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ORIGINAL PAPER

# Genetic analysis of photoperiod sensitivity in a tropical by temperate maize recombinant inbred population using molecular markers

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**Abstract** Photoperiod sensitivity is an important consideration in maize cultivation. Flowering time is affected by photoperiod and sensitivity to it limits the potential for successful exchange of germplasm across different latitudes. For resolving the genetic basis of photoperiod sensitivity in maize, a set of 207 recombinant inbred lines derived from a temperate and tropical inbred line cross was evaluated for 2 years in a long-day and short-day environment. Genetic linkage maps were constructed using 237 SSR markers with a total length 1,974.3 cM, and an average space between two makers of 8.33 cM. Twenty-nine QTL were detected for the five measured photoperiod sensitivity traits using composite interval mapping and multiple interval mapping. QTL for flowering time, plant height and leaf number, under long-day conditions, were found clustered on chromosome 10, while QTL for short-day conditions resided on chromosome 3. The QTL in the bin 10.04 region of chromosome 10 were detected associated with photoperiod sensitivity and related traits during long days. These results indicated that this region might contain an important photoperiod sensitivity element.

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## Introduction

Commercial maize hybrids are largely produced from crosses between elite inbred lines. Over time, this breeding program has resulted in a gradually narrowed maize germplasm, particularly in temperature growing regions (Peng et al. 1993; Troyer 1999). The limited genetic variability for temperate maize has not only hindered maize breeding development but also increased the cultivar's susceptibility to disease and pests, among other risks (Tallury and Goodman 1999; Tarter et al. 2004). Many studies propose tropical maize germplasm as a viable source of favorable alleles to increase the genetic diversity available for temperate maize breeding programs (Uhr and Goodman 1995; Holland and Goodman 1995; Tarter et al. 2004). However, in temperate zones, most tropical germplasm sources generate taller plants with an increased number of leaves and delayed flowering. Furthermore, due to photoperiod sensitivity (PS), some tropical varieties do not flower under temperate environmental regimes (Goodman 1985; Giauffret et al. 2000; Gouesnard et al. 2002). Therefore, it is vital for maize breeders to understand the genetic basis of PS in their efforts to integrate tropical germplasm into temperate zone maize breeding.

It is generally considered that maize is sensitive to photoperiod for the last 4–8 days prior to tassel initiation, or very shortly thereafter, and a long-day photoperiod may delay tassel initiation (Kiniry et al. 1983; Ellis et al. 1992; Bonhomme et al. 1994; Ren et al. 2006). Tassel initiation is defined as the timing transition from vegetative to

reproductive stages accomplished by the shoot apical meristem. This process is comprised of two complex progressions, beginning with the end of leaf primordia initiation leading to the onset of flower bud differentiation (Ellis et al. 1992). Previous studies proposed that the photoperiod critical threshold is 12–13 h. Beyond that time period, the thermal time necessary for the photoperiod-sensitive maize germplasm to flower increases linearly as day length increases, accompanied by plant height and leaf number increase (Kiniry et al. 1983; Ellis et al. 1992; Bonhomme et al. 1990, 1994; Birch et al. 1998). Gouesnard et al. (2002) regressed thermal time from sowing to 50% anthesis against photoperiod as the criteria to define maize PS. Results from classical genetic research and breeding programs demonstrated that PS is quantitative, and to a large extent, controlled by additive genes (Russel and Stuber 1983; Giauffret et al. 2000; Ellis et al. 1992; Moutiq et al. 2002; Chen et al. 2003). The development of high-density molecular marker linkage maps and QTL detection approaches have made it possible to map quantitative trait loci associated with important agronomic trait variation. A large body of QTL data for flowering time, plant height and leaf number, associated with different environmental parameters are presently available (Stuber et al. 1992; Koester et al. 1993; Ribaut et al. 1996; Austin et al. 2001; Veldboom et al. 1994; Veldboom and Lee 1996; Vladutu et al. 1999; Bohn et al. 1997; Salvi et al. 2002; Chardon et al. 2005; Balint-Kurti et al. 2007; Szalma et al. 2007). Chardon et al. (2004) employed a metadata-analysis methodology on 312 publicly available QTL for flowering time and generated a synthetic genetic model with 62 consensus QTL, and determined that hot-spot loci were located on chromosomes 1, 8, 9 and 10. However, few QTL resolved in previous studies directly addressed PS. On the other hand, QTL research associated with PS in other cereal crops has been conducted and genes controlling the trait have been identified in rice, barley and wheat (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Worland et al. 1998; Izawa et al. 2003; Hanocq et al. 2004; Turner et al. 2005). To the best of our knowledge, reports of photosensitivity genes in maize are lacking with the exception of Koester et al. (1993) and Moutiq et al. (2002). To establish the genetic basis of maize photoperiods, Koester et al. (1993) identified flowering time, plant height and leaf number QTL under different photoperiod environments and speculated that maturity QTL on chromosome 8 may represent a photoperiod response element. Moutiq et al. (2002) compared flowering time QTL in different photoperiod environments, and suggested that QTL on chromosomes 8 and 10 had the greatest additive effects during long days, while those on chromosomes 3 and 9 had increased additive effects during short days. Despite these photosensitivity studies, a direct QTL index to evaluate PS in maize is yet to be reported.

