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Full Length Research Paper

Quantitative genetic analysis of flowering time, leaf number and photoperiod sensitivity in maize (*Zea mays* L.)

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Photoperiod sensitivity is a major difficulty of broadening the resources of breeding germplasms and widening the planting area of varieties in maize. The objective of this study was to dissect the quantitative genetic control of days to pollen shed (DPS), days to silking (DS), anthesis-silking interval (ASI), LNB (the leaf number below the top ear), LN (the total leaf number), and photoperiod sensitivity index (PSIs) of these traits into the main-effect quantitative trait loci (QTL) in an F_2 population consisting of 232 $F_{2:3}$ lines from the cross between HZ32 (low sensitive to photoperiod) and Huangzao4 (high sensitive to photoperiod). DPS, DS, LNB and LN of the $F_{2:3}$ families and parents were investigated in two locations, Beijing (with a longer photoperiod regime, east longitude 117, northern latitude 41) and Wuhan (with a shorter photoperiod regime, east longitude 113, northern latitude 29) in 2007. Further QTL analysis resolved the genetic components of DPS, DS, ASI, LNB and LN into the main-effect QTLs and gene × environment interactions. A total of fifty-nine QTLs for all PS related traits were found in the two photoperiod environments, twenty-two QTLs for PSIs and twenty-two QTLs for QTL × environment were included, respectively. Some important genes associated with photoperiod response and flowering time are found in the regions. The known QTLs and the implications of the results for maize breeding have been discussed.

Key words: Maize (*Zea mays*), photoperiod sensitivity, quantitative trait loci.

INTRODUCTION

In most plant species, light components, such as light quality, intensity, and photoperiod (length of the light period), appear to be the main environmental factors affecting flowering time (Austin et al., 2001), thus plant species have been divided into long-day (LD) plants and short-day (SD) plants. As the same with many other agronomic traits, flowering time is quantitative and varies among plants adapted to different environments (Murfet, 1977; Austin et al., 2001). In recent years, more information has been accumulated on the molecular, biochemical, physiological and morphological response to flowering time and photoperiod sensitivity in plants: rice(Yano et al., 2000; Yamamoto et al., 2000; Takahashi et al., 2001; Izawa et al., 2002; Lee et al., 2004; Ryu et al., 2009; Wu et al., 2008; Fornara et al., 2008; Takahashi et al., 2009),wheat (Kane et al., 2005; Shimada et al., 2009),sorghum (Lee et al., 1998; Finlayson et al., 1998; Finlayson et al., 1999; Searle and Coupland, 2004), arabidopsis (Hicks et al., 2001), soybean (Wong et al., 2008), barley (Faure et al., 2007), pea (Hecht et al., 2007) and others(Fowler et al., 1999; Danyluk et al., 2003; Griffiths et al., 2003;Colasanti et al., 2006; Gardner et al., 2006; McClung, 2006; Salvi et al., 2007; Hotta et al., 2007; Jackson, 2009; Buckler et al., 2009; Mathieu et al., 2009; Langdon et al., 2009; Moccia et al., 2009).

Aside from the studies of a few circadian clock components and specific photoreceptors, to our

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Abbreviations: DPS, Days to pollen shed; **DS,** days to silking; **ASI,** anthesis-silking interval; **LNB,** leaf number below the top ear; **LN,** total leaf number; **PSIs,** photoperiod sensitivity index; **QTL,** quantitative trait loci; **LD,** long-day; **SD,** short-day; **DTF,** date to flowering time; **PH,** plant height; **SSR,** simple sequence repeat; **CIM,** composite interval mapping; **cM,** centimorgan; **LOD,** logarithm of odds; **PS,** photoperiod sensitivity; **MAS,** marker-assisted selection; **NILs,** near isogenic lines.

knowledge there has been almost no study of maize genes directly involved in the photoperiod pathway to date (Sheehan et al., 2004; Camus-Kulandaivelu et al., 2006; Bomblies et al., 2003; Sheehan et al., 2007; Miller et al., 2008).

Photoperiod controls many developmental responses and photoperiod sensitivity is a major difficulty of broadening the resources of breeding germplasms and widening the planting area of varieties in maize (Goodman, 1985; Giauffret et al., 2000; Gouesnard et al., 2002; Jackson, 2009). Hence, how to resolve the difficulty is one of the main objectives of many maize breeding programs. In recent years, Leaf number, flowering time and plant height were found to be highly associated with photoperiod sensitivity in maize and other crops (Ellis et al., 1992a, 1992b; Koester et al., 1993; Yano et al., 2000; Giauffret et al., 2000; Gouesnard et al., 2002; Moutiq et al., 2002; Adams et al., 2003; Ren et al., 2006; Liang et al., 2008; Wang et al., 2008). And the quantitative genetic control of flowering time and photoperiod sensitivity has been preliminarily studied in maize using quantitative genetic analysis method (Koester et al., 1993; Moutiq et al., 2002; Wang et al., 2008). Conservatively, all these studies identified, are located in different chromosomal segments, six genomic regions affecting date to flowering time (DTF), the total leaf number LN, plant height (PH) and anthesis-silking interval ASI. Because of the complexity of the photoperiod response of maize, quantitative genetic analysis using different maize accessions and different traits is a good method to explore more elements associated with photoperiod response and to prove the accuracy of known QTLs (Holland, 2007).

In this work, we investigated the genetic control of the variation of the traits associated with photoperiod response and their PSIs (location and effects of QTLs involved) present in an F_2 population derived from a cross between two elite maize inbred lines, which differ in DPS, DS, ASI, LNB and LN. To our knowledge, LNB was firstly used to identify QTL as the photoperiod sensitivity related trait. The aims of this study were to (1) identify QTLs associated with photoperiod sensitivity related traits, including flowering time, leaf number under different day length conditions, and the QTLs of PSIs of the traits，(2) detect QTL groups associated with photoperiod sensitivity, (3) explore markers associated with photoperiod insensitive to carry out molecular assisted selection in breeding program. This quantitative genetic analysis has allowed the identification of new QTLs responsible for photoperiod response variation and as putative candidate genes for some of those loci.

MATERIALS AND METHODS

Plant material and population development

An F_2 population was developed from a cross between two maize inbred lines, Huangzao4 (a photoperiod relatively sensitive inbred line derived from a local Chinese germplasm, Tangsipingtou, a heterotic group used broadly in China), and HZ32 (a photoperiod relatively insensitive inbred line bred by Maize group of Huazhong Agriculture University from a foreign germplasm, Lancaster, a heterotic group used broadly in China). More than two hundred and thirty-two F_2 seeds derived from a single F_1 parent were planted and 232 of the subsequent F_2 plants were successful self-pollinated at the experiment farm of Huazhong Agricultural University. The seeds of the 232 $F_{2:3}$ ears were harvested from the F_2 selfed-plants in the 2005 maize-growing season. The F_2 plants were used for genotyping simple sequence repeat (SSR) loci and the $F_{2:3}$ seeds harvested from each F_2 plants were utilized to conduct the photoperiod experiments.

Evaluation of sensitivity to photoperiod

The parental inbreds and the 232 $F_{2:3}$ families for the QTL mapping were grown in the field under a short-relative-day environment in Wuhan and long-day environment in Beijing during the spring of 2007. The experiments were laid out in a randomized complete-block design with three replications at each location. Each family was planted in a one-row plot (0.6m apart and 4 m in length) with a total of 15 plants per row; the density was 50, 000 plants/ha.

Phenotype data collection

DPS, DS, LNB and LN were measured from ten consecutive plants beginning with the third plant of each row. DPS were recorded as the number of days from sowing to the emergence of the first pollen shed from anthers on the central spike. ASI was calculated using the following formula: ASI = DPS-DS; Photoperiod sensitivity index (PSIs) = ± (value of trait in LD-value of trait in SD)/ value of trait in SD]; “LD” means long day and “SD” means short day; “-” means the traits are DPS and DS; “+” means the traits are ASI, LNB and LN.

