Synthetic Genetic Circuits to Engineer Water Use Efficiency, Photosynthetic Efficiency, and Biocontainment

Jennifer A. N. Brophy[1], Degao Liu[2], Matt Zinselmeier[2], José R. Dinneny[1], Daniel F. Voytas[2*] (voytas@umn.edu) and **Ivan Baxter**[3]

[1]Stanford University, Palo Alto, CA; [2]University of Minnesota, St. Paul, MN; [3]The Donald Danforth Plant Science Center, St. Louis, MO

www.foxmillet.org

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.

Improving *Sorghum bicolor* as a biofuel crop requires methods to manipulate gene expression in ways that alter important agronomic traits. To achieve precise control over gene expression, we are building synthetic genetic circuits. These circuits enable precise spatial and magnitudinal control over gene expression and offer an exciting means to reprogram plant development and control growth. To build circuits, we've generated libraries of transcriptional regulators composed of bacterial DNA binding proteins or catalytically inactive dCas9 fused to transcriptional activation or repression domains. We tested thirty-six plant transcription factors in Arabidopsis and Setaria protoplasts for their ability to activate both reporters and native plant genes. A subset of the best performing transcriptional activators were introduced as transgenes into Arabidopsis and shown to activate expression of native genes. The best synthetic transcription factors were also used to construct circuits that perform Boolean logic operations. These circuits can regulate spatial patterns of gene expression across root tissues and, as a proof of concept, we used them to selectively modify branching in Arabidopsis roots. In parallel, synthetic transcription factors are being deployed in an innovative strategy for biocontainment of transgenes. We are identifying genes (target genes) that compromise the viability of *Sorghum bicolor* when overexpressed. We plan to introduce mutations into the target gene so that it is no longer recognized by a programmable transcription factor, then we will transform the mutant *Sorghum bicolor* with a synthetic circuit that leads to overexpression of the lethal gene, such that when the plant is outcrossed, its progeny are inviable. Taken together, these data show how synthetic genetic circuits can be built and used to successfully modify important plant traits.

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0018277.