13 Photosynthesis

13.1 Photosynthesis as an energy transfer process

a) *energy transferred as ATP and reduced NADP from the light dependent stage is used during the light independent stage (Calvin cycle) of photosynthesis to produce complex organic molecules*

Three basic steps of photosynthesis:

1) light-dependent stage
   - energy capture
   - chemiosmosis (generation of ATP)

2) light-independent stage
   - carbon fixation: enzyme catalysed reactions using ATP formed in light reactions to fix carbon dioxide into sugars

3) pigment regeneration
   - electron replacement from the splitting of H_2O in oxygenic photosynthesis

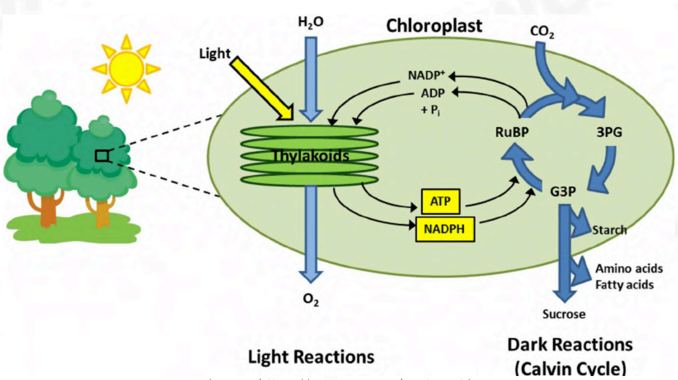


Image: https://www.researchgate.net/

b) reaction sites

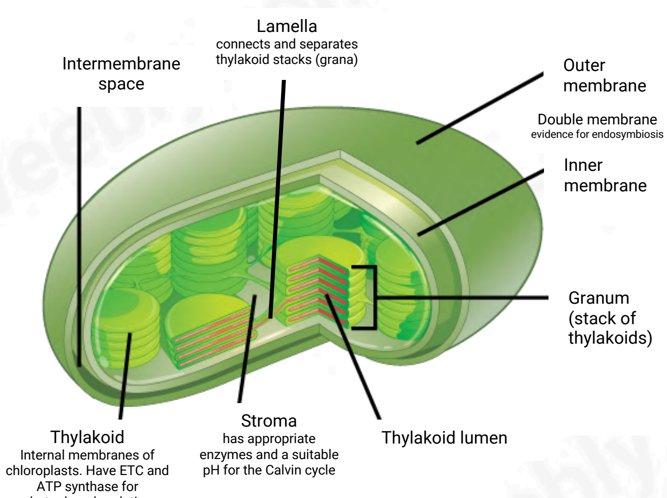
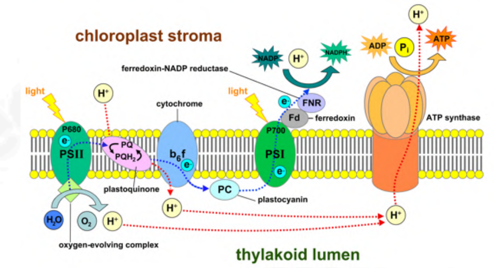


Image: https://openoregon.pressbooks.pub/mhccmajorsbio/chapter/8-2-main-structures-and-summary-of-photosynthesis/

<table>
<tr><th>STAGE</th><th>REACTION SITE</th></tr>
<tr><td>light-dependent</td><td>thylakoid (membrane)


https://en.wikipedia.org/wiki/Photosynthesis#/media/File:Thylakoid_membrane_3.svg</td></tr>
<tr><td>light-independent</td><td>stroma
Colourless colorless hydrophilic matrix surrounding the grana within the chloroplast. It contains DNA, ribosomes and some temporary products of photosynthesis.</td></tr>
</table>

c) role of chloroplast pigments in light absorption in the grana

- a pigment is any substance that absorbs light
- colour of the pigment comes from different wavelengths of light reflected (*not* absorbed)
- the light absorption pattern of a pigment is called the absorption spectrum

GROUP	PIGMENT	COLOUR	ABSORBS
chlorophylls	chlorophyll *a*	green	red, blue-violet
	chlorophyll *b*		
carotenoids	carotene	orange	blue-violet
	xanthophyll	yellow	

d) absorption and action spectra of chloroplast pigments

➢ absorption spectrum – graph showing the absorbance of different wavelengths of light by a pigment

