Comparative evolution of vegetative branching in sorghum

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Abstract

Tillering and secondary branching are two plastic traits with high agronomic importance, especially in terms of the ability of plants to adapt to changing environments. We describe a quantitative trait analysis of tillering and secondary branching in two novel BC$_1$F$_2$ populations totaling 246 genotypes derived from backcrossing two *Sorghum bicolor* x *S. halepense* F$_1$ plants to a tetraploidized *S. bicolor*. A two-year, two-environment phenotypic evaluation in Bogart, GA and Salina, KS permitted us to identify major effect and environment specific QTLs. Significant correlation between tillering and secondary branching followed by discovery of overlapping sets of QTLs continue to support the developmental relationship between these two organs and suggest the possibility of pleiotropy. Comparisons with two other populations sharing *S. bicolor* BTx623 as a common parent but sampling the breadth of the Sorghum genus, increase confidence in QTL detected for these two plastic traits and provide insight into the evolution of morphological diversity in the Eusorghum clade. Correlation between flowering time and vegetative branching supports other evidence in suggesting a pleiotropic effect of flowering genes. We propose a model to predict biomass weight from plant architecture related traits, quantifying contribution of each trait to biomass and providing guidance for future breeding experiments.

Introduction

Plant architecture is the three-dimensional organization of a plant body. The above-ground architecture includes the pattern of vegetative branching, sizes and shapes of stalks, leaves and floral organs, and plant height. The expression of plant architecture varies during different developmental stages by a series of highly regulated endogenous genetic factors [1–3] and exogenous constraints exerted by environments. Genetic factors impart the biodiversity of plant architecture, contributing to adaptation to different niches, are often utilized in the classification of taxa. On the other hand, responsiveness to biotic and abiotic stresses tailors plant architecture to fitness under different environments [4,5].

Important aspects of plant architecture are tillering and vegetative branching, which are considered to be medium to low heritability traits with a high degree of plasticity [6,7]. The
complexity of these traits is due in part to their non-deterministic and genetic pathways controlling axillary meristem initiation and outgrowth that affect the number of tillers and patterns of vegetative branching [2,3,8]. Many of these genes are involved in the production, signal transduction, transport, degradation and interactions of hormones such as auxin, cytokinin and strigolactone. Those hormones act directly and locally to promote or repress axillary meristem activity [7,9–12].

Recent studies have also suggested that genes involved in controlling flowering time also influence the activity of axillary meristems and thus influence tillering and vegetative branching. For example, the flowering locus T (Ft) gene that regulates flowering time in many species, has recently been found to trigger storage organ formation through direct interaction with the TCP factors [13]. The rice homolog of Leafy (Lfy) from Arabidopsis, expressed during the development of axillary bud and inflorescence branch primordia, is also required to produce tillers and panicle branches (Rao, 2008) [37].

As a C4 model plant, sorghum has a relatively small genome (~730 Mb) with a high quality reference genome sequence [14] and provides many avenues to study traits related to plant architecture. Using co-linearity, results from sorghum may be extrapolated to many other C4 plants with large genomes, such as sugarcane. The flexibility to make crosses between the five main sorghum races (bicolor, guinea, caudatum, durra and kafir), and with wild relatives such as S. propinquum and S. halepense which vary widely in plant architecture, makes sorghum particularly attractive to dissect and compare genetic components of plant architecture. Compared to voluminous studies of plant height and flowering [15–24], understanding of genetic components for tiller number and vegetative branching in sorghum has been relatively limited [6,25–28], possibly due to difficulties in phenotyping and the lower heritability of these traits.

Here, we describe a quantitative trait study of two important components of plant architecture, tillering and vegetative branching, in two half-sib tetraploid BC1F2 populations derived from crossing Sorghum bicolor BTx623 and Sorghum halepense Gypsum 9E. A two-year, two-environment phenotypic evaluation in Bogart, GA and Salina, KS permitted us to identify major effect and environment specific QTLs [29,30]. Quantitative trait loci (QTLs) discovered in these two populations are compared to those from two diploid sorghum recombinant inbred line (RIL) populations sharing BTx623 as a common parent but sampling the breadth of the Sorghum genus, one a cross to S. bicolor IS3620C [31], and the other to S. propinquum [32]. QTLs identified in this study and their comparison elucidate morphological evolution in the Sorghum genus, are of practical use for marker-assisted breeding, and provide a foundation for molecular cloning and functional analysis.

Materials and methods

Population development is shown in Fig 1. Genetic maps of two BC1F1 populations derived from crosses of S. bicolor (sorghum) and S. halepense were produced with totals of 722 and 795 single nucleotide polymorphism (SNP) markers. These maps respectively span 37 and 35 linkage groups, with 2–6 for each of the 10 basic sorghum chromosomes due to fragments covering different chromosomal portions or independent segregation from different S. halepense homologs. Details of population development, genotyping methods and methods for QTL analysis were discussed in Kong, Nabukalu [29] and [30].

Phenotyping

We evaluated tillering (TL) and secondary branching per tiller (BRCH) in the BC1F2 families with three subsamples for each genotype in two fields in two years, 2013 and 2014; and at two locations, on May 29th 2013 and Jun 9th 2014 at the University of Georgia Plant Science Farm,
Watkinsville, GA, USA (33.87˚, -83.53˚, Athens 2013 and Athens 2014 hereafter), and on Jun 3rd 2013, and Jun 17th 2014 at The Land Institute, Salina, KS, USA (38.77˚, -97.57˚, Salina 2013 and Salina 2014 hereafter). Tillering (TL) was measured by counting the number of tillers with mature seed heads before plants were senesced. Secondary branches per tiller (BRCH) was calculated by taking the average number of secondary branches from two representative tillers (S1 and S2 Files).

Phenotyping of vegetative branching in the IS-RIL population was consistent with our system applied to the *S. bicolor* × *S. propinquum* RILs described in Kong, Guo [6]. To compare secondary branching across population and environments, we used the number of mature tillers (TL), and calculated the average number of secondary branches per mature tiller (BRCH) in the IS-RIL and PQ-RIL population. The variance component method was used to calculate broad-sense heritability \[ H = \frac{V_G}{V_G + V_{GE}/e + V_{residual}/r} \] where \( V_G \) is the variance estimate for genotype, \( V_E \) is the variance estimate for environment, \( V_{GE} \) is the genotype by environment interactions, \( e \) is the number of environments and \( r \) is the number of subsamples.

