Final Technical Report

Unraveling the Genetics of Two Key Biomass Traits that Differentiate Upland and Lowland Tetraploid Switchgrass Ecotypes, Colonization by Mycorrhizal Fungi and Frost Tolerance

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Project Title: Unraveling the Genetics of Two Key Biomass Traits that Differentiate Upland and Lowland Tetraploid Switchgrass Ecotypes, Colonization by Mycorrhizal Fungi and Frost Tolerance

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Summary:
The overall objectives of the proposal were to dissect the genetics of the ability of switchgrass to interact with arbuscular mycorrhizal fungi (AMF) and to tolerate subzero temperatures, and to investigate the effect of AMF colonization on freezing tolerance and biomass production. In order to achieve this, we generated genetic maps in an F_2 mapping population derived from a cross between the lowland genotype AP13 and the upland genotype VS16. In addition to providing a framework for identification of quantitative trait loci (QTL), the genetic maps were used to validate and improve the switchgrass genome assembly. We mapped phenotypic quantitative trait loci (QTL) for AMF colonization and cold tolerance in the F_2 population. We demonstrated that identification of QTL for AMF colonization was achieved only if AMF genus-specific colonization levels were used as phenotypes. No QTL were obtained when the overall level of AMF colonization was used as a trait. We conducted RNASeq on a subset of the F_2 progeny following cold acclimation, and developed statistical methods to conduct expression QTL (eQTL) analyses in a polyploid organism. A preliminary analysis of the results identified eQTL for a range of genes that were differentially regulated in the lowland AP13 compared to the upland VS16 under cold acclimation. Once sequencing of the transcriptomes of a temporal replicate of the mapping population that was cold-acclimated using the same conditions has been completed, a final eQTL analysis will be conducted.

Achievements:
Objective 1: To genetically map QTL for AMF colonization, frost tolerance and biomass production

1.1 Mapping population development and genetic mapping

Two F_1 sib lines, PV458 and PV317, from a cross between the lowland genotype AP13 and the upland genotype VS16 that differ in their level of AMF colonization (PV458: low AMF levels; PV317: high AMF levels) were crossed to yield Pop1. Two other F_1 sibs, PV346 (low AMF) and PV304 (high AMF) were crossed to generate Pop2 as a backup population. A total of 475 progeny from Pop1 were genotyped using a modified genotyping-by-sequencing (GBS) protocol (Qi et al. 2018). Several thousand robust SNPs were obtained at a sequence depth of at least 8X that could be scored in at least 80% of the progeny. The SNPs were used for the construction of AH maps (consisting of markers that were homozygous in the female parent and heterozygous in the male parent), HA maps (markers heterozygous in the female parent and homozygous in the male

parent) and HH maps (markers heterozygous in both parents). The summary statistics for the Pop1 maps are given in Table 1.

Three replications of Pop1 and one copy of Pop2 were planted in 2015 at the University of Georgia (UGA)'s Iron Horse Farm in a complete randomized design. Because this was the first year after UGA transitioned from the old Plant Sciences Farm to the new Iron Horse Farm, and irrigation was not yet in place, transplant mortality for Pop1 was very high. The majority of the Pop2 plants (330 progeny) survived because only a single replicate was planted and hence the transplanted plants had higher tiller numbers. Therefore, Pop1 was abandoned, and research was switched to Pop2. In Summer 2016, the Pop2 plants were split to provide material for an additional three replicates. One copy of Pop2 progeny was also maintained in the UGA Plant Biology greenhouse. The 330 F_2 progeny from Pop2 were genotyped using GBS and the generated SNP markers were used for genetic map construction as described for Pop1. The summary statistics for the Pop2 maps are given in Table 1. The genetic maps generated for Pop1 and Pop2 were incorporated as part of a larger manuscript that was recently submitted on the identification of a candidate gene cluster that underlies a QTL for leaf wax (Qi et al. 2020).

Table 1: Summary statistics for the Pop1 and Pop2 genetic maps

Pop1						Pop2					
HH map		AH map		HA map		HH map		AH map		HA map	
No. of markers	Map Length	No. of markers	Map Length	No. of markers	Map Length	No. of markers	Map Length	No. of markers	Map Length	No. of markers	Map Length
6028	1381.8	1185	895.3	1088	1047.7	5154	1453.8	1192	969.3	1135	1008.3

Both the Pop1 and Pop2 maps were used to improve the switchgrass AP13 reference genome assembly from v4 to v5.1. To assign switchgrass linkage groups to the switchgrass K and N subgenomes, we obtained seed from the switchgrass relative, *Panicum rudgei*, from collaborators in Costa Rica. *Panicum rudgei* had been shown by Triplett and colleagues (Triplett et al. 2012) to be more closely related to one of the switchgrass subgenomes (A, now called K) than to the other (B, now called N). We subsequently confirmed this using switchgrass subgenome-specific primers that amplified orthologous loci in *P. rudgei*. The *P. rudgei* DNA for the preliminary experiments was provided by E. Kellogg (Danforth Center). While it took more than two years to (1) obtain the *P. rudgei* seed and (2) successfully germinate the seed, we ultimately obtained two *P. rudgei* plants. DNA from those plants as well as the original DNA sample provided by E. Kellogg was sent to the Joint Genome Institute. A total of 509,837,736 reads were generated (paired-end 150 bp) for a total of 77.5 Gb of DNA. Sequence information confirmed the identity of the two plants as *P. rudgei*. *P. rudgei* sequence reads were aligned to the *P. virgatum* genome assembly by JGI, and were key in assigning the switchgrass chromosomes to the correct subgenome (Lovell et al. 2020).

