately breaks down** to **2** molecules of 3C compound known as **glycerate-3-phosphate (GP)**.

Rubsico thus regulates the rate of photosynthesis.

GP can be **converted to pyruvate** which is used to synthesize **fatty acids**. The pyruvate that is formed can also be converted to **acetyl coenzyme A** which undergoes the Krebs Cycle (covered in 'Respiration') to form **α-ketoglutarate**. α-ketoglutarate can undergo further reactions to form **amino acids** in plants.



(b) <u>Carbon Reduction</u>



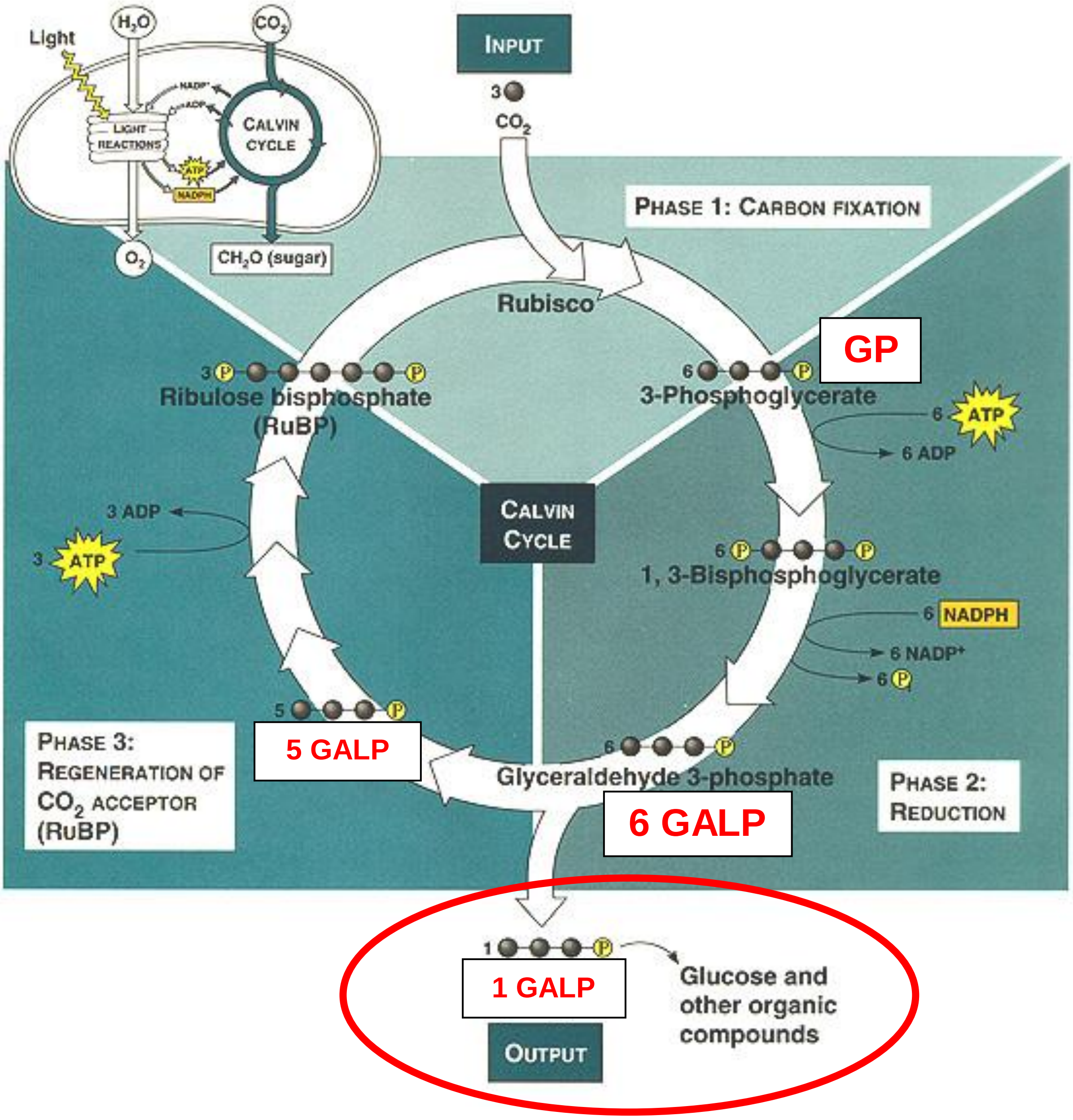
GALP = **<u>glyceraldehyde-3-phosphate</u>** (3C sugar)
previously known as Triose Phosphate (TP)