In this paper, we present a novel genetic investigation of photoperiod response in maize based on a recombinant inbred line (RIL) population and molecular markers. The objective of this study was to (1) identify the QTL traits related to PS, including flowering time, leaf number and plant height under long-day and short-day conditions, (2) analysis of QTL differences detected in the two photoperiod environments and (3) characterize PS QTL.

## Materials and methods

### Plant material and field trials

The study population consisted of 207 $F_8$ RILs, derived from a cross between two inbred lines, Huangzao4 and CML288, using a single-seed descent method under short-day conditions (Sanya, China, 18°45′N, 109°30′E). The parent, Huangzao4, is a temperate photoperiod insensitive inbred line derived from a local Chinese germplasm, Tangsipingtou, a heterotic group used broadly in China. CML288 is a tropical photoperiod sensitive flint inbred line introduced from CIMMYT.

### Field evaluation

Evaluation of the 207 RILs, two parents and F1 were conducted in the field under a long-day environment at Zhengzhou (34°43′N, 113°43′E) and short-day environment at Sanya (18°45′N, 109°30′E) during 2005 and 2006. The field experiment was designed according to a complete randomized block design with three replications at each location. Each RIL was planted in one row, 0.67 m apart and 4 m long with a total of 15 plants per row; the density was 45,000 plants/ha. Field management was in accordance with local practices.

Maize is sensitive to photoperiod at the stage of tassel initiation (Kiniry et al. 1983). Considering the slow rate of change of photoperiod, the photoperiod during tassel initiation was assumed as the photoperiod of sensitive stage in each environment. However, tassel initiation could not be determined directly; in this study, it was estimated as half the average thermal time necessary from sowing to silking according to the method of Bonhomme et al. (1994) and Gouesnard et al. (2002). The average photoperiod of sensitive stage is 14.25 h at Zhengzhou and 12.67 h at Sanya.

### Data collection

Traits were measured from ten consecutive plants beginning with the third plant of each row. Days to pollen shed (DPS) were recorded as the number of days from sowing

to the first pollen shed from anthers on the central spike. The arithmetic mean values for DPS were subsequently transformed into thermal time, and used to detect flowering time QTL in different day-length environments. Thermal time (TT) was calculated as follows:

$$\mathrm{TT} = \sum_{1}^{n} [(\mathrm{TX} + \mathrm{TN})/2 - \mathrm{Tb}]$$

where TT is the thermal time accumulated over $n$ days, TX is the maximum daily temperature, TN is the minimum daily temperature and Tb is the base temperature (Bonhomme et al. 1994; Gouesnard et al. 2002). PS was calculated as follows: $\mathrm{PS} = (\mathrm{TT_{LD}} - \mathrm{TT_{SD}})/(D_{\mathrm{LD}} - D_{\mathrm{SD}})$, where $\mathrm{TT_{LD}}$ and $\mathrm{TT_{SD}}$ are the thermal times of each RIL from sowing to DPS in long days and short days, respectively. $D_{\mathrm{LD}}$ and $D_{\mathrm{SD}}$ are the average day length of PS stage in long-day environment and short-day environment, respectively (PS/h; Gouesnard et al. 2002). For both locations, Zhengzhou and Sanya, Tb was set at 10°C. Plant height was measured from the ground to tassel top. The fifth, tenth and fifteenth leaf were marked when the first, fifth and tenth leaf were still visible, respectively, and the 15th leaf marked was clearly visible when the total number of leaf was counted. The data obtained from ten consecutive plants were averaged to obtain trait values for each plot, and three replications were averaged to obtain trait values for each line in each experiment. The arithmetic mean values of each line for the two experimental years at each location were averaged to obtain RIL trait values for each photoperiod environment. The mean PS over 2 years was used to detect main-effect QTL associated with PS.