1.2. Genetic mapping of AMF colonization levels and freezing tolerance

Introduction

Switchgrass is an obligate outcrossing species, and genetically identical plants are typically obtained by splitting the plants into individual tillers. However, because we

needed AMF-free plants for our research, we aimed to propagate plants through nodal culture. We spent a significant amount of time testing and optimizing different protocols. While we used nodal propagation in experiments with a limited number of plants, this approach was ultimately not pursued for propagation of the entire mapping population because the ability to generate nodal shoots proved to be genotype dependent and segregated in the Pop2 mapping population. We therefore decided to generate replicate genotypes by splitting and, because AMF were already present in the roots of the split plants, to only map cold tolerance in the presence of AMF. This decision was further driven by the fact that (1) the presence of AMF had little effect under our test conditions on the overall cold tolerance of switchgrass accessions (see next section) and (2) we were limited by the number of tillers available for each plant and opted for increased replication for a single experiment. In order to increase the throughput of the freezing experiments, initial testing of the effect of different parameters (presence of AMF, cold acclimation, day length during acclimation) on an accession's tolerance to artificial freezing conditions was done on seedlings grown from commercially obtained seed of the tetraploid switchgrass cultivars Alamo (lowland), Kanlow (lowland), Summer (upland) and Dacotah (upland).

Generating AMF inoculum

We generated whole inoculum of three AMF species in a replicated, semi-closed culture pot system in the UGA Plant Biology greenhouse. We constructed pots from 12.7 cm diameter, 35 cm long polyvinyl chloride (PVC) piping, with elevated mesh bottoms, filled them with a 2:2:1 autoclaved sand:turface:vermiculite mix, and inoculated the soil with 25 grams of a single species of whole dried AMF inoculum, with 7 to 10 replicates per species. Initial AMF cultures were obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, for *Paraglomus occultum* (IA702) and *Rhizophagus irregularis* (FL208), and from Richard Lankau, University of Wisconsin, Madison, for *Funneliformis mosseae*. We planted ~10 seeds of the sorghum inbred line BTx623 in each culture pot and covered the pots with clear polypropylene bags with micro perforations to allow gas exchange but decrease air-borne spore contamination. Individual pots were watered every other day and fertilized with ½X Hoagland solution every 14 days using a gravity-fed irrigation system. Inoculated sorghum were grown for 4-5 months, then watering was slowed and finally ceased to promote AMF sporulation and plant senescence. We harvested and discarded aboveground biomass, and collected all dried belowground biomass and soil in multiple paper bags. These were stored at 4 ^{0}C until use. For inoculations of experiments, cultures were homogenized and roots cut into ~1 cm sections. This served as our species specific "whole inoculum". For our AMF experiments, equal masses of *Paraglomus occultum, Rhizophagus irregularis,* and *Funneliformis mosseae* were mixed and used as inoculum.

Preliminary freezing tolerance experiments

We conducted eight freezing tolerance experiments using the two upland and two lowland accessions, manipulating day length and temperature acclimation, freezing temperature, and presence of arbuscular mycorrhizal fungi. These experiments were designed to set a baseline for ecotypes and identify efficient and, to the extent possible, realistic acclimation conditions that likely upregulate gene expression and physiological processes to induce over-wintering survival.

Surface sterilized seeds from the switchgrass accessions Dacotah, Summer, Alamo and Kanlow were germinated in the lab in petri dishes, individually transferred to single 18 cm cone-tainers, and allowed to grow to at least the V3 stage (Moore et al. 1991) in either a greenhouse or growth chamber (depending on the trial). Plants were then exposed to a 7-10 day acclimation period, with varying combinations of photoperiod (12 or 16 hr light), and temperature (4 ^{0}C or 24 ^{0}C) depending on the experiment. Initial acclimation exposure was done in Conviron growth chambers; later runs were done in a cold room with Aurora LED light bars with 100% blue, 100% white and 50% red light wavelengths. Immediately prior to freezing tests, plants were removed from cone-tainers, plant tissue was trimmed 30 cm above and 5 cm below the crown, and placed within sealed plastic bags with a damp 3 cm paper filter to maintain moisture. The bags were spaced on racks within an ESPEC Platinum test chamber. The temperature of the chamber was reduced by 2 ^{0}C per hour until the target temperature was reached. After 1 hour at the target temperature, 5-8 plants were removed from the test chamber and returned to 4 ^{0}C for 4 to 6 hours. Multiple temperatures were tested within a single experiment, holding the temperature for 1 hour at each level and removing a subset of plants before dropping the temperature to the next target level. In initial experiments, freezing tolerance was tested at 0, -2, -4, -6, -8, -10, and -12 ^{0}C. In later trials, freezing tests were conducted only at -2 and -8 ^{0}C. After acclimation to 4 ^{0}C, plant crowns were replanted and returned to the growth chamber or greenhouse. Regrowth was monitored over the next 6 to 8 weeks. A failure to regrow after this period was considered mortality due to cold stress.

