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RESEARCH

Quantitative Trait Loci Mapping of Agronomic and Yield Traits in Two Grain Sorghum Biparental Families

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ABSTRACT

The animal industry is a major sector of agriculture in the southeastern United States, but a large deficit exists in regional feed grains needed to support the industry. An increase in production of sorghum [*Sorghum bicolor* (L.) Moench], a water- and nutrient-use-efficient cereal, on marginal lands could lead to an alternative crop option for growers and reduce the current grain deficit. Quantitative trait locus (QTL) mapping of grain yield components in two sorghum biparental recombinant inbred line (RIL) populations was performed to better understand the genetic basis of grain yield and characterize these traits in a marginal environment. A more robust knowledge of the genetics underlying these complex traits could provide insights into molecular breeding strategies that aim to increase genetic gain. Specific yield traits investigated were grain number per primary panicle (GNP), 1000-grain weight (TGW), and grain yield per primary panicle (YPP). Two-year phenotyping in the South Carolina coastal plain revealed greater than threefold variation for both GNP and YPP, whereas TGW variation was just above twofold in both RIL families. There were 16 total yield trait QTL identified across both populations. Of the 16, eight QTL colocated with previously published QTL for yield-related traits, including a QTL on chromosome 1 that was significant for all three grain yield components. A novel QTL for TGW was identified on chromosome 5 that explained >21% of the phenotypic variance observed in one RIL population. This QTL and the seven additional novel QTL identified in this study provide new targets for grain yield improvement in sorghum.

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Abbreviations: BLUP, best linear unbiased predictor; GBS, genotyping-by-sequencing; GNP, grain number per primary panicle; GVE, genotypic variance explained; G × E, genotype-by-environment; LD, linkage disequilibrium; LOD, logarithm of the odds; PVE, phenotypic variance explained; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SNP, single-nucleotide polymorphism; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

Grain sorghum [*Sorghum bicolor* (L.) Moench] is a hardy, C_4 cereal crop that can produce yield in locales where other cereal species cannot, a tribute to its immense natural diversity. Sorghum domestication in sub-Saharan Africa provided landraces with excellent drought and heat tolerance (Borrell et al., 2014). These traits allow sorghum production on marginal lands throughout the semiarid tropical and temperate zones, including US and Australian regions where natural rainfall can be unreliable. While grain sorghum hybrids can yield in unfavorable environments, this cereal crop can also generate yields exceeding 10,000 kg ha^{-1} when grown under ideal conditions (Jordan et al., 2011). This can be attributed to sorghum's genetic and physiological diversity, as this resilient species is adapted to variable climates across regions of Africa and southern Asia (Morris et al., 2013). Identifying useful

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genetic variation underlying agronomic and yield traits would help exploit this genetic diversity for crop improvement and allow marker-assisted selection and breeding strategies to develop sorghums for specific environments.

Final grain yield in sorghum is correlated with a multitude of traits, including anthesis, vegetative biomass, and abiotic and biotic stress resistance (Borrell et al., 2014; Boyles et al., 2016a). The magnitude of effect that each trait has on grain yield is dependent on genotype, environmental conditions throughout the growing season, and specific genotype-by-environment (G × E) interactions (Chapman et al., 2000). Genetically, grain yield is a highly quantitative and complex trait; however, genetic mapping strategies have been able to identify quantitative trait loci (QTL) for grain yield and yield-related traits in sorghum (Rami et al., 1998; Brown et al., 2006; Murray et al., 2008; Srinivas et al., 2009). Genetic mapping of three grain yield components—grain number per primary panicle (GNP), 1000-grain weight (TGW), and grain yield per primary panicle (YPP)—that were evaluated in the southeastern United States was implemented to compare grain yield QTL found in a new production region, which has low organic matter sandy soils and a high average humidity, with existing QTL found previously. Additionally, six traits—plant color, pericarp color, days to anthesis, plant height, sugarcane aphid (*Melanaphis sacchari*) prevalence, and crop injury—were evaluated in the study to ascertain their correlations with grain yield components and identify QTL colocalizations between traits. Most US grain sorghum is developed, produced, and grown in the Great Plains from Kansas to Texas, but there has been interest in sorghum production on marginal dry lands in the southeastern United States as a feed grain alternative to the more nutrient- and water-intensive maize (*Zea mays* L.). Sorghum can be productive for growers in absence of heavy nutrient inputs and can importantly help to reduce the large feed grain deficit in the region (Hollis, 2002). A better understanding of the genetics of yield components in general and G × E interaction that exists within the southeastern United States could aid breeding efforts to increase sorghum productivity in the region and additional growing regions with similar land quality and precipitation patterns, such as eastern South America, central Africa, and southern Asia (USDA-NRCS-Soils, 2003).

Quantitative trait locus mapping was implemented to identify regions of the genome that are linked to grain yield-related traits. This study used two sorghum recombinant inbred line (RIL) populations that shared a common parent. Next-generation sequencing technology and its rapid decrease in cost have enabled genotyping of segregating populations at much higher density (Metzker, 2010). In addition, establishment of the 'BTx623' reference genome (Paterson et al., 2009) and use of genotyping-by-sequencing (GBS) technology (Elshire et al., 2011) further facilitate genotyping of individuals for thousands of single-nucleotide polymorphisms (SNPs) in *S. bicolor*. This marker density allows for sufficient coverage of all linkage disequilibrium (LD) blocks within a segregating population, potentially narrowing down the search window for causal genes (Boyles et al., 2016b). In this study, the development of high-density SNP datasets for two RIL populations and 2-yr phenotyping of agronomic and yield traits allowed linkage mapping to detect robust QTL for complex grain yield traits.

MATERIAL AND METHODS

Plant Material

Both RIL populations in this study share BTxARG-1 (PI561072) as a common parent. This breeding line has desirable attributes for food-grade products (Miller et al., 1992). BTxARG-1 has a tan plant color, white pericarp color, and waxy endosperm (low amylose) (Miller et al., 1992). The male-sterile near-isogenic line of PI561072 is used as a female parent for hybrid production (Hayes and Rooney, 2014). For yield components evaluated in this study, BTxARG-1 has above average GNP at 1827 but a low TGW of 17.5 g in South Carolina (Boyles et al., 2016a). The second parent of one population was P850029 (PI656056), which was derived from the mutant line P721Q (Weaver et al., 1998). P850029 is a high-lysine sorghum breeding line that possesses the high-digestible protein trait (Weaver et al., 1998; Jampala et al., 2012; Winn et al., 2009). Relative to grain yield, P850029 previously displayed the highest GNP (1859) and TGW (26.1 g) among the three parent lines used in this study. The second parent of the additional RIL population was BTx642 (PI656029), a yellow-pericarp sorghum with postflowering drought resistance (Harris et al., 2007). BTx642, a derivative of the stay-green conversion line SC35, was developed by Rosenow et al. (2002) as a parent line for hybrid grain sorghum development. This line has low average GNP of 1585 and a TGW of 23.5 g (Boyles et al., 2016a).

There were a total of 279 individuals in the BTxARG-1/P850029 population and 191 individuals in the BTx642/BTxARG-1 population after removal of lines with poor genetic data or missing phenotypes. Both populations were phenotyped in the $F_{4:5}$ generation. The two populations, BTx642/BTxARG-1 and BTxARG-1/P850029, are henceforth referred to as BTx642 and P850029, respectively.

