

PHOTOSYNTHESIS

- **Photosynthesis** is a **physico-chemical process** by which green plants use **light energy (solar energy)** to synthesise organic compounds. So they are autotrophs.
- It is the basis of life on earth.

- Ultimately, all living forms depend on sunlight for energy.

Importance of Photosynthesis

- It is the primary source of all food on earth.
- It releases oxygen into the atmosphere.

EXPERIMENTS RELATED WITH PHOTOSYNTHESIS

1. Variegated leaf experiment

- Take a variegated leaf (or leaf partially covered with black paper) that was exposed to light.
- Test the leaves for starch. It shows that photosynthesis occurs only in green parts of the leaves in presence of light.

2. Half-leaf experiment

- A part of a leaf is enclosed in a test tube containing KOH soaked cotton (which absorbs CO₂).
- The other half of leaf is exposed to air.
- Place this setup in light for some time.
- Test the leaf for presence of starch. Exposed part shows positive for starch and portion in the tube shows negative. This proves that CO₂ is required for photosynthesis.

EARLY EXPERIMENTS

Experiments by Joseph Priestley (1770)

- Priestley performed experiments to prove the role of air in the growth of green plants.
- He discovered oxygen in 1774.
- He observed that a candle burning in a closed bell jar gets extinguished. Similarly, a mouse suffocated in closed jar. He concluded that a burning candle or a breathing animal damage the air.
- He placed a mint plant in the same bell jar. He found that the mouse stayed alive and the candle continued to burn.
- He hypothesised that plants restore to the air whatever breathing animals and burning candles remove.

Experiments by Jan Ingenhousz (1730-1799)

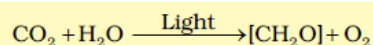
- He conducted the same experiment by placing in darkness and sunlight.
- He showed that sunlight is essential to the plant for purifying the air fouled by burning candles or animals.
- He repeated this experiment with an aquatic plant. It showed that in bright sunlight, small bubbles were formed around green parts while in the dark they did not.
- Later he identified these bubbles to be of oxygen. Thus he showed that only the green part of plants release O₂.

Experiments by Julius von Sachs (1854)

- He proved that
 - Glucose is produced when plants grow and it is usually stored as starch.
 - **Chlorophyll** is located in special bodies (**chloroplasts**).
 - Glucose is made in the green parts of plants.

Experiments by T.W Engelmann (1843 – 1909)

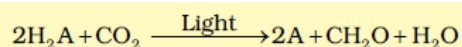
- He split the light using a prism into its spectral components and illuminated a green alga (*Cladophora*) placed in a suspension of aerobic bacteria.
- The bacteria were used to detect the sites of O₂ evolution.
- He observed that the bacteria accumulated mainly in the region of blue and red light of the split spectrum.
- It was a first described **action spectrum** of photosynthesis. It resembles the absorption spectra of chlorophyll *a* & *b*.
- By the middle of 19th century, it is discovered that plants use light energy to make carbohydrates from CO₂ & H₂O.
- Empirical equation of the process of photosynthesis is



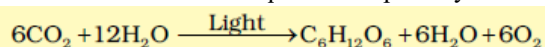
Where, [CH₂O] represents a carbohydrate (e.g. glucose).

Experiments by Cornelius van Niel (1897-1985)

- Van Niel (microbiologist) conducted some studies in purple and green bacteria.
- He demonstrated that photosynthesis is a light-dependent reaction in which hydrogen from an oxidisable compound reduces CO₂ to carbohydrates.



