**The genetic basis for panicle traits variation in switchgrass (*Panicum virgatum*)**

**Running head: Panicle genetics in switchgrass**

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**Abstract**

Grass species exhibit large diversity in panicle architecture, variations of which are often controlled by genes, the environment, and their interaction. The genetic study of panicle architecture in perennial grasses is limited. In this study, we evaluate the genetic basis of panicle architecture including panicle length, primary branching number, and secondary branching number in an outcrossing switchgrass population grown across ten field sites in the central United States, through multi-environment mixed QTL analysis. Furthermore, we searched for candidate genes underlying panicle trait QTL in switchgrass. Overall, 18 QTL were detected for the three panicle traits. Twelve of the QTL exhibited consistent effects (i.e., no QTL by environment interactions or no QTL x E), and most (4 of 6) of the effects with QTL x E exhibited condition-specific effects. Panicle QTL co-localized with previously identified flowering time QTL and candidate genes associated with flowering, supporting a pleiotropic model of panicle development based on shared developmental genetics and responses to environmental signals.

Key words: panicle, QTL x E interaction, phenotypic plasticity, , condition-specific effect, *Panicum virgatum*, switchgrass

**Declarations**

**Funding**

This research was supported and funded by the National Science Foundation Plant Genome Research Program (IOS-1444533) and by the US Department of Energy, Office of Science, Office of Biological and Environmental Research Award DESC0014156 to T.E.J.

**Conflicts of interest**

We declare that there is no conflicts of interest.

**Data Availability**

The data used in this manuscript in included in supplementals.

**Author contributions**

D.B.L., J.B., F.B.F., and T.E.J. designed research; D.B.L., J.B., P.A.F., R.B.M., J.L.-R., A.R.B., Y.W., F.M.R., R.L.W., X.W., K.D.B., A.L., D.B., A.S., F.B.F., and T.E.J. performed research; L.Z., X.W., A.H.M. and K.D.B. analyzed data; and L. Z., X.W. and A.H.M. wrote the paper with comments and editing by all authors.

**Key Message**

Our investigation suggests that panicle trait variation in switchgrass is due to a combination of QTL and the environment, with some QTL displaying stable effects while others exhibiting differential effects across geographic regions.

**Introduction**

As the bearers of grain, grass panicles (or inflorescences) have been targets of selection for thousands of years (Doust 2007). There is enormous diversity in panicle architecture within and among grass species (Coen and Nugent 1994). Panicle architecture is a critical determinant of interspecies differences in plant morphology and life history, which is often measured as variation in panicle length, branching structure (number, length, and pattern), and flower number and size borne on each branch type. Simple panicles may have only primary branches, while complex panicles can possess many secondary and tertiary branches (Bommert and Whipple 2018; Glemin and Bataillon 2009). In wild grasses, branching pattern plays an important role in wind pollination and affects the number and size of seeds, which ultimately influences seed yield and plant fitness (Brown et al. 2006; Friedman and Harder 2004). In domesticated species, there is a direct association between panicle architecture and seed productivity (Brown et al. 2006; Crowell et al. 2016; Wang and Li 2005). Analysis of the phylogenetic distribution of panicle variation in the grasses suggests that different panicle architecture have arisen independently many times, and homoplasy across the grass phylogeny has obscured the mechanisms of panicle diversity (Doust and Kellogg 2002; Kellogg 2000). These traits likely evolve in response to natural selection mediated by pollinator (Friedman and Harder 2004) and environmental variation such as light (Vogler et al. 1999), drought (Mal and Lovett-Doust 2005), nutrient availability (Dorken and Barrett 2004), soil water availability (Caruso 2006), and intraspecific competition (Wolfe and Mazer 2005). Given the importance of inflorescence architecture to the fitness of wild species and the productivity of domesticated species, it is of great interest to understand genetic variation in panicle architecture.

Genetic variation in phenotypic plasticity in response to the environment is better known as genotype-by-environment interactions (G x E) (Des Marais et al. 2013). Quantitative studies of G x E in many plant species (e.g., maize, rice) have identified important quantitative trait loci (QTL) impacting many panicle traits (Adriani et al. 2016; Doust et al. 2005; Leng et al. 2017; Liu et al. 2008; Miura et al. 2010). For example, Doust et al. (2015) detected 14 replicated QTL for four inflorescence traits under two trials with high density and low density of plants, and these QTL were suggested to represent genes controlling differences between foxtail millet and green millet. G x E is common in QTL studies and identifying G x E and the pattern of interactions is of great interest to understand the genetic architecture underlying phenotypic traits. In addition to quantitative studies of G x E, genome-wide association studies (GWAS) on panicle architecture and GWAS of G x E on panicle architecture are also of great interest on various crops (Zhao et al., 2016; Liu et al., 2018; Ta et al., 2018; Thapa et al., 2021; Zhong et al., 2021). It will be of great value to evaluate the genetic basis of panicle traits, and evaluate the evidence for G x E using both quantitative studies and GWAS, which will further empower the confidence for identifying the genetic regions responsible for panicle architecture.

