

## PHOTOSYNTHESIS

### 10.1 THE C<sub>4</sub> PHOTOSYNTHETIC CARBON ASSIMILATION (PCA) CYCLE

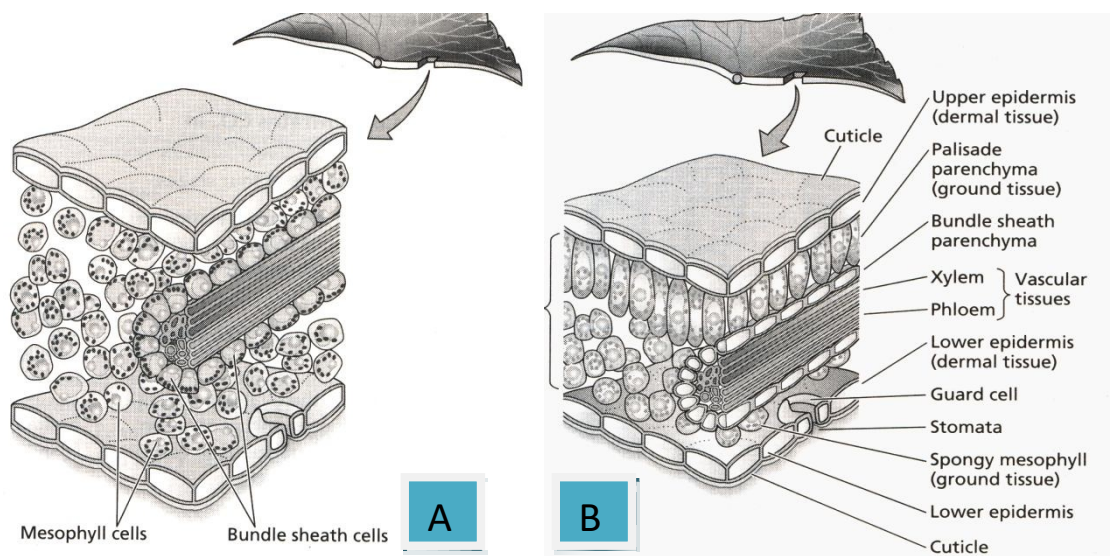
**Hal Hatch and Roger Slack** elucidated what is now known as the C<sub>4</sub> PCA cycle. Using sugarcane leaves, they established that the C<sub>4</sub> acids malic acid and aspartic acid were the first stable detectable intermediates of photosynthesis in leaves of C<sub>4</sub> plants. The primary carboxylation in these leaves was not catalyzed by Rubisco but by PEP carboxylase.

The basic C<sub>4</sub> PCA cycle consists of four stages.

1. Assimilation of CO<sub>2</sub> involving carboxylation of phosphoenol pyruvate (PEP) in the mesophyll cells to form C<sub>4</sub> acids (Malate and / or aspartate)
2. Transport of C<sub>4</sub> acids to the bundle sheath cells.
3. Decarboxylation of C<sub>4</sub> acids within in the bundle sheath cells and generation of CO<sub>2</sub> which is reduced to carbohydrate via the C<sub>3</sub> PCR cycle.
4. Transport of the C<sub>3</sub> acid formed by the decarboxylation (pyruvate or alanine) back to the mesophyll cell and regeneration of the CO<sub>2</sub> acceptor, phosphoenol pyruvate.

#### **Anatomical differences**

A cross section of a typical C<sub>3</sub> leaf reveals essentially one type of photosynthetic, chloroplast containing cell, the mesophyll. In contrast, a typical C<sub>4</sub> leaf has two distinct chloroplast containing cell types, the mesophyll and the bundle sheath cells. The extensive network of **Plasmodesmata** connects mesophyll and bundle sheath cells, providing a pathway for the flow of metabolites between cells. This is called as **KRANZ ANATOMY**. (Fig.9)