Broad-sense heritability ($h^2$) for each trait was computed according to Knapp et al. (1985). The heritability was calculated as follows: $h^2 = {\sigma_g}^2/(\sigma_g^2 + \sigma_{gy}^2/n + \sigma_e^2/nr)$, where $\sigma_g^2$ is the genetic variance, $\sigma_{gy}^2$ is genotype-by-year interaction, $\sigma_e^2$ is the error variance, $r$ is the replication number and $n$ is the number of years. The estimates of $\sigma_g^2$, $\sigma_{gy}^2$ and $\sigma_e^2$ were obtained by analysis of variance (ANOVA) using the general linear model procedure of the statistical software SPSS 12.0. Simple Pearson correlation coefficients ($r$) were calculated between the traits using the adjusted means of the RIL families.

### Molecular linkage construction and QTL mapping

In accordance with bin location, a total of 713 SSR markers were chosen from the maize genome database to detect parental polymorphisms, according to the protocol available at http://www.maizegdb.org/documentation/maizemap/ssr_protocols.php, with minor modifications. The codominant segregation SSR markers were used to genotype the RIL population. The genetic linkage map was constructed with Mapmaker/Exp 3.0 at the LOD threshold > 3.0 (Lander et al. 1987).

Composite-interval mapping method of Windows QTL cartographer version 2.5 software (Wang et al. 2007) was performed initially to map QTL for all measured traits. Model 6 of the Zmapqtl module was employed, scanning intervals of 2 cM between markers and putative QTL with a window of 10 cM. Ten control markers were identified using forward and backward regression. The appropriate LOD threshold value to identify QTL at a 5% significance level was determined by 1,000 random permutations. QTL positions from CIM were used to supply the initial models for multiple-interval mapping (MIM). The MIM models were created and tested in an iterative fashion and the BIC were used for model selection. After identifying the best model, QTL effects were estimated using the summary option (Balint-Kurti et al. 2006). On a given chromosome, QTL for different traits were declared as colocated QTL when their one-LOD support interval overlapped.

## Results

### Phenotypic measurement of photoperiod sensitivity, and flowering time, leaf number and plant height in maize

Huangzao4 and CML288, under both environmental conditions, were significantly different for the three related traits ($P < 0.01$). Flowering time, leaf number and plant height for both Huangzao4 and CML288 were significantly increased during long-day compared with short-day conditions (Table 1). The difference in thermal time, leaf number and plant height between the two parents was 114.95°C, 2.43 and 36.86 cm, respectively, in the short-day environment, and 520.75°C, 8.96 and 74.20 cm in the long-day environment. Thermal time, leaf number and plant height of CML288 increased by 59.28, 47.80 and 49.31%, respectively, under long-day compared with short-day conditions, while Huangzao4 increased by only 21.03, 17.98 and 34.35%, respectively. In addition, Huangzao4 and CML288 were significantly different for PS ($P < 0.01$). CML288 did not silk in long-day conditions over the study period. PS values of CML288 were approximately three times that observed in Huangzao4, which indicated that CML288 exhibited increased PS.

A similar trend in PS and flowering time, leaf number and plant height, in response to day length, was observed in the RIL population compared with the parents. The results showed a clear, continuous normal distribution pattern as expected for quantitative traits (Table 1, Fig. 1). The RIL

