Journal of Experimental Botany, Vol. 63, No. 15, pp. 5451–5462, 2012
doi:10.1093/jxb/ers205 Advance Access publication 1 August, 2012

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Identification of QTLs for eight agronomically important traits using an ultra-high-density map based on SNPs generated from high-throughput sequencing in sorghum under contrasting photoperiods

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Received 21 February; Revised 11 June 2012; Accepted 15 June, 2012

Abstract

The productivity of sorghum is mainly determined by agronomically important traits. The genetic bases of these traits have historically been dissected and analysed through quantitative trait locus (QTL) mapping based on linkage maps with low-throughput molecular markers, which is one of the factors that hinder precise and complete information about the numbers and locations of the genes or QTLs controlling the traits. In this study, an ultra-high-density linkage map based on high-quality single nucleotide polymorphisms (SNPs) generated from low-coverage sequences (~0.07 genome sequence) in a sorghum recombinant inbred line (RIL) population was constructed through new sequencing technology. This map consisted of 3418 bin markers and spanned 1591.4 cM of genome size with an average distance of 0.5 cM between adjacent bins. QTL analysis was performed and a total of 57 major QTLs were detected for eight agronomically important traits under two contrasting photoperiods. The phenotypic variation explained by individual QTLs varied from 3.40% to 33.82%. The high accuracy and quality of this map was evidenced by the finding that genes underlying two cloned QTLs, *Dw3* for plant height (chromosome 7) and *Ma1* for flowering time (chromosome 6), were localized to the correct genomic regions. The close associations between two genomic regions on chromosomes 6 and 7 with multiple traits suggested the existence of pleiotropy or tight linkage. Several major QTLs for heading date, plant height, numbers of nodes, stem diameter, panicle neck length, and flag leaf width were detected consistently under both photoperiods, providing useful information for understanding the genetic mechanisms of the agronomically important traits responsible for the change of photoperiod.

Key words: Agronomically important traits, genome sequencing, photoperiod, QTL mapping, SNP map, *Sorghum bicolor* (L.) Moench.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the world's fifth most important cereal crop, after wheat, rice, maize, and barley (Doggett, 1988). The overall goal for sorghum breeding programmes around the world is to develop varieties with the most desired agronomically important traits such as better quality, higher yield, and stronger tolerance to different kinds of

stresses (Klein *et al*., 2008; Knoll *et al*., 2008, Mace *et al*., 2009). Progress in genetic manipulation of agronomic traits is essential for the sustained improvement of sorghum.

Many studies have been undertaken to investigate the genetic variants and quantitative trait loci (QTLs) controlling >150 traits in sorghum, including stem morphology (Lin *et al*., 1995; Pereira and Lee, 1995; Hart *et al*., 2001; Feltus *et al*., 2006; Brown *et al*., 2008; Murray *et al*., 2008; Shiringani *et al*., 2010), grain and panicle traits (Pereira *et al*., 1995; Rami *et al*., 1998; Brown *et al*., 2006; Feltus *et al*., 2006; Murray *et al*., 2008; Srinivas *et al*., 2009), leaf morphology (Hart *et al*., 2001; Feltus *et al*., 2006), maturity (Crasta *et al*., 1999; Chantereau *et al*., 2001; Hart *et al*., 2001; Kim, 2003; Brown *et al*., 2006), stem composition (Murray *et al*., 2008; Ritter *et al*., 2008; Shiringani *et al*., 2010), stay-green drought tolerance (Tuinstra *et al*., 1996; Crasta *et al*., 1999; Subudhi *et al*., 2000; Tao *et al*., 2000; Xu *et al*., 2000; Kebede *et al*., 2001; Hausmann et al., 2002; Harris *et al*., 2007), fertility restoration (Klein *et al*., 2001; Jordan *et al*., 2010), aluminium tolerance (Magalhaes *et al*., 2004), and biotic stress resistance (Klein *et al*., 2001; Tao *et al*., 2003; Mohan *et al*., 2009; Perumal *et al*., 2009). Most of the linkage maps used in these studies were low-density maps based on low-throughput marker systems such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers.

For a given trait in a particular population, increasing marker density can increase the resolution of the genetic map, thus enhancing the precision of QTL mapping. The development of new high-throughput sequencing technologies provides the capacity for massively parallel re-sequencing of genomes, and thus can be used to develop single nucleotide polymorphism (SNP) markers for population genotyping (Mardis, 2008; Schuster, 2008; Varshney *et al*., 2009). Using the bar-coded multiplexed sequencing technology and Illumina Genome Analyzer, the whole-genome genotyping of 150 rice recombinant inbred lines (RILs) based on SNPs generated from the whole-genome re-sequencing was performed. A sliding window approach was adopted to call genotypes of RILs, and a QTL for plant height was located in a 100 kb region containing the rice 'green revolution' gene, *sd1*. This sequencing-based method was ~20 times faster in data collection and 35 times more precise in recombination breakpoint determination compared with the genetic map constructed with 287 PCR-based markers (Huang *et al*., 2009). Similarly, a rice parent-independent genotyping method was also successfully developed to identify SNP markers using only low-coverage RIL sequences, which greatly reduced the cost for deep sequencing of the two parents. The validation of the locations of several cloned genes, *GS3*, *GW5/qSW5*, and *OsC1*, indicated the high quality and accuracy of the map (Xie *et al*., 2010; Yu *et al*., 2011). These high-density maps generated from a sequencing-based genotyping method have been applied for QTL mapping of agriculturally important traits and proved to be powerful and accurate for QTL mapping (Wang *et al*., 2010; Yu *et al*., 2011).

The sequencing of the sorghum genome (accession BTx623; Paterson *et al*., 2009) provides the opportunity for genotyping mapping populations using whole-genome sequencing approaches. In this study, a RIL population developed from a cross between grain and sweet sorghum, previously utilized to investigate the genetic variability and correlation of stalk yield-related traits and sugar concentration of stalk juice (Zou *et al*., 2011), was genotyped based on SNPs generated from whole-genome re-sequencing, and an ultra-high-quality SNP map was constructed and used for QTL analysis of eight agronomically important traits under two contrasting photoperiods. This research will facilitate fine mapping and cloning of quantitative trait genes, providing foundations enabling the development of superior sorghum varieties. Furthermore, it will become possible to illustrate the genetic mechanisms of complex traits in sorghum functional genomics.

