**Improvement of Sorghum Transformation and Genome Editing for the Development of Stable Lines for the Analysis of Photosynthetic and Water Use Efficiencies**

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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.

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 have established reliable protocols for the *Agrobacterium*-mediated introduction of experimental genetic constructs into Sorghum cv BTx430, and collaborate to generate the viable transgenics required for the ongoing investigations on this project. 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 rapid transformation method using MMRT technology with the potential to increase through-put and decrease time for our projects. Working with the Voytas lab, we have evaluated a public version of the MRMT vectors. The Voytas lab is also testing novel methods for delivering genome editing reagents, specifically the use of RNA viral vectors to deliver gRNAs through infection. Heritable gene editing through infection has been achieved in several dicot species, and we are working to implement the technology in *Setaria* and sorghum.

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