

Genome-wide association studies of drought tolerance and water use efficiency related traits in switchgrass

Yongqin Wang^{*1,2,3} (yqwang@uga.edu), Hari B. Chhetri³, Kyle Sullivan³, David Kainer³, Larry M. York^{2,3}, Malay C. Saha^{2,3}, Michael K. Udvardi^{2,3}, Daniel Jacobson³, Yun Kang^{2,3}, Ali Missaoui^{1,3} and **Gerald A. Tuskan³**

¹University of Georgia, Athens, Georgia; ²Noble Research Institute, LLC, Ardmore, OK; ³Center for Bioenergy Innovation, Oak Ridge National Laboratory, TN.

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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 addresses 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 biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

Switchgrass (*Panicum virgatum* L.) is a promising feedstock for biofuels in the United States. As for most crops, periodic drought often limits its productivity, especially in marginal lands where it is likely to be planted. Improving drought tolerance and water use efficiency (WUE) in crops through breeding and biotechnology strategies is essential to meet global food and fuel demands and to achieve sustainable agriculture. In this CBI project, we aim to evaluate drought tolerance and WUE related physiological and biochemical traits of a switchgrass genome-wide association study (GWAS) population (Juenger et al, 2016), and to understand mechanisms and identify key genes underlying drought tolerance and WUE in switchgrass through GWAS.

Phenotyping experiments were conducted under greenhouse conditions using a switchgrass GWAS panel of 299 genotypes with five biological replicates (two tillers per replicate) each for drought-stressed and well-watered treatments per genotype. Three weeks after tiller transplanting, drought stress was applied by withholding watering, and drought stress levels were monitored with a soil moisture sensor. When the soil volumetric water content dropped to 5% (or at the wilting point), which usually occurred in two to four weeks after withholding water, phenotypic data on related traits were collected. For well-watered plants, phenotypic data were collected eight weeks after tiller transplanting. Traits characterized included leaf area, specific leaf weight, stomatal density, leaf cuticular wax, leaf osmotic pressure, shoot and root biomass/ratio, water use efficiency, and root traits. Large genotypic variations were observed in all the traits characterized. GWAS analyses have been performed using the standard GCTA MLMA model, and multiple significant marker associations were identified. Top candidate genes have been evaluated by networks analysis using Random Walk with Restart/Lines of Evidence on Multiplex Graphs (Wen

et al, 2021), and will be further evaluated by PCR, RT-PCR, and functional genomics tools such as CRISPR, stable transformation or virus-mediated gene silencing or overexpression.

References/Publications

1. Juenger TE, Schmutz J, Wolke T, Fritschi F, Zare A, Bartley L, et al. Climate adaptation and sustainability in switchgrass: Exploring plant-microbe-soil interactions across continental scale environmental gradients. IN: Genomic Science Contractors–Grantees Meeting XIV and USDA-DOE Plant Feedstock Genomics for Bioenergy Meeting. 2016.
2. Wen Y, Song X, Yan B, Yang X, Wu L, Leng D, et al. Multi-dimensional data integration algorithm based on random walk with restart. BMC Bioinformatics. 2021, 22: 97.

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