**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**

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**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.M and K.D.B. analyzed data; and L. Z., X.W. and A.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, the grass panicle (or inflorescence) has been the target 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 architectures are critical determinant of interspecies differences in plants morphology and life history, and are often measured as variation in panicle length, branching (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 and productivity of both wild and domesticated species, it is of great interest to understand genetic variation in panicle architecture.

Organisms often respond to changing resource availability and environmental signals through phenotypically plastic changes (Sultan 2000). Panicle traits often display plasticity in response to different environmental cues, as panicle development involves complex regulatory mechanisms and these mechanisms interact with environmental signals (Adriani et al. 2016; Bai et al. 2016; Tu et al. 2019). For example, Adriani et al. (2016) found that secondary branch number was the most variable (or plastic) trait of panicle architecture in response to light resources in rice. Secondary branching was also found to be influenced by water deficit more than primary branching in a rice recombinant inbred family (Liu et al. 2008). Abiotic components such as heat, drought, and light affect panicle development and ultimately panicle architecture (Adriani et al. 2016; Wu et al. 2017; Wu et al. 2016). In addition to environmental effects on variation in panicle traits, standing genetic variation within species in panicle traits is also common (Brown et al. 2006; Jamal et al. 2009; Ungerer et al. 2002).

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. Leng et al. (2017) identified 17 QTL for five panicle related traits in a double haploid population in rice. Among these QTL, six QTL showed QTL-by-environment interactions, indicating that panicle related traits are susceptible to environmental influence. Zhao et al. (2017) found that 11 out of 19 QTL were involved in QTL-by-environment interactions for tassel primary branching number in maize under different watering environments. 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.

Pleiotropy is the phenomenon of a single gene affecting multiple distinct traits (Williams 1957) and is an important driver of trait integration and modularity (Armbruster et al. 2014; Klingenberg 2008). Pleiotropy contributes to the genetic correlation among traits and therefore has broad implications in genetics, development and adaptive evolution (Armbruster et al. 2014; Auge et al. 2019; Pigliucci and Preston 2004). In plants, the formation of all aboveground organs, such as leaves, tiller, internodes, and inflorescences, is mainly dictated by the activity and determinacy of the shoot apical and axillary meristems. During the vegetative-to-reproductive transition, many developmentally related traits originate from the same meristem and complex environmental signals may affect the different type of meristems by similar regulatory networks (Wang and Li 2005; Xue et al. 2020). Therefore, the loci for vegetative and reproductive development-related traits frequently show pleiotropy. For example, most flowering time pathway genes show pleiotropic effects on tiller number and yield potential in crops (Auge et al. 2019). Genes involved in hormone pathways frequently affect both vegetative growth and reproductive development, in part through their developmental impacts on meristems (Azizi et al. 2015). QTL mapping is one of the many approaches that have been used to estimate genome-wide pleiotropy, and fits squarely in the context of developmental pleiotropy (Paaby and Rockman 2013). Pleiotropic effects identified through overlapping QTL locations have been observed in panicle development in sorghum, rice, and Poaceae (Brown et al. 2006; Doust et al. 2005; Endo-Higashi and Izawa 2011; Komatsu et al. 2001; Miura et al. 2010; Yu et al. 2017), but these patterns have not been investigated in native perennial grasses.

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 filling 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., 2020,accepted).

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 across 10 field sites in the central US. 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. Three panicle traits including panicle length (PL), primary branching number (PBN) per panicle, and secondary branching number (SBN) on the panicle, were assessed at each site to investigate: (1) the genetic architecture underlying these three traits, (2) the sensitivity of QTL and their effects across different environments, and (3 searched for candidate genes on the identified QTL regions involved in regulating 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**

The details of the creation of this population were 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. In three of the 10 sites, 370 extra genotypes were planted. 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 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 in our study. 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**

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.

