Seteria viridis as a Model System to Accelerate Gene Discovery in Panicoid Grasses

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Project Goals: This project aims to develop genetic resources and tools in *Setaria viridis* for gene identification and functional characterization. Outcomes from this research include the identification of genes that can be manipulated in target feedstocks for the improvement of agronomic traits including plant architecture, disease/pest resistance and flowering time. We are also developing new genome editing technologies and methods of genetic analysis to accelerate gene discovery and genome engineering efforts.

Setaria viridis is a C_4 panicoid grass with a diploid genome, rapid life cycle, small stature and high seed production that together facilitate genetic analyses. It serves as an ideal model system to accelerate gene discovery in bioenergy feedstocks including maize, sorghum, Miscanthus, switchgrass and sugarcane. Multiple forward genetics, reverse genetics, as well as high throughput phenotyping resources have been generated in *S. viridis*, including a chemically mutagenized population of over 15,000 families and a diverse panel of ~600 wild accessions. To demonstrate the utility of the system, we have conducted several reverse and forward genetic screens. In a forward genetic screen, we identified *SvAUX1* as a regulator of inflorescence branching and gravitropism in *S. viridis*. We identified four single gene recessive sparse panicle phenotypes characterized by reduced and uneven branching of the inflorescence from approximately 2700 M2 families. A bulked segregant analysis was performed to identify the gene underlying the *sparse panicle1* (*spp1*) phenotype, and *spp1* was fine mapped to a ~1 Mb interval. Through complementation tests and deep sequencing of another mutant, *spp3*, a causal gene *SvAUX1* was identified. This gene is one of the two genes in the ~1Mb interval and the only gene disruption shared between *spp1* and *spp3*. We further show that the maize ortholog of *SvAUX1*, *ZmAUX1* plays similar role in inflorescence and root development, highlighting the utility of *S. viridis* in accelerating functional genomic studies in maize. In a second forward genetic screen, we identified a candidate gene (*SvCO-like 1*) that may control flowering time, a key trait for bioenergy feedstocks. This gene was found through bulked segregant analysis in a prolonged flowering mutant family. Further functional characterization of *SvCO-like1* is currently ongoing. Through reverse genetics, we are characterizing the jasmonate (JA) signaling pathway in *S. viridis* that contributes to growth and defense responses that must be optimized for high yielding feedstocks that are resistant to pest pressures. In grasses, there are three genes encoding the jasmonate receptor *CORONATINE INSENSITIVE* (*COI*). Using CRISPR-Cas9

technology, we generated mutants in *COI1b*, and identified three independent alleles that exhibit an early flowering phenotype, similar to what has been described in *Arabidopsis* where only a single *COI* gene is present. Additional screens to identify *COI1a* and *COI2* mutants are underway to dissect *COI* gene function and gain deeper insight into the role of JA in regulating growth and defense responses in *S. viridis*. Taken together, multiple toolsets have been developed in *S. viridis* for rapid gene candidate identification and functional characterization, which will accelerate functional genomics studies in panicoid grasses.