DNA isolation and SSR analysis

Genomic DNA from each of the F_2 plants and the parental lines was isolated from fresh leaf tissue following a procedure similar to that used by Saghai-Maroof et al. (1984). The modifications in the procedure were (1) addition of boiled CTAB extraction buffer to the 50 mL polypropylene centrifuge tube, and (2) a reduction of the incubation time to 30 min. In accordance with bin location, a total of 550 SSR markers were chosen from the maize genome database to detect parental polymorphisms according to the procedure similar to that used by Qiu et al. (2007). The co-dominant segregation SSR markers were used to genotype the F2 populations.

Linkage analysis and map construction

The genetic linkage map was constructed using Mapmaker/Exp 3.0b (Lander et al., 1987; Lincoln et al., 1993). All the markers were assigned at LOD≥3.0 to ten linkage groups. By means of the Kosambi mapping function (Kosambi, 1944), the values of recombination fractions were converted into genetic map distances (cM). The map was drawn according to Liu and Meng (2003).

Statistical analysis

Means, Kurtosis, Skewness, and P values of all traits in the $F_{2:3}$ population and parents were analyzed for both locations by the analysis tools in Excel. The genetic ANOVA, the broad-sense

heritability (h^2) and correlation coefficiency of traits were carried out with SAS ver. 8.02 (SAS Institute Inc., Cary, North Carolina, 1991 to 2001). Main QTLs were identified by using Windows QTL Cartographer Version 2.0 (North Carolina State University, Raleigh, NC) programmed by Wang et al. (2002). Composite interval mapping (CIM) was used to map the QTLs. The parameters were set as follows. Map function: Kosambi; distance units: centimorgan (cM); distance type: position; cross-information: SF3 (self-cross F3); walk speed: 2 cM; LOD=2.5; CIM mode selection: model 6, that is, standard model; background controls: 5 of control marker numbers and 10.0 cM of window size. Significance thresholds were determined by permutation tests (n=1000 permutations; Churchill and Doerge, 1994). QTL×Environment (Q×E) interaction and digenic epistatic QTLs analysis were conducted by using QTLMapper V2.0 based on a mixed model approach (Wang et al., 1999). *P≤0.005* for Type-I errors and a log10 likelihood ratio (LOD) value of 2.5 were used as criteria to declare the putative main effect QTL position, digenic epistatic QTLs and QTL×Environment (Q×E) interaction. Epistasis effect was estimated according to the definition of Mather and Jinks (1982). The R^2 value (coefficient of determination) from this analysis indicated the percentage of phenotypic variance explained by the marker genotypes at the locus.

RESULTS

Phenotypic data and phenotypic variation

All five PS related traits except for LN between Huangzao4 and HZ32 showed significant differences at Wuhan at the level of 0.05 or 0.01. All five PS related traits between Huangzao4 and HZ32 showed significant differences at Beijing at the level of 0.05 or 0.01. All traits of Huangzao4 under two photoperiod environments showed significant differences at the level of 0.01. DPS and DS in HZ32 between two locations showed significant differences at the level of 0.01, and LNB showed significant difference at the level of 0.05. In addition, the PSIs of the five traits in Huangzao4 and HZ32 were significantly different. PSIs in Huangzao4 were more than that observed in HZ32, which indicated that Huangzao4 exhibited increased photoperiod sensitivity (PS). The phenotypic variations of all traits and their PSIs in the $F_{2:3}$ families were fit for normal distribution indicated by the values of Kurtosis and Skewness, which suggested that these traits should be quantitative traits (Table 1). Broad-sense heritability of these traits differed for the two photoperiod conditions (Table 1). DPS, DS, LNB and LN had relatively higher heritability (69, 75, 63 and 81%, respectively) under the LD condition and lower heritability (57, 52, 36 and 59%, respectively) under the SD condition, while the heritability of ASI was relatively close.

It was 57 and 47% under the SD and LD condition, respectively. The broad-sense heritability of PSIs of DPS, DS, ASI, LNB and LN were 88, 87, 74, 83 and 87%, respectively. Correlation of the traits in the $F_{2:3}$ families between in Beijing and in Wuhan were detected. Strongest positive significant correlations for each trait were detected between DPSw and DPSb, DSw and DSb, ASIw and ASIb, LNBw and LNBb, LNw and LNb. LNw and LNBw showed stronger significant correlations with all other traits but ASI (Table 2). Correlation among the PSIs of the five traits in the $F_{2:3}$ families were also detected. Strong, positive correlations were detected between DPS and DS (r=0.89) and between LN and LNB (r=0.86) (Table 3). Significant and positive correlation was also detected between ASI and DPS. PSIs of the traits of DPS and DS were significantly and negatively correlated to PSIs of the traits of LNB and LN.

Linkage analysis and map construction

One hundred and seventy-nine SSR markers showing co-dominant segregation were employed to construct a linkage map (Figure 1). Of which, one hundred and fifty-eight informative markers were assigned to ten chromosomes based on LOD values exceeding 3.0. The linkage map had a total length of 1407.9 cM with an average interval length of 8.91 cM between adjacent markers. The chromosome location of locus arrangement for informative markers in the linkage map was consistent with the location released in SSR bin map except for 4 loci (bnlg1091, umc1774, phi93225 and umc1916).

QTLs detection for photoperiod sensitivity related traits

Fifty-nine QTLs for all PS related traits were found in two photoperiod environments. Of those, 31 QTLs in Beijing and 28 QTLs in Wuhan were detected; seven QTLs were common detected in the two locations. These QTLs were distributed on all chromosomes except linkage group chromosome 7 in Beijing, while these QTLs were on chromosomes 1, 2, 3, 4, 5, 8 and 10 in Wuhan. The detected QTL individually accounted for 0.17 to 30.71% of the phenotypic variation. Out of 59 QTLs, 19 QTLs individually accounted for more than 10% of the phenotypic variation. A list of the putative QTLs flanked by SSR markers along with their phenotypic variance, additive effects and peak LOD scores, were presented in Tables 4, 5 and 6. A graphical presentation of QTLs locations on the linkage map was shown in Figure 1.

Days to pollen shed (DPS)

Thirteen QTLs were identified for DPS. Seven (dps1-4b, dps1-5b, dps1-7b, dps2-5b, dps3-8b, dps9-8b, dps9-10b) and six QTLs (dps1-10w, dps1-13w, dps1-15w, dps2-10w, dps3-3w, dps3-17w) were detected in Beijing and Wuhan, respectively. Of all these, only one QTL (dps1-7b and dps1-10w shared partially common position) was detected in both environments. QTLs for DPS in Beijing were identified on chromosomes 1, 2, 3 and 9, accounting for a phenotypic variance ranged from 2.49 to

Table 1. phenotype evaluation of the two parents and the F2:3 families in the two environments.

Character	DPS		DS		ASI		LNB		LN	
Location	**Beijing**	**Wuhan**	**Beijing**	**Wuhan**	**Beijing**	**Wuhan**	**Beijing**	**Wuhan**	**Beijing**	**Wuhan**
Huangzao4(P_1)(mean)	68±0	42.33±1.53	70.67±0.58	42.67±1.53	2.67±0.58	0.33±0.58	17.67±0.58	14.67±0.58	22.33±0.58	18.67±0.58
HZ32(P_2)(mean)	71.67±1.15	56.67±1.53	77±1.73	62.67±2.31	5.33±1.53	6±1	14.67±0.58	13±1	20.33±0.58	20±3
P_1 versus P_2†	*	**	*	**	*	**	**	*	*	ns
P_1 Beijing vs. Wuhan	**		**		**		**		**	
P_2 Beijing vs. Wuhan	**		**		ns		*		ns	
$F_{2:3}$										
Mean	65.71±1.54	77.33±1.81	69.67±1.63	80±2.11	3.96±0.9	2.68±0.88	15.26±0.49	13.67±0.6	21.05±0.59	18.74±0.72
Range	60.36-69.75	72.97-82.04	64.06-73.25	75.14-86.03	1.84-6.57	0-6.32	13.97-16.91	11.91-15.66	19.41-22.63	16.49-21.1
Skewness	-0.28	0.12	-0.51	0.15	0.09	-0.09	0.24	-0.12	0.05	-0.06
Kurtosis	0.37	-0.17	0.73	-0.36	-0.21	1.17	0.36	0.57	0.09	0.49
h^2	0.69	0.57	0.75	0.52	0.47	0.57	0.63	0.36	0.81	0.59
Note:										
PS index	PSIs of DPS		PSIs of DS		PSIs of ASI		PSIs of LNB		PSIs of LN	
P_1(mean)	0.608±0.05		0.658±0.053		0.667±0.333		0.206±0.054		0.197±0.042	
P_2(mean)	0.265±0.035		0.23±0.047		-0.094±0.263		0.133±0.085		0.032±0.138	
P_1 vs P_2	**		**		**		*		**	
$F_{2:3}$ Mean	0.149±0.030		0.128±0.031		0.302±0.238		0.103±0.037		0.108±0.034	
$F_{2:3}$ Range	0.058-0.227		0.046-0.207		-0.30-0.782		0.000-0.208		0.010-0.200	
Skewness	-0.030		-0.009		-0.361		-0.046		-0.211	
Kurtosis	-0.200		-0.072		-0.248		-0.125		0.036	
h^2	0.88		0.87		0.74		0.83		0.87	

