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⁻ (Kₜ₃) 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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