Theor Appl Genet (2014) 127:2387–2403
DOI 10.1007/s00122-014-2384-x

ORIGINAL PAPER

Genetic analysis of vegetative branching in sorghum

Wenqian Kong · Hui Guo · Valorie H. Goff · Tae-Ho Lee · Changsoo Kim · Andrew H. Paterson

Received: 12 March 2014 / Accepted: 15 August 2014 / Published online: 28 August 2014

Abstract

Key message **We identified quantitative trait loci influencing plant architecture that may be valuable in breeding of optimized genotypes for sustainable food and/or cellulosic biomass production, and advancing resilience to changing climates.**

Abstract We describe a 3-year study to identify quantitative trait loci (QTLs) for vegetative branching of sorghum in a recombinant inbred line population of 161 genotypes derived from two morphologically distinct parents, *S. bicolor* × *S. propinquum.* We quantify vegetative branching based on morphological position and physiological status. Different sets of QTLs for different levels of branching were identified. QTLs discovered on chromosomes 1, 3, 7 and 8 affect multiple vegetative branching variables, suggesting that these regions may contain genes that control general axillary meristem initiation. Other regions that only influence one vegetative branching trait could contain genes that influence developmental processes contributing to divergent patterns of plant architecture. We investigate the relationship between vegetative branching patterns and dry biomass, and conclude that tillers with mature panicles and immature secondary branches each show consistent positive correlation with dry biomass. Among 19 branching-related genes from rice, eight sorghum homologs of seven rice genes are in syntenic blocks within branching-related QTL likelihood intervals. Five of these eight genes are within 700 kb of SNPs significantly associated with differences in branching in genome-wide association study of a diversity panel of 377 sorghum accessions, and three contain striking allelic variations between *S. bicolor* and *S. propinquum* that are likely to impact gene functions. Unraveling genetic determinants for vegetative branching may contribute to deterministic breeding of optimized genotypes for sustainable food and cellulosic biomass production in both optimal and marginal conditions, which are resilient to future climates that are more volatile and more stressful.

Communicated by Antonio Augusto Franco Garcia.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2384-x) contains supplementary material, which is available to authorized users.

W. Kong · H. Guo · V. H. Goff · T.-H. Lee · C. Kim · A. H. Paterson (✉)
Plant Genome Mapping Laboratory, University of Georgia, Athens, GA 30602, USA
e-mail: paterson@plantbio.uga.edu

Introduction

Plant architecture is determined by the sizes and shapes of plant organs, patterns of above-ground branching of stalks, and underground growth by roots and rhizomes (subterranean stems). Plant architecture decides the dispositions of vegetative organs that capture light, and the synchrony of inflorescence and seed development that are important factors for grain production. The temporal and spatial development of axillary buds is believed to be largely genetically controlled (Wang and Li 2006; Doust 2007b). Therefore, plant architecture frequently contributes to classification of different genotypes into taxa and genera. On the other hand, environmental factors such as density, humidity, temperature and nutrition allow those vegetative organs to achieve a high level of plasticity, making the body plan of a single species variable.

Understanding the genetics of plant architecture has taken on new importance with invigorated efforts to

develop plant genotypes optimized for production of biomass for use in fuels or chemical feedstocks. Increases in yield of one of the best studied biomass crops, sugarcane, have been achieved primarily by increasing source and sink capacity (Moore and Maretzki 1996; Moore et al. 1997). While tillering is an important element of sink capacity, additional factors including higher-order branching (i.e., 'secondary' branches from tillers) as well as stalk (tiller) dimensions must also be considered.

The timing of occurrence of plant architectural components such as branching can be of high importance. A degree of early season branching may confer some resilience to weather variations such as transient temperature extremes, for example by providing for some compensatory seed set if pollen viability on the primary inflorescence(s) is damaged. Late-season branching or post-harvest regrowth may be adaptive in the tropics toward a 'ratoon' crop, but is likely to be a futile waste of resources in temperate climates where cold temperatures prohibit maturation. This tradeoff may be of even greater importance under perennial production systems, which promise to mitigate many constraints to biomass production (Tilman et al. 2009) but which require a balance between single-harvest and overall-life-cycle productivity.

Analysis of genetically determined differences in branching of divergent genotypes provides a potential complement and supplement to physiological study of hormonal or nutritional factors that contribute to the high environmental plasticity of plant architecture (Alam et al. 2014). Identifying genes and discovering genetic pathways responsible for axillary meristem initiation and outgrowth have been a fertile research topic in tomato (Groot et al. 1994), rice (Komatsu et al. 2003; Li et al. 2003; Takeda et al. 2003), Arabidopsis (Sorefan et al. 2003), maize (Doebley et al. 1997; Gallavotti et al. 2004), pea, petunia (Simons et al. 2007) and barley (Dabbert et al. 2009, 2010). There is also growing insight into hormonal regulation of vegetative branching (McSteen 2009): auxin and cytokinin have long been known to affect vegetative branching (Leyser 2003, 2006; Shani et al. 2006; Kyozuka 2007), and the newly discovered hormone, strigolactone, has increased knowledge of molecules that influence vegetative branching (Gomez-Roldan et al. 2008; Umehara et al. 2008; Waldie et al. 2010). However, even the primary and best studied component of vegetative branching, tillering, is considered to be among the most 'plastic' of traits affecting biomass accumulation (Kim et al. 2010a, b). Genetic variation in tillering affects the dynamics of canopy development and hence the timing and nature of crop water limitations, with high tillering advantageous when water is plentiful but imparting vulnerability in water-limited circumstances (Hammer et al. 2006). This genotype-by-environment (GE) interaction applies broadly in breeding of modern cereals (Doust 2007a), with high tillering generally maximizing yield potential under high-input conditions but risking inefficient resource use under water-limited environments.

Sorghum (*Sorghum bicolor*) has rich morphological diversity from naturally occurring variation and divergent artificial selection regimes, making it an excellent system to study plant architecture. Sorghum uses C4 photosynthetic metabolism that is more water efficient and thought to be better adapted to tropical areas than C3 photosynthesis used by plants such as rice and wheat. The relatively small genome size (~730 Mb) of *Sorghum bicolor* among C4 plants has made it a botanical model and a valuable complement to rice as a C3 model (Paterson et al. 2009). To date, quantitative studies of plant architecture in sorghum have been limited to discover quantitative loci (QTLs) responsible for the number of tillers (Lin et al. 1995; Paterson et al. 1995; Hart et al. 2001; Murray et al. 2008a, b; Shiringani et al. 2010; Takai et al. 2012; Upadhyaya et al. 2012). Genome-wide association study (GWAS) has been broadly applied to unraveling the genetic basis for complex traits in crops, such as maize (Hufford et al. 2012), rice (Huang et al. 2010) and sorghum (Morris et al. 2013). We are not aware of priorQTL or GWAS research focused on identifying genetic determinants of vegetative branching patterns in sorghum, and little has been done in other species (Doust et al. 2004; Doust and Kellogg 2006).

We report a quantitative study to discover genomic regions that underlie different vegetative branching traits based on morphological positions and physiological status in sorghum. A cross between *Sorghum bicolor* and *S. propinquum* (Paterson et al. 2009), and their progenies has proved to offer rich information for a wide range of traits (Chittenden et al. 1994; Lin et al. 1995; Paterson et al. 1995; Bowers et al. 2003; Hu et al. 2003; Feltus et al. 2006).The genetic map of a recombinant inbred line (RIL) population has demonstrated its improved power to detect QTLs (relative to the F2 population from which it was derived by single-seed descent) in an example of detecting flowering QTLs (Kong et al. 2013). Since the RIL population was advanced in a temperate area, eliminating a short-day flowering gene from *S. propinquum* has reduced factors that would otherwise confound development of many traits, and may reveal QTLs more salient to growth and productivity in temperate regions. Exploring QTL intervals for causal genes has been accelerated by evaluating sorghum orthologs of published rice genes for vegetative branching, and by a genome-wide association study in a diversity panel consisting of 377 accessions including exotic genotypes converted to day-neutral flowering ($n = 228$) and elite breeding lines ($n = 149$). Characterizing the morphological and physiological distribution of vegetative branching patterns permits us to distinguish genomic regions that may exert general control over vegetative branching,

from those that may conferring specific levels or patterns of branching. Better understanding the genetic determinants of different branching patterns and their relationships may facilitate a variety of applications ranging from plant growth control to breeding for optimized genotypes in different environments.

Materials and methods

Plant materials

A total of 161 recombinant inbred lines (RIL) derived from a previously described F2 population (Paterson et al. 1995) of two morphologically different parents, *Sorghum bicolor* BTx623 and its wild relative, *Sorghum propinquum* (unnamed accession) were planted at the University of Georgia Plant Science Farm, Watkinsville, GA, USA, in 2009, 2010 and 2011. Single 1.5-m plots of each RIL were transplanted (on 20 May 2009 and 16 May 2011) or directly seeded (28 May 2010) in completely randomized designs.

Genetic map

A total of 161 RILs were assayed with 141 SSR markers with non-significant deviations from 1:1 segregation ratios. The linkage map constructed using MAPMAKER (Lander et al. 1987) collectively spanned 773.1 cM on 10 linkage groups. The average interval between consecutive loci is 5.48 cM, ranging from 0.0 cM between cosegregating markers to 25.7 cM in the largest gap on chromosome 5 (Kong et al. 2013).

Phenotype analysis

Our phenotyping system for vegetative branching integrates the morphological locations and physiological status of each branch; i.e., for each plant, we quantify the number of primary, secondary and tertiary branches based on their morphological locations, and the number of mature floral, immature floral, and vegetative branches based on their physiological status. Primary branches emanate from basal nodes, while secondary branches emanate from primaries, and tertiary branches emanate from secondaries. Higher-order branches, such as quaternary, occurred rarely and were recorded as tertiaries. The total of nine types of branches, mature primary (M1), mature secondary (M2), mature tertiary (M3), immature primary (IM1), immature secondary (IM2), immature tertiary (IM3), vegetative primary (V1), vegetative secondary (V2), and vegetative tertiary (V3), was recorded for two representative plants from each plot in each year (2009, 2010, 2011). Plants were measured at physiological maturity of most mature primary branches.