**Table 1** Phenotypic evaluation of the two parents and the RIL population in two photoperiod environments

| | Trait | | | | | | |
|---|---|---|---|---|---|---|---|
| | TT (degrees) | | LN | | PH (cm) | | PS (degrees/h) |
| | Zhengzhou | Sanya | Zhengzhou | Sanya | Zhengzhou | Sanya | |
| Huangzao4 ($P_1$) (mean) | 1,068.43 | 882.80 | 21.23 | 17.99 | 172.15 | 128.14 | 116.02 |
| CML288 ($P_2$) (mean) | 1,589.18 | 997.75 | 30.18 | 20.42 | 246.35 | 164.99 | 369.64 |
| $P_1$ versus $P_2$[a] | ** | ** | ** | ** | ** | ** | ** |
| $F_1$ (mean) | 1,190.92 | 910.43 | 25.84 | 19.05 | 289.48 | 180.34 | 175.36 |
| RIL | | | | | | | |
| Mean ± SD | 1,309.23 ± 118.34 | 932.30 ± 45.23 | 25.90 ± 1.43 | 19.62 ± 0.68 | 223.04 ± 26.43 | 154.60 ± 18.51 | 235.52 ± 68.40 |
| Range | 974.20–1,690.20 | 750.80–1,197.20 | 20.00–34.90 | 16.00–23.38 | 159.50–315.60 | 110.80–202.40 | 96.44–517.13 |
| Skewness | 0.25 | −0.14 | 0.29 | −0.13 | 0.40 | 0.15 | 0.44 |
| Kurtosis | −0.20 | 0.06 | −0.21 | −0.13 | 0.20 | 0.23 | −0.34 |
| $\sigma_g^2$ | 12,773.31 | 1,405.16 | 5.68 | 1.10 | 555.65 | 145.62 | 2,442.74 |
| $\sigma_{gy}^2$ | 2,255.71 | 800.78 | 0.88 | 0.40 | 314.65 | 76.13 | 853.83 |
| $\sigma_e^2$ | 193.86 | 182.77 | 1.00 | 0.32 | 73.94 | 76.05 | 140.06 |
| $h_B^2$ | 91.67 | 76.53 | 90.37 | 81.35 | 76.61 | 74.16 | 84.44 |
| Confidence interval | 89.52–93.38 | 70.47–81.35 | 87.88–92.35 | 76.53–85.18 | 70.56–81.41 | 67.49–79.46 | 80.42–87.63 |

*TT* flowering time (estimated as the sum of effective temperature from sowing to days to pollen shed), *LN* the total leaf number, *PH* plant height, *PS* photoperiod sensitivity (estimated as $PS = (TT_{LD} - TT_{SD})/(D_{LD} - D_{SD})$), $\sigma_g^2$ genotypic variance of measured traits, $\sigma_{gy}^2$ genotype and environment interaction variance of measured traits, $\sigma_e^2$ residual error variance of measured traits, $h_B^2$ the broad-sense heritability of measured traits, *confidence interval* the confidence intervals of broad-sense heritability between 5 and 95% significance levels

[a] Statistical test for difference between two parents at 0.05 (*) and 0.01 (**) levels of probability

families exhibited a wide range of variability for the traits measured, and the thermal time, leaf number and plant height means in two photoperiod environments differed substantially. Transgressive segregation in both directions was observed for all measured traits under the two photoperiod environments.

Broad-sense heritability differed for the two photoperiod conditions (Table 1). Plant height had similar broad-sense heritability, 76.61 and 74.14% in both photoperiods, while flowering time and leaf number had relatively high heritability, 91.67 and 90.37%, under long-day conditions and lower heritability, 76.53 and 81.35%, under short-day conditions. Broad-sense heritability for PS was 84.4%.

Thermal time, leaf number and plant height showed a significant positive correlation to each other in both photoperiod conditions (Table 2). However, correlation coefficients for all traits measured were higher in long-day than short-day environments. PS, and flowering time, leaf number and plant height under a long-day environment revealed a significant positive correlation, while no significant correlation was found for PS and the three traits in short-day environment. Each pair of traits between the two photoperiod environments also showed significantly positive correlations.

## Genetic linkage map construction

A total of 713 SSR markers were used to screen polymorphisms between the two parental inbred lines. Two hundred and seventy-nine distinct codominant markers were employed to construct a genetic linkage map. Two hundred and thirty-seven informative markers were assigned to 10 chromosomes using Mapmaker 3.0 at LOD > 3.0. The linkage map had a total length of 1,974.3 cM with an average interval of 8.33 cM between adjacent makers (Fig. 2).

## QTL detection for flowering time, leaf number and plant height

QTL for PS and its related traits were mapped to all maize chromosomes but chromosome 5 (Table 3, Fig. 2). Five putative QTL were found associated with PS and 23 QTL with its related traits.

Seven QTL were identified for DPS thermal time. Only one QTL (*qDPS9*) was detected under both photoperiod conditions and the other six QTL were detected under each environment, respectively. QTL for DPS thermal time under long-day conditions were identified on chromosomes

