Project Title: "The Genetics of Biofuel Traits in Panicum Grasses: Developing a Model System with Diploid Panicum Hallii"

SC#: SC0008451

Below we provide a detailed description of our activities and outcomes on various projects related to our DOE feedstock genomics grant. Our grant supports 6 interrelated projects, and as such I provide a short summary of each below.

Project 1

This project involves the establishment of common gardens of P. virgatum and P. hallii to explore the standing quantitative genetic variation in both species for growth architecture and related phenotypes. We have accumulated a large sampling of clonal material to date and have established garden sites at the Brackenridge Field labs in Austin Texas (Figure 1). The existing garden includes a number of well studies agronomic cultivars (Alamo, Kanlow, VS16) as well as many new collection made by the Juenger lab. In addition, we have requested additional germplasm from USDA GRIN and plan to incorporate a more global sampling as we receive this material. In addition, we have requested clonal cuttings of Panicum virgatum genotypes that are being sequenced by the JGI community sequencing program - ideally, our common garden will act as a backup of this valuable material and possibly allow studies associating measured phenotypes with sequenced variants in the future. We have extensively phenotyped the garden and it has formed the core of a new panel being developed for genomewide association studies. One of the most striking discoveries from these experiments was the detection of patterns of leaf traits related to fast/slow leaf economics spectrum and associations with growing season lengths at sites of origin.

In the context of Panicum hallii, we have collect material from over 130 locations across the southwestern US and have over 400 inbred accessions for study. This material has been resequenced and genotyped using ddRAD methods and has formed the core of a graduate dissertation project by Juan Diego Palacio. Juan and other students in the Juenger lab have focused on studies of freezing tolerance, leaf characteristics, stress physiology, and seed biology in the panel.

Figure 1. BFL common garden rows – each spaced plant corresponds to a clonal replicate of a study genotype of Panicum virgatum.

Project 2

This project involves developing a new 4-way genetic mapping population for Panicum virgatum that will segregate both upland and lowland traits. We have made great progress on this project - to date, we have generated 800 F2 progeny derived from reciprocal crosses of F1 parents. F1 parents were derived from 4 grandparental lines including Alamo AP13 (lowland, JGI sequencing reference), Summer/VS16 (upland), WBC (lowland), and Dakotah (upland). All of these genotypes are currently being resequenced by DOE JGI. We have collected basic growth and morphological measurements from these progeny, have genotyped individuals, have created a dense linkage map, and initiated QTL mapping studies. This primary effort has been published in the Genes-Genomes-Genetics.

Milano, E., Lowry, D. and T. Juenger. 2016. The genetic basis of upland/lowland ecotype divergence in switchgrass (Panicum virgatum). Genes-Genomes-Genetics 6: 3561.

This mapping population has been clonally propagated and from a core set of mapping material for collaborative common gardens. The population is currently planted at 11 locations spanning the North American latitudinal gradient.

Project 3

This project involves the continuation of QTL mapping efforts with Panicum hallii and the development of a new recombinant inbred mapping population. We have made extensive use of crosses between two subspecies (filipes and hallii) to develop tools for better evaluating upland/lowland divergence in C4 perennial grasses. We have developed a set of 300 F2 plants that were genotyped using ddRAD methodology and studies in greenhouse and field conditions. This work has been published in the New Phytologist.

Lowry, D., Hernandez, K., Taylor, S., Meyer, E., Logan, T., Barry, K. Chapman, J., Rokhsar, D., Schmutz, J. and T. Juenger. 2015. The genetics of divergence and reproductive isolation between ecotypes of Panicum hallii. New Phytologist 205. 402-414.

In addition, we have expanded this population with an additional 500 progeny and genotyped these using large indel markers. The genome referenced indel markers provide a nice community resource for cheap and rapid genotyping, and the additional progeny will increase mapping resolution. Plants from this population have been studies for life history characteristics, flowering time, growth architecture, seed traits, and tissue characterization.

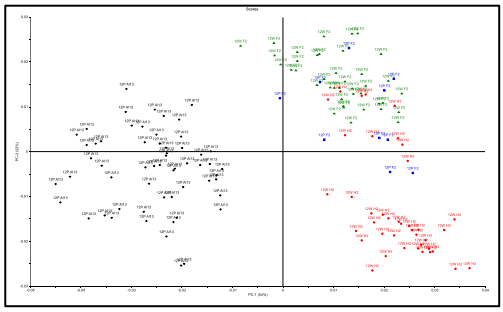
We have further bred our filipes x hallii derived population to an F7 recombinant inbred population. This population has been genotyped utilizing shallow whole genome resequencing and studied in greenhouse and field conditions. This population has substantially improved mapping resolution and power for understanding the genetic architecture of key grass traits. A number of manuscripts are in preparation.