In general, we found that we could differentiate upland and lowland ecotypes by their tolerance to freezing temperature exposure. Upland ecotypes (Dacotah, Summer) had high survivorship after exposure to freezing temperatures above -9 ^{0}C, while lowland ecotype survivorship suffered significantly below -6 ^{0}C (Figure 1). As such, we settled on -8 ^{0}C as a threshold to differentiate the two switchgrass ecotypes, and -2 ^{0}C as a control treatment. However, we did find variation to this pattern, generally driven by poor upland performance across treatments during winter months in the greenhouse, despite providing the plants with supplemental lighting. Later experiments were conducted in growth chambers or initial growth was done under natural long days in the greenhouse.

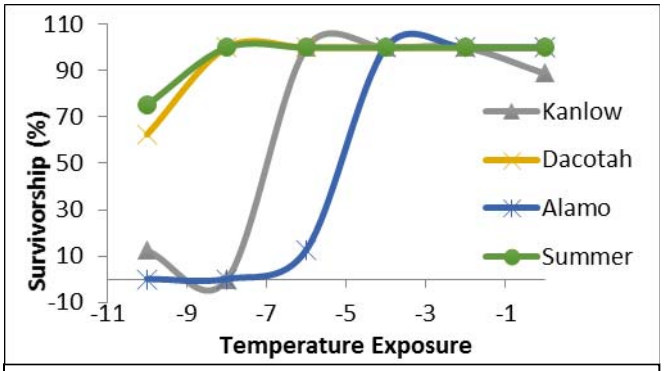

Figure 1. Ecotype variation in survivorship upon exposure to different levels of freezing stress in test chambers following 7 days of 4 ^{0}C acclimation under long days (16 hrs light). Dacotah and Summer accessions are upland ecotypes; Kanlow and Alamo accessions are lowland.

We also tested acclimation under varying combinations of day length and temperature. Although results varied by trial, overall, acclimation temperature significantly affected post-freezing survival. For example, lowland Alamo and upland Dacotah plants both had high survivorship when exposed to -2 ^{0}C, regardless of acclimation, supporting the use of -2 ^{0}C as a control treatment. However, upland Dacotah plants had significantly higher survivorship following 10 days of acclimation at 4 ^{0}C compared to 24 ^{0}C in a -8 ^{0}C freezing trial (Figure 2). The lowland ecotype had low

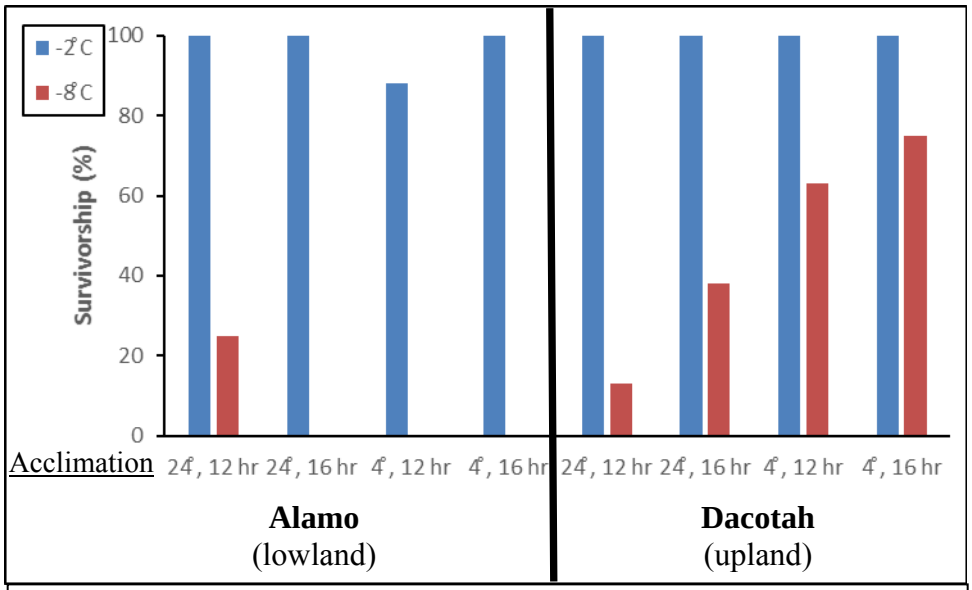

Figure 2. Survivorship of Alamo (lowland) and Dacotah (upland) accessions following 10 day of acclimation at different temperatures and photoperiods (X-axis) followed by freezing at -2 ^{0}C or -8 ^{0}C in test chambers.

survival in all -8 ^{0}C treatments, irrespective of the acclimation treatment. In this and other experiments, day length and mycorrhizal status had minor and inconsistent effects on survivorship. Based on the preliminary experiments, we decided on subjecting plants, grown in the presence of AMF, to 7-10 day acclimation at 4 ^{0}C under a 12-hour photoperiod before subjecting them to a temperature of -8 ^{0}C to phenotype the Pop2 F_2 mapping population for cold tolerance.