Genotyping

Genotyping was first and fully described in Boyles et al. (2016b). Briefly, leaf tissue was harvested from ~14-d-old seedlings of each F_5 RIL and the three parents. DNA was extracted from harvested tissue and genotyped at the Cornell University Genomic Diversity Facility. DNA was digested with the ApeKI restriction enzyme, and DNA fragments from 96 individuals were pooled together for sequencing. Genotype-by-sequencing libraries were sequenced on an Illumina HiSeq 2500.

Reads were aligned to the most recent reference genome version, *Sorghum bicolor v3.1* (https://phytozome.jgi.doe.gov/). The TASSEL 5.0 GBS pipeline (Glaubitz et al., 2014) was used to call and impute SNPs. Imputation was performed with the

TASSEL plugin FSFHap (Swarts et al., 2014) with the parameters described in Boyles et al. (2016b). The SNPs with a minor allele frequency <0.05 were treated as missing. The final number of SNPs retained after culling was 71,856 for BTx642 and 49,617 for P850029.

Recombination Bin and Genetic Map Construction

Existing genetic maps for each population were available from Boyles et al. (2016b). To highlight, SNPs for each RIL population were placed into recombination bins using the Huang et al. (2009) method to reduce the computational burden and accommodate software memory limitations. Following the Huang et al. (2009) protocol, there were 4601 recombination breakpoints in BTx642 and 4154 breakpoints in the P850029 population identified using a 15-SNP sliding window. Of these total breakpoints, 1423 and 777 were classified as homozygous-to-heterozygous in BTx642 and P850029, respectively. For map construction, recombination breakpoints were treated as individual markers. The Kosambi mapping function (Kosambi, 1943) was used to convert physical positions into genetic distances on the basis of a maximum iteration number of 1000 and an error probability of 1×10^{-4}. The genetic maps were converted to cross type "riself," which is an R/qtl abbreviation for "RIL by selfing." This cross type riself does not allow for heterozygosity (Broman et al., 2003); therefore, heterozygous markers were treated as missing. Individual markers with a minor allele frequency <0.05 as a result of both high heterozygosity and missing data were removed. Markers with severe segregation distortion ($p < 10^{-20}$) were also eliminated to result in a total of 4589 markers for BTx642 and 4149 markers for P850029 in the final dataset (Supplemental Table S1). Genetic map lengths were 1574.2 and 1416.7 cM for BTx642 and P850029, respectively. For each RIL population, average marker spacing per chromosome was ≤0.5 cM for all 10 chromosomes. The chromosomal mean intermarker physical distance was 199,363 bp for BTx642 and 173,805 bp for P850029.

Field Design

Field experiments were planted on 13 May 2014 and 6 May 2015. Populations were arranged in a twice-replicated randomized complete block design in Blackville, SC. Soil type classification both years was Barnwell loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludults; ~20% loam, ~80% sand), a coarse soil type with a low water- and nutrient-holding capacity. Plot dimensions consisted of 0.965-m row spacing and row lengths of 6.1 m, except the BTx642 population in 2014, which required row lengths of 3.05 m as a result of limited available seed. Seeding rate was 170,000 seeds ha^{-1}. Prior to planting, granular N, P, and K were applied at variable rates across field locations based on soil sample recommendations in effort to normalize soil nutrient concentration. Bicep II Magnum (*S*-metolachlor + atrazine) was applied preemergence at 3.5 L ha^{-1} both years. A postemergent application of atrazine at a rate of 4.7 L ha^{-1} was applied each year when average plant height reached 40 cm. During the 120-d growing season (planting through harvest), total accumulated rainfall was 52.6 and 29 cm in 2014 and 2015, respectively. Plots received minimal supplemental irrigation only to prevent plants from becoming severely drought stressed. The 2014 field experiment was cultivated 44 d after planting to reduce Texas panicum (*Panicum texanum*). A single lay-by-N application at 67 kg ha^{-1} was administered both years ~45 d after planting. During grain fill, 0.36 L ha^{-1} of Endigo ZC (pyrethroid) in 2014 and 1.2 L ha^{-1} of Prevathon (chlorantraniliprole) in 2015 were applied to control corn earworm (*Helicoverpa zea*) populations. A BroadBand Pro (Bird-X) noise repeller was used to prevent birds from causing severe grain yield loss. There was a severe infestation of sugarcane aphids (*Melanaphis saccari*) in 2015. In effort to minimize aphid populations, Nufos 4E (chlorpyrifos) and Dimethoate 4E (dimethoate), two organophosphate insecticides at 1.2 L ha^{-1} each, were simultaneously applied 62 d after planting. After application of this chemical mixture, noticeable crop injury of variable degrees was observed across RILs. Large sugarcane aphid populations still remained; therefore, two applications of Transform WG (sulfoxaflor) at 0.1 L ha^{-1} were administered 21 d apart to avoid yield loss. Quilt Xcel at 1 L ha^{-1} was applied during grain fill to minimize fungal pressure.

Phenotyping

Detailed collection of grain yield component traits is described in Boyles et al. (2016a). In addition to grain yield traits, number of days to anthesis was recorded from planting date to when 50% of the plants in the plot reached mid-bloom. Height (cm) was measured from ground to apex of main panicle at physiological maturity in the P850029 population. Population BTx642 did not segregate for plant height. Crop injury from organophosphate insecticide applications in 2015 was recorded on a nominal scale from 1 to 5, with 1 = no injury and 5 = severe injury. Visual estimations of sugarcane aphid prevalence within each plot were also recorded on a 1-to-5 scale in 2015. Plant color and pericarp color (BTx642 only) were also recorded for quality control purposes and to investigate the relationships of these qualitative traits with individual grain yield components. For a minority of RILs, observations for these traits were inconsistent across years and replications of data. This inconsistency is likely a result of outcrossing during population development, seed carryover between plots during planting, and simple misclassification. Therefore, to improve accuracy, these traits were imputed on the basis of the most frequently observed color for each individual.

Three randomly selected main panicles were harvested at physiological maturity within each plot for grain-yield component measurements. Physiological maturity was determined when black layer was present on grains at the basal portion of the main panicle. After harvest, panicles were air dried for 10 to 14 d in a greenhouse until reaching a constant weight. Panicles were threshed manually by hand to avoid grain loss caused by mechanical threshers. The grain obtained after threshing was first cleaned with an air aspirator (AT Ferrell Company) to remove loose glumes and plant debris. Grain number per primary panicle was measured by running the grain from each panicle through a Model 900-2 seed counter (Old Mill). Grain yield per primary panicle was measured with a Discovery series scale (Ohaus), and TGW was estimated on the basis of GNP and YPP.

Phenotypic Analysis

The simple mean from three data values (one value for each panicle) was calculated within each field replicate for YPP,

GNP, and TGW. Variance components (σ^2) using multiyear and replicated data were calculated with the "lme4" R package (Bates et al., 2014), as described previously (Boyles et al., 2016a). All effects were treated as random. The trait correlation matrix was generated by the Pearson method with the cor() function in R software (R Core Development Team, 2015). For correlation analysis, best linear unbiased predictors (BLUPs) were generated using the ranef() function within the same "lme4" R package (Bates et al., 2014) according to Merk et al. (2012). Because the sugarcane aphid prevalence and crop injury phenotypes were only evaluated in 2015, mean replicated observational ratings were used for correlations instead of BLUPs. The cor.test() function in R was used to determine significance for each correlation. The replicate component was used in place of location when calculating broad-sense heritability (H^2), as shown below:

$$H^2 = \frac{\sigma_G^2}{\left[\sigma_G^2 + \left(\frac{\sigma_{G\times R}^2}{R}\right) + \left(\frac{\sigma_{G\times Y}^2}{Y}\right) + \left(\frac{\sigma_E^2}{RY}\right)\right]}$$

where G is genotype, R is replicate, Y is year, and E is error.

