

## Phenomics of stomata and water use efficiency in C<sub>4</sub> species

Andrew D.B. Leakey<sup>1\*</sup> (leakey@illinois.edu), John Ferguson<sup>1</sup>, Jiayang Xie<sup>1</sup>, Charles Pignon<sup>1</sup>, Niteen Kadam<sup>1</sup>, Ashish Rajukar<sup>1</sup>, Dustin-Mayfield Jones<sup>1</sup>, Maximilian Feldman<sup>2</sup>, Parthiban Prakash<sup>1</sup>, Darshi Banan<sup>1</sup>, Rachel Paul<sup>1</sup>, Scott Lee<sup>2</sup>, Greg Ziegler<sup>2</sup>, **Ivan Baxter**<sup>2</sup>, Thomas E. Clemente<sup>3</sup>

<sup>1</sup>University of Illinois at Urbana-Champaign, Urbana, IL; <sup>2</sup>Donald Danforth Plant Science Center, St Louis, MO; <sup>3</sup>University of Nebraska, Lincoln, NE

### 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.

url: [www.foxmillet.org](http://www.foxmillet.org)

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 CO<sub>2</sub> 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 C<sub>4</sub> crops. 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 C<sub>4</sub> crops is still poor. Understanding of natural diversity in stomatal traits is limited by the lack of high-throughput phenotyping methods. In response, a rapid method to assess stomatal patterning in model C<sub>4</sub> species grown in the field 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. We identified trait correlations as well as 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. Plants were grown in a new field facility for comprehensive evaluation of leaf, root and canopy WUE traits under Midwest growing conditions.

**Funding statement:** This work was supported by the Office of Biological and Environmental Research in the DOE Office of Science (DE-SC0008769).