# 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 $CO_2$).
- 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 $CO_2$ 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 $O_2$.

### 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 $O_2$ 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 19$^{th}$ century, it is discovered that plants use light energy to make carbohydrates from $CO_2$ & $H_2O$.
- Empirical equation of the process of photosynthesis is

$$CO_2 + H_2O \xrightarrow{\text{Light}} [CH_2O] + O_2$$

Where, $[CH_2O]$ 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 $CO_2$ to carbohydrates.

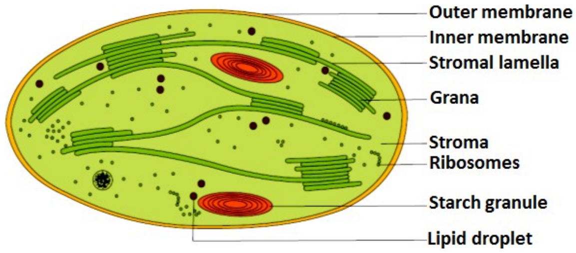
$$2H_2A + CO_2 \xrightarrow{\text{Light}} 2A + CH_2O + H_2O$$

- In plants, $H_2O$ is the hydrogen donor and is oxidised to $O_2$.
- Purple & green sulphur bacteria use $H_2S$ as H-donor. So the 'oxidation' product is sulphur or sulphate and no $O_2$ is produced.
- Thus, he inferred that the $O_2$ evolved by the green plant comes from $H_2O$, not from $CO_2$. This was later proved by using radio isotopic techniques.
- Therefore overall correct equation for photosynthesis is:

$$6CO_2 + 12H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

## PHOTOSYNTHESIS: SITE AND PIGMENTS

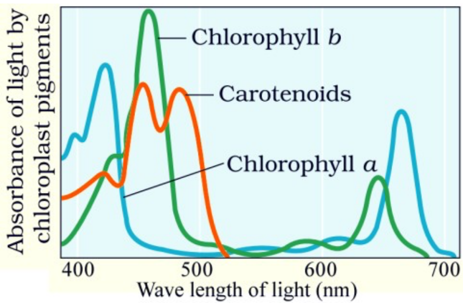
- Photosynthesis occurs in green leaves & other green parts.
- **Chloroplasts** present in the walls of **mesophyll cells** of leaves. It helps to get optimum quantity of incident light.
- Chloroplast contains a **membranous system.** It consists of **grana, stroma lamellae** and **matrix stroma.**
- 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 synthesize 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.



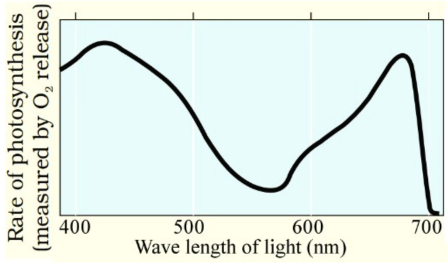

## PIGMENTS INVOLVED IN PHOTOSYNTHESIS

- 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)

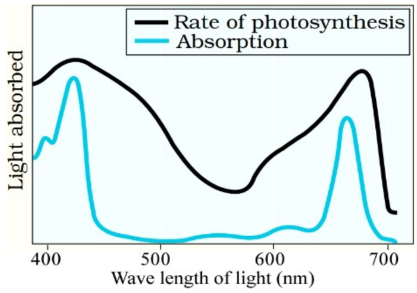
  (Chlorophyll *b*, Xanthophylls, Carotenoids: **Accessory pigments**)
- **Functions of accessory pigments:**
  - They absorb light at different wavelength and transfer the energy to chlorophyll *a*.
  - They protect chlorophyll *a* from photo-oxidation.
- The **absorption spectrum & action spectrum** 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**



