PEPC kinetics and the efficiency of C_4 photosynthesis in *Sorghum bicolor*

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Overall 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. Here we specifically focus on Objective #1*: Engineering photosynthesis to improve performance under water stress.*

Abstract: Due to the predicted increase in food demand, studying the biochemical components of C_4 photosynthesis may provide insight into enhancing photosynthesis in crop plants to increase yield. Currently, photosynthesis can be reduced in C_4 crops by drought conditions which reduce intercellular CO_2 concentrations (C_i) in the plant. The initial carboxylation reaction in C_4 plants is catalyzed by phospho*enol*pyruvate carboxylase (PEPC) and leads to elevated CO_2 around Rubisco. The C_4 isozyme of PEPC originated from a non-photosynthetic PEPC and it has been suggested that specific amino acid substitutions in PEPC confer differences in the affinity of the enzyme for PEP (K_{PEP}). These changes in K_{PEP} may be an unavoidable side effect of selecting for a higher affinity for HCO_3^- (K_{HCO3}) to maintain rates of PEPC when stomatal conductance (g_s) is low. However, experimental evidence for amino acid changes influencing *in planta* kinetic properties of PEPC and rates of C_4 photosynthesis is lacking. Therefore, the objective of this aim is to determine how specific amino acid differences between the C_3 and C_4 isozymes of PEPC influence the efficiency of C_4 photosynthesis when the availability of atmospheric CO_2 is low. To accomplish this objective, we are measuring the kinetic properties of 28 PEPC isozymes from both C_3 and C_4 plants from members of the Poaceae family. These enzymes were overexpressed and purified from the PEPC-less *PCR1 Escherichia coli* strain. The kinetic measurements have been compared to determine if there is a tradeoff between K_{PEP} and K_{HCO3}. These PEPC kinetic parameters were measured in a temperature-controlled cuvette linked to a mass spectrometer. The ultimate goal of this research is to introduce an enhanced PEPC enzyme into sorghum to increase photosynthesis under drought conditions. The outcome of this research will enhance C_4 photosynthetic efficiency and will lead to an increase in whole plant water use efficiency.

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