The **<u>reducing power of reduced NADP</u>** and **<u>energy from the hydrolysis of ATP</u>** are used to convert GP to **<u>GALP</u>**. GALP contains more chemical energy than GP, and is the **<u>first carbohydrate</u>** made in photosynthesis. **<u>About 1/6</u>** of the total amount of GALP is used to synthesize **<u>other carbohydrates</u>** (e.g. sucrose and starch) and **<u>glycerol</u>**.

(c) <u>Regeneration of RuBP</u>

<u>About 5/6</u> of the total amount of GALP has to be used to **<u>regenerate the RuBP</u>** consumed in the first reaction. This process **<u>requires energy from the hydrolysis of ATP</u>**.

This regeneration of RuBP makes this process a cycle.



In order to generate 1 molecule of GALP (3C sugar) from the Calvin cycle, we require:

- 3 CO_2
- 9 ATP
- 6 reduced NADP

Therefore, in order to generate 1 molecule of glucose (6C sugar), we require:

- 6 CO_2
- 18 ATP
- 12 reduced NADP

6. Limiting Factors in Photosynthesis

A **<u>limiting factor</u>** is one which **<u>at its minimum value will limit the rate of the overall reaction</u>**.

The rate of photosynthesis is an important factor in crop production since it affects yields. An understanding of these factors affecting the rate is likely to lead to an improvement in crop management.

Rate of photosynthesis can be measured in terms of:

- dry mass formed per unit time;
- volume of oxygen evolved per unit time;
- volume of carbon dioxide absorbed per unit time.

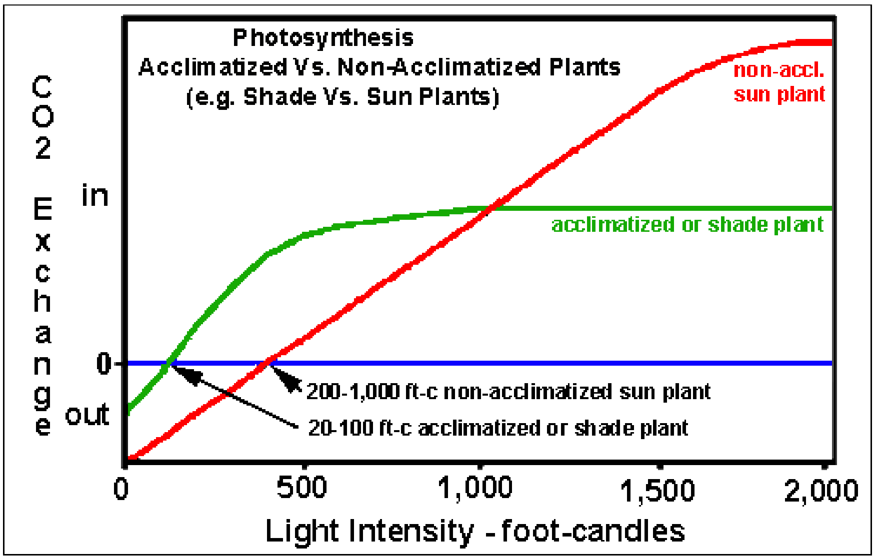
(a) <u>Light</u>

(i) Light intensity

<u>Light intensity</u> is an important limiting factor in the light-dependent stage to **<u>excite the special chlorophyll a molecules for photophosphorylation</u>** to occur. However, it is seldom the limiting factor during daylight hours (except in the case of shaded plants).