**Genetic analysis**

To fully utilize the available data while protecting against false-positive results, genetic analysis employed two approaches. Using genetic maps that were constructed as described [29] from selected well-groomed SNP segregation data for each of the two SBSH-BC1F2 populations, interval mapping was conducted [33]. Permutation tests (with \( \alpha = 0.10 \)) suggested LOD scores of 2.9 and 3.1 for H4 and H6 populations, respectively. QTLs with LOD scores of 2.5 were listed as marginal QTLs. Additional QTLs were added to the model after considering the larger effect QTL as a fixed effect. For each trait, percentage of variance explained were calculated based on an additive QTL model with QTL positions refined.

In addition, single marker analysis was conducted using each SNP marker that met quality standards described (whether in the genetic map or not), using hierarchical clustering to separate SNP markers on potentially different homologous chromosomes and inferring QTLs only if more than 4 SNPs were found within a cluster cut at height of 0.3 in recombination frequency to mitigate spurious associations [30]. Similarities and differences in the results of these analyses were addressed in results.
Mixed modeling for biomass

We constructed a mixed effect model with Biomass as the response variable; FL, PH, TL, BRCH, mid-stalk diameter (MD), the number of nodes (ND), and population (H4 or H6) as fixed explanatory variables; and the environment (ENV) as a random effect. MD was the stalk diameter at the middle of a plant. The average of the six phenotypes from two blocks and three subsamples was taken for the mixed effect modeling. A natural log transformation was used for Biomass to normalize the data. Mixed effect modeling and model selection used the lme4 and lmerTest packages in R [34,35]. We used a modified method to calculate R-squared for the fixed and model effects [36] for the mixed effect modeling.

Results

Summary statistics and heritability analysis

The average number of mature tillers (ML) of S. halepense G9E was 16, higher than the 2.6 of diploid S. bicolor BTx623 (S1 Table). Tetraploid BTx623 had an average of 0.77 more tillers than diploid BTx623 in 2013 (t = 2.91, p = 0.006) and 1.58 more in 2014 (t = 3.82, p = 0.0005). In the BC1F2 population, average TL for most lines fell within the range of those of their parents, showing less transgressive segregation than plant height (PH) and flowering time (FL) [30]. The average TL was 1.46 more in Salina than Athens (t = 14.07, p<0.001). Average TL in Athens was 2.24 (t = -21.87, p<0.001) fewer in 2013 than 2014; and in Salina 2013 was 2.14 fewer in 2013 than 2014 (t = 19.07, p<0.001). Average TL of the BC1F2 population is 0.30 greater than that of the PQ-RILs (t = 2.52, p = 0.020, S2 Table), and 2.83 greater than that of the IS-RILs (t = 36.19, p<0.001, S3 Table). Broad sense heritability estimates for TL were intermediate for all three populations, at 35%, 36%, and 30% for the PQRIL, ISRIL and SH-BC1F2 populations, respectively (S1–S3 Tables).

The number of secondary branches per primary tiller (BRCH) is sensitive to environmental changes and is also a fail-safe for a plant in case the primary seed head is damaged. Average BRCH of S. halepense is 13, dramatically higher than the 0.286 of S. bicolor BTx623 (S1 Table). There were no statistically significant differences for BRCH between diploid and tetraploid BTx623 in Athens 2014, Salina 2013 and 2014, while there was 2.1 more BRCH in tetraploid BTx623 than in diploid BTx623 (t = 4.16, p = 0.0011) in Athens 2013. The average number of BRCH of most progeny lines fell within the range of the respective parents. For the SH-BC1F2 progeny lines, the average number of BRCH in Athens was 1.29 more than in Salina (t = 25.50, p<0.001). Average BRCH in Athens was 0.45 more in 2013 than 2014 (t = 7.70, p<0.001); and in Salina was 0.60 more in 2013 than 2014 (t = 7.98, p<0.001). The average number of BRCH of the SH-BC1F2 population was 2.28 smaller than that of the PQ-RIL population (t = -14.38, p<0.001, S2 Table), and 0.99 smaller than that of the ISRI population (t = -0.99, p<0.001, S3 Table). Broad-sense heritability estimates for BRCH are relatively low, 7% and 10% for the PQRIL and SH-BC1F2 populations, respectively, but intermediate for the ISRI population, 40.9% (S1–S3 Tables).

Trait correlations

In all four environments, FL is positively and significantly correlated with PH (Fig 2) [30], i.e., late flowering individuals are generally taller than early flowering ones. FL and TL are negatively correlated in both the H4 (p = 0.034) and H6- derived populations (p = 0.032) in Athens in 2013, and positive in the other three environments, although not significant (p>0.05) for H4-derived populations in Athens 2014 or Salina 2013 and the H6-derived population in Salina 2014. In three out of four environments, Athens 2013, Salina 2013 and 2014, FL and
BRCH are negatively correlated, with a non-significant positive correlation in Athens 2014. Correlations between TL and BRCH are generally positive, except for the H6 population in Athens 2013 where the correlation is negative but not significant.
Genetic analysis

Number of tillers. We detected a total of two marginal QTLs, qTL.4A.H4.1 and qTL.4D.H4.1, for TL in the H4-derived population (Table 1). qTL.4A.H4.1 is significant in both Athens 2013 and Salina 2014, and qTL.4D.H4.1 is significant in Salina 2013 and Salina 2014. An additive model of the two QTLs, qTL.4A.H4.1 and qTL.4D.H4.1 explains 13.9% of the total phenotypic variance in Salina 2014. Although the peaks of qTL.4A.H4.1 are ~26 cM apart in Athens 2013 and Salina 2014, their corresponding physical locations of one-lod interval in genetic distance overlap. No QTLs for TL were detected in Athens 2014. Both QTLs have positive allele effects, indicating that S. halepense alleles increase TL.

We detected a total of two QTLs and five marginal QTLs for the number of TL in the H6-derived population with only qTL.2C.H6.1 significant in both Athens 2013 and Salina 2013 (Table 1). Five QTLs detected in Athens 2013, qTL.2C.H6.1, qTL.6A.H6.1, qTL.6B.H6.1, qTL.9B.H6.1 and qTL.10C.H6.1, collectively explain 34% of the total phenotypic variance, one QTL detected in Athens 2014 explains 11.42% of the total phenotypic variance, and two QTLs detected in Salina 2013 explain 13.9% of the total phenotypic variance. No QTLs were found in Salina 2014. While most QTL alleles have positive allele effects, indicating that S. halepense alleles increase TL, two marginal loci (qTL.6B.H6.1 and qTL.10C.H6.1) detected in Athens 2013 have negative allele effects, with S. halepense alleles decreasing the number of tillers.

