5. Nitrogen nutrition

Nitrogen, in its organic or mineral form, makes up **1 to 5% of dry matter**. Nitrogen is found in proteins, which contain an average of **16%** nitrogen. It is also present in nucleic acids, coenzymes, vitamins, hormones…
When nitrogen is in mineral form, it appears as ions such as NH_4^+ or NO_3^-.

A. Atmospheric nitrogen (N_2)

It represents **78%** of the air, making it the main source. However, only a few plants living in symbiosis with bacteria or algae are capable of directly using atmospheric nitrogen.

B. Soil nitrogen

Nitrogen has five electrons in its outer shell, three of which are unpaired and can form covalent bonds. The oxidation number of nitrogen ranges from **–3 to +5**.

Mineral nitrogen is found in three forms: NO_3^-, NO_2^-, NH_4^+.
Organic nitrogen occurs in complex molecules such as proteins or amino acids, primarily found in humus.

Waste materials and dead organisms release amino compounds that are recycled by various decomposer microorganisms, mainly aerobic bacteria and some fungi. This process is called mineralization, and it occurs in two steps:

- **Ammonification**, which produces ammonium (NH_4^+). It is carried out by a large and diverse microflora.
- **Nitrification**, which leads to the production of nitrates (NO_3^-). It takes place in two steps:
  - **Nitrosation**: ammonium (NH_4^+) is converted into nitrite (NO_2^-) by *Nitrosomonas*.
  - **Nitratation**: nitrite is converted into nitrate (NO_3^-) by *Nitrobacter*.

The nitrogen cycle includes other stages, such as **denitrification**, which is the conversion of ammonium into gaseous nitrogen (N_2) by denitrifying bacteria.

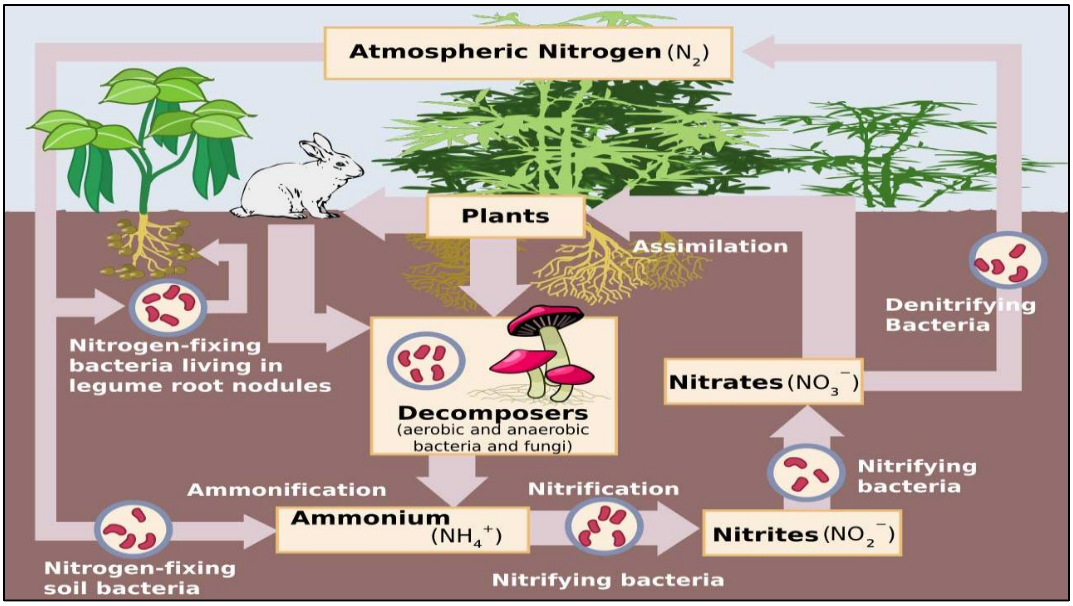


Figure 1 Nitrogen cycle

Nitrogen metabolism in plants

Nitrogen metabolism includes all the processes by which plants take up, transform, and use nitrogen to build essential molecules such as amino acids, proteins, and nucleic acids.

Plants cannot use atmospheric nitrogen (N_2). They use nitrate (NO_3^-) or ammonium (NH_4^+) from the soil.