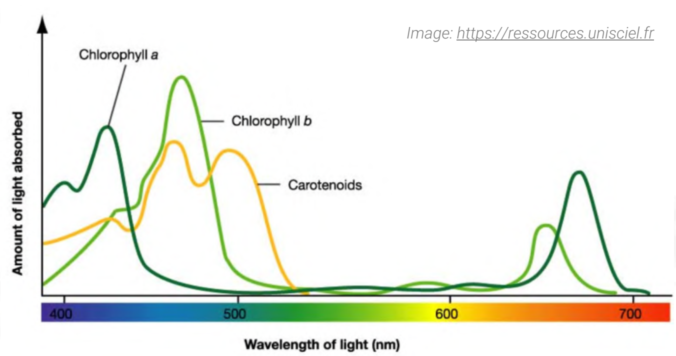


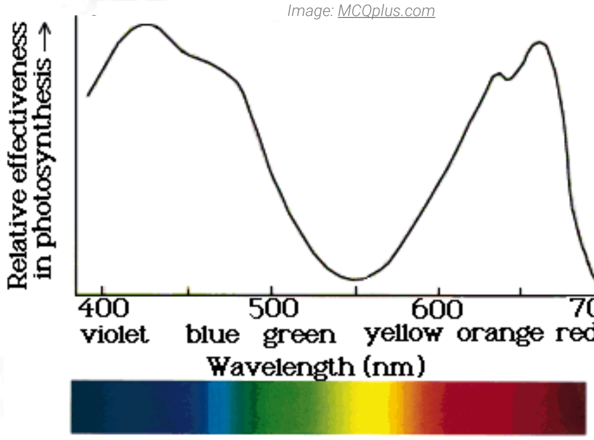
Image: https://ressources.unisciel.fr

Interpreting this absorption spectrum with respect to chlorophyll a & b
- best absorption with blue-violet light
- good absorption with red-orange light

- poor or no absorption with green light; most green light is reflected ∴ not absorbed

➢ **action spectrum** – graph showing the rate of photosynthesis at different wavelengths of light

Image: MCQplus.com



Interpreting the action spectrum

- the rate of photosynthesis is not the same for every wavelength of light
- rate is the least with green-yellow light
- good rate with red-orange light
- best rate is with violet-blue light

Comparing the absorption and action spectra

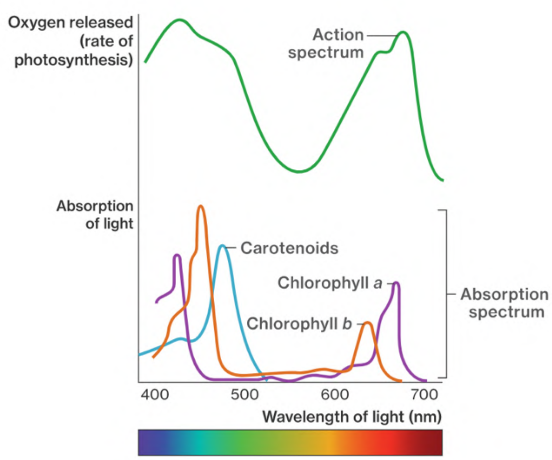
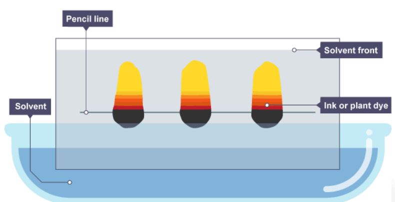


Image: coursehero.com

e) chromatography

- used to separate and identify chloroplast pigments
- used to carry out investigations to compare chloroplast pigments in different plants
- was used to identify the intermediates of the Calvin cycle



Procedure: Image: bbc.co.uk/bitesize/guides/z9dfxfr/revision/4

1) spot sample on base line (drawn with a pencil)
2) concentrate the spot with the sample by applying the sample multiple times, drying the chromatogram each time
3) suspend the base of the chromatograph in an organic solvent (e.g., ethanol)
4) ensure that the chromatography chamber is covered with a lid (to prevent the evaporation of the solvent) and the chromatogram is removed before the solvent front reaches the top
5) calculate the R_f value using:

$$R_f = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$

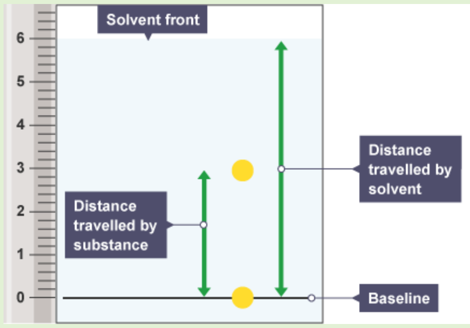


Image: bbc.co.uk/bitesize/guides/z9dfxfr/revision/4

6) compare the result with a known R_f value to identify the sample

f) the light dependent stage of photosynthesis

This is the photoactivation of chlorophyll resulting in the photolysis of water and the transfer of energy to ATP and reduced NADP.