To investigate potential pleiotropic relationships between panicle traits and other traits, we ran the multi-environment QTL model for flowering (FL50), tiller count (TC), and biomass (BIO) in switchgrass and examined if the QTL identified for panicle traits share the same QTL with these traits. FL50, TC and BIO data were collected in the same year as panicle data and previously reported in Lowry et al. (2019). FL50 is the day of the year when 50% of the tillers on a plant have panicles that have begun flowering. TC and BIO are the total tiller count and dry biomass at the end of the growing season, respectively. Traits with overlapping QTL confidence interval were considered as potentially pleiotropic.

**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 clones 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), using effect estimates from univariate genome-wide association studies (GWAS). <add more here>

**Genomic prediction**

We further conducted a genomic prediction on the core genotypes grown at the 10 sites and validate for the 370 extra genotypes grown at the three sites (Pickle, TX or PKLE; Columbia, MO or CLMB; and Hickory Corners, MI or KBSM). Specifically, we first implemented the Bayesian multi-trait and multi-environment (BMTME) model in R package ‘BMTME’ (Montesinos-López et al. 2019) on the core genotypes grown at the 10 field sites. BMTME package includes multivariate statistical models for genome-based prediction and is used for analyzing breeding data with multiple traits and multiple environments. It allows parameter estimation and evaluates the prediction performance in a reliable, efficient and user-friendly way. We used the BMTME model in the package to take into account the genetic effect (i.e., genomic relationship matrix derived from marker or pedigree), the environment and interactions between these two. Details on the models and the package can be found in Montesinos-López et al. (2019). Second, we used the BMORS (Bayesian multi-output regressor stacking) function embedded in the package to cross validate and evaluate the prediction performance for the core genotypes. After checking the prediction performance using measurements of R2 between observed and predicted phenotypes and Pearson’s correlations at each field site, the model was used to predict for the extra 370 genotypes at the three sites. Percentage of bias (bias%) and the prediction accuracy (*r*, or correlation coefficient) between the model predictions and field observations were used as statistical measures for model performance.

**Candidate genes and GO enrichment analysis**

The genes located in the confidence intervals of the discovered QTL were considered candidate genes. All candidate genes were compared with the rice (v7) and Arabidopsis annotation databases (TAIR 10), and annotated with Gene Ontology (GO). The annotation file for switchgrass was accessed on JGI (Joint Genome Institute) Phytozome 13 website: https://njp-spin.jgi.doe.gov/. The GO enrichment analysis was tested using fisher’s exact test for each GO term using R package ‘topGO’ (Alexa and Rahnenfuhrer 2020). The GOs with adjusted *p* values of less than 0.05 were considered as significant.

**Results**

**Phenotypic variation and heritability**

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).

**Pleiotropic effect**

We hypothesized that loci impacting panicle traits might show strong pleiotropic relationships with other important characteristics of switchgrass growth and development. To test this idea, we examined whether the QTL identified for panicle traits shared the same QTL with flowering (FL50), tiller count (TC), and biomass (BIO) in switchgrass. Our results (Figure 4 and Table 4) showed that two QTL (2K@77.89, 3N@62.06) for PL overlapped with QTL for BIO, one QTL (4K@26.26) overlaps with FL50, and one QTL on 9N@38.02 clusters with FL50, TC, and BIO. Out of the seven QTL for PBN, six QTL shared the same QTL with either FL50, TC or BIO, with five QTL (2N@66.12, 3K@38, 5N@84.04, 7N@54.06, 9N@26.03) exhibiting pleiotropy with FL50, two QTL (3K@38, 5N@84.04) with TC and one (2K@74.02) with BIO. One QTL of SBN (9N@38.02) clustered with QTL of TC and BIO. Among these pleiotropic QTL, some showed similar patterns of QTL x E, providing further support for a pleiotropy hypothesis. For example, the QTL 3N@62.06 for PL exhibited condition-specific effects (Figure 5) and the overlapping QTL for BIO showed a similar pattern (data not shown). The QTL 9N@38.02 for PL displayed the same pattern (i.e., no QTL x E, figure 5) as the overlapping QTL for FL50 and BIO, but not for TC, suggesting there may be different loci controlling PL and TC.

