Switchgrass Field Experiments to Enable Rapid Domestication, Increased Sustainability, and a Genome-wide Association Study

Reginald J. Millwood,[1,2*] (rmillwood@utk.edu), Mitra Mazarei,[1,2] C. Neal Stewart Jr.,[1,2] and **Gerald A. Tuskan**[2]

[1]University of Tennessee Institute of Agriculture, Knoxville; The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee

https://cbi.ornl.gov

Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Biomass production is the single most important factor impacting economic sustainability of biofuels and bioproducts. A diverse panel of 500 switchgrass accessions is planned to be planted in Knoxville in spring, 2018. This panel will consist largely of clonal replicates of lowland (tetraploid) switchgrass accessions provided by Dr. Thomas Juenger. The experimental common garden study in Knoxville, Tennessee will consist of six replicates of each switchgrass accession, using a randomized complete block experimental design, which includes a nitrogen fertility treatment.

For total biomass, the aerial parts of each genotype will be harvested in late fall, dried, and weighed. Tissue from selected lines will be used to assess lignin valorization potential. To dissect yield into its genetic components, we will record flowering time (50% of the first panicle emerged), plant height at flowering and at maturity, total biomass, leaf-to-stem ratio, lodging, and timing of spring regrowth using a combination of manual and automated phenotyping. Automated plant architecture measures will be acquired through the use of an unmanned aerial vehicle (UAV) using LIDAR-based methods.

The end-of-season shoot-to-root nitrogen will be measured to determine the nitrogen remobilization, which is a critical trait for long-term NUE. The nitrogen status of plants will be determined via elemental analysis at two growth stages: mid-season when plants are mature but still green and end-of-season when plants are senesced. We will employ UAV-based multispectral sensors to estimate the level of nitrogen in leaves and stems. Five spectral bands, including near-infrared, and various predictive algorithms will be used to estimate nitrogen presence and use. These predictive algorithms will be “ground-proofed” against lab-based plant nitrogen analyses. Nitrogen uptake, utilization, and use efficiencies of each genotype will be determined.

We will also evaluate disease severity with focus on rust caused by fungal *Puccinia emaculata*, which is the most prevalent pathogen of switchgrass in the southern US. The plants will be examined for rust severity at different time points. The coverage of leaf surface with rust uredia will be visually assessed using the following scale: 0=0%, 1≤5%, 2≤10%, 3≤25%, 4≤40%, 5≤55%, 6≤70%, and 7≤100% of leaf area coverage with uredia. Ultimately, these data should also be collected by remote sensing using the UAV. All data will be incorporated in the GWAS analysis to determine the loci associated with rust resistance, which can be integrated into the genomic selection models.

Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.