1. Nitrogen uptake (Absorption)

Plants absorb nitrogen in two main mineral forms:

- Nitrate (NO_3^-) : most common in aerobic soils
- Ammonium (NH_4^+) : abundant in acidic or anaerobic soils

Root transporters:

- NRT transporters for NO_3^-
- AMT transporters for NH_4^+

2. Reduction of nitrate (if the plant absorbs NO_3^-)

Nitrate must be reduced before it can be used.

Step 1: Nitrate to Nitrite (NO_3^- to NO_2^-)

Enzyme: **Nitrate reductase** (cytosol)

Step 2: Nitrite to Ammonium (NO_2^- to NH_4^+)

Enzyme: **Nitrite reductase** (chloroplasts in leaves, plastids in roots)

Result: NH_4^+, the usable form.

3. Ammonium assimilation (NH_4^+ to Organic molecules)

Ammonium is incorporated into organic molecules:

GS–GOGAT (main pathway): Most important and universal.

1. **GS:** Glutamine synthetase : NH_4^+ + glutamate → glutamine
2. **GOGAT:** Glutamate synthase : Glutamine + α-ketoglutarate → 2 glutamate

Glutamate and glutamine serve as nitrogen donors to synthesize all other amino acids (aspartate, alanine, serine, cysteine etc.). From amino acids, plants build proteins, enzymes, chlorophyll, nucleic acids (DNA, RNA).

Special case: Legumes (biological nitrogen fixation)

Legumes form symbiosis with rhizobium bacteria in root nodules. Bacteria convert **N_2** to **NH_4^+** using the enzyme nitrogenase. This provides plants with a high nitrogen supply.

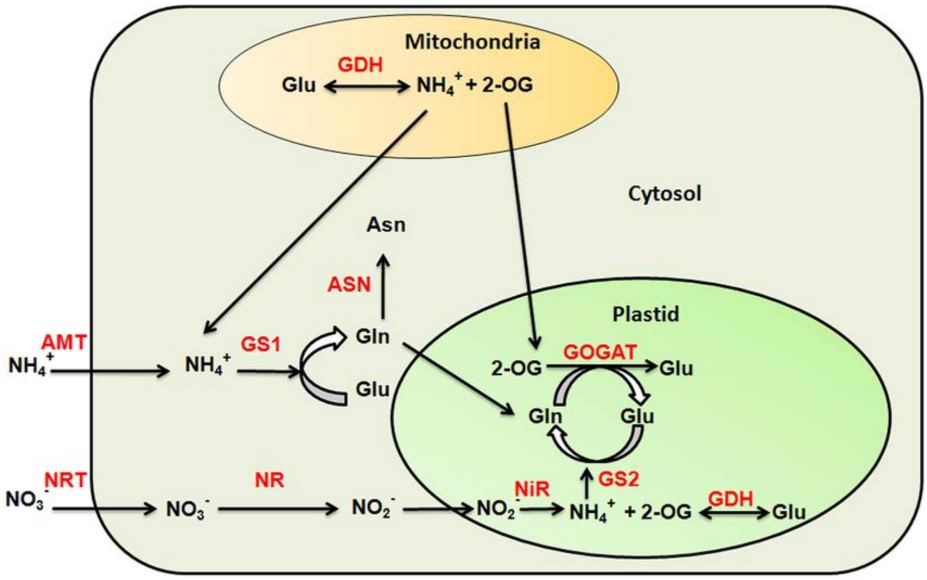


Figure 2 Nitrogen metabolism in plants

6. Carbon nutrition (Photosynthesis)

Photosynthesis is a physiological process by which plants that contain certain pigments (especially chlorophyll) are able to capture light energy and convert it into chemical energy in order to carry out carbon nutrition using atmospheric CO_2. This process is accompanied by the release of oxygen.

Plants synthesize their organic matter from simple molecules (CO_2 + H_2O) and light energy (sunlight).
Carbon dioxide and water combine (a reduction reaction) to form carbohydrates.

The general formula of photosynthesis is: **CO_2 + H_2O + light energy --------> (CH_2O) + O_2**

Location

In green plants, the photosynthetic apparatus is mainly located in the leaves. Leaf chlorophyll-containing cells contain several hundred chloroplasts. Depending on the species, there are **10 to 100 chloroplasts per cell** (the more numerous they are, the smaller they become).

Chloroplasts are generally found in the leaves, but they are also present in petioles, herbaceous stems, and some floral organs.

Chloroplast

The chloroplast is a double-membrane organelle, oval-shaped, and several tens of micrometers long.