Phenotyping the F_2 mapping population for freezing tolerance

A subset (153 genotypes) of the Pop2 F_2 mapping population was used to identify genomic regions associated with freezing tolerance. Five tillers from each of the 153 F_2 genotypes as well as the parents PV304 and PV346, and the grandparents AP13 and VS16, were obtained from the field, trimmed, and inoculated with equal whole cultures of *Funneliformis mosseae, Paraglomus occultum,* and *Rhizophagus irregularis*. These plants were grown in the UGA Plant Biology greenhouses for 12 weeks. Emerging panicles were removed to delay senescence. As described in the previous section, plants were then exposed to a 7-day acclimation period in a 4 ^{0}C cold room with a 12 hour photoperiod provided by Aurora LED light bars with 100% blue, 100% white and 50% red light wavelengths. Immediately prior to the freezing tests, beginning 1.5 hours before the lights were due to shut off in the acclimation room, the most recent leaf from each

replicate plant of an F_2 genotype was harvested and immediately frozen in liquid nitrogen. The remaining plant tissue was trimmed 30 cm above and 5 cm below the crown. Crowns, placed in sealed plastic bags with a damp 3 cm paper filter, were spaced on racks within an ESPEC Platinum test chamber. The test chamber's temperature was reduced by 2 ^{0}C/hour until reaching -8 ^{0}C. After 1 hour at -8 ^{0}C, the chamber's temperature was increased by 2 ^{0}C/hour until it reached 4 ^{0}C, which was maintained overnight. Plant crowns were replanted, returned to the greenhouse and monitored for regrowth over the next 6 to 8 weeks. This experiment was run in 2018 and repeated in 2019.

Our freezing test resulted in significant mortality of the F_2 progeny: 65% and 73% of all individuals failed to regrow in 2018 and 2019, respectively. Indeed, 64% and 60% of all F_2 genotypes had 0 or only 1 tiller survive our freezing trial. However, 20% of our genotypes had greater than 50% survival and 5% of our genotypes had high survivorship across tests. Using composite interval QTL mapping and 500 LOD threshold permutations, we found a significant peak for freezing tolerance using the arcsine square root transformation of the frequency of surviving tillers for each genotype as phenotype at ~40 cM on chromosome 4N (Figure 3).

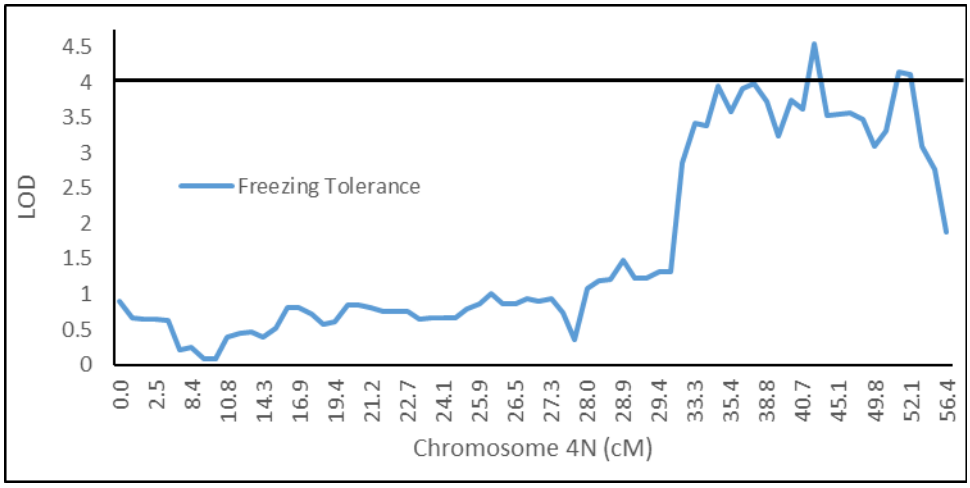

Figure 3. QTL for freezing tolerance after exposing the AP13 x VS16 F_2 mapping population to -8 ^{0}C. The LOD threshold at a significance level of 0.05 is 4.0.

Field testing of the F_2 mapping population for cold tolerance in Wisconsin

In early June of 2017, we made three replicates of a subset of 292 F_2 progeny and established them in the UGA Plant Biology greenhouses. We provided these clones to Dr. Michael Casler (UW-Madison, USDA ARS), who planted the F_2 population at the Marshfield, Wisconsin field site, which had demonstrated strong over-winter selection (Casler and Vogel, 2014). Replicates were planted in three blocks, and were monitored for overwintering survival, number of tillers, and height in the summer of 2018.

Eliminating plants that died before the winter, likely from transplant shock, overwintering survival was high, with 75% of all genotypes having at least two clones survive. From the resulting QTL analyses, we found significant overlapping peaks for

overwintering survival and June height on chromosome 2K (Figure 4), explaining 10% and 6.5% of the variation, respectively. This suggests that plants with higher cold tolerance had better growth potential the following season. There are a large number of possible gene candidates on chromosome 2K, including CBF, COR and CAS15a genes that appear to be upregulated with cold and drought exposure.

