Engineering Tissue Succulence to Improve Water-use Efficiency of Bioenergy Feedstocks

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Project Goals: Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that exploits a temporal CO_2 pump with nocturnal CO_2 uptake to improve the water-use efficiency (WUE) and the adaptability of plants to hotter, drier climates. The long-term goal of the CAM Biodesign project is to introduce CAM into C_3 photosynthesis plants, such as *Arabidopsis* and *Populus,* and thereby enhance WUE and photosynthetic performance. Major project goals include: 1) defining the genetic basis of key CAM modules in both eudicot and monocot CAM species; 2) characterizing the regulation of 'carboxylation', 'decarboxylation', and 'inverse stomatal control' gene modules of CAM using a wide variety of functional genomic approaches including loss-of-function studies and transcriptome profiling in model CAM species; 3) deploying advanced genome engineering technologies to enable transfer of fully functional CAM modules into C_3 species; and 4) analyzing the effects of these transgenic modules on 'stomatal control', CO_2 assimilation and transpiration rates, biomass yield, and WUE in *Arabidopsis* and *Populus*. Successful transfer of CAM into target C3 photosynthesis species might allow expansion of biofuel feedstock product into water-limited, semi-arid or seasonally dry environments.

Introducing the CAM photosynthetic machinery into C_3 plants (CAM biodesign) is expected to confer improved WUE in order to assist in the expansion of crop production into semi-arid regions (1, 2, 3). Tissue succulence is an important plant trait that allows plants to withstand long episodes of drought. Some degree of tissue succulence is typically correlated with the optimal performance of CAM. Thus, for CAM biodesign, tissue succulence engineering in C_3 photosynthesis plants may be a key anatomical attribute for enhancing the efficient operation of engineered CAM in C_3 photosynthesis species. Specifically, increased tissue succulence is expected to afford increased mesophyll cell size in order to increase malate storage capacity in the vacuole and to reduce intercellular air space (IAS) to limit the diffusion of CO_2 out of the leaf during the day for refixation by ribulose 1,5-bisphosphate carboxylase oxygenase (RUBISCO). A method for the genetic engineering of tissue succulence was developed involving the overexpression of a modified basic helix-loop-helix (bHLH) transcription factor from *Vitis vinifera*. The engineered tissue succulence in *Arabidopsis* resulted in up to a 2.2-fold increase plant leaf fresh weight, a 2.4-fold increase in leaf dry weight, root, flower biomass, and seed production relative to controls. The increased cell size does not appear to be associated with an increase in ploidy level of the plants. The increased size of all organs also resulted in up to a 1.6-fold increase in leaf thickness, up to a 1.8-fold increase in leaf succulence, up to a 2.9-fold increase in leaf water amount, and up to a 37% reduction in intracellular air space (IAS). This reduction in IAS is a key feature of the innovation because it limits CO_2 diffusion out of the leaf and is thought to be critical for recapture of photorespiratory CO_2 loss and CO_2 recapture by CAM during the day. Importantly, plants with engineered succulent exhibited up to a 35% increase in seed number per silique, up to a 21% increase in seed area, up to a 38% increase in

100-seed weight, and up to a 2.6-fold increase in overall seed yield per plant. Lastly, the engineered succulent plants displayed greater tolerance to salinity and osmotic stress, and water-deficit stress and greater survival and regrowth following acute water-deficit stress likely due to their ability to retain and store water within their tissues.

References

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