Genetic variance explained (GVE) by individual QTL was calculated according to Broman et al. (2003). Using the maximum logarithm of odds (LOD) score within the QTL interval, GVE was calculated as follows:

$$\text{GVE} = 1 - 10^{-2\text{LOD}/n}$$

where LOD is LOD score and n is number of RILs included in the analysis.

To estimate phenotypic variance explained (PVE) by each QTL, GVE was multiplied by the overall broad-sense heritability (H^2) (Broman et al., 2003):

$$\text{PVE} = H^2 (1 - 10^{-2\text{LOD}/n})$$

QTL Mapping

Linkage analysis was performed with the software R/qtl (Broman et al., 2003) within the R environment (R Core Development Team, 2015). The scanone() R function performed simple interval mapping to detect significant QTL for each trait using BLUPs calculated from replicated data within the 2 yr, as described above. Mean replicated observational ratings for sugarcane aphid prevalence and crop injury were used instead of BLUPs for mapping these two traits because they were only evaluated in 2015. The QTL model incorporated phenotypic covariates (Supplemental Table S2), which removed potential confounding effects on grain yield traits. With $\alpha = 0.05$, a genomewide LOD significance threshold of 3.3 was determined by running $n = 1000$ permutations of the expectation-maximization algorithm. The LOD = 3.3 significance threshold was the same for each biparental population (Boyles et al., 2016b). Marker effects were estimated to generate a directed QTL plot. The effect of a marker was considered positive when the BLUP mean from RILs possessing the unique parent allele was greater than the BLUP mean from RILs possessing the allele from BTxARG-1. A negative marker effect was estimated when RILs carrying a unique parent allele had a lower BLUP mean. The LOD score at each positive- and negative-effect marker was subsequently multiplied by 1 and –1, respectively, to obtain directed LOD scores.

RESULTS

Genomic Features Influencing QTL Mapping

Details regarding the total number of SNP markers, recombination breakpoints, and the genetic map for each RIL population have been previously described in Boyles et al. (2016b). The P850029 HapMap contained 22,239 fewer genomewide SNPs than the BTx642 HapMap, as well as 447 fewer recombination breakpoints. This finding was expected, as BTx642 is a Milo-Durra type sorghum, whereas P850029 and BTxARG-1 both group primarily within the Caudatum race (Supplemental Fig. S1). The SNP sites segregating within each population were slightly overrepresented with alleles from BTxARG-1.

The pairwise LD average of BTx642 and P850029 fell below $r^2 = 0.2$ after 5.7 and 5.1 Mb, respectively, but regional LD varied genomewise. Recombination maps for each RIL family clearly show that recombination was much more frequent at the distal ends of chromosomes (Fig. 1). In BTx642, there were two regions, one on chromosome 2 and the other on chromosome 6, where a high proportion of lines retained heterozygosity (Fig. 1a). The ~10-Mb region on chromosome 2 contained nearly a twofold increase in heterozygous markers compared with the genomewide average and a significant increase in missing data (Supplemental Fig. S2). Smaller regions on chromosomes 2, 3, 5, and 6 in P850029 also contained higher than expected residual heterozygosity (Fig. 1b). In the P850029 population, there was a large pericentromeric region on chromosome 9 that contained very little polymorphisms detected by GBS (Fig. 1b).

Trait Characterization

Of the 484 RILs evaluated, 17% were still segregating for plant color. Approximately 48% of lines were red or purple (pigmented), and 35% were tan. In the BTx642 population, 40% of RILs had a white pericarp, 19% red, and 10% yellow, and 31% segregated for pericarp color. The 38 red pericarp lines in BTx642 had a greater mean GNP and TGW than the 79 RILs with a white pericarp, and thus red pericarp lines had a higher final grain yield (Supplemental Table S3). When simultaneously observing plant and pericarp color in BTx642, RILs with pigmented plant and red pericarp color possessed highest YPP at 61.3 g, followed by lines with segregating plant and red pericarp color (57.3 g). The 14% of RILs in BTx642 with tan plant and white pericarp color had a lower mean YPP at 51.4 g. This drop in yield was attributed to tan plant, white pericarp RILs having both fewer GNP and a significantly lower TGW (Supplemental Table S3).

Plant maturity and height across parental genotypes were very similar (Table 1). The three parents reached anthesis at ~70 to 75 d after planting when data were averaged across years. Parent lines matured faster and were slightly shorter in height at maturity in 2015. Mean height in the P850029 population was 25 cm shorter in 2015.

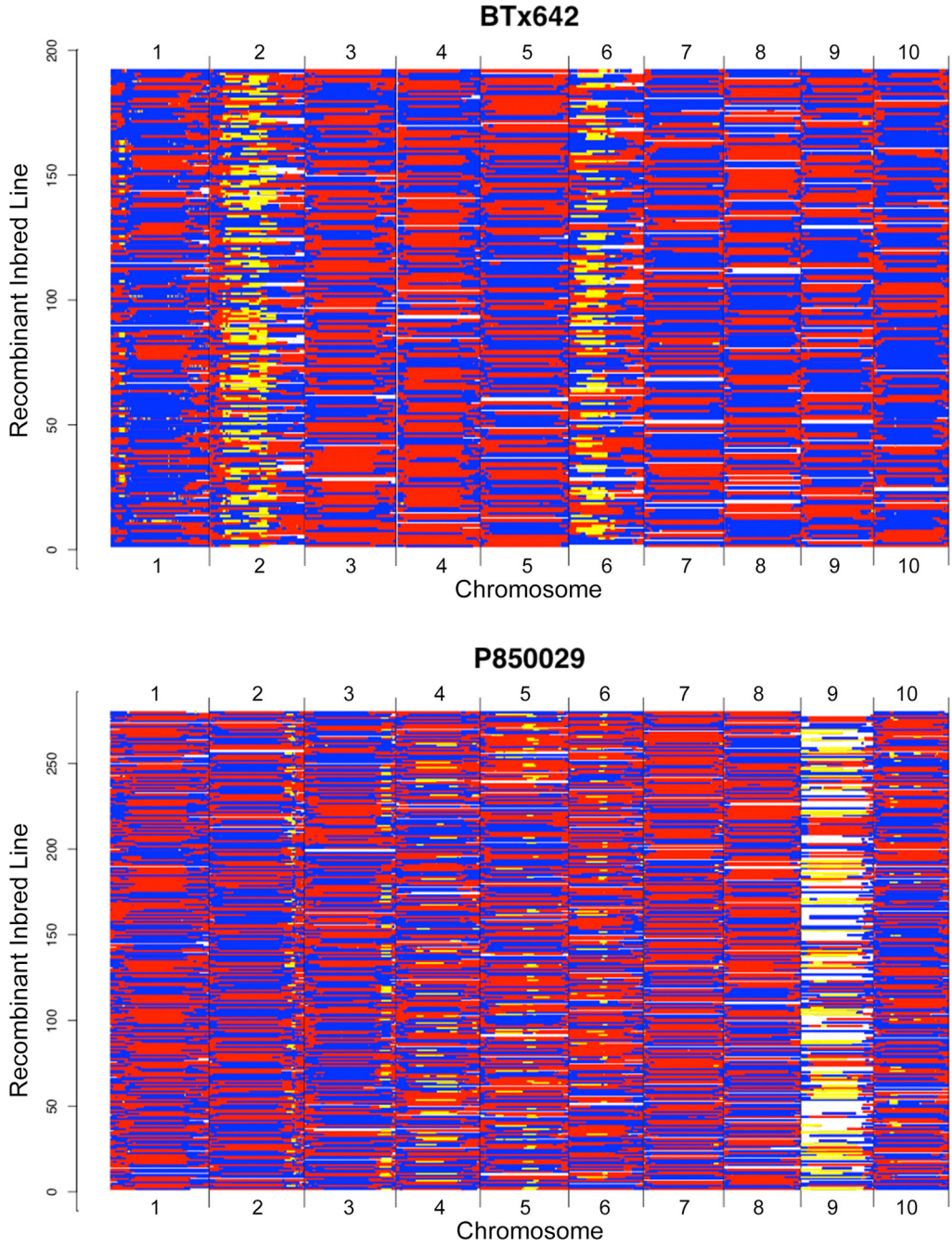

Fig. 1. Genomewise recombination blocks are shown for each RIL in (a) BTx642 and (b) P850029. (a) Blue, BTxARG-1 allele; red, BTx642 allele; yellow, heterozygous. (b) Blue, BTxARG-1 allele; red, P850029 allele; yellow, heterozygous.

