# The Centrality of the Development of Transgenic Lines 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.

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**Abstract:** 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. A required but often underappreciated technology for this goal involves the capability to create, test and cultivate transgenic and genome edited plants. The need and centrality of transgenic lines in sorghum is technically difficult yet essential to the analysis of traits genes. Standard plant transformation protocols are often limited by various factors including: (1) tissue culture expertise and facility intensive resources; (2) genotype and explant dependence, (3) low efficiencies; and, (4) time and labor intensive efforts. We have established reliable protocols for the standard *Agrobacterium*-mediated introduction for stable genetic constructs into sorghum cv BTx430. Using these protocols, we have established lines of transgenic sorghum to phenotypically study various genes of interest. Stable lines of sorghum 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 have explored current applications using transient expression of *Baby boom* and *Wuschel2* delivered by microprojectile bombardment in sorghum. Our program supports the central and essential aspects to provide the transgenic lines to investigate photosynthetic and water use efficiencies in sorghum.

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