**Genomic prediction**

The model performance on the core genotypes at the 10 field sites showed average R2 of 0.47, 0.53, and 0.53 for PL, PBN, and SBN, respectively. The cross validation showed decent Pearson’s correlation at the majority of the sites (0.3-0.8) for the three panicle traits except for Stillwater, OK (Figure 6). The subsequent evaluation on the extra 370 genotypes at the three sites (PKLE, CLMB, and KBSM) displayed moderate prediction accuracy for each trait (Figure 7). PL had prediction accuracy ranging from 0.50 to 0.61 with slight overprediction at KBSM and CLMB (i.e., positive %bias) and slight underpredictions (i.e., negative %bias) at PKLE. PBN had prediction accuracy of around 0.6 at the three sites, with minor underprediction at KBSM and slight overpredictions at CLMB and PKLE. SBN had slightly lower prediction accuracy at CLMB (*r* = 0.45), and overpredictions at KBSM and CLMB.

**Candidate gene identification and GO enrichment analysis**

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). For the 18 panicle architecture QTL identified in our study, the confidence intervals ranged from 2 to 32 cM and from 0.9 to 35 Mb. We linked the QTL discovered here with known candidate genes that have been reported in previous studies (Supplemental Table S2).

Among these candidate genes, 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.

GO enrichment analysis identified 380 significant GO terms for genes within the QTL intervals for panicle traits. ‘Response to Auxin’ was one of the significantly enriched GO terms (*p*=0.0012). This is an exciting result as the auxin signaling pathway has been previously shown to be important in panicle meristem development and affects primary branching number in rice and other grasses (He et al. 2018; Zhang and Yuan 2014). Several other significant terms that are relevant to our study traits include ‘response to oxidative stress’ and ‘H4/H2A histone acetyltransferase complex’ (Boycheva et al. 2014; Deng et al. 2007; Peng et al. 2018; Wu et al. 2017; Yano et al. 2019). These results point to potentially interesting candidate genes and hormone-related pathways that are likely important in panicle development.

**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.

Inflorescence architecture is influenced by the vegetative-to-reproductive phase transition, which also largely determines patterns of vegetative growth and resource allocation. In our study, 11 of 18 inflorescence QTLs co-localized with flowering time or vegetative growth genomic intervals, which supports the hypothesis that pleiotropy impacts the phenotypic integration of these vegetative and reproductive structures. An exciting opportunity lies in the search for the candidate genes that may underlie this integration. Fortunately, extensive genetic mapping efforts in crops and model systems have identified a number of candidate genes and a basic understanding of their role in the development of the inflorescence. For example, a locus on chromosome 9N (at 38.02 cM) was associated with the whole process of vegetative-to-reproductive transition (PL, PBN, SBN, FL50, TC and BIO). This QTL cluster is in the vicinity of homologs of *OsCOL10* and *OsTB1*, which are known as the key regulators in flowering and branch development (Takeda et al. 2003; Tan et al. 2016). Specifically, *OsCOL10* functions as a flowering time repressor downstream of *Ghd7* and the *OsTB1* gene negatively regulates lateral branching in rice. Moreover, the locus on chromosome 3K (at 38 cM) was clustered with QTLs for PBN, FL50 and TC. Significantly, this QTL clustering region had large eﬀects for PBN, suggesting a major QTL that coordinates vegetative and reproductive processes. We identified a homolog of GA2ox3 in this region, which is considered as a key factor in gibberellin catabolism and plays a central role in plant development (Sakamoto et al. 2004). These results imply that there may be a shared genetic basis between vegetative and reproductive divergence within switchgrass populations.

Genomic selection and prediction have been widely used in plant breeding and implemented in may crops (Crossa et al. 2014; Rutkoski et al. 2011; Spindel et al. 2015) since it was proposed (Meuwissen et al. 2001). Recent research has begun to develop and improve existing statistical models for genomic selection as the successful genomic selection implementation is closely connected to the accuracy of the model predictions (Montesinos-López et al. 2019). The Bayesian multi-trait and multi-environment model implemented in our study not only considers variance-covariance matrices of traits in comparison to the other existing multi-trait analyses software, but also considers the generic covariance (correlation) between environments, which can help improve parameter estimation and prediction and prediction accuracy. The moderate prediction accuracy (0.45-0.61) for the independent genotypes at the three field sites was largely improved compared to the prediction based on the multi-environment QTL model (0.19-0.32) we built (data not shown). This is because genomic prediction makes use of all markers simultaneously in the training of the statistical models based on genotyping and phenotyping of a reference population, and predicts genomic breeding values or phenotypic values of the validation population. While QTL model would only make use of the significant QTL detected, and not accounting for the epistatic or dominance effects to some degree. However, QTL model is still useful for downstream fine mapping of candidate genes.