Using single marker analysis, we detected a total of 63, 46, 26 and 48 significant SNP markers (p < 10\(^{-3}\)) for TL for pooled (H4 and H6) data in Athens 2013, Athens 2014, Salina 2013 and Salina 2014, respectively, with only one SNP marker, S4_58879601, significant in all environments (S1 Fig and Fig 3). Fewer signals detected for TL in multiple environments reflect lower heritability and large genotype by environment interactions. In the H4 population, we detected two QTLs for TL, qTL2.H4.1 and qTL.H7.1 in addition to the two QTLs on chromosome 4 detected by interval mapping (S4 Table). As was true for H4 QTLs found by interval mapping, S. halepense alleles increase the number of TL. In the H6 population, we detected a total of 14 QTLs for TL on chromosomes 1 (2), 2, 3 (2), 4 (2), 6 (3), 9 (2), 10 (2) with three new QTLs (qTL4.H6.1, qTL4.H6.2 and qTL10.H6.2) not overlapping with any QTLs detected in the interval mapping, all with S. halepense alleles increasing the number of TL (S4 Table). The other 11 QTLs from the single-marker analysis overlap with seven QTLs from interval mapping based on their physical positions. We consider all marginal TL QTLs from interval

| QTL Name     | Pos (CM) | Pos (Mb) | LOD | % of Variance explained | Effect | Left (Mb) | Right (Mb) | Env   |
|--------------|----------|----------|-----|-------------------------|--------|-----------|------------|-------|
| qTL.4A.H4.1  | 123.2    | 53.4     | 2.6 | 7.94                    | 0.58   | 52.5      | 61.2       | AT13  |
| qTL.4D.H4.1  | 149.0    | 58.9     | 2.6 | 8.53                    | 1.01   | 57.4      | 61.2       | SL14  |
| qTL.1D.H6.1  | 100.4    | 65.3     | 2.8 | 11.42                   | 1.38   | 19.2      | 65.3       | AT14  |
| qTL.2C.H6.1  | 108.0    | 9.0      | 3.0 | 8.34                    | 1.21   | 8.4       | 65.8       | AT13  |
| qTL.2C.H6.1  | 110.0    | 9.0      | 2.5 | 10.68                   | 1.19   | 8.4       | 65.8       | SL13  |
| qTL.3E.H6.1  | 205.0    | 59.7     | 2.9 | 11.15                   | 1.05   | 4.5       | 59.7       | SL13  |
| qTL.6A.H6.1  | 186.0    | 57.5     | 3.0 | 11.81                   | 0.73   | 56.5      | 60.5       | AT13  |
| qTL.6B.H6.1  | 121.0    | 47.2     | 6.7 | 24.58                   | -1.09  | 47.2      | 50.9       | AT13  |
| qTL.9B.H6.1  | 55.0     | 53.6     | 3.2 | 12.57                   | 0.78   | 47.9      | 55.8       | AT13  |
| qTL.10C.H6.1 | 87.7     | 6.0      | 2.8 | 11.69                   | -0.74  | 1.2       | 12.8       | AT13  |

LOD scores in bold suggest significance beyond the permutation test.

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mapping to be real QTLs, since results from single-marker analysis suggested lower P-values (smaller than 0.0001) for the peak SNPs (S4 Table).

**Number of secondary branches per primary branch (BRCH).** We detected a total of five QTLs and two marginal QTLs for BRCH in the H4-derived population, including six from Athens 2014 and one from Salina 2013 (Table 2). No QTLs were found in Athens 2013 or Salina 2014. The six QTLs detected in Athens 2014 together explain 22.0% of the total phenotypic variance, while the one QTL detected from Salina 2013 explains about 8.28% of the phenotypic variance. It is interesting that six out of seven QTLs show negative allele effects (Table 2), suggesting that *S. halepense* alleles contribute to decreased BRCH, which is unexpected and contrary to the difference between parents. Those QTLs with negative additive effect might reflect late release of apical dominance from *S. halepense*, which is associated with fewer BRCH.

We detected a total of five QTLs and two marginal QTLs for BRCH in the H6-derived population, with one QTL, qBRCH.3E.H6.1, significant in two environments, Salina 2013 and 2014 (Table 2). Two BRCH QTLs found in Athens 2014, qBRCH.1C.H6.1 and qBRCH.10C.H6.2, three BRCH QTLs found in Salina 2013, qBRCH3E.H6.1, qBRCH6B.H6.1, qBRCH10C.H6.1, and two BRCH QTLs found in Salina 2014, collectively explain 19.3%, 19.5% and 26.4% of the total phenotypic variance, respectively. For four QTLs, qBRCH.1C.H6.1, qBRCH.3E.H6.1, qBRCH.5C.H6.1 and qRBCH10C.H6.1, *S. halepense* alleles increase BRCH as predicted based on the parental phenotypes, while *S. halepense* alleles decrease BRCH for the other three QTLs, qBRCH6b.H6.1, qBRCH.6B.H6.2 and qBRCH.10C.H6.2.