Materials and methods

Plant materials

A mapping population consisting of 244 RILs derived from a cross between the inbred lines 654 (grain sorghum) and LTR108 (sweet sorghum) was developed and used in this study. Both 654 and LTR108 were developed by the Chinese National Sorghum Improvement Center. Line 654 is high grain yielding and used as the parental line for several commercial sorghum hybrids in China, and is characterized by being insensitive to photoperiod, early maturing, having short internodes and a thin stem, and dwarf. LTR108 is a sweet sorghum restorer line and is characterized by being sensitive to photoperiod, late maturing, having long internodes and a thick stem, and tall under long days, although the line performs quite differently under short days (Zou *et al*., 2011). Each RIL was derived from a single F_2 plant following the single seed descent (SSD) method until the F_8 generation.

Experimental design

The field trials to evaluate phenotypic performance of RILs and the parents were conducted in two growing seasons (summer and winter) at four locations. Summer-sown trials (long-day photoperiod as the maximum day length is 14 h within the plant growing season) were established on 30 April 2008 at Hangzhou and 6 May 2009 at Yangdu in Zhejiang province, and winter-sown trials (short-day photoperiod as the maximum day length is 10 h within the plant growing season) on 18 November 2009 at Yacheng and 5 December 2010 at Linshui in Hainan province, respectively. The characteristics of the locations are presented in Supplementary Table S1 at *JXB* online. All genetic materials in each experiment were planted in a randomized complete block design (RCBD) with two replications. The experimental design and field management were the same as described by Zou *et al*. (2011).

Phenotyping for agronomically important traits

Heading date (HD) was recorded when 50% of plants in the plot showed 50% flowering. Three individual plants from the middle of each plot were selected for phenotype evaluation. Plant height (PH), number of nodes (NN), stem diameter (SD), panicle neck length (PNL), panicle length (PL), flag leaf length (FLL), and flag leaf width (FLW) were evaluated at maturity as previously described (Zou *et al*., 2011).

High-throughput genotyping using whole-genome re-sequencing method

Total genomic DNAs of all the 244 RILs and the two parents, 654 and LTR108, were isolated from leaf tissues using a DNeasy Plant Mini Kit (Qiagen). A high-throughput genotyping method was employed and the genotypes of the whole RIL population were determined based on SNPs generated from the whole-genome re-sequencing by the Illumina Genome Analyzer IIx as described by Huang *et al.* (2009).

Briefly, the genome sequence of BTx623 (Paterson *et al*., 2009) was used as the sorghum reference sequence in this study. The genomes of 654 and LTR108 were re-sequenced using the Illumina GAIIx for 2 × 76 bp paired-end sequencing, and ultimately gaining ~25- and 28-fold coverage of the sorghum genome, respectively. Then, the 76 bp paired-end reads of the two parents were mapped to the sorghum reference genome (version 1.0, http://genome.jgi-psf.org/Sorbil/Sorbil) using Ssaha2 software v2.3 (http://www.sanger.ac.uk/Software/analysis/SSAHA2/). Finally, after removing the low-quality bases (Q score in Phred scale <25) and those sites with conflicting genotypes among different reads, only uniquely aligned reads which were mapped to unique locations in the reference genome were retained and used to call the single base pair genotypes of the consensus sequences across the whole genome using the Ssaha_Pileup package (v0.5). A total of 1 243 151 unique SNPs were identified from comparing the genome sequences of the two parents and used as potential markers for genotyping.

Genomes of the RILs were also re-sequenced on the Illumina Genome Analyzer IIx using the multiplexed sequencing and paired-end strategy. Sequences were sorted and aligned with the pseudo-molecules of the parental genome sequences for SNP detection. For each RIL, all paired short reads were mapped against both parental pseudomolecules by SMALT (http://www.sanger.ac.uk/resources/software/smalt/), with the threshold '-i 900 -j 50 -m 20'. Then a Perl script, Smalt2rlt.pl in the SEG-Map package (Zhao *et al*., 2010, http://www.ncgr.ac.cn/software/SEG), was used to obtain the reads which covered the SNP sites between parents. Each collected read was uniquely aligned to the pseudomolecules, and was perfectly matched to one parent while containing one mismatched base pair (candidate SNP site) compared with the other parent. Detected SNPs were arranged along the chromosomes according to their physical locations. A sliding window approach was used for genotype calling. Consecutive SNP sites with the same genotype were lumped into blocks and a recombination breakpoint was assumed at the transition between two different genotype blocks. Blocks with length <300 kb in which the number of sequenced SNPs was fewer than five were masked as missing data to avoid false double recombinations (Huang *et al*., 2009). A Perl script, Seq2bin (Zhao *et al*., 2010, http://www.ncgr.ac.cn/software/SEG), was adopted in order to identify the genotype for each genomic region and the recombination breakpoints accurately.

Bin map construction

After all genotypes of the RILs were called and recombination breakpoints were determined, the recombination map of each RIL was generated and converted into a skeleton bin map in order to conduct genetic analyses. The methodology for the construction of the high-density bin map as described by *Huang* et al. *(2009)* was followed. Briefly, the recombination maps of the RILs were aligned and split into recombination bins according to the recombination breakpoints. Adjacent 100 kb intervals with the same genotype across all RILs were combined into a recombination bin. Resulting bins were then treated as genetic markers for linkage map construction using MAPMAKER/EXP V3.0 (Lincoln *et al*., 1992).

Data analysis and QTL mapping

Analysis of variance (ANOVA) was performed based on a fixed effect model by using S-Plus for Windows V6.1 (Insightful Corporation 2001, Seattle, WA). Phenotypic correlation coefficients were calculated using the mean values. Broad-sense heritability (H^2) was calculated as in *Zou* et al. *(2011)*.

QTL mapping was conducted with the composite interval mapping (CIM) implemented in the software package windows QTL Cartographer V2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm; Wang *et al*., 2007) using this linkage bin map and phenotypic data. The CIM analysis was run using Model 6 with forward and backward stepwise regression, a window size of 10 cM, and a step size of 2 cM. Experiment-wide significance ($P < 0.05$) thresholds for QTL detection were determined with 1000 permutations. The location of a QTL was described according to its LOD (logarithm of odds) peak location and the surrounding region, with 95% confidence interval calculated using WinQTLCart. Adjacent QTLs on the same chromosome for the same trait were considered as different QTLs when the support intervals were non-overlapping. The contribution rate (R^2) was estimated as the percentage of variance explained by each QTL in proportion to the total phenotypic variance. Major QTLs were named following the popular nomenclature, and alphabetic order was also used for QTLs on the same chromosome (McCouch *et al*., 1997). QTLs with a positive or negative additive effect for a specific trait imply that the increase in the phenotypic value of the trait is contributed by the alleles from 654 or LTR108, respectively.

Results

Analysis of phenotypic data

The mean values of the traits measured of the parents and the population under long days and short days are given in Tables 1 and 2, respectively. The phenotypic values of the traits measured in the RIL population had a continuous distribution, which approximately fitted normality with skewness <1.0, indicating that all measured traits were quantitatively inherited. ANOVA showed significant phenotypic variation for all of the traits among the lines. The location effect was also significant for all traits under both long-day and short-day conditions. Significant effects caused by location×genotype interaction were also observed for all traits except for PL under long days (Tables 1, 2).