The outer membrane is relatively permeable and continuous, while the inner membrane is impermeable (a selective barrier) and folded inward to form sacs called **thylakoids**, where the photosynthetic pigments are located.

The chloroplast is composed of **grana** and **stroma**:

- A **granum** is a stack of several thylakoid sacs or discs.
- A granum may contain **2 to 100 discs**, and these grana are connected to each other by stroma lamellae, forming a continuous internal network.

The stroma also contains ribosomes and circular DNA.

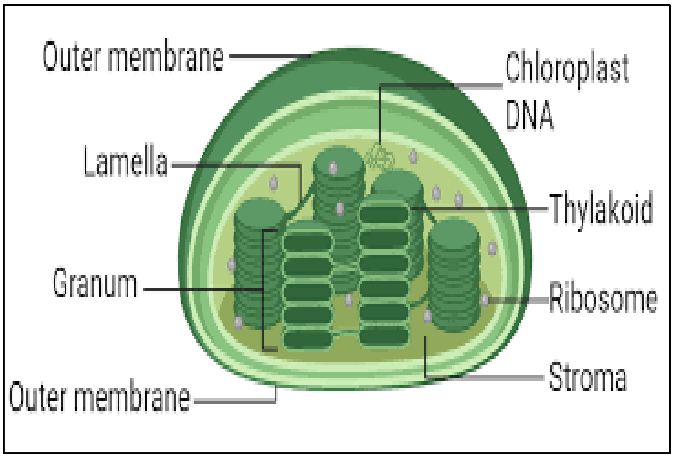


Figure 3 chloroplast

Photosynthesis takes place in two major phases: *the light phase* and *the dark phase*

I. The light phase (Photochemical reactions)

These are the steps that convert solar energy into chemical energy. Light triggers a transfer of electrons and protons. The photochemical reactions take place in the **thylakoids**.

II. The dark phase (Calvin cycle)

This is the carbon fixation phase, during which CO_2 is incorporated and then reduced to produce a carbohydrate. The Calvin cycle takes place in the stroma of the chloroplasts.

The synthesis of the energy molecules NADPH and ATP, produced by the conversion of light into chemical energy, requires the functioning of the photosystems.

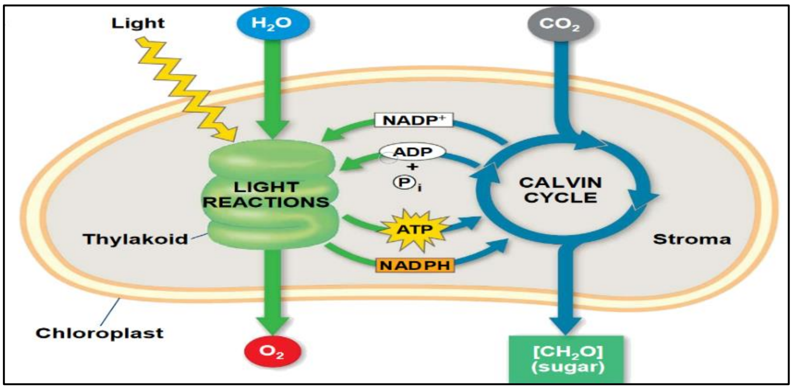


Figure 4 Light and dark phases of photosynthesis

I. The light phase (Photochemical reactions)

What is a photosystem?

Photosystems are complexes of proteins and pigments that capture and convert light energy into chemical energy within the thylakoid membranes. They consist of **antennae** and a **reaction center**.

The **antenna** is made up of pigments (chlorophylls and carotenoids). Each pigment molecule absorbs a photon and is excited to a higher energy state. This excitation is then transferred from one chlorophyll molecule to another via resonance energy transfer (without energy conversion).

The excitation eventually reaches a specific *chlorophyll a molecule* associated with the reaction center at the core (a large molecular complex). This chlorophyll molecule becomes excited and transfers an electron to a primary electron acceptor. After being reduced, this acceptor passes the electron to a secondary acceptor, and the process continues sequentially.