Light energy absorbed by chloroplast pigments in the light dependent stage of photosynthesis is used to drive reactions of the light independent stage that produce complex organic compounds.

Light dependent reactions only take place in the presence of **pigments** that absorb wavelengths of light.

PIGMENTS

PRIMARY PIGMENTS

- act as reaction centres
- e.g., chlorophyll a

ACCESSORY PIGMENTS

- several hundred surround a primary pigment molecule
- light energy absorbed is passed onto the primary pigment molecule

pigments are arranged in light harvesting clusters in the thylakoid membrane called **PHOTOSYSTEMS**

PHOTOSYSTEM I

- absorb light of wavelength 700 nm
- present in bacteria as well as plants

PHOTOSYSTEM II

- absorb light of wavelength 680 nm
- present in plants only

(a) Photosystem II (P680)

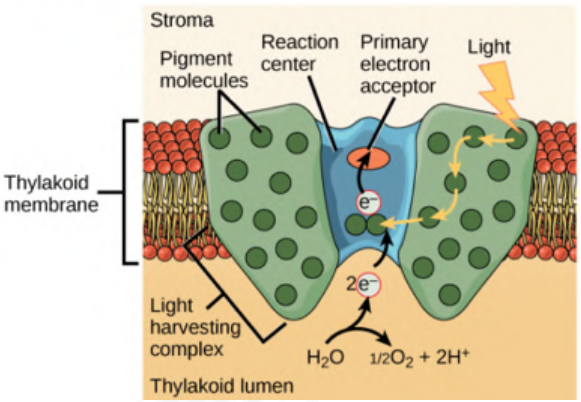


Image: courses.lumenlearning.com

(b) Photosystem I (P700)

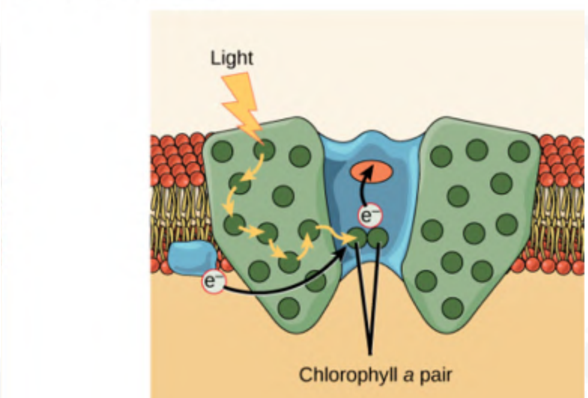


Image: courses.lumenlearning.com

Light energy is required for:

a) photolysis (only in photosystem II)
b) ATP production in photophosphorylation (for the reduction of CO_2 to carbohydrates in light independent stages)

Summary of the light dependent stage

a) photolysis of water (only in photosystem ii ∴ plants)

- photosystem II includes a water-splitting enzyme that catalyses the breakdown of water
- oxygen is a waste product of this process

$$H_2O \rightarrow 2H^+ + 2e^- + \tfrac{1}{2}O_2$$

- hydrogen ions combine with electrons from photosystem I and NADP to give reduced NADP

$$2H^+ + 2e^- + NADP \rightarrow \text{reduced NADP}$$

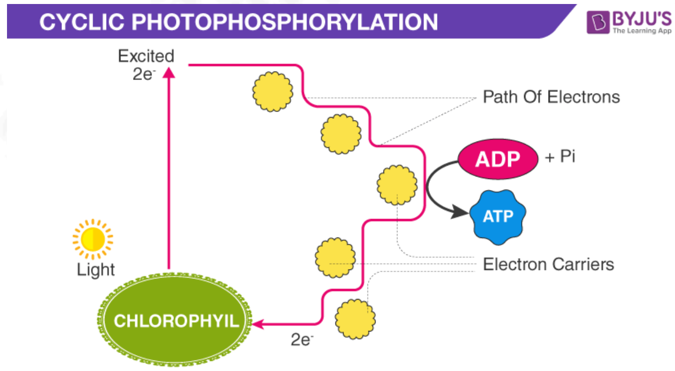
- reduced NADP passes to the light independent reactions and is used in the synthesis of carbohydrate
- the photolysis of water can be demonstrated by the Hill reaction *(see 13.2d)*

b) photophosphorylation (of ADP to ATP)

This is of two types – cyclic and non-cyclic.