The performances of eight agronomically important traits were all influenced by photoperiod (Tables 1, 2). The average HDs of 654 and LTR108 were 75 d and 97 d under a long-day photoperiod, and 71 d and 59 d under a short-day photoperiod, respectively. The HD for LTR108 was decreased by ~38 d, while that for 654 remained almost the same. The PLs of the two parental lines were also significantly affected by photoperiod, and line 654 was always shorter than LTR108 under both photoperiods. The PH of LTR108 and RILs was decreased dramatically while that of 654 declined slowly with the short-day photoperiod. Line 654 produced fewer NN than LTR108 (15 versus 20) under long days. The difference of NN between the two parents (7 versus 8) could be almost eliminated under short days. The average values of NN of the RILs were also greatly reduced from long days to short days (19 versus 8). The SD was also significantly different between the two parents under both photoperiods. Line 654 had thinner stems than LTR108 under long days, while the opposite was observed under short days. The SD of LTR108 was decreased, with a larger magnitude than that of 654 and RILs when the day was short. Line 654 had a longer panicle neck than LTR108 under both photoperiods. The PNLs of the two parents and the RILs under long days were shorter than those under short days. Line 654 produced longer panicles and larger flag leaves than LTR108 under both photoperiods. The panicle and flag leaf

Table 1. Descriptive statistics and mean squares of ANOVA and H^2 for the traits across two long-day trials

Category	Source of variation	DF	HD	PH	NN	SD	PNL	PL	FLL	FLW
Descriptive statistics	654 (±Std)		75 (7)[a]	191.5 (19.1)[a]	15 (1)[a]	1.7 (0.3)[a]	40.5 (3.5)[a]	28.7 (2.1)[a]	48.2 (7.8)[a]	8.5 (1.5)[a]
	LTR108 (±Std)		97 (9)[b]	334 (14.2)[b]	20 (0)[b]	2.1 (0.3)[b]	30.4 (4.9)[b]	21(0.7)[b]	38.8 (5.7)[b]	6.8 (2.0)[b]
	RIL (±Std)		91 (16)	312.0 (70.3)	19 (5)	2.0(0.2)	36.9 (7.0)	26.6 (4.6)	47.7 (12)	7.1 (1.2)
	RIL range		62–129	104.5–480.0	10.8–33.0	1.4–2.9	15.0–54.8	15.0–44.5	16.0–91.5	3.7–10.7
	Skewness		0.3	–0.5	0.7	0.4	–0.2	0.5	0.6	0.1
ANOVA	Location	1	44 540.1***	274 139.2***	21 19.8***	6.4***	220.4**	282.7***	4914.3***	147.7***
	Genotype	243	853.1***	20719.1***	56.9***	0.2***	166.9***	61.0***	374.8***	4.1***
	Replication	1	10.2	1361.7	5.9	0.8***	102.4	20.2	173.6***	2.5
	Location× genotype	243	215.6***	3083.6***	15.0***	0.2***	68.7***	19.3	194.2***	2.5***
	Error	487	50.3	723.6	5.8	0.1	41.5	16.1	75.1	1.3
	H^2 (%)		74.73	85.12	73.71	23.96	58.83	68.36	48.20	38.46

DF, degrees of freedom; HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.
[a] and [b] indicate significant differences of the trait mean values between the two parents.
Std, standard deviation; ***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$.

Table 2. Descriptive statistics and mean squares of ANOVA and H^2 for the traits across two short-day trials

Category	Source of variation	DF[c]	HD	PH	NN	SD	PNL	PL	FLL	FLW
Desceiptive statistics	654 (±Std)		71 (8) [a]	166.0 (22.6)[a]	7 (1)	1.5 (0.2)[a]	50.5 (3.4)[a]	25.9 (2.8)[a]	42.5 (4.6)[a]	7.7 (2.0)[a]
	LTR108 (±Std)		59 (6)[b]	193.3 (34.6)[b]	8 (2)	1.3 (0.2)[b]	41.3 (2.8)[b]	18.8 (1.4)[b]	37 (5.3)[b]	6.4 (1.8)[b]
	RIL (±Std)		64 (3)	187.1 (34.5)	8 (1)	1.4 (0.2)	46.8 (6.5)	23.0 (3.1)	39.3 (6.4)	7.1 (1.0)
	RIL range		53–74	86.8–273.8	5.0–11.8	0.8–2.0	27.8–62.8	15.2–32.4	21.0–55.8	4.8–9.9
	Skewness		–0.3	–0.6	0.1	0.2	0.0	0.3	–0.1	0.0
ANOVA	Location	1 (0)	52 911.6***	199 233.1***	4.2***		14 197.3***	3795.5***		
	Genotype	243	50.6***	4693.2***	3.8***	0.1***	161.5***	36.3***	78.1***	1.9***
	Replication	1	6.2	21.2	0.1	0.02	95.1*	34.6**	31.2	1.1
	Location×genotype	243	12.3***	874.8***	1.4***		46.0***	8.2***		
	Error	487 (243)	6.6	93.2	0.4	0.01	17.6	4.0	36.9	0.6
	H^2 (%)		75.67	82.89	63.63	83.09	71.52	77.34	52.72	67.62

DF, degrees of freedom; HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.
[a] and [b] indicate significant differences of the trait mean values between the two parents.
[c]Because the phenotypic values for SD, FLL, and FLW were investigated only at Yacheng in 2010, the DF are different. The numbers in parentheses indicate the DF for SD, FLL, and FLW, and the numbers outside the parentheses indicate the DF for other traits.
Std, standard deviation; ***$P < 0.0001$; ** $P < 0.01$; *$P < 0.05$.

of the two parents and the RILs under a long-day photoperiod were larger than those under a short-day photoperiod.

The broad sense heritability (H^2) was estimated for each trait measured under both photoperiods (Tables 1, 2). Under long days, the heritabilities of the eight traits ranged from 23.96% to 85.12%. PH had the highest H^2 value, followed by HD, NN, PL, PNL, FLL, and FLW. The H^2 of SD was lowest. Under short days, the heritabilities of the traits ranged from 52.72% to 83.09%. The SD had the highest H^2 value, followed by PH, PL, HD, PNL, FLW, and NN. The H^2 of FLL was lowest. Higher H^2 values were observed under a short-day photoperiod than under a long-day photoperiod, except those for PH and NN.