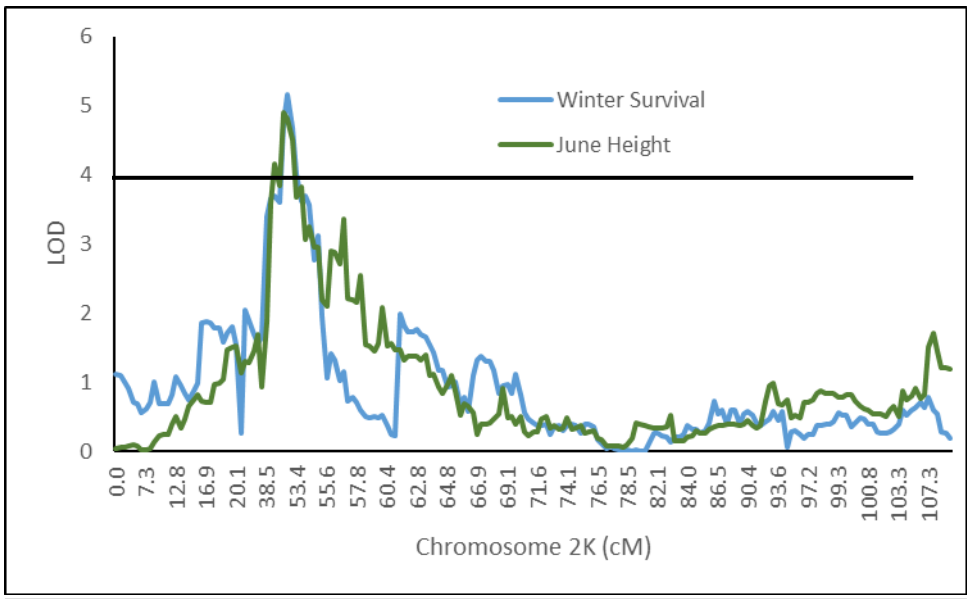

Figure 4. QTL for overwintering survival and June height in AP13 x VS16 F_2 mapping population grown in northern Wisconsin. Significance LOD thresholds are 3.9 and 4.1, respectively.

AMF quantification in the roots of the Pop2 switchgrass F_2 population

While we had previously used semi-quantitative PCR (semi-qPCR) using AMF-specific primers combined with gel-based quantification of amplicons using the switchgrass galacturonosyltransferase 3 (GAUT3) gene as reference to determine levels of mycorrhizal colonization in roots, we decided to switch to next-generation sequencing of amplicons so that we could map levels of individual AMF species in the F_2 population. To assess AMF diversity in roots of the Pop2 F_2 mapping population, roots were harvested from the field in May. A crowbar was used to dig at the base of the switchgrass plant to loosen the soil, and the plants were gently pulled up to expose the roots up to a length of 10 inches below the crown. Five gram of harvested roots were placed in 50 ml centrifuge tubes, stored in ice, and taken to the lab where they were rinsed gently with sterile water to remove the adhering rhizosphere soil. The washed roots were quickly frozen in liquid nitrogen. DNA was extracted according to the protocol of Stoffel et al. (2012) modified to suit medium-throughput DNA extraction. The DNA was quantified and stored at -20 ^{0}C until further use. 100 ng of root tissue DNA was used for PCR amplification.

Amplicons were generated using the 28S rRNA-based FLR3 and FLR4 primers (Gollotte et al. 2004), which amplified diverse mycorrhizal genera both from switchgrass root samples and commercial mycorrhizal formulations. In addition, this primer set

allowed us to capture a high degree of interspecific diversity within the AMF genera (Figure 5). The primers did not amplify host plant ribosomal RNA sequences. GAUT3 primers, which were specific for the switchgrass genome, were used to generate reference amplicons. Amplicon libraries were prepared from each root DNA sample according to Chaluvadi and Bennetzen (2018) and Bahulikar et al. (2020). The AMF and GAUT primers were tailed, allowing the incorporation of Illumina indices and Illumina adaptor sequences during a second round of PCR in which 5 µl of the first-round PCR product was used as template. PCR products were purified with Sera-Mag magnetic beads (GE Life Sciences, USA). After Picogreen quantification, 10 µl of each FLR amplicon library and 2 µl of each GAUT amplicon library were pooled for a total of 192 samples (96 FLR amplicons and 96 GAUT amplicons per pool) and subjected to paired-end 250 bp sequencing on an Illumina MiSeq platform at the Georgia Genomics and Bioinformatics Core (GGBC), Athens, GA.

