Phenomics of stomata and water use efficiency in C_4 species

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Genetically tractable model systems closely related to bioenergy grasses need to be developed to drive the crop improvement required for large scale, ecologically sustainable bioenergy production. *Setaria viridis* is an ideal candidate C_4 panacoid grass. The over-arching objectives of this large, collaborative project are to utilize genomic, computational and engineering tools to begin the genetic dissection of drought response in *S. viridis*. This will be achieved through: 1) Quantitative trait and association genetics; 2) novel controlled environment and field phenotyping combined with molecular and chemical profiling; 3) development of metabolic and gene networks; 4) development of transformation technologies*;* 5) reverse genetic testing of candidate genes.

Water use efficiency (WUE), which is physiologically distinct from drought tolerance, is a key target for improving crop productivity, resilience and sustainability. This is because water availability is the primary limitation to crop yield globally and irrigation uses the largest fraction of our limited and diminishing freshwater supply. The exchange of water and CO_2 between a leaf and the atmosphere is regulated by the aperture and pattern of stomata. Mechanistic modeling indicates that stomatal conductance could be reduced or stomatal movements accelerated to improve water use efficiency in important C4 crops such maize, sorghum and sugar cane. While molecular genetics has revealed much about the genes regulating stomatal patterning and kinetics in Arabidopsis, knowledge of the genetic and physiological control of WUE by stomatal traits in C4 crops is still poor. Understanding of natural diversity in stomatal traits is limited by the lack of high-throughput phenotyping methods. To this end two novel phenotyping platforms were developed. First, a rapid method to assess stomatal patterning in three model C_4 species grown in the field – maize, sorghum and setaria has been implemented. Here the leaf surface is scanned in less than two minutes with a modified confocal microscope, generating a quantitative measurement of a patch of the leaf surface. An algorithm was designed to automatically detect stomata in 10,000s of these images via a training of a pattern-recognition neural network approach. Second, a thermal imaging capture strategy, to rapidly screen the kinetics of stomatal closure in response to light has been developed. We are gaining insight on the underlying genetics governing stomatal stomatal patterning through quantitative trait loci and genome wide association studies in addition to phenotypic evaluations of sorghum with transgenically modified expression of stomatal patterning genes. These multifaceted approaches are complemented by a recently established field facility for comprehensive evaluation of leaf, root and canopy WUE traits under Midwest growing conditions.

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