Modification of PEPC kinetics to enhance the efficiency of C4 photosynthesis

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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 C4 photosynthesis may provide insight into enhancing photosynthesis in crop plants to increase yield. Currently, photosynthesis can be reduced in C4 crops by drought conditions which reduce intercellular CO2 concentrations (Ci) in the plant. The initial carboxylation reaction in C4 plants is catalyzed by phosphoenolpyruvate carboxylase (PEPC) and leads to elevated CO2 around Rubisco. The C4 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 (KPEP). These changes in KPEP may be an unavoidable side effect of selecting for a higher affinity for HCO3- (KHCO3) to maintain rates of PEPC when stomatal conductance (gs) is low. However, experimental evidence for amino acid changes influencing in planta kinetic properties of PEPC and rates of C4 photosynthesis is lacking. Therefore, the objective of this aim is to determine how specific amino acid differences between the C3 and C4 isozymes of PEPC influence the efficiency of C4 photosynthesis when the availability of atmospheric CO2 is low. To accomplish this objective, we are measuring the kinetic properties of 28 PEPC isozymes from both C3 and C4 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 C4 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 HCO3- and enhance C4 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 C4 photosynthetic efficiency and will lead to an increase in whole plant water use efficiency.

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