The heritability was computed as $h^2=\delta_g^2/(\delta_g^2+\delta_e^2/n)$, where δ_g^2 and δ_e^2 were the estimates of genetic and residual variances, respectively, derived from the expected mean-squares of the analysis of variance, and n was the number of replications. Photoperiod sensitivity index [estimated as PSI=± (value of trait in LD-value of trait in SD)/ value of trait in SD]. "-": DPS and DS; "+": ASI, LNB and LN. Data are mean + S.e.m. † Statistical test for difference between two parents at 0.05 (*) and 0.01 (**) levels of probability; ns, not significant.

13.82% (Tables 4, 5 and Figure 1). For five of the QTLs (dps1-4b, dps1-5b, dps1-7b, dps9-8b and dps9-10b), alleles from 'Huangzao4' contributed an increase of the trait values. For the other two QTLs, alleles from 'HZ32' tended to increase the trait values. Six putative QTLs for DPS in Wuhan were identified on chromosomes 1, 2 and 3, accounting for 0.72 to 12.45% of the phenotypic variance. Trait values at all detected QTLs except for dps3-17w were increased from the allelic contributions of HZ32 in Wuhan.

Days to silking (DS)

For DS, seven (ds1-4b, ds2-4b, ds2-17b, ds3-11b, ds4-4b, ds9-8b, ds9-10b) and six QTLs (ds1-13w, ds1-15w, ds2-15w, ds3-3w, ds4-4w, ds4-7w) were resolved in Beijing and in Wuhan, respectively. Only one QTL (ds4-4b and ds4-4w shared partially common position) was detected in both environments. QTLs for DS in Beijing were identified on chromosomes 1, 2, 3, 4 and 9, accounting for a phenotypic variance ranged from

Table 2. Correlations between PS related traits in the $F_{2:3}$ families under two environmental regimes.

Traits	DPSw	DSw	ASIw	LNBw	LNw
DPSb	0.39**	0.35**	0.01	0.24**	0.24**
DSb	0.37**	0.43**	0.24**	0.19**	0.26**
ASIb	-0.01	0.2**	0.46**	-0.08	0.06
LNBb	0.01	0	-0.03	0.54**	0.41**
LNb	0.09	0.1	0.05	0.5**	0.58**

The vertical values stands for the phenotype in the long-day environment, and the horizontal values are the phenotype in the short-day environment. DPSw stands for the value of DPS in Wuhan, DPSb stands for the value of DPS in Beijing. The other traits are similar name rule. * Significant at $P<0.05$; ** significant at $P<0.01$.

Table 3. Correlations between PSIs of different traits.

PSIs	DS	ASI	LNB	LN
DPS	0.89**	0.14**	-0.44**	-0.52**
DS		-0.22**	-0.38**	-0.48**
ASI			-0.08	-0.10
LNB				0.86**

* Significant at $P<0.05$; ** significant at $P<0.01$.

3.87 to 16.27% (Tables 4, 5 and Figure 1). For four of the QTLs (ds1-4b, ds4-4b, ds9-8b and ds9-10b), alleles from 'Huangzao4' contributed an increase of the trait values, whereas for the other three QTLs the alleles from 'HZ32' contributed to the increase in the trait score. Six putative QTLs for DPS in Wuhan were identified on chromosomes 1, 2, 3 and 4, accounting for 2.02 to 10.61% of the phenotypic variance. Trait values at all detected QTLs, except for ds4-4w and ds4-9w, were increased from the allelic contributions of HZ32 in Wuhan.

Anthesis-silking interval (ASI)

Nine QTLs were identified for ASI. Five (asi3-12b, asi4-4b, asi4-7b, asi5-9b, asi10-10b) and four QTLs (asi1-2w, asi4-4w, asi5-7w, asi10-7w) were detected in Beijing and Wuhan, respectively. Out of them, only one QTL (dps4-4b and dps4-4w shared partially common position) was detected in both environments. QTLs for ASI in Beijing were identified on chromosomes 3, 4, 5 and 10, accounting for a phenotypic variance ranged from 2.16 to 19.48% (Table 4, 5 and Figure 1). For three of the QTLs (asi4-4b, asi4-7b and asi10-10b), alleles from 'Huangzao4' contributed an increase of the trait values. The other two QTLs, alleles from 'HZ32' also increased the trait values. Four putative QTLs for ASI in Wuhan were identified on chromosomes 1, 4, 5 and 10, accounting for 3.42 to 13.42% of the phenotypic variance. Increased trait values at all detected QTLs, except asi5-7w and asi10-7w, were seen from the allelic contributions of Huangzao4 in Wuhan.

The leaf number below the top ear (LNB)

For LNB, seven (lnb1-10b, lnb1-15b, lnb2-16b, lnb5-11b, lnb6-11b, lnb8-6b, lnb10-10b) and four QTLs (lnb1-17w, lnb4-5w, lnb8-5w, lnb10-6w) were resolved in Beijing and Wuhan, respectively. Two QTLs (ds1-15b and ds1-17w, lnb8-6b and lnb8-5w shared partially common position) were detected in both environments. QTLs for LNB in Beijing were identified on chromosomes 1, 2, 5, 6, 8 and 10, accounting for a phenotypic variance ranged from 0.17 to 30.71% (Table 4, 5 and Figure 1). For five of the QTLs (lnb1-10b, lnb5-11b, lnb6-11b, lnb8-6b and lnb10-10b), alleles from 'Huangzao4' contributed an increase of the trait values, whereas for the other two QTLs the alleles from 'HZ32' contributed to the increase in the trait score. Four putative QTLs for LNB in Wuhan were identified on chromosomes 1, 4, 8 and 10, accounting for 2.24 to 12.49% of the phenotypic variance. Trait values at all detected QTLs except for lnb1-17w were increased from the allelic contributions of Huangzao4 in Wuhan. The QTL (lnb1-10b), mapped in the region of bin 1.05-1.06, could explain 30.71% of the phenotypic variation, and demonstrated the highest additive effects with values of 0.77.

The total leaf number (LN)

For LN, five (ln1-10b, ln1-16b, ln2-5b, ln5-11b, ln6-11b, lnb8-5b) and eight QTLs (ln1-15w, ln2-12w, ln4-4w, ln4-10w, ln4-11w, ln8-6w, ln8-16w, ln10-9w) were observed in Beijing and Wuhan, respectively. Of all these, two QTLs