Switchgrass (*Panicum virgatum* L.) has been championed as a potential biofuel crop since it was selected by the US Department of Energy (US DOE) as a model grass species for bioenergy in the early 1990s (Hohenstein and Wright 1994; McLaughlin 1993). Its potential for high biomass production on marginal land, adaptation to a wide range of environments, and ecosystem service such as carbon sequestration, water flow management and erosion control, makes switchgrass an excellent candidate for meeting bioenergy needs (Mitchell et al. 2012; Robertson et al. 2017). Switchgrass is a warm-season C4 perennial grass native to the North America, with a range that extends from the eastern seaboard west to the Rocky Mountains and from southern Canada south to the Texas Coastal Plain and Northern Mexico (Casler 2007; Hopkins 1995). Two major distinctive populations have been classified in the past based on morphology and habit preference, northern upland and southern lowland ecotypes (Porter Jr 1966). A very recent study based on a resequenced switchgrass diversity panel was able to define a third coastal ecotype, which is broadly sympatric with the lowland ecotype but possesses upland leaf characters and lowland plant morphotype (Lovell et al., 2021).

Information on panicle morphology is limited in switchgrass, although panicle length differences have been reported between switchgrass ecotypes and cultivars (Porter Jr 1966; Price 2014; Van Esbroeck 2003). We hypothesize that panicle evolution in switchgrass may be related to selection on aspects of mating system and degree of investment in vegetative versus sexual reproduction, especially in the context of seedling establishment in differing habitats. For example, lowland switchgrass has a restricted bunch grass growth form and occurs primarily in patchy distributions along riparian areas. In contrast, upland switchgrass has a rhizomatous spreading growth form that occurs in many prairie habitats. Pattern of pollen dispersal across patches, or aspects of seed establishment (e.g. seed size/number tradeoffs or disturbance regimes) likely differ in these habitats and may have driven divergence in panicle form. Panicle morphology and its relationship to seed quality may be important targets of selection and breeding, as consistent seed production will be critical to meet the demands for large-scale biofuel production (Das and Taliaferro 2009; Vogel 2000).

In this study, we evaluated the genetic architecture of switchgrass panicle traits using a four-way population grown across 10 field sites (or common gardens) in the central US and a natural population of switchgrass (i.e., a diversity panel) grown at three common gardens out of the 10 sites. To accomplish this goal, we planted clonal divisions of progeny from a four-way outbred mapping population derived from upland and lowland germplasm, along with the four grandparents and F1 hybrids, at 10 field sites spanning a large latitudinal gradient. Also, we planted a diversity panel of switchgrass at three of the 10 field sites. Three panicle traits including panicle length (PL), primary branching number (PBN) per panicle, and secondary branching number (SBN) on the panicle, were assessed for each population at their planting sites in the end of 2019 growing season. These datasets were used to investigate: (1) the genetic architecture underlying these three traits through quantitative analysis and GWAS analysis, (2) the sensitivity of QTL and their effects across different environments from the four-way population, and (3) the single nucleotide polymorphism (SNP) effects on the traits in the three common gardens from the diversity panel, and to search for candidate genes that are overlapping between the two populations and are in regulation of panicle architecture in switchgrass.

**Materials and Methods**

**Data availability**

Whenever possible, plant material will be shared upon request. Source data and code to replicate these analyses are available at: <https://github.com/lzhangUT/PanicleData.git>. Large genetic data files to replicate these analyses are available from the UT dataverse at: *make\_stable\_doi\_link*.

**Field experiment and phenotyping of the four-way**

The details of the creation of the four-way population are described in Milano et al. (2016). Briefly, the grandparents of the mapping population were derived from highly divergent southern lowland and northern upland ecotypes. The population was developed by initial crosses between AP13 (A) x DAC6 (B) and WBC3 (C) x VS16 (D). AP13 and WBC3 are genotypes clonally derived from an individual selected from the lowland cultivar ‘Alamo’ (southern Texas accession) and an individual from naturally occurring population ‘West Bee Cave’ (central Texas accession), respectively. DAC6 and VS16 are genotypes clonally derived from individuals selected from the upland cultivars ‘Dacotah’ and ‘Summer’ (both northern upland accessions), respectively. The F1 hybrids of each of those crosses were then intercrossed reciprocally to produce the four-way outbred mapping population.

