

QTL x environment interactions for panicle traits in switchgrass (*Panicum virgatum*)

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Abstract

Panicle traits exhibit quantitative variation controlled by genes, the environment, and their interaction. In switchgrass, a perennial biofuel crop, identification of quantitative trait loci (QTL) and QTL x E interactions controlling panicle architecture could aid breeding efforts and cultivar development by impacting seed productivity. In this study, we evaluate the genetic architecture of panicle traits 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. We evaluated pleiotropic relationships between panicle traits and flowering time, tiller production and biomass. We also identified environmental factors correlated with QTL x E interactions and potential candidate genes underlying panicle trait QTL in switchgrass. Overall, our multi-environment mixed QTL model detected 18 QTL for panicle traits. Twelve of the QTL exhibited consistent effects (i.e., no QTL x E), and most (4 of 6) of the effects with QTL x E exhibited condition-specific effects. Many of the QTL x E effects were associated with yearly mean temperature and photoperiod. 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.

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 & 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 & Whipple, 2018; Glemin & 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 (Friedman & 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 & 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 & Kellogg, 2002; Kellogg, 2000). These traits likely evolve in response to natural selection mediated by pollinator (Friedman & Harder, 2004) and environmental variation such as light (Vogler et al., 1999), drought (Mal & Doust, 2005), nutrient availability (Dorken & Barrett, 2004), soil water availability (Caruso, 2006), and intraspecific competition (Wolfe & 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., 2010). 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; Mitchell et al., 1997). In addition to environmental effects on variation in panicle traits, standing genetic variation within species in panicle traits is also common (Jamal et al., 2009; Brown et al., 2006; Hong & Yan, 2004; 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 Marias et al., 2013). Quantitative studies of G x E in many crops (e.g., maize, rice) have identified important quantitative trait loci (QTL) impacting many panicle traits (Kovi et al., 2011; Leng et al., 2017; Zhao et al., 2017). For example, 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 & Li, 2008; 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 (Lee et al., 2019), 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 & Rockman, 2013). Pleiotropic effects identified through overlapping QTL locations have been observed in panicle development in sorghum and rice (Brown et al., 2006; Endo-Higashi & 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 & 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, Vogel, & Uden, 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, in review).

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 & Taliaferro, 2009; Vogel, 2000).

In this study, we evaluated the genetic architecture of panicle traits across ten field sites in the central US to investigate the importance and nature of G x E for panicle traits. 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 ten 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 (i.e., QTL x E), (3) the extent of pleiotropy between panicle and other traits, and (4) the environmental factors contributing to QTL x E interactions. Finally, we identified candidate genes potentially involved in regulating panicle architecture in switchgrass.

Materials and Methods

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, 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.ghx3ffb/v1> (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 (h^2) for each trait at each environment and the genetic correlation between traits among sites and across sites 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 h^2 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. (2014) 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 multi-environment mixed model was fit for each trait as shown in Eq. 1:

$$trait = \mu + E + \sum QTL + \sum (QTL \times E) + e \text{ Eq. (1)},$$

where μ is the population mean; E represents the environment effect; $\sum QTL = \sum (a^{a1} + a^{a2} + a^d)$, denoting the total effect from the additive effect from the first grandparent (i.e., the difference between *A* and *B* alleles, a^{a1}), the second grandparent (i.e., the difference between *C* and *D* alleles, a^{a2}), and the dominance effect (i.e., the intralocus interaction, a^d); $\sum (QTL \times E)$ represents the QTL \times 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.

The quality of the predictions from the full G x E model (Eq. 1) was evaluated with an independent dataset. In three of the 10 field sites (CLMB, KBSM, PKLE), 370 extra genotypes were planted and panicle morphology data were collected for these genotypes in 2016. The multi-environment QTL x E model was evaluated by 1) extracting the final full model from Genstat based on the core set of progeny, 2) reconstructing the model in R, 3) predicting the panicle traits for these additional genotypes at these 3 field sites, and 4) comparing the model predictions with field observations. 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.

Our Genstat modeling of QTL x E considered sites as fixed effect. To further explore the possible environmental drivers of the QTL x E, we explored a variety of regression models using best subset generalized linear models ('bestglm' package, McLeod et al., 2020) in R. Here, the QTL effects (i.e., the difference of A versus B or C versus D alleles) were predicted with the yearly mean temperature (Tmean), the yearly total rainfall (Rainfall), the day length (DL), and the yearly average solar radiation (SRAD) as predictors as shown in Equation 2.

$$\text{effect} = \mu + \text{Tmean} + \text{Rainfall} + \text{DL} + \text{SRAD} + e \quad \text{Eq. (2)}$$

These environmental variables were selected because temperature, water availability and light have been identified as interacting with panicle development, as mentioned in the Introduction. Interaction terms between variables were not included in our models because they were highly correlated with the individual variable. We used the bestglm function and performed all-subset linear regression based on Bayesian information criteria (BIC) to find the best subset of variables to fit the model. Environmental variables retained from the model were considered as candidate environmental factors that interacted with specific QTL, resulting in different effects at different sites.