We detected a total of 4, 110, 65 and 20 significant SNP markers (p<10−3) for BRCH in Athens 2013, Athens 2014, Salina 2013 and Salina 2014 for pooled data with very little
correspondence among different environments. This observation is consistent with low heritability estimates and large genotype by environment interactions (S2 Fig and Fig 4). In the H4-derived population, we detected a total of 11 QTLs for BRCH on chromosomes 1 (2), 3, 4

Table 2. Parameters of branching (BRCH) QTLs from interval mapping of the H4 and H6 SBSH-BC1F2 populations.

| QTL Name       | POS (cM) | POS (Mb) | LOD | % Var Explained | Effect | Left (Mb) | Right (Mb) | Env    |
|----------------|----------|----------|-----|-----------------|--------|-----------|------------|--------|
| qBRCH.1F.H4.1  | 4.0      | 1.6–3.2  | 3.7 | 11.56           | -0.47  | 1.6       | 8.6        | AT14   |
| qBRCH.2D.H4.1  | 122.0    | 74.5     | 3.0 | 9.98            | -0.38  | 66.1      | 75.5       | AT14   |
| qBRCH.4C.H4.1  | 8.0      | 4.8      | 2.6 | 8.48            | -0.34  | 3.7       | 6.9        | AT14   |
| qBRCH.4D.H4.1  | 102.7    | 61.2     | 3.2 | 10.22           | -0.38  | 20.7      | 61.8       | AT14   |
| qBRCH.5C.H4.1  | 59.8     | 11.6     | 3.6 | 11.46           | -0.38  | 1.7       | 57.9       | AT14   |
| qBRCH.6B.H4.1  | 8.2      | 0.9      | 2.6 | 8.28            | -0.48  | 0.9       | 37.2       | SL13   |
| qBRCH.7C.H4.1  | 86.0     | 61.6     | 3.1 | 9.96            | 0.36   | 56.5      | 62.8       | AT14   |
| qBRCH.1C.H6.1  | 142.0    | 70.2     | 3.5 | 13.82           | 0.41   | 69.1      | 72.5       | AT14   |
| qBRCH.3E.H6.1  | 203.0    | 59.7     | 3.8 | 13.72           | 0.95   | 4.5       | 59.7       | SL13   |
| qBRCH.5C.H6.1  | 218.0    | 59.7     | 3.9 | 7.91            | 0.88   | 2.7       | 59.7       | SL14   |
| qBRCH.3E.H6.1  | 6.0      | 54.5     | 6.1 | 19.19           | 1.37   | 2.6       | 3.1        | AT13   |
| qBRCH.6B.H6.1  | 20.0     | 3.1      | 3.5 | 13.72           | -0.63  | 2.0       | 42.2       | SL13   |
| qBRCH.6B.H6.2  | 95.0     | 47.0     | 2.9 | 11.66           | -0.65  | 3.3       | 50.9       | SL14   |
| qBRCH.10C.H6.1 | 19.2     | 2.4      | 4.1 | 16.55           | 1.11   | 2.4       | 58.3       | SL13   |
| qBRCH.10C.H6.2 | 91.0     | 6.0      | 2.8 | 11.76           | -0.38  | 1.2       | 53.4       | AT14   |

LOD scores in bold suggest significance beyond the permutation test.

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Fig 4. Single marker analysis for the number of secondary branches per tiller in the H4, H6-derived and the pooled BC1F2 populations.

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(2), 5, 6(2), 7, 9, 10, with three negative effect QTLs, suggesting that *S. halepense* alleles at these loci decrease BRCH (*S5 Table*). A total of four QTLs, qBRCH1.H4.2, qBRCH3.H4.1, qRBCH9.H4.1 and qBRCH10.H4.1 were newly detected only in the single-marker analysis, all with *S. halepense* alleles increasing BRCH. In the H6 population, we detected a total of 11 QTLs on chromosomes 1, 3 (2), 4, 5, 6(3), 7, 9 10 with only one negative effect QTL, qBRCH.H6.2 (*Table 2*). A total of three QTLs, qBRCH4.H6.1, qRBCH7.H6.1 qRBCH9.H6.1, were newly detected in the single-marker analysis, with all three increasing BRCH. The other 8 QTLs detected in single markers analysis overlap with the seven BRCH QTLs from interval mapping by comparing their physical positions. We consider all marginal BRCH QTLs from interval mapping to be the real QTL, since single-marker analysis suggested lower P-values (smaller than 0.0001) for the peak SNPs (*S5 Table*).

QTL correspondence across traits in the BC1F2 population

In most environments, TL and BRCH are significantly and positively correlated (*Fig 2*), therefore some QTL regions are expected to overlap due to their developmental relationship [6]. Indeed, we found two TL QTLs, qTL2.H4.1 and qTL4D.H4.1 overlapping with qBRCH.2D.H4.1 and qBRCH.4D.H4.1 in the H4-derived population based on their physical positions. Four QTLs, qTL3E.H6.1, qTL6B.H6.1, qTL6A.H6.1 and qTL10C.H6.1 overlap with qBRCH.3E.H6.1, qBRCH.6B.H6.2, qBRCH.6H.6.3 and qBRCH.10C.H6.2 in the H6-derived population, respectively. Interestingly, *S. halepense* contributed opposite allele effects for the two pairs of overlapping QTLs in the H4-derived population (*S. halepense* alleles increased TL but decreased BRCH), but the same effect for all overlapping pairs in the H6-derived population.

Recent studies have suggested that genes controlling days to flowering might also influence tillering and vegetative branching [1,13,37,38]. We found a total of six TL QTLs overlapping with FL QTLs in the H6-derived population, with two pairs of QTLs, qTL.4.H6.1 with qFL4A.H6.1 and qTL6B.H6.1 with qFL6B.H6.2, showing opposite effects from *S. halepense* (*Table 3*).

Similarly, a total of two and five QTLs for BRCH show possible correspondence to FL in the H4 and H6-derived BC1F2 populations, respectively (*Table 4*), with four pairs of overlapping QTLs showing opposite allele effect from *S. halepense*. Additional QTLs that overlap but are not limited to the same population are qBRCH.4C.H4.1 and qFL4A.H6.1; qTL6B.H6.1 and qFL6B.H6.2, showing opposite effects from *S. halepense* (*Table 3*).