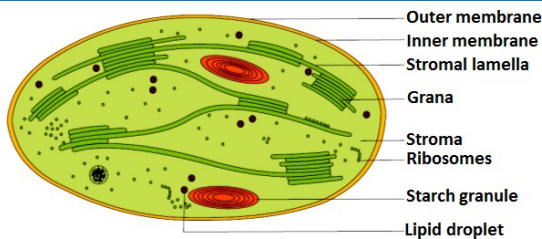
- In plants, H₂O is the hydrogen donor and is oxidised to O₂.
- Purple & green sulphur bacteria use H₂S as H-donor. So the 'oxidation' product is sulphur or sulphate and no O₂ is produced.
- Thus, he inferred that the O₂ evolved by the green plant comes from H₂O, not from CO₂. This was later proved by using radio isotopic techniques.
- Therefore overall correct equation for photosynthesis is:



PHOTOSYNTHESIS: SITE AND PIGMENTS

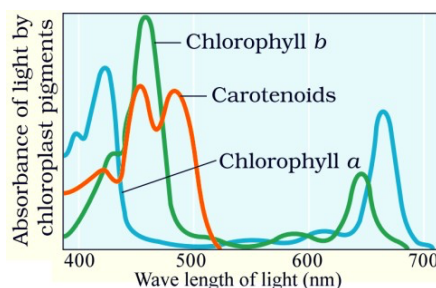
- Photosynthesis occurs in green leaves & other green parts.
- **Chloroplasts** present in the walls of **mesophyll cells** of leaves. It helps to get optimum quantity of incident light.
- Chloroplast contains a **membranous system**. It consists of **grana**, **stroma lamellae** and **matrix stroma**.
- Each granum is a group of membrane-bound sacs called **thylakoids (lamellae)**. They contain leaf pigments.

- The **membrane system** traps light energy and synthesise ATP and NADPH. It is called **light reactions**.
- In **stroma**, enzymatic reactions synthesise sugar, which in turn forms starch. It is called **dark reactions (carbon reactions)**. It does not mean that they occur in darkness or that they are not light dependent.

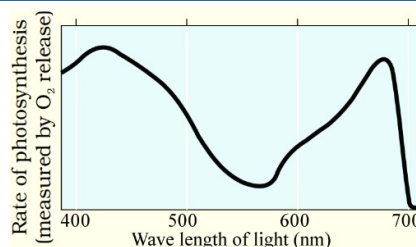


PIGMENTS INVOLVED IN PHOTOSYNTHESIS

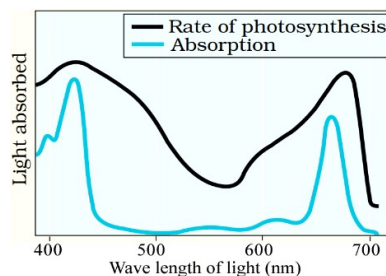
- Pigments are substances that have ability to absorb light at specific wavelengths.
 - Chromatography shows the following leaf pigments:
 - **Chlorophyll a** (bright or blue green in chromatogram)
 - **Chlorophyll b** (yellow green)
 - **Xanthophylls** (yellow)
 - **Carotenoids** (yellow to yellow-orange)
- Accessory pigments**
- **Functions of accessory pigments:**
 - They absorb light at different wavelength and transfer the energy to chlorophyll a.
 - They protect chlorophyll a from photo-oxidation.
 - The **absorption spectrum & action spectrum** coincide closely showing that photosynthesis is maximum at the **blue & red regions** of the spectrum.
 - The graphs also show that chlorophyll a is the chief pigment associated with photosynthesis.



Graph showing absorption spectrum of chlorophyll a, b & carotenoids



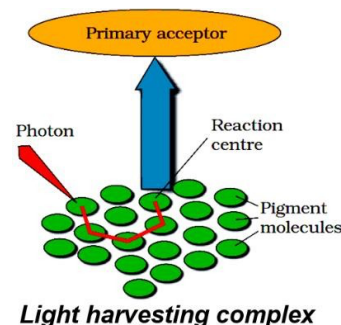
Graph showing action spectrum of photosynthesis



Graph showing action spectrum of photosynthesis superimposed on absorption spectrum of chlorophyll a

Photosystems

- Pigments are organised into two **Photosystems** called **Photosystem I (PSI) & Photosystem II (PSII)**. These are named in the sequence of their discovery.
- Each photosystem has a **chlorophyll a** and **accessory pigments** bound by proteins.
- All pigments (except one molecule of chlorophyll a) form a **light harvesting complex (LHC or antennae)**.
- Single chlorophyll a acts as **reaction centre**.
- In **PS I**, the reaction centre absorbs light at **700 nm**, and so called **P700**.
- In **PS II**, the reaction centre absorbs light at **680 nm**, and so called **P680**.