Correlations of the traits

Correlations among the eight measured traits based on the line means under both photoperiods were evaluated at $P < 0.05$ and $P < 0.01$, showing that the correlations, in terms of the magnitude and direction, were significantly different under the different photoperiods (Table 3). Significant positive correlations between long-day and short-day conditions for PH ($r = 0.49$, $P < 0.01$), PNL ($r == 0.52$, $P < 0.01$), PL ($r = 0.58$, $P < 0.01$), and FLW ($r = 0.21$, $P < 0.01$), and significant negative correlations for HD ($r = –0.23$, $P < 0.01$) and NN ($r = –0.14$, $P < 0.05$) were observed. No significant correlations were observed for SD and FLL. Under a long-day photoperiod, significant positive correlations were observed among HD, PH, NN, and SD ($P < 0.01$). In addition, PH had significant positive correlations with PNL and PL. PL had significant positive correlations with PNL and FLL. FLL and FLW were significantly and positively correlated with SD, while they were negatively correlated with PNL. FLL was also significantly and positively correlated with PL and FLW. FLW was significantly and positively correlated with HD. Under short-day conditions, significantly positive

Table 3. Correlation coefficient of the traits investigated

	Long day versus short day	HD	PH	NN	SD	PNL	PL	FLL	FLW
HD	–0.23**		0.32**	0.55**	0.30**	0.02	0.24**	0.10	0.12
PH	0.49**	0.60**		0.54**	–0.10	0.48**	0.47**	0.24**	–0.08
NN	–0.14*	0.75**	0.51**		0.25**	–0.15	0.22**	0.10	–0.09
SD	–0.03	0.43**	0.21**	0.29**		–0.19**	0.27**	0.17	0.46**
PNL	0.52**	–0.07	0.27**	–0.11	–0.16		0.47**	0.22**	0.07
PL	0.58**	–0.05	0.23**	–0.04	–0.04	0.36**		0.36**	0.16
FLL	0.12	0.01	–0.10	–0.10	0.30**	–0.14	0.17		0.41**
FLW	0.21**	0.19**	–0.08	0.13	0.23**	–0.46**	–0.12	0.40**	

The numbers below the diagonal are correlation coefficients under a long-day photoperiod and the numbers above the diagonal are correlation coefficients under a short-day photoperiod.

HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.

$^{**}P < 0.01$; $^{*}P < 0.05$.

correlations were observed among HD, PH, NN, SD, and PL, with the exception of a weakly negative correlation between SD and PH. Further, PH had significant positive correlations with PNL and FLL. SD and PL were significantly and positively correlated with FLL and FLW. PNL was significantly and positively correlated with PL and FLL, while it was negatively correlated with NN and SD. The highest correlation coefficient was observed between HD and NN under both photoperiods (0.75 and 0.55, respectively).

Genotyping RILs with high-density SNPs and constructing the bin map

About 48 Mb sequences were generated for each RIL, equivalent to ~0.07 coverage of the sorghum genome. When a 36-mer read of a RIL was aligned to a region where an SNP was detected between the two parents, the genotype of the RILs was assigned to this nucleotide position. Using the quality score of each SNP base as a filter, a total of 7.76 million high-quality SNPs were detected. Therefore, each RIL had ~31 800 SNPs, with a range from 1023 to 108 507. The average SNP density of the RILs was 0.045 SNPs per kb, or one SNP every 22.2 kb. These SNPs were used to detect break points, and a total of 8905 break points for the 244 RILs were detected using the sliding window approach. After recombination breakpoints were determined, the genotype of each RIL was called.

To conduct genetic analyses, the recombination map of each line was converted into a skeleton bin map by aligning all chromosomes of the 244 RILs together and comparing them for a minimum of 100 kb intervals (Supplementary Fig. S1A at *JXB* online). By taking adjacent 100 kb intervals with the same genotype across the entire RIL population as a single recombination bin (e.g. Supplementary Fig. S1B), a total of 3418 recombination bins were obtained from the 244 RILs. The physical lengths of the bins ranged from 50 kb to 5.58 Mb, with an average of 192 kb and a median of 139 kb. In total, 77.4% of bins were 0.1–0.2 Mb in length, 11.7% of bins were 0.2–0.3 Mb in length, with 10.9% of bins remaining (Supplementary Table S2).

Using the allele call within each bin as a genetic marker for genotyping, a genetic linkage map was constructed based on recombination frequency, giving a genetic distance of 1591.4 cM in length, ~0.5 cM per bin. For all the 10 chromosomes, the average genetic distances between adjacent bins ranged from 0.4 cM to 0.5 cM, with the maximal distances between 2.1 cM and 4.0 cM (Supplementary Table S4 at *JXB* online).

QTL analysis using the high-density SNP map

QTL mapping was conducted and a total of 57 QTLs were identified for the eight traits investigated. Of these QTLs, nine were detected consistently under both photoperiods. The others were detected only under either a long-day or short-day photoperiod, implying that some QTLs controlled the corresponding traits only under one photoperiod. Under a long-day photoperiod, a total of 25 major QTLs were detected for the traits studied (Table 4, Fig. 1). Two of these QTLs (*qHD6a* and *qHD6b*) were detected for HD and the LTR108 alleles resulted in delayed heading of >6 d. Three QTLs (*qPH6a*, *qPH6b*, and *qPH7*) were found to influence PH, and the LTR108 alleles contributed to increase height by >20 cm. Two QTLs (*qNN1b* and *qNN6*) were identified for NN. Three QTLs (*qSD4*, *qSD6a*, and *qSD6b*) were detected for SD. The effects in improving NN and SD were also attributed to LTR108 alleles. Three QTLs (*qPNL1a*, *qPNL6a*, and *qPNL6b*) were identified for PNL, and the effects in improving PNL were caused by the 654 alleles, while for *qPNL3*, the effect on increasing PNL was caused by the LTR108 allele. Four QTLs (*qPL6c*, *qPL6d*, *qPL8a*, and *qPL9b*) were detected for PL on chromosomes 6, 8, and 9, and the 654 alleles contributed to the increased PL. For FLL, only one QTL (*qFLL10*) was detected and the LTR108 allele was responsible for this increase in the flag leaf. Two QTLs (*qFLW1a* and *qFLW1b*) for FLW were located on chromosome 1, and the effects for increasing leaf width were contributed by 654 alleles, while the effects for the other four QTLs (*qFLW2a*, *qFLW4*, *qFLW6a*, and *qFLW6b*) on chromosomes 2, 4, and 6 were contributed by the LTR108 alleles. The contribution rate of a single QTL varied from 4.02% to 33.82%. The QTL *qHD6a* had the largest effect.