Data exploration

To utilize the nine branching measurements for effective QTL mapping, we used the following trait combinations. We classify the morphological positions of the branches of each plant based on the number of tillers (TL), which is the sum of primary branches from the basal nodes, and the number of axillary branches (AX), which is the sum of secondary and tertiary branches. We pooled secondary and tertiary branch numbers since both develop from the nodes of tillers or higher-order branches and they generally initiate at later developmental stages than primary branches. To distinguish the physiological maturity of each branch, we measured the numbers of mature (MA), immature (IM), and vegetative branches (VG). To investigate the genetic potential for forming axillary branches, we devised two more measurements, the secondary ratio (SR) and the tertiary ratio (TR). SR is the ratio of the number of secondary branches per node (determined by counting nodes on the most mature tiller and assuming that the number of nodes was similar on other mature tillers). TR is the ratio of the number of tertiary branches per secondary branch, since the number of nodes on secondary branches was not recorded. Trait means, standard deviations and correlation coefficients using phenotypic values were calculated with SAS®9.2 (SAS Institute, Cary NC).

We analyzed the impact of genotype (G), environment (E) and genotype by environment interaction (G × E) using analysis of variance with the type III sums of squares. Different years (from 2009 to 2011) were treated as different environments. Lines, environments, and their interactions were considered random factors. Variance components were used to calculate the broad-sense heritability $H = V_G \Big/ \left(V_G + \frac{V_{G\times E}}{E} + \frac{V_{residual}}{ER}\right)$, in which E is the number of environments and R is the number of replications. We conducted QTL analysis using both overall BLUP values across three different environments (years, Table 4) and single year values (Supplementary Table 1). The statistical analysis used SAS software PROC MIXED, Version 9.2 of the SAS system for Windows. Copyright© 2002–2008 SAS Institute Inc.

QTL analysis

Single marker analysis and composite interval mapping (CIM) were performed using Win QTL Cartographer V2.5_010 (Wang et al. 2011). CIM analysis used the standard model (model 6) with a walking speed of 1 cM and 10 cM window size. Significance thresholds (0.05 experiment-wise) were calculated by 1,000 permutation tests.

Multiple interval mapping (MIM) was used to estimate the total phenotypic variance explained with Win QTL Cartographer.

QTL nomenclature used a system that was described in rice (McCouch et al. 1997), starting with a '*q*', followed by an abbreviation of each trait (TL, AX, MA, IM, VG, SR, TR, M1 and IM2), then the year in which QTLs were detected (if not the overall BLUP values), then the chromosome number, and then a decimal number to differentiate multiple QTLs on the same chromosome.

Biomass analysis

To investigate the relationship between vegetative branching pattern and dry biomass, we conducted a regression study using phenotypic data from 2010 and 2011 for both the RIL population (biomass data were not collected in 2009), and a GWAS population (described below). Total dry vegetative biomass, which consists of both stem and leaf weights, was the response variable, and vegetative branching variables described above, together with plant height (PH) and stalk middle diameters (MD), were evaluated as explanatory variables. We controlled both year and replication in the analysis. To understand the contribution of each vegetative branching pattern to total dry vegetative biomass, we first predicted the total biomass with the total number of branches, and then dissected the vegetative branching pattern based on the seven branching variables, M1, IM1, IM2, IM3, V1, V2, V3 (the variables M2 and M3 were not informative, being highly skewed with the vast majority of values being zero). A backward selection was performed to eliminate insignificant variables at the alpha level of 0.05, and Bayesian information criterion (BIC) was used to select the best model. All statistical analysis used the R program (R Core Team 2013).

Identification of sorghum homologs of rice genes controlling vegetative branching

A total of 19 rice genes are known (to us, at the time of writing) that affect either axillary meristem initiation or outgrowth. We used the "Locus Search" function in the Plant Genome Duplication Database (Lee et al. 2013) to identify corresponding sorghum genes and investigate their proximity to QTLs for vegetative branching based on syntenic relationships between rice and sorghum. For rice genes that could not be traced to corresponding syntenic blocks on the sorghum genome, homologous sorghum genes were identified based on protein sequence similarity (MOC1 and RCN1). For those sorghum genes that locate in the likelihood intervals of QTLs conferring vegetative branching, we examined single-nucleotide variation (SNV) between the two mapping parents. The *S. propinquum* accession was sequenced by Illumina whole genome sequencing at 30× read depth, with ~22 % of reads mapped to the *S. bicolor* reference genome with mapping quality ≥20 using bwa (Li and Durbin 2009) and revealing ~5 million SNPs between *S. propinquum* and *S. bicolor* using reads of ≥29 mapping quality. Single-nucleotide variations (SNVs) are identified by aligning Illumina reads to the sorghum reference genome using bwa/samtools (Li and Durbin 2009; Li et al. 2009). Non-synonymous SNVs (nsSNVs) and SNVs inferred to have striking effects on protein function are identified using sorghum gene models from annotation version 1.4. The effect of nsSNP songene function is evaluated using a Function Index Score (FIS), which measures functional impact of a mutation on protein function using a gene evolution conservation profile as described by Paterson et al. (2012).

Genome-wide association study (GWAS)

Published SNPs based on genotyping-by-sequencing were used (Morris et al. 2013). Evaluation of nearby SNPs using genome-wide association data employed a diversity panel of 377 *S. bicolor* accessions (Morris et al. 2013) that we phenotyped as described for the RIL set in 2009–2010, and their distances to the sorghum genes have been listed. $\mathrm{Log}(n + 1/e)$ transformation was applied to TL, AX, MA, IM, VG, M1 and IM2; square-root transformation was applied to SR and TR for GWAS study. GWAS used a compressed mixed linear model (Zhang et al. 2010b) with the GAPIT R package (Lipka et al. 2012).

Results

Phenotypic distribution of traits

The means and ranges of the seven branching variables of one of the parents, BTx623, and the RILs are shown in Table 1. RIL means for both positions and maturities (TL, AX, MA, IM and VG) are larger than BTx623 (parental) means. The other parent, *S. propinquum*, is native to tropical or subtropical regions. Grown in a temperate region in this experiment, *S. propinquum* just starts to flower when the temperature reaches the freezing point. Therefore, its vegetative branching patterns were considered not representative of its mature state and were not used in this analysis. The short-day flowering trait was previously eliminated from the RILs (Kong et al. 2013).

A correlation matrix of seven vegetative branching traits is listed in Table 2. Two variables indicating the positions of vegetative branches, TL and AX, are correlated with each other (Table 2, $r = 0.5432$, $P < 0.0001$). Variables indicating the maturity of branches, MA, IM, and VG are also significantly correlated with each other ($r_{\mathrm{MA:IM}} = 0.6302$,

Table 1 Trait values for *S. bicolor* (BTx623) × *S. propinquum* recombinant inbred lines (RILs) and BTx623 in 3 years

Trait	2009			2010			2011		
	BTx623	RILs		BTx623	RILs		BTx623	RILs	
	Mean (SD)	Mean (SD)	Range	Mean (SD)	Mean (SD)	Range	Mean (SD)	Mean (SD)	Range
TL	2.25 (0.95)	14.41 (8.74)	1–45	5.13 (2.22)	16.80 (10.96)	2–61	4.50 (1.27)	7.80 (5.06)	1–33
AX	2.85 (1.27)	19.48 (15.69)	1–121	5.38 (1.73)	36.32 (31.67)	0–185	5.90 (2.60)	25.65 (27.26)	0–171
MA	1.75 (0.54)	4.06 (3.07)	1–16	1.56 (0.86)	6.53 (5.44)	1–35	1.80 (0.63)	5.52 (4.29)	1–29
IM	2.55 (1.61)	19.06 (14.94)	0–119	7.25 (2.74)	38.11 (31.49)	0–194	6.20 (3.22)	23.49 (23.95)	0–157
VG	0.80 (0.35)	10.77 (7.50)	0–42	1.69 (1.46)	8.49 (8.12)	0–55	2.40 (2.22)	4.44 (4.65)	0–29
SR	0.12 (0.06)	0.087 (0.056)	0.0067–0.35	0.10 (0.06)	0.11 (0.064)	0–0.38	0.067 (0.02)	0.18 (0.11)	0–0.64
TR	0.07 (0.12)	0.61 (0.63)	0–4.4	0.31 (0.19)	0.72 (0.53)	0–3	0.52 (0.44)	0.77(0.79)	0–4.63

TL Tillers, *AX* axillary (high-order) branches, *MA* mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches)

Table 2 Correlation coefficients of seven vegetative branching traits in the *S. bicolor* (BTx623) × *S. propinquum* RIL population

	TL	AX	MA	IM	VG	SR	TR
TL	1						
AX	0.54***	1					
MA	0.46***	0.68***	1				
IM	0.63***	0.98***	0.63***	1			
VG	0.76***	0.50***	0.25***	0.48***	1		
SR	−0.28***	0.36***	0.26***	0.28***	−0.17***	1	
TR	0.044^{NS}	0.49***	0.23***	0.43***	0.19***	0.25***	1

TL tillers, *AX* axillary (high-order) branches, *MA* Mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches), *NS* not significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

$r_{MA:VG} = 0.2480$, $r_{IM:VG} = 0.4759$, $P < 0.0001$). Variables SR and TR are indicators of the potential of a plant to form secondary or tertiary branches. Unlike the high positive correlation between AX and TL, SR and TL are negatively correlated ($r = -0.2831$, $P < 0.001$), and TR and TL are not significantly correlated ($r = 0.04378$, $P = 0.26$). The negative correlation of SR and TL indicates that a high number of primary branches were associated with formation of secondary branches from a lower percentage of the available nodes, perhaps reflecting a resource limitation.

It is not surprising that the effect of genotype, environment and genotype by environment interactions are statistically significant (at 0.05) for most traits, since vegetative branching is thought to be among the most plastic of traits (Sultan 2000) (Table 3). An exception is the variable MA, where both genotype and environment effects are not significant. The large residual of this trait might be due to variation in the numbers of mature secondary and tertiary branches, which are highly variable among years. Heritability varies widely among different branching traits, implying different levels of plasticity.

QTL detection of overall BLUP values vs single year values

We conducted QTL analysis using both overall BLUP values across three different environments (years, Table 4) and single year values (Supplementary Table 1). All chromosomes except chromosome 10 contain QTLs for vegetative branching based on the phenotypic system that we devised. Chromosomes 1, 3, 7, and 8 are 'hotspots' that confer QTLs controlling multiple vegetative branching traits. These results suggest that overall BLUP values are powerful in detecting QTLs mainly for two reasons: first, QTLs with large additive effects are easily detected with overall BLUP values; second, 'putative' QTLs with small effects repetitively among years may be detected by overall BLUP values.

