

Phenomics of stomata and water use efficiency in C₄ species

Andrew D.B. Leakey^{1*} (leakey@illinois.edu), John Ferguson¹, Nathan Miller², Jiayang Xie¹, Charles Pignou¹, Gorka Erice¹, Timothy Wertin¹, Nicole Choquette¹, Maximilian Feldman³, Funda Ogut⁴, Parthiban Prakash¹, Peter Schmuker¹, Anna Dmitrievna¹, Dylan Allen¹, Elizabeth A. Ainsworth^{5,1}, **Ivan Baxter**³, Thomas P. Brutnell³, Edward S. Buckler^{6,7}, Patrick J. Brown¹, Thomas E. Clemente⁸, Stephen P. Long¹, Lauren M. McIntyre⁴, Edgar Spalding²

¹University of Illinois at Urbana-Champaign, Urbana, IL; ²University of Wisconsin, Madison, WI; ³Donald Danforth Plant Science Center, St Louis, MO; ⁴University of Florida, Gainesville, FL; ⁵ USDA Agricultural Research Service, Urbana, IL; ⁶USDA Agricultural Research Service, Ithica, NY; ⁷Cornell University, Ithica, NY; ⁸University of Nebraska, Lincoln, NE

<http://foxmillet.org/>

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 C₄ panacoid grass. The overarching 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 freshwater supply. The exchange of water and CO₂ 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 as sorghum and sugarcane. 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. Two novel phenotyping platforms were developed. First, a rapid method to assess stomatal patterning in three model C₄ species grown in the field – maize, sorghum and *setaria* has been implemented. The leaf surface is scanned in less than two minutes with an optical tomographer, 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 training of a neural network approach. Second, a thermal imaging strategy, to rapidly screen the kinetics of stomatal closure in response to light has been developed. We identified genotype to phenotype associations for stomatal patterning, leaf gas exchange and canopy water use through quantitative trait loci and genome wide association studies. Transgenically modified expression of stomatal patterning genes has produced sorghum with greater WUE. These plants

were grown in a new field facility for comprehensive evaluation of leaf, root and canopy WUE traits under Midwest growing conditions in summer of 2018.

This research was funded through Subaward No. 23009-UI, CFDA # 81.049 between University of Illinois and Donald Danforth Plant Science Center Under Prime Agreement No. DE-SC0008769 from Department of Energy.