Under a short-day photoperiod, a total of 32 QTLs were detected for the traits studied (Table 5, Fig. 1). Among them, three QTLs (*qHD6a*, *qHD6c*, and *qHD8*) were associated with

Table 4. QTLs identified for eight traits using the high-density SNP bin map (showing significant QTLs) across two long-day trials

Trait	QTL	Chrom.	Bin	Position (cM)	Interval (cM)[a]	LOD score	Additive[b]	R^2 (%)[c]
HD	*qHD6a*	6	Bin_2054	48.5	47.59–48.73	22.7	–9.7	33.82
	qHD6b	6	Bin_2071	57.22	56.99–57.96	10.9	–6.7	18.05
PH	*qPH6a*	6	Bin_2054	48.5	47.23–49.83	7.7	–24.8	10.73
	qPH6b	6	Bin_2067	53.86	53.42–54.75	6.4	–22.0	9.03
	qPH7	7	Bin_2454	113.36	110.81–114.63	8.9	–30.6	12.61
NN	*qNN1b*	1	Bin_0312	121.59	120.47–122.49	4.5	–1.1	5.22
	qNN6	6	Bin_2054	48.5	47.83–48.82	21.7	–2.7	29.65
SD	*qSD4*	4	Bin_1535	104.44	102.46–106.22	3.9	–0.1	6.17
	qSD6a	6	Bin_2047	47.14	45.99–48.50	3.4	–0.1	5.45
	qSD6b	6	Bin_2064	51.17	51.00–53.40	3.9	–0.1	6.14
PNL	*qPNL1a*	1	Bin_0377	158.05	157.83–160.93	4.6	1.8	6.44
	qPNL3	3	Bin_1234	146.91	145.33–147.99	3.3	–1.5	4.61
	qPNL6a	6	Bin_2124	88.33	87.32–89.24	6.6	2.2	9.53
	qPNL6b	6	Bin_2141	98.18	97.52–98.59	3.9	1.7	5.81
PL	*qPL6c*	6	Bin_2131	94.16	93.19–96.54	10.4	1.9	15.18
	qPL6d	6	Bin_2143	100.19	98.61–100.98	10.7	1.8	14.97
	qPL8a	8	Bin_2532	18.13	16.99–18.56	5.2	1.2	6.94
	qPL9b	9	Bin_3037	116.6	115.31–117.45	3.2	1.0	4.22
FLL	*qFLL10*	10	Bin_3102	16.55	15.41–18.6	2.9	–2.7	4.78
FLW	*qFLW1a*	1	Bin_0050	16.47	15.85–17.32	3.0	0.3	4.02
	qFLW1b	1	Bin_0078	26.07	25.06–28.83	4.0	0.3	5.38
	qFLW2a	2	Bin_0836	148.17	146.11–150.01	4.9	–0.3	6.81
	qFLW4	4	Bin_1293	6.72	5.62–8.01	3.5	–0.3	4.85
	qFLW6a	6	Bin_2053	47.83	45.22–49.17	4.3	–0.3	5.78
	qFLW6b	6	Bin_2212	142.42	141.00–143.34	4.6	–0.3	6.27

HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.

[a] 1.5-LOD support interval of the QTL.

[b] Addictive effect: positive values of the additive effect indicate that alleles from 654 were in the direction of increasing the trait score.

[c] Percentage of the variation explained by the QTL.

HD and the 654 alleles caused the delay of heading by >1 d. Three QTLs for PH were identified. The 654 alleles for *qPH1* on chromosome 1 and *qPH6a* on chromosome 6 resulted in increased height, while the LTR108 allele for *qPH7* on chromosome 7 contributed to increased height. Six QTLs were detected for NN on chromosomes 1, 6, 7, and 8. The LTR108 alleles for *qNN1a*, *qNN1c*, and *qNN7* were helpful to promote NN, while the 654 alleles for *qNN6*, *qNN8a*, and *qNN8b* were useful to increase NN. Four QTLs (*qSD1*, *qSD6a*, *qSD7a*, and *qSD7b*) were indentified for SD. The LTR108 allele for *qSD1* resulted in increased SD, and the 654 alleles for the other QTLs improved SD. Four QTLs (*qPNL1b*, *qPNL4*, *qPNL6a*, and *qPNL6b*) were found for PNL on chromosomes 1, 4, and 6, and the 654 alleles contributed to increase the PNL. Six QTLs for PL (*qPL6a*, *qPL6b*, *qPL6c*, *qPL8b*, *qPL8c*, and *qPL9a*) were found on chromosomes 6, 8, and 9, and the 654 alleles for these QTLs increased the PL. Three QTLs for FLL (*qFLL2*, *qFLL3*, and *qFLL7*) were found on chromosomes 2, 3, and 7. The LTR108 allele for *qFLL2* caused the lengthening of the leaf, and the 654 allele for the other QTLs resulted in lengthening of the leaf. Three QTLs for FLW (*qFLW1c*, *qFLW2a*, and *qFLW2b*) were found on chromosomes 1 and 2. The 654 allele for *qFLW1c* was responsible for widening the leaf, and the LTR108 alleles for the other QTLs widened the leaf. The additive effect of a single QTL could explain 3.40–32.72% of the total variance.

Discussion

The features of the ultra-high-density SNP map

For the first time in sorghum, an ultra-high-density genetic map, comprising 3418 bins and spanning a genetic distance of 1591.4 cM, was constructed based on the sequence-based genotyping for a RIL population. The total map length is comparable with that of other recently reported sorghum genetic maps. It is slightly shorter than the BTx623/IS3620C map spanning 1713 cM based on 2926 AFLP, RFLP, and SSR markers (Menz *et al*., 2002), the consensus map spanning 1603.5 cM consisting of 2029 unique loci (1190 DArT loci and 839 other loci) (Mace *et al*., 2009), and the expanded consensus map through the addition of 1243 markers comprising an additional 888 DArTs, 229 SSRs, 81 RFLPs, and 45 genes (Mace and Jordan, 2011), and longer than the BTx623/*S. propinquum* map spanning 1059.2 cM based on 2512 loci (Bowers *et al*., 2003). Comparing the marker density of the present ultra-high SNP map with all of these previously published maps, 2.1 markers per cM in the present ultra-high-density SNP map was similar to the 2.1 markers per cM found by *Mace and Jordan (2011*), and a higher overall marker density than 2.4 markers per cM found by *Bowers* et al. *(2003)*, 1.7 markers per cM by *Menz* et al. *(2002)* and 1.3 markers per cM by *Mace* et al. *(2009*). Compared with other genotyping methods, the sequencing-based high-throughput method,