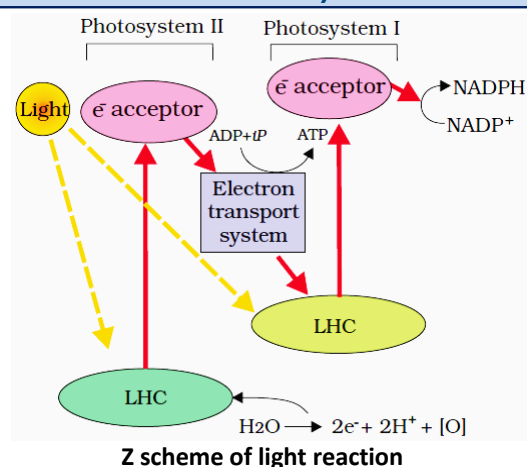


LIGHT REACTION (PHOTOCHEMICAL PHASE)

- Light reactions include **light absorption, water splitting, oxygen release** and **formation of ATP & NADPH** (high-energy chemical intermediates).

The Electron Transport

- When **PS II** absorbs **red light of 680 nm** wavelength, electrons are excited and transferred to an electron acceptor.
- The electron acceptor passes them to a chain of **electrons transport system** consisting of **cytochromes**.
- This movement of electrons is downhill, in terms of redox potential scale.
- The electrons are transferred to the pigments of **PS I**.
- Simultaneously, electrons in **PS I** are also excited when they receive **red light of 700 nm** and are transferred to another acceptor molecule having a greater redox potential.
- These electrons are moved downhill to a molecule of **NADP⁺**. As a result, **NADP⁺** is reduced to **NADPH + H⁺**.
- Transfer of electrons from **PS II** to **PS I** and finally downhill to **NADP⁺** is called the **Z scheme**, due to its zigzag shape. This shape is formed when all the carriers are placed in a sequence on a redox potential scale.



Splitting of Water (Photolysis)

- The **water splitting complex** in **PS II** is located on the inner side of the thylakoid membrane.
- Water is split into **H⁺, [O]** and electrons.

$$2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + \text{O}_2 + 4\text{e}^-$$
- So **PS II** can supply electrons continuously by replacing electrons from water splitting.

- Thus PS II provides electrons needed to replace those removed from PS I.
- The protons (H^+) are used to reduce NADP to NADPH.
- Oxygen is liberated as a by-product of photosynthesis.

Photo-phosphorylation

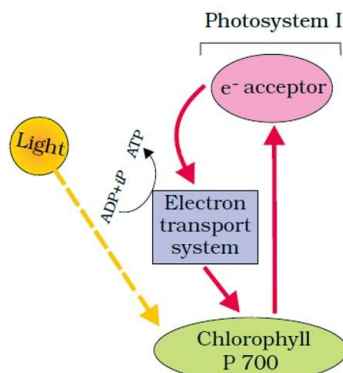
- The synthesis of ATP by cells (in mitochondria & chloroplasts) is called **phosphorylation**.
- **Photo-phosphorylation** is the synthesis of ATP from ADP in chloroplasts in presence of light.
- It occurs in 2 ways: **Non- cyclic** and **Cyclic**.

a) Non-cyclic photo-phosphorylation

- It occurs when the two photosystems work in a series, (first PS II and then PS I) through an electron transport chain as seen in the Z scheme.
- Here, ATP & NADPH + H^+ are synthesised.
- It is a non-cyclic process because the electrons lost by PS II do not come back to it but pass on to NADP $^+$.