Additionally, 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.

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 Rahnenhruer, 2019). 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 F_2 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 (h^2) for PL, PBN and SBN varied by site (Table 2). The h^2 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 h^2 for PBN ranged between 0.45 and 0.66 for 9 out of the 10 sites, with Stillwater, OK (STIL) having low heritability ($h^2 = 0.20$). The h^2 for SBN ranged from 0.02 to 0.62, where Stillwater, OK (STIL) had h^2 close to zero ($h^2 = 0.02$), Columbia, MO (CLMB) had low heritability ($h^2 = 0.15$), and four sites had heritability at approximately 0.50. 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 (Table 3). The phenotypic correlation between traits across all sites ranged from 0.50 to 0.61. The genetic correlation between PL and PBN across all sites was close to zero (0.03), while the genetic correlation between PBN and SBN was high (0.73) (Table 3). Together, these results suggest independence in panicle length and branching characteristics.

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.

QTL model evaluation

The evaluation of the QTL and QTL x E model (Eq. 1) with the data collected on extra genotypes at the three sites showed low to moderate prediction accuracy (Figure 6). Panicle length had relatively low prediction accuracy ($r = 0.34$) but also low percentage of bias (%bias = 0.3%). Primary branching number had similar results as panicle length ($r = 0.36$, %bias = 0.3%). Secondary branching number had a moderate prediction accuracy ($r = 0.62$) but with slightly larger percentage of bias (%bias = 0.6%).

Environmental factor exploration

To evaluate possible environmental drivers of G x E, we used a regression-based approach. Here, we evaluated yearly mean temperature, annual total rainfall, day length (photoperiod), and yearly average solar radiation as possible drivers of QTL x E for each of the six QTL with site interactions. Overall, the main drivers of the QTL x E identified in this study are yearly mean temperature and photoperiod, with impacts on eight QTL (Table 5). Rainfall was also identified as a driver of the QTL x E for the QTL 6N@54.19 of LEN and 5K@14.06 of PBN, and solar radiation as a driver of the QTL x E for the QTL 9K@51.96 of SBN (Table 5). We failed to identify candidate environmental factors for some of the QTL x E. This is likely related to the fact that not all the environmental factors (such as soil) were accounted for in our analyses.

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 (Miura et al., 2010; Doust, 2007; McSteen, 2006; 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. 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., 2010). 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 & 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, Vassileva, & Iantcheva, 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 and across sites (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 and the potential environmental factors underlying the QTL x E, 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 are relatively easy to incorporate into breeding programs because the selected favorable alleles will confer an advantage in some environments, without a negative 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 (Wadgyamar et al., 2017). QTL with antagonistic pleiotropy can result in tradeoffs and challenges in breeding if the preferred allele depends strongly on the environment (El-Soda et al., 2014; Lowry et al., 2019). 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-analysis which found that asymmetry of QTL effect is more often caused by conditional neutrality than it is by trade-offs (Wadgyamar et al., 2017). Overall, our results show that panicle traits are controlled by a combination of QTL and the environment and, in a number of cases, their complex 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 (Tan et al., 2016; Takeda et al., 2003). 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.

The low to moderate prediction accuracy (0.34-0.62) of the multi-environment mixed model (Eq. 1) is likely due to two factors. Our model only accounts for significant QTL, while there are likely many smaller QTL, that were below the threshold for detection, which contribute to variation in these traits. Unfortunately, our power to detect these small effects is likely low due to our modest sample sizes (380 progeny). Additionally, the QTL model does not consider epistatic effects or dominance effects between QTL. Epistasis is known to be an important factor that affects genetic variation and phenotypic expression in populations, especially for developmentally regulated traits like inflorescence architecture. Epistatic effects on panicle related traits have been identified in several studies (Leng et al., 2017; Ye et al., 2009). Further inclusion of epistasis into the multi-environment QTL model may help improve model prediction. However, our approach provides a way of predicting the performance of new genotypes under environments similar to the tested environments, and can potentially help with suitable genotype selection for traits of interest under a specific environment.