Comparison to QTLs found in IS-RIL and PQ-RIL

We found a total of five TL QTLs in the SH-BC1F2, qTL.1D.H6.1, qTL.3E.H6.1, qTL6A.H6.1, qTL6B.H6.1, and qTL7.H4.1, corresponding in physical position on the sorghum genome sequence to four IS-RIL TL QTLs, qTL._1.1, qTL._3.1, qTL._6.1 and qTL7.1; and a total of seven SBSH-BC1F2 TL QTLs corresponding to five PQ-RIL QTLs (*Table 3*). Curiously, qTL6A.H6.1 and qTL6B.H6.1, not overlapping with each other but both overlapping with QTLs found in the IS-RIL and PQ-RIL populations, display opposite allele effects. qTL6B.H6.1 from the SH-BC1F2 population shows a negative effect, indicating that fewer tillers may be associated with late flowering, which might be associated with the *Ma1* [18] gene on chromosome 6. However, there appears to be another QTL region on chromosome 6 significant in all three populations, roughly spanning 50–60 Mb, and suggesting that *S. bicolor* alleles reduce the number of tillers.
Despite that BRCH is a plastic trait with low heritability, we still found two BRCH QTLs in the H4-derived SBSH-BC\textsubscript{1}F\textsubscript{2}, qBRCH4D.H4.1 and qBRCH5C.H4.1 overlapping with two IS-RIL QTLs, qRBCH4.1 and qRBCH5.1; and three H6-derived SBSH-BC\textsubscript{1}F\textsubscript{2} QTLs overlapping with two IS-RIL QTLs, qRBCH4.2 and qRBCH10.1 (Table 4). Two pairs of QTLs, qRBCH.5C.H4.1 and qRBCH5.1, from IS-RIL show opposite allele effects from S. halepense, suggesting that S. bicolor alleles increase BRCH in the SBSH-BC\textsubscript{1}F\textsubscript{2} population but decrease it in the IS-RIL. This implies that IS3620c has an allele conferring abundant branching, with the BTx623 allele conferring less branching than IS3620c but more than S. halepense. In addition, a total of five and four H4 and H6–derived SBSH-BC\textsubscript{1}F\textsubscript{2} QTLs for BRCH overlap with QTLs for various degrees (primary, secondary or tertiary) of vegetative branching in PQRIL described in Kong, Guo [6]. Most overlapping pairs of QTLs of SBSH-BC\textsubscript{1}F\textsubscript{2} and the PQRIL show the same direction of effect, from S. halepense and S. propinquum, respectively, except one case on chromosome 7 where QTLs within PQRIL show different directions of effects (Table 4; within the PQRIL population, qSR\textsubscript{7}.1 showed negative effect while qVG7.1 and qIM2\textsubscript{7}.1 showed positive effect).

**A regression model to predict biomass**

We performed a regression analysis to predict biomass weight (Biomass, using natural log transformation) with respect to traits related to plant architecture while controlling for population structure and environmental factors. Our final model consists of a total of seven variables, with plant height (PH), mid-stalk diameter (MD), number of mature tillers (TL), number of secondary branches (BRCH), flowering time (FL), and population (H4 or H6) as fixed effects and environmental factors as a random effect (Table 5). Fixed effects (the environmental factor) in this model collectively explain about 71.76% of the total variance using a modified method for estimating R-squared in mixed models [36]. The typical log error in this model is about 0.3148, and can be decomposed into environmental error that is estimated to be normally distributed with a mean of zero and standard deviation of 0.1260; and the inherent residual error that is estimated to be normally distributed with a mean of zero and standard deviation of 0.2885 (Table 5A). The model suggests that PH, TL and MD are the three most important variables in predicting Biomass, followed by FL and BRCH (Table 5B). For

**Table 3. Comparisons of TL and FL QTL in the SBSH-BC\textsubscript{1}F\textsubscript{2}, IS-RIL and PQRIL population.**

| QTL Name | ISRIL | PQRIL | FLQTL |
|----------|-------|-------|-------|
| qTL2.H4.1 (+) | qM1.2.1 (+) | | |
| qTL4A.H4.1 (+) | qTL4.1 (-) | | |
| qTL4D.H4.1 (+) | qTL4.1 (-) | | |
| qTL7.H4.1 (+) | qTL7.1 (+) | qTL7.1 (+) | |
| qTL1D.H6.1 (+) | qTL1.1 (+) | | qFL1C.H6.1 (+) |
| qTL2C.H6.1 (+) | | qM1.2.1 (+) |
| qTL3E.H6.1 (+) | qTL3.1 (+) | | |
| qTL4.H6.1 (+) | | qFL4A.H6.1 (-) |
| qTL4.H6.2 (+) | | qFL4D.H6.1 (+) |
| qTL6A.H6.1 (+) | qTL6.1 (+) | qM1.6.1 (+) | |
| qTL6B.H6.1 (-) | qTL6.1 (+) | qM1.6.1 (+) | qFL6B.H6.2 (+) |
| qTL9B.H6.1 (+) | | | |
| qTL10C.H6.1 (-) | | qFL10A.H6.1 (-) |
| qTL10.H6.2 (+) | | qFL10.H6.1 (+) |

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example, a 10 cm increase in plant height leads to 6.4% increase in Biomass weight, keeping other variables constant, while an increase of one TL leads to a 15.1% increase in Biomass weight, keeping other variables constant.

### Discussion

The present study offers several new insights into the genetic control of tillering and vegetative branching. First, it adds more information to current knowledge of vegetative branching in sorghum, an under-studied trait, especially providing early insights into QTL polymorphism in *S. halepense*. Correspondence of QTL regions between three populations sharing *S. bicolor* BTx623 as a common parent, with the other parents being morphologically and genetically distinct genotypes that represent cultivated (IS3620C), wild diploid (*S. propinquum*) and wild polyploid (*S halepense*) sorghums, provides information about common QTLs shared between or among populations and taxon-specific QTLs that contribute to divergence. Finally, constructing a mixed model to predict dry biomass with respect to various traits associated with plant architecture and the environmental factors provides a framework to prioritize each trait in selection for biomass, as well as quantifying environmental influences.
QTL mapping

QTL mapping results for two relatively plastic traits, TL and BRCH, suggest high genotype by environment interactions and population differences. We only found three and one QTLs significant in multiple environments for TL and BRCH with interval mapping, respectively, with 6 and 13 significant in only single environments. Overlapping SNP sets from single marker analysis are much lower for these traits than for highly heritable traits such as plant height and flowering time (S1 and S2 Figs).

QTL results are very different in the two populations derived from two different sibling S. halepense x S. bicolor F1 plants, possibly due to Ma and Dw genes on chromosome 6. We detected fewer TL QTLs in the H4 than the H6-derived population, as was also true of FL and PH QTLs [30]. The number of BRCH QTLs for the two populations does not follow this pattern, but most H4-derived QTLs had negative effects in interval mapping, indicating the S. halepense allele reduced BRCH. Unexpected cases in which S. halepense alleles reduce TL associated with FL QTLs, qTL4.H6.1 and qFL4A.H6.1, qTL6B.H6.1 and qFL6B.H6.2; and S. halepense alleles that reduce BRCH associated with FL QTLs, qBRCH1.H4.1 and qFL1A.H4.1, qBRCH6B.H4.1, and qFL6B.H4.1, qBRCH6B.H6.1 and qFL6B.H6.1, and qBRCH6B.H6.2 and qFL6B.H6.2 (Tables 3 and 4). This finding suggests that delaying flowering might reduce tillers and branching, perhaps due to late release of apical dominance.

