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Full Length Research Paper

Optimization of Enzyme Properties in C3 and C4 and CAM plants

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C3, C4 and CAM are the three different processes that plants use to fix carbon during the process of photosynthesis. Fixing carbon is the way plants remove the carbon from atmospheric carbon dioxide and turn it into organic molecules like carbohydrates. Crassulacean acid metabolism (CAM) plants minimize photorespiration and save water by separating these steps in time, between night and day. C4 plants occur in various taxonomic groups of monocot and dicot plants. C4 plants use a particular mechanism to concentrate CO₂ at the reaction site of Rubisco and thereby suppress photorespiration. In C3 photosynthesis, the atmospheric CO₂ is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). C4 plants photosynthesize more efficiently than C3 plants under conditions of high light intensity and temperature, and low CO₂ conductance. We demonstrated that C4 plants have better modularity with complex mechanism coordinates the reactions and pathways than that of C3 plants. C4 plants can be classified to three subtypes according to decarboxylation modes: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME) and PEP carboxykinase (PCK). We explored the influence of each subtype on biomass synthesis and CO₂ fixation, by blocking the flux of other two enzymes and giving enough supply of water and nitrogen. C4 plants can be annual or perennial. Annual C4 plants include corn, sudan grass, and pearl millet. Perennial C4 plants include big bluestem, Indian grass, Bermuda grass, switch grass, and old world bluestem.

Keywords: Plant C3, plant C4, Cam,

INTRODUCTION

C3 and C4 plants both use the process of photosynthesis to convert light to energy and atmospheric CO₂ into plant food energy (carbohydrates). The plants differ in the leaf anatomy and enzymes used photosynthesis. These differences are important with respect to their optimal growing conditions, nitrogen and water-use efficiency, forage quality, and seasonal production profile.

Plants exchange gases, CO₂ and O₂, with their environment through pores known as stomata. When the stomata are open CO₂ can diffuse in to be used in photosynthesis and O₂, a product of photosynthesis can diffuse out. However, when the stomata are open the plant also loses water due to transpiration, and this problem is enhanced in hot and dry climates. Plants that perform C4 photosynthesis can keep their stomata closed more than their C3 equivalents because they are more efficient in incorporation CO₂. This minimizes their water loss.

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C3 Plants

The C3 pathway gets its name from the first molecule produced in the cycle (a 3-carbon molecule) called 3-phosphoglyceric acid. About 85% of the plants on Earth use the C3 pathway to fix carbon via the Calvin Cycle. During the one-step process, the enzyme Ru BisCO (ribulosebis phosphate carboxylase/oxygenase) causes an oxidation reaction in which some of the energy used in photosynthesis is lost in a process known as photorespiration. The result is about a 25% reduction in the amount of carbon that is fixed by the plant and released back into the atmosphere as carbon dioxide. The carbon fixation pathways used by C4 and CAM plants have added steps to help concentrate and reduce the loss of carbon during the process. Some common C3 plant species are spinach, peanuts, cotton, wheat, rice, barley and most trees and grasses.

C4 Plants

The C4 process is also known as the Hatch-Slack pathway and is named for the 4-carbon intermediate molecules that are produced, malic acid or aspartic acid. It wasn't until the 1960s that scientists discovered the C4 pathway while studying sugar cane. C4 has one step in the pathway before the Calvin Cycle which reduces the amount of carbon that is lost in the overall process. The carbon dioxide that is taken in by the plant is moved to bundle sheath cells by the malic acid or aspartic acid molecules (at this point the molecules are called malate and aspartate). The oxygen content inside bundle sheath cells is very low, so the Ru Bis CO enzymes are less likely to catalyze oxidation reactions and waste carbon molecules. The malate and aspartate molecules release the carbon dioxide in the chloroplasts of the bundle sheath cells and the Calvin Cycle begins. Bundle sheath cells are part of the Kranz leaf anatomy that is characteristic of C4 plants.