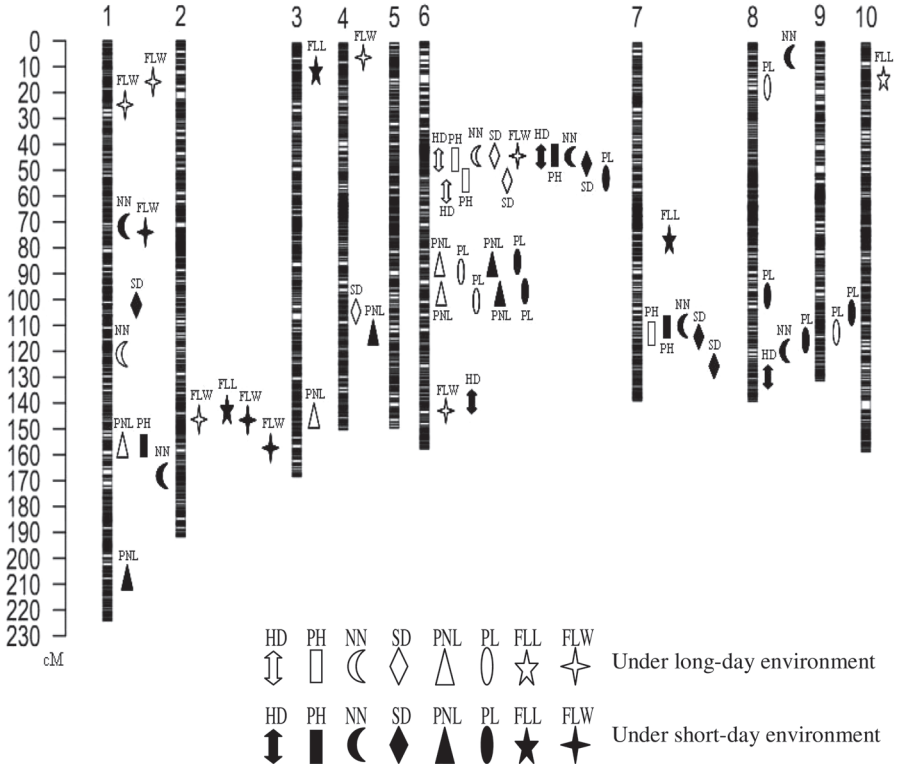

Fig. 1. QTL locations in the bin map for eight agronomically important traits under long-day and short-day photoperiods. HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.

taking only 4 weeks to genotype 244 RILs in this study, is proved to be a very powerful approach which is significantly more time efficient, cost-effective and less laborious.

The quality and accuracy of the bin map

The quality and accuracy of this map for genetic analysis were verified from the QTL mapping studies in two particular cases where the underlying genes responsible for the QTL are known, *Dw3* for plant height (Multani *et al*., 2003) and *Ma1* for flowering time in *Sorghum bicolor* (Murphy *et al*., 2011).

Classical genetic studies in sorghum identified four major dwarfing genes, designated *Dw1*, *Dw2*, *Dw3*, and *Dw4* (Quinby, 1974). Only *Dw3* has been cloned based on a sequence mapping strategy and determined to be Sb07g023730. *Dw3* is the homologue of maize *Br2* and *Arabidopsis PGP1*, and encodes a protein similar to ATP-binding cassette transporters of the multidrug resistant (MDR) class of Pglycoproteins (PGPs) (Multani *et al*., 2003). In this study, a major QTL for PH on chromosome 7 corresponding to the *Dw3* gene was identified stably across four locations, with LOD scores ranging from 5.8 to 16.2, and explained 9.43–23.59% of the phenotypic variation in the RIL population. The LTR108 allele had the genetic effect of increasing mean PH from 14.2 to 35.9 cm (Fig. 2A; Supplementary Table S3 at *JXB* online). The co-location of *Dw3* with this QTL provided strong evidence showing the high accuracy of the bin mapping strategy.

The other evidence came from the mapping of the gene for photoperiod sensitivity (*Ma1*). Sorghum genotypes show a wide range of photoperiod sensitivity and critical floral-inductive day lengths (Quinby, 1974; Craufurd *et al*., 1999). Historic genetic studies uncovered six flowering time (maturity) loci, designated *Ma1*, *Ma2*, *Ma3*, *Ma4*, *Ma5*, and *Ma6* (Quinby and Karper, 1945; Quinby, 1966, 1967; Rooney and Aydin, 1999). Of the six original maturity loci, *Ma1* with the largest impact on flowering time has been located in a small region on chromosome 6 and narrowed down to an 86 kb interval (Quinby, 1974; Lin *et al*., 1995; Rooney and Aydin, 1999; Klein *et al*., 2008; Murphy *et al*., 2011). *SbPRR37*, the sole gene present among the stretches of repetitive DNA in this region, was eventually confirmed as the best candidate for *Ma1*. The structure of *SbPRR37* contained three untranslated and eight protein-coding exons. This transcript encodes a 739 amino acid, ~93 kDa protein that contains a predicted N-terminal pseudoreceiver domain (residues 99–207) and a C-terminal CCT domain (residues 682–727), present in all known plant PRR proteins (Murphy *et al*., 2011). At the amino acid level, *SbPRR37* is very closely related to *Arabidopsis* PRR7, two maize PRR37-like proteins (encoded by GRMZM2G033962 and GRMZM2G005732), rice PRR37 (LOC_Os07g49460), and PRR proteins encoded by barley *Ppd-H1* and wheat *Ppd-D1a* (Turner *et al*., 2005; Beales *et al*., 2007). The regulation mechanism of flowering by the *SbPRR37* gene has been studied. It is revealed that output from the circadian clock activates *SbPRR37* expression in the morning and evening, and the continuous expression of PRR37 in long days is proposed to repress flowering, while lack of increased *SbPPR37* expression during the evening phase is

Table 5. QTLs identified for eight traits using the high-density SNP bin map (showing significant QTLs) across two short-day trials