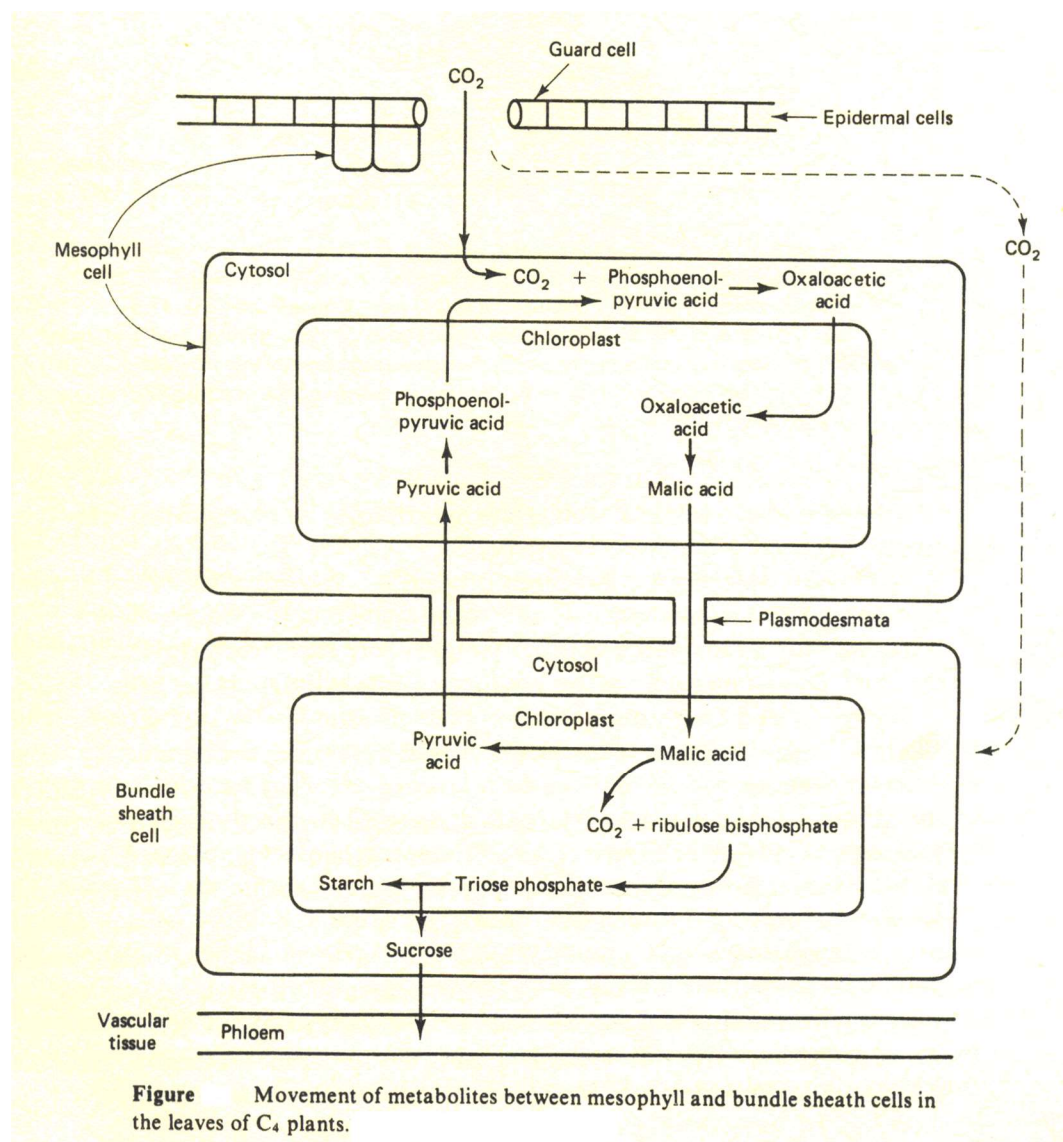
\*Figure 9 is from reference 6

The figure 'A' shows that in a C<sub>4</sub> leaf both mesophyll and bundle sheath cells possess chloroplasts.

Discovered in tropical grasses (e.g., sugarcane and maize), the C<sub>4</sub> cycle is now known to occur in 16 families of both monocotyledons and dicotyledons, and it is particularly prominent in Gramineae (sugarcane, corn, sorghum), Chenopodiaceae (Atriplex), and Cyperaceae (sedges). About 1% of the characterized species have C<sub>4</sub> metabolism.