About 3% or 7,600 species of plants use the C4 pathway, about 85% of which are angiosperms (flowering plants). C4 plants include corn, sugar cane, millet, sorghum, pineapple, daisies and cabbage. Edwards and Ku 1987.

CAM Plants

Plants that use crassulacean acid metabolism, also known as CAM plants, are succulents that are efficient at storing water due to the dry and arid climates they live in. The word crassulacean comes from the Latin word *Crassus* which means "thick." There are over 16,000 species of CAM plants on Earth including cacti, sedum, jade, orchids and agave. Succulent plants like cacti have leaves that are thick and full of moisture and can also have a waxy coating to reduce evaporation.

CAM plants keep their stoma close during the day to

prevent water loss. Instead, the stoma are opened at night to take in carbon dioxide from the atmosphere. The carbon dioxide is converted to a molecule called malate which is stored until the daylight returns and photosynthesis begins via the Calvin Cycle. Most plants open their stomata during the day because that is when energy is received from the Sun. The energy from the Sun is harvested by the chloroplasts and used to make ATP and NADPH. These short-term energy storage molecules are then used to power the fixation of carbon into sugar.

In plants living in very dry environments, however, dangerous amounts of water can be lost if the stomata are open during the hot, dry days. During the night, which tends to be much cooler in dry environments, far less water is lost by opening the stomata.

In order to meet their needs to combine the Sun's energy with CO₂ from the air, CAM plants take in CO₂ at night and store it in the form of a four-carbon acid called "malate." Then the malate is released during the day, where it can be combined with the ATP and NADPH created by the Sun's energy.

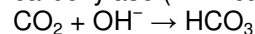
This allows the plants to conserve their water by closing their stomata during the hot daytimes.

The name "Crassulacean Acid Metabolism" comes from the *Crassula* plant, which was the first place that CAM metabolism was discovered and studied.

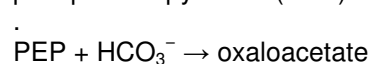
Steps of CAM Photosynthesis:

1. CAM photosynthesis begins at night, when the plant's stomata open and CO₂ gas is able to diffuse into the cytoplasm of CAM mesophyll cells.

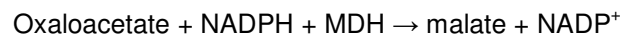
In the cytoplasm of those cells, the CO₂ molecules encounter hydroxyl ions, OH⁻, which they combine with to become HCO₃⁻ the enzyme phosphoenolpyruvate carboxylase (PEP carboxylase).



2. The PEP carboxylase enzyme catalyzes the following reaction to add the CO₂ to a molecule called phosphoenolpyruvate (PEP) Arnon 1949.



3. Oxaloacetate then receives an electron from NADH and becomes a molecule of malate. This reaction is catalyzed by the enzyme Malate Dehydrogenase (MDH). That reaction looks like:



Interestingly, malate dehydrogenase catalyzes a reversible reaction, meaning that it can either add electrons to oxaloacetate, or take electrons away from molecules of malate.