Project 4

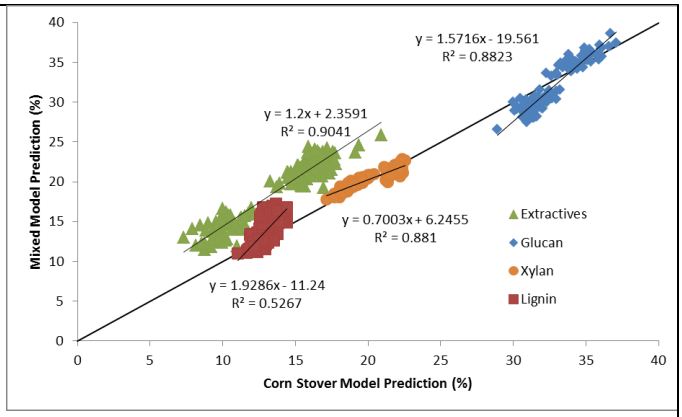
An important thrust of the funded work has been to develop near-infrared spectroscopy methods for characterizing tissue in Panicum grasses. This work is in close collaboration with Ed Wolfrum at NREL. Our strategy has been to screen a large sampling of P. hallii tissues for NIRs and to pick outlier samples for wet lab chemistry. These samples will be used to generate calibration models that link quick and cheap NIRs with accurate estimation of lignin, cellulose, and sugars from sampled tissues. We have completed our NIRs run and wet chemistry efforts and have built robust calibration models. We have screened material from several Panicum hallii genetic mapping populations and have generated a manuscript (in review) detailing these results. This effort formed an important chapter in dissertation research by Liz Milano, a graduate student in the Juenger lab.

Milano, E., Payne, C., Wolfrum, E., Lovell, J., Jenkins, J., Schmutz, J. and T. Juenger. 2016.. Quantitative Trait Loci For Cell Wall Composition Traits Using Near-Infrared Spectroscopy in The Model C4 Perennial Grass *Panicum hallii.* In review

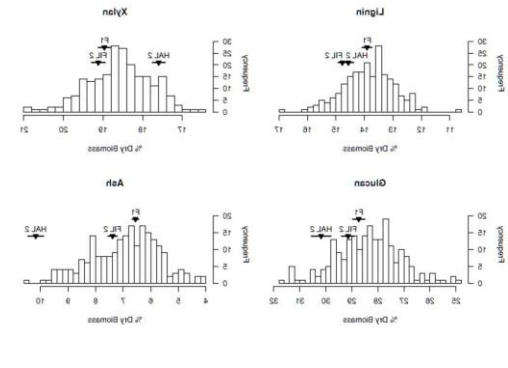
An exciting discovery from this effort is the occurrence of "pleiotropic" QTL controlling aspect of tissue characterization. We have initiated additional studies to fine-map these loci.

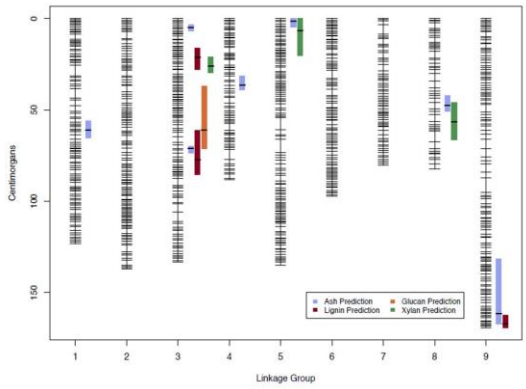


PCA plots of NIRs data on P. hallii test samples separating genotypes and growth conditions based on tissue characteristics



Comparisons of existing calibration models (mixed forage and corn stover) in predicting P. hallii tissue composition