The grandparents, F1 hybrid parents, and the F2 progeny were propagated by dividing plants manually to produce 10 clones, each of which was maintained in a 3.8-L pot at the Brackenridge Field Laboratory, Austin, TX in 2013-2015. One replicate of each of the mapping progeny genotypes (i.e., 380 core genotypes), along with multiple replicates of grandparents and F1 parents, were transplanted from May to July of 2015 at 10 field sites. The 10 field sites cover 17 degrees of latitude from South Texas to South Dakota (Figure 1A). Detailed information of the 10 field sites, including latitude, longitude and soil type is provided in Table 1. The annual mean temperature at the 10 sites in 2016 ranged from 10.4 °C in the north to 20.7 °C in the south, and the total rainfall varied from 574 mm to 1440 mm (Figure 1B, data are from local weather station or from NOAA if local weather data are not available; the weather station or NOAA link is included in Table 1). To control weeds, each field site was covered with one layer of weed barrier cloth (Dewitt, Sikeston, MO). Holes were cut into the weed cloth in a honeycomb fashion. Plants were randomized into the holes, with each plant having four nearest neighbors each located 1.56m away from each other. A row of border plants was planted at every edge position of the field to minimize edge effects. The border plants were derived from rhizome plugs obtained from an approximately 10-year-old stand of Alamo switchgrass. Plants were well watered in the field during the summer of 2015 to facilitate establishment and all phenotypes were collected in 2016.

Three panicles were cut from each plant at full maturity. Panicle length (PL in mm), primary branching number (PBN), and secondary branching number (SBN) were assessed at the end of the 2016 growing season. A diagram depicting these phenotypes is presented in Figure 2, with representative images of panicles from the four grandparents. PL was measured on the primary panicle from the base of the first primary branch to the top of the panicle. PBN was counted as the total number of branches along the primary rachis. Due to the numerous secondary branches in switchgrass, SBN in our study referred to the total number of secondary branches on the lowest primary branch of the panicle (Figure 2). In total, over 10,000 separate panicle morphology measurements were collected for the four-way population. The phenotypic data (i.e., average values) for each genotype at each field site are provided in Supplemental Table S1.

**Genotyping and multi-environment QTL modelling of the four-way**

Illumina fragment paired end libraries, representing each of the four grandparents (A: AP13, B: DAC; C: WBC; D: VS16) were sequenced and genotyped in the context of the *P.virgatum* reference genome v5 as detailed in (Bragg et al. 2020). The genetic map spans 750 recombinant 4-way progeny genotyped at 4700 markers. Details on the genetic map construction, map polishing and fine-scale reordering can be accessed on https://datadryad.org/stash/dataset/doi:10.5061/dryad.ghx3ffbjv (Lovell et al., 2020). For computational efficiency in G x E analysis, the genetic map was reduced into 738 markers, with an average intermarker distance of to 2cM.

Narrow-sense heritability (*h2*) for each trait and the genetic correlation between traits at each environment were estimated using the additive kinship matrix based on marker genotypic information using the ‘sommer’ package (Covarrubias-Pazaran 2016) in R (2018). Briefly, we used a multivariate mixed model (mmer) that takes the kinship matrix to estimate the variance components for each trait under each environment, and calculates *h2* as the proportion of additive genetic variance to the total variance.

Details of the mapping scheme and application in the outbred four-way population are described in Malosetti et al. (2013) and Lowry et al. (2019). In brief, ‘single trait under multiple environments’ QTL mapping for each panicle trait in the cross-pollinated (CP) family was implemented in Genstat (2019). The QTL approach with CP family resulted in four possible QTL alleles designated *A* and *B* corresponding to marker alleles of the first pair of grandparents (AP13 x DAC) and QTL alleles *C* and *D* corresponding to marker alleles of the second pair of grandparents (WBC x VS16). A multienvironment mixed model was fit for each trait as shown in Eq. 1:

Eq. (1),

where *μ* is the population mean; *E* represents the environment effect; , denoting the total effect from the additive effect from the first grandparent (i.e., the difference between *A* and *B* alleles, , the second grandparent (i.e., the difference between *C* and *D* alleles, , and the dominance effect (i.e., the intralocus interaction, ; represents the QTL × environment interactions; and *e* represents the error term that was modeled by an unstructured variance–covariance matrix. The unstructured model was used to specify the data structure in the genome-wide QTL scan of simple interval mapping (SIM) and composite interval mapping (CIM). A backward selection procedure was used to retain significant fixed terms (*p* < 0.05) after three consecutive runs of CIM to confirm stability of QTL. The QTL with highest LOD peaks were considered as the most significant QTL, and the flanking markers associated with 1.5 LOD drop around the most significant QTL were considered as confidence interval for the QTL peaks.