QTLs controlling morphological distribution of vegetative branching

A total of four QTLs controlling tillering [on chromosomes 1, 7 (2), and 8] and four QTLs controlling axillary branches

Table 3 Heritability and variance components for vegetative branching traits in the *S. bicolor* (BTx623) × *S. propinquum* RILs, based on genotype, year, and genotype by year interaction percentage

Traits	Rep (year) (%)	Genotype (%)	Year (%)	Genotype × year (%)	Residual	Heritability (%)
TL	NS	19.5***	33.8***	13.3***	33.5	66.0
AX	NS	16.0***	6.6*	19.4***	58.0	49.8
MA	NS	4.3 NS	1.6 NS	27.8***	66.3	17.3
IM	NS	13.2***	13.3***	16.2***	57.3	47.0
VG	5.6***	22.6***	14.6	15.9***	41.3	64.9
SR	NS	7.2*	24.0***	19.9***	49.0	32.7
TR	1.1*	34.7***	NS	18.5***	45.7	71.6

TL tillers, *AX* axillary (high-order) branches, *MA* mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches), *Rep (year)* replication effect was nested within years, *NS* not significant

[on chromosomes 1 (2), 3, and 8] are significant after 1,000 permutation tests (Fig. 1; Table 4) based on the overall BLUP values, explaining 34.4 and 58.9 % of phenotypic variation considering all QTLs in the model (described in "Materials and methods"), respectively. Two additional environment-specific QTLs that do not overlap with those from the BLUP values were detected on chromosomes 1 and 3 for tillering (qTL11_1.1, qTL10_3.2, Supplementary Table 1), and two for AX were detected on chromosomes 2 and 4 (qAX09_2.1 and qAX09_4.1). One QTL for AX (qAX1.1) requires further validation since it is located at the most segregation distorted region in the genome, on chromosome 1 (Kong et al. 2013). After removing this QTL from our model, the QTLs for AX collectively explained 21.9 % of phenotypic variation. *S. propinquum* alleles increase the number of tillers and axillary branches for all QTLs detected except qTL10_3.2 and qAX09_4.1 (Supplementary Table 1).

We are surprised to find that no QTLs for tillering and axillary branching locate in the same genomic regions based on the overall BLUP values, in spite of the morphological similarities of tillers and axillary branches. However, a few environment-specific QTLs on chromosomes 3 and 4 suggest some overlapping genomic regions for TX and AX. The few occurrences of QTL co-localization could also result from the plasticity of these vegetative branching traits and limited statistical power of QTL detection. Thresholds determined by permutation tests might be too stringent and thus lower the power of QTL detection (Chen and Storey 2006). Two additional 'putative' QTLs for tillering (i.e., that reach LOD 2 but not the higher level indicated by permutation tests) are found on chromosomes 4 and 6 based on BLUP values (Table 4). For one of these putative QTLs, on chromosome 4 (qTL4.1), the *S. bicolor* allele exhibits a positive additive effect for increased tillering, differing from the other QTLs. Additional environment-specific putative QTLs are listed in Supplementary Table 1.

Tillering QTLs detected here on chromosomes 1 and 7 overlap with tillering QTLs found in a previous F2 population (Paterson et al. 1995), and with QTLs found in other sorghum populations (Hart et al. 2001; Shiringani et al. 2010; Mace and Jordan 2011), as revealed using the Comparative Quantitative Trait Locus Database for Saccharinae Grasses (Zhang et al. 2013). One QTL for tillering located on chromosome 7 covers a large physical area, arousing suspicion that it may be a rediscovery of the dwarf 3 (dw3) gene. However, the peak of this QTL is ~51 Mb in physical distance from the start of the chromosome, ~9 Mb away from dw3. Thus, we cannot rule out the possibility that branching and plant height were controlled by separate genes. The QTL discovered on chromosome 6 falls in the same genomic region as one found in a sweet sorghum study (Shiringani et al. 2010) and also in the vicinity of a SSR marker that shows significance for tillering in an association study (Upadhyaya et al. 2012). The QTL detected on chromosome 8 is near one found in a BTx623 × IS3620C population (Hart et al. 2001). Tillering QTLs on chromosomes 1 and 7 fall into high QTL density regions for many other agronomic traits in sorghum (Mace and Jordan 2011).

QTLs controlling physiological maturity of vegetative branching

It is widely known that not all vegetative branches mature in synchrony. Selection for high yield of grain crops generally favors synchrony of tiller maturity, and senescence, remobilizing resources into the seed before mechanical harvest. In contrast, genotypes with immature and vegetative tillers or branches may have higher yields of biomass. Perennial plants are often at least somewhat indeterminate, producing moderate numbers of vegetative branches that may flower throughout their growing season. In this experiment, we provide empirical evidence that physiological maturity

Table 4 QTLs affecting vegetative branching in the *S. bicolor* and *S. propinquum* RILs based on the overall BLUP values for each trait

Trait	QTL name	Ch	Position (cM)[a]	LOD	Additive	R^2 (%)	Start (Mb)[b]	End (Mb)
TL	qTL1.1	1	51.8	6.8	−1.49	12.1	28.1	60.8
TL	qTL7.1	7	16.9	3.3	−0.95	7.0	0.9	8.4
TL	qTL7.2	7	32.7	2.8	−0.79	4.5	8.4	58.2
TL	qTL8.1	8	53.2	4.8	−1.02	8.3	4.9	51.5
TL[†c]	qTL4.1	4	62.0	2.3	0.71	3.6	58.8	64.6
TL[†]	qTL6.1	6	55.4	2.4	−0.75	4.3	60.8	62.1
AX	qAX1.1	1	40.3	6.0	−8.89	30.0	28.2	57.5
AX	qAX1.2	1	68.9	2.5	−2.00	5.9	64.0	70.0
AX	qAX3.1	3	50.2	6.4	−2.89	12.3	6.2	7.8
AX	qAX8.1	8	0.0	3.0	−1.74	5.4	0.2	3.0
MA	qMA8.1	8	1.0	5.2	−0.12	12.1	0.2	3.0
IM	qIM1.1	1	40.3	3.7	−6.43	22.2	28.2	57.5
IM	qIM3.1	3	50.2	5.4	−2.22	10.0	6.2	7.8
IM	qIM8.1	8	0.0	4.1	−1.75	7.3	0.2	3.0
IM[†]	qIM4.1	4	56.5	2.2	1.32	4.1	51.2	58.8
IM[†]	qIM5.1	5	24.5	2.0	−1.94	7.8	0.2	4.5
VG	qVG2.1	2	50.6	3.3	−0.87	5.8	4.7	63.2
VG	qVG3.1	3	50.2	3.3	−0.72	6.0	6.2	7.8
VG	qVG7.1	7	34.7	3.2	−0.68	6.3	8.4	58.3
VG	qVG8.1	8	52.2	5.2	−0.85	9.6	4.5	51.5
VG[†]	qVG1.1	1	48.8	2.5	−0.80	5.1	28.2	60.8
VG[†]	qVG1.2	1	67.9	2.4	−0.66	5.0	64.0	66.9
SR	qSR3.1	3	66.1	4.5	−0.0045	9.8	13.8	51.2
SR	qSR7.1	7	24.3	3.0	0.0035	6.0	0.9	37.7
SR	qSR8.1	8	7.5	2.5	−0.0032	5.2	0.2	3.0
TR	qTR3.1	3	50.2	12.0	−0.16	21.6	6.2	7.8
TR	qTR5.1	5	51.7	2.8	0.087	5.6	4.8	42.0
TR	qTR9.1	9	29.5	2.7	−0.077	5.0	4.2	54.5
M1	qM1_2.1	2	56.9	2.6	−0.11	6.0	59.1	63.2
M1	qM1_7.1	7	41.4	2.5	−0.082	5.3	51.1	58.6
M1[†]	qM1_6.1	6	22.7	2.0	−0.082	4.6	45.8	58.5
M1[†]	qM1_8.1	8	5.0	2.3	−0.08	5.3	0.2	3.0
IM2	qIM2_1.1	1	68.9	4.4	−0.68	9.5	64.0	70.0
IM2	qIM2_2.1	2	55.9	3.4	−0.06	6.5	59.1	63.2
IM2	qIM2_7.1	7	41.4	4.7	−0.60	9.2	51.1	58.3

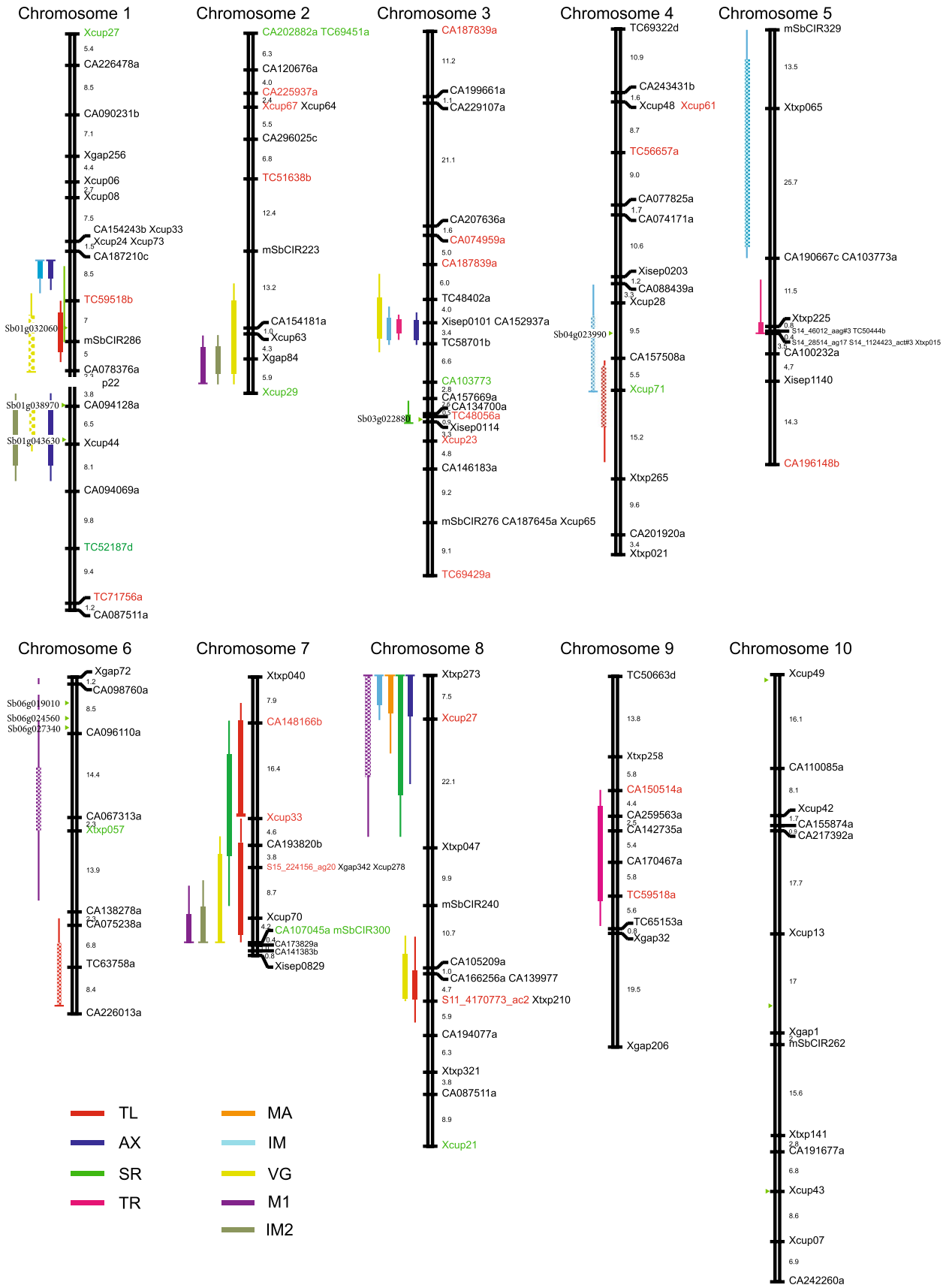
TL tillers, *AX* axillary (high-order) branches, *MA* mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches), *M1* mature primary branches, *IM2* immature secondary branches