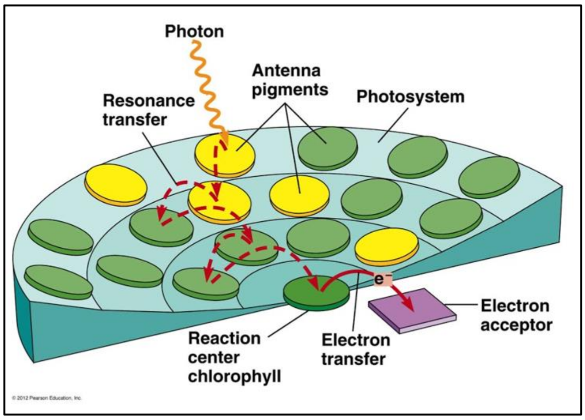


Figure 5 photosystem

Two types of photosystems

There are two types of photosystems in the thylakoid membrane.

1 – Photosystem II (PSII)

Light energy is first absorbed by the light-harvesting antenna, which then transfers this energy to the **P680** reaction-center complex.

The chlorophyll a molecule in P680 becomes excited and releases an electron, which is captured by the primary electron acceptor (A_0 = a modified chlorophyll a).

The electron is then transported along an electron transport chain made of pheophytin, quinone, and plastoquinone.

The electron continues to the cytochrome complex, where its passage drives the pumping of protons from the stroma into the thylakoid lumen.
The accumulation of these protons creates a proton gradient, which will be used by ATP synthase to produce ATP (photophosphorylation).

After leaving the cytochrome complex, the electron is transferred to **Photosystem I (PSI)**.

At the level of PSII, the chlorophyll (a) molecule of P680 has lost electrons (it becomes photo-oxidized), so it must replace them through the photolysis of water, which occurs during non-cyclic electron transport.

Water is therefore the primary electron donor of photosynthesis.
The reaction is as follows:

$$\frac{1}{2} H_2O \text{ ---------> } \frac{1}{2} O_2 + H^+ + 1 e^-$$

The electron released by water splitting is captured by PSII,
the protons produced accumulate in the thylakoid lumen to contribute to the proton gradient,
and the oxygen is released into the atmosphere.

Thus, oxygen is a waste product of photosynthesis.

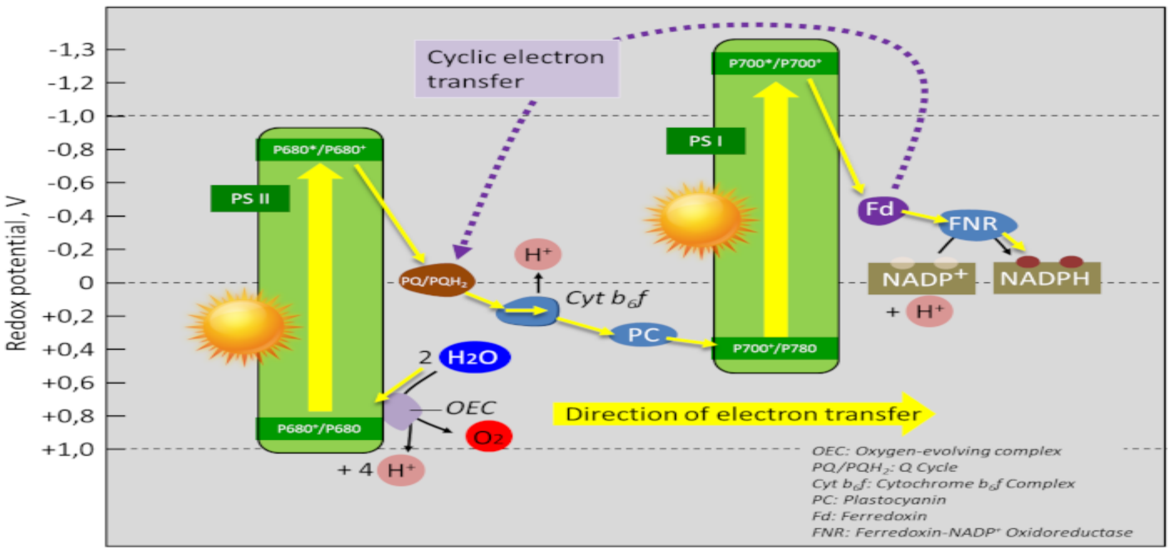


Figure 6 Z-Scheme Diagram of Photosynthesis

Photosystem I (PSI)

The continuation of photosynthesis requires additional light energy, which is absorbed by the light-harvesting antenna of PSI and transferred to the P700 reaction center. The chlorophyll *a* molecule in the P700 complex then releases an electron, which is carried through the electron acceptor chain to ferredoxin. Reduced ferredoxin transfers the electron via ferredoxin–NADP reductase (FNR) to oxidized $NADP^+$, reducing it to NADPH.