**Genome-wide association and multivariate adaptive shrinkage in the switchgrass diversity panel**

The formation and resequencing of the switchgrass diversity panel has been described previously (Lovell et al 2020). Briefly, hundreds of tetraploid switchgrass plants were resequenced, and these genotypes were clonally replicated and planted at multiple common gardens spanning a latitudinal gradient across the continental United States. We phenotyped panicle length (PL in mm), primary branch number (PBN), and secondary branch number (SBN) as above for three panicles cut from each plant at full maturity at the end of the 2019 growing season, in a subset of genotyped individuals and common gardens. We phenotyped 381 genotyped individuals that had clonally replicated individuals present at each of three common garden locations (Pickle, TX or PKLE; Columbia, MO or CLMB; and Hickory Corners, MI or KBSM).

We analyzed SNP effects on three panicle traits in three common gardens using multivariate adaptive shrinkage (mash), starting with effect estimated via univariate genome-wide association studies (GWAS). GWAS were conducted using the bigsnpr R package (Privé et al 2018), which performs fast statistical analysis of large SNP arrays encoded as matrices, and which implements the current best practices in human genetics for principal component analysis of population genetic data (Privé et al 2020). Only SNPs with <20% missing data and minor allele frequencies >0.05 at all three gardens were used in univariate GWAS, resulting in 18.7M SNPs retained for the analysis. We then ran mash on the effect estimates and standard errors generated from univariate GWAS, following [mash documentation](https://stephenslab.github.io/mashr/articles/eQTL_outline.html): first, 100K SNPs randomly sampled from a set of SNPs unlinked at an r2 of 0.2 were used as a ‘random’ set to learn the background correlation structure; second, 5K SNPs with the maximum -log10p-values in any of the univariate GWAS were used to construct data-driven covariance matrices; third, the random set was used to fit the mash model; fourth, posterior summaries using the model fit on the random set were computed on all 18.7M SNPs. We determined which SNPs had evidence of significant phenotypic effects using local false sign rates (lfsr), which are analogous to false discovery rates but more conservative (in that they also reflect the uncertainty in the estimation of the sign of the effect)(Stephens 2016). These lfsr were condition-specific measures of significance; for an overall measure of significance for each SNP on the trait effects included in mash, we used the log10(Bayes Factor) computed by mash.

**Enrichment tests to find candidate genes in both mapping populations**

To determine if SNPs with significant trait effects on panicles in our diversity panel (assessed using mash) were enriched in panicle QTL intervals in the four-way population, we compared SNP enrichment in the QTL intervals to SNP enrichment of 1000 permutations of the QTL regions. First, the 18.7M SNPs used in mash were clumped to keep only the most significant SNP in each LD block, using a linkage threshold of r2 < 0.2 (Privé et al 2019). Significance was assessed using the log10(Bayes Factor) and resulted in retention of 2.7M SNPs. Second, 1000 permutations of the QTL regions were created of the same size (in bp) of the 18 QTL found using the pseudo-F2 mapping cross. For both the QTL intervals and these 1000 permutations, we assessed the number of QTL that had significant enrichments of mash SNPs in the top 1% quantile of the 2.7M unlinked SNPs using hypergeometric tests.

Third, we identified genes that were located both in the confidence intervals of the discovered QTL from the four-way and within 20kb of the 6149 mash SNPs with log10(Bayes Factor) > 1.3, equivalent to a FDR-adjusted p-value of 0.05, from the diversity panel. Because these genes were identified in two independent mapping panels, we have increased confidence that they are involved in panicle architecture in switchgrass. These genes were compared with the rice (v7) and Arabidopsis annotation databases (TAIR 10) to further identify candidate genes with functional validation in panicle and flower architecture, or bolt architecture after the transition to flowering, in other species. The annotation file for switchgrass was accessed on JGI (Joint Genome Institute) Phytozome 13 website: https://njp-spin.jgi.doe.gov/.

**Results**

**Phenotypic variation and heritability of the four-way**

There was a general trend of increasing trait values in the F2 with latitude, exhibiting latitudinal plasticity of the measured panicle traits (Figure 3). The violin plot for each trait at each site displayed approximately normal distribution and transgressive behavior in the F2 generation. Lowland genotypes, AP13 and WBC, always had larger values of panicle length (PL in mm), primary branching number (PBN), and secondary branching number (SBN) than upland genotypes, DAC and VS16 (Figure 3).