[a] Positions refer to the beginning of the genetic map (Kong et al. 2013)

[b] Based on DNA marker locations flanking 1-LOD interval in the published genome sequence (Paterson et al. 2009)

[c†] Significant at an LOD score of 2.0

is genetically controlled. Our system permits detection of QTLs controlling the numbers of tillers and branches at different physiological status when the primary tillers reach maturity, i.e., we differentiate the number of mature, immature, and vegetative branches (Fig. 1; Table 4). Only one QTL was discovered for the number of mature branches based on the overall BLUP values, accounting for 12.07 % of phenotypic variance. Although QTLs for MA on chromosome 1 are significant for all three environments (Supplementary Table 1), no QTL was detected by the overall BLUP values. The interval CA154243b–CA187210c on chromosome 1 is under severe segregation distortion (Kong et al. 2013), so QTLs detected here require further validation. A total of three and four QTLs for the number of immature and vegetative branches accounted for 47.8 and 28.8 % of phenotypic variance considering all QTLs in the models based on the overall BLUP values. After deleting the QTL for IM that falls in the severely segregation-distorted region, the model explains 15.9 % of the phenotypic variation. We also detected two environment-specific QTLs on chromosomes 1 (overlapping qIM10_1.1 and qIM11_1.1) and 2 (qIM09_2.1) for IM, and two for VG (qVG11_5.1 and qVG09_8.1) that eluded detection by the overall BLUP values (Supplementary Table 1). We detected two more 'putative' QTLs controlling IM on chromosomes 4 and 5, and two controlling VG on chromosome 1


Chromosome 1
Xcup27
5.4
CA226478a
8.5
CA090231b
7.1
Xgap256
4.4
Xcup06
2.7
Xcup08
7.5
CA154243b Xcup33
Xcup24 Xcup73
1.5
CA187210c
8.5
TC59518b
Sb01g032060
7
mSbCIR286
5
CA078376a
p22
3.8
Sb01g038970
CA094128a
6.5
Sb01g043630
Xcup44
8.1
CA094069a
9.8
TC52187d
9.4
TC71756a
1.2
CA087511a
Chromosome 2
CA202882a TC69451a
6.3
CA120676a
4.0
CA225937a
2.4
Xcup67 Xcup64
5.5
CA296025c
6.8
TC51638b
12.4
mSbCIR223
13.2
CA154181a
1.0
Xcup63
4.3
Xgap84
5.9
Xcup29
Chromosome 3
CA187839a
11.2
CA199661a
1.1
CA229107a
21.1
CA207636a
1.6
CA074959a
5.0
CA187839a
6.0
TC48402a
4.0
Xisep0101 CA152937a
3.4
TC58701b
6.6
CA103773
2.8
CA157669a
1.6
CA134700a
Sb03g022880
0.9
TC48056a
3.3
Xisep0114
Xcup23
4.8
CA146183a
9.2
mSbCIR276 CA187645a Xcup65
9.1
TC69429a
Chromosome 4
TC69322d
10.9
CA243431b
1.6
Xcup48 Xcup61
8.7
TC56657a
9.0
CA077825a
1.7
CA074171a
10.6
Xisep0203
1.2
CA088439a
3.3
Xcup28
Sb04g023990
9.5
CA157508a
5.5
Xcup71
15.2
Xtxp265
9.6
CA201920a
3.4
Xtxp021
Chromosome 5
mSbCIR329
13.5
Xtxp065
25.7
CA190667c CA103773a
11.5
Xtxp225
0.8
S14_46012_aag#3 TC50444b
0.4
S14_28514_ag17 S14_1124423_act#3 Xtxp015
3.5
CA100232a
4.7
Xisep1140
14.3
CA196148b
Chromosome 6
Xgap72
1.2
CA098760a
Sb06g019010
Sb06g024560
Sb06g027340
8.5
CA096110a
14.4
CA067313a
2.3
Xtxp057
13.9
CA138278a
2.3
CA075238a
6.8
TC63758a
8.4
CA226013a
Chromosome 7
Xtxp040
7.9
CA148166b
16.4
Xcup33
4.6
CA193820b
3.8
S15_224156_ag20 Xgap342 Xcup278
8.7
Xcup70
4.2
CA107045a mSbCIR300
0.4
CA173829a
CA141383b
0.8
Xisep0829
Chromosome 8
Xtxp273
7.5
Xcup27
22.1
Xtxp047
9.9
mSbCIR240
10.7
CA105209a
1.0
CA166256a CA139977
4.7
S11_4170773_ac2 Xtxp210
5.9
CA194077a
6.3
Xtxp321
3.8
CA087511a
8.9
Xcup21
Chromosome 9
TC50663d
13.8
Xtxp258
5.8
CA150514a
4.4
CA259563a
2.5
CA142735a
5.4
CA170467a
5.8
TC59518a
5.6
TC65153a
0.8
Xgap32
19.5
Xgap206
Chromosome 10
Xcup49
16.1
CA110085a
8.1
Xcup42
1.7
CA155874a
0.8
CA217392a
17.7
Xcup13
17
Xgap1
2.5
mSbCIR262
15.6
Xtxp141
2.8
CA191677a
6.8
Xcup43
8.6
Xcup07
6.9
CA242260a
TL
AX
SR
TR
MA
IM
VG
M1
IM2

◀ **Fig. 1** QTL mapping of vegetative branching in *S. bicolor* × *S. propinquum* RILs. QTLs are shown with 1-LOD (*box*) and 2-LOD (*whiskers*) intervals. *Solid boxes* indicate QTLs significant based on (1,000) permutation tests. *Dotted boxes* are 'putative' QTLs significant only at an LOD score of 2. QTL-associated sorghum orthologs of rice branching-related genes are shown, with approximate locations marked on the genetic map by *green triangles*. A special case is the gene Sb01g032060 (see Table 5 footnote), where an additional line indicates the range of possible positions. *TL* tillers, *AX* axillary (high-order) branches, *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches), *MA* mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *M1* mature primary branches, *IM2* immature secondary branches

Table 5 Correlation coefficients of biomass, plant height (PH), stalk middle diameter (MD) and total number of vegetative branches (TBCH) in *S. bicolor* (BTx623) × *S. propinquum* RILs (left) and in a GWAS population (right)

	Biomass	PH	MD	TBCH
Biomass	1			
PH	0.52***/0.45***	1		
MD	0.30***/0.52***	0.17***/0.31***	1	
TBCH	0.50***/0.45***	0.29***/0.12**	-0.082^{NS}/0.055^{NS}	1

NS not significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

based on the overall BLUP values (Table 4), and additional environment-specific 'putative' QTLs are listed in Supplementary Table 1. The 'putative' QTLs on chromosomes 1 and 4 overlap with TL and AX and show the same direction of additive effects. For example, qVG1.1 overlaps with qTL1.1, and qVG1.2 with qAX1.2. This further supports the validity of these QTLs, albeit not reaching the thresholds of permutation tests. *S. propinquum* alleles increased the number of branches of all VG and IMQTLs except qIM09_4.1, qIM_4.1 and qVG11_5.1.

One overlapping QTL region was found at the interval Xtxp237–Xcup27 on chromosome 8 controlling both MA (qMA8.1) and IM (qIM8.1). Another overlapping interval was on chromosome 3 controlling IM (qIM3.1) and VG (qVG3.1). QTLs found on chromosomes 1, 3, 4,7, and 8 controlling the maturity of vegetative branching also overlap with QTLs underlying tillers and axillary branches, indicating that overlapping sets of genes and biochemical pathways may influence axillary meristem initiation related to different levels in the hierarchy of vegetative branching patterns.