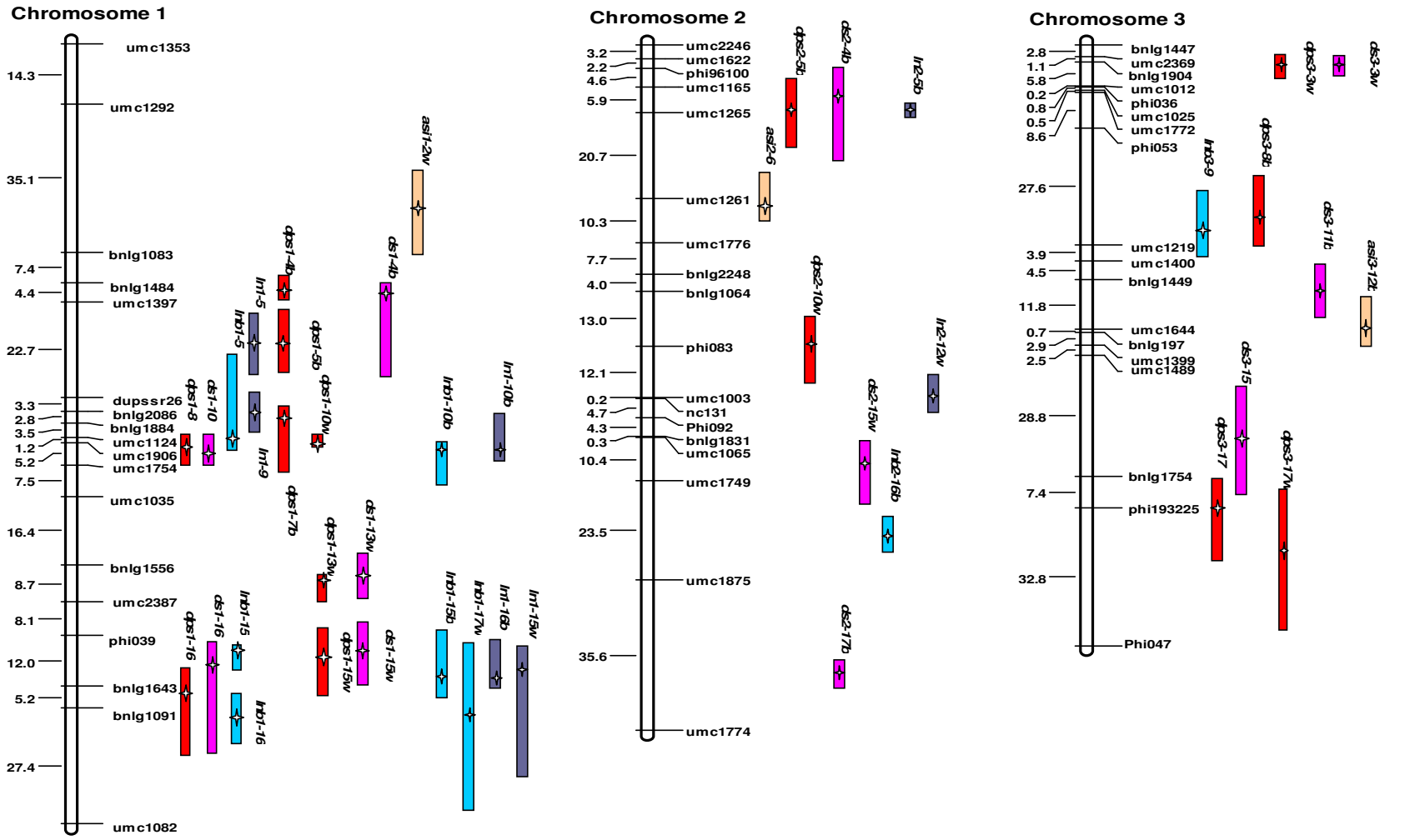

Figure 1. Molecular linkage map of the F_2 population derived from a cross between 'Huangzao4' and 'HZ32', and summary of QTL for all traits responsive to photoperiod in the mapping population of maize in Beijing and Wuhan. *dps* days to pollen shed ; *ds* days to silking; *asi* anthesis to silking interval; *lnb* the leaf number below the top ear; *ln* the total leaf number. For all the QTL names, the first number following the letters represents the chromosome locations of the QTL, the second number represents the orders of the nearest marker with the peak position of the QTLs located on the same chromosome and the last letter represents the photoperiod environment of the QTL ("*w*" represents Wuhan; "*b*" represents Beijing; nothing represents PSIs). The distances between markers (cM) are listed to the left of each figure part. Red box denotes the relative position of QTLs for DPS under two photoperiod environments and its PSIs. Pink box denotes the relative position of QTLs for DS under two photoperiod environments and its PSIs. Brown box denotes the relative position of QTLs for ASI under two photoperiod environments and its PSIs. Azure box denotes the relative position of QTLs for LNB under two photoperiod environments and its PSIs. Blue grey box denotes the relative position of QTLs for LN under two photoperiod environments and its PSIs. Cross denotes the peak position of QTLs for PS related traits under two photoperiod environments and their PSIs.

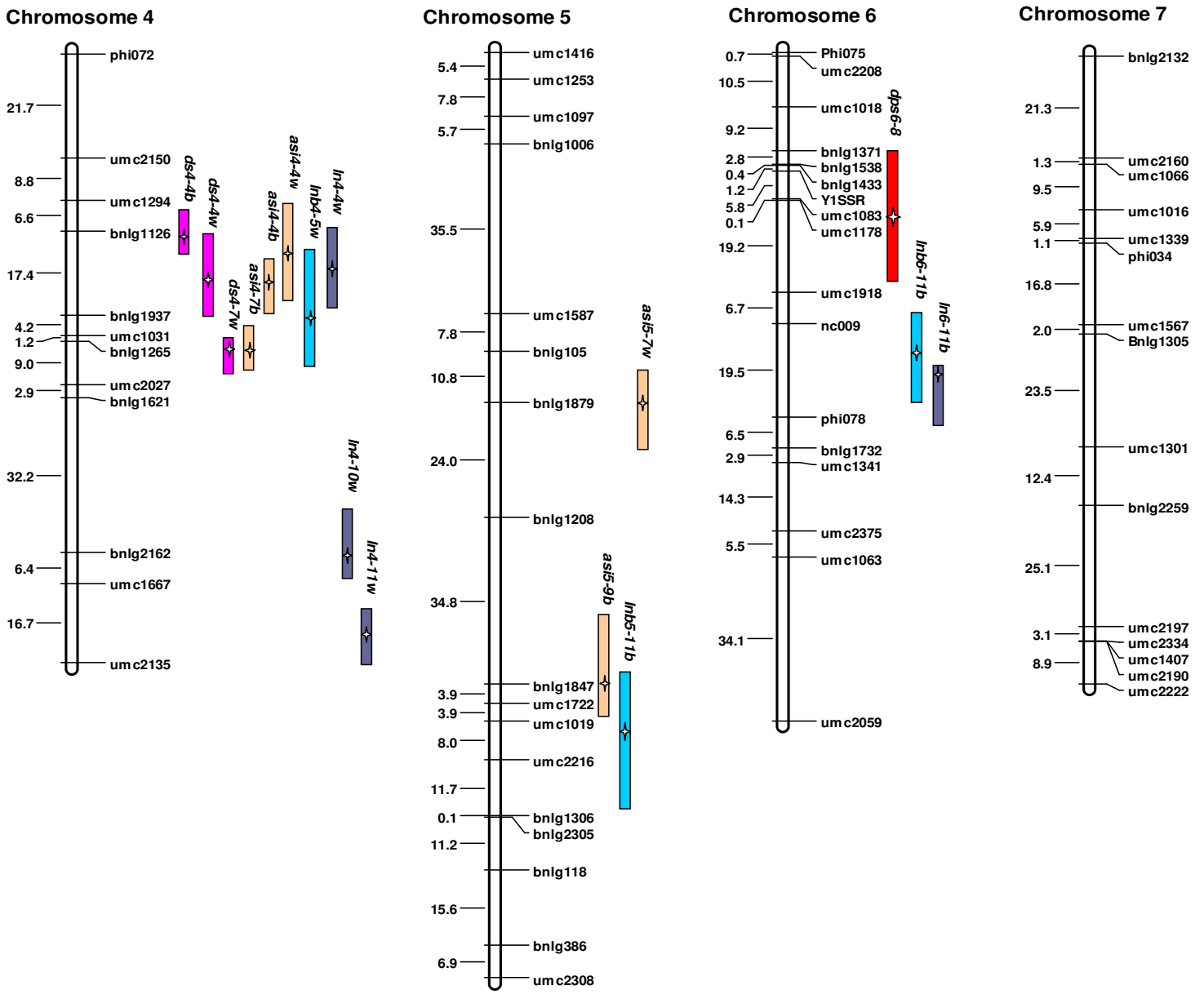

Figure 1. Contd.