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.

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**References**

Adriani DE, Dingkuhn M, Dardou A, Adam H, Luquet D, Lafarge T (2016) Rice panicle plasticity in Near Isogenic Lines carrying a QTL for larger panicle is genotype and environment dependent. Rice 9:28

Alexa A, Rahnenfuhrer J (2020) topGO: enrichment analysis for gene ontology

Armbruster WS, Pélabon C, Bolstad GH, Hansen TF (2014) Integrated phenotypes: understanding trait covariation in plants and animals. Philosophical Transactions of the Royal Society B: Biological Sciences 369:20130245

Auge GA, Penfield S, Donohue K (2019) Pleiotropy in developmental regulation by flowering-pathway genes: is it an evolutionary constraint? New Phytologist 224:55-70

Azizi P, Rafii M, Maziah M, Abdullah S, Hanafi M, Latif M, Rashid A, Sahebi M (2015) Understanding the shoot apical meristem regulation: a study of the phytohormones, auxin and cytokinin, in rice. Mechanisms of development 135:1-15

Bai X, Zhao H, Huang Y, Xie W, Han Z, Zhang B, Guo Z, Yang L, Dong H, Xue W, Li G, Hu G, Hu Y, Xing Y (2016) Genome-Wide Association Analysis Reveals Different Genetic Control in Panicle Architecture Between Indica and Japonica Rice. The Plant Genome 9

Bommert P, Whipple C (2018) Grass inflorescence architecture and meristem determinacy. Seminars in Cell & Developmental Biology 79:37-47

Boycheva I, Vassileva V, Iantcheva A (2014) Histone acetyltransferases in plant development and plasticity. Curr Genomics 15:28-37

Bragg J, Tomasi P, Zhang L, Williams T, Wood D, Lovell JT, Healey A, Schmutz J, Bonnette JE, Cheng P, Chanbusarakum L, Juenger T, Tobias CM (2020) Environmentally responsive QTL controlling surface wax load in switchgrass. Theoretical and Applied Genetics 133:3119-3137

Brown PJ, Klein PE, Bortiri E, Acharya CB, Rooney WL, Kresovich S (2006) Inheritance of inflorescence architecture in sorghum. Theoretical and Applied Genetics 113:931-942

Caruso CM (2006) Plasticity of Inflorescence Traits in Lobelia siphilitica (Lobeliaceae) in Response to Soil Water Availability. American journal of botany 93:531-538

Casler MD (2007) Genetic Diversity, Plant Adaptation Regions, and Gene Pools for Switchgrass. Crop science v. 47:pp. 2261-2260-2007 v.2247 no.2266

Coen ES, Nugent JM (1994) Evolution of flowers and inflorescences. Development 1994:107

Covarrubias-Pazaran G (2016) Genome-assisted prediction of quantitative traits using the R package sommer. PLOS ONE 11:e0156744

Crossa J, Pérez P, Hickey J, Burgueño J, Ornella L, Cerón-Rojas J, Zhang X, Dreisigacker S, Babu R, Li Y, Bonnett D, Mathews K (2014) Genomic prediction in CIMMYT maize and wheat breeding programs. Heredity (Edinb) 112:48-60

Crowell S, Korniliev P, Falcão A, Ismail A, Gregorio G, Mezey J, McCouch S (2016) Genome-wide association and high-resolution phenotyping link Oryza sativa panicle traits to numerous trait-specific QTL clusters. Nature Communications 7:10527

Das MK, Taliaferro CM (2009) Genetic variability and interrelationships of seed yield and yield components in switchgrass. Euphytica 167:95-105