QTLs controlling the potential for forming axillary branches

Not every node undergoes axillary meristem initiation and outgrowth. Most nodes on the tillers may remain dormant until certain genetic or environmental factors trigger growth at specific developmental stages. For grain crops, secondary and tertiary branches are usually arrested during early developmental stages and may lack photosynthate to grow if the primary inflorescences set seed and mature normally. In addition, plants may respond differently when they encounter environmental changes such as shading and grazing (Whipple et al. 2011). We found genetic variation in potential for forming secondary and tertiary branches (SR and TR) by QTL mapping. Three QTLs for SR (on chromosomes 3, 7, and 8) and three QTLs for TR (on chrs. 3, 5, and 9) were identified based on the overall BLUP values, explaining 17.6 and 25.2 % of phenotypic variance considering all QTLs in the models (Fig. 1; Table 4). *S. propinquum* alleles confer increased

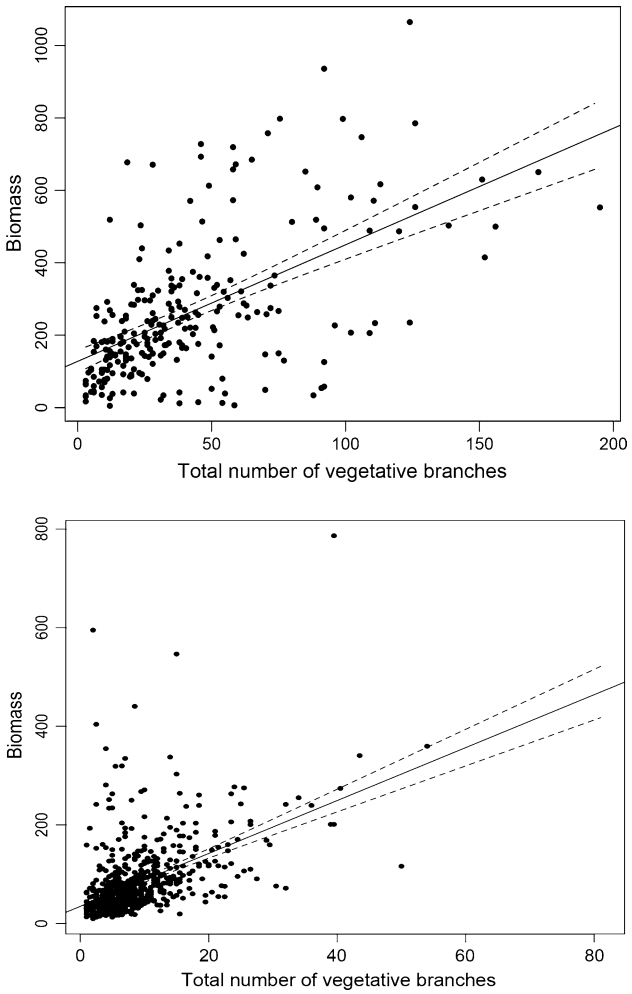


Fig. 2 Scatter plots of biomass vs. total number of vegetative-branches in *S. bicolor* (BTx623) × *S. propinquum* RILs (*top*) and in a GWAS population (*bottom*). *Solid line* shows the regression lines, and *dotted lines* show the 95 % confidence interval of the regression line

branching at four of these loci, with *S. bicolor* alleles increasing branching at the chr. 5 (qTR5.1) and 7 loci (qSR7.1). One environment-specific QTL was detected for SR on chromosome 2 (qSR10_2.1) that does not overlap with QTLs detected

Table 6 Vegetative branching variables related to biomass in both *S. bicolor* (BTx623) × *S. propinquum* RILs and in a GWAS population

Population	Response variable[b]	*N*	Final model (significant at alpha = 0.01)	RMSE	R^2
RIL	Wt10	126	Wt10 = 180.67 + 24.29 × M1	242.8	0.102
RIL	Wt11	127	Wt11 = 87.13 + 29.81 × M1 + 3.75 × IM3	96.4	0.475
GWAS	Wt	690	Wt = 43.41 + 11.75 × M1 + 9.77 × IM2 − 4.30*IM3	63.49	0.265

RMSE root mean square error, *Wt10* dry vegetative biomass collected in 2010 in *S. bicolor* (BTx623) × *S. propinquum*RILs, *Wt11* dry vegetative biomass collected in 2011 in *S. bicolor* (BTx623) × *S. propinquum* RILs, *Wt* dry vegetative biomass collected in 2010 and 2011 in a GWAS population, *M1* Mature primary branches, *IM2* Immature secondary branches, *IM3* Immature tertiary branches

using overall BLUP values, or one for TR (qTR09_4.1 and qTR11_4.1, Supplementary Table 1). Other environment-specific 'putative' QTLs are also listed in Supplementary Table 1.

Biomass components related to vegetative branching

A drought-tolerant crop, sorghum is an excellent plant for cellulosic biofuel feedstock production on marginal land that is not suitable for food production. Total dry vegetative biomass (Biomass) is significantly correlated with total number of vegetative branches (TBCH), plant height (PH), and stalk middle diameter (MD) (Table 5). We evaluated the association of total dry biomass, TBCH, PH and MD using regression analysis, with biomass as response variable, and total number of vegetative branches (TBCH), plant height (PH) and stalk middle diameter (MD) being significant explanatory variables in both the RIL population and the GWAS population. Environments and replications are not significant in both models. The models are:

$$\text{For the RIL population : Biomass} = -272.93 + 2.44 \times \text{TBCH} + 1.70 \times \text{PH} + 19.29 \times \text{MD} \quad (1)$$

$$\text{For the GWAS population : Biomass} = -99.27 + 2.97 \times \text{TBCH} + 0.51 \times \text{PH} + 9.63 \times \text{MD} \quad (2)$$

The models demonstrated significant associations between dry biomass weight and vegetative branching, plant height and middle diameter, with root mean squared deviations of 151.6 and R^2 of 0.47 for the RIL population; and 51.35 and R^2 of 0.52 for the GWAS population. TBCHis an indispensible component in each of these two models, with elimination of this variable dramatically reducing explanatory power.

To investigate the effects of different types and levels of branching on biomass production, we performed a regression study to evaluate the different branching variables as explanatory variables, with total biomass as the response variable in both RIL and GWAS populations (Fig. 2). This investigation used all vegetative branching elements except M2 and M3, as noted above because their distributions are very skewed in this dataset with most values being zero. Parsimonious models to predict dry biomass were separately obtained for data collected in 2010 and 2011, respectively, using backward elimination and Bayesian Information Criterion (Table 6). In the RIL population, M1 and IM2 are highly correlated with each other (0.81 in 2010, and 0.69 in 2011) for both years, so IM2 was excluded in the regression analysis. The model predicting dry biomass of 2011 is much better than that of 2010, with a smaller root mean squared deviation (96.4) and a much higher R^2 value (0.475). Both models show that M1 or highly correlated IM2 is the most important component in predicting dry biomass, whereas the data from 2011 also show some evidence of IM3 being a significant variable, though its coefficient is much lower than M1. For the GWAS population, the model likewise indicates that M1, IM2 and IM3 are significant variables in predicting dry biomass (Table 6). The regression analysis has provided some general trend of the relationship between dry biomass and vegetative branching and also indicates that QTLs increase in the number of M1 and IM2 may improve dry biomass production in sorghum.

Based on their contributions to stalk biomass, we conducted further QTL analysis for M1 and IM2. Two QTLs for M1 and three QTLs for IM2 are significant at an LOD score of 2.5 based on overall BLUP values, accounting for 17.2 and 24.3 % of phenotypic variance considering all QTL in the models (Table 4). For all M1 and IM2 QTLs found, *S. propinquum* alleles increase the number of branches. QTLs on chromosomes 2 and 7 control both traits, indicating that the high correlation between these traits may be a result of overlapping sets of determinant genes.

Identification of candidate branching genes

For 19 genes controlling axillary meristem initiation and outgrowth in rice (Nakagawa et al. 2002; Komatsu et al. 2003; Li et al. 2003; Goto et al. 2005; Ishikawa et al. 2005; Zou et al. 2005; Xiong et al. 2006; Arite et al. 2007; Mao et al. 2007; Nagasaki et al. 2007; Itoh et al. 2008; Rao

Table 7 Genomic positions of candidate sorghum genes that correspond to characterized rice genes controlling axillary meristem initiation and outgrowth

Gene name	Rice ID	Sorghum ID	RIL QTL
MONOCULM 1 (*MO1C*)	Os06g0610300	Sb10g023950[a]	No
LAX PANICLE (*LAX*)	Os01g0831000	Sb03g038820	Out of range
Class III HD-Zip (*OsHB3*)	Os12g0612700	Sb08g021350	Out of range
		Sb01g013710	No
SHOOTLESS 2 (*SHL2*)	Os01g0527600	Sb03g022880	qSR3.1
SHOOTLESS 4/SHOOT ORGANIZATION 2 (*SHL4/SHO2*)	Os03g0449200	Sb01g032060	qTL1.1, qAX1.1, qIM1.1, qVG1.1[b]
LAX PANICLE 2 (*LAX2*)	Os04g0396500	No synteny	N.A.
TILLER ENHANCER (*TE*)	Os03g0123300	Sb01g048980	Out of range
TILLERING 1 (*OsTIL1*)	Os04g0460600	Sb04g023990	qIM4.1
		Sb06g019010	qM1_6.1
DWARF 3 (*D3*)	Os06g0154200	Sb10g003790	No
DWARF 17/HIGH TILLERING AND DWARF1 (*D17/HTD1*)	Os04g0550600	Sb06g024560	qM1_6.1
DWARF 10 (*D10*)	Os01g0746400	Sb03g034400	Out of range
FINE CULM 1/TEOSINTE BRANCHED 1 (*FC1/OsTB1*)	Os03g0706500	Sb01g010690	No
DWARF 27 (*D27*)	Os11g0587000	Sb05g022855	Out of range
DRARF 88/DWARF 14/HIGH TILLERING AND DWARF 2 (*D88/D14/HTD2*)	Os03g0203200	Sb01g043630	qAX1.2, qVG1.2, qIM2_1.1
LEAF AND TILLER ANGLE INCREASED CONTROLLER (*OsLIC*)	Os06g0704300	Sb10g029250	No
LEAFY HEAD2 (*LHD2*)	Os01g0907900	Sb03g043230	Out of range
REDUCED CULM NUMBER 1 (*RCN1*)	Os03g0281900	Sb01g038970[a]	qAX1.2, qVG1.2, qIM2_1.1
Rice FLO-LFY homolog (*RFL*)	Os04g0598300	Sb06g027340	qM1_6.1
LAGGING GROWTH AND DEVELOPMENT 1 (*LGD1*)	Os09g0502100	Sb02g029070	Out of range

[a] Synteny of MOC1 and RCN1 is not found in PGDD database. However, two corresponding homologous genes have been found in sorghum genome showing a similarity of approximately 80 % of their protein sequence

[b] The exact position of Sb01g032060 is uncertain due to an incongruity in alignment of the genetic and physical maps in this region (Kong et al. 2013). It is either located within the interval qAX1.1 and qIM1.1, or within the interval qTL1.1 and qVG1.1, but not both

et al. 2008; Wang et al. 2008; Arite et al. 2009; Lin et al. 2009; Tabuchi et al. 2011; Lin et al. 2012; Thangasamy et al. 2012), we identified colinear locations in sorghum (Table 7) using the Plant Genome Duplication Database (Lee et al. 2013). The corresponding sorghum genes were searched for their relationships with QTLs for vegetative branching based on their physical positions. Most of these rice genes have corresponding sorghum genes. For both rice genes *MOC1* and *RCN1*, we failed to find corresponding syntenic blocks in the sorghum genome, but their best sorghum homologs display approximately 80 % protein sequence similarity so were included in the study.