The primary carboxylation reaction, catalyzed by PEP carboxylase, which is common to all three variants, occurs in the cytosol of the mesophyll chloroplasts by NADPH using NADP malate dehydrogenase. The malate formed enters the chloroplast of the bundle sheath cell and there undergoes oxidative decarboxylation, yielding pyruvate. The CO<sub>2</sub> released within the bundle sheath cells is converted to carbohydrate by the Calvin cycle. ( Fig.10)

The residual C<sub>3</sub> acid is transported back to the mesophyll as pyruvate and converted to phosphoenolpyruvate in the mesophyll chloroplast.



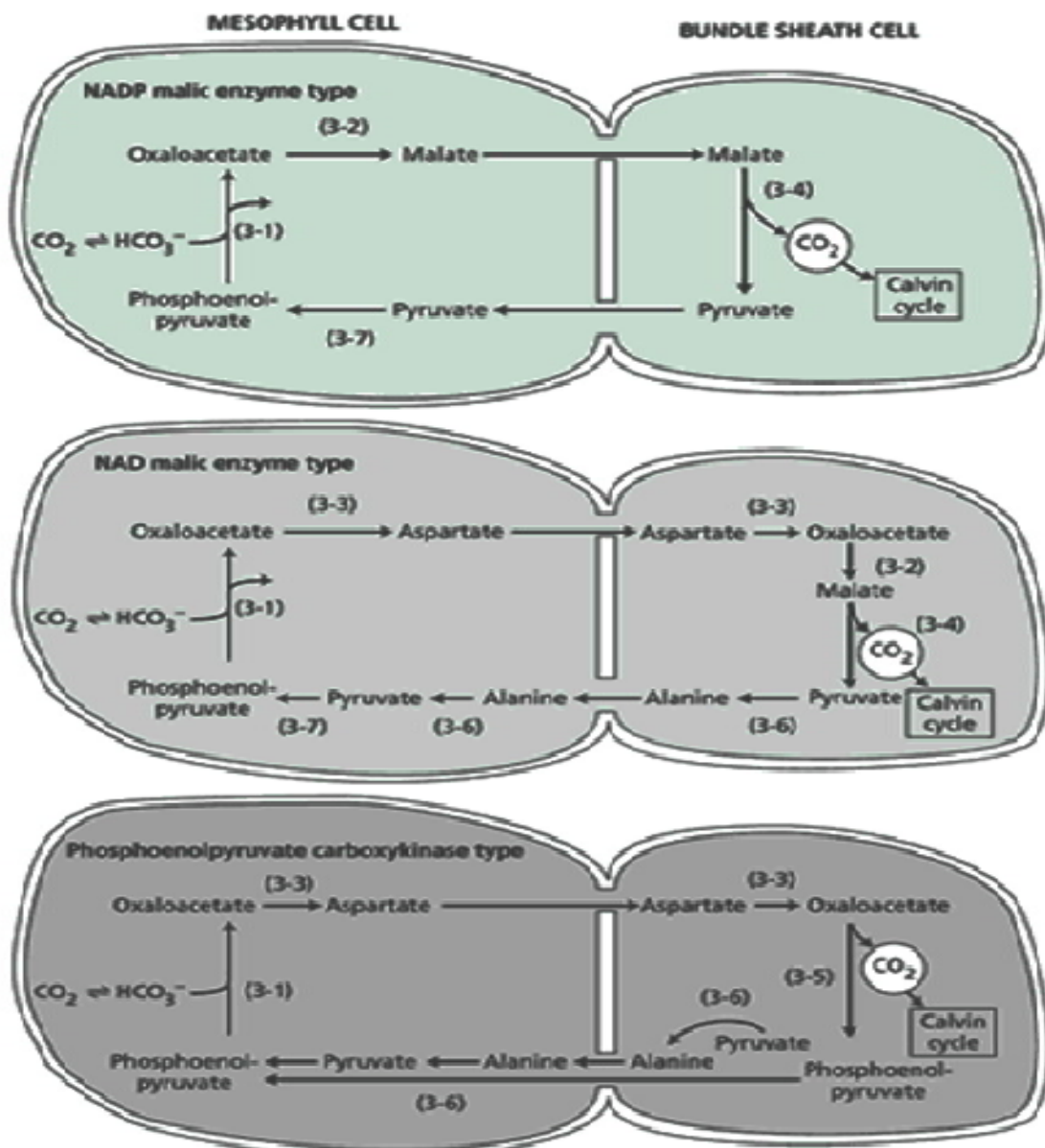
**Figure** Movement of metabolites between mesophyll and bundle sheath cells in the leaves of C<sub>4</sub> plants.