Deng W, Liu C, Pei Y, Deng X, Niu L, Cao X (2007) Involvement of the Histone Acetyltransferase AtHAC1 in the Regulation of Flowering Time via Repression of &lt;em&gt;FLOWERING LOCUS C&lt;/em&gt; in Arabidopsis. Plant Physiology 143:1660

Des Marais DL, Hernandez KM, Juenger TE (2013) Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. Annual Review of Ecology, Evolution, and Systematics 44:5-29

Dorken ME, Barrett SCH (2004) Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of Sagittaria latifolia (Alismataceae): a clonal aquatic plant. Journal of Ecology 92:32-44

Doust A (2007) Architectural evolution and its implications for domestication in grasses. Ann Bot 100:941-950

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EA (2005) The Genetic Basis for Inflorescence Variation Between Foxtail and Green Millet (Poaceae). Genetics 169:1659

Doust AN, Kellogg EA (2002) Inflorescence diversification in the panicoid "bristle grass" clade (Paniceae, Poaceae): evidence from molecular phylogenies and developmental morphology. American journal of botany 89:1203-1222

El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, Aarts MGM (2014) Genotype × environment interaction QTL mapping in plants: lessons from Arabidopsis. Trends in Plant Science 19:390-398

Endo-Higashi N, Izawa T (2011) Flowering time genes Heading date 1 and Early heading date 1 together control panicle development in rice. Plant Cell Physiol 52:1083-1094

Friedman J, Harder LD (2004) Inflorescence architecture and wind pollination in six grass species. Functional Ecology 18:851-860

Glemin S, Bataillon T (2009) A comparative view of the evolution of grasses under domestication. New Phytol 183:273-290

He Q, Yang L, Hu W, Zhang J, Xing Y (2018) Overexpression of an auxin receptor OsAFB6 significantly enhanced grain yield by increasing cytokinin and decreasing auxin concentrations in rice panicle. Scientific Reports 8:14051

Hohenstein WG, Wright LL (1994) Biomass energy production in the United States: an overview. Biomass and Bioenergy 6:161-173

Hopkins AA (1995) Genotypic Variability and Genotype × Environment Interactions among Switchgrass Accessions from the Midwestern USA. Crop science v. 35:pp. 565-560-1995 v.1935 no.1992

Hubbard L, McSteen P, Doebley J, Hake S (2002) Expression patterns and mutant phenotype of teosinte branched1 correlate with growth suppression in maize and teosinte. Genetics 162:1927-1935

Jamal, Khalil IH, Bari A, Khan S, Zada I (2009) Genetic variation for yield and yield components in rice. Journal of agricultural and biological science 4:60-64

Kellogg EA (2000) Molecular and morphological evolution in the Andropogoneae, CSIRO,Melbourne,Australia

Klingenberg CP (2008) Morphological Integration and Developmental Modularity. Annual Review of Ecology, Evolution, and Systematics 39:115-132

Komatsu M, Maekawa M, Shimamoto K, Kyozuka J (2001) The LAX1 and FRIZZY PANICLE 2 Genes Determine the Inflorescence Architecture of Rice by Controlling Rachis-Branch and Spikelet Development. Developmental Biology 231:364-373

Leng Y, Xue D, Huang L, Chen L, Ren D, Yang Y, Zhang G, Hu J, Zhu L, Guo L, Lin Y, Qian Q, Zeng D (2017) Mapping QTL with main effect, digenic epistatic and QTL × environment interactions of panicle related traits in rice (Oryza sativa). International Journal of Agriculture and Biology 19:1608-1614

Liu G, Zhang Z, Zhu H, Zhao F, Ding X, Zeng R, Li W, Zhang G (2008) Detection of QTLs with additive effects and additive-by-environment interaction effects on panicle number in rice (Oryza sativa L.) with single-segment substitution lines. Theor Appl Genet 116:923-931

Lowry DB, Lovell JT, Zhang L, Bonnette J, Fay PA, Mitchell RB, Lloyd-Reilley J, Boe AR, Wu Y, Rouquette FM, Wynia RL, Weng X, Behrman KD, Healey A, Barry K, Lipzen A, Bauer D, Sharma A, Jenkins J, Schmutz J, Fritschi FB, Juenger TE (2019) QTL × environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient. Proceedings of the National Academy of Sciences 116:12933