4. Malate is now stored in vacuoles within the plant cells, until the sun rises and photosynthesis begins. When that

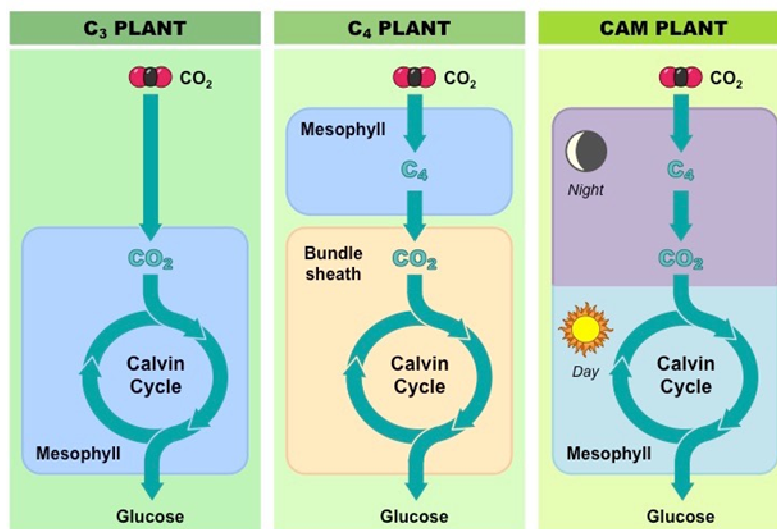


Figure 1: Show Glucose production in C₃ and C₄ and Cam plants

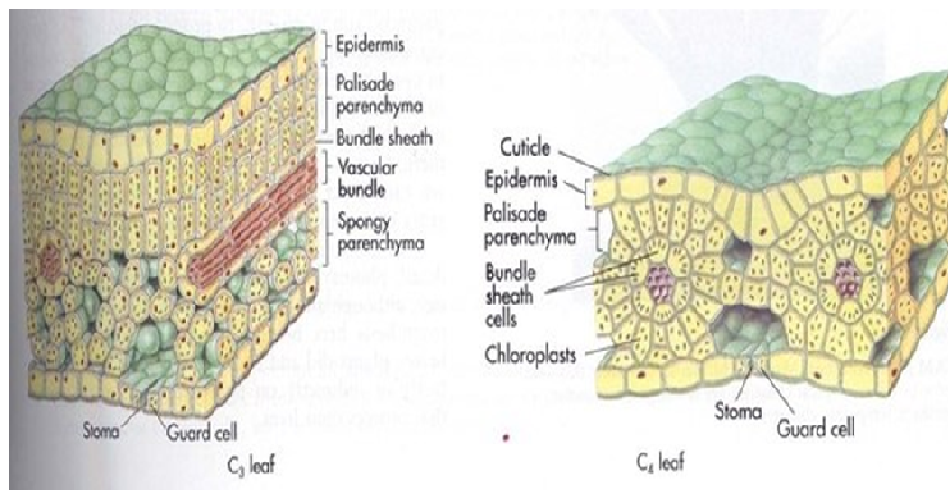


Figure 2: show difference between C₃ and C₄ Leaf

happens, malate enters the Calvin Cycle, just like 3-phosphoglycerate would in a plant using a 3-carbon, or "C₃" pathway for carbon fixation. Yoshimura et al. 2004

Examples of CAM Plants

CAM metabolism is common in plants that live in hot, dry environments where water is difficult to gain and conserve. Examples include:

Cacti

The stereotypical "desert plant" is the cacti. These plants, which look very different from your average leafy green, are ideally designed to survive in deserts. Typical cacti have a rounded shape, which minimizes the surface area through which they can lose water during the day. Many

also have spines to stab any animals that might want to eat them and consume their delicious water.

It makes sense, then, that cacti would also make use of the CAM cycle to prevent them from opening their stomata and losing water during the day!

Agave – a plant which has become popular because it is used to make tequila and the sweet agave nectar – also uses CAM to survive in desert environments. It looks more like a leafy green plant than a cactus, but like cacti, it has developed thick flesh to reduce its surface area and conserve water, and spines along the edges of its leaves to discourage animals from eating them. Hibberd et al. 2008. Figure 4 show that C₄ photosynthesis is a physiological syndrome resulting from multiple anatomical and biochemical components, which function together to