Among the 19 genes controlling axillary meristem initiation and outgrowth in rice, eight sorghum orthologs for sevenrice genes (*SHL2*, *SHL4/SHO2*, *OsTIL1*, *D17/HTD1*, *D88/D14/HTD2*, *RCN1* and *RFL*) are within a total of 10different sorghum QTL likelihood intervals influencing vegetative branching, with qM1_6.1 containing three candidates, and qAX1.2 and qVG1.2 each containing two candidates (Table 7). We detected different QTL intervals on chromosome 1that confer different levels of vegetative branching, corresponding to three different rice genes, *SHL4*/*SHO2*, *D88*/*D14*/*HTD2*, and *RCN1*. *SHL4*/*SHO2* homologs are found within two possibleQTL intervals (either qTL1.1 and qVG1.1, or qIM1.1 and qAX1.1, see Fig. 1 legend) conferring different vegetative branching components, and function in rice in controlling axillary meristems, suggesting that the corresponding sorghum gene may have a similar function. Similarly, the correspondence of *D88/D14/HTD2* and *RCN1* with sorghum QTLs controlling higher-order and vegetative branches also suggests that the corresponding sorghum genes may function similarly in controlling axillary meristem outgrowth. Sorghum genes syntenic with *LAX*, *OsHB3*, *TE*, *D10* and *D27* are located at the distal regions of their respective chromosomes, slightly beyond the range of this genetic map—however we see no evidence of QTLs in these regions based on the nearest markers that are mapped.

Some of the eight QTL-associated sorghum homologs of rice branching-related genes contain striking allelic differences between the parents of our RIL set. Of 261 single-nucleotide variants (SNVs) identified between *S.*

Table 8 Allelic variations between *S. bicolor* and *S. propinquum*in eight sorghum genes that fall in the regions of QTLs conferring vegetative branching

Gene ID	No. of SNV between Sb-Sp	No. non-synonymous SNV	No. synonymous SNV	No. intronic SNV	Highest FIS[a]	GWAS SNP[b]	*P* value of SNP	Distance (bp)[c]
Sb01g032060	27	7	9	11	3.26	S1_54630641	9.49E−05	−254,619
Sb01g038970	21	7	12	2	2.73	S1_63145321	6.25E−05	705,978
Sb01g043630	28	3	7	18	0.16	S1_66130482	9.52E−05	−649,840
Sb03g022880	70	5	11	54	3.40	N.A.		
Sb04g023990	30	7	6	17	1.07	S4_54157098	6.57E−05	490,611
Sb06g019010	26	7	7	12	3.50	N.A.		
Sb06g024560	34	11	7	16	1.13	N.A.		
Sb06g027340	25	3	3	19	0.49	S6_56411801	3.81E−05	166,102
Total	261	50	62	149				

SNV single-nucleotide variation, *Sb sorghum bicolor* BTx623, *Sp sorghum propinquum* (unnamed accession)

[a] Function index score which measures functional impact of a mutation on protein function using a gene evolution conservation profile as described by Paterson et al. (2012)

[b] Closest GWAS SNP to the candidate gene

[c] The distance of the GWAS SNP to the physical position of the gene

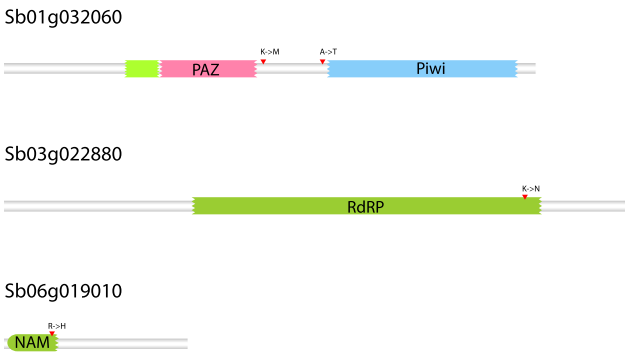


Fig. 3 Striking mutations in three sorghum genes that correspond to characterized rice genes controlling vegetative branching. The reference nucleotide sequences come from *S. bicolor* (*left*), and the mutated nucleotides are from *S. propinquum* (*right*). Protein domains of each gene are shown in *boxes*

bicolor BTx623 and *S. propinquum*in the 8 genes, 50are non-synonymous, 62 are synonymous and 149 are intronic (Table 8). We further assessed the potential impact of the non-synonymous SNVs (nsSNV) on gene functions based on evolutionary conservation profiles of plant protein sequences (see "Materials and methods"). Three SNVs, in different genes (Sb01g032060, Sb03g022880 and Sb06g019010), are highly likely to affect gene functions based on the FIS value (false discovery rate <0.05; Table 8; Fig. 3). Information of the protein domain is predicted by the Pfam database (Finn et al. 2014). Both Sb03g022880 and Sb06g019010 contain striking SNVs in protein functional domains, while Sb01g032060 contains an SNV located between two functional domains (Fig. 3). A fourth candidate gene, Sb04g023990, contains a G to C mutation in an mRNA splicing motif (i.e., GT-AG). This mutation might cause inclusion of the intron sequence in the final mRNA product and premature termination or frameshift of the protein sequence in *S. propinquum*.

Genome-wise association study in a sorghum diversity panel

We evaluated vegetative branching in a diversity panel of 377 *S. bicolor* accessions (Casa et al. 2008; Morris et al. 2013) in 2 years (2009 and 2010, Supplementary Figs. 1–9). Trait means, standard deviations, ranges and heritability for vegetative branching are listed in Table 9. The nine measured vegetative branching traits were significantly (*P* value $<10^{-4}$) associated with a total of 410 SNPs in 2009, and 214 in 2010. Of those significant SNPs, 326 and 177 found in 2009 and 2010, respectively, are within the range of the genetic map, and 242(74.2 %) and 114 (64.4 %) are within QTL intervals based on overall BLUP values.

The 19 genes controlling axillary meristem initiation and outgrowth in rice correspond to 21 sorghum genes, with 13 within the range of the genetic map, 7 out of range, and 1 showing no syntenic relationship between rice–sorghum. QTL likelihood intervals cover about 25.3 % of the genome based on genetic distance, and 69 % based on physical distances, while 8/13 (61.5 % of) genes are within the QTL interval. A total of 25,369 genes are within the range of the genetic map, with 14,286 within the QTL intervals. The probability of having 8 out of 13 genes within the QTL interval does not provide enough evidence to be nonrandom (*P* value = 0.21). However, among the

Table 9 Summary statistics for vegetative branching traits in a GWAS population

Trait	2009			2010			Heritability (%)
	Mean	SD	Range	Mean	SD	Range	
TL	3.69	2.5	1–17.5	4.42	4.20	1–52	65.1
AX	3.29	2.74	0–20	6.51	9.95	0–146	40.9
MA	1.69	1.25	1–11	2.12	2.11	1–30	57.0
IM	1.86	2.03	0–13.5	6.08	9.44	0–138	30.7
VG	3.42	2.66	0–16	2.73	2.96	0–29.5	52.4
SR	0.103	0.08	0–0.5	0.12	0.09	0–0.51	35.6
TR	0.188	0.33	0–2	0.24	0.32	0–2.87	32.1
M1	1.33	0.62	1–6	1.86	1.48	1–17.5	53.0
IM2	1.21	1.26	0–7.5	3.48	4.78	0–68	30.7

TL tillers, *AX* axillary (high-order) branches, *MA* mature branches, *IM* immature branches (with floral induction), *VG* vegetative branches (without floral induction), *SR* secondary ratio (potential of forming secondary branches), *TR* tertiary ratio (potential of forming tertiary branches), *M1* mature primary branches, *IM2* immature secondary branches

eight QTL-associated sorghum homologs of rice branching-related genes to explain branching patterns within *S. bicolor*, five (62.5 %) are within 700 Kb (totaling 1 % of the genome for the 5 candidates) of SNPs that show significant (*P* value $<10^{-4}$) association with branching by GWAS (Table 8). There is one significant GWAS SNP located ~250 Kb upstream of one of the genes containing striking SNVs, Sb01g032060.

We note that a widely studied maize gene associated with apical dominance, *tb1* (Doebley et al. 1997), is not associated with sorghum QTLs or with a significant signal from GWAS in the sorghum diversity panel.

Discussion

A recombinant inbred line (RIL) population derived from two divergent parents, *S. bicolor* and *S. propinquum,* provides new insights into the genetic control of vegetative branching in sorghum. Replication over multiple environments and little heterozygosity of RILs facilitate the analysis of genotype by environment interactions, as well as precision and validation of QTLs. Advanced in a temperate region (Lubbock, TX), the RIL population improves the ability of discovering QTLs relative to a previously studied F2 population from the same parents (Paterson et al. 1995), by eliminating confounding factors that are correlated with short-day flowering from *S. propinquum*. This principle was exemplified by identifying two flowering QTLs (Kong et al. 2013) that eluded detection in the F2 population (Lin et al. 1995). Compared with the F2 generation of this RIL set (Paterson et al. 1995), we validated two previously discovered tillering QTLs and detected two new ones that are validated by independent studies (Hart et al. 2001; Shiringani et al. 2010). However, eliminating the short-day alleles from *S. propinquum* leads to selection towards *S. bicolor* alleles. For example, it is unlikely to detect the tillering QTL on chromosome 6 that was found in the F2 population near the short-day flowering locus (Paterson et al. 1995). Segregation distortion due to selection against short-day flowering might be beneficial to the detection power of QTLs (Xu 2008), but the estimated positions and phenotypic effects of QTL might be altered (Zhang et al. 2010a).

We introduce a phenotyping system to dissect the genetic control of different levels of vegetative branching and demonstrate its efficiency to detect QTLs for each trait in this study. A genomic region on chromosome 3 (TC48402a-TC58702b) shows some evidence of QTLs overlapping many traits, including TR, AX, IM and VG. Another "hotspot" in the interval Xtxp273–Xcup27 on chromosome 8 influenced four vegetative branching traits (qAX8.1, qMA8.1, qIM8.1 and qSR8.1). Genomic regions on chromosomes 1 and 7 also influenced at least 5 vegetative branching traits. The QTL regions influencing many branching traits support our expectation that different levels of branching may share some common genetic control for axillary meristem initiation. This could be either due to pleotropic effects of single genes in the identified genomic regions, or to high concentrations of different genes related to branching in particular chromosomal regions. Another reason for some genomic regions to contain multiple vegetative branching traits could be inter-relationships between traits. For example, since secondary and tertiary branches are mostly immature or vegetative, it may be possible to find a common QTL that controls all these traits. However, there is also clear evidence that some traits, such as tillering and high-order branching, might have degrees of distinct genetic control based on the discovery of non-overlapping QTLs.