Table 1. Characterization of agronomic and yield-related traits within the two recombinant inbred line (RIL) populations.

	Parents			BTx642 RIL Population			P850029 RIL Population		
Trait†	BTx642	BTxARG-1‡	P850029	H^2§	Range	Mean	H^2	Range	Mean
Anthesis	72	75.3/68.8	76.3	0.19	(66.5–80.5)	72.8	0.32	(65.8–79)	72.4
Height	110.5	118.5	118.5	NA	NA	NA	0.81	(78–224)	137
Crop injury¶	3.5	1.5/1	2.5	NA	(1–5)	2.5	NA	(1–5)	2.4
SCA	2.5	2.5/2.5	2	NA	(1–4.5)	3.2	NA	(1–5)	3
GNP	1209	2184/2080	2194	0.72	(999–3716)	1940	0.57	(1132–4026)	2245
TGW	30.9	24.3/25.9	27	0.79	(19.3- 39)	27.9	0.71	(18.6–39)	29
YPP	37.9	52.6/53.9	65.8	0.64	(26.5–99.7)	53.6	0.53	(34.1–103)	64.6

† SCA, sugarcane aphid prevalence; GNP, grain number per primary panicle; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

‡ BTx642 population average/P850029 population average.

§ Broad-sense heritability.

¶ 2015 data only.

Parent line P850029 was only 5 cm taller than BTxARG-1, but the range in height across the population was much larger (Table 1). The lack of observed variation in height in the BTx642 population justified omitting this trait from the dataset. Number of days to anthesis contained a significant percentage of trangressive segregation within both populations. Regarding crop injury, there was very little damage from insecticide application observed on parent BTxARG-1 compared with other parental genotypes.

There were considerable differences in individual yield components among the three parental lines: BTx642, BTxARG-1, and P850029 (Table 1). Common parent BTxARG-1 possessed the median GNP (2132) and YPP (53.3 g) but had a lower mean TGW (25.1 g) than the other parents. Parent BTx642 had the lowest average GNP (1209) and YPP (37.9 g) but the highest TGW (30.9 g), whereas P850029 at nearly 2200 grains and 65.8 g per main panicle was the highest-yielding parental line. Within the two RIL populations, there was greater than threefold variation for both GNP and YPP (Fig. 2). Variation in TGW was just above twofold in BTx642 and P850029. The progeny mean of each yield component was nearly equal to or greater than the parent line with the highest value, with the exception of a lower mean TGW in the BTx642 population (Table 1).

Variance Components and Trait Heritability

Similar error variance (~25%) was found across yield components in BTx642, whereas the P850029 population error variance ranged from 18% for TGW to 31% for GNP. Variation attributable to year was relatively high for GNP (42%) and YPP (54%) in BTx642. In both populations, greater variance was explained by year (environment) than the genetic (line) component for GNP and YPP, whereas the genetic component accounted for the highest variance in TGW (Supplemental Table S4). The broad-sense heritability of anthesis was low in BTx642 (H^2 = 0.19) and P850029 (H^2 = 0.32), which may be attributed to anthesis similarity among parental lines (Table 1) and hence lower population variation (1.2-fold) in anthesis compared with other traits evaluated. This result is supported by a higher percentage of error variance than other traits (Supplemental Table S4). Plant height at physiological maturity in P850029 was highly heritable, as previously reported (Brown et al., 2006; Boyles et al., 2016a). The heritabilities of all three grain-yield traits were higher in BTx642 than in P850029 (Table 1). In both populations, TGW had the highest heritability, followed by GNP. The YPP had lower heritability than GNP in BTx642 and P850029.

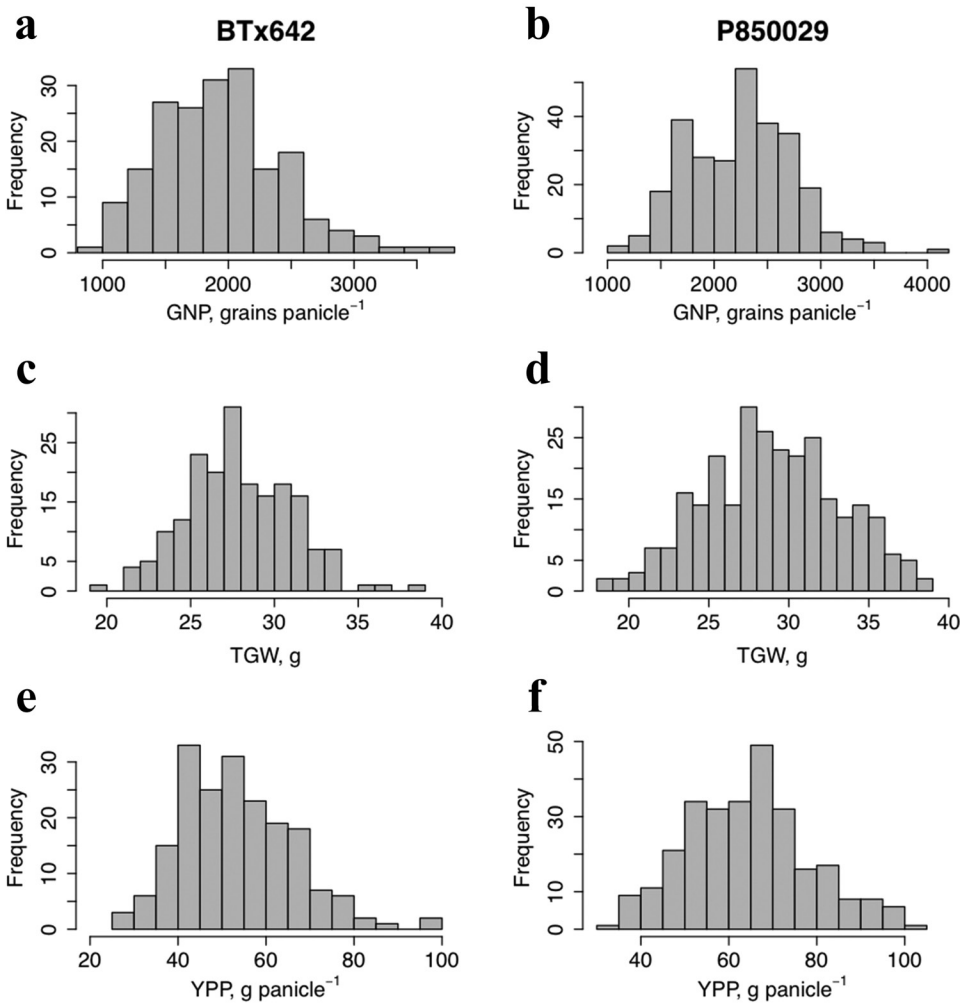

Fig. 2. Histograms for the three grain yield components in (a, c, e) BTx642 and (b, d, f) P850029. Values represent the phenotypic mean between years for each recombinant inbred line. GNP, grain number per primary panicle; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

