Targeted Mutagenesis and Programmed Transcriptional Regulation in Setaria and Sorghum

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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 edit genes and manipulate gene expression *in vivo*. We are optimizing mutagenesis strategies using CRISPR/Cas and CRISPR/Cpf1 nucleases to achieve targeted gene knockouts, gene replacements and transgene insertions. Further, we are implementing base editor technology to achieve precise sequence changes without the need for a DNA double strand break. To achieve regulated gene expression, we are optimizing the use of programmable transcription factors (activators and repressors) derived from nuclease inactive dCas9 and dCpf1. The programmable transcription factors will be deployed in an innovative strategy for biocontainment of transgenes. To achieve genetic containment, we will identify genes (target genes) that compromise viability of *Sorghum bicolor* when overexpressed by the programmable transcription factors. We plan to introduce mutations into the target gene so that it is no longer recognized by the transcription factors. We will then combine all components of the synthetic circuit needed for genetic containment and test efficacy.

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