Vegetative branching is a highly plastic trait, with the effects of genotype, environment and their interactions

generally significant. Different vegetative branching traits might differ in plasticity from each other as they respond differently to environmental fluctuation, resulting in different sets of QTLs. For example, the number of tillers might be more consistent among different environments than higher-order branches, since the latter trait may be more likely to respond to changing environments. QTLs for certain vegetative branching trait might be significant while others remain under the threshold level, perhaps due to hormonal or nutritional factors that contribute to the high environmental plasticity of plant architecture (Alam et al. 2014). To determine whether the effects of QTLs are caused by different genes or environments requires multi-environment testing, comparison to other populations, and ideally positional cloning of genes and testing of gene functions.

A drought-tolerant plant, sorghum is a promising candidate for biomass-dedicated feedstock to be grown on marginal land not suitable for food production (Rooney et al. 2007). Vegetative branching is an important component of biomass yield. This study provides guidance for improving vegetative architecture of biomass-dedicated crops. Together with plant height and middle stalk diameter, vegetative branching pattern is significantly correlated to biomass yield. Although the result is variable in different environments, mature tillers and immature secondary branches are both consistently correlated to dry biomass, implying that efforts to increase these two traits may improve biomass production.

Syntenic relationships with rice genes controlling axillary meristem initiation implicate corresponding sorghum genes within QTL likelihood intervals as attractive candidate genes. For example, the sorghum gene corresponding to *SHL4*/*SHO2* is within two possible QTL intervals conferring different vegetative branching patterns, and also has allelic variations between *S. bicolor* and *S. propinquum*, suggesting that it might control axillary meristem initiation. Three sorghum genes (Sb01g032060, Sb03g022880 and Sb06g019010) with striking SNVs either in protein domain or inter domain regions (Fig. 3), all encode regulatory proteins functioning during developmental stages (Punta et al. 2012), consistent with important roles in vegetative branching patterns. Further functional analyses may validate these candidate genes and elucidate their functions.

Intersections between synteny and GWAS data may improve upon the resolution of QTL mapping, toward identifying causal genes. QTL likelihood intervals identified by mapping in a bi-parental population often occupy a relative long physical distance, especially if they include the recombinationally recalcitrant pericentromeric regions. Candidate genes implicated by synteny, and significant SNPs implicated by GWAS, may delineate a small subset of the QTL region as being most likely to be causal. For example, the candidate gene Sb01g032060 is located in a QTL interval for multiple traits spanning a long physical distance (28.1–60.8 Mb). Syntenic relationship to a characterized rice gene has pinpointed a corresponding sorghum gene that is also ~250 k downstream of a significant SNP discovered by GWAS, narrowing the most likely location of causal genes.

QTLs for vegetative branching revealed in this study may be valuable in several ways for different sorghum breeding programs. For most branching QTLs, alleles increasing vegetative branching are coming from S. *propinquum*, and only rarely from *S. bicolor*, consistent with the general increase in apical dominance associated with selection of sorghum (and other cereals) for monocarpy and synchronous harvest. Grain sorghum breeders may utilize this QTL information to further increase apical dominance and suppress the growth of axillary meristems. However, some degree of branching and temporal dispersal of flowering may improve yield stability—for example, providing the ability to compensate for transient 'temperature spikes' that reduce seed set, by having several inflorescences that flower over some period of time. Such a period of time probably needs to be relatively short—perhaps a few weeks—so as to permit efficient harvest of the crop. Prolonged branching and flowering associated with early efforts to breed ratooning/perennial sorghums tend to result in 'wasteful' production of flowers that bloom too late to mature in temperate climates. On the other hand, correlations with dry matter yield (above) suggest that breeding sorghum for biofuel feedstocks or forage production might benefit from such additional branching.

The high degree of common genetic control of many traits across Poaceae grasses suggests that identification of specific genes related to elements of plant architecture may have value in diverse contexts, for example, in improvement of a wide range of grain, forage, biomass, and turfgrasses. Candidate genes for some QTLs identified in this population have been deduced based on evidence from rice and new QTLs identified here may contribute to identifying genetic determinants of branching in other grasses, also providing a start toward identifying genes responsible for these components of vegetative branching in sorghum.

Author contribution W. K. performed experiments, analyzed data and wrote the manuscript; H. G. and T. L. wrote computer codes and developed analytical tools; V. H. G. and C. K. performed experiments; A. H. P designed, supervised and performed experiments and analyses, and edited the manuscript.

Acknowledgments We thank the members of the Paterson lab for valuable help and precious suggestions in this work. We appreciate financial support from the DOE-USDA Plant Feedstock Genomics Program (2012-03304).

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of the USA.

References

Alam MM, Hammer GL, Oosterom EJ, Cruickshank AW, Hunt CH, Jordan DR (2014) A physiological framework to explain genetic and environmental regulation of tillering in sorghum. New Phytol 203(1):155–167

Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J (2007) DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. Plant J 51:1019–1029

Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J (2009) d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiol 50:1416–1424

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, Jessup R, Lemke C, Lennington J, Li ZK, Lin YR, Liu SC, Luo LJ, Marler BS, Ming RG, Mitchell SE, Qiang D, Reischmann K, Schulze SR, Skinner DN, Wang YW, Kresovich S, Schertz KF, Paterson AH (2003) A high-density genetic recombination map of sequence-tagged sites for Sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–386

Casa AM, Pressoir G, Brown PJ, Mitchell SE, Rooney WL, Tuinstra MR, Franks CD, Kresovich S (2008) Community resources and strategies for association mapping in sorghum. Crop Sci 48:30–40

Chen L, Storey JD (2006) Relaxed significance criteria for linkage analysis. Genetics 173:2371–2381

Chittenden LM, Schertz KF, Lin YR, Wing RA, Paterson AH (1994) A detailed Rflp map of Sorghum-bicolor X S-propinquum, suitable for high-density mapping, suggests ancestral duplication of sorghum chromosomes or chromosomal segments. Theor Appl Genet 87:925–933

Dabbert T, Okagaki RJ, Cho S, Boddu J, Muehlbauer GJ (2009) The genetics of barley low-tillering mutants: absent lower laterals (als). Theor Appl Genet 118:1351–1360

Dabbert T, Okagaki RJ, Cho S, Heinen S, Boddu J, Muehlbauer GJ (2010) The genetics of barley low-tillering mutants: low number of tillers-1 (lnt1). Theor Appl Genet 121:705–715

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488

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

Doust AN (2007b) Grass architecture: genetic and environmental control of branching. Curr Opin Plant Biol 10:21–25

Doust AN, Kellogg EA (2006) Effect of genotype and environment on branching in weedy green millet (*Setaria viridis*) and domesticated foxtail millet (*Setaria italica*) (Poaceae). Mol Ecol 15:1335–1349

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EA (2004) Genetic control of branching in foxtail millet. Proc Natl Acad Sci USA 101:9045–9050

Feltus FA, Hart GE, Schertz KF, Casa AM, Brown P, Klein PE, Kresovich S, Paterson AH (2006) Genetic map alignment and QTL correspondence between inter- and intra-specific sorghum populations. Theor Appl Genet 112:1295–1305

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M (2014) Pfam: the protein families database. Nucl Acids Res 42:D222–D230

Gallavotti A, Zhao Q, Kyozuka J, Meeley RB, Ritter M, Doebley JF, Pe ME, Schmidt RJ (2004) The role of barren stalk1 in the architecture of maize. Nature 432:630–635

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189-U122

Goto Y, Tanabe M, Ishibashi T, Tsutsumi N, Yoshimura A, Nemoto K (2005) Tillering behavior of the rice fine culm 1 mutant. Plant Prod Sci 8:68–70

Groot SPC, Keizer LCP, Deruiter W, Dons JJM (1994) Seed and fruit-set of the lateral suppressor mutant of tomato. Sci Hortic-Amst 59:157–162

Hammer G, Cooper M, Tardieu F, Welch S, Walsh B, van Eeuwijk F, Chapman S, Podlich D (2006) Models for navigating biological complexity in breeding improved crop plants. Trends Plant Sci 11:587–593

Hart GE, Schertz KF, Peng Y, Syed NH (2001) Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. Theor Appl Genet 103:1232–1242

Hu FY, Tao DY, Sacks E, Fu BY, Xu P, Li J, Yang Y, McNally K, Khush GS, Paterson AH, Li ZK (2003) Convergent evolution of perenniality in rice and sorghum. Proc Natl Acad Sci USA 100:4050–4054

Huang XH, Wei XH, Sang T, Zhao QA, Feng Q, Zhao Y, Li CY, Zhu CR, Lu TT, Zhang ZW, Li M, Fan DL, Guo YL, Wang A, Wang L, Deng LW, Li WJ, Lu YQ, Weng QJ, Liu KY, Huang T, Zhou TY, Jing YF, Li W, Lin Z, Buckler ES, Qian QA, Zhang QF, Li JY, Han B (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42:961-U976

Hufford MB, Xu X, van Heerwaarden J, Pyhajarvi T, Chia JM, Cartwright RA, Elshire RJ, Glaubitz JC, Guill KE, Kaeppler SM, Lai JS, Morrell PL, Shannon LM, Song C, Springer NM, Swanson-Wagner RA, Tiffin P, Wang J, Zhang GY, Doebley J, McMullen MD, Ware D, Buckler ES, Yang S, Ross-Ibarra J (2012) Comparative population genomics of maize domestication and improvement. Nat Genet 44:808-U118

Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J (2005) Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiol 46:79–86

Itoh JI, Hibara KI, Sato Y, Nagato Y (2008) Developmental role and auxin responsiveness of class III homeodomain leucine zipper gene family members in rice. Plant Physiol 147:1960–1975

Kim HK, Luquet D, van Oosterom E, Dingkuhn M, Hammer G (2010a) Regulation of tillering in sorghum: genotypic effects. Ann Bot 106:69–78

Kim HK, van Oosterom E, Dingkuhn M, Luquet D, Hammer G (2010b) Regulation of tillering in sorghum: environmental effects. Ann Bot 106:57–67

Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, Okamoto H, Shimamoto K, Kyozuka J (2003) LAX and SPA: major regulators of shoot branching in rice. Proc Natl Acad Sci USA 100:11765–11770