Trait Correlations

Anthesis date was positively correlated with GNP and YPP in P850029 (Table 2). Although less significant than in P850029, positive correlations between these traits were detected in BTx642. Conversely, TGW in both populations had a slight negative relationship with anthesis. Sugarcane aphid prevalence had a low negative correlation with all yield components in each population, but only its correlation with GNP in BTx642 was significant at the $\alpha = 0.05$ probability level. Sugarcane aphid prevalence was not correlated with any additional traits except anthesis, where these traits displayed a slight negative correlation in BTx642 and P850029. Less sugarcane aphid prevalence in later-maturing accessions suggested that aphids might prefer sorghum at advanced developmental stages. Pigmented plant color was correlated with increased crop injury from insecticides in both populations; however, this might be observational bias by detecting injury more easily in RILs with pigmented rather than tan colors. Crop injury negatively affected grain yield-related traits in both populations, except TGW in BTx642. The GNP and YPP in BTx642 shared the strongest negative correlations with crop injury at $r = -0.42$.

Grain number per primary panicle had a stronger positive correlation with YPP than with TGW, irrespective of RIL population (Table 2). In fact, the correlation between these traits was $r = 0.89$ in BTx642 and $r = 0.79$ in P850029. The tradeoff observed between GNP and TGW was nearly identical between populations. Between populations, TGW contributed significantly more to final YPP in P850029 than among BTx642 progeny.

Crop Injury QTL and Candidate Genes

A negative relationship between crop injury from insecticides and grain yield components prompted investigation of the genetics underlying the trait. It was also important to determine the confounding effect crop injury had on

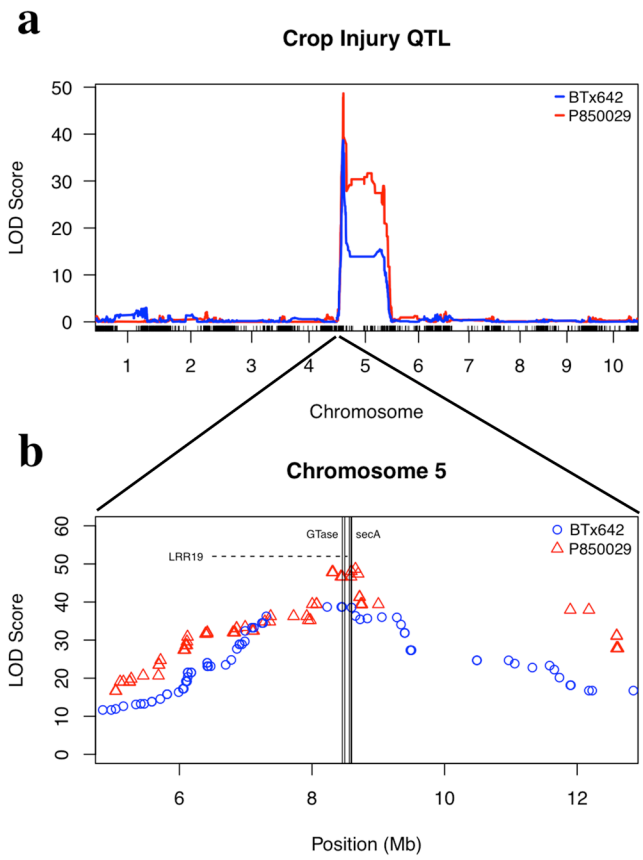

Fig. 3. A strong quantitative trait locus (QTL) was detected for crop injury from a simultaneous application of two organophosphates, dimethoate and chlorpyrifos, in 2015. (a) The crop injury QTL was easily detected by interval mapping in both recombinant inbred line populations. The QTL peaks between BTx642 and P850029 were separated by ~200 kb. (b) Within the 200-kb interval between QTL peaks, there were six transcripts encoding three LRR19-like proteins, UDP-glycosyl transferase 83A1 (GTase), and translocase secA (secA). Each blue circle and red triangle represents genetic marker locations from BTx642 and P850029, respectively. LOD, logarithm of odds.

Table 2. Phenotypic correlations using best linear unbiased predictors (BLUPs) among agronomic and yield traits in BTx642 (upper right) and P850029 (lower left).

	Plant color	Pericarp color†	Anthesis	Height‡	Crop injury§	SCA¶	GNP	TGW	YPP
Plant color	–	0.18*	−0.11	NA	0.23**	0.04	−0.05	0.3***	0.12
Pericarp color	NA	–	0.01	NA	−0.05	0.01	0.06	0.19**	0.17*
Anthesis	−0.22	NA	–	NA	0.04	−0.2**	0.18**	−0.09	0.15*
Height	−0.07	NA	0.06	–	NA	NA	NA	NA	NA
Crop injury	0.16**	NA	−0.01	−0.03	–	0.1	−0.42***	−0.01	−0.42***
SCA	0.03	NA	−0.16**	−0.05	0.06	–	−0.04	−0.02	−0.05
GNP	−0.08	NA	0.36***	0.18**	−0.21***	−0.14*	–	−0.4***	0.89***
TGW	−0.11	NA	−0.14*	0.32***	−0.33***	0.05	−0.35***	–	0.03
YPP	−0.15**	NA	0.3***	0.4***	−0.37***	−0.11	0.79***	0.27***	–

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probabiltiy level.

† Not segregating in P850029.

‡ 2015 phenotypic data only (no BLUPs).

§ Not segregating in BTx642.

¶ SCA, sugarcane aphid prevalence; GNP, grain number per primary panicle; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

yield trait QTL analysis. Interval mapping analysis identified a large-effect crop injury QTL on chromosome 5 in both RIL populations (Fig. 3a and 3b). This QTL on chromosome 5, which explained 61% of the genetic variance in BTx642 and 55% in P850029, was the only significant QTL identified in either population. This QTL colocated with the major-effect *opr* gene (resistance to organophosphates) that has been mapped previously (Tao et al., 1998; Tao et al., 2000; Xu et al., 2000). The marker with the maximum LOD score in BTx642 was located at 8,455,739 bp. In P850029, the most significant marker was positioned 200 kb away at 8,655,283 bp. There were six genes within this 200-kb window, with three encoding leucine rich repeat (LRR) proteins. These three *LRR19*-like genes in tandem likely arose from duplication events, as each protein similarity between them was >75%. Based on sequence homology, two additional genes encoded UDP-glycosyltransferase 83A1 and preprotein translocase SecA. The final gene, *Sobic.005G071800*, had no functional annotation.

Genetic Mapping Approach and Correlations

It was evident that crop injury confounded interval mapping of yield components based on QTL colocalization on chromosome 5 (Fig. 4a and 4b); therefore, the crop injury phenotype was incorporated into the model as a covariate for all grain-yield traits (Supplemental Table S2) with the exception of TGW in BTx642, which did not have a negative correlation with crop injury (Table 2). The QTL analysis incorporating the crop injury covariate eliminated the spurious chromosome 5 QTL in both RIL populations and increased LOD significance of additional QTL (Fig. 4c and 4d).

