Modification of PEPC kinetics to enhance the efficiency of C_4 photosynthesis

Asaph B. Cousins[1]* (acousins@wsu.edu), Robert J. DiMario[1]*(robert.dimario@wsu.edu), Ryan L. Wessendorf[1], Kuenzang Om[1], and **Ivan Baxter**[2]

[1]Washington State University, Pullman, WA 99163, [2]Donald Danforth Plant Science Center, St. Louis, MO 63132

url: http://foxmillet.org

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 are being overexpressed and purified from the PEPC-less *PCR1 Escherichia coli* strain. The kinetic measurements will be compared to protein alignments to find specific amino acid residues contributing to the variation in PEPC kinetic properties. To test how these specified amino acids influence PEPC kinetics and C_4 photosynthetic efficiency we will use targeted mutagenesis with base editors and targeted gene replacement to modify specific amino acid residues. PEPC kinetics will be conducted in a temperature-controlled cuvette linked to a mass spectrometer, as previously described. The outcome from this research will determine if changes in specific amino acids confer kinetic differences of PEPC affinity for HCO_3^- and enhance C_4 photosynthesis. Ultimately, the goal 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.

Funding statement: This work was supported by the Office of Biological and Environmental Research in the DOE Office of Science (DE-SC0018277).