The heritability (*h2*) for PL, PBN and SBN varied by site (Table 2). The *h2* for PL ranged from 0.20 to 0.71, with an average of 0.46 and values greater than 0.50 at four northern sites. The *h2* for PBN ranged between 0.45 and 0.66 for 9 out of the 10 sites, with Stillwater, OK (STIL) having low heritability (*h2*=0.20). The *h2* for SBN ranged from 0.02 to 0.62, where Stillwater, OK (STIL) had *h2* close to zero (*h2*=0.02), Columbia, MO (CLMB) had low heritability (*h2*=0.15), and four sites had heritability at approximately 0.50. The low heritabilities of panicle traits at Stillwater, OK may be related to the less rainfall it received (~700 mm), compared to Overton, TX (OVTN) and Manhattan, KS (MNHT) which also have sandy loam soil but received ample rain (~1400mm at OVTN and ~1000mm at MNHT, Figure 1), and had slightly cooler temperature in MNHT (Figure 1). These changes in heritability by environment indicate G x E, which primarily is the result of changes in variances across the common garden environments. The phenotypic and genetic correlations between traits were generally positive but varied by site, ranging from 0.21 to 0.63 for phenotypic correlation and from 0.35 to 0.88 for genetic correlation (genetic correlation of Stillwater did not converge, Table 3).

**Multi-environment mixed QTL model**

A total of 18 QTL were identified for panicle morphology traits with the multi-environment mixed model analyses (Figure 4, Table 4). Seven QTL were identified for PL, distributed across seven different chromosomes. Among these, five QTL (2K@77.89, 4K@26.26, 5K@76.02, 5N@36.27 and 9N@38.02) had consistent effects across field sites (Figure 5a). In contrast, two QTL (3N@62.06 and 6N@54.19) show interaction with the environment (QTL x E). The additive effects for QTL 3N@62.06 changed in magnitude across geographic regions. Further, QTL 6N@54.19 had the largest effects at the most northern and southern site and smaller effect at mid-latitude sites. QTL 6N@54.19 (A x B cross) also had a trade-off pattern, with the allelic effects changing sign from southern to northern sites.

Seven QTL were identified for PBN that are also distributed on 7 chromosomes. Four QTL (2K@74.02, 2N@66.12, 5N@84.04 and 9N@26.03) had consistent effects across locations, while three QTL (3K@38, 5K@14.06, and 7N@54.06) had QTL x E interactions, including both changes of magnitude (3K@38 and 7N@54.06) and direction (5K@14.06) from the allelic effect across geographic regions (Figure 5b). Four QTL were identified for SBN. Three QTL (2N@72.03, 5K@95.5 and 9N@36.02) had consistent effects across locations, while there was a magnitude changing interactions for QTL 9K@51.96 (Figure 5c). We also observed that two QTL for PBN (2K@74.02 and 9N@26.03) co-localized with PL QTL on chromosome 2K and 9N, based on overlapping confidence intervals (Figure 4). QTL 9N@38.02 for SBN co-localized with the QTL of PL and PBN on chromosome 9N (Figure 4).

**Genome-wide association and multivariate adaptive shrinkage in the switchgrass diversity panel**

Our mash model of three panicle traits in three common gardens found that 6149 (0.23%) of LD blocks unlinked at r2 = 0.2 (hereafter ‘unlinked SNPs’) had significant effects on at least one panicle trait by garden condition. 76.1% of significant unlinked SNPs had significant effects in all nine combinations of panicle trait and garden. Most of the 18.7M SNPs (56.2%) had high mash model weights on covariance matrices with equal effects in all nine conditions; most remaining SNPs (43.7%) had high model weights on covariance matrices with no effects in any condition. Thus, the diversity panel data showed little evidence for GxE for panicle traits at these three gardens, supporting our QTL findings that there was little GxE for panicle traits across ten common gardens.

**Enrichment tests to find candidate genes in both mapping populations**

All QTL regions from the pseudo-F2 cross also contained significant unlinked SNPs in the diversity panel which fell within 20kb of genes that had functionally validated roles in panicle, spikelet, or grain traits in rice (Table S#2). Because QTL regions could be comprised of one or more linked effect on panicle architecture, we additionally determined that 10 of the 18 QTL regions also had a significant enrichment of unlinked SNPs (p hypergeometric test < 0.05, Table S#1). 0.2% of permuted genomic intervals had as many or more permuted QTL regions enriched for unlinked SNPs (p = 0.002), while no permuted genomic intervals had more than 10 regions significantly enriched for unlinked SNPs. Thus, even with the very different population makeup of the GWAS panel, we could confirm a higher than expected overlap between SNP effects and QTL effects on panicle traits.

Panicle traits have been well-studied in crop plants and model systems and many candidate genes have been reported (Doust 2007; Doust et al. 2005; McSteen 2006; Miura et al. 2010; Vollbrecht et al. 2005). We found 131 overlapping candidate genes located in the confidence intervals of the identified regions from the four-way population and the diversity panel (Supplemental Table S2).