b) Cyclic photo-phosphorylation

- It occurs in stroma lamellae when only PS I is functional.
- The electron is circulated within the photosystem and the ATP synthesis occurs due to cyclic flow of electrons.
- The lamellae of grana have PS I & PS II. The stroma lamellae membranes lack PS II and *NADP reductase*.
- The electron does not pass on to NADP $^+$ but is cycled back to PS I complex through electron transport chain.
- Here, only ATP is synthesised (no NADPH + H^+).
- Cyclic photophosphorylation also occurs when only light of wavelengths beyond 680 nm are available.

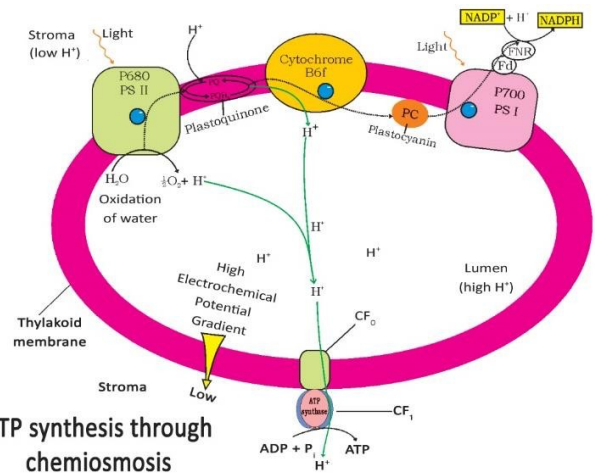


Chemiosmotic Hypothesis

- It explains **mechanism of ATP synthesis** in chloroplast.
- **Chemiosmosis**: Movement of ions across a semipermeable membrane. It occurs in chloroplast and mitochondria.
- Chemiosmosis needs a membrane, a proton pump, a proton gradient (across thylakoid membranes) and *ATP synthase*.
- Splitting of water occurs on the inner side of the membrane. So the protons accumulate in the lumen of thylakoids.
- As electrons move through the photosystems, protons are transported across the membrane. It is due to the removal

of protons from the stroma for the following reasons:

- **Primary electron acceptor** is located towards the outer side of the membrane. It transfers its electron to an **H carrier**. So this molecule removes a proton from the stroma while transporting an electron. When this molecule passes on its electron to the **electron carrier** on the inner side of the membrane, proton is released into the lumen of the membrane.
- The **NADP reductase** enzyme is located on the stroma side of the membrane. Along with electrons coming from PS I, protons are necessary to reduce NADP $^+$. These protons are also removed from the stroma.
- Hence, protons in stroma are decreased but in lumen, protons are accumulated. It creates a proton gradient across the thylakoid membrane and decrease in pH in the lumen.



ATP synthesis through chemiosmosis

- Breakdown of proton gradient leads to synthesis of ATP by *ATP synthase* enzyme.
- The *ATP synthase* consists of two parts:
 - **CF₀**: It is embedded in the membrane and forms a trans-membrane channel. It carries out facilitated diffusion of protons across the membrane to the stroma. It results in breakdown of proton gradient.
 - **CF₁**: It protrudes on the outer surface of the thylakoid membrane. The energy due to breakdown of gradient causes a conformational change in the CF₁ particle. It makes the enzyme to synthesise ATP molecules.
- Energy is used to pump protons across a membrane, to create a gradient or a high concentration of protons within the thylakoid lumen.
- *ATP synthase* has a channel for the diffusion of protons back across the membrane. This releases energy to activate *ATP synthase* that catalyses formation of ATP.