\*Figure 10 is from reference 7

10.1.1 There are three variants in PCR cycle of C<sub>4</sub> plants:

Variant Name	Principal C <sub>4</sub> acid transported to the bundle sheath cells	Decarboxylating enzyme	Examples.
NADP – ME	Malate	NADP-dependent Malic enzyme	Maize, Sugarcane, Sorghum
NAD-ME	Aspartate	NAD-dependent Malic enzyme	Millet, Pigweed, Panicum milliaceum (variga) Amaranthus
PEP-CK	Aspartate	Phosphoenol pyruvate carboxy kinase	Guinea grass (Panicum maximum), chloris gayana

These variants differ principally in the C<sub>4</sub> acid transported into the bundle sheath cells and decarboxylating enzyme.(Fig.11)



\*Figure 11 is from reference 6

### **A) NADP-ME Type**

The primary carboxylation reaction occurs in the cytosol of mesophyll cells and is catalyzed by phosphoenol pyruvate carboxylase (PEP carboxylase) using  $\text{HCO}_3^-$  rather than  $\text{CO}_2$  as a substrate.

Oxalo acetate formed in this reaction rapidly reduced to malate in the mesophyll chloroplasts by NADPH. The  $\text{C}_4$  acid (Malate) then transported to the chloroplasts of bundle sheath cells and undergoes oxidative decarboxylation by NADP-ME. The  $\text{CO}_2$  released within the bundle sheath cells is reduced to carbohydrate by the PCR cycle. The  $\text{C}_3$  acid (Pyruvate) formed in the decarboxylation is transported back to the mesophyll. In the final step of PCA cycle, the pyruvate is converted to phosphoenol pyruvate with in the mesophyll chloroplast.

**B) NAD-ME Type** :The primary carboxylation reaction occurs in the cytosol of the mesophyll cells and is catalyzed by PEP carboxylase using  $\text{HCO}_3^-$ .

Oxalo acetate formed undergoes transamination in the cytosol, with glutamate as the amino donor and forms into Aspartate. The  $\text{C}_4$  acid (Aspartate) then transported to the bundle sheath cells. In the bundle sheath cells, the aspartate is first reconverted into oxalo acetate by transamination in the mitochondria. The oxalo acetate in the mitochondria is then reduced and decarboxylated by the NAD-Malic enzyme. The  $\text{CO}_2$  released within the bundles sheath cells is reduced to carbohydrate by PCR cycle. The  $\text{C}_3$  acid pyruvate undergoes transamination and formed in to alanine which will be transported back to mesophyll cells. In the measophyll cytosol alanine appears to be converted to pyruvate via transamination. In the final step the pyruvate is converted to phosphoenol pyruvate within the mesophyll chloroplast.

### **C) PEP-CK type**

The primary carboxylation reaction occurs in cytosol of mesophyll cells and is catalyzed by PEP carboxylase using  $\text{HCO}_3^-$ . The Oxalo acetate undergoes transamination and formed into aspartate a  $\text{C}_4$  acid. The  $\text{C}_4$  acid then transported to the bundle sheath cell and is reconverted into oxalo acetate. The oxalo acetate is decarboxylated by PEP carboxy knase and forms into phosphoenol pyruvate. The PEP then transported to mesophyll cells.

### 10.1.2 Significance of C<sub>4</sub> Photosynthesis

C<sub>4</sub> PCA cycle effectively shuttles CO<sub>2</sub> from the atmosphere into the bundle sheath cells. This transport process generates a much higher concentration of CO<sub>2</sub> in the bundle sheath cells than would occur in equilibrium with the external atmosphere. This elevated concentration of CO<sub>2</sub> at the site of carboxylation of ribulose 1,5 biphosphate by RUBISCO results in the suppression of ribulose biphosphate oxygenation or Photorespiration. This is called as CO<sub>2</sub> concentration mechanism.