Overall, yield trait LOD scores were positively correlated in P850029, and BTx642 correlations were similar except there was no relationship found between TGW and YPP (Supplemental Table S5). The positive correlation between GNP and YPP in P850029 was stronger than in BTx642. The LOD scores were strongly correlated between GNP and YPP in BTx642 and P850029 (Supplemental Table S5), which follows the positive phenotypic relationship observed between these two traits (Table 2).

Yield Component QTL

BTx642

With high phenotypic variability in GNP, it was unexpected to find only a few significant QTL in BTx642. Only one QTL for GNP surpassed the LOD significance threshold. This QTL, located near 1 Mb on chromosome 1, colocated with a grain number QTL in P850029 (Table 3, Supplemental Table S6). The RILs with the BTxARG-1 allele on chromosome 1 for increased grain number had a mean GNP of >400 grains over the progeny average possessing the unfavorable BTx642 allele.

There were four significant QTL identified for TGW located on chromosomes 1, 2, 6, and 8 (Table 3). Each of these QTL accounted for 5 to 7% of the PVE. The

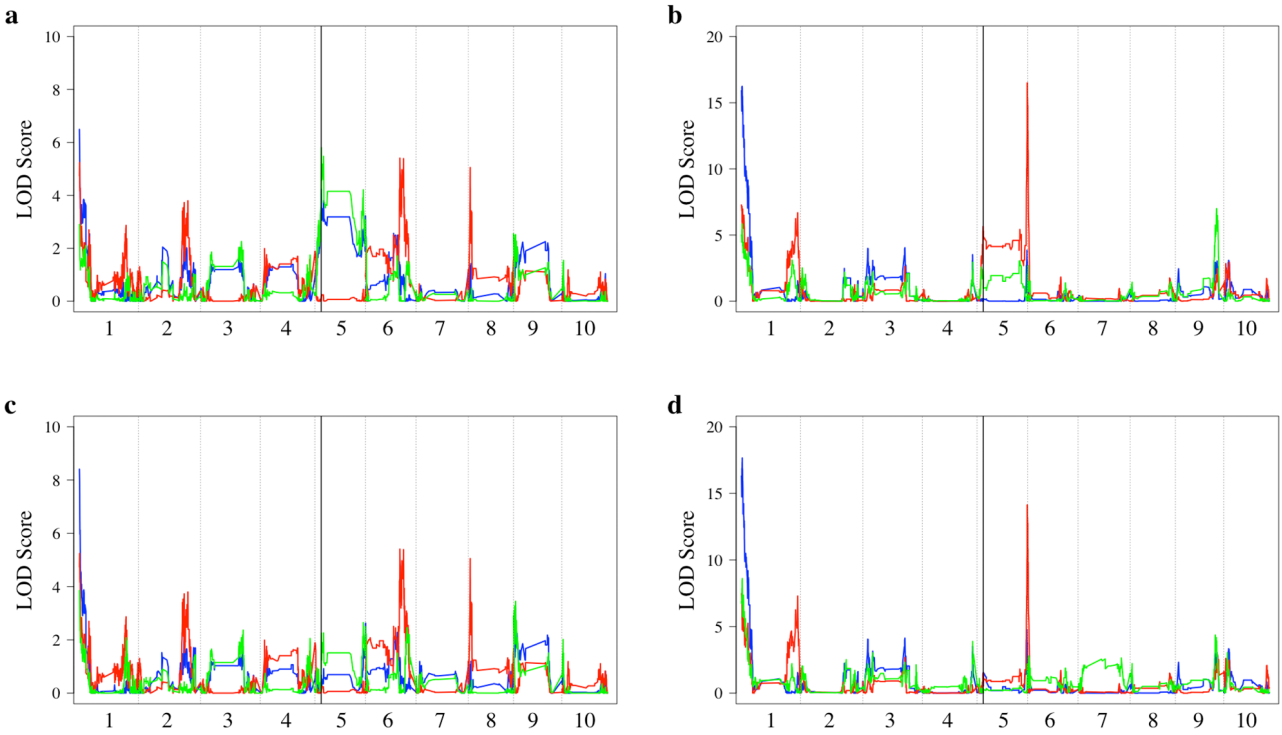

Fig. 4. The crop injury phenotype was included within the model for quantitative trail loci (QTL) mapping of yield components to eliminate the false-positive QTL on chromosome 5. The black vertical line denotes the crop injury QTL peak. Additional QTL for grain number per primary panicle (GNP), 1000-grain weight (TGW), and grain yield per primary panicle (YPP) were found consistently across models that (a, b) included or (c, d) excluded the crop injury covariate. Refer to Supplemental Table S6 for exact QTL positions. LOD, logarithm of odds.

Table 3. List of significant quantitative trait loci (QTL) identified in each biparental population.

	Trait†	Chromosome	Start	End	Peak	LOD‡	GVE§	PVE¶
			——— Mb ———		bp			
BTx642	Plant color	6	49.9	60.77	56,650,607	49.47	0.697	0.424
	Pericarp color	1	62.54	77.04	68,387,980	30.13	0.516	0.314
	Pericarp color	3	53.77	58.51	56,719,439	5.23	0.118	0.072
	Anthesis	7	5.16	5.16	5,161,061	3.54	0.082	0.05
	GNP	1	0.78	8.12	778,962	8.41	0.183	0.112
	TGW	1	0.78	1.94	1,010,720	5.24	0.119	0.072
	TGW	2	59.13	66.62	66,456,799	3.79	0.087	0.053
	TGW	6	45.45	51.89	46,083,025	5.41	0.122	0.074
	TGW	8	2.87	5.38	3,157,564	5.05	0.115	0.07
	YPP	1	0.78	1.01	778,962	3.85	0.089	0.054
	YPP	9	3.2	3.22	3,214,625	3.45	0.08	0.049
P850029	Plant color	6	50.91	60.6	56,635,333	55.71	0.601	0.508
	Anthesis	5	66.61	67.53	66,674,886	3.78	0.06	0.051
	Height	1	60.9	61.89	61,226,796	4.33	0.069	0.058
	Height	7	5.3	58.11	56,493,308	9.97	0.152	0.128
	Height	9	54.16	58.54	57,076,974	9.34	0.143	0.121
	GNP	1	0.26	14.45	1,473,698	17.66	0.253	0.214
	GNP	3	6.74	7.46	7,134,234	4.05	0.065	0.055
	GNP	3	55.7	56.55	56,549,055	4.13	0.066	0.056
	GNP	4	67.36	67.44	67,388,724	3.52	0.056	0.048
	GNP	5	66.01	67.29	66,806,978	4.76	0.076	0.064
	GNP	10	6.82	6.85	6,849,249	3.34	0.054	0.045
	TGW	1	0.26	9.84	434,057	7.48	0.116	0.098
	TGW	1	61.89	77.28	75,387,139	7.29	0.113	0.096
	TGW	5	65.85	69.94	67,294,479	14.13	0.208	0.176
	YPP	1	0.26	8.23	1,473,698	8.61	0.132	0.112
	YPP	4	67.44	67.57	67,459,999	3.89	0.062	0.052
	YPP	9	53.36	55.55	53,618,584	4.37	0.07	0.059

† GNP, grain number per primary panicle; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

‡ LOD, logarithm of odds.

§ GVE (genetic variance explained) = $1 - 10^{-2LOD/n}$.

¶ PVE (phenotypic variance explained) = $H^2(1 - 10^{-2LOD/n})$.

QTL on chromosome 1 colocated with QTL for GNP and YPP. Parent BTx642 possessed an allele for increased TGW at the relevant loci on chromosomes 1 and 6 (Fig. 5). BTxARG-1, the parent with the lower TGW, contained the favorable allele on chromosome 8. The RIL progeny with this allele had a mean TGW of 29.2 g compared with an average of 26.7 g among lines with the BTx642 allele. This chromosome 8 QTL did not colocate with GNP.