Table 5. A mixed-effect model and parameter estimations for predicting Biomass (natural log transformation) in the SBSH-BC1F2 population.

| (a) Variance components |
|-------------------------|
| Groups                      | Variance | Std. dev. |
| Env                        | 0.01589  | 0.1260    |
| Residual                   | 0.08324  | 0.2885    |

| (b) Modeling |

|            | Sum Sq | Mean Sq | DF  | F-stat  | P value |
|------------|--------|---------|-----|---------|---------|
| PH         | 32.561 | 32.561  | 1   | 391.19  | < 2.2e-16*** |
| MD         | 10.609 | 10.609  | 1   | 127.46  | < 2.2e-16*** |
| TL         | 25.421 | 25.421  | 1   | 305.42  | < 2.2e-16*** |
| BRCH       | 2.303  | 2.303   | 1   | 27.67   | 1.901e-07 ***|
| FL         | 4.892  | 4.892   | 1   | 58.77   | 4.396e-14 ***|

| (c) Parameter estimation |

|            | Estimate | Std. Error | df  | t-stat  | P-value |
|------------|----------|------------|-----|---------|---------|
| Intercept  | 2.6460   | 0.1066     | 19.8| 24.815  | 2.22E-16*** |
| PH         | 0.006197 | 0.000313   | 936.3| 19.779  | < 2e-16*** |
| MD         | 0.02962  | 0.002624   | 953.4| 11.290  | < 2e-16*** |
| TL         | 0.1409   | 0.008061   | 675.1| 17.476  | < 2e-16*** |
| BRCH       | 0.06978  | 0.01327    | 720.6| 5.260   | 1.9E-07 ***|
| FL         | 0.007882 | 0.001028   | 942.7| 7.666   | 4.4E-14*** |
| Population | -0.09472 | 0.01960    | 954.8| -4.833  | 1.56E-06*** |

PH: Plant height.  
MD: Mid-stalk diameter.  
TL: Number of mature tillers.  
BRCH: Number of secondary branches per tiller.  
Env: Environmental effects.

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QTL correspondence

Two TL QTLs and one BRCH QTL overlapped in all three populations (this study, ISRIL and PQRIL) with the same direction of allele effect (Tables 3 and 4), suggesting a parsimonious hypothesis that *S. halepense*, *S. propinquum* and *S. bicolor* IS3620C share an ancestral allele, while a different recently-derived allele has been selected in the elite cultivar *S. bicolor* BTx623. Cases in which overlapping QTLs have different directions of allele effect are more complex, possibly suggesting more than two alleles, or perhaps representing spurious correspondence due to relatively large QTL intervals.

The *S. halepense* data continue to support the hypothesis that TL and BRCH are developmentally related [6]—six QTL pairs (qTL2.H4.1 and qBRCH.2D.H4.1, qTL4D.H4.1 and qBRCH.4D.H4.1, qTL3E.H6.1 and qBRCH.3E.H6.1, qTL6B.H6.1 and qBRCH.6B.H6.2, qTL6A.H6.1 and qBRCH6.H6.3, qTL10C.H6.1 and qBRCH.10C.H6.2) overlapped, perhaps harboring genes influencing axillary meristem development at early stages.

A surprising number of genomic regions were significant for FL and TL or FL and BRCH, perhaps suggesting pleiotropic relationships (Tables 3 and 4, Fig 5). For example, genes regulating flowering such as MADS box proteins also influence determinacy of other meristems [39]. Further, the flowering locus T (*FT*) gene that regulates flowering time in many species, has recently been found to trigger storage organ formation through direct interaction with the TCP factors [13]. We found a total of six genomic regions harboring QTLs responsible for both FL and TL, and four regions for both FL and BRCH in their respective populations. Previous study [21,27,40] has suggested that regions on chromosome 6 that harbor *Ma1* also contain QTLs for tiller number. One explanation might be that *Ma1*, which appears to be a homolog of the Arabidopsis *Ft* and Rice *Hd3a* genes [18], influences organ formation. The *Ma1* associated region in this study affected both TL and BRCH, while another QTL region at ~47.2Mb on chromosome 6 affecting all three traits, FL, TL and BRCH. This QTL (~47.2Mb) might be related to the Sb06g019010 or (Sobic.006G107400.1 in *Sorghum bicolor* v3.11) gene encoding the ‘number of apical meristem’ (NAM) protein [6,41].
Regression model for predicting biomass

Plant architecture related traits can predict Biomass with relatively high accuracy. A mixed model for predicting dry biomass weight (Biomass) retained a total of five traits, plant height (PH), mid-stalk diameter (MD), mature tillers (TL), number of secondary branches (BRCH), and flowering time (FL) as significant predictors of dry biomass. The fixed effects explains 71.76% of the total variance, and a log error of 0.3148. Application of this model might be a cost-efficient method for predicting Biomass for future experiments, quantifying the contribution of individual traits to Biomass and providing guidance for improving genotypes aimed at biomass production.

Supporting information

S1 Fig. Venn diagram of the number of SNP markers for tillering (TL) significant at a p value < 10^{-3} in different environments for pooled SBSH BC1F2 populations.

S2 Fig. Venn diagram of the number of SNP markers for secondary branches per tiller (BRCH) significant at a p value < 10^{-3} in different environments for SBSH BC1F2 pooled populations.

S1 Table. Summary statistics for number of mature tillers (TL) and number of secondary branches (BRCH) in the SBSH-BC1F2 [S. halepense derived (S. bicolor BTx623× S. halepense G9E) backcross] population and parents.

S2 Table. Summary statistics for the number of mature tillers (TL) and number of secondary branches (BRCH) in the PQ-RIL [propinquum derived (S. bicolor BTx623× S. propinquum) recombinant inbred line] population and parents.

S3 Table. Summary statistics for number of mature tillers (TL) and number of secondary branches (BRCH) in the IS-RIL [IS3620C derived (S. bicolor BTx623× S. bicolor IS3620C) recombinant inbred line] population and parents.

S4 Table. Parameters of tillering (TL) QTLs from single marker analysis of the H4 and H6 SBSH-BC1F2 populations.