Among these overlapping candidate genes, XXXX and XXX stood out. ADD MORE HERE (Xiaoyu) key transcription factors and hormone related genes associated with panicle development were identified in the intervals of most QTL exhibiting environmental interactions. For example, key regulators involved in GA metabolism (*Pavir.3KG352627* as the homolog of *GA2ox3* in 3K@38; *Pavir.5KG065800* as the homolog of *GA3ox2* in 5K@14.06) and CK signaling pathways (*Pavir.7NG435700* as the homolog of *ARR6* in 7N@54.06; *Pavir.9KG213000* as the homolog of *ARR1* in 9K@51.96) were found in four branching QTL x E intervals. Another two candidate genes, *Pavir.9NG142700* and *Pavir.5NG572850*, which are homologs of *Tb1* and *ba1,* respectively, in maize, were found in panicle traits QTL regions. *Tb1* and *ba1* genes are involved in tassel and branching development in maize (Hubbard et al. 2002; Neuffer et al. 1968), and were suggested as candidate genes for inflorescence variation in Poaceae (Doust et al. 2005). A number of key flowering genes were also found in our panicle QTL intervals. These genes are known to be involved in the photoperiodic flowering pathway and control panicle morphology in other grasses (Shrestha et al. 2014; Tsuji et al. 2011). However, these candidate genes were not co-localized with flowering QTL that we identified, indicating the potentially complex functions of flowering time genes in the transition from vegetative to reproductive phases.

**Discussion**

There has been considerable interest in the molecular mechanisms of G x E across a diversity of phenotypes, species, and environments. G x E is common and is often driven by differential sensitivity of alleles and may play an important role in adaptive plasticity and local adaptation (Des Marais et al. 2013). With its large scale, our study evaluated the genetic basis and examined the QTL x E of panicle morphological traits in switchgrass grown at 10 field sites in the central United States (Figure 1). Overall, we detected moderate heritability (except for the field site Stillwater, OK) for panicle traits (Table 2) and positive phenotypic and genetic correlations between traits at each site (Table 3). These data suggest considerable standing genetic variation in inflorescence characteristics available for natural or artificial selection to act upon. We identified several QTL with significant QTL x E effects, indicating that panicle traits in switchgrass result from the combination of QTL and environment. We also detected pleiotropic effects between panicle traits and flowering time as well as tiller count and biomass, suggesting a possible shared genetic basis between different traits.

Our study identified genomic regions (QTL) that contribute to panicle trait variation across a broad latitudinal gradient. These QTL exhibited constant effects (i.e., no QTL x E), antagonistic pleiotropy, or condition-specific effects across the studied environmental gradients. QTLs with condition-specific effects often confer an advantage in some environments but no effect in other environments (El-Soda et al. 2014). Antagonistic pleiotropy is a genetic trade-off at an individual locus or QTL, that results in opposite effects (i.e., sign change) on a trait in different environments (Wadgymar et al. 2017). Studying the molecular genetic basis of specific QTL should greatly contribute to the mechanistic understanding of such QTL x E. In our study, most of the QTL are conditionally neutral. This is consistent with a recent meta-analyses which found that asymmetry of QTL effect is more often caused by conditional neutrality than it is by trades-offs (Wadgymar et al. 2017). For the few cases of putative QTL x E in our results, it could be possibly due to the differential survival of plants at the various locations, especially in the northern site South Dakota and in the southern site TX. Differential mortality of non-cold tolerant genotypes in South Dakota and non-heat tolerant genotypes in Texas could cause differential effects across locations. However, further examination of the survivorship found that more than 80% of plants survived at least at seven field sites. Overall, our results show that panicle traits are controlled by a combination of QTL and the environment and, in a number of cases, their interaction with the environment.

In summary, our results suggest that variation of panicle traits in switchgrass is due to a combination of QTL and the environment, with QTL displaying different effects across geographic regions. Future work focusing on identifying the driver of QTL by environment interactions and understanding the mechanisms underlying them will facilitate the selection of suitable genotypes of switchgrass for specific environments.

**Supplemental Files**

The phenotyping data (panicle length, PL; primary branching number, PBN; and secondary branching number, SBN) for genotypes at each of the 10 field sites (Table S1), and the candidate gene lists (Table S2) are included in the supplemental excel files.

**Acknowledgements**

We thank the numerous field technicians, students, and postdocs who worked in the field helping out with data collection. Special thanks go to Alice MacQueen and other Juenger Lab members for helping improve the manuscript with their comments. This research was supported and funded by the National Science Foundation Plant Genome Research Program (IOS-1444533) and by the US Department of Energy, Office of Science, Office of Biological and Environmental Research Award DESC0014156 to T.E.J. This material is based upon work supported in part by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494. Support for this research was provided by the National Science Foundation Long-term Ecological Research Program (DEB 1832042) at the Kellogg Biological Station and by Michigan State University AgBioResearch. We thank the Joint Genome Institute and collaborators for prepublication access to the *Panicum virgatum* v5 AP13 genome reference.