### 10.1.3 Energy Requirement

One interesting feature of the cycle is that the enzyme that catalyzes the regeneration of phospho enol pyruvate by the enzyme pyruvate-orthophosphate dikinase, consumes two “high-energy” phosphate bonds through the conversion of ATP to AMP. There by, explaining the high energy requirement of C<sub>4</sub> cycle.

Total energy requirement for fixing one molecule of CO<sub>2</sub> in C<sub>4</sub> plants is 5 ATP plus 2 NADPH. This includes the cost of concentrating CO<sub>2</sub> within the bundle sheath cell i.e. 2ATP per CO<sub>2</sub>

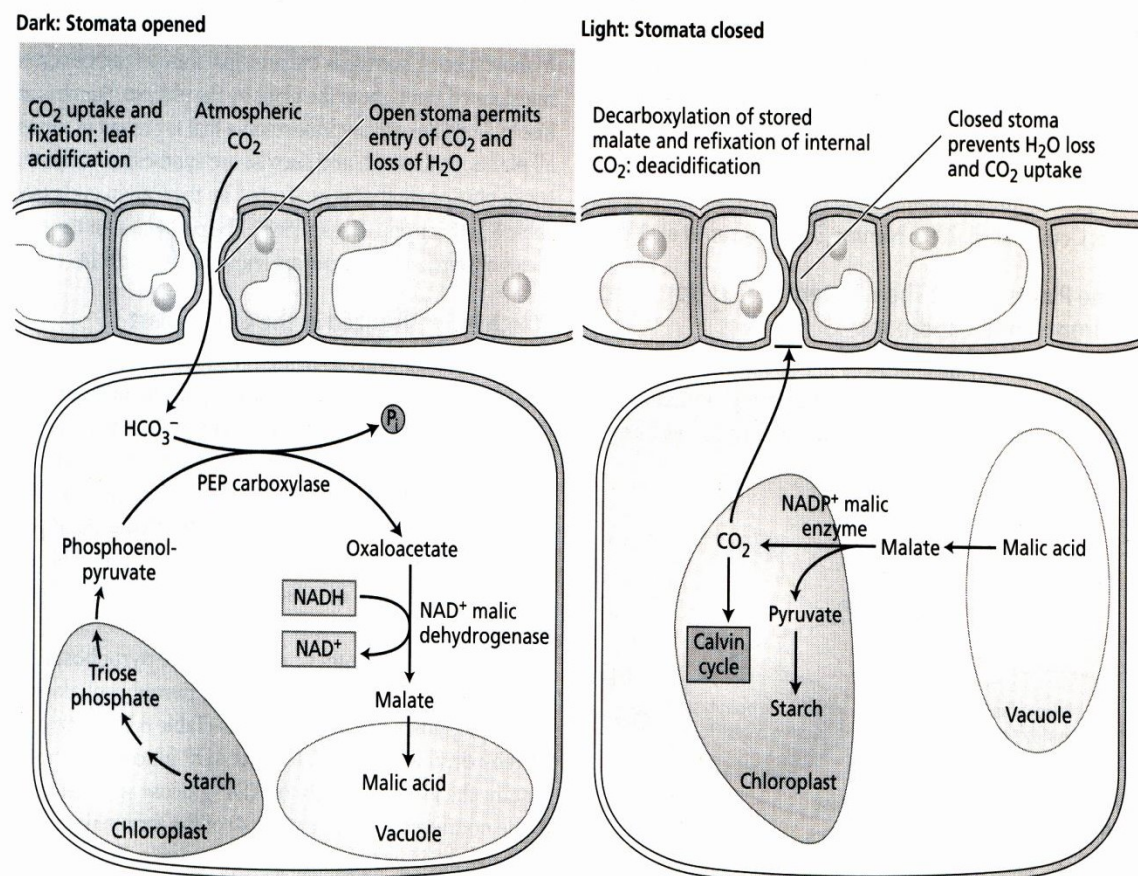
## 10.2 CRASSULACEAN ACID METABOLISM (CAM)

**CAM** is an acronym for crassulacean acid metabolism, but the mechanism is not restricted to the family crassulaceae alone, like the C<sub>4</sub> PCA cycle, it is found in many angiosperm families.

