Investigation of Carbonic Anhydrase to Improve Sorghum C_4 Photosynthetic Efficiency

Joshua Rosnow[1]*(jrosnow@danforthcenter.org), Indrajit Kumar[1], Matthew Zinselmeier[2], Dan Voytas[2], Asaph Cousins[3], Todd Mockler[1], **Ivan Baxter**[1]

[1]Donalad Danforth Plant Science Center, St. Louis, MO

[2] College of Biological Sciences, University of Minnesota, St. Paul, MN

[3] The School of Biological Sciences, Washington State University, Pullman, WA

www.foxmillet.org

Project Goal: 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:

One objective of this project is to optimize the expression of carbonic anhydrase (CA) to increase the residence time of carbon dioxide in the leaf to improve C_4 photosynthesis and drought resilience. Phylogenetic and gene expression analysis of CA across the grasses demonstrates that there are several α, β, γ - CA genes present in all grass genomes that exhibit a conserved expression in leaf tissue regardless of photosynthetic type. We are working to understand the role of alternative splicing of the most abundant leaf expressed β-CA, with the working hypothesis that two transcript isoforms facilitate CA being localized to different sub-cellular locations. On-going experiments include investigation of CA protein targeting and biochemical analysis of the alternatively spliced protein products. Results suggest that translation of the most abundant C_4 CA transcript does not begin in exon 1 as previously annotated. These results provide insights for designing constructs to optimize CA expression in Sorghum. Constructs that are currently being made for Sorghum include a β-CA overexpression construct and a transcriptional activator, dCas9-TV, for all β-CA isoform promoters with 1-2 sgRNAs per isoform. Further research on CA promoter elements that drive elevated and cell-specific gene expression, will help refine strategies for editing CA promoters to improve photosynthesis in Sorghum.

Funding Statement: This project is funded by grant DE-SC0018277 from The DOE Department of Biological and Environmental Research