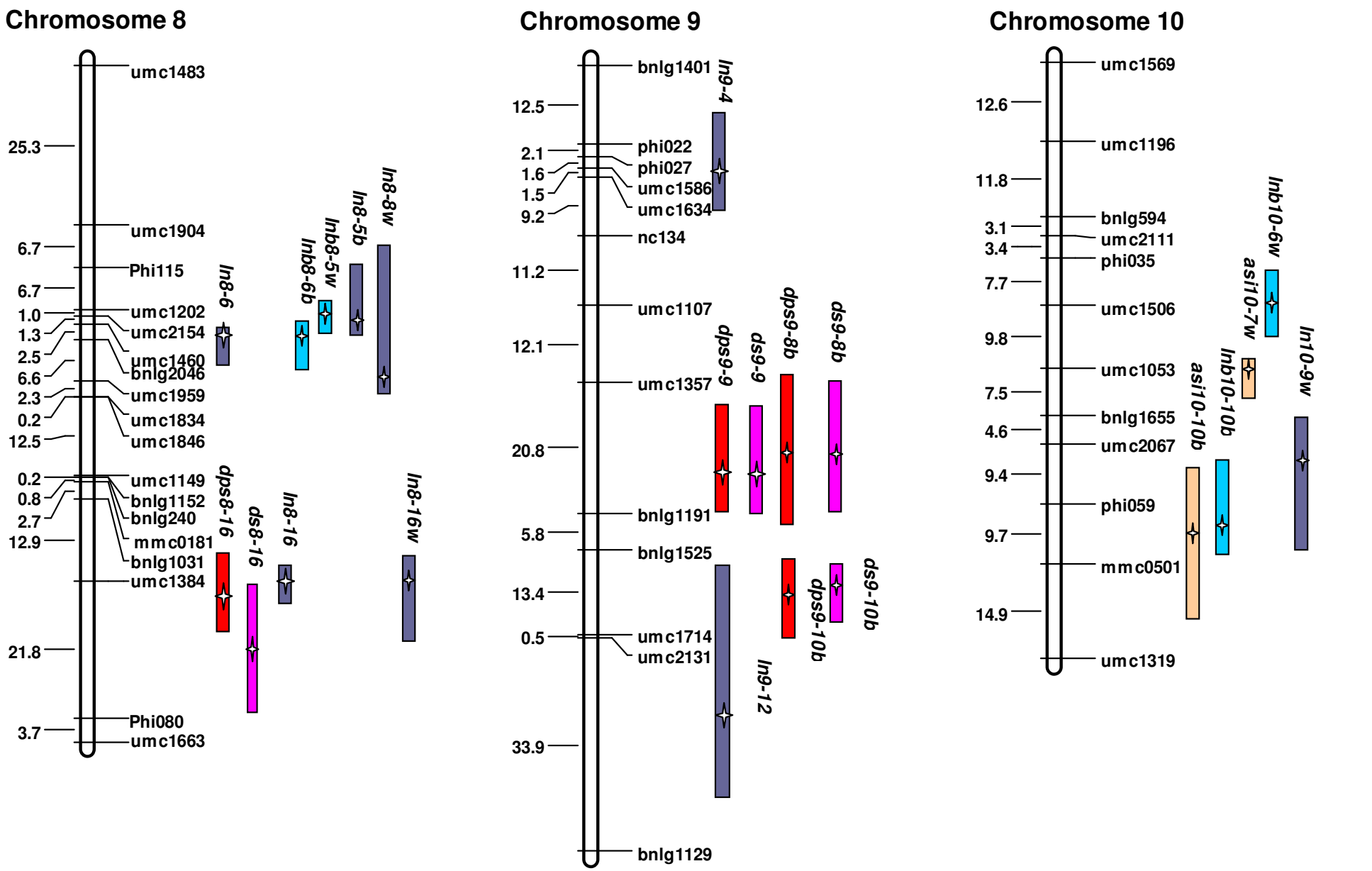

Figure 1. Contd.

Table 4. QTL detected for PS related traits in Wuhan (shorter photoperiod regime).

Trait	QTL[a]	chromosome number	cM[b]	Range[c]	Nearest marker	LOD[d]	R^2 (%)[e]	Additivity[f]
Days to pollen shed	*dps1-10w*	1	96.7	95-97	umc1906	2.545	6.11	-0.881
	dps1-13w	1	127.9	127-133	bnlg1556	5.5485	8.59	-0.9794
	dps1-15w	1	144.7	138-151	phi039	8.2731	10.89	-1.1086
	dps2-10w	2	71.7	66-80	phi083	3.1849	0.72	-0.2688
	dps3-3w	3	3.9	2-8	bnlg1904	5.5383	1.75	-0.4512
	dps3-17w	3	121.8	108-133	phi93225	3.4604	12.45	1.1519
Days to silking	*ds1-13w*	1	129.9	124-133	bnlg1556	5.268	6.48	-0.9768
	ds1-15w	1	142.7	136-150	phi039	8.45	8.4	-1.1109
	ds2-15w	2	101.4	96-112	umc1065	3.2835	4.95	-0.8495
	ds3-3w	3	3.9	3-7	bnlg1904	5.8247	2.02	-0.528
	ds4-4w	4	47.1	38-55	bnlg1126	3.4778	9.58	1.1435
	ds4-7w	4	61.9	59-66	bnlg1265	4.6588	10.61	1.1657
Anthesis silking interval	*asi1-2w*	1	42.3	33-52	umc1292	4.9374	3.42	0.3122
	asi4-4w	4	43.1	33-52	bnlg1126	6.2275	13.42	0.5865
	asi5-7w	5	73	67-83	bnlg1879	3.7888	8.39	-0.4818
	asi10-7w	10	48.5	46-52	umc1053	2.8144	4.64	-0.3516
The leaf number below the top ear	*lnb1-17w*	1	163.9	143-182	bnlg1091	4.3987	12.49	-0.4257
	lnb4-5w	4	56.5	41-65	bnlg1937	4.0177	3.38	0.206
	lnb8-5w	8	39.6	38-43	umc2154	5.8658	2.24	0.1664
	lnb10-6w	10	38.6	34-44	umc1506	4.7601	3.99	0.2238
The total leaf number	*ln1-17w*	1	163.9	145-175	bnlg1091	8.5729	21.54	-0.6289
	ln2-12w	2	84.1	79-87	nc131	3.0417	1.82	-0.1777
	ln4-4w	4	45.1	36-53	bnlg1126	6.4839	14.67	0.4875

[a]The first number following the letters represents the chromosome locations of the QTL, the second number represents the orders of the nearest marker with the peak position of the QTLs located on the same chromosome and the last letter represents the photoperiod environment of the QTL (“w” represents Wuhan; “b” represents Beijing; nothing represents PSIs). [b]Position of the peak of the QTL in centimorgans. [c]Range of the QTL above the threshold LOD score. [d]LOD score calculated by WinQTLCart 2.0. [e]Percentage of the phenotypic variance explained by genotype class at QTL peak. [f]Additivity: positive additivity indicates that the high values of the trait were inherited from the photoperiod sensitive parent (‘Huangzao4’); negative additivity means. that the high values of the trait were inherited from the photoperiod insensitive parent (‘HZ32’).

(ds1-16b and ds1-17w, lnb8-5b and lnb8-6w shared partially common position) were uncovered in both environments. QTLs for LN in Beijing were identified on chromosomes 1, 2, 6, and 8, accounting for a phenotypic variance ranged from 3.88 to 26.19% (Table 4, 5 and Figure 1). For three of the QTLs (ln1-10b, lnb6-11b and lnb8-5b), alleles from ‘Huangzao4’ contributed an increase of the trait values, whereas for the other two QTLs the alleles from ‘HZ32’ contributed to the increase in the trait score. Eight putative QTLs for LN in Wuhan were

Table 5. QTL detected for PS related traits in Beijing (longer photoperiod regime).