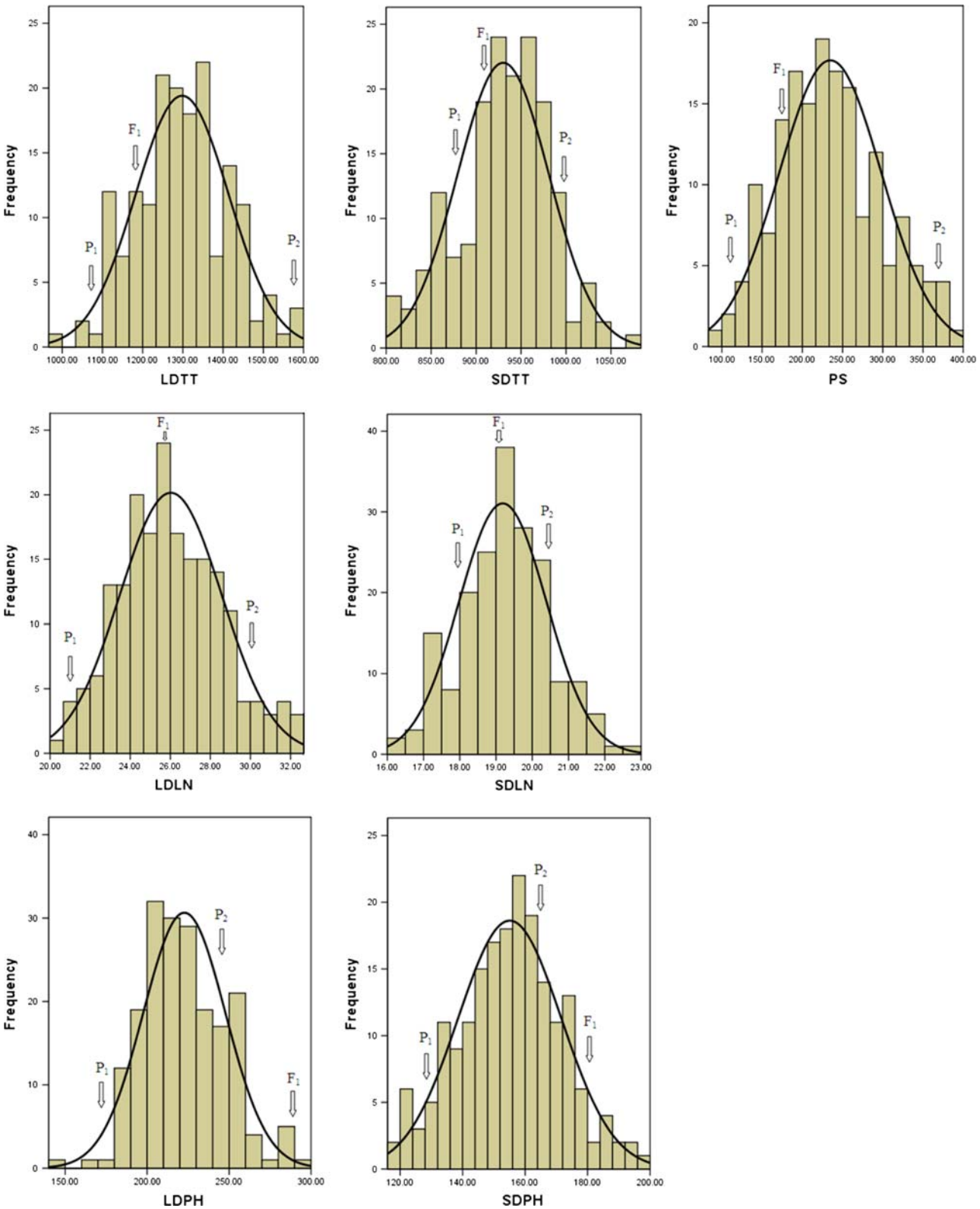


**Fig. 1** Frequency distribution for photoperiod sensitivity and related traits in the 207 $F_8$ recombinant inbred lines (RILs) derived from the cross Huangzao 4 × CML288. *LDTT, SDTT* flowering time in long-day and short-day environments, respectively (estimated as the sum of effective temperature from sowing to days to pollen shed); *LDLN, SDLN* leaf number in long-day and short-day environments, respectively; *LDPH, SDPH* plant height in long-day and short-day environments, respectively; *PS* photoperiod sensitivity (estimated as $PS = (TT_{LD} - TT_{SD})/(D_{LD} - D_{SD})$)

**Table 2** Correlations between photoperiod sensitivity, and flowering time, leaf number and plant height in the RIL population under two environmental regimes

| Traits | TT | LN | PH | PS |
|---|---|---|---|---|
| TT | 0.3162** | 0.8512** | 0.5232** | 0.8975** |
| LN | 0.4354** | 0.3630** | 0.6631** | 0.7967** |
| PH | 0.3068** | 0.5758** | 0.5106** | 0.5452** |
| PS | −0.1189 | −0.0524 | −0.0945 | |