Like GNP, interval mapping of YPP revealed only a few QTL surpassing the LOD = 3.3 significance threshold. Only two QTL were found to be significant, including the QTL that colocated with GNP on chromosome 1 (Table 3). The second QTL was located on chromosome 9. These QTL for YPP accounted for ~8 and 5% of the GVE and PVE, respectively.

P850029

There were six GNP QTL identified in P850029 on five different chromosomes. Five of the six loci explained between 5 and 6% of phenotypic variance. Two loci, located on chromosomes 1 and 5, colocated with QTL for TGW. The QTL with the largest effect on GNP was located near 1 Mb on chromosome 1 (Table 3), explaining 21% of total phenotypic variance. The other QTL for GNP that colocated with TGW was on chromosome 5 at 66 Mb. The additional minor-effect GNP QTL were located on chromosomes 3 (two QTL), 4, and 10.

There were only three QTL for TGW that reached significance in P850029; however, these three QTL accounted for 37% of the PVE for the trait. Two of these three QTL were located on chromosome 1, with individual peaks on opposite ends at 434 kb and 75.4 Mb (Table 3). Having a maximum LOD score >14, the most significant QTL was at 67.3 Mb on chromosome 5. This QTL explained 21% of the genetic variance for TGW, which is high for a quantitative trait. Of the previously mapped QTL for grain weight in sorghum, none corresponded to chromosome 5. Interestingly, the favorable allele at this locus was from BTxARG-1, the parent with the lowest

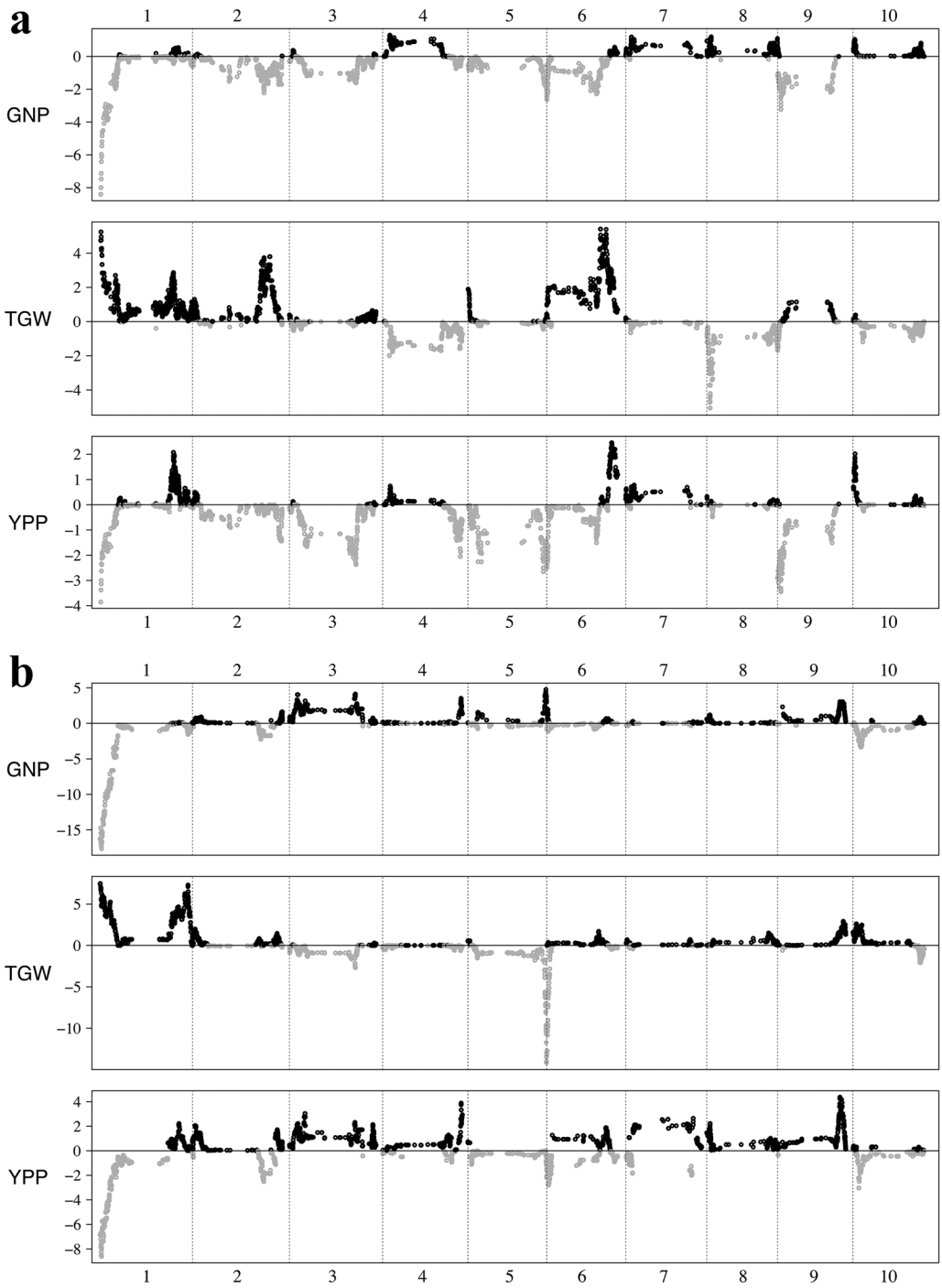

Fig. 5. Directed quantitative trait loci (QTL) plot of grain yield components. Logarithm of odds scores were multiplied by ±1 according to the predicted effect of the unique parent allele (+1, positive effect, –1, negative effect). (a) BTx642/BTxARG-1. (b) BTxARG-1/P850029. GNP, grain number per primary panicle; TGW, 1000-grain weight; YPP, grain yield per primary panicle.

TGW (Table 1). Upon closer examination, the highest-ranked marker was located within *Sobic.005G188400*.

Three total grain yield QTL were significant in the P850029 RIL population. The QTL of greatest significance colocated with the chromosome 1 QTL for GNP and TGW. There was a QTL at 67.5 Mb on chromosome 4. The allele for increased grain yield at this QTL came from the high-yielding P850029 parent (Fig. 5). The final significant QTL was found on chromosome 9, which colocated with the major height gene *Dw1* (Hilley et al., 2016). These two QTL on chromosomes 4 and 9 each explained ~5% of phenotypic variance for YPP.

DISCUSSION

Residual Heterozygosity in Recombinant Inbred Lines

Several distinct regions on chromosomes 2 and 6 in BTx642 contained much higher heterozygosity than expected (Fig. 1). In P850029, there was a large pericentromeric region on chromosome 9 that was genotyped at very low density. Genomic regions with excess residual heterozygosity have been found previously in an intraspecific sorghum F_5 population (Kong et al., 2013; Truong et al., 2014). The markers that were identified in these regions retained high levels of heterozygosity according to recombination breakpoint analysis using the method

of Huang et al. (2009). However, because this method uses a 15-SNP sliding window, it should be stated that this finding is subject to sampling bias, as the 15-SNP window potentially spanned many megabases as a result of missing data (Supplemental Fig. S2). At the two locations in BTx642 and the chromosome 9 region in P850029, a parental genotype tended to be heterozygous, resulting in marginal heterozygous base-calls and high relative missing data in both the parent and RILs. Poor parent genotyping likely resulted in increased imputation error throughout these regions, which would increase heterozygous markers by default using the sliding SNP window. This potential bias likely did not affect QTL mapping results, as heterozygous markers were treated as missing and markers with low coverage were subsequently removed.