Cyclic photophosphorylation

- only involves photosystem I
- no reduction of NADP takes place



1) light is absorbed by photosystem I and is passed onto the primary pigment
2) photoactivation (an electron present in the chlorophyll molecule is excited to a higher energy level and is emitted from the chlorophyll molecule)
3) the excited electron is captured by an electron acceptor and passed back to the chlorophyll molecule via a chain of electron carriers
4) energy released from this is used to synthesise ATP (from ADP and P_i by chemiosmosis)
5) ATP then passes to the light independent reactions

Non-cyclic photophosphorylation

- involves both photosystems *(in the so-called 'Z scheme' of electron flow)*
- NADP is reduced

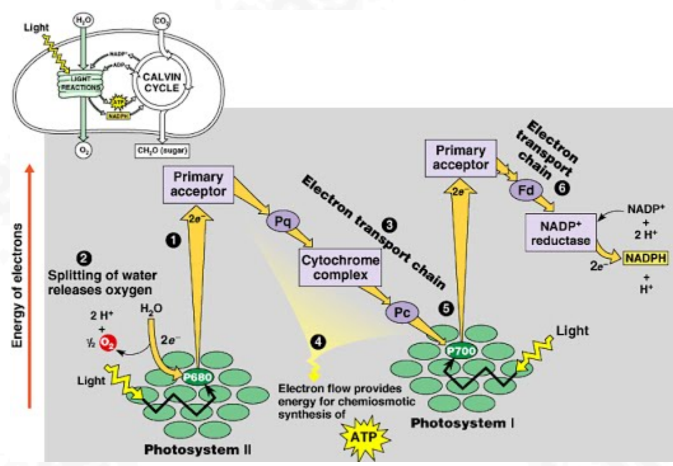


Image: http://thelessonlocker.com/kvhs/biology/noncyclic_electron_flow.jpg

1) light is absorbed by both photosystems and excited electrons are emitted from the primary pigments of both reaction centres
2) these electrons are absorbed by electron acceptors and pass along electron carriers
3) photosystem I's electrons are passed onto NADP, forming reduced NADP
4) photosystem I's primary pigment absorbs the electron from photosystem II
5) photosystem II receives a replacement electron from the photolysis of water (a)
6) ATP is synthesised as electrons lose energy passing the carrier chain

Comparing cyclic and non-cyclic photophosphorylation

CYCLIC	NON-CYCLIC
Involves PSI only	Involves both PSI and PSII
P700 is the active reaction centre	P680 is the active reaction centre
Water is not required	Water is required for photolysis
Electrons revert to PSI	Electrons from PSI are accepted by NADP
Reduced NADP is not synthesised	Reduced NADP is synthesised
Oxygen is not evolved as a by-product	Oxygen is evolved as a by-product
This process is predominant only in bacteria	This process is predominant in all green plants

g) the light independent stage of photosynthesis (Calvin cycle)

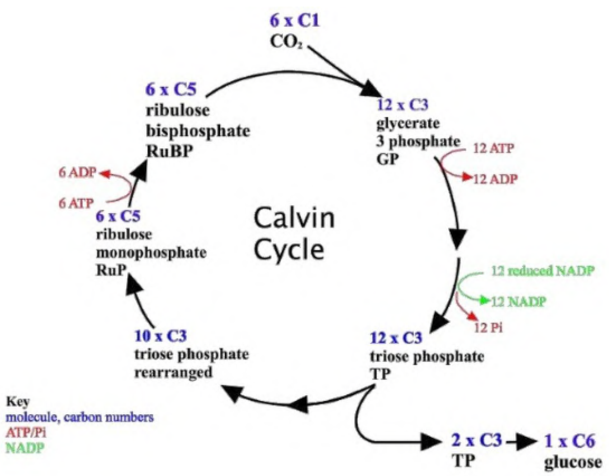


Image: https://home.infomasif.com/light-independent-reactions.html

The three main stages of the Calvin cycle:

1) RuBP (ribulose bisphosphate), a 5C compound, combines with carbon dioxide
   - this is catalysed by the enzyme rubisco
   - an unstable 6C compound is formed, which splits to form two molecules of GP (glycerate-3-phosphate), a.k.a PGA (phosphoglyceric acid), a 3C compound

CO_2 + RuBP —rubisco→ 2 GP *or* PGA

2) GP/PGA is reduced using reduced NADP and ATP to form TP (triose phosphate)
   - 1/6 of TP produced is used to make glucose, carbohydrates, lipids, amino acids, etc.

2 GP → 2 TP (triose phosphate) (reduced NADP → NADP; ATP → ADP + P_i)

3) 5/6 of TP produced is used in the regeneration of RuBP, using ATP

2 TP → RuBP (ATP → ADP + P_i)

h) conversion of Calvin cycle intermediates

Most of the TP produced is used to make glucose and other organic substances such as:

1) carbohydrates

TP (3C) + TP (3C) → hexose (6C)

hexose (6C) —polymerisation→ carbohydrates

Other carbohydrates include cellulose for making cell walls, sucrose for transport, starch for energy storage, etc.

2) amino acids (for making proteins)

GP (+ Krebs cycle intermediates) —ACoA→ amino acids

3) lipids (for energy storage)

glycerol (from TP) + fatty acids (from GP) → lipids

13.2 Investigation of limiting factors

a) limiting factors

➢ limiting factor – factor that has the greatest effect in reducing the rate of photosynthesis

b) factors affecting photosynthesis

1) light intensity and wavelength

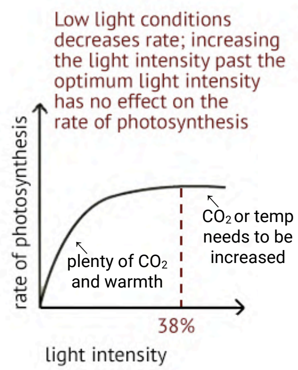


Image: studyrocket.co.uk/revision/gcse-biology-triple-aqa

2) temperature

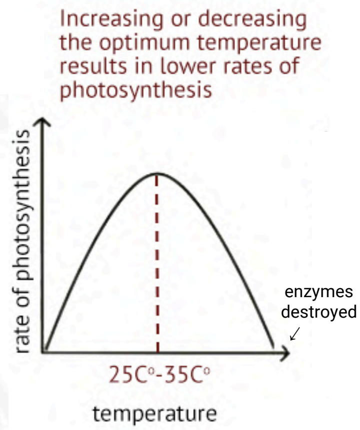


Image: studyrocket.co.uk/revision/gcse-biology-triple-aqa

3) carbon dioxide concentration

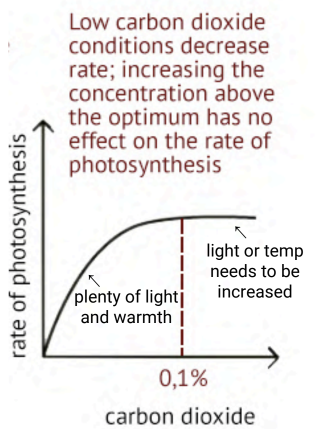


Image: studyrocket.co.uk/revision/gcse-biology-triple-aqa

Features of a dicotyledonous leaf that can affect the rate of photosynthesis:

1) stomata number/size
2) chloroplast number/size
3) surface area of leaf/thinness of lamina
4) number/size of intracellular air spaces
5) rubisco concentration
6) age/senescence

c) how limiting factors can be used to increase crop yields (in protected environments e.g., glasshouses)

Environmental factors influence the rate of photosynthesis. Investigating these shows how they can be managed in protected environments used in crop production.

In a glasshouse –

1) using artificial light allows photosynthesis to continue beyond daylight hours
   - this can be further adjusted by using a particular wavelength of light which is absorbed more, thereby increasing the photosynthesis rate
2) using artificial heating so photosynthesis can occur at an accelerated rate (using the optimum temperature for the plant)
3) additional CO_2 being released into the atmosphere (e.g., from dry ice) also increases the photosynthesis rate

d) investigating the effect of light intensity or light wavelength on the rate of photosynthesis of a plant, using a redox indicator such as DCPIP

The Hill reaction

This Hill reaction investigates the light dependent reactions of photosynthesis which take place in the thylakoid membranes of chloroplasts. The reaction can only occur if the thylakoid membranes are illuminated as the light-dependent stage stops in the dark.