Traits	QTL[a]	Chromosome number	cM[b]	Range[c]	Nearest marker	LOD[d]	R^2(%)[e]	Additivity[r]
	dps1-4b	1	56.8	54-59	bnlg1484	3.0383	2.49	0.612
	dps1-5b	1	73.2	67-80	umc1397	8.4787	16.77	1.4449
	dps1-7b	1	87.2	85-99	bnlg12086	4.3022	6.39	1.0158
Days to pollen	dps2-5b	2	15.9	8-23	umc1265	3.0708	5.87	-0.7827
	dps3-8b	3	39.8	32-48	phi053	8.1743	7.76	-0.9349
	dps9-8b	9	62.2	50-73	umc1357	6.4291	13.82	1.2677
	dps9-10b	9	82.8	77-89	bnlg1525	5.2919	10.61	1.1289
	ds1-4b	1	56.8	53-75	bnlg1484	10.08	12.9	1.1589
	ds2-4b	2	14.1	6-27	umc1165	3.2586	6.15	-0.8608
	ds2-17b	2	143.3	140-146	umc1875	2.8491	3.87	-0.684
Days to silking	ds3-11b	3	57.7	51-64	bnlg1449	7.9605	10.34	-1.1318
	ds4-4b	4	39.1	35-46	bnlg1126	2.7867	5.06	0.7787
	ds9-8b	9	62.2	50-70	umc1357	7.7685	16.27	1.4424
	ds9-10b	9	80.8	77-86	bnlg1525	6.6811	13.1	1.3435
	asi3-12b	3	67.5	62-72	umc1644	3.4292	2.16	-0.2595
	asi4-4b	4	47.1	42-53	bnlg1126	9.3587	19.48	0.7501
Anthesis-silking interval	asi4-7b	4	61.9	57-66	bngl1265	10.8056	15.92	0.6544
	asi5-9b	5	131.8	118-139	bnlg1847	3.9519	5.9	-0.4368
	asi10-10b	10	74	63-87	phi059	3.1339	2.27	0.2692
	Lnb1-10b	1	96.7	94-104	umc1906	13.1285	30.71	0.7651
	lnb1-15b	1	150.7	141-155	phi039	3.847	4.1	-0.2771
	lnb2-16b	2	117.8	114-122	umc1749	2.5796	0.17	-0.0519
The leaf number below the top ear	nb5-11b	5	141.7	129-154	umc1019	3.557	4.66	0.2893
	lnb6-11b	6	64.7	55-73	nc009	5.3886	13.31	0.4921
	lnb8-6b	8	40.9	40-48	umc1460	13.9806	16.71	0.5367
	lnb10-10b	10	72	66-75	phi059	2.6251	8.67	0.3002
	ln1-10b	1	96.7	88-99	umc1906	13.7937	26.19	0.7506
	ln1-16b	1	152.7	144-155	bnlg1643	4.6052	9.32	-0.4396
The total leaf number	ln2-5b	2	15.9	14-18	umc1265	2.6407	3.88	-0.2622
	ln6-11b	6	66.7	66-78	nc009	4.387	9.48	0.4415
	ln8-5b	8	39.6	32-43	umc2154	11.8899	12.4	0.4541

[a]The first number following the letters represents the chromosome locations of the QTL, the second number represents the orders of the nearest marker with the peak position of the QTLs located on the same chromosome and the last letter represents the photoperiod environment of the QTL ("w" represents Wuhan; "b" represents Beijing; nothing represents PSIs). [b]Position of the peak of the QTL in centimorgans. [c]Range of the QTL above the threshold LOD score. [d]LOD score calculated by WinQTLCart 2.0. [e]Percentage of the phenotypic variance explained by genotype class at QTL peak. [f]Additivity: positive additivity indicates that the high values of the trait were inherited from the photoperiod sensitive parent ('Huangzao4'); negative additivity means that the high values of the trait were inherited from the photoperiod insensitive parent ('HZ32').

identified on chromosomes 1, 2, 4, 8 and 10, accounting for 0.54 to 21.54% of the phenotypic variance. Trait values at all QTLs except for ln1-15w, ln2-12w, ln4-10w and ln4-11w were increased from the allelic contributions from Huangzao4 in Wuhan. The QTL (ln1-10b), mapped in the region of bin 1.05-1.06, could explain 26.19% of the phenotypic variation, and demonstrated the highest additive effects with values of 0.75.

QTL detection for PSIs of the traits

A total of 22 putative QTLs were found to be associated with the PSIs of the five photoperiod sensitivity related traits, and mapped on chromosomes 1, 2, 3, 6, 8 and 9. The detected QTLs individually accounted for 0.28 to 22.56% of the phenotypic variation. Out of them, nine QTLs individually accounted for more than 10% of the

Table 6. QTL detected for PSIs of PS related traits.

Traits	QTL[a]	Chromosome number	cM[b]	Range[c]	Nearest maker	LOD[d]	R^2(%)	Additivity[f]
Days to pollen shed	dps1-8	1	92.1	90-95	bnlg11884	15.3641	22.56	-0.0227
	dps1-16	1	154.7	148-168	bnlg1643	4.607	0.63	-0.0037
	dps3-17	3	109.8	106-123	umc3225	5.1498	8.6	0.0129
	dps6-8	6	30.7	20-48	umc1083	3.7108	6.37	-0.0114
	dps8-16	8	89.7	79-101	umc1384	3.6274	7.16	0.0116
	dps9-18	9	60.2	53-66	umc1357	10.0707	17.43	-0.0186
Days to silking	ds1-10	1	96.7	92-99	umc1906	12.4064	22.19	-0.023
	ds1-16	1	152.7	143-167	bng1643	3.9385	0.28	-0.0025
	ds3-15	1	91.6	82-107	umc1489	5.0776	12.06	0.016
	ds8-16	8	91.7	89-104	umc1384	3.1485	7.42	0.0122
	ds9-8	9	58.2	89-104	umc1357	9.2534	16.23	-0.0185
Anthesis-silking interval	asi2-6	2	40.6	31-43	umc1261	2.6492	7.88	-0.102
The leaf number below the top ear	lnb1-9	1	93.5	69-96	umc1124	7.4574	17.31	0.024
	lnb1-15	1	142.7	141-147	phi039	2.6183	6.04	0.0141
	lnb1-17	1	163.9	156-169	bnlgl091	2.6933	6.92	0.0153
	lnb3-8	3	41.8	33-49	phi053	3.2744	2.62	0.0089
The total leaf number	ln1-5	1	75.2	68-82	umc1124	7.2923	16.22	0.0244
	lnl1-9	1	93.5	90-95	umc11397	7.2923	16.22	0.0212
	ln8-6	8	40.9	40-45	umc1460	2.7794	1.66	0.0065
	ln8-16	8	81.7	80-86	umc1384	2.6008	3.11	-0.0094
	ln9-4	9	16.2	7-23	umc586	3.4459	0.49	0.0037
	ln9-12	9	98.7	81-110	umc2131	3.2259	10.11	0.016

[a]The first number following the letters represents the chromosome locations of the QTL, the second number represents the orders of the nearest marker with the peak position of the QTLs located on the same chromosome and the last letter represents the photoperiod environment of the QTL (“w” represents Wuhan; “b” represents Beijing; nothing represents PSIs). [b]Position of the peak of the QTL in centimorgans. [c]Range of the QTL above the threshold LOD score. [d]LOD score calculated by WinQTLCart 2.0. [e]Percentage of the phenotypic variance explained by genotype class at QTL peak. [f]Additivity: positive additivity indicates that the high values of the trait were inherited from the photoperiod sensitive parent (‘Huangzao4’); negative additivity means that the high values of the trait were inherited from the photoperiod insensitive parent (‘HZ32’).

phenotypic variation (Table 6). Six QTLs (dps1-8, dps1-16, dps3-17, dps6-8, dps8-16, and dps9-9) for PSIs of DPS were mapped on chromosomes 1, 3, 6, 8 and 9. Each of them could explain 0.63 to 22.56% of the total phenotypic variation. Four of the six QTLs (dps1-8, dps1-16, dps6-8and dps9-9), alleles from ‘HZ32’ contributed an increase in the trait values. Whereas for the other two QTLs the alleles from ‘Huangzao4’ contributed to the increase in the trait score. The QTL (dps1-8),mapped in the region of bin 1.05-1.06, could explain 22.56% of the phenotypic variation, and demonstrated the highest additive effects with values of 0.02. For PSIs of DS, five QTLs (ds1-10, ds1-16, ds3-15, ds8-16 and ds9-9) were uncovered on chromosomes 1, 3, 8 and 9. They possibly explained 0.28 to 22.19% of the total phenotypic variation. Three QTLs alleles (ds1-10, ds1-16, and ds9-9) from ‘HZ32’ gave an increase of the trait values, whereas the other two QTLs alleles (ds3-15 and ds8-16) from

'Huangzao4' increased the trait score.

