Agave as an Ethanol Source, the CAM Photosynthetic Path of the Agave

Overview

The blue agave, Agave americana, also known as the "Century Plant" is a familiar garden plant throughout the American Southwest. It is a relative of A. tequilana, Figure 1, the agave plant used for the production of tequila. The majority of agave tequila comes from the Mexican state of Jalisco with most of the distilleries located around the city of Tequila. Over 50% of the agave crop used for tequila is also grown in the same area. Somewhat less than 50% of the agave comes from "Los Altos", a region 600-990 meters above the plain of the city of Tequila.



Figure 1: A field of *Agave* tequilana

The *Agave* genus is an interesting plant system in its adaption to areas having rainfall variability over the year. The agaves, with their adaptability to survive in areas with less than ideal growing conditions, have been put forth as a potential source of bioethanol (ethanol from now on) which would not displace food crops¹.

Agave's Interesting Biology

Photosynthesis

The most important biological function on the planet is photosynthesis by green plants; in the process called photosynthesis plants use light energy to convert (reduce) carbon dioxide to sugars. In doing so, plants are the ultimate source of all food on the planet. The photosynthetic process is a collection of chemical reactions, some driven by light and others just chemical

¹ For a general review see: "Feasibility of Agave as a Feedstock for Biofuel Production in Australia", RIRDC Publication no. 10/104, <u>www.rirdc.gov.au</u>

reactions which occur independent of light. The overall reaction pathways are shown in Figure 2². The 2 light absorbing centers absorb photons, at 680 nm and 700 nm³, to produce the high energy intermediates ATP and NADPH₂, molecular oxygen is also produced as a by-product.



Figure 2: Summary of the Light Reactions of Photosynthesis

These high energy species are used for the reduction of CO2 in 2 pathways, the C3 path, known as the Calvin Cycle, for the first reaction product, the 3 carbon glyceraldehyde 3-phosphate (PGA). The complete (C3) Calvin cycle is shown in Figure 3⁴. The C3 cycle has direct contact of atmospheric CO2 with Rubisco⁵. The C4 cycle uses the Calvin cycle for the reduction of CO₂ as well, except now there is an intermediate CO₂ carrier between atmospheric CO₂ and Rubisco. C4 plants are less efficient than C3 plants in terms of energy used to fix CO2. C4 plants use 5 ATP's for one CO₂; C3 plants need only use 3 ATP's. However, C4 plants have higher photosynthetic rates compared to C3 plants and can continue to run the photosynthetic process when C3 plants cannot.

(Rubisco is an enzyme involved in the C3 and C4 photosynthetic pathways. It is the CO2 assimilating enzyme and is linked to the light driven reactions. Rubisco constitutes about ½ the protein in the chloroplast and is thought to be the most abundant protein on the planet.)

² G. Acquaah, Horticulture, Principles and Practices, 2nd Ed'n, Prentice-Hall, 2002, ISBN 0-13-033125-2, Chapter 5

³ Both wavelengths are considered as "red" light

⁴ B. J. Tipple & M. Pagani, Annu. Rev. Earth Planet. Sci., 2007, 35:435-61

⁵ Ribulose-1,5-bisphosphate carboxylase/oxygenase, said to be the most abundant protein in nature







Figure 4: The C4 cycle for CO2 fixation

The differing responses of C3 plants and 4 plants to environmental conditions are shown in Figure 5⁶. In general, the C4 plants are better adapted to tropical conditions compared to C3 plants. As C4 plants use CO2 more efficiently than C3 plants they are able to function with partially closed stomata which happens on hot, sunny days. All plants known to use the C4 pathway are flowering plants. Notable members of the C4 class include: corn, sugar cane, sorghum, crabgrass and millet. Notable C3's include: peanut, soybean, rice, wheat, rye and oats.

Just to complicate life a little bit, there is another photosynthetic pathway called Crassulacean acid metabolism (CAM) in which the capture of CO2 and its conversion to sugars are temporally and spatially separated. This pathway has this unusual (and long) name owing to the fact that this pathway was first described for the genus Crassulaceae. Only about 6% of vascular plants use this metabolic pathway. The CAM plants appear to be concentrated among cacti, and succulents. CAM is of interest to us because the Agave genus uses this pathway.

⁶ Reference 2, page 154



Figure 5: Relative Rates of Photosynthesis in C3 vs C4 Plants at Different Temperatures and Light Levels

The metabolic paths used in the CAM cycle are shown in Figure 6. Most CAM plants are found in environments where moisture stress and intense light are the norm. CAM plants are relatively slower growing compared to C3 or C4 plants under favorable conditions owing to their tendency to conserve water by closing their stomata in the heat of the day thus limiting CO2 uptake to the night. CAM plant family includes pineapples, Spanish moss, prickly pear cactus and the agaves.



Figure 6: The CAM Pathway. CO2 Captured at Night and Reacted during the Day

There is some confusion about the nature of the CAM pathway in the agaves. One source⁷ states that the "Agave genus is composed exclusively of obligate CAM plants". Other reports⁸ have stated that agaves operate with both C3 and CAM metabolic pathways which allow the plants to add carbon at night and during the day as well. This duality has been established for A. tequilana, the tequila agave.

⁷ S C. Davis et. al., GCB Bioenergy (2011) 3, 68-78. The global potential for Agave as a biofuel feedstock ⁸ Reference 1 and references therein