Trait	QTL	Chrom.	Bin	Position (cM)	Interval (cM)[a]	LOD score	Additive[b]	R^2 (%)[c]
HD	*qHD6a*	6	Bin_2051	47.14	46.45–49.12	25.1	2.1	32.72
	qHD6c	6	Bin_2214	144.26	143.34–146.65	3.3	0.6	3.44
	qHD8	8	Bin_2786	129.42	128–130.69	3.3	0.6	3.4
PH	*qPH1*	1	Bin_0377	158.05	157.71–161.1	5.4	8.9	6.5
	qPH6a	6	Bin_2051	47.14	46.45–47.76	8.2	11.8	10.12
	qPH7	7	Bin_2453	111.78	111.33–113.36	11.9	–20.3	16.24
NN	*qNN1a*	1	Bin_0163	72.52	69.38–73.3	4.0	–0.2	4.12
	qNN1c	1	Bin_0391	169.84	168.76–172.14	4.1	–0.2	4.43
	qNN6	6	Bin_2051	47.14	46.45–48.9	22.5	0.6	29.0
	qNN7	7	Bin_2452	111.78	110.31–112.92	5.5	–0.3	6.2
	qNN8a	8	Bin_2505	0.21	0.01–1.13	5.5	0.2	6.04
	qNN8b	8	Bin_2770	120.4	119.27–123.03	4.8	0.2	5.22
SD	*qSD1*	1	Bin_0253	103.62	102.71–105.31	5.1	–0.1	7.26
	qSD6a	6	Bin_2058	49.39	47.73–50.03	7.1	0.1	9.98
	qSD7a	7	Bin_2461	116.23	115.05–116.44	5.9	0.1	8.19
	qSD7b	7	Bin_2478	125.12	122.74–126.39	3.1	0.5	4.3
PNL	*qPNL1b*	1	Bin_0469	209.06	208.64–210.1	5.7	1.9	7.92
	qPNL4	4	Bin_1553	114.16	113.28–116.6	3.2	1.5	4.54
	qPNL6a	6	Bin_2123	87.88	86.98–89.24	5.0	1.7	6.99
	qPNL6b	6	Bin_2141	98.18	96.86–98.67	3.2	1.4	4.69
PL	*qPL6a*	6	Bin_2065	52.53	50.96–53.9	5.1	0.9	6.78
	qPL6 b	6	Bin_2123	87.88	86.98–88.33	7.5	1.0	10.25
	qPL6c	6	Bin_2131	94.38	93.48–94.78	8.6	1.1	11.79
	qPL8b	8	Bin_2742	99.44	98.48–101.49	3.1	0.6	4.02
	qPL8c	8	Bin_2753	107.34	106.25–110.9	3.2	0.6	4.08
	qPL9a	9	Bin_3023	109.12	108.44–111.67	3.0	0.6	3.82
FLL	*qFLL2*	2	Bin_0830	145.77	144.6–147.26	3.6	–1.6	5.69
	qFLL3	3	Bin_0955	12.96	12.47–14.62	3.6	1.6	5.93
	qFLL7	7	Bin_2403	76.83	75.04–77.51	3.2	1.5	5.07
FLW	*qFLW1*[c]	1	Bin_0164	73.61	71.43–74.89	3.3	0.3	4.81
	qFLW2[a]	2	Bin_0835	147.73	147.2–148.17	8.2	–0.4	13.1
	qFLW2[b]	2	Bin_0859	157.48	156.58–157.95	4.2	–0.3	7.03

[a] 1.5-LOD support interval of the QTL.
[b] Addictive effect: positive values of the additive effect indicate that alleles from 654 were in the direction of increasing the trait score.
[c] Percentage of variation explained by the QTL.
HD, heading date; PH, plant height; NN, numbers of nodes; SD, stem diameter; PNL, panicle neck length; PL, panicle length; FLL, flag leaf length; FLW, flag leaf width.

proposed to reduce the level of the repressor PRR37, allowing floral initiation (Murphy *et al*., 2011).

In the present study, *Ma1* was consistently mapped within a 700 kb interval using 244 RILs across four locations, and explained 21.28–33.59% of total variance (Fig. 2B; Supplementary Table S3 at *JXB* online). It is worth noting that in long days, an additive effect for delaying the flowering time originates from the *Ma1* allele (*SbPRR37* photoperiod-sensitive) carried by LTR108 rather than the *ma1* allele (*Sbprr37* photoperiod-insensitive) carried by 654. In short days, however, photoperiod sensitivity has nothing to do with delay of flowering, but the plant height could become an influential factor. The QTL mapping result that *qHD6a* and *qPH6a*, both with an additive genetic effect from 654, co-located at Bin2051 on chromosome 6 provides a strong suggestion that it is the 654 allele of *qPH6a* that is responsible for the delayed flowering in short days (Supplementary Table S3).

These results support the assumption that the SNP bin map constructed in this study is accurate and suitable for gene mapping and QTL identification; the sequencing-based method is perhaps an effective approaches to genotype a large mapping population that provides accurate information.

Comparison of chromosomal locations of QTLs under contrasting photoperiods

In recent decades, there has been a remarkable increase in the use of QTL mapping as a tool to uncover the genetic control of agronomically important traits, and >700 QTLs have been identified in sorghum (http://www.gramene.org), but very few studies have been reported on the underlying genetic mechanisms of these traits for photoperiod response. In this study, QTLs were identified using an ultra-high-density SNP map for eight agronomically important traits under two contrasting photoperiods. Numerous major QTLs were detected under both photoperiods. However, almost half of these QTLs were only detected under one type of photoperiod, indicating that the agronomically important traits were controlled by different genes under different photoperiod conditions.

Interestingly, it was also indicated, from QTL mapping conducted in this study, that the agronomically important traits shared