The CAM mechanism is similar in many respects to the C<sub>4</sub> PCA cycle, but differs from it in two important features.

1) In C<sub>4</sub> plants the formation of C<sub>4</sub> acids is spatially, but not temporally, separated from the decarboxylation of the C<sub>4</sub> acids and re fixation of the resulting CO<sub>2</sub> by the PCR cycle. In CAM plants, the formation of C<sub>4</sub> acids is temporally but not spatially, separated from decarboxylation and re fixation. CAM plants lack the specialized leaf anatomy **kranz leaf anatomy** typical of C<sub>4</sub> plants.

2) CAM plants open their stomata during the cool, desert nights and closed during the hot, dry days. This minimizes water loss. The CO<sub>2</sub> is assimilated at night. (Fig.12)



\*Figure 12 is from reference 6

The CO<sub>2</sub> assimilation is accomplished by carboxylation of phosphoenol pyruvate to oxalo acetate, which is then reduced to malate. The PEP originates from the breakdown of starch and other sugars by the glycolytic pathway. The C<sub>4</sub> acid accumulates as malic acid in large vacuoles. The accumulation of substantial amounts of malic acid has long been recognized as **dark acidification of leaf**.

With the onset of day, the stomata close, preventing loss of water and further acquisition of CO<sub>2</sub>. The leaf cells become deacidified as the reserves of vacuolar malic acid are consumed. Decarboxylation is achieved by the action of NADP malic enzyme on malate. Because the stomata are closed, the internally released CO<sub>2</sub> cannot escape from the leaf and instead is reduced to carbohydrate by operation of the C<sub>3</sub> PCR cycle. The C<sub>3</sub> acid remaining after decarboxylation is thought to be converted into starch or sucrose thus recovering the original starting material.

### 10.2.1 Significance:

The CAM mechanism enables plants to maximize their water – use efficiency due to **skoto active opening of stomata**. Typically a CAM plant loses 50-100 g of water for every gram of CO<sub>2</sub> gained, compared with the values of 250-300 and 400-500g for C<sub>4</sub> and C<sub>3</sub> plants respectively. Thus CAM plants are specially adapted to arid environment. Like in C<sub>4</sub> plants, the elevated internal concentration of CO<sub>2</sub> effectively suppresses the photo respiratory oxygenation of ribulose biphosphate and favors carboxylation.

### **10.3 MEASUREMENT OF PHOTOSYNTHESIS:**

There are different methods by which photosynthesis can be measured.

#### **A. Assay of Chloroplast activity (Hill reaction)**

Either intact or isolated chloroplasts of leaf fragments evolve oxygen and electrons during photosynthesis. Photosynthesis hence is measured by the changes in the absorption spectra of 2,6-dichlorophenol indophenol (DCPIP) which is reduced by the evolved electrons. It represents chloroplast activity. This method is convenient to identify photosynthetic inhibitors.

#### **B. Gas exchange Measurement (Infra – red Gas Analysis IRGA)**

Photosynthetic gas exchange refers to the fluxes or flows of CO<sub>2</sub> between **the plant** and environment.

Infrared gas analysis is the most popular, accurate and simple technique determining photosynthetic or respiratory CO<sub>2</sub> exchange technique in air. It is based on the principle that the hetero atomic gas molecules typically absorb radiation at specific infrared wave bands and that each gas has got a characteristic absorption spectrum and not interfered by gas molecules of identical atoms. Hence IRGA can be used for accurate determination of concentrations of CO<sub>2</sub> uptake by small leaves.

#### **C. Monitoring with <sup>14</sup>CO<sub>2</sub> :** Photosynthesis true to the field, is measured by

##### **a) Exposing Photosynthetic tissue to <sup>14</sup>CO<sub>2</sub>**

In this photosynthetic tissue is exposed to a gas of <sup>14</sup>CO<sub>2</sub>. By killing the tissue after a definite time <sup>14</sup>CO<sub>2</sub> fixed is determined and the rate of Photosynthesis is estimated.

##### **b) Exposing leaf to a gas mixture in a closed space:**

In this method the leaf is exposed in a closed space to a gas mixture containing the carbon isotopes and the rate of decrease of radioactivity of the gas is measured by a built in β- counter.

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