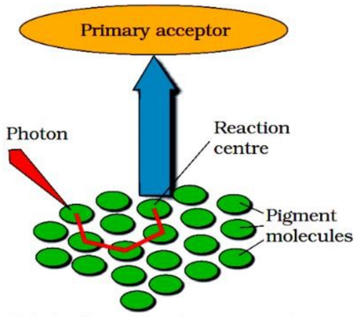

**Graph showing action spectrum of photosynthesis**




**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.**



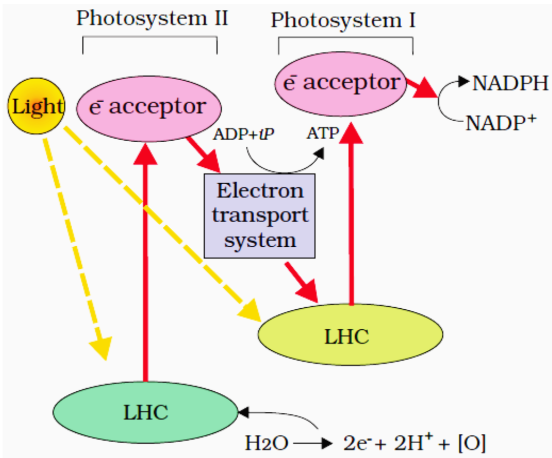

***Light harvesting complex***

## 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 accepter 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.




**Z scheme of light reaction**

### 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.

$$2H_2O \rightarrow 4H^+ + O_2 + 4e^-$$

- 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

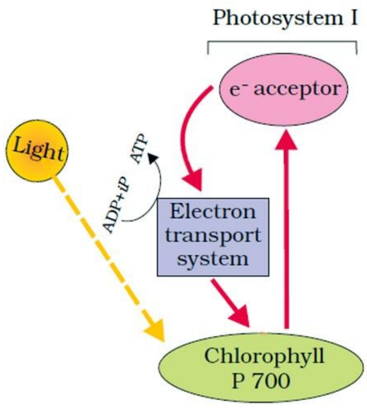
- The synthesis of ATP by cells (in mitochondria & chloroplasts) is called **phosphorylation.**
- **Photo-phosphorylation** is the synthesis of ATP from ADP in chloroplasts in presence of light.
- It occurs in 2 ways: **Non- cyclic** and **Cyclic.**

### 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

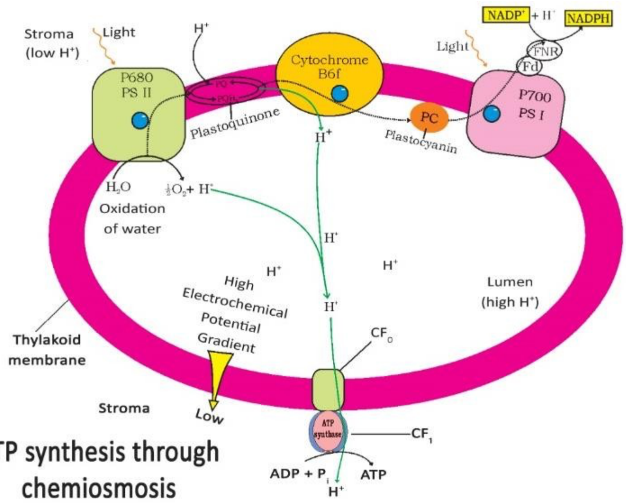
- It occurs in stroma lamellae when only PS I is functional.
- The electron is circulated within the photosystem and the ATP synthesis occurs due to cyclic flow of electrons.
- The lamellae of grana have PS I & PS II. The stroma lamellae membranes lack PS II and *NADP reductase*.




- 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 accepter** 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:
  - **$CF_0$:** 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.
  - **$CF_1$:** It protrudes on the outer surface of the thylakoid membrane. The energy due to breakdown of gradient causes a conformational change in the $CF_1$ 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.

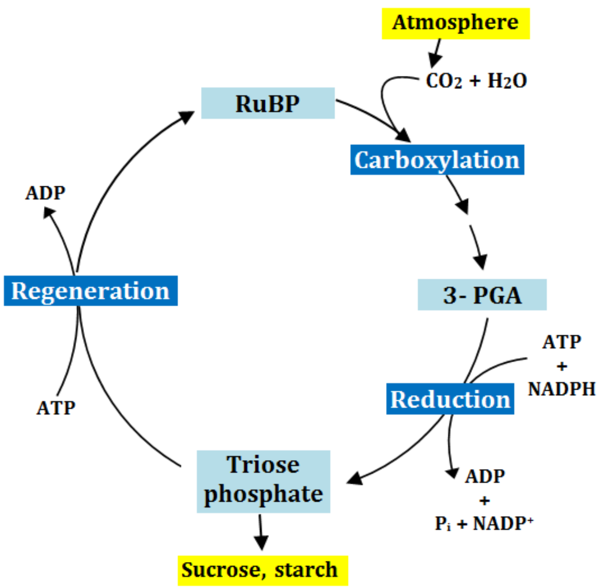
# DARK REACTION (BIOSYNTHETIC PHASE) - USE OF ATP & NADPH

- Products of light reaction are ATP, NADPH and $O_2$.
- **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.
- $CO_2$ combines with $H_2O$ to form $(CH_2O)_n$ or sugars.
- $CO_2$ assimilation during photosynthesis is 2 types:
  - **$C_3$ pathway:** In this, first stable product of $CO_2$ fixation is a $C_3$ acid **(3-phosphoglyceric acid - PGA). Melvin Calvin** discovered this using $^{14}C$ in algal photosynthesis.

- $C_4$ **pathway:** In this, first stable product is **oxaloacetic acid (OAA),** a 4-carbon ($C_4$) organic acid.

## $C_3$ PATHWAY (CALVIN CYCLE)

- It occurs in **all photosynthetic plants** ($C_3$ or $C_4$pathways).
- It has 3 stages: carboxylation, reduction and regeneration.