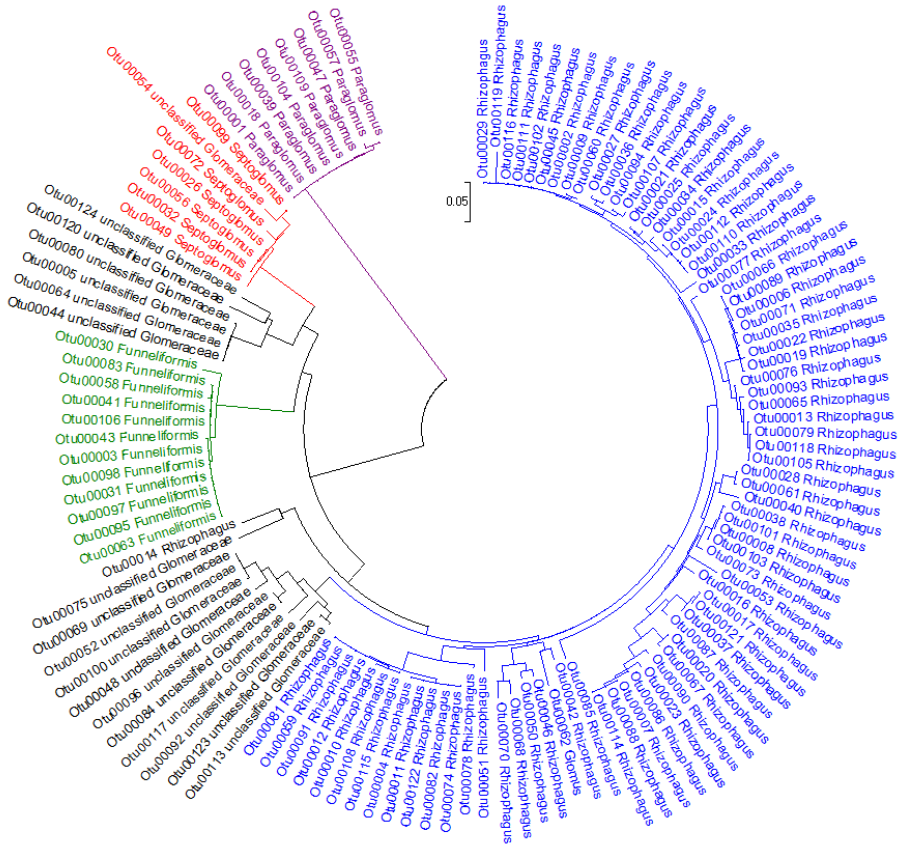

Figure 5: AMF diversity in the roots of Pop2 F_2 progeny as assessed by Illumina sequence analysis of the 28S rRNA gene. Two sequences were considered to belong to the same operational taxonomic unit (OTU) if their similarity was ≥ 97%.

MiSeq data were analyzed using the MOTHUR MiSeq SOP (https://mothur.org/wiki/miseq_sop/). Briefly, sequences were trimmed, clustered and classified in MOTHUR (Schloss et al. 2009) using the parameters '*minimum length =200*

bp; maximum length = 500 bp; average quality score = 25; nucleotide mismatches in the primer sequence = 0; maximum number of Ns = 0'. The trimmed 28S and GAUT reads were further cleaned using UCHIME in MOTHUR. The 28S sequences were combined across samples and aligned in MOTHUR using the 28S sequences of the Ribosomal Dataset Project (RDP) database (http://rdp.cme.msu.edu/misc/resources.jsp) as template. The MOTHUR program was also used for preclustering, rarefaction analysis, distance calculations, operational taxonomic unit (OTU) clustering, and further analyses based on OTUs.

For the QTL mapping, metagenomic analyses were performed on 105 F_2 progeny. Amplicons were generated using the 28S ribosomal DNA primers (FLR3/FLR4) and, after addition of barcoded sequencing primers, were sequenced on an Illumina MiSeq platform (Paired-end 250 bp). The ribosomal DNA sequences were grouped into specific mycorrhizal OTUs by clustering them at ≥97% homology. For the purpose of QTL mapping, the OTUs were further grouped into AMF genera using a BLASTN analysis against a curated fungal 28S RDP database. The AMF OTU count from each sample was normalized against the GAUT3 count from that sample, and the normalized read counts obtained for each AMF genus were used as phenotypes for QTL analysis. Significant QTL were obtained for *Funneliformis, Septoglomus, Paraglomus, Claroideoglomus* and unclassified *Glomeraceae* (Figure 6). The QTL explained between 8% and 23% of the

