

“Breeding Resilient, Disease-Resistant Switchgrass Cultivars for Marginal Lands”

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Project Goals: This project is expanding on previous projects for the development of disease-resistant switchgrass cultivars for cultivation in the northeastern US, while expanding knowledge of underlying genotype-by-environment interactions. This involves: 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 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 existing research, populations, and genomics tools from previous projects 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 focuses on improvement of resistance to anthracnose (caused by *Colletotrichum navitas*), *Bipolaris* leaf spot (caused by *Bipolaris oryzae*), and environmental stress. In addition to cultivar development, we strive to better understand the sources of genetic and environmental variation affecting yield and disease susceptibility in switchgrass, including soil metagenome variation. To study Genotype-by-Environment interactions in-depth, we have established both cultivar yield trials and a GWAS family at 3 agronomically distinct field trial sites in NY, PA, and NJ.

Expanded selection and testing of disease-resistant switchgrass cultivars: A *seeded yield trial established at the NY, NJ, and PA field trial sites* in year 1, consisting of 13 cultivar selections replicated 4 times in randomized block design, was evaluated for disease incidence and biomass yield. Little anthracnose was observed, with exception of the NJ site. Vigor of plants at the mine reclamation site in PA was poor compared to the NY and NJ sites. A subset of plants from each site were prepared for evaluation of biomass composition by NIR. The *mature nursery at Ithaca NY*, consisting of 178 half-sibling progeny from two populations (one upland ecotype and one lowland ecotype) of switchgrass were evaluated for disease incidence. Leaf damage from unseasonably early frost in September made a second disease evaluation impractical. *Large progeny trials of 5,760 seedlings* from 180 advanced breeding lines, established in year 1 at the Rutgers and Ithaca field trial sites, were scored for vigor and disease incidence. Results of phenotypic data in years 1 and 2, including comparisons of years and sites, will be presented.

Three years of phenotypic data collection was obtained from the *QTL mapping population* of 240 full-sib plants at Rutgers. Overall, the mapping family exhibited a relatively normal distribution

of anthracnose disease severity, with a skew towards more disease susceptibility within the population. The family was transferred from the field site to the greenhouse over winter to provide healthy leaves for DNA isolation. After delays due to the pandemic, ddRADseq sequencing of the samples is underway. The data will soon be available for map construction and identification of QTL for disease-resistance.

Association mapping panel: A second year of phenotypic data (height, circumference, vigor, and anthracnose severity ratings) was collected from all 552 genotypes of the [Lu et al. 2013] association panel planted in 3 replicates at the NY, NJ, and PA trial sites in 2018. Bipolaris infections were not observed in 2019, however smut infections were wide-spread and thus severity ratings were also collected at all 3 trials sites. In general, growth, volume and vigor values were lower at the PA mine reclamation site relative to the NJ and NY field sites, although several genotypes did perform well there. Normal distributions of data were generally observed, with similar means in both 2019 and 2020. Anthracnose infection levels in the GWAS family were found to be negatively correlated with growth across the 3 test sites, as expected.

GWAS analyses were conducted to identify SNPs associated with anthracnose disease ratings and growth. A preliminary GWAS analysis for SNP-trait associations conducted with Tassel v5.0 software (Mixed Linear Model, using GBS genotypes (Lu et al 2013) and minimum SNP presence of 25%) was conducted for volume and anthracnose disease ratings collected in year 1 from all 540 association panel genotypes, replicates, and field sites in NY, NJ and PA. At a significance threshold of 5×10^{-8} , 6 SNP markers were associated with anthracnose disease ratings, and 2 SNP markers were associated with calculated plant volumes. Several additional SNP markers just below the threshold are also being investigated. After year 2 growth phenotypes and disease ratings were collected, new GWAS studies were conducted to compare significance of associations among years and sites (Method = FDR, $MAF \geq 0.05$, $nSNPs = 67,721$) using the statgenGWAS package in R and exome-capture genotypes (Evans et al., 2017). For severity of anthracnose infection, 92 SNPs were found to be significant above the fixed threshold of $LOD = 4$. Genes harboring significant SNPs included those related to known plant immunity and stress response gene families; such as NAC TranscriptionFactor-Like 9, Quinolinate Synthase, and GDP-D-mannose 3', 5'-epimerase (GME). Additionally, genes including those belonging to the Divergent CCT motif (relating to flowering time) and NAALAD (relating to plant cell wall regulation) were found to be significant and represented by a high LOD score (>4.5).

Rhizosphere microbiome composition: A further component of the assessment of genotype-by-environment interactions involves analysis of the rhizosphere microbiome composition and if the microbiome changed after transfer of the GWAS family to the 3 trial sites. The first step was characterization of the rhizosphere microbiome at the initial common garden site of the GWAS family at Cornell. Metagenomic sequence data (16S and ITS amplicons) from 382 rhizosphere soil samples revealed a rich rhizosphere microbial community of at least 493 bacterial genera and 57 fungal genera, distributed across 8 major phyla. Alpha diversity analyses indicated an influence of host switchgrass genotypes on rhizosphere bacterial diversity, and overall that rhizosphere diversity differed along three modes of switchgrass stratification - ploidy, ecotype, and populations. To assess microbiome composition after transplant and establishment at the 3 trial sites, soil core samples were taken for 128 selected switchgrass genotypes at all 3 trial sites

(Ithaca NY, Freehold NJ, and Philipsburg PA). Bacterial 16S amplicon libraries were generated from each sample. However, sequencing was delayed by covid pandemic issues. Sequencing and metagenomic analyses should be completed early in project year 3, barring further interruptions.

Reference cited:

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