## 113. Engineering Crassulacean Acid Metabolism (CAM) to Improve Water-use Efficiency of Bioenergy Feedstocks

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**Project goals: The long-term goal of this research is to enhance the water-use efficiency (WUE) and adaptability to hotter, drier climates of species that normally perform $C_3$ photosynthesis by introducing crassulacean acid metabolism (CAM). Four major objectives will be pursued to enhance photosynthetic performance and WUE in *Arabidopsis* and *Populus*: 1) Define the genetic basis of key CAM modules in both eudicot and monocot CAM species *via* network modeling using 'omics (transcriptome and metabolome) technologies; 2) characterize the regulation of 'carboxylation', 'decarboxylation', and 'inverse stomatal control' modules of CAM using comparative genomics, network and molecular dynamics modeling, and loss-of-function testing; 3) deploy advanced genome engineering technologies to enable stacking of a large number of transgenes into a single genomic locus to improve transgene persistence and transfer fully functional 'carboxylation' and 'decarboxylation' modules from CAM species to $C_3$ species that can accommodate overnight malic acid storage in the vacuole; and 4) analyze the effects of these transgenic modules on 'stomatal control', $CO_2$ assimilation and transpiration rates, biomass yield, and WUE in *Arabidopsis* and *Populus*.**

Increasing demands for food, feed, fiber, and fuels due to future human population growth, coupled with decreasing arable land area; predicted increasing severity and frequency of extreme weather conditions including higher temperatures and drought; and overreliance on groundwater for crop irrigation indicate the need for novel strategies to improve WUE of plants. One potential strategy is to move CAM into $C_3$ plants (Borland et al., 2014). CAM is a temporally controlled plant inorganic CCM that maximizes WUE by shifting all or part of the $CO_2$ uptake to the nighttime, when evapotranspiration rates are reduced compared with the daytime. CAM is distinguished by two major features: (i) nocturnal $CO_2$ uptake and fixation by phospho*enol*pyruvate carboxylase (PEPC), which leads to the formation of $C_4$ organic acids that are stored in the vacuole, and (ii) an inverse stomatal behavior, in which stomata are closed during all or part of the day and are open at night. The organic acids accumulated overnight are subsequently decarboxylated during the day to release $CO_2$ and concentrate it around ribulose-1- 5-bisphosphate carboxylase/oxygenase (RUBISCO), favoring carboxylase activity and carbohydrate production *via* the $C_3$ Calvin–Benson cycle.

A fundamental requirement for engineered CAM is to define the minimal set of genes and proteins required for its efficient establishment and operation. Genomic sequences and transcriptome atlases have become available from cycling, facultative, or obligate CAM species sampled from diverse phylogenetic origins including monocot orchids (*Phalaenopsis),* pineapple (*Ananas comosus*), several *Agave* species (*A. Americana*, *A. deserti*, *A. sisalana*, and *A. tequilana*), and core eudicots including the common ice plant (*Mesembryanthemum crystallinum*), *Kalanchoë fedtschenkoi*, *K. laxiflora*, *Sedum album,* and *Opuntia*

*ficus-indica, a* widely cultivated member of the cactus family. Comparative transcriptomic and genomic approaches are used to discern CAM gene function by comparing gene expression patterns of known CAM components across $C_3$, $C_4$, and CAM species. Co-expression network modeling incorporating transcriptional data, functional genomics annotation, and genetics information is also used to discover genes comprising functional CAM modules. In addition, loss-of-function studies of individual enzymes, regulatory proteins, or transcription factors are used to provide critical insights into the basic genetic requirements for CAM. The above information is then combined to guide the design and empirical testing of minimal functional modules for carboxylation and decarboxylation, malate influx and efflux into and out of the vacuole, stomatal control, and anatomical requirements for CAM. A set of genes with coordinate function, rather than gene-by-gene testing, will be used to accelerate the empirical testing process (DePaoli et al., 2014).

Initial CAM biodesign functional testing efforts will target the genetic model *Arabidopsis* owing to its rapid growth rate and ease of transformation. With regard to bioenergy feedstocks, fast-growing woody plants within the *Populus* genus, which are used extensively in the timber, pulp, and paper industries, and more recently as a bioenergy crop, will be targeted. CAM modules will be expressed under the control of circadian clock-controlled, drought-inducible promoters to ensure proper temporal expression of the CAM gene sets and promote maximal productivity. Resulting plants will be tested for transgene expression, biochemical signatures of CAM, $CO_2$ assimilation, stomatal conductance and transpiration rates, leaf carbon balance, level/mode of CAM activity, biomass productivity and quality, and integrated WUE. The effective transfer of CAM photosynthetic machinery into the important bioenergy crop *Populus* could significantly increase WUE for biofuels production in water-limited environments. If successful, the basic design principles outlined here can be extended to increase significantly the WUE of other bioenergy crops. Thus, this research is expected to have broad potential for ensuring sustainable biofuel feedstock production and for expanding production into semi-arid land areas.

**References**

Borland AM, Hartwell J, Weston D, Schlauch KA, Tschaplinski TJ, Tuskan GA, Yang X, Cushman JC. (2014) Engineering crassulacean acid metabolism to improve water-use efficiency. Trends Plant Sci. *In press.*

DePaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. (2014) Synthetic biology as it relates to CAM photosynthesis: Challenges and opportunities. J. Exp. Bot. *In press.*

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