Mal TK, Lovett-Doust J (2005) Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, Lythrum salicaria (Lythraceae), in response to soil moisture. American journal of botany 92:819-825

Malosetti M, Ribaut J-M, van Eeuwijk FA (2013) The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. Frontiers in Physiology 4

McLaughlin S (1993) New switchgrass biofuels research program for the southeast. In: Proceedings of the annual automative technology development contractors coordinating meeting Nov. 2–5, 1992, Dearborn:111–115

McSteen P (2006) Branching Out: The &lt;em&gt;ramosa&lt;/em&gt; Pathway and the Evolution of Grass Inflorescence Morphology. Plant Cell 18:518

Meuwissen TH, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829

Milano ER, Lowry DB, Juenger TE (2016) The genetic basis of upland/lowland ecotype divergence in switchgrass (*Panicum virgatum*). G3 (Bethesda) 6:3561-3570

Mitchell R, Vogel KP, Uden DR (2012) The feasibility of switchgrass for biofuel production. Biofuels 3:47-59

Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M (2010) OsSPL14 promotes panicle branching and higher grain productivity in rice. Nat Genet 42:545-549

Montesinos-López OA, Montesinos-López A, Luna-Vázquez FJ, Toledo FH, Pérez-Rodríguez P, Lillemo M, Crossa J (2019) An R Package for Bayesian Analysis of Multi-environment and Multi-trait Multi-environment Data for Genome-Based Prediction. G3: Genes|Genomes|Genetics 9:1355

Neuffer M, Jones L, Zuber MS (1968) The mutants of maize. Crop Science Society of America Madison, Wisconsin

Paaby AB, Rockman MV (2013) The many faces of pleiotropy. Trends in Genetics 29:66-73

Peng Y, Hou F, Bai Q, Xu P, Liao Y, Zhang H, Gu C, Deng X, Wu T, Chen X, Ali A, Wu X (2018) Rice Calcineurin B-Like Protein-Interacting Protein Kinase 31 (OsCIPK31) Is Involved in the Development of Panicle Apical Spikelets. Front Plant Sci 9:1661-1661

Pigliucci M, Preston K (2004) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford University Press

Porter Jr CL (1966) An analysis of variation between upland and lowland switchgrass, *Panicum virgatum* L., in central Oklahoma. Ecology 47:980-992

Price DL (2014) Predictive Relationships between Plant Morphological Traits and Biomass Yield in Switchgrass. Crop science v. 54:pp. 637-630-2014 v.2054 no.2012

Robertson GP, Hamilton SK, Barham BL, Dale BE, Izaurralde RC, Jackson RD, Landis DA, Swinton SM, Thelen KD, Tiedje JM (2017) Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. Science 356

Rutkoski JE, Heffner EL, Sorrells ME (2011) Genomic selection for durable stem rust resistance in wheat. Euphytica 179:161-173

Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Agrawal GK, Takeda S, Abe K, Miyao A, Hirochika H, Kitano H, Ashikari M, Matsuoka M (2004) An Overview of Gibberellin Metabolism Enzyme Genes and Their Related Mutants in Rice. Plant Physiology 134:1642

Shrestha R, Gómez-Ariza J, Brambilla V, Fornara F (2014) Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals. Annals of botany 114:1445-1458

Spindel J, Begum H, Akdemir D, Virk P, Collard B, Redoña E, Atlin G, Jannink J-L, McCouch SR (2015) Genomic Selection and Association Mapping in Rice (Oryza sativa): Effect of Trait Genetic Architecture, Training Population Composition, Marker Number and Statistical Model on Accuracy of Rice Genomic Selection in Elite, Tropical Rice Breeding Lines. PLOS Genetics 11:e1004982

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5:537-542

Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M, Ueguchi C (2003) The OsTB1 gene negatively regulates lateral branching in rice. The Plant journal : for cell and molecular biology 33:513-520