Since chlorophyll *a* in P700 has lost an electron, it must regain one for the system to function. This electron is supplied by PSII via plastocyanin.

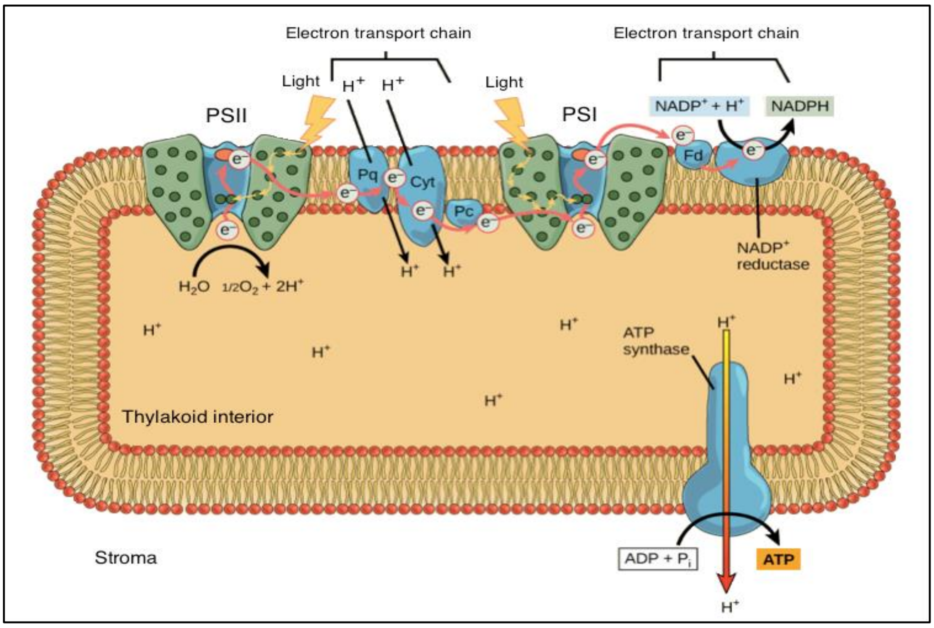


Figure 7 Light dependent reactions

The overall equation for the light-dependent reactions (photochemical phase) of photosynthesis is written as:

Light

$$2H_2O + 2NADP^+ + 3ADP + 3Pi \text{ ---------------------------> } O_2 + 2NADPH + 3ATP$$

II. The dark phase (Biochemical phase) (The Calvin cycle)

This phase occurs at the same time as the photochemical (light-dependent) phase, but it does not require light energy. The biochemical phase allows the carbon from atmospheric CO_2 to be fixed and combined with hydrogen atoms from water molecules.

1. CO_2 Fixation

The first molecule of the Calvin cycle is ribulose bisphosphate (RuBP, also called RuDP**)**, which contains five (05) carbon atoms. The fixation of CO_2 onto this molecule requires an enzyme called Rubisco (Ribulose Bisphosphate Carboxylase/Oxygenase).

This enzyme enables the formation of an unstable 6-carbon compound, which quickly splits into two molecules of 3-phosphoglycerate (3-PGA), each containing 3 carbon atoms.

2. Reduction of the Fixed Carbon

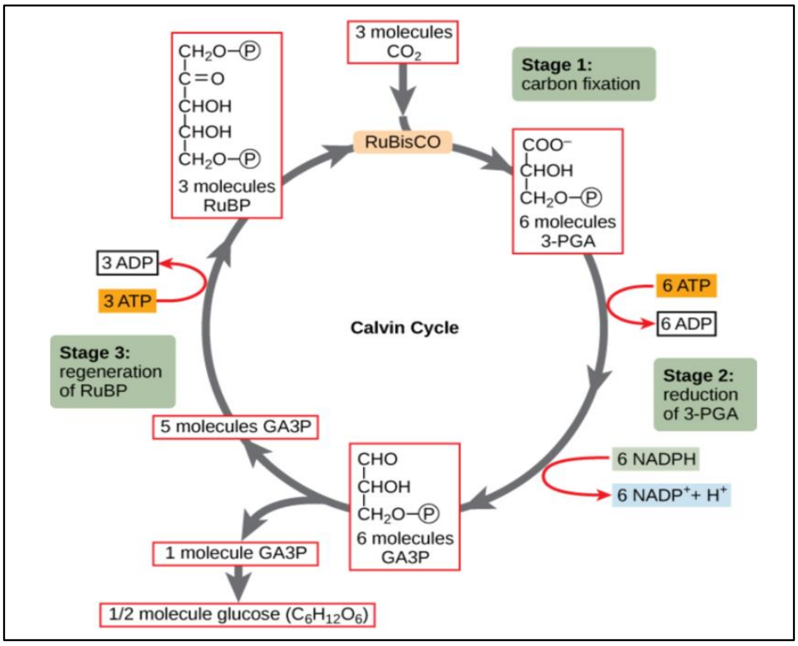
The second phase of the Calvin cycle corresponds to the reduction of 3-phosphoglycerate. First, ATP phosphorylates 3-PGA to form 1,3-bisphosphoglycerate, which is then reduced by NADPH to produce glyceraldehyde-3-phosphate (G3P, also called PGAL), which is a sugar.