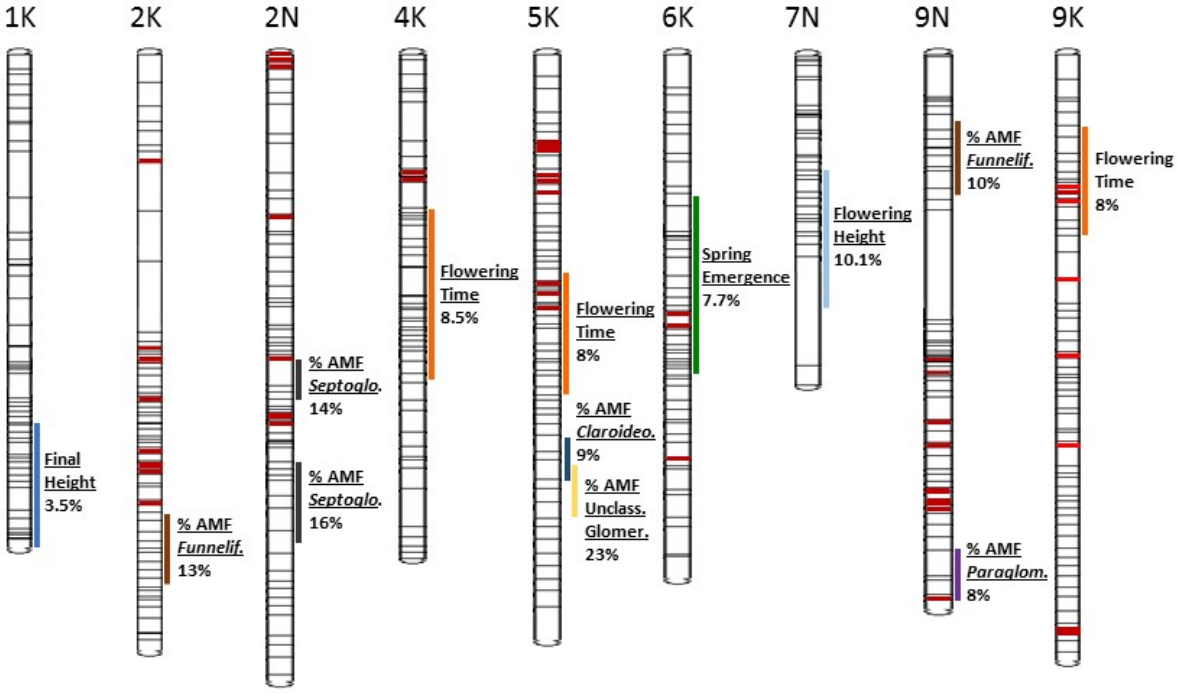

Figure 6: Chromosome location of AMF and a few other QTL on the switchgrass genetic map. All chromosomes with identified AMF QTL are depicted.

genetic variation for genus-specific AMF colonization level. Interestingly, no QTL were obtained for *Rhizophagus*. The genus *Rhizophagus* was the most diverse (Figure 5) and it is possible that the colonization potential differs for the different clades of *Rhizophagus* OTUs. Indeed, while we found QTL for the other genera, combining all data into a

single AMF phenotype did not yield any QTL. We are therefore planning the repeat the QTL analysis for individual OTU clades identified in the phylogenetic tree (Figure 5). These results are from a single season; amplicon sequencing of mycorrhizal 28S genes from F_2 root samples harvested during a second season is underway.

2. Objective 2: To map switchgrass gene expression differences associated with the analyzed phenotypes

RNASeq: Leaves from replicate plants were pooled, ground and used for RNA extraction using the Trizol method for a total of 133 (replicate 1) and 100 (replicate 2) progeny. Genomic DNA was removed using the Turbo DNA-free kit (Invitrogen) and the resulting RNA was used to generate barcoded libraries using the KAPA stranded mRNA-Seq kit (Roche). Following quality control, pools of ~24 libraries were sequenced on an Illumina NextSeq Platform (paired-end 75 bp) at UGA's GGBC facility. We currently have sequencing data in hand for ~130 progeny for replicate 1 and ~100 progeny for replicate 2. We are currently in the process of resequencing ~30 libraries that have less than ten million reads per sample as a single pool.

A preliminary analysis has been conducted for the replicate 1 data. This analysis will be repeated once sequencing for all samples has been completed (at least 10 million reads/sample). Sequence reads were trimmed using TrimGalore v0.45 (https://github.com/FelixKrueger/TrimGalore) to remove adaptors and low-quality sequences (phred <33). The trimmed reads were aligned against the switchgrass reference genome assembly v5.1 with HISAT2 version 2.1.0 (Kim et al. 2015). The alignments were converted to .bam format with SAMtools (Li et al. 2009), assembled into transcripts based on the switchgrass reference genome annotation v5.1 and quantified using StringTie (Pertea et al. 2016). The quantified coverage of each transcript was converted to Fragments Per Kilobase per Million reads (FPKM) using Ballgown (Pertea et al. 2016) for eQTL analyses.

eQTL analyses: In a preliminary analysis, eQTL (gene–SNP marker pairs) were identified using the high-density Pop2 linkage map (4028 SNP markers) and the FPKM values of each transcript across the 133 F_2 progenies through MatrixeQTL (Shabalin et al., 2012) and Composite Interval Mapping (CIM) in R/qtl (Broman et al., 2003). The p-value threshold of the eQTL analysis through MatrixeQTL was set at 0.05. The eQTL identified at a p-value <0.05 were filtered manually using the threshold False Discovery Rate (FDR) of 0.05. The eQTL identified by CIM in R/qtl were manually filtered to have a Logarithm of Odds (LOD) score ≥ 4. eQTL were defined as '*cis*' and '*trans*' eQTL if the distance between a marker and the gene from which the transcript was derived was smaller or greater than 1 Mb, respectively. The eQTL analysis will be repeated, integrating the eQTL approaches that were developed by Co-PI Schliekelman (see section below). Nevertheless, the preliminary analyses of replicate 1 identified a large number of eQTL, including several hundred that were highly upregulated during cold acclimation with the expression in the upland VS16 genotype being at least five-fold higher than the expression in the lowland AP13 genotype. Examples are eQTL for the gene ERD2 (endoplasmic reticulum lumen protein-retaining receptor) which plays a role in misfolded protein quality control, for a gene annotated as chaperone ClpD1, also involved in protein quality control, for pyruvate decarboxylase, a key enzyme in ethanol fermentation under hypoxia, and a gene with homology to momilactone A synthase.

Momilactone A is an allopathic phytoallexin that has recently been shown to be associated with drought tolerance. GO-term analysis of the genes with *cis* eQTL that had ≥2-fold higher transcript levels in the cold-tolerant upland VS16 compared to the cold-sensitive lowland AP13 showed significant enrichment for the terms 'histone H3-K3 methylation' (32-fold enrichment at a false discovery rate (FDR) of 0.03 and 'protein quality control' (16-fold enrichment; FDR=0.03). The top two enriched GO terms for genes that had had ≥2-fold higher transcript levels in AP13 compared to VS16 were 'hexose metabolic process' (8-fold enrichment, FDR=0.0002) and 'monosaccharide metabolic process (8-fold enriched, FDR=0.00001).