Photosynthesis results in uptake of carbon dioxide and evolution of oxygen. At the same time respiration uses oxygen and produces carbon dioxide. There will come a point when the light intensity causes photosynthesis and respiration to exactly balance each other. This is called the **<u>light compensation point</u>** (i.e. **<u>light intensity at which net gas exchange is zero</u>**).

The light compensation points of plants grown in abundant sunlight is higher than those grown in shade.



(Source: http://generalhorticulture.tamu.edu/lectsupl/Light/light.html)

(ii) Wavelength of light

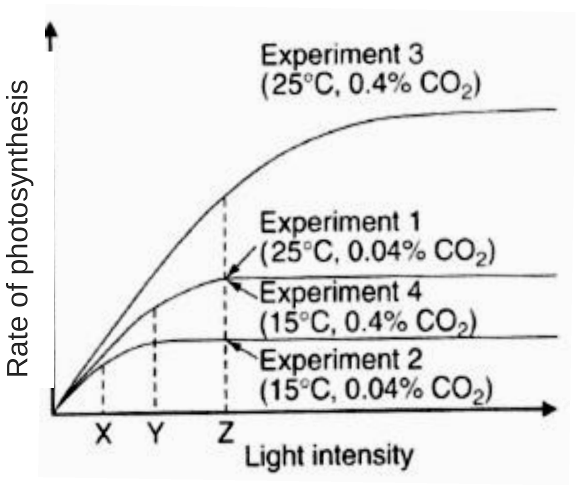
<u>Wavelength of light</u> is also a limiting factor as demonstrated by comparing the action and absorption spectra for photosynthesis. The rate of photosynthesis is **<u>highest at the red and blue-violet regions</u>** of the action spectrum and **<u>lowest at the green region</u>**.

(b) Temperature

Temperature is an important limiting factor as it affects the **rate of enzyme reactions** during light-dependent (e.g. **NADP reductase**) and light-independent stages (e.g. **Rubisco**).

(c) Carbon Dioxide

Carbon dioxide is a major limiting factor as its **concentration in the atmosphere is low** (0.03 – 0.04%). It is the **raw material for the Calvin cycle** and its increased concentration will increase the rate of photosynthesis.



Comparing the following experiments, identify the limiting factors.

Experiments	Limiting Factor
1 & 2	
1 & 3	
2 & 4	
3 & 4	

Appendix

C_3,C_4 and CAM plants

In hot and dry conditions, plants will close their stomata to prevent loss of water. Under these conditions, oxygen gas, produced by the light reactions of photosynthesis, will concentrate in the leaves causing photorespiration to occur. Some plants have evolved mechanisms to increase the CO2 concentration in the leaves under these conditions.

C4 plants capture carbon dioxide using an enzyme called PEP Carboxylase that adds carbon dioxide to the three carbon molecule Phosphoenolpyruvate (PEP) creating the 4-carbon molecule oxaloacetic acid. Plants without this enzyme are called C3 plants because the primary carboxylation reaction produces the three-carbon sugar 3-phosphoglycerate directly in the Calvin-Benson Cycle. When oxygen levels rise in the leaf, C4 plants reverse the reaction to release carbon dioxide thus preventing photorespiration. By preventing photorespiration, C4 plants can produce more sugar than C3 plants in conditions of strong light and high temperature. Many important crop plants are C4 plants including maize, sorghum, sugarcane, and millet.
Xerophytes such as cacti and most succulents also can use PEP Carboxylase to capture carbon dioxide in a process called Crassulacean acid metabolism (CAM). They store the CO2 in different molecules than the C4 plants (mostly they store it in the form of malic acid via carboxylation of phosphoenolpyruvate to oxaloacetate, which is then reduced to malate). Nevertheless, C4 plants capture the CO2 in one type of cell tissue (mesophyll) and then transfer it to another type of tissue (bundle sheath cells) so that carbon fixation may occur via the Calvin cycle. They also have a different leaf anatomy than C4 plants. They grab the CO2 at night, when their stomata are open, and they release it into the leaves during the day to increase their photosynthetic rate. C4 metabolism physically separates CO2 fixation from the Calvin cycle, while CAM metabolism temporally separates CO2 fixation from the Calvin cycle.