S5 Table. Parameters of number of secondary branches per tiller (BRCH) QTLs from single marker analysis of the H4 and H6 SBSH-BC1F2 populations.

S6 Table. Parameters of tillering and vegetative branching related QTLs from interval mapping of the IS-RIL population.

S7 Table. Parameters of plant architecture related QTLs from interval mapping of the PQ-RIL population.

S1 File. Phenotype of the H4 population.
S2 File. Phenotype of the H6 population.
(XLSX)

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References
1. Rameau C, Bertheloot J, Leduc N, Andrieu B, Foucher F, Sakr S. Multiple pathways regulate shoot branching. Front Plant Sci. 2014; 5:741. https://doi.org/10.3389/fpls.2014.00741 PMID: 25628627; PubMed Central PMCID: PMC4292231.
2. McSteen P. Hormonal Regulation of Branching in Grasses. Plant Physiology. 2009; 149(1):46–55. https://doi.org/10.1104/pp.108.129056 WOS:000262261500007. PMID: 19126694.
3. Kebrom TH, Spielmeyer W, Finnegan EJ. Grasses provide new insights into regulation of shoot branching. Trends in Plant Science. 2013; 18(1):41–8. https://doi.org/10.1016/j.tplants.2012.07.001 WOS:000314022000006. PMID: 22858267.
4. Krishna Reddy S, Finlayson SA. Phytochrome B promotes branching in Arabidopsis by suppressing auxin signaling. Plant Physiol. 2014; 164(3):1542–50. PubMed Central PMCID: PMC3938639. https://doi.org/10.1104/pp.113.234021 PMID: 24492336.
5. Kebrom TH, Brutnell TP, Finlayson SA. Suppression of sorghum axillary bud outgrowth by shade, phyB and defoliation signalling pathways. Plant Cell Environ. 2010; 33(1):48–58. https://doi.org/10.1111/j.1365-3040.2009.02050.x ISI:000272661000005. PMID: 19843258.
6. Kong W, Guo H, Goiff V, Lee T-H, Kim C, Paterson A. Genetic analysis of vegetative branching in sorghum. Theoretical and Applied Genetics. 2014; 127(11):2387–403. https://doi.org/10.1007/s00122-014-2384-x PMID: 25163936.
7. Doust AN. Grass architecture: genetic and environmental control of branching. Current Opinion in Plant Biology. 2007; 10(1):21–5. https://doi.org/10.1016/j.pbi.2006.11.015 ISI:000244006000004. PMID: 17140840.
8. Wang YH, Li JY. Branching in rice. Current Opinion in Plant Biology. 2011; 14(1):94–9. https://doi.org/10.1016/j.pbi.2010.11.002 ISI:000275551000015. PMID: 21144796.
9. Mueller D, Leyer O. Auxin, cytokinin and the control of shoot branching. Annals of Botany. 2011; 107 (7):1203–12. https://doi.org/10.1093/aob/mcr069 WOS:000290590700014. PMID: 21504914.
10. Gallavotti A. The role of auxin in shaping shoot architecture. Journal of Experimental Botany. 2013; 64(9):2593–608. https://doi.org/10.1093/jxb/er1141 WOS:000319433800006. PMID: 23709672

11. Waldie T, Hayward A, Beveridge CA. Auxillary bud outgrowth in herbaceous shoots: how do strigolactones fit into the picture? Plant Molecular Biology. 2010; 73(1–2):27–36. https://doi.org/10.1007/s11103-010-9599-2 WOS:000276440900004. PMID: 20112050

12. Waldie T, McCulloch H, Leyer S. Strigolactones and the control of plant development: lessons from shoot branching. Plant J. 2014; 79(4):607–22. https://doi.org/10.1111/tpj.12488 WOS:000340499200006. PMID: 24612082

13. Navarro C, Cruz-Oro E, Prat S. Conserved function of FLOWERING LOCUS T (FT) homologues as signals for storage organ differentiation. Current Opinion in Plant Biology. 2015; 23:45–53. https://doi.org/10.1016/j.pbi.2014.10.008 WOS:000349880900008. PMID: 25449726

14. Paterson A, H., Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, et al. The Sorghum bicolor genome and the diversification of grasses Nature. 2009; 457:551–6. https://doi.org/10.1038/nature07723 PMID: 19189423

15. Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, et al. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(2):453–8. https://doi.org/10.1073/pnas.1215985110 WOS:000319306600023. PMID: 23267105

16. Yang SS, Murphy RL, Morishige DT, Klein PE, Rooney WL, Mullet JE. Sorghum Phytochrome B Inhibits Flowering in Long Days by Activating Expression of SbPBR37 and SbGHD7, Repressors of SbEHD1, SbCN8 and SbCN12. Plos One. 2014; 9(8). ARTN e105352 https://doi.org/10.1371/journal.pone.0103532 WOS:000341017000118. PMID: 25122453

17. Yang SS, Weers BD, Morishige DT, Mullet JE. CONSTANS is a photoperiod regulated activator of flowering in sorghum. Bmc Plant Biology. 2014;14. Art148 https://doi.org/10.1186/1471-2229-14-14 WOS:000338156700005. PMID: 24405939

18. Cuevas HE, Zhou C, Tang H, Khadikc PP, Das S, Lin YR, et al. The Evolution of Photoperiod-Insensitive Flowering in Sorghum, A Genomic Model for Panicoid Grasses. Mol Biol Evol. 2016; 33(9):2417–28. https://doi.org/10.1093/molbev/msw120 PubMed Central PMCID: PMC4989116. PMID: 27335143

19. Zhang D, Kong W, Robertson J, Goff VH, Epps E, Kerr A, et al. Genetic analysis of inflorescence and plant height components in sorghum (Panicoidae) and comparative genetics with rice (Oryzoidae). BMC Plant Biol. 2015; 15:107. https://doi.org/10.1186/s12870-015-0477-6 PMID: 25896918; PubMed Central PMCID: PMC4404672.

20. Murphy RL, Klein RR, Morishige DT, Brady JA, Rooney WL, Miller FR, et al. Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(19):16469–74. https://doi.org/10.1073/pnas.1106212108 WOS:000285255300006. PMID: 21930910

21. Lin YR, Schertz KF, Paterson AH. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics. 1995; 141(1):391–411. Epub 1995/09/01. PMID: 8536986; PubMed Central PMCID: PMC1206736.