Tan J, Jin M, Wang J, Wu F, Sheng P, Cheng Z, Wang J, Zheng X, Chen L, Wang M, Zhu S, Guo X, Zhang X, Liu X, Wang C, Wang H, Wu C, Wan J (2016) OsCOL10 , a CONSTANS-Like Gene, Functions as a Flowering Time Repressor Downstream of Ghd7 in Rice. Plant and Cell Physiology 57:798-812

Tsuji H, Taoka K, Shimamoto K (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. Curr Opin Plant Biol 14:45-52

Tu C, Li T, Liu X (2019) Genetic and epigenetic regulatory mechanism of rice panicle development. AIP Conference Proceedings 2079:020001

Ungerer MC, Halldorsdottir SS, Modliszewski JL, Mackay TFC, Purugganan MD (2002) Quantitative Trait Loci for Inflorescence Development in &lt;em&gt;Arabidopsis thaliana&lt;/em&gt. Genetics 160:1133

Van Esbroeck GA (2003) Variation between Alamo and Cave-in-Rock Switchgrass in Response to Photoperiod Extension. Crop science v. 43:pp. 639-630-2003 v.2043 no.2002

Vogel K (2000) Improving warm-season forage grasses using selection, breeding, and biotechnology. In: Moore KJ, Anderson BE (eds) Native warm-season grasses: research trends and issues 30:83-106

Vogler DW, Peretz S, Stephenson AG (1999) Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in Campanula rapunculoides (Campanulaceae). American journal of botany 86:482-494

Vollbrecht E, Springer PS, Goh L, Buckler Iv ES, Martienssen R (2005) Architecture of floral branch systems in maize and related grasses. Nature 436:1119-1126

VSN I (2019) Genstat for Windows 19th Edition. VSN International, Hemel Hempstead, UK

Wadgymar SM, Lowry DB, Gould BA, Byron CN, Mactavish RM, Anderson JT (2017) Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation. Methods in Ecology and Evolution 8:738-749

Wang Y, Li J (2005) The plant architecture of rice (Oryza sativa). Plant molecular biology 59:75-84

Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398-411

Wolfe LM, Mazer SJ (2005) Patterns of Phenotypic Plasticity and Their Fitness Consequences in Wild Radish (Raphanus sativus: Brassicaceae). International Journal of Plant Sciences 166:631-640

Wu C, Cui K, Wang W, Li Q, Fahad S, Hu Q, Huang J, Nie L, Mohapatra PK, Peng S (2017) Heat-Induced Cytokinin Transportation and Degradation Are Associated with Reduced Panicle Cytokinin Expression and Fewer Spikelets per Panicle in Rice. Front Plant Sci 8:371-371

Wu C, Cui K, Wang W, Li Q, Fahad S, Hu Q, Huang J, Nie L, Peng S (2016) Heat-induced phytohormone changes are associated with disrupted early reproductive development and reduced yield in rice. Scientific Reports 6:34978

Xue Z, Liu L, Zhang C (2020) Regulation of Shoot Apical Meristem and Axillary Meristem Development in Plants. International Journal of Molecular Sciences 21:2917

Yano K, Morinaka Y, Wang F, Huang P, Takehara S, Hirai T, Ito A, Koketsu E, Kawamura M, Kotake K, Yoshida S, Endo M, Tamiya G, Kitano H, Ueguchi-Tanaka M, Hirano K, Matsuoka M (2019) GWAS with principal component analysis identifies a gene comprehensively controlling rice architecture. Proceedings of the National Academy of Sciences:201904964

Yu H, Qiu Z, Xu Q, Wang Z, Zeng D, Hu J, Zhang G, Zhu L, Gao Z, Chen G, Guo L, Qian Q, Ren D (2017) Fine mapping of LOW TILLER 1, a gene controlling tillering and panicle branching in rice. Plant Growth Regulation 83:93-104

Zhang D, Yuan Z (2014) Molecular Control of Grass Inflorescence Development. Annual Review of Plant Biology 65:553-578

Zhao X, Peng Y, Zhang J, Fang P, Wu B (2017) Mapping QTLs and meta-QTLs for two inflorescence architecture traits in multiple maize populations under different watering environments. Molecular Breeding 37:91

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 |