QTL mapping results of tissue quality metrics

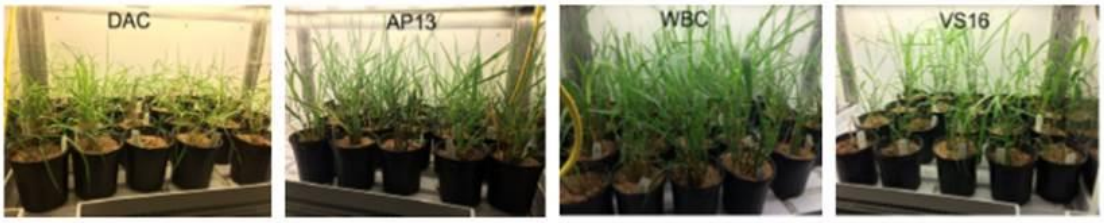
Project 5

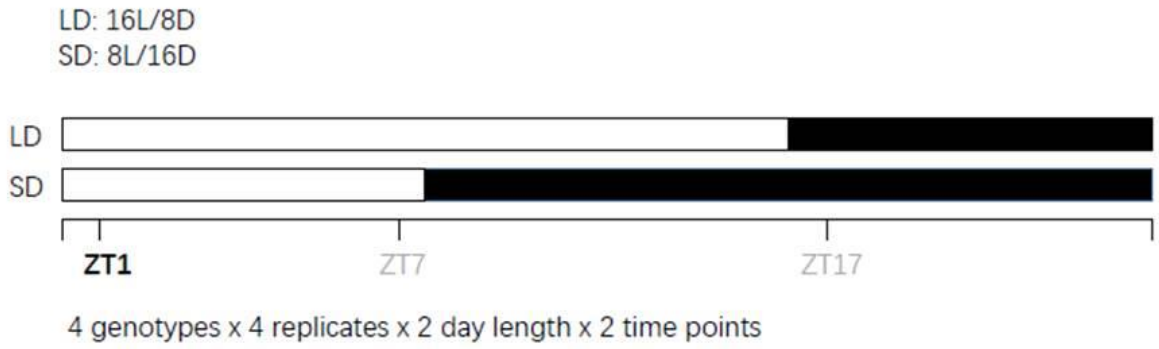
A key component of the project is to study the plasticity of growth architecture of P. virgatum and P. hallii grown under differing nitrogen and harvest environments. This experiment will be conducted in the field using large cylinders (4 ft x 32 inch diameter). This project will focused on clonal replicates of the parents of the 4-way cross (AP13, VS16, WBC, Dahkota) as well as single seed decent lines of the two core P. hallii genotypes (FIL2 and HAL2). The design plan is 4 genotypes x 4 treatments x 6 biological replicates= 96 cylinders for P. virgatum and 2 x 4 x 6 = 48 cylinders for P. hallii. We have collected data from several field seasons related to the experimental plants, including analyses involving the transcriptome responses to the imposed treatments. In addition, we added a component studying belowground rooting characteristics and an evaluation of the microbial communities associated with these plantings. These data are currently being analyzed and manuscripts are targeted for submission in 2017.

Cylinders for nitrogen and harvest studies

Existing P. virgatum clonal replicates (AP13 genotype) for drought response studies

In addition, we have utilized new growth chambers for more detailed physiological studies centered on switchgrass responses to light quality and photoperiod. Here, we have utilized the 4-way mapping population parents and potted plants growth under 16 and 8-hr day length. These studies have better characterized the circadian and photoperiod responses of flowering time and growth regulators in switchgrass.





Project 6

An important component of our project is to facilitate the development of Panicum hallii as a model genomic system for perennial grasses. To this end, we continue to develop protocols, share materials, and facilitate ongoing sequencing efforts in collaboration with the DOE Joint Genome Institute. These projects include sequencing of genomic DNA for genome assembly, total RNA-sequencing for transcriptome development, genotyping-by-sequencing of genetic mapping projects, resequencing of natural accessions, and RNA-sequencing to quantify gene expression in a natural populations. In addition, we plan to use our linkage mapping information to confirm and improve sequence assembly efforts. In addition to our DOE feedstocks funding, these efforts are currently supported by a DOE Community Sequencing Program (CSP) support.

The assembly of the P. hallii genome is going well – we have helped create and release 2 versions of the P. hallii genome with collaborators at DOE JGI

https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Phallii

At UT, we have developed bioinformatics pipelines to genotype individuals using 2bRAD and ddRAD genotyping methods and to quantify gene expression using 3 prime TAG sequencing protocols. We continue to improve the technical procedures and bioinformatics pipelines to improve the quality and speed of analysis.

We have shared P. hallii seeds from several accessions with many researchers – to date, we have sent materials to Zeng-Yu Wang (Noble Foundation), James Schnable (Danforth Center, Brutnell lab), Zack Nimchuk (Cal Poly, Meyerwitz lab), Geoff Morris (University of South Carolina, Kresovich lab), and Neil Stewart (UT Knoxville, Bioenergy Center) and are in the process of submitting many accessions to USDA GRIN.