- This reaction involves isolating chloroplasts from living cells and suspending them in a coloured electron acceptor such as DCPIP.
- The reaction then depends on the electrons released during the light-dependent stage of photosynthesis being picked up by DCPIP.

When oxidised, DCPIP is blue, and when reduced, it's colourless. Therefore, it is possible to monitor the loss of blue colour as an indication that DCPIP has accepted electrons.

In this experiment, DCPIP takes the place of NADP (which is usually reduced in the light-dependent reaction), allowing photolysis to continue even when the supply of NADP has been exhausted because the DCPIP can continue to accept the electrons from the electron transport chain.

- independent variable – light intensity/wavelength reaching the chloroplast samples
- dependent variable – amount of DCPIP reduced (can be judged based on the colour of the mixture.
- control variables –
  1) use chloroplasts from the same species of plant
  2) amount of chloroplast/buffer solution added to each test tube
  3) duration – all test tubes must be left for the same amount of time before comparing the colour of the mixture
  4) pH should be the same – add buffer solution to maintain the pH at 7.0

Q) Describe how you would carry out an investigation into the effect of wavelength of light (/light intensity) on the rate of photosynthesis of a plant, using a redox indicator such as DCPIP. [8 marks]

1) grind spinach leaves with ice-cold buffer solution using a pestle and mortar, centrifuge the resulting suspension to remove unwanted debris
2) this buffer solution is added to maintain pH at 7.0
3) add 5 cm^3 of the buffered chloroplast suspension with 10 cm^3 of DCPIP into test tubes

4) place the test tubes in different light intensities or in different wavelengths of light
5) the rate can be measured using one of two methods:

method 1

- measure time for blue colour of DCPIP to go colourless (getting reduced)
- calculate rate using 1/t

method 2

- leave for a fixed amount of time and measure with a colorimeter
- calculate rate as change in colour value divided by time

6) repeat and take the average for accuracy or calculate the mean
7) plot a graph (method 1 – wavelength on x-axis, and calculated rate on y-axis // method 2 – time on x-axis, and colorimeter reading/calculated rate on y-axis)
8) this is known as the Hill reaction

e) investigating the effects of light intensity, carbon dioxide and temperature on the rate of photosynthesis using whole plants (e.g., aquatic plants such as *Elodea* and *Cabomba*)

These experiments can be carried out using a variety of different apparatus such as –

1) micro burette

Image: https://www.philipharris.co.uk/product/biology/material-cycles-and-energy/cellular-respiration/microburette/b8a21439

2) photosynthometer

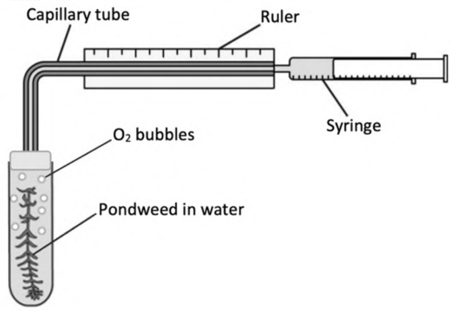


Image: https://www.elevise.co.uk/gab4d.html

- **independent variable** – light intensity / wavelength of light / CO_2 concentration / temperature
- **dependent variable** – number of bubbles produced per minute
- **control variables** – concentration of $NaHCO_3$ solution / temperature / type of plant used, its size, age, etc.

Q) Describe how you would carry out an investigation into the effect of temperature on the rate of photosynthesis of an aquatic plant. [8 marks]

1) the experiment can be carried out with the use of a photosynthometer (or micro burette or gas syringe)