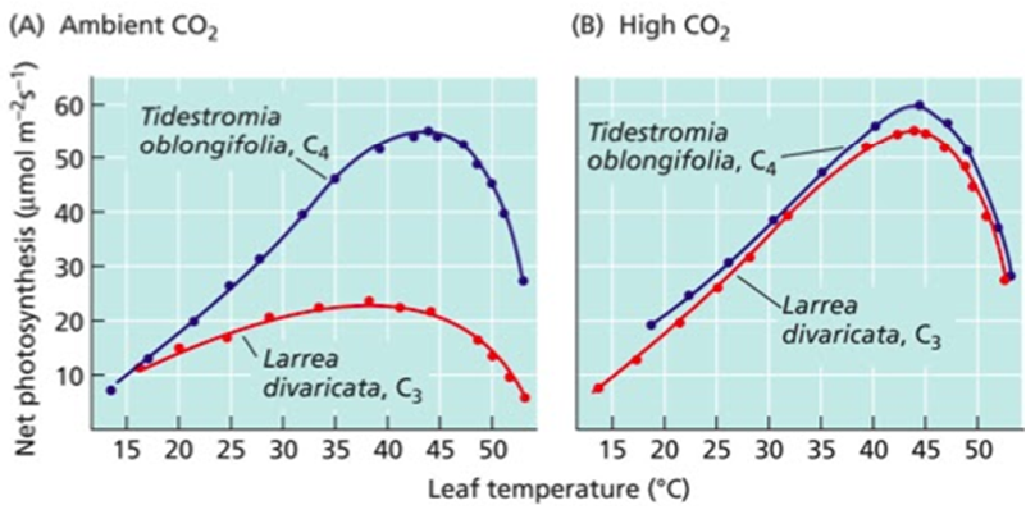


Figure 3: Leaf temperature in C3 and C4 and photosynthesis

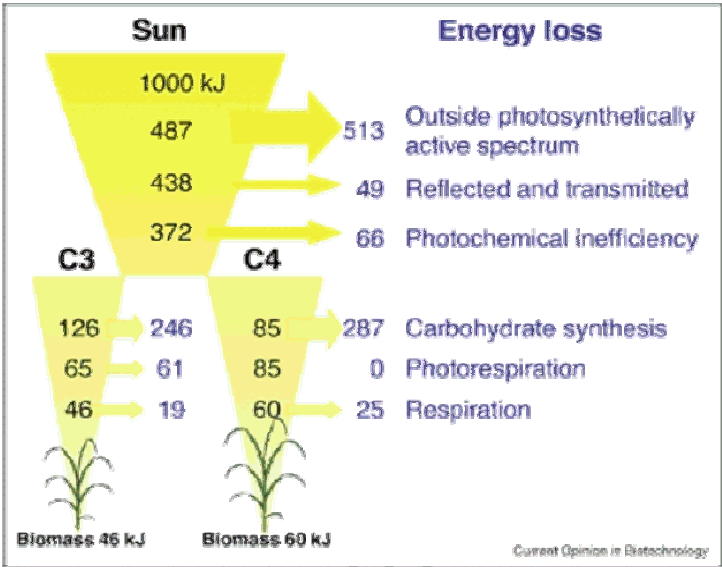


Figure 4: Use of Sun energy in C3 and C4 plants

Table 1 Fundamental differences between photosynthetic metabolic types.

	C3	C4	CAM
Stomata open during the day	Yes	Yes	No
Photosynthetic enzyme	RUBISCO	PEPCO	PEPCO
Inhibition of photosynthesis by oxygen	Yes	No	Yes during the day No at night
Seperation of photosynthetic process	None	Spatial	Temporal
Adapted climate	Cool, Moist	Warm, Moist-Dry	Hot, Dry

Presented in 2013 at 5th International Conference on Modelling, Identification and Control, Cairo, Egypt Aug 31-Sept 2 ©

Table 2: Differences between calvin plant and C4 palnts

Differences between calvin (C3) and C4	
C3	C4
<ul style="list-style-type: none">• Temp 15-25° C• Absence of malate• One carboxylation reaction• CO2 is the substrate• Usual leaf structures	<ul style="list-style-type: none">• Temp 30-35° C• Presence of malate• 2 carboxylation reactions• HCO3 is the substrate• Closed stomata to reduce water loss and concentrating CO2 in the bundle sheet cells• Additional ATP is required

increase the CO2concentration around Rubisco and reduce photorespiration. C4photosynthesis is a complex phenotype, formed from multiple anatomical and

biochemical components that together increase the concentration of CO2around Rubisco.

CONCLUSION

The evolutionary history of each C₄ taxon is rich and unique. It starts with the acquisition by its ancestors of characters that are required to build a C₄ system, but which evolve for completely unrelated reasons. Once all the characters exist in a given plant, these can be co-opted to create a weak C₄ cycle following an increase of PEPC activity.

