81. A Systems-Level Analysis of Drought and Density Response in the Model C4 Grass *Setaria viridis*

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http://foxmillet.org/

Project Goals: To develop *S. viridis* as a model system to molecularly dissect traits that limit current bioenergy feedstock grasses. This includes developing an informatics toolkit to enable the interrogation of the genome, mapping genes that underlie water use efficiency and density tolerance, developing new gene knockout and replacement technologies and investigating gene flow for biocontainment. A web portal will host transcriptomic and QTL datasets generated over the course of this project as well as metabolic and gene annotation data for the *S. viridis* genome. The tools and datasets will not only serve this project but also provide a resource for the *Setaria* research community to mine and display community datasets.

Abstract text: Bioenergy grasses promise to provide a sustainable source of renewable fuels for the US bioenergy economy. These dedicated second generation bioenergy crops can be grown on marginal lands and with fewer inputs than traditional row crops such as corn which requires energy intensive annual planting and the addition of chemical fertilizers. To engineer bioenergy grasses with the desirable traits needed for large scale production, it will be necessary to develop model systems that are closely related to bioenergy feedstocks, but which are more amenable to genetic analysis. One of the most promising model species is *Setaria viridis*. *S. viridis*, like all major feedstock targets, is a C_4 panicoid grass. However, it is much smaller in stature, flowers within 6 weeks of planting, and can be easily transformed with genes of interest. The objectives of this project are to utilize genomic, computational and engineering tools to begin the genetic dissection of drought and density response in *S. viridis*. The ability of bioenergy feedstocks to utilize water efficiently and to produce abundant yields at high density will be major drivers in the development of improved varieties. Through the use of *S. viridis*, we hope to discover the molecular mechanisms that underlie this response and in doing so identify candidate genes and pathways for improving the closely related feedstock grasses. Importantly, we will also begin field studies to assess the ethical, legal and societal implications (ELSI) in anticipation of broad adoption of these technologies.

The specific aims of the proposal are to: 1) Identify QTL for the effect of drought and density on biomass and seed yield components of *S. viridis*. 2) Conduct in–depth physiological profiles in roots and leaves of a subset of selected lines 3) Integrate datasets and develop metabolic and gene networks for *S. viridis* to prioritize candidate genes for transgenic characterizations 4) Develop transformation technologies for *S. viridis* 5) Functionally examine the role of candidate genes deduced by network models; and 6) Develop protocols and best practices for monitoring gene flow in transgenic *S. viridis*. To achieve these aims we will produce one of the most extensive molecular characterizations of plant growth in the field to date, generating several million data points that will be collected from physiological and molecular genetic studies. We will develop novel informatics models and network tools that will guide future molecular characterization in *S. viridis* and guide breeding efforts in major feedstock targets. The ELSI activity will lay the foundation for future studies and establish *S. viridis* as the model system for bioenergy grass research. Work over the past year of this project has included: 1) Conducted replicated field trials of *S. viridis* x. *S. italica* RIL populations to examine the response of plant growth to water stress and density 2) Developed

a pipeline and performed QTL analysis of several traits examined in field studies 3) Established SetariaCyc, a genome-wide metabolic network of *Setaria italica* (available from the Plant Metabolic Network website) 4) Generated an *S. viridis* genome assembly and established a framework to visualize SNP, QTL and mutant datasets 5) Established a *Setaria viridis* focused web-portal that provides access to the *S. viridis* genome assembly and annotation including a genome browser (JBrowse), and BLAST, FTP functionalities 6) Constructed and transformed several constructs to perform site directed mutagenesis in *S. viridis* 7) Developed resources and protocols for conducting genetic analysis in *S. viridis* in field and laboratory settings 8) Conducted developmental, physiological and QTL studies of water stress responses in the root system.