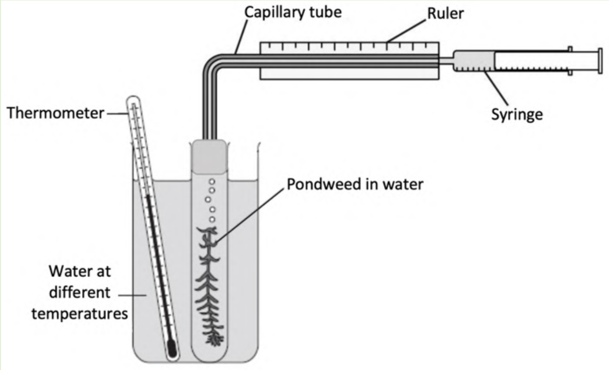


Image: https://www.elevise.co.uk/gab4d.html

2) the shoot of the plant should be cut and placed in hydrogen carbonate solution, to provide carbon dioxide
   - ensure that the plant is well illuminated before use
3) use a water bath for maintaining the temperature (use at least five different temperatures e.g., 10°, 20°, 30°, 40°, 50°)
4) allow sufficient time for the shoots to acclimatise to the conditions
5) keep the lamp at a fixed distance from the shoots
6) count the number of bubbles produced during a fixed amount of time
   - to prevent the gases in the bubbles given off from dissolving in water, aerate the water well (by bubbling air through it) before use
7) repeat the experiment twice more and calculate the mean values
8) use these values to calculate the rate of photosynthesis
9) plot a graph to show the results

Image: https://www.elevise.co.uk/gab4d.html

To investigate the other factors that affect photosynthesis, the same experiment setup can be used, with the following changes made –

- light intensity – by altering distance, d, of a small light source from the plants (light intensity is proportional to $1/d^2$)
    - the light needs to be a white light and should not get hot (LED lights are the best for this)
    - set up the experiment as shown:

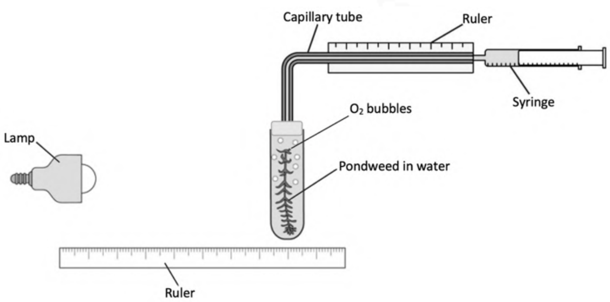


Image: https://www.elevise.co.uk/gab4d.html

    - plot a graph to show the results:

Image: https://www.elevise.co.uk/gab4d.html

- wavelength of light – by using different colour filters, making sure that they each transmit the same light intensity

- concentration of carbon dioxide – by adding different quantities of sodium hydrogencarbonate to the water surrounding the plant

13.3 Adaptations for photosynthesis

a) structure and function of chloroplasts

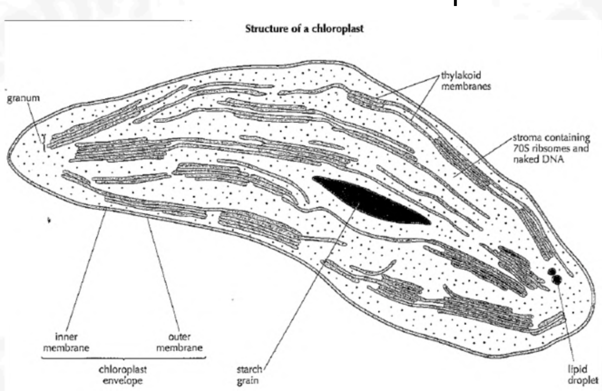
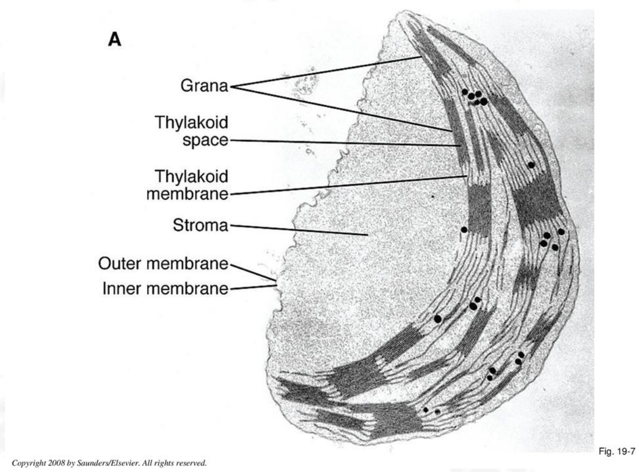