3. Regeneration of the CO_2 Acceptor

The G3P produced can have several fates:

- One sixth of it is used by the cell as a carbohydrate component.
- The remaining five sixths are used to continue the Calvin cycle.

The regeneration of RuBP, which will be used to fix new CO_2, occurs through several steps and requires ATP. The glyceraldehyde-3-phosphate produced in the chloroplast is rapidly transported to the cytoplasm, where it is used to synthesize sucrose.



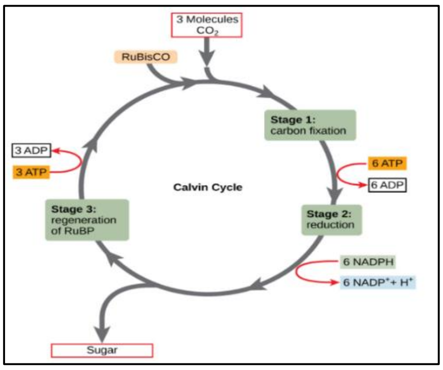


Figure 8 Calvin cycle

The overall equation for photosynthesis is:

Light, chlorophyll

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

Different types of carbon fixation

There are three known mechanisms for carbon dioxide fixation during photosynthesis: **C3**, **C4**, and **CAM**. These three mechanisms differ in the efficiency of carbon fixation.
The C3 pathway corresponds to the "basic" mechanism and is used by **98% of green plants**.

The **C4** and **CAM** types are less common, but they occur in well-known species (maize (corn) is a C4 plant and pineapple is a CAM plant). These mechanisms are adaptations to water stress or to reduced CO_2 availability during the day.

<u>C3 plants (Calvin Cycle)</u>

The first step of the Calvin cycle is the carboxylation (fixation of a CO_2 molecule) onto ribulose-1,5-bisphosphate (RuBP), catalyzed by **Rubisco**.
This reaction produces two molecules of a 3-carbon compound, 3-phosphoglyceric acid **(3-PGA).**

However, this enzyme can also catalyze the reaction of oxygen with RuBP (oxygenase activity), a process called photorespiration.
Photorespiration blocks the cycle and occurs when CO_2 levels in the leaf air spaces become too low. Rubisco does have a stronger affinity for CO_2 than for O_2, but low CO_2 makes oxygenation more likely.

<u>C4 plants</u>

The problem caused by stomatal closure in a hot, dry atmosphere (leading to CO_2 shortage in the chlorenchyma) is solved by C4 plants, such as maize or sugarcane.

In these plants:

1. Mesophyll cells fix CO_2 using PEP carboxylase (PEPcase).
2. CO_2 is fixed onto a 3-carbon compound (phosphoenolpyruvate, PEP) to form a 4-carbon compound (malate, or oxaloacetate depending on the plant).
3. Malate is transported to the bundle-sheath cells.
4. There, it is decarboxylated, releasing CO_2.
5. The released CO_2 enters the Calvin cycle, while pyruvate returns to the mesophyll.

This spatial separation greatly reduces photorespiration.

<u>CAM plants (Crassulacean Acid Metabolism)</u>

The CAM mechanism differs from C_4 because the separation is not spatial but temporal (night/day).

- **At night:**
  - Stomata are open.
  - CO_2 is fixed into malate, which is stored in the vacuole.
- **During the day:**
  - Stomata close to prevent water loss.
  - Malate is converted back into CO_2.
  - The Calvin cycle continues using this internally released CO_2.

This mechanism greatly limits water loss by transpiration. It is found especially in Crassulaceae ("succulent plants"), such as **cacti**.