Title: Advanced Phenomic Approaches to Improving Water-Use Efficiency in Bioenergy Grasses

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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. Through the advancement of phenotyping platforms we are developing a quantitative and integrative understanding of how multiple shoot and root traits vary genetically and in response to water-deficit.

Abstract Text: Crop yields are frequently limited by the availability of water during the growing season; however, efforts to improve the acquisition and efficient use of water by crops have been challenged by the difficulty of rapidly phenotyping traits that control these processes.We have established a comprehensive portfolio of tools, methodologies and phenotyping platforms that are enabling new levels of insight into root, photosynthetic and stomatal traits. The resulting knowledge of associations between genotype, phenotype and environment are informing our efforts to engineer greater water use efficiency.

We have leveraged automated imaging, weighing and watering to monitor biomass, water use and other growth parameters over time. We have combined these approaches with biochemical profiling (transcriptomic, metabolomic, elements and isotopic ratios) and pan-genome variation analyses to get an in depth understanding of how plants respond to water deficit.

Advances in phenotyping leaf morphological and physiological traits important to water use efficiency and drought stress of field-grown sorghum and setaria have been achieved by leveraging a suite of imaging technologies. Stomatal patterning on the leaf epidermis has been phenotyped by optical tomography combined with an AI-enabled automated analysis pipeline. Leaf specific leaf area and nitrogen content have been phenotyped by leaf hyperspectral reflectance. Leaf rolling in response to drought and plant productivity has been phenotyped by hemispherical photography below the canopy. Canopy water use and the rate of stomatal closure has been phenotyped by thermal imaging at two different scales. In each case, (1) the fidelity of the rapid phenotyping method has been demonstrated against traditional gold-standard methods and (2) the resulting trait data has been applied to understanding the genetic architecture of the traits in setaria and /or sorghum. GWAS coupled with analysis of pan-genomic sequence

variation in putative candidate genes has revealed new sets of candidate genes and loci as targets for engineering enhanced water use efficiency and drought response in C4 bioenergy crops. It is also revealing how interactions among traits create opportunities and constraints for crop improvement.

The characterization of root system architecture has been an obsession for plant physiologists and developmental biologists. Recent work across several labs have established a wide range of different methods that bring with them strengths and weaknesses related to throughput, physiological relevance, resolution, and amenable species. The GLO-Roots method has been a successful entree to this portfolio due to the non-sterile soil-like media that is used to grow the root systems, the span of the plant life cycle that can be explored, and the ease of applying this approach to the model genetic systems such as Setaria, Brachypodium and Arabidopsis. Bottlenecks in the GLO-Roots imaging pipeline have been addressed recently, and as a consequence, we have been able to generate data of a magnitude that is several fold greater than was envisioned before. In LaRue and Lindner et al. we present the GLO-Bot I robotics platform, which enabled the first characterization of species-level differences in root system architecture in a soil-like growth environment beyond the seedling stage. We established a platform for automated plant root growth, imaging and image analysis that involved innovations in plant growth systems, robotics and computer vision approaches. This integrated solution to root phenotyping provides a stunning model for future studies and will influence the field of plant phenomics. We identify the major contributors to variation in the spatiotemporal control of root system architecture. New traits we define, such as average lateral root angle per day, show significant correlations to climate variables and suggest potential adaptive advantages for plants having shallow root systems when temperature variation is high in a location. Through continued funding from DOE-BER we have established the GLO-Bot II system, which is constructed with industry-standard robotics components and promises a new level of reliability and throughput compared to its predecessor.

References/Publications

1. LaRue T, Lindner H, Srinivas A, Exposito-Alonso M, Lobet G, Dinneny JR (2021) Uncovering natural variation in root system architecture and growth dynamics using a robotics-assisted phenomics platform. bioRxiv preprint doi: https://doi.org/10.1101/2021.11.13.468476

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