The values above the diagonal are the phenotypic correlation in the long-day environment, and the values below the diagonal are the phenotypic correlation in the short-day environment. Values along the diagonal are the phenotypic correlations between long-day and short-day environments

*TT* flowering time (estimated as the sum of effective temperature from sowing to days to pollen shed), *LN* the total leaf number, *PH* plant height, *PS* photoperiod sensitivity (estimated as $PS = (TT_{LD} - TT_{SD})/(D_{LD} - D_{SD})$)

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

4, 9 and 10, accounting for a phenotypic variance range from 3.4 to 37.3% (Table 3, Fig. 2). The QTL, *qDPS10* located on chromosome 10.04 between markers umc1873 and umc1053, demonstrated the highest additive effects with values of 72.3°C and accounted for 37.3% of the phenotypic variance for DPS thermal time in the long-day environment. Four putative QTL for DPS thermal time in the short-day environment were identified on chromosomes 3, 6, 7 and 9, accounting for 6.8–15.5% of the phenotypic variance. Trait values at all detected QTL were increased from the allelic contributions of CML288.

Seven putative QTL for leaf number were mapped on chromosomes 1, 3, 4, 7, 9 and 10. Out of these seven QTL, three QTL (*qLN4, qLN7* and *qLN9*) were detected in both environments. *qLN1-1* and *qLN10* were only detected in long-day conditions, while *qLN1-2*and *qLN3* were only detected in short-day conditions. For two of the QTL (*qLN1-1* and *qLN7*), alleles from Huangzao 4 tend to increase the trait value. Alleles from CML288 increased trait values at other five QTL. The QTL, *qLN10*, mapped in the region of bin 10.04, demonstrated the highest additive effects with values of 1.72, and explained 38.5% of the phenotypic variation under long-day conditions. The QTL, *qLN3* accounted for 10.5% of the phenotypic variance in the short-day environment.

A total of nine putative QTL were found associated with plant height on chromosomes 1, 2, 3, 4, 7, 8 and 10. Out of these nine QTL, four QTL (*qPH1-2, qPH3, qPH4* and *qPH10*) were detected in both environments and others were only detected in one photoperiod environment. The alleles derived from CML288 contributed towards an increase in the trait values for six QTL (*qPH2*, *qPH3*, *qPH4*, *qPH7-1*, *qPH8* and *qPH10*), and alleles from Huangzao4 generally increased the trait values for three additional QTL. Two QTL, *qPH4* and *qPH10*, showed additive effects of 8.37 cm and 11.18 cm, and accounted for 12.6 and 20.1% of the phenotypic variance for plant height under long-day conditions. The QTL, *qPH3*, had the highest additive effect measured at 9.32 cm. This locus explained 28.9% of the phenotypic variance in the short-day environment.

### QTL detection for photoperiod sensitivity

For PS, a total of five QTL were identified on chromosomes 3, 4, 7, 9 and 10. For two of the detected QTL, *qPS3* and *qPS7*, alleles from Huangzao4 contributed an increase in trait values. Alleles from CML288 tended to increase the trait values for the remaining three QTL. Of these, QTL *qPS3* had a similar position to *qDPS3*, *qLN3* and *qPH3*, all of which were significantly associated with DPS thermal time, leaf number and plant height under short-day conditions. The QTL *qPS10* shared a similar position with DPS thermal time, leaf number and plant height QTL in the long-day environment (Table 3, Fig. 2).

## Discussion

### Phenotypic variation

Because of the effects of photoperiod on flowering time, many researchers have used flowering time as an indicator to indirectly study photoperiod response in cereal crops (Koester et al. 1993; Moutiq et al. 2002; Yano et al. 2000; Laurie et al. 1995, 1997). To further elucidate the characteristics of PS, this study followed the definition of Gouesnard et al. (2002) as a criterion to estimate the effects of photoperiod on maize. Our results demonstrated that lines that flowered later in the short-day environment had increased PS, and lines that flowered earlier had decreased PS. This result was expected considering a significant positive correlation between flowering time and PS was revealed. However, three earlier lines with increased sensitivity and eight later lines with low sensitivity were observed, congruent with previous reports (Francis et al. 1969; Russel and Stuber 1983). These results indicated that the two components of flowering time, base maturity and PS were possibly governed by different genetic mechanisms.

### Trait correlation and QTL cluster

Related traits are often mapped to similar genome regions and phenotypic correlations can be attributed to three causes: pleiotropy, linkage and environment (Aastveit et al. 1993). In the present study, a strong phenotypic correlation

