**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.

The high rates of photosynthesis and the carbon concentrating mechanism in $C_4$ plants is initiated by the enzyme phospho*enol*pyruvate carboxylase (PEPC). A decrease in the $K_m$ for $HCO_3^-$ ($K_{HCO3}$) has been proposed as a selective advantage for maintaining high rates of $C_4$ photosynthesis, particularly when carbon substrate availability is low due to restricted stomatal conductance under drought. We will present our work showing significant variability in $K_{HCO3}$ among PEPC enzymes from different $C_4$ grass species. Additionally, our data provides new insight into the structural components responsible for $K_{HCO3}$ that can be used to engineer increased photosynthetic efficiency in $C_4$ plants. This research builds the foundation for engineering a kinetically enhanced PEPC and the next step is using gene editing to enhance the kinetic properties of PEPC in Sorghum.

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 leave 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 characterization 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.

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