### 1. Carboxylation of RuBP

- RuBP (**ribulose bisphosphate - a 5-carbon ketose sugar)** is the primary $CO_2$ acceptor.
- It is the most crucial step. $CO_2$ 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 $CO_2$ molecule are used.
- Fixation of 6 $CO_2$ 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 $CO_2$ 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.

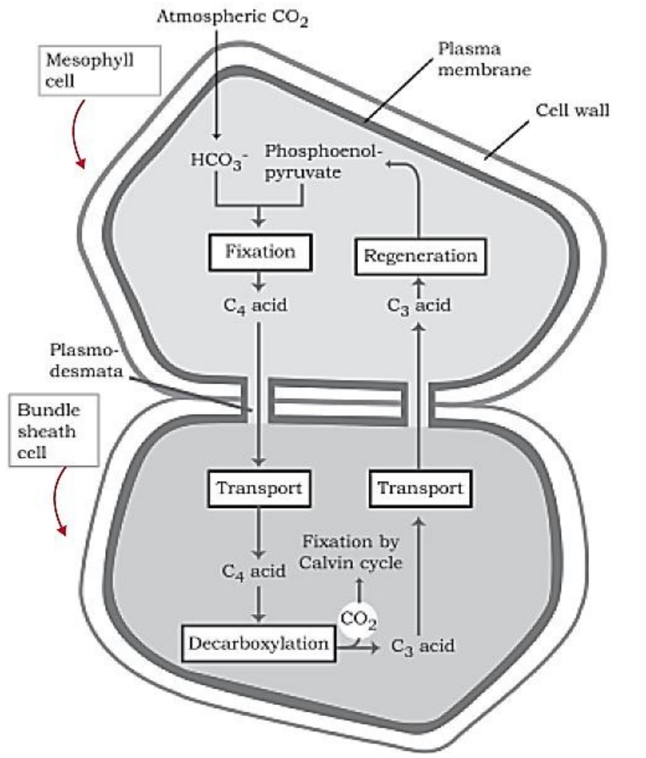
| **What does go in and come out of the Calvin cycle?** | **In** | **Out** |
|---|---|---|
| | 6 $CO_2$ | 1 glucose |
| | 18 ATP | 18 ADP |
| | 12 NADPH | 12 NADP |

## $C_4$ PATHWAY (HATCH & SLACK PATHWAY)

- It is present in plants adapted to dry tropical regions.
- They also use $C_3$ pathway as main biosynthetic pathway.
- The large cells around the vascular bundles of the $C_4$ 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 $CO_2$ 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 $C_4$ 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, $C_4$ acids are broken down to release $CO_2$ and a $C_3$ molecule.
- The $C_3$ molecule is transported back to mesophyll where it is converted to PEP again.
- The released $CO_2$ enters the $C_3$pathway.
- Bundle sheath cells are rich in ***RuBisCO,*** but lack *PEPcase.* Thus $C_3$ pathway is common to $C_3$ & $C_4$ plants.

$C_4$ 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 $CO_2$.

$$RuBP + CO_2 \xrightarrow{Rubisco} 2 \times 3PGA$$

- Active site of RuBisCO can bind to $CO_2$ & $O_2$ – so the name.
- RuBisCO has a greater affinity for $CO_2$ than for $O_2$. This binding is competitive. Relative concentration of $O_2$ and $CO_2$ determines which one will bind to the enzyme.
- In $C_3$ plants, some $O_2$ bind to RuBisCO. Hence $CO_2$ fixation is decreased. Here RuBP binds with $O_2$ 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

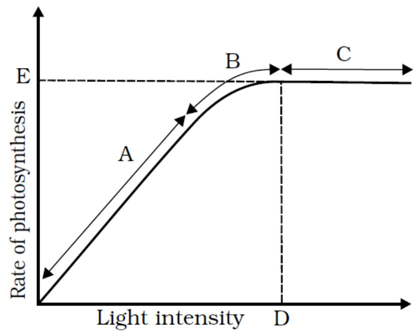
| $C_3$ plants | $C_4$ plants |
|---|---|
| 1. Photosynthesis occurs in mesophyll cells. | In mesophyll and bundle sheath cells. |
| 2. Kranz anatomy is absent. | Present. |
| 3. RuBP is the primary $CO_2$ acceptor. | PEP is the primary $CO_2$ acceptor. |
| 4. 3-PGA, a 3-C compound is the first stable 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 (25-100 μl. $CO_2$ $l^{-1}$). | Low $CO_2$ compensation point (0-10 μl. $CO_2$ $l^{-1}$). |
| 8. Optimum temperature for photosynthesis is about 25°C. | About 35°C - 45°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 (0.03-0.04%). 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 μl$L^{-1}$.
- $C_3$ plants respond to increased $CO_2$ concentration and saturation is seen only beyond 450 μl$L^{-1}$. 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.