Yield Component Variation

The progeny mean of yield components was nearly equal to or greater than that of the highest parent in BTx642 and P850029 (Table 1). This finding may be a consequence of residual heterosis among $F_{4:5}$ RILs or evidence of directional selection. In general, the variation and heritability for grain-yield components were similar to those found in the grain sorghum diversity panel (Boyles et al., 2016a), except that the broad-sense heritabilities for GNP and YPP in BTx642 were higher than the heritabilities found in the diversity panel. These higher heritabilities could have resulted from the BTx642 population possessing less variation in agronomic traits, such as anthesis and plant height, which could confound grain yield components. A stronger positive correlation between GNP and YPP than between TGW and YPP supports previous findings, strengthening the assumption that GNP is more critical than TGW in determining final grain yield (Borrell et al., 1999; Boyles et al., 2016a). The same three yield components under study were previously characterized in a diverse grain association panel, and all three grain-yield traits contained extensive variation across the 390 accessions evaluated (Boyles et al., 2016a). Similar to the diversity panel, GNP possessed more phenotypic variation than TGW in the biparental families. However, GNP is less heritable than TGW and thus more difficult to achieve significant genetic gain and increase grain yield. Targeting specific factors that regulate final grain number, such as fertile spikelet development, fertilization efficiency, or ovule abortion, may provide opportunities to identify heritable traits and associated genes to increase GNP in sorghum. Brown et al. (2006) found GNP to be significantly correlated with panicle branch number, which could serve as another phenotype involved in determining final grain number.

A higher than expected number of lines in both biparental populations (16% in BTx642, 18% in P850029) were classified as segregating for plant color. Plant color in sorghum is determined by an epistatic interaction between two loci, *P* and *Q* (Rooney and Miller, 1982; Dykes and Rooney, 2006), which can result in tan, red, or purple pigmentation. This unexpected finding may be attributed to classifying individuals as segregating when one or few plants within the plot were outcrosses or carryovers from a different plot. Similarly, more RILs than expected in BTx642 were also still segregating for pericarp color (31%). Pericarp color is also determined by an epistatic interaction between two genes, *Y* and *R* (Rooney and Miller, 1982). Individuals with two recessive alleles at the *Y* locus (rryy or R-yy) will have a white pericarp. Dominance at both *R* and *Y* loci results in red pericarp color, whereas RILs with homozygous recessive *R* and a dominant *Y* alleles will possess a yellow pericarp. Like plant color pigmentation, it is difficult to differentiate between white and yellow pericarp color, which could have resulted in misclassifying lines as segregating, when in reality they were fixed for white or yellow color. In the BTx642 population, RILs with a red pericarp color outperformed yellow and white pericarp lines. The pigmentation found in the pericarp of the grain is created by various flavonoid compounds (Dykes and Rooney, 2007). These compounds have multiple roles in plants, including disease resistance (Treutter, 2006), and specific flavonoids are induced in sorghum on anthracnose (*Colletotrichum sublineolum*) infection (Snyder and Nicholson, 1990). High humidity and intermittent rainfalls common to the southeastern United States provide an ideal climate for fungal pathogens, including anthracnose and *Fusarium* spp. that can dramatically reduce grain sorghum yields (Tesso et al., 2011). Breeding for host-plant resistance using flavonoid levels within reproductive tissues may be one way to feasibly and effectively combat high disease pressure in the southeastern United States.

Correspondence between New and Existing Grain Yield QTL

Yield component QTL identified in the current study were compared with QTL that have been detected in previous studies. Despite the complex genetic architecture of grain yield and related traits, this comparison provided evidence of stable QTL across contrasting environments. In addition, yield-related QTL identified in new genomic regions are potential selection targets to improve sorghum yield on marginal sandy soils in a humid environment. Overall, 8 of 16 grain yield-related QTL identified across two RIL populations colocated with previously published yield trait QTL within the CGSRqtl database (Zhang et al., 2013). Thus, half of the identified QTL were novel, which could be a consequence of a unique environment or family structure (i.e., genetic background effects) used in this study. Neither of these underlying reasons for finding novel QTL is surprising, given the complex nature of grain-yield components.

Only one QTL for GNP was found in BTx642, whereas six GNP QTL were found in P850029. The sole BTx642 QTL on chromosome 1 was also identified in P850029. Two QTL for GNP, one each on chromosomes 3 and 4, colocated with existing QTL for total grain weight (Brown et al., 2006) and/or grain yield (Murray et al., 2008). Aside from the QTL on chromosome 1, each of the QTL for GNP accounted for <6% of total phenotypic variance. Both of these GNP QTL colocated with YPP, but not with TGW. The QTL on chromosome 4 overlapped with existing QTL for panicle length, primary branch number (Brown et al., 2006), and dry grain yield (Murray et al., 2008). The chromosome 1 QTL for GNP, which respectively accounted for 11 and 21% PVE in BTx642 and P850029, colocated with a YPP QTL. Colocalization between these two traits has also been observed in a diverse association panel (Boyles et al., 2016a). This grain yield QTL on chromosome 1 was also associated with TGW. Another minor-effect QTL for GNP that was identified in the P850029 population on chromosome 10 at 6.8 Mb also colocated with a QTL for YPP.

Of the six QTL for TGW, four colocated with previously identified QTL for grain weight according to information published in CGSRqtl (Zhang et al., 2013). The chromosome 1 QTL colocated with grain weight QTL found by Rami et al. (1998), Murray et al. (2008), and Srinivas et al. (2009) in different RIL populations. The identification of this QTL in different genetic backgrounds provides evidence that a common genetic variant or multiple variants exist within this chromosome 1 region that control grain weight in sorghum. However, contrary to association mapping results (Boyles et al., 2016a), the chromosome 1 QTL effects between GNP and TGW were found to act inversely and create a tradeoff between yield components. The most significant marker within the novel chromosome 5 QTL for TGW was located within a putative remorin-encoding gene (*Sobic.005G188400*). *Sobic.005G188400* is primarily expressed in early inflorescence tissues and developing embryo (Makita et al., 2015). In rice (*Oryza sativa* L.), *grain setting defect1* (*GSD1*) transcribes a plant-specific remorin protein that affects TGW via regulation of plasmodesmatal conductance, which controls movement of photoassimilates that ultimately reach the grains (Gui et al., 2014, 2015). Two other QTL on chromosomes 6 and 8 overlapped with kernel weight QTL identified by Feltus et al. (2006). Each of these QTL explained 7% of the phenotypic variance in TGW.

Conclusions

Although several studies have revealed QTL for yield-related traits in sorghum, no experiments have taken place in the southeastern United States, where the environment can be drastically different from traditional regions of US production. The southeastern United States has a soil profile and weather patterns similar to some important international sorghum production areas, including parts of central Africa, southern Asia, and eastern South America (USDA-NRCS-Soils, 2003), which could provide a suitable US breeding location for these regions. Eight of the sixteen QTL identified for grain yield-related traits did not colocalize with existing loci. Although this result could certainly be attributed to parental genotype differences, the relatively high number of published QTL mapping studies for yield components suggests that environmental disparities likely affect QTL or gene effects. Using these environment-specific genetic markers to assist in genotype selections for sorghum production in high-humidity regions with marginal sandy soils may help to increase genetic gain and improve regional breeding efficiency. Introgression of favorable alleles identified in this study into elite genotypes will be needed to evaluate their potential to increase grain-yield components and ultimately affect sorghum improvement.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

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