The QTL (ds1-10), mapped in the region of bin1.05-1.06, could explain 22.19% of the phenotypic variation, and demonstrated the highest additive effects with values of 0.02. Only one QTL (asi2-6) was noted for the PSIs of ASI and accounted for 7.88% of the total phenotypic variation and the primary effect was negative-additive, meaning that allele from 'HZ32' at asi2-6 operate in the direction of increasing the PSIs of ASI. For PSIs of LNB, four QTLs (lnb1-9, lnb1-15, lnb1-17 and lnb3-8) were mapped on chromosomes 1 and 3. They could explain 2.62 to 17.31% of the total phenotypic variation. Trait values at all detected QTLs were acquired from the allelic contributions of Huangzao4. The QTL (lnb1-9), mapped in the region of bin 1.05-1.06, could explain 17.31% of the phenotypic variation, and demonstrated the highest additive effects with values of 0.02. Six QTLs (ln1-5, ln1-9, ln8-6, ln8-16, ln9-4 and ln9-12) for PSIs of LN were revealed on chromosomes 1, 8 and 9. They could explain 0.49 to 21.25% of the total phenotypic variation. Trait values at all detected QTLs except for ln8-16 were increased from the allelic contributions of Huangzao4. The QTL (ln1-9), mapped in the region of bin 1.05-1.06, could explain 16.22% of the phenotypic variation, and demonstrated the second highest additive effects with values of 0.02.

QTL congruence

Taken together, only eleven QTLs (asi1-2w, ds2-15w, lnb2-16b, ds2-17b, ln4-10w, ln4-11w, asi5-7w, dps6-8, lnb10-6w, asi10-7w, and ln9-4) were not covered close to other QTLs (Figure 1). The remaining 70 QTLs were overlapped in 14 chromosome regions (Table 8). The most highly clustered QTL were found in the region of umc1397-umc1754 on chromosome 1, in the bin of 1.04-1.06, QTLs for DPSb, DPSw, DSb, LNBb, LNb, PSIs of DPS, DS, LNB and LN were detected. Other important QTL groups were distributed on all chromosomes except for chromosome 7, where QTL for more than two traits were detected (Figure 1, Table 8). The results indicated that these regions are under related genetic control and respond to the same environmental change (Figure 1).

QTL-by-environment interactions (QEs)

Significant epistatic loci ($P < 0.005$) for all target traits combining LD and SD conditions were detected by the software QTLmapper2.0 (Wang et al., 1999). For DPS, environmental interactions were detected for five main-effect QTLs (Table 7). In total, the QEs explained 1.92% of the phenotypic variation. Six DS, three ASI, four LNB, and three LN main-effect QTLs interacted in the environments. Each of them could explain 4.51, 14.19, 5.87 and 7.71% of the phenotypic variation, respectively.

DISCUSSION

Phenotypic variation, trait correlation and QTL groups

Because of the effects of photoperiod on flowering time and leaf number, many studies have used flowering time and leaf number as indicators to study photoperiod response in plants (Ellis et al., 1992b; Koester et al., 1993; Moutiq et al., 2002; Adams et al., 2003; Wang et al., 2008). To further elucidate the genetic characteristics of PS, this study investigated five PS related traits, one of them are firstly used, and computed the PSIs of each trait to estimate the effects of photoperiod on maize (Zhang et al., 1995; Moutiq et al., 2002; Guo, 2005). Our results demonstrated that the PSIs could directly exhibit differences of the traits of the parents and families under different environments, have advantages over the exhibit of effects of photoperiod and enable these effects to be identified the genetic control of PSIs. Except the two ungrouped QTLs for PSIs (dps6-8 and ln9-4), the other 20 QTLs for PSIs were clustered into 8 QTL groups, which represented 57% of all 14 QTL groups. Moreover, five of the eight QTL groups (1, 2, 7, 12 and 13) showed clustering for PSIs and other traits period. Three groups (3, 6 and 11) contained QTL for one PSIs and other traits simultaneously (Table 8).

In the present study, the LNB, firstly taken as the PS related traits to exploit genetic components of LNB and its PSIs, presented the considerable evidence that it was fit for the representative of photoperiod response due to the genetic analysis of phenotype (Table 1). In this research, the results of QTL mapping supported that LNB and its high correlation to PSIs was helpful in the discovery of more loci related to photoperiod response, which included 2 QTL groups (9 and 10), and two ungrouped QTLs (lnb2-16b and lnb10-6w). Out of the eight groups (Table 8), three groups (8, 9 and 10), belonged to the novel hot regions determined in the study. Related traits are often mapped to similar genome regions and phenotypic correlations can be caused by pleiotropy, linkage and environmental effects (Aastveit and Aastveit, 1993). In this study, significant correlations of the traits were detected between the two flowering time related traits, DPS and DS were highly correlated, and stronger correlations were also detected between the two leaf number related traits, LNB and LN. However, the correlation coefficients between the leaf number related traits and the flowering time related traits were much smaller.

The similar results were provided through the correlation analysis of PSIs. The coincidence of the genetic correlation to the similarity of morphological traits indicated that the data presented in this study was reliable. This was also explained by the results of QTL

Table 7. Environmental interactions of QTLs for PS related traits.

Traits	[a]Ch-Ini	Flanking marker	[a]Ch-Inj	Flanking marker	LOD	[b]AEi	[c]H∧2(AEi)	bAEj	[c]H∧2(AEj)	[b]AAEij	[c]H∧2(AAEij)
DPS	1-12	umc1035-bnlg1556	9-8	umc1357-bnlg1191	9.73			0.4953	0.63		
	1-15	phi039-bnlg1643	8-3	phi115umc1202	12.21	0.5073	0.66				
	3-8	Phi053-umc1219	5-7	bnlg1879-bnlg1208	12.49	-01934	0.1				
	3-9	umc1219umc1400	7-4	umc1016umc1339	13.34					0.2071	0.11
DS	1-10	umc1906-umc1754	2-5	umc1265-umc1261	7.54	0.5019	0.75	-0.216	0.14		
	1-13	bnlg1556-umc2387	9-8	umc1357-bnlg1191	12.38					0.7373	1.61
	1-14	umc2387-phi039	7-3	umc1016-umc1016	13.18	0.5936	1.04				
	3-3	bnlg1904-umc1012	8-15	bnlg1032-umc1384	12.06	0.5428	0.87				
	3-8	phi053-umc1219	7-14	umc2190-umc2222	9.78					0.1828	0.1
ASI	1-2	umc1292-bnlg1083	7-6	phi1043-umc1567	12.2					0.1082	0.67
	3-8	pho053-umc1219	4-5	bnlg1937-umc14.51				0.2983	5.08		
	3-11	bnlg1449-umc1644	10-1	umc1569-umc1196	8.58	-0.3318	6.28				
	5-16	bnlg386-umc2308	7-7	umc1567-bnlg1301	8.3					-0.1945	2.16
LNB	1-1	umc1353-umc1292	10-1	umc1569-umc1196	9.4	0.0708	0.34				
	1-8	bnlg1884-umc1124	5-15	bnlg115-bnlg386	20.76	0.241	3.97				
	2-9	bnlg1064-phi1083	8-6	umc1460-bnlg2046	27.98					0.1511	1.56
LN	1-8	bnlg1884-umc1124	8-3	phi115-umc1202	21.15	0.2376	2.51	0.0957	0.41	-0.0704	0.22
	1-10	umc1906-umc1754	4-9	bnlg1621-bnlg2162	15.67	0.0903	0.36				
	3-7	umc1772-phi1053	9-8	umc1357-bnlg1191	10.01			0.1374	0.84	-0.0619	0.17
	3-8	phi054-umc1219	9-10	bnlg1525-umc1714	8.09	0.1637	1.19				
	8-6	umc1460-bnlg2046	9-7	umc1107-umc1357	15-32			0.2127	2.01		

[a]Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis. [b]AEi, AEj and AAEij are effects of the environmental interaction of locus i, j and epistasis, respectively. [c]H^2(AEi), H^2(AEj) and H^2(AAEij) are the percentages of the phenotypic variations explained by AEi, AEj and AAEij, respectively.

mapping. Of the QTL groups, the group 1, 2, 3, 5, 6, 7, 12 and13 were related to DPS, DS or their PSIs, and the other six QTL groups, 1, 2, 8, 10, 11 and 14 were associated with LNB, LN or their PSIs. Furthermore, all the 14 QTL groups could be divided into three types: LD response groups (No. 1, 3, 6, 9, 10 and 13), SD responsgroups (No. 2, 4, 5, 7 and 8) and no response groups (No. 11 and 14). This results were not surprising given the fact that flowering time, leaf number were dictated mainly by the timing transition from vegetative to reproductive development, determined by photoperiod (Irish and Nelson, 1991). Except for QTL groups No. 7, 10, 14, the other eleven QTL groups were detected to be involved into GE interactions. These results indicated that mechanisms governing flowering time and related traits in maize differed substantially in different photoperiod environments. Among the 14 QTL groups, in the regions of eight groups, No. 1, 2, 5, 6, 11, 12, 13 and 14,

Table 8. QTL Groups of all QTLs for traits and their PSIs.

