Title: The Development of Transgenic Lines and Improved Technologies for the Analysis of Photosynthetic and Water Use Efficiencies in 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.

Abstract text: The development of a genome-level knowledge base linking genes to phenotypes in sorghum for bioenergy goals through the use of genome editing and stable plant transformation technology is critical to understanding fundamental physiological functions and important to crop improvement. We contribute the central hub capability to create, test and cultivate transgenic and genome edited plants with the various laboratories involved with this project. We harness this capability to help meet the Overall Project Goal to develop novel technologies to redesign the bioenergy feedstock *Sorghum bicolor* and to enhance its water use and photosynthetic efficiencies. We have established reliable protocols for the *Agrobacterium*-mediated introduction of experimental genetic constructs into sorghum cv BTx430, and deliver the viable transgenics required for the ongoing investigations with our collaborators on this project. We show the timeline of stable lines of sorghum that have been and are currently being produced to investigate the selected target genes for the analysis of photosynthetic and water use efficiencies. For example, these experiments include:; (1) sorghum RNAi constructs for knockdowns such as for voltage-gated chloride channel proteins, alpha carbonic anhydrase 7 (CA) and nine-cis-epoxycarotenoid dioxygenase 4, and myb domain protein 60; (2) constructs to test the fidelity of phosphoenol pyruvate carboxylase (PEPC) promoter expression, CA overexpression and PEPC with altered kinetics; (3) additional versions of CA overexpression aimed to test a range of increased mesophyll CA activity; (4) Ta Cas 9, dTa Cas9, and, dCas9 transcriptional activator for improved editing, and; (5) constructs to evaluate improvements to the transgenic process with the intent to increase transformation frequencies and shorten the time to T1 seed. These lines are currently in various stages of the transgenic process. The recent developments using morphogenic regulator-mediated

transformation (MRMT) is a breakthrough toward enabling rapid transformation and genome editing. We report the development of an improved transformation method using MMRT technology with the potential to increase through-put and decrease time for our projects. We will be working with the Voytas lab that will allow MMRT to be more broadly utilized within the group. We also report the development of qPCR methods for the quantification of transgene insert copy number which further improves our capabilities for molecular analysis of the transgenic sorghum lines prior to shipment to our collaborators. Our program continues to support the central and essential aspects to provide the transgenic lines to investigate photosynthetic and water use efficiencies in sorghum.

Funding statement: This work was supported by the Office of Biological and Environmental Research in the DOE Office of Science (DE-SC0018277)