Temperature and photoperiod were the most significant predictors of QTL x E interactions. This is consistent with the pattern of additive effects of most of the QTL (Table 3), where QTL displayed conditional

neutrality with effects either in the northern or the southern sites (Figure 5). Previous study also showed that temperature-based growing degree days and photoperiod affected switchgrass morphology (Mitchell et al., 1997). Solar radiation was also a significant driver of QTL x E for secondary branching number (SBN) QTL. This is consistent with a rice study in which SBN was more plastic in response to different light resources (Adriani et al., 2016). No environmental factors were detected for some of the QTL x E interactions, possibly because the appropriate environmental factors (i.e., growing degree days, soil moisture etc.) were not explored or were obscured by complex interactions between environmental factors. The relative low heritability for panicle traits at the field site Stillwater, OK (STIL, Table 2) also suggested that there may be other environmental factor affecting panicle traits but not being accounted for, such as effective soil moisture. As noted in Table 1, Stillwater, OK, Overton, TX (OVTN), and Manhattan, KS (MNHT) all have sandy loam soil which may impact water status and subsequently plant growth and organ expansion. In our study year, OVTN received ample rain (~1400mm), MNHT had slightly cooler temperature and received approximately 1000mm rain, while STIL only received around 700mm rain (Figure 1). This study could be expanded in the future to include more field sites, multiple years, and more environmental data collection such as soil composition and nutrient availability to better capture the environmental drivers underlying the QTL x E interactions and trait plasticity across large geographic regions and across multiple years.

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 for specific environments in switchgrass breeding programs.

Data Availability and 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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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

Field Site	Site Code	Latitude	Longitude	Soil Texture	Weather Data Source
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/
Stillwater, OK	STIL	35.991	-97.05	Sandy loam	https://www.mesonet.org/index.php/weathe
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/templ
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 (h^2), and its one standard error ($\pm 1SE$), 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		Phenotypic correlation		Phenotypic correlation		Genetic correlation	
		PL	PBN	SBN	PL	PBN	SBN	
BRKG	PL	1	-	-	1	-	-	
	PBN	0.41	1	-	0.68	1	-	
	SBN	0.42	0.39	1	0.82	0.72	1	
KBSM	PL	1	-	-	1	-	-	
	PBN	0.30	1	-	0.44	1	-	
	SBN	0.42	0.54	1	0.62	0.79	1	
LINC	PL	1	-	-	1	-	-	
	PBN	0.43	1	-	0.49	1	-	
	SBN	0.52	0.60	1	0.79	0.80	1	
MNHT	PL	1	-	-	1	-	-	
	PBN	0.56	1	-	0.62	1	-	
	SBN	0.68	0.67	1	0.74	0.80	1	
CLMB	PL	1	-	-	1	-	-	
	PBN	0.36	1	-	0.50	1	-	
	SBN	0.40	0.50	1	0.88	0.97	1	
STIL	PL	1	-	-	1	-	-	
	PBN	0.42	1	-	0.35	1	-	
	SBN	0.42	0.57	1	-	-	1	

Sites		Phenotypic correlation	Phenotypic correlation	Phenotypic correlation	Genetic correlation	Genetic correlation
OVTN	PL	1	-	-	1	-
	PBN	0.52	1	-	0.58	1
	SBN	0.59	0.58	1	0.72	0.73
TMPL	PL	1	-	-	1	-
	PBN	0.35	1	-	0.54	1
	SBN	0.53	0.46	1	0.69	0.63
PKLE	PL	1	-	-	1	-
	PBN	0.27	1	-	0.61	1
	SBN	0.21	0.51	1	0.47	0.74
KING	PL	1	-	-	1	-
	PBN	0.52	1	-	0.56	1
	SBN	0.63	0.61	1	0.70	0.73
Across Sites	PL	1	-	-	1	-
	PBN	0.50	1	-	0.03	1
	SBN	0.55	0.61	1	0.38	0.72

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

Table 5. The environmental factor(s) selected by best subset general linear regression model based on Bayesian information criteria for the effects of QTL by environment interaction (QTL x E) for panicle traits. PL: panicle length; PBN: number of primary branches; SBN: number of secondary branches. A x B and C x D denote the cross from AP13 x DAC and WBC x VS16, respectively. Tmean, Rainfall, DL, and SRAD

are annual mean temperature, total rainfall, day length (photoperiod) and average solar radiation in 2016, respectively. * indicates the environmental factor that was considered as the candidate factor for the QTL x E based on the model.

Trait	QTL	Cross	Tmean	Rainfall	DL	SRAD
PL	3N@62.06	A x B				
		C x D	*		*	
PL	6N@54.19	A x B		*		*
		C x D		*		
PBN	3K@38	A x B	*		*	
		C x D				
PBN	5K@14.06	A x B		*	*	
		C x D		*	*	
PBN	7N@54.06	A x B	*	*		*
		C x D			*	
SBN	9K@51.96	A x B				*
		C x D	*			

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