22. Murray SC, Rooney WL, Hamblin MT, Mitchell SE, Kresovich S, Sweet Sorghum Genetic Diversity and Association Mapping for Brix and Height. Plant Genome-U s. 2009; 2(1):48–62. https://doi.org/10.3835/plantgenom2008.10.0111 WOS:000208575800008.

23. Brown PJ, Rooney WL, Franks C, Kresovich S. Efficient mapping of plant height quantitative trait loci in a sorghum association population with introgressed dwarfing genes. Genetics. 2008; 178(1):629–37. https://doi.org/10.1534/genetics.108.092239 PMID: 18757942; PubMed Central PMCID: PMC2535712.

24. Upadhyaya HD, Wang Y-H, Sharma S, Singh S. Association mapping of height and maturity across five environments using the sorghum mini core collection. Genome. 2012; 55(6):471–9. https://doi.org/10.1139/g2012-034 WOS:000305294800007. PMID: 22680231

25. Upadhyaya HD, Wang YH, Sharma S, Singh S, Hasenstien KH. SSR markers linked to kernel weight and tiller number in sorghum identified by association mapping. Euphytica. 2012; 187(3):401–10. https://doi.org/10.1007/S10681-012-0726-9 WOS:000308355000010.

26. Hart GE, Schertz KF, Peng Y, Syed NH. Genetic mapping of Sorghum bicolor (L) Moench QTLs that control variation in tillering and other morphological characters. Theoretical and Applied Genetics. 2001; 103(8):1232–42. WOS:000173261300015.

27. Paterson AH, Schertz KF, Lin YR, Liu SC, Chang YL. The Weediness of Wild Plants—Molecular Analysis of Genes Influencing Dispersal and Persistence of Johnsongrass, Sorghum-Halepense (L) Pers. Proceedings of the National Academy of Sciences of the United States of America. 1995; 92(13):6127–31. https://doi.org/10.1073/pnas.92.13.6127 WOS:A1995RF050000076. PMID: 11607551
28. Alam MM, Mace ES, van Oosterom EJ, Cruickshank A, Hunt CH, Hammer GL, et al. QTL analysis in multiple sorghum populations facilitates the dissection of the genetic and physiological control of tillering. Theoretical and Applied Genetics. 2014; 127(10):2253–66. https://doi.org/10.1007/s00122-014-2377-9 WOS:000342494500016. PMID: 25163934

29. Kong W, Nabukalu P, Cox TS, Goff VH, Pierce GJ, Lemke C, et al. Transmission Genetics of a Sorghum bicolor × S. halepense Backcross Populations. Front Plant Sci. 2020; 11(467). https://doi.org/10.3389/fpls.2020.00467 PMID: 32425964

30. Kong W, Nabukalu P, Cox T, Goff VH, Robertson JS, Pierce GJ, et al. Quantitative trait mapping of plant architecture in two BC 1 F 2 populations of Sorghum Bicolor × S. halepense and comparisons to two other sorghum populations. Theoretical and Applied Genetics. 2021:1–16. https://doi.org/10.1007/s00122-020-03709-7 PMID: 33136168

31. Kong W, Kim C, Zhang D, Guo H, Tan X, Jin H, et al. Genotyping by Sequencing of 393 Sorghum bicolor BTx623 x IS3620C Recombinant Inbred Lines Improves Sensitivity and Resolution of QTL Detection. G3 (Bethesda). 2018; 8(8):2563–72. Epub 2018/06/02. https://doi.org/10.1534/g3.118.200173 PMID: 29853656; PubMed Central PMCID: PMC6071585.

32. Kong W, Jin H, Franks CD, Kim C, Bandopadhayay R, Rana MK, et al. Genetic Analysis of Recombinant Inbred Lines for Sorghum bicolor × Sorghum propinquum. G3 Genes| Genomes| Genetics. 2013; 3:101–8. https://doi.org/10.1534/g3.112.004499 PMID: 23316442

33. Lander ES, Botstein D. Mapping Mendelian Factors Underlying Quantitative Traits Using Rflp Linkage Maps. Genetics. 1989; 121(1):185–99. WOS:A1989 R676700018. PMID: 2563713

34. Kuznetsova A, Brockhoff PB, Christensen RHB. Package ‘lmerTest’. R package version. 2015;2.

35. Bates D, Mächler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using Lme4. J Stat Softw. 2015; 67(1):1–48. WOS:000365981400001.

36. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution. 2013; 4(2):133–42. https://doi.org/10.1111/j.2041-210x.2012.00261.x

37. Rao NN, Prasad K, Kumar PR, Vijayraghavan U. Distinct regulatory role for RFL, the rice LFY homolog, in determining flowering time and plant architecture. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(9):3646–51. https://doi.org/10.1073/pnas.0709059105 WOS:000253846500083. PMID: 18305171

38. Peng M, Cui Y, Bi YM, Rothstein SJ. AtMBD9: a protein with a methyl-CpG-binding domain regulates flowering time and shoot branching in Arabidopsis. Plant J. 2006; 46(2):282–96. https://doi.org/10.1111/j.1365-313X.2006.02691.x PMID: 16623890.

39. Melzer S, Lens F, Gennenn J, Vanneste S, Rohde A, Beeckman T. Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. Nat Genet. 2008; 40(12):1489–92. https://doi.org/10.1038/ng.253 PMID: 18997783.

40. Feltus FA, Hart GE, Schertz KF, Casa AM, Brown P, Klein PE, et al. Genetic map alignment and QTL correspondence between inter- and intra-specific sorghum populations. Theoretical and Applied Genetics. 2006; 112:1295–305. https://doi.org/10.1007/s00122-006-0232-3 PMID: 16491426

41. Finn RD, Bateman A, Clements J, Coggil P, Eberhardt RY, Eddy SR, et al. Pfam: the protein families database. Nucleic Acids Research. 2014; 42(D1):D222–D30. https://doi.org/10.1093/nar/gkt1223 WOS:000331139800034. PMID: 24288371

42. Lee TH, Tang HB, Wang XY, Paterson AH. PGDD: a database of gene and genome duplication in plants. Nucleic Acids Research. 2013; 41(D1):D1152–D8. https://doi.org/10.1093/Nar/Gks1104 WOS:000312893300163. PMID: 23180799