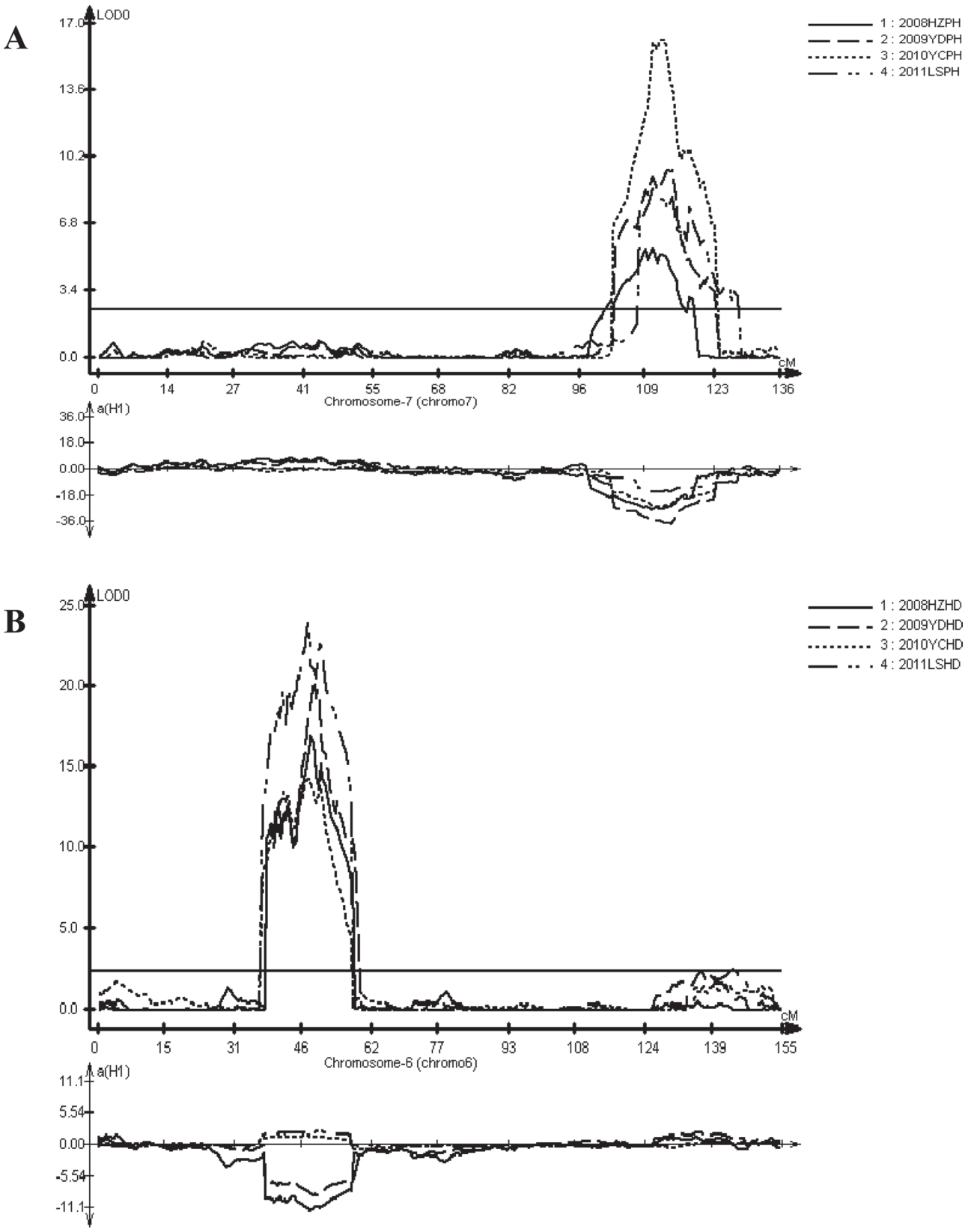

Fig. 2. QTL profiles for PH and HD in the SNP bin map. (A) LOD curves of QTL mapping for plant height on chromosome 7 across four locations. (B) LOD curves of QTL mapping for heading date on chromosome 6 across four locations.

a similar genetic basis under different photoperiods. Several major QTLs (*qHD6a*, *qPH6a*, *qPH7*, *qNN6*, *qSD6a*, *qPNL6a*, *qPNL6b*, *qPNL6c*, and *qFLW2a*) were detected consistently under both photoperiods in this study. The genetic effects for *qHD6a*, *qPH6a*, *qNN6*, and *qSD6a* came from different parents under two contrasting photoperiods. The LTR108 alleles worked for delaying heading and improving height, numbers of nodes, and stem diameter under long days, while the 654 alleles worked for delaying heading and improving height, numbers of nodes, and stem diameter under short days. This phenomenon may be attributed to the *Ma1* gene. The *Ma1* gene carried by LTR108 caused the varied phenotypes for LTR108 and RILs under the two contrasting photoperiods. From a long-day environment to a short-day environment, HD, PH, NN, and SD of LTR108 and RILs were decreased dramatically, while few changes happened in 654. The dramatically varied phenotypes resulted in the change in the strength of correlations among HD, PH, NN, and SD. Higher correlations were observed under a long-day photoperiod than under a short-day photoperiod. The LTR108 alleles for *qPH7* and *qFLW2a* resulted in increased height and widened leaves under both photoperiods, indicating that the effects of these QTLs were independent of photoperiod.

Co-locations of QTLs for multiple traits

Phenotypically correlated traits are known to be mapped together (Lebreton *et al*., 1995; Shashidhar *et al*., 1999; Hittalmani *et al*., 2002). The co-localizations of QTLs for different traits are very often observed. Murray *et al.* (2008) found co-localization of QTLs associated with fibre-related traits. It was also detected that QTLs for plant height and flowering date were co-localized with those of sugar and fibre components across the genome (Lin *et al*., 1995; Pereira and Lee, 1995; Murray *et al*., 2008; Ritter *et al*., 2008; Shiringani and Friedt, 2011). In this study, the co-localizations of QTLs for several traits investigated were clearly observed in some chromosomal intervals (Fig. 1). For example, 12 QTLs were detected and located in the interval Bin_2046–Bin_2068 (~2.9 Mb) on chromosome 6, including *qFLW6a* (in long days), *qPL6a* (in short days) and QTLs for HD, PH, NN, and SD under both photoperiods. The additive effects for most of 12 QTLs were from LTR108. At the corresponding interval (Bin_2046–Bin_2068), *Lin* et al. *(1995*) and *Klein* et al. *(2008)* detected the QTLs for maturity and height. *Srinivas* et al. *(2009)* reported the QTL cluster for plant height, days to anthesis, green leaf area at maturity, panicle length, grain yield, panicle weight, and seed weight. Similarly, *qPH7*, *qSD7a*, and *qNN7* under short days were located at the bottom of chromosome 7. In this genomic region, Rami *et al.* (1998) found major QTLs for yield components and morphological traits such as germination rate, number of kernels per panicle, kernel weight per panicle (grain yield), thousand-kernel weight (seed weight), panicle compactness, plant height, and panicle length. *Srinivas* et al. *(2009)* detected the QTLs for plant height and panicle length. When comparing the present QTL mapping with other QTL studies based on population sequencing, syntenic regions were also found between sorghum and other species. For example, the QTL region for panicle neck length on chromosome 3 in sorghum was syntenic to the one for plant height on chromosome 1 in rice (Wang *et al*., 2010). These results provide very useful information about target chromosomal intervals for candidate gene analysis and marker-assisted selection breeding since these intervals could be regarded as hotspots with agronomical importance. Taking such hotspots based on QTL results as prior chromosomal regions, a strategy was recently suggested for candidate gene isolation. Because the relationship between the genetic bin map and the physical position of SNP is consistent, it is easy to anchor the physical interval and find the putative genes in this region. Moreover, it was also proposed to transfer large size chromosomal intervals from a donor parent (such as LTR108 in this study) into a recurrent parent. Marker-assisted backcross procedures are underway in our group to transfer LTR108 alleles of chromosomal regions mentioned above into 654 and BTx623.

Supplementary data

Supplementary data are available at *JXB* online.
Figure S1. Recombination bin map constructed using high-density SNPs from sequencing genotyping of the RIL population.
Table S1. Location and sowing date of the four trials undertaken from 2008 to 2011.
Table S2. Distribution of size ranges of recombination bins in the ultra-high-density SNP map constructed using RILs of the 654/LTR108 cross based on population sequencing.
Table S3. QTLs for plant height (PH) and heading date (HD) under four trials.
Table S4. Map information for all 3418 bins for the 244 RILs from the 654/LTR108 cross based on high-quality SNPs obtained from population sequencing (XLS).

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant no. 31000739) and the Key Subject Construction Program of Zhejiang for Modern Agricultural Biotechnology and Crop Disease Control.

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