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Table 1. The latitude, longitude, site code, soil texture, and source of weather data for the 10 experimental fields in the study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Field Site | Site Code | Latitude | Longitude | Soil Texture | Weather Data Source |
| Brookings, SD | BRKG | 44.307 | -96.67 | Clay loam | https://www.ncdc.noaa.gov/cdo-web/results |
| Hickory Corners, MI | KBSM | 42.42 | -85.37 | Loam | https://lter.kbs.msu.edu/datatables/7 |
| Lincoln, NE | LINC | 41.154 | -96.42 | Loam | https://www.ncdc.noaa.gov/cdo-web/results |
| Manhattan, KS | MNHT | 39.141 | -96.64 | Sandy loam | mesonet.k-state.edu/weather/historical |
| Columbia, MO | CLMB | 38.897 | -92.22 | Loam | http://agebb.missouri.edu/weather/history/index.asp?station\_prefix=bfd |
| Stillwater, OK | STIL | 35.991 | -97.05 | Sandy loam | https://www.mesonet.org/index.php/weather/local/perk |
| Overton, TX | OVTN | 32.303 | -94.98 | Sandy loam | https://www.ncdc.noaa.gov/cdo-web/results |
| Temple, TX | TMPL | 31.043 | -97.35 | Clay | https://www.ars.usda.gov/plains-area/temple-tx/grassland-soil-and-water-research-laboratory/docs/temple-climatic-data/ |
| Austin, TX | PKLE | 30.384 | -97.73 | Clay | https://www.ncdc.noaa.gov/cdo-web/results |
| Kingsville, TX | KING | 27.55 | -97.88 | Sandy clay loam | https://www.ncdc.noaa.gov/cdo-web/results |

Table 2. Narrow-sense heritability (*h2*), and its one standard error (±1SE), for panicle length (PL), primary branching number (PBN), and secondary branching number (SBN) at each of the 10 field sites (ordered from north to south).

|  |  |  |  |
| --- | --- | --- | --- |
| Sites/Traits | PL | PBN | SBN |
| BRKG | 0.55±0.07 | 0.64±0.06 | 0.40±0.08 |
| KBSM | 0.71±0.06 | 0.62±0.07 | 0.56±0.07 |
| LINC | 0.58±0.07 | 0.65±0.07 | 0.47±0.08 |
| MNHT | 0.38±0.08 | 0.66±0.06 | 0.38±0.08 |
| CLMB | 0.57±0.07 | 0.64±0.07 | 0.15±0.08 |
| STIL | 0.20±0.08 | 0.13±0.08 | 0.02±0.07 |
| OVTN | 0.44±0.08 | 0.63±0.06 | 0.62±0.07 |
| TMPL | 0.49±0.08 | 0.54±0.07 | 0.30±0.08 |
| PKLE | 0.24±0.09 | 0.45±0.09 | 0.29±0.09 |
| KING | 0.47±0.08 | 0.58±0.07 | 0.48±0.08 |

Table 3. The phenotypic correlation and genetic correlation between panicle traits among sites and across sites. PL, panicle length, PBN, primary branching number, and SBN, secondary branching number.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sites |  | Phenotypic correlation | | |  | Genetic correlation | | |
|  |  | PL | PBN | SBN |  | PL | PBN | SBN |
|  | PL | 1 | - | - |  | 1 | - | - |
| BRKG | PBN | 0.41 | 1 | - |  | 0.68 | 1 | - |
|  | SBN | 0.42 | 0.39 | 1 |  | 0.82 | 0.72 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| KBSM | PBN | 0.30 | 1 | - |  | 0.44 | 1 | - |
|  | SBN | 0.42 | 0.54 | 1 |  | 0.62 | 0.79 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| LINC | PBN | 0.43 | 1 | - |  | 0.49 | 1 | - |
|  | SBN | 0.52 | 0.60 | 1 |  | 0.79 | 0.80 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| MNHT | PBN | 0.56 | 1 | - |  | 0.62 | 1 | - |
|  | SBN | 0.68 | 0.67 | 1 |  | 0.74 | 0.86 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| CLMB | PBN | 0.36 | 1 | - |  | 0.50 | 1 | - |
|  | SBN | 0.40 | 0.50 | 1 |  | 0.88 | 0.97 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| STIL | PBN | 0.42 | 1 | - |  | 0.35 | 1 | - |
|  | SBN | 0.42 | 0.57 | 1 |  | - | - | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| OVTN | PBN | 0.52 | 1 | - |  | 0.58 | 1 | - |
|  | SBN | 0.59 | 0.58 | 1 |  | 0.72 | 0.73 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| TMPL | PBN | 0.35 | 1 | - |  | 0.54 | 1 | - |
|  | SBN | 0.53 | 0.46 | 1 |  | 0.69 | 0.63 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| PKLE | PBN | 0.27 | 1 | - |  | 0.61 | 1 | - |
|  | SBN | 0.21 | 0.51 | 1 |  | 0.47 | 0.74 | 1 |
|  | PL | 1 | - | - |  | 1 | - | - |
| KING | PBN | 0.52 | 1 | - |  | 0.56 | 1 | - |
|  | SBN | 0.63 | 0.61 | 1 |  | 0.70 | 0.73 | 1 |