No group	Interval	Traits or PSIs of traits	Character	Additive parent	Max R2 (%)
1	bnlg1083-umc1754	DPSb,DPSw,DSb,LNBb,LNb,PSIs of DPS,DS, LNB and LN	LONG	huangzao4	30.71
2	bnlg1556-nlg1091	DPSw,DSw, LNBb,LNBw, LNb,PSIs of DPS,DS and LNB	SHORT	HZ32	21.54
3	phi96100-mc1261	DPSb, DSb, LNb, PSIs of ASI	LONG	HZ32	6.15
4	phi083-phi092	DPSw and LNw	SHORT	HZ32	10.89
5	bnlg1447-umc1399	DPSw and LNw	SHORT	HZ32	2.02
6	phi053-umc1399	DPSb, DSb,ASIb, PSIs of LNB	LONG	HZ32	10.34
7	umc1489-phi93225	DPSw, PSIs of DPS,DS	SHORT	huangzao4	12.45
8	umc1294-umc2027	DSw, DSb, LNBw, LNw, ASIw and ASIb	SHORT	huangzao4	19.48
9	bnlg1847-umc2216	LNBb and ASIb	LONG	huangzao4/HZ32	4.66/5.9
10	nc009-phi078	LNBb and LNb	LONG	huangzao4	13.3
11	phi115-umc1959	LNBb, LNb, LNBw, PSIs of LN	L/S	huangzao4	16.71
12	bnlg1030-phi080	LNw, PSIs of DPS, DS and LN	SHORT	huangzao4	7.42
13	umc1357-umc1714	DPSb, DSb, PSIs of DPS, DS and LN	LONG	huangzao4	17.43
14	bnlg 1655-mmc0501	ASIb, LNBb and LNw	L/S	huangzao4	8.67

Note: groups 3, 4, 7, 8, 9, 10 are novel hot regions; groups 1, 2, 3, 6, 7, 11, 12 and13 PSIs involve.

QTLs for some similarly traits were reported by Koester et al. (1993), Moutiq et al. (2002) and Wang et al. (2008); however, for the other six QTL clustered regions, no QTL associated with photoperiod response were reported. The major QTL controlling DPSb (R^2= 16.77%), DSb (R^2 =12.9%), LNBb (R^2= 30.71%), LNb (R^2 =26.19%) and PSIs of DPS (R^2 =22.6%), DS (R^2= 22.2%), LNB (R^2= 17.31%) and LN (R^2= 21.25%) were mapped in a similar position, and these traits are highly related (Tables 4, 5 and 6). LN could be used as the most stable and representative indicator of photoperiod sensitivity in maize for two reasons. The first reason is that stronger correlations were detected (r=0.58 between LNw and LNb, and r=0.86 between PSIs of LN and PSIs of LNB). The second reason is that the trait of LN was involved into the large number of the major QTLs (R^2 is more than 10%), QTLs and QTL clusters.

The major QTL cluster on chromosome 1

In the present study, the largest QTL, associated with LNB and LN in Beijing (LD environment), was detected in the 1.05-1.06 bin region between the markers bnlg1884 and umc1754. Furthermore, QTLs for DS in Beijing (LD environment), and DPS in both environment, and PSIs of DPS, DS, LNB and LN were also mapped to the same region on chromosome 1. In this region, Wang et al. (2008) also reported a QTL for leaf number and plant height in the LD environment. Koester et al. (1993) detected QTLs for DPS, PH and LN. These results suggested that this region might harbor some important photoperiod pathway components. A synteny conservation approach based on comparative mapping between a maize genetic map and japonica rice physical map showed *OsSOC1* associated with QTL for flowering time in bin 1.05 of maize chromosome 1 (Chardon et al., 2004). OsMADS50 (OsSOC1) shares 50.6% of amino acid identity with the *Arabidopsis* MADS-box gene SUPPRESSOR OF OVEREXPRESSION OF CO 1/AGAMOUS-LIKE20 (SOC1/AGL20) (Lee et al., 2004). In *Arabidopsis*, *SOC1* is the floral integrator gene induced by *CO,* which promotes flowering (Onouchi et al., 2000; Samach et al., 2000; Yamaguchi et al., 2005). *OsSOC1* is an upstream regulator of OsMADS1, OsMADS14, OsMADS15, OsMADS18, and HD (Heading data) *3a,* but worked either parallel with or downstream of *Hd1* and *O. sativa* GIGANTEA (OsGI) (Lee et al., 2004). Recent study showed OsMADS50 (OsSOC1) functioned as a LD-specific flowering activator and proposed that OsMADS50 (OsSOC1) regulated Ehd1 via OsLFL1 (Ryu et al., 2009). This coincidence in map position suggested that the maize ortholog to OsSOC1 might be a candidate gene of a QTL detected

here in the bin 1.05 region on chromosome 1. However, finer mapping and a gene-specific marker are required to determine that this QTL is in fact orthologous to OsSOC1.

Another important QTL associated with photoperiod sensitivity related traits and their PSIs were detected on chromosome 9

In this study, the major QTL for PSIs of DPS, dps9-9 (R^2=17.43%), located in the bin of 9.05 to 9.07 on the chromosome 9, was detected. Moreover, QTL for DS and DPS under LD photoperiod environments and QTL for PSIs of LN and DS were also detected in this region; so, we presume that there is a specific photoperiod response gene in this region. Various authors also found QTLs for DPS (Ribaut, 1996; Bohn et al., 1997; Kozumplik et al., 1996), heat units to pollen (Veldboom et al., 1994, Veldboom and Lee, 1996), DS and ASI (Szalma et al, 2007), and DPS thermal time, LN and PS (Wang et al., 2008) in the bin 9.05 region. Phytochrome B2 (Phy B2) was located in the region of 9.05 to 9.06 on maize chromosome 9 (Sheehan et al., 2004). Phytochrome B2 is one of the primary photoreceptors mediating photoperiod-dependent floral transition and was necessary to repress flowering under long day photoperiods (Sheehan et al., 2007). However, fine mapping and a genespecific marker are needed to prove if this QTL actually is PhyB2.

Maize PS maker-assisted selection breeding

Photoperiod sensitivity limits the evaluation and exchange of germplasm between temperate and tropical breeding programmes. For example, some tropical varieties are unable to be cultivated in temperate areas due to be difficulty in harvesting seeds because of adverse photoperiod response (Goodman, 1985; Giauffret et al., 2000; Gouesnard et al., 2002; Wang et al., 2008). Therefore, maize genetic diversity has been limited (Collard and Mackill, 2008). In general, the temperate varieties were crossed with tropical varieties to decrease photoperiod sensitivity of the later firstly in the SD environment. Within the lines, early flowering plants would be selected as candidate materials. Although many QTLs were expressed in LD environment, to increase the leaf numbers and delay transition to the floral meristem in the present study. Marker-assisted selection (MAS) has been superior to conventional selection when alleles are not expressed in the selection environments (Holland et al., 2004). Furthermore, our study indicates a favored photoperiod controlling allele comes from the same parent in the same QTL for many traits in this study (Table 8); therefore these results can be utilized for MAS in maize breeding. In the future, NILs (near isogenic lines) will be constructed for major QTL fine mapping.

Meanwhile, the markers which developed from the candidate genes located on major QTL regions will be useful for the major QTL fine mapping.

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