**Title: Engineering enhanced photosynthesis and water use efficiency in Sorghum**

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**Project Goals:** This project aims to leverage *Setaria viridis* as a model system to develop novel technologies and methodologies to redesign the bioenergy feedstock *Sorghum bicolor* to enhance water use and photosynthetic efficiencies.

**Abstract:** The yields of $C_4$ bioenergy crops are increasing through breeding and improved agronomy but the amount of biomass produced for a given amount of water use (water use efficiency) remains unchanged. Therefore, our research focuses on three major control points of water-limited production, with a systems-approach to biodesign: (1) greater photosynthetic carbon assimilation; (2) reduced water use through greater stomatal resistance to water loss; and (3) enhanced acquisition of available water by roots.

Plant cell wall structural polymers (e.g., cellulose, hemicelluloses, and lignins) influence biofuel digestibility as well as $CO_2$ and water movement within the leaf. For example, both $CO_2$ and water conductance are influenced by cell wall thickness and porosity. We will present data testing the hypothesis that changes in the chemical properties of mesophyll and bundle sheath cell walls will influence $CO_2$ and water conductance within the leaf, and therefore photosynthetic and whole plant water use efficiency. We have conducted experiments with grasses genetically modified for cell walls with differences in glucuronoarabinoxylan composition and mixed-linkage glucans. This research will improve our understanding of how cell wall modifications needed for cellulosic biofuel production influence photosynthetic water use efficiency in important $C_4$ crops.

Recent increases in atmospheric [$CO_2$] means that $C_4$ crops increasingly have greater $CO_2$ supply than is needed to saturated photosynthesis. Therefore, reducing stomatal conductance by reducing the number or size of stomata can increase intrinsic water use efficiency without necessarily suffering a trade-off of reduced photosynthetic $CO_2$ fixation. We are testing a series of orthologs of Arabidopsis stomatal developmental genes to determine the best solution for reducing stomatal conductance without unwanted pleiotropic effects that can alter stem or reproductive development. In addition, we have performed transcriptomic profiling of developing leaves to identify the network of genes controlling differentiation of epidermal cells.

Roots represent the supply side of plant-water relations. Root architecture, which is the branching pattern of the root system in soil, determines the efficiency that water and nutrients are accessed, but also represent a cost to the plant in terms of carbon. Previous work by our group has shown that grasses exhibit a suppression of crown root development under drought and a dramatic induction of their growth upon rewatering. Our current work is focused on identifying the genes necessary for these responses and the design of a synthetic biology approach to finely tune root branching. Work that will be presented includes the cloning of a novel locus controlling the initiation of Setaria root development under well-watered conditions and the establishment of a synthetic biology toolkit that enables two-input logic gates to be constructed in plants that create predictable changes in gene expression and modify root architecture.

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