Image: https://heenabhakta17.wordpress.com/2013/11/06/warm-up-11413/

Electron micrograph of a thin section of a chloroplast



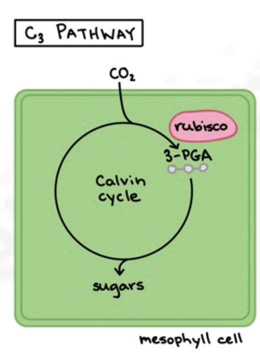
1) biconvex discs, 3-10 μm (in dicots)

2) ground substance is stroma
    - site of light dependent reactions
    - contains the enzymes of Calvin cycle, sugars, organic acids
    - bathes membranes of grana to receive products of light dependent reactions
    - contains small (70S) ribosomes which can produce some chloroplast proteins which are coded by the DNA loop
    - contains lipid droplets and starch granules

3) thylakoids (series of flattened fluid-filled sacs which form stacks called grana)
    - grana are linked via membranes
    - membranes provide large surface area, which holds pigments, electron carriers, and enzymes for light dependent reactions
    - membranes facilitate a large number of pigment molecules to be arranged for maximum light absorption
    - pigments are arranged in light harvesting clusters
    - pigments are arranged in a funnel structure in the thylakoids
    - membranes of grana hold ATP synthase and is the site of ATP synthesis

b) C4 plants

C4 plants are called C4 plants because the first stable intermediate product of carbon fixation is a 4-carbon compound (oxaloacetate) instead of a 3-carbon compound (GP/PGA) in C3 or 'normal' plants.



The anatomy and physiology of C4 plants (such as maize or sorghum) differs to C3 plants.

Some tropical crops have C4 metabolism and adaptations to maximise carbon dioxide fixation, this mostly involves avoiding photorespiration.

- photorespiration – when RuBisCo catalyses the reaction between RuBP and oxygen instead of RuBP with carbon dioxide
- this reaction happens most readily in high temperature and high light intensity
- as a result, oxygen us taken up in the light, and some carbon dioxide is given out, contrary to the general pattern of photosynthesis

C4 adapted for high rates of carbon fixation at high temperatures via:

a) the spatial separation of initial carbon fixation from the light dependent stage

The light dependent reactions and Calvin cycle are physically separated –

- light dependent reactions occur in the **mesophyll cells**
- the Calvin cycle occurs in the **bundle-sheath cells** (these cells have no direct contact with air inside the leaf, reducing the chance that RuBisCo catalyses the wrong reaction)

1) CO_2 is fixed in the mesophyll cells to form oxaloacetate (4C) by the enzyme PEP carboxylase which has no tendency to bind to O_2

CO_2 + PEP — PEP carboxylase → **oxaloacetate**

2) oxaloacetate is then converted to malate which is transported to bundle-sheath cells

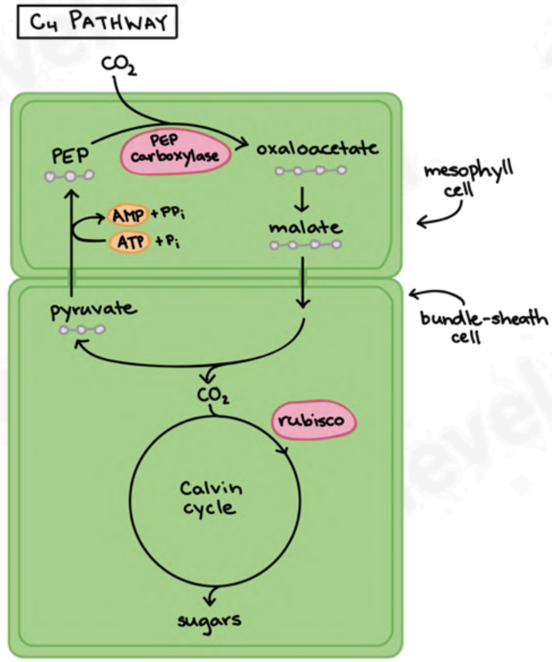
oxaloacetate → malate

3) malate breaks down in the bundle-sheath cells releasing a CO_2 molecule

malate → CO_2

4) the CO_2 molecule released is fixed by rubisco into RuBP and made into sugars via the Calvin cycle

O_2 + RuBP — RuBisCo → **sugars**



Images: khanacademy.org

b) the high optimum temperatures of enzymes involved

C4 plant species have a higher temperature optimum for photosynthesis than C3 plants.