Table 4. The identified QTL, along with their marker name (chromosome with physical distance in mega base pair), maximum LOD values, and flanking markers with a LOD drop of 1.5 for panicle morphology traits (PL: panicle length; PBN: number of primary branches; SBN: number of secondary branches). The presence of genotype by environmental interaction is marked as ‘Yes’ or ‘No’ in column Q x E. The overlapping QTL confidence interval between traits indicates pleiotropic effect, and what other traits (FL50, TC, and BIO) have pleiotropy with panicle traits at each identified QTL position is marked in column Pleiotropy. FL50, TC, and BIO are flowering time, tiller count and biomass at the end of season, respectively.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| trait | QTL | MARKER | LOD | Left flanking marker | Right flanking\_marker | QxE | Pleiotropy |
| PL | 2K@77.89 | Chr02K\_62.598826 | 6.18 | Chr02K\_60.739957 | Chr02K\_64.045891 | No | BIO |
| PL | 3N@62.06 | Chr03N\_26.099983 | 4.29 | Chr03N\_24.521536 | Chr03N\_30.30364 | Yes | BIO |
| PL | 4K@26.26 | Chr04K\_13.041487 | 4.66 | Chr04K\_9.916183 | Chr04K\_29.613196 | No | FL50 |
| PL | 5K@76.02 | Chr05K\_56.620419 | 4.82 | Chr05K\_44.678143 | Chr05K\_58.488157 | No |  |
| PL | 5N@36.27 | Chr05N\_16.511689 | 3.63 | Chr05N\_11.735767 | Chr05N\_47.154718 | No |  |
| PL | 6N@54.19 | Chr06N\_48.768076 | 3.56 | Chr06N\_43.871788 | Chr06N\_51.935176 | Yes |  |
| PL | 9N@38.02 | Chr09N\_18.617122 | 5.82 | Chr09N\_10.880731 | Chr09N\_20.831824 | No | FL50, TC, BIO |
| PBN | 2K@74.02 | Chr02K\_59.503978 | 4.09 | Chr02K\_56.436103 | Chr02K\_63.664705 | No | BIO |
| PBN | 2N@66.12 | Chr02N\_55.500715 | 5.52 | Chr02N\_50.387752 | Chr02N\_56.445418 | No | FL50 |
| PBN | 3K@38 | Chr03K\_17.77051 | 8.83 | Chr03K\_13.323286 | Chr03K\_20.786505 | Yes | FL50, TC |
| PBN | 5K@14.06 | Chr05K\_7.188103 | 4.90 | Chr05K\_4.388419 | Chr05K\_8.204815 | Yes |  |
| PBN | 5N@84.04 | Chr05N\_64.047349 | 4.73 | Chr05N\_60.974614 | Chr05N\_65.990782 | No | FL50, TC |
| PBN | 7N@54.06 | Chr07N\_49.904749 | 4.17 | Chr07N\_49.035214 | Chr07N\_49.904749 | Yes | FL50 |
| PBN | 9N@26.03 | Chr09N\_12.531268 | 4.89 | Chr09N\_7.913256 | Chr09N\_21.588445 | No | FL50 |
| SBN | 2N@72.03 | Chr02N\_58.696003 | 9.46 | Chr02N\_54.556579 | Chr02N\_60.798034 | No |  |
| SBN | 5K@95.5 | Chr05K\_60.232411 | 6.22 | Chr05K\_58.583292 | Chr05K\_60.232411 | No |  |
| SBN | 9K@51.96 | Chr09K\_24.465322 | 10.37 | Chr09K\_19.959778 | Chr09K\_28.697896 | Yes |  |
| SBN | 9N@38.02 | Chr09N\_18.617122 | 9.29 | Chr09N\_17.684245 | Chr09N\_19.333648 | No | TC, BIO |