The upcoming discussion will update you about the difference between C₃ Plants and C₄ Plants.

Difference # C₃ Plants:

1. Examples of these plants are wheat, oats, barley, rice, cotton, beans, spinach, sunflower, Chlorella etc..
2. Carbon pathway in photosynthesis is C₃ pathway i.e. Calvin cycle only.

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3. First stable product of above carbon pathway is 3-C compound phosphoglyceric acid (PGA).
4. The leaves have diffused mesophyll and only one type of chloroplasts.
5. Optimum temp, for photosynthesis is low to high.
6. Photorespiration occurs.

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7. Photosynthetically less efficient.
8. Carbon dioxide compensation point is high, about 50 ppm.
9. Rate of CO₂ evolution in light is higher.
10. Carbonic anhydrase activity is higher.
11. Rate of translocation of end products of photosynthesis is low.
12. Optimum temperature for growth is low to high.

Difference # C₄ Plants:

1. Example of these plants are sugarcane, maize, sorghum, Atriplex, Amaranthus etc..
2. Carbon pathway in photosynthesis is C₄—di-carboxylic acid pathway (Hatch-Slack pathway).
3. First stable product of above carbon pathway is 4—C compound Oxaloacetic acid (OAA).

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4. The leaves have 'cane type' of anatomy (Krantz anatomy) with compact mesophyll around the bundle sheath of vascular bundles and dimorphic chloroplasts. Those of bundle sheath are large and lack grana, while those of mesophyll are smaller and contain grana.
5. Optimum temperature for photosynthesis is high.

6. No photorespiration (or very little photorespiration).
7. Photosynthetically more efficient.
8. Carbon dioxide compensation point is low, 2 to 5 or even 0 ppm.
9. Rate of CO₂ evolution in light is apparently none.
10. Carbonic anhydrase activity is low.
11. Rate of translocation of end products of photosynthesis is high.
12. Optimum temperature for growth is high.

We demonstrated that in contrast to C₃, C₄ plants have less dense topology, higher robustness, better modularity, and higher CO₂ and radiation use efficiency. In addition, preliminary analysis indicated that the rate of CO₂ fixation and biomass production in PCK subtype are superior to NADP-ME and NAD-ME subtypes under enough supply of water and nitrogen. Kajala et al. 2011. C₄ cells in C₃ plants

The ability to use the C₄ pathway has evolved repeatedly in different families of angiosperms — a remarkable example of convergent evolution. Perhaps the potential is in all angiosperms.

A report in the 24 January 2002 issue of Nature (by Julian M. Hibbard and W. Paul Quick) describes the discovery that tobacco, a C₃ plant, has cells capable of fixing carbon dioxide by the C₄ path. These cells are clustered around the veins (containing xylem and phloem) of the stems and also in the petioles of the leaves. In this location, they are far removed from the stomata that could provide atmospheric CO₂. Instead, they get their CO₂ and/or the 4-carbon malic acid in the sap that has been brought up in the xylem from the roots.

If this turns out to be true of many C₃ plants, it would explain why it has been so easy for C₄ plants to evolve from C₃ ancestors.

These are also C₄ plants but instead of segregating the C₄ and C₃ pathways in different parts of the leaf, they separate them in time instead. (CAM stands for crassulacean acid metabolism because it was first studied in members of the plant family Crassulaceae.)

At night,

CAM plants take in CO₂ through their open stomata (they tend to have reduced numbers of them). The CO₂ joins with PEP to form the 4-carbon oxaloacetic acid. This is converted to 4-carbon **malic acid** that accumulates during the night in the central vacuole of the cells.

In the morning, the stomata close (thus conserving moisture as well as reducing the inward diffusion of oxygen). The accumulated malic acid leaves the vacuole and is broken down to release CO₂. The CO₂ is taken up into the Calvin (C₃) cycle.

These adaptations also enable their owners to thrive in conditions of high daytime temperatures- intense sunlight - low soil moisture.

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