Kong W, Jin H, Franks CD, Kim C, Bandopadhyay R, Rana MK, Auckland SA, Goff VH, Rainville LK, Burow GB, Woodfin C, Burke JJ, Paterson AH (2013) Genetic Analysis of Recombinant Inbred Lines for *Sorghum bicolor* × *Sorghum propinquum*. G3: Genes| Genomes|Genetics 3:101–108

Kyozuka J (2007) Control of shoot and root meristem function by cytokinin. Curr Opin Plant Biol 10:442–446

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA (1987) MAPMAKER: an interactive computer

package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lee TH, Tang HB, Wang XY, Paterson AH (2013) PGDD: a database of gene and genome duplication in plants. Nucl Acids Res 41:D1152–D1158

Leyser O (2003) Regulation of shoot branching by auxin. Trends Plant Sci 8:541–545

Leyser O (2006) Dynamic integration of auxin transport and signalling. Curr Biol 16:R424–R433

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760

Li XY, Qian Q, Fu ZM, Wang YH, Xiong GS, Zeng DL, Wang XQ, Liu XF, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li JY (2003) Control of tillering in rice. Nature 422:618–621

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Proc GPD (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141:391–411

Lin H, Wang RX, Qian Q, Yan MX, Meng XB, Fu ZM, Yan CY, Jiang B, Su Z, Li JY, Wang YH (2009) DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. Plant Cell 21:1512–1525

Lin QB, Wang D, Dong H, Gu SH, Cheng ZJ, Gong J, Qin RZ, Jiang L, Li G, Wang JL, Wu FQ, Guo XP, Zhang X, Lei CL, Wang HY, Wan JM (2012) Rice APC/C-TE controls tillering by mediating the degradation of MONOCULM 1. Nat Commun 3:752

Lipka AE, Tian F, Wang QS, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang ZW (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397–2399

Mace ES, Jordan DR (2011) Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. Theor Appl Genet 123:169–191

Mao CZ, Ding WN, Wu YR, Yu J, He XW, Shou HX, Wu P (2007) Overexpression of a NAC-domain protein promotes shoot branching in rice. New Phytol 176:288–298

McCouch SR, Cho YG, Yano M, Paul E, Blinstrub M, Morishima H, Kinoshita T (1997) Report on QTL nomenclature. Rice Genet Newsl 14:11–13

McSteen P (2009) Hormonal regulation of branching in grasses. Plant Physiol 149:46–55

Moore PH, Maretzki A (1996) Photoassimilate distribution in plants and crops—source-sink relationships. Marcel Dekker, New York

Moore PH, Botha FC, Furbank RT, Grof CRL (1997) Potential for overcoming physio-biochemical limits to sucrose accumulation. Intensive sugarcane production: meeting the challenge beyond 2000. Sugar 2000 symposium, Intensive sugarcane production: meeting the challenge beyond 2000; pp 141–155. CAB International, Wallingford, UK

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown PJ, Acharya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci USA 110:453–458

Murray SC, Rooney WL, Mitchell SE, Sharma A, Klein PE, Mullet JE, Kresovich S (2008a) Genetic improvement of sorghum as a biofuel feedstock: II. QTL for stem and leaf structural carbohydrates. Crop Sci 48:2180–2193

Murray SC, Sharma A, Rooney WL, Klein PE, Mullet JE, Mitchell SE, Kresovich S (2008b) Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. Crop Sci 48:2165–2179

Nagasaki H, Itoh JI, Hayashi K, Hibara KI, Satoh-Nagasawa N, Nosaka M, Mukouhata M, Ashikari M, Kitano H, Matsuoka M, Nagato Y, Sato Y (2007) The small interfering RNA production pathway is required for shoot meristern initiation in rice. Proc Natl Acad Sci USA 104:14867–14871

Nakagawa M, Shimamoto K, Kyozuka J (2002) Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice. Plant J 29:743–750

Paterson AH, Schertz KF, Lin YR, Liu SC, Chang YL (1995) The weediness of wild plants—molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum-Halepense* (L) Pers. Proc Natl Acad Sci USA 92:6127–6131

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Lyons E, Maher C, Narechania A, Penning B, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein PE, Kresovich S, McCann MC, Ming R, Peterson DG, Ware D, Westhoff P, Mayer KFX, Messing J, Rokhsar DS (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457:551–556

Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin DC, Llewellyn D, Showmaker KC, Shu SQ, Udall J, Yoo MJ, Byers R, Chen W, Doron-Faigenboim A, Duke MV, Gong L, Grimwood J, Grover C, Grupp K, Hu GJ, Lee TH, Li JP, Lin LF, Liu T, Marler BS, Page JT, Roberts AW, Romanel E, Sanders WS, Szadkowski E, Tan X, Tang HB, Xu CM, Wang JP, Wang ZN, Zhang D, Zhang L, Ashrafi H, Bedon F, Bowers JE, Brubaker CL, Chee PW, Das S, Gingle AR, Haigler CH, Harker D, Hoffmann LV, Hovav R, Jones DC, Lemke C, Mansoor S, Rahman MU, Rainville LN, Rambani A, Reddy UK, Rong JK, Saranga Y, Scheffler BE, Scheffler JA, Stelly DM, Triplett BA, Van Deynze A, Vaslin MFS, Waghmare VN, Walford SA, Wright RJ, Zaki EA, Zhang TZ, Dennis ES, Mayer KFX, Peterson DG, Rokhsar DS, Wang XY, Schmutz J (2012) Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature 492:423

Punta M, Coggill PC, Eberhardt RY, Mistry J, Tate J, Boursnell C, Pang N, Forslund K, Ceric G, Clements J, Heger A, Holm L, Sonnhammer ELL, Eddy SR, Bateman A, Finn RD (2012) The Pfam protein families database. Nucl Acids Res 40:D290–D301

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing

Rao NN, Prasad K, Kumar PR, Vijayraghavan U (2008) Distinct regulatory role for RFL, the rice LFY homolog, in determining flowering time and plant architecture. Proc Natl Acad Sci USA 105:3646–3651

Rooney WL, Blumenthal J, Bean B, Mullet JE (2007) Designing sorghum as a dedicated bioenergy feedstock. Biofuels Bioprod Biorefining-Biofpr 1:147–157

Shani E, Yanai O, Ori N (2006) The role of hormones in shoot apical meristem function. Curr Opin Plant Biol 9:484–489

Shiringani AL, Frisch M, Friedt W (2010) Genetic mapping of QTLs for sugar-related traits in a RIL population of *Sorghum bicolor* L. Moench. Theor Appl Genet 121:323–336

Simons JL, Napoli CA, Janssen BJ, Plummer KM, Snowden KC (2007) Analysis of the DECREASED APICAL DOMINANCE genes of petunia in the control of axillary branching. Plant Physiol 143:697–706

Sorefan K, Booker J, Haurogne K, Goussot M, Bainbridge K, Foo E, Chatfield S, Ward S, Beveridge C, Rameau C, Leyser O (2003) MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in *Arabidopsis* and pea. Gene Dev 17:1469–1474

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

Tabuchi H, Zhang Y, Hattori S, Omae M, Shimizu-Sato S, Oikawa T, Qian Q, Nishimura M, Kitano H, Xie H, Fang XH, Yoshida H, Kyozuka J, Chen F, Sato Y (2011) LAX PANICLE2 of rice encodes a novel nuclear protein and regulates the formation of axillary meristems. Plant Cell 23:3276–3287

Takai T, Yonemaru J, Kaidai H, Kasuga S (2012) Quantitative trait locus analysis for days-to-heading and morphological traits in an RIL population derived from an extremely late flowering F-1 hybrid of sorghum. Euphytica 187:411–420

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

Thangasamy S, Chen PW, Lai MH, Chen J, Jauh GY (2012) Rice LGD1 containing RNA binding activity affects growth and development through alternative promoters. Plant J 71:288–302

Tilman D, Socolow R, Foley JA, Hill J, Larson E, Lynd L, Pacala S, Reilly J, Searchinger T, Somerville C, Williams R (2009) Beneficial Biofuels-the food, energy, and environment trilemma. Science 325:270–271

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195-U129

Upadhyaya HD, Wang YH, Sharma S, Singh S, Hasenstein KH (2012) SSR markers linked to kernel weight and tiller number in sorghum identified by association mapping. Euphytica 187:401–410

Waldie T, Hayward A, Beveridge CA (2010) Axillary bud outgrowth in herbaceous shoots: how do strigolactones fit into the picture? Plant Mol Biol 73:27–36

Wang YH, Li JY (2006) Genes controlling plant architecture. Curr Opin Biotech 17:123–129

Wang S, Basten CJ, Zeng Z-B (2011) Windows QTL Cartographer 2.5. Department of statistics. North Carolina State University, Raleigh. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Whipple CJ, Kebrom TH, Weber AL, Yang F, Hall D, Meeley R, Schmidt R, Doebley J, Brutnell TP, Jackson DP (2011) Grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. Proc Natl Acad Sci USA 108:E506–E512

Xiong GS, Hu XM, Jiao YQ, Yu YC, Chu CC, Li JY, Qian Q, Wang YH (2006) LEAFY HEAD2, which encodes a putative RNA-binding protein, regulates shoot development of rice. Cell Res 16:267–276

Xu SZ (2008) Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201–2208

Wang L, Xu Y, Zhang C, Ma Q, Joo SH, Kim SK, Xu ZH, Chong K (2008) OsLIC, a Novel CCCH-type zinc finger protein with transcription activation, mediates rice architecture via brassinosteroids signaling. Plos One 3(10):e3521

Zhang LY, Wang SQ, Li HH, Deng QM, Zheng AP, Li SC, Li P, Li ZL, Wang JK (2010a) Effects of missing marker and segregation distortion on QTL mapping in F-2 populations. Theor Appl Genet 121:1071–1082

Zhang ZW, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu JM, Arnett DK, Ordovas JM, Buckler ES (2010b) Mixed linear model approach adapted for genome-wide association studies. Nat Genet 42:355-U118

Zhang D, Guo H, Kim C, Lee TH, Li JP, Robertson J, Wang XY, Wang ZN, Paterson AH (2013) CSGRqtl, a comparative quantitative trait locus database for saccharinae grasses. Plant Physiol 161:594–599

Zou JH, Chen ZX, Zhang SY, Zhang WP, Jiang GH, Zhao XF, Zhai WX, Pan XB, Zhu LH (2005) Characterizations and fine mapping of a mutant gene for high tillering and dwarf in rice (*Oryza sativa* L.). Planta 222:604–612