Development of statistical methods for eQTL analyses: We developed statistical methodology for eQTL mapping in polyploid organisms using RNASeq data. Polyploids present special challenges for eQTL mapping because genes exist in multiple copies and thus it can be uncertain which copy of a gene a read comes from. Prior to our work, there were no methods that accounted for this uncertainty and thus we developed a pipeline for conducting eQTL mapping in polyploids. We combined simulations with data to test several methods of dealing with reads of ambiguous genomic origin. We concluded that the strategy that best balances false positive and false rates in eQTL identification is to discard reads of ambiguous origin and thus only use reads that contain a SNP between homoeologous genes. We determined the required sequencing depth and explored various other statistical issues related to polyploid eQTL mapping. This work was published this year (Fan et al. 2020).

Deliverables

Publications

Fan KH, Devos KM, Schliekelman P (2020) Strategies for eQTL mapping in allopolyploid organisms. Theor Appl Genet 133:2477-2497

Lovell JT, MacQueen AH, Mamidi S, Bonnette J, Jenkins J, Napier JD, Sreedasyam A, Healey A, Session A, Shu S, Barry K, Bonos S, Boston L, Daum C, Deshpande S, Ewing A, Grabowski PP, Haque T, Harrison M, Jiang J, Kudrna D, Lipzen A, Pendergast THI, Plott C, Qi P, Saski CA, Shakirov EV, Sims D, Sharma M, Sharma R, Stewart A, Singan VR, Tang Y, Thibivillier S, Webber J, Weng X, Williams M, Wu GA, Yoshinaga Y, Zane M, Zhang L, Zhang J, Behrman KD, Boe AR, Fay PA, Fritschi FB, Jastrow JD, Lloyd-Reilley J, Martínez-Reyna JM, Matamala R, Mitchell RB, Rouquette FMJ, Ronald P, Saha M, Tobias CM, Udvardi M, Wing R, Wu Y, Bartley LE, Casler M, Devos KM, Lowry DB, Rokhsar DS, Grimwood J, Juenger TE, Schmutz J (2020) Polyploidy and genomic introgressions facilitate climate adaptation and biomass yield in switchgrass. Nature (Accepted)

Qi P, Pendergast TH, Johnson A, Bahri BA, Choi S, Missaoui A, Devos KM (2020) Quantitative trait locus mapping combined with variant and transcriptomme analyses identifies a cluster of gene candidates underlying the variation in leaf wax between upland and lowland switchgrass ecotypes. Theor Appl Genet (submitted)

Oral/Poster Presentations:

Devos KM, Chaluvadi S, Qi P, Schwoyer C, Young J, Missaoui A, Lindstrom O, Schliekelman P, Bennetzen JL (2015) Improving cold tolerance in lowland switchgrass. Plant Feedstocks PI/PD meeting, Tysons Corner, VA, February 2015 (Poster).

Johnson Q, Qi P, Chaluvadi S, Schwoyer C, Pendergast IV TH, Missaoui A, Lindstrom O, Bennetzen JL, Devos KM (2015) Understanding the genetic relationship between cold-tolerance and colonization by mycorrhizal fungi in order to improve the cultivation range of switchgrass. The Plant Center Retreat, Helen, GA, October 2015 (Poster).

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Bahri B, Pendergast TH, Choi S, Johnson AC, Qi P, Missaoui A, Devos KM (2018) Plasticity of QTL across years in a perennial F_2 population using a GBS-SNP based linkage map. The Plant Center Retreat, Helen, GA, September 2018 (Poster)

Community Resources Generated:

- Contributed to the switchgrass genome sequence assembly
- Methodology for eQTL mapping in polyploid species

Other products/ outcomes:

- Genetic maps (publication submitted)
- QTL for cold tolerance and AMF (not yet published)
- RNASeq data (will be released upon publication)

Training

Post-docs:

Peng Qi: GBS analyses, genetic mapping

Thomas Pendergast: AMF culturing and analyses, organization of field trials, led phenotyping for cold tolerance, conducted QTL mapping

Liliam Martinez Bello: Participated in trait mapping

Bochra Bahri: Participated in trait mapping

Srinivasa Chaluvadi: AMF colonization tests and analyses

Graduate students:

Ruyue Ding: Developed the two F_2 mapping populations and worked with the field technician on field establishment and maintenance of the populations

Christian Schwoyer: Initial optimization of freezing tests (left program without notification)

Alex Johnson: Nodal propagation, participated in phenotyping for cold tolerance, conducted miRNA analyses of roots in presence and absence of AMF in switchgrass F2 population (This project was funded by a pre-doctoral NIFA grant to Alex Johnson)

John Spiekerman: Generated RNASeq libraries from cold-acclimated leaf material

Soyeon Choi: Generated RNASeq libraries from cold-acclimated leaf material and analyzed RNASeq data

Kang-Hsien Fan: Developed the statistical methodology for eQTL mapping in polyploids, including writing computer code, conducting simulations and performing the data analysis

Ruyue Ding: Developed the two F_2 mapping populations and worked with the field technician on field establishment and maintenance of the populations

Technical personnel:
Olliff Weldon: Provided technical assistance with freezing tests

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