"Breeding Resilient, Disease-Resistant Switchgrass Cultivars for Marginal Lands"

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Project Goals: Our primary goals are to accelerate the development of superior switchgrass cultivars and to expand the range of switchgrass cultivation in the Northeast. We are building on the cultivars, populations, and field trials resulting from previous projects by expanding progeny trials and creating higher levels of associations from genomics information. To this end we are addressing the following specific objectives: 1) Expanding the selection and testing of superior, disease-resistant switchgrass cultivars for marginal environments in the Northeast; 2) Mapping QTL for anthracnose resistance, Bipolaris resistance, and yield; 3) Identifying associations of SNPs and candidate genes with anthracnose and Bipolaris disease ratings; and 4) Identifying genome-wide and metagenome-wide variations associated with Genotype-by-Environment interactions affecting yield characters and disease susceptibility in switchgrass.

Abstract:

Switchgrass is a fast growing, perennial, warm-season grass, native to North America with great potential for development as a bioenergy crop. In the humid Northeast, fungal diseases are prevalent, and these can reduce the yield and quality of harvests. We are building upon previous research, populations and genomics tools to accelerate the development of superior, disease-resistant, climate-resilient switchgrass (*Panicum virgatum* L.) cultivars for expanding the range of biomass cultivation in the Northeast. The project is specifically focusing on improvement of resistance to anthracnose (caused by *Colletotrichum navitas*), *Bipolaris* leaf spot (caused by *Bipolaris oryzae*), and environmental stress-resistance. In this poster, the results of the metagenomics and phenotypic data collections and analyses in year 1 will be presented.

In year 1 (December 2018 – November 2019), progress was made on all project objectives. This included seeding new plots of advanced experimental populations and standard check cultivars at all 3 test locations, developing a uniform set of methods for phenotyping and conducting disease ratings across the project, and identifying superior breeding lines for growth and disease tolerance from mature cultivar nurseries at Cornell University and Rutgers University. New progeny trials were established with 5,760 seedlings of progeny from 180 advanced breeding lines selected in the USDA NEWBio project. These progeny trials will be evaluated for disease resistance and growth phenotypes in years 2 and 3. The [Lu et al. 2013] association panel was well established at all 3 trial sites by the start of the project, and growth was abundant at the field sites during the 2019 growing season. The Cornell, Rutgers, and PSU groups completed data collection for plant growth (height and circumference in cm), plant vigor (rated from 1 to 5), and

anthracnose severity (rated from 1 to 5) from replicates for all 552 genotypes in the panel. Bipolaris infections were not observed in 2019. Values for plant volume were computed as a proxy for biomass yield. Heritability estimates for each trait in 2019 were calculated from the phenotypic data. All replicates at the 3 trials will be scored for disease ratings and growth traits again in project year 2 (the 2020 growing season). We observed substantial, normally-distributed quantitative phenotypic variation between the sites and among genotypes for all of the traits studied. A trend in site-productivity of Rutgers>Cornell> PSU was observed for height, circumference and volume. Anthracnose incidence was severe at all 3 sites. Furthermore, heritability levels were high for all of the traits at all 3 trial sites. The results in year 1 indicate strong genetic and site effects on variation in traits that should allow GWAS and GxE analyses to uncover genes and alleles important in biomass productivity and disease-resistance.

A third year of phenotypic data was collected from Rutgers University's QTL mapping family, including scores for anthracnose severity and plant vigor. The 3 years of phenotypic data will next be used to construct a genetic linkage map and to identify QTL. Most of the plants were too severely infected in the 2019 growing season to permit high quality and contamination-free DNA to be purified from field-collected samples. Thus, after disease ratings were taken, the mapping population was cut back and all 240 plants were brought into the greenhouse for re-growth under controlled conditions for the collection of fresh tissues for DNA isolations.

In pre-award work, triplicate soil and root samples were collected from all of the association population plants within 2 days after the clonal "plugs" of each genotype were collected in triplicate from the switchgrass provenance trial common garden site at Cornell University near Ithaca, NY [Lu et al. 2013]. DNA was isolated from all rhizosphere soil samples. The 382 samples with highest DNA quality and representing all wild population groups [Lu et al. 2013] were selected for the initial "pre-transplant" metagenome sequencing. Amplicons for the 16S rRNA gene and ITS loci were generated, and separate NGS libraries produced, for each of the 382 samples. From the pooled libraries, app. 60Gb of sequence was produced on an Illumina HiSeq 2000. The pre-transplant metagenome data revealed an established rhizosphere microbial community with a rich composition of at least 493 bacterial genera and 57 fungal genera. This provides the composition of rhizosphere microbiomes representing past interactions between the switchgrass genotypes and both aboveground and belowground environmental conditions at the original nursery at Cornell, representing the common starting point of our project. From the 382 samples at the transplant stage, we selected 128 association panel genotypes in which to conduct detailed monitoring of changes in rhizosphere microbiome composition that may occur as the association population becomes re-established in the 3 different field trial sites in PA, NJ, and NY. The 128 genotypes were selected to maximize the range of switchgrass source populations represented. In July 2019, triplicate rhizosphere soil samples were collected from all 3 replicates of the 128 core set of genotypes at all 3 sites, to assess changes in the microbiomes that may have occurred during the establishment of the genotypes at the 3 trial sites. DNA isolations, amplifications, and sequencing will be completed early in year 2 (2020).

References cited:

Lu, F., Lipka, A.E., Glaubitz, J., Elshire, R., Cherney, J.H., Casler, M.D., Buckler, E.S. and Costich, D.E., 2013. Switchgrass genomic diversity, ploidy, and evolution: novel insights from a network-based SNP discovery protocol. *PLoS genetics*, *9*(1), p.e1003215.

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