DARK REACTION (BIOSYNTHETIC PHASE) - USE OF ATP & NADPH

- Products of light reaction are ATP, NADPH and O₂.
- **Dark reaction** is the use of ATP and NADPH to drive the processes for the synthesis of food (sugars).
- This phase does not directly depend on the light but is dependent on the products of the light reaction.
- It can be verified as follows: Immediately after light becomes unavailable, the biosynthetic process continues

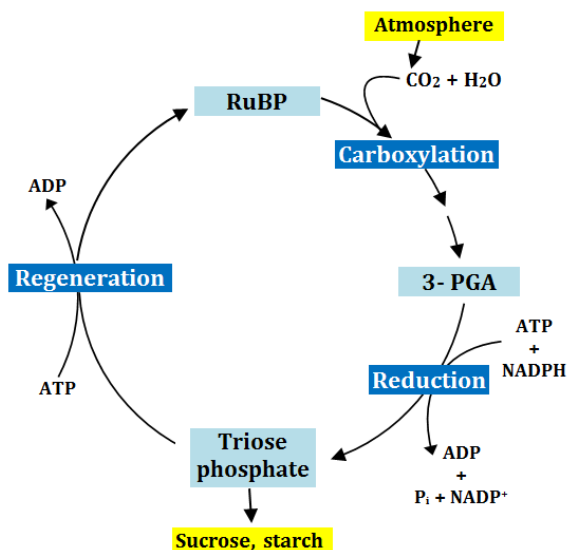
for some time, and then stops. If light is available, the synthesis starts again.

- CO₂ combines with H₂O to form (CH₂O)_n or sugars.
- CO₂ assimilation during photosynthesis is 2 types:
 - **C₃ pathway**: In this, first stable product of CO₂ fixation is a C₃ acid (**3-phosphoglyceric acid - PGA**). **Melvin Calvin** discovered this using ¹⁴C in algal photosynthesis.

- **C₄ pathway:** In this, first stable product is **oxaloacetic acid (OAA)**, a 4-carbon (C₄) organic acid.

C₃ PATHWAY (CALVIN CYCLE)

- It occurs in **all photosynthetic plants** (C₃ or C₄ pathways).
- It has 3 stages: carboxylation, reduction and regeneration.



1. Carboxylation of RuBP

- RuBP (**ribulose biphosphate** - a 5-carbon ketose sugar) is the primary CO₂ acceptor.
- It is the most crucial step. CO₂ is fixed by **RuBP** to two 3- PGA in presence of the enzyme **RuBP carboxylase**.
- Since this enzyme also has an oxygenation activity it is called **RuBP carboxylase-oxygenase (RuBisCO)**.
- RuBisCO is the most abundant enzyme in the world.

2. Reduction

- It is a series of reactions leading to the glucose formation.
- Here, 2 ATP molecules for phosphorylation and two of NADPH for reduction per CO₂ molecule are used.
- Fixation of 6 CO₂ molecules and 6 turns of the cycle are needed to remove one glucose molecule from the pathway.

3. Regeneration of RuBP

- It is crucial for continuation of the cycle.
- It requires one ATP for phosphorylation to form RuBP.
- Hence for every CO₂ molecule, 3 ATP molecules and 2 NADPH are required.
- It is probably to meet this difference in number of ATP and NADPH used in the dark reaction that the cyclic phosphorylation takes place.
- To make 1 glucose molecule, 6 turns of the cycle are needed.

What does go in and come out of the Calvin cycle?	In	Out
	6 CO ₂	1 glucose
	18 ATP	18 ADP
	12 NADPH	12 NADP

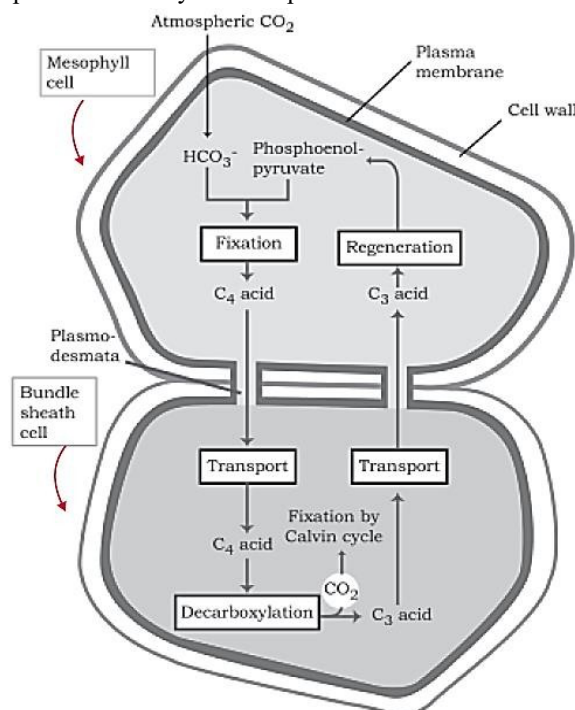
C₄ PATHWAY (HATCH & SLACK PATHWAY)

- It is present in plants adapted to dry tropical regions.
- They also use C₃ pathway as main biosynthetic pathway.
- The large cells around the vascular bundles of the C₄ plants are called **bundle sheath cells**. Such anatomy is called '**Kranz**' anatomy ('Kranz' = 'wreath').

- The bundle sheath cells may form **several layers** around the vascular bundles.
- They have large number of chloroplasts, thick walls impervious to gas exchange and no intercellular spaces.

Steps of Hatch and Slack Pathway

- Primary CO₂ acceptor is **phosphoenol pyruvate (PEP)** - a 3-carbon molecule seen in mesophyll cells. The enzyme for this fixation is **PEP carboxylase (PEPcase)**.
- The mesophyll cells lack **RuBisCO** enzyme.
- The C₄ acid OAA is formed in the mesophyll cells.
- It then forms other 4-carbon acids like malic acid or aspartic acid. They are transported to bundle sheath cells.



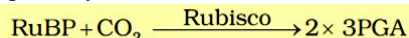
- In the bundle sheath cells, C₄ acids are broken down to release CO₂ and a C₃ molecule.
- The C₃ molecule is transported back to mesophyll where it is converted to PEP again.
- The released CO₂ enters the C₃ pathway.
- Bundle sheath cells are rich in **RuBisCO**, but lack **PEPcase**. Thus C₃ pathway is common to C₃ & C₄ plants.

C₄ plants are special because:

- They have a special type of leaf anatomy (Kranz).
- They tolerate higher temperatures.
- They show a response to highlight intensities.
- They lack photorespiration.
- They have greater productivity of biomass.

PHOTORESPIRATION

- In Calvin pathway, RuBP combines with CO₂.



- Active site of RuBisCO can bind to CO₂ & O₂ - so the name.
- RuBisCO has a greater affinity for CO₂ than for O₂. This binding is competitive. Relative concentration of O₂ and CO₂ determines which one will bind to the enzyme.
- In C₃ plants, some O₂ bind to RuBisCO. Hence CO₂ fixation is decreased. Here RuBP binds with O₂ to form one

molecule of phosphoglycerate and phosphoglycolate. This pathway is called **photorespiration**.

- In this, there is no synthesis of sugars, ATP and NADPH. Hence **photorespiration is a wasteful process**. Rather it causes the release of CO_2 by using ATP.
- In **C_4 plants, photorespiration does not occur** because they can increase CO_2 concentration at the enzyme site.

This takes place when C_4 acid from the mesophyll is broken down in the bundle cells to release CO_2 . This minimises the oxygenase activity of RuBisCO.

- Due to the lack of photorespiration, productivity and yields are better in C_4 plants. Also, these plants show tolerance to higher temperatures.

Differences between C_3 and C_4 plants

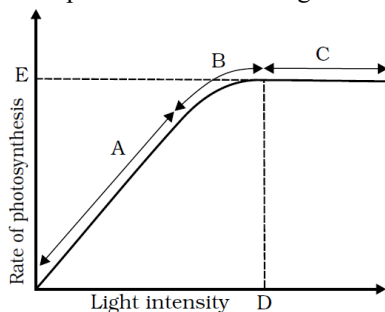
C_3 plants	C_4 plants
1. Photosynthesis occurs in mesophyll cells.	In mesophyll and bundle sheath cells.
2. Kranz anatomy is absent.	Present.
3. RuBP is the primary CO_2 acceptor.	PEP is the primary CO_2 acceptor.
4. 3-PGA, a 3-C compound is the first stable product.	OAA, a 4-C compound is the first stable product.
5. Chloroplasts are of only one type (granal).	Dimorphic (granal in mesophyll and agranal in bundle sheath).
6. Photorespiratory loss is high.	Photorespiration is absent or negligible.
7. High CO_2 compensation point ($25\text{--}100 \mu\text{L CO}_2 \text{ l}^{-1}$).	Low CO_2 compensation point ($0\text{--}10 \mu\text{L CO}_2 \text{ l}^{-1}$).
8. Optimum temperature for photosynthesis is about 25°C .	About $35^\circ\text{C} - 45^\circ\text{C}$.
9. Photosynthetically less efficient and productivity low.	Photosynthetically more efficient and productivity high.
10. E.g. Rice, wheat, bean, potato.	E.g. Maize, sugarcane, amaranth, sorghum.

FACTORS AFFECTING PHOTOSYNTHESIS

- **Internal (plant) factors:** The number, size, age and orientation of leaves, mesophyll cells and chloroplasts, internal CO_2 concentration and amount of chlorophyll. Plant factors depend on the genes and growth of the plant.
- **External factors:** Sunlight, temperature, CO_2 concentration and water.
- **Blackman's Law of Limiting Factors (1905):** *"If a biochemical process is affected by more than one factor, its rate is determined by the factor nearest to its minimal value: it is the factor which directly affects the process if its quantity is changed."*
- E.g. a plant with green leaf, optimal light & CO_2 conditions may not photosynthesize if the temperature is very low. If optimal temperature is given, it will start photosynthesis.

Light

- **Light quality, light intensity and duration of exposure to light** influence photosynthesis.
- There is a linear relationship between incident light and CO_2 fixation rates at low light intensities.
- At higher light intensities, the rate does not show further increase because other factors become limiting.
- Light saturation occurs at 10% of the full sunlight. Hence, except for plants in shade or in dense forests, light is rarely a limiting factor in nature.
- High increase in incident light breaks down chlorophyll. It decreases photosynthesis.



Carbon dioxide Concentration

- CO_2 is the major limiting factor for photosynthesis.
- CO_2 concentration is very low in the atmosphere ($0.03\text{--}0.04\%$). Increase up to 0.05% cause increase in CO_2 fixation rates. Beyond this level can become damaging over longer periods.
- At low light, C_3 and C_4 plants do not respond to high CO_2 . At high light, they show increased rate of photosynthesis.
- C_4 plants show saturation at about $360 \mu\text{L l}^{-1}$.
- C_3 plants respond to increased CO_2 concentration and saturation is seen only beyond $450 \mu\text{L l}^{-1}$. Thus, current availability of CO_2 levels is limiting to the C_3 plants.
- Due to response to higher CO_2 concentration, C_3 plants show increased photosynthesis and higher productivity. This fact is used for some greenhouse crops (tomatoes, bell pepper etc). They are grown in CO_2 enriched atmosphere.

Temperature

- Dark reactions, being enzymatic, are temperature controlled. Influence of temperature on Light reactions is very less.
- The C_4 plants respond to higher temperatures and show higher rate of photosynthesis.
- C_3 plants have a much lower temperature optimum.
- The temperature optimum of plants also depends on their habitat. Tropical plants have a higher temperature optimum than the plants adapted to temperate climates.

Water

- Water stress closes the stomata hence reduce the CO_2 availability.
- Water stress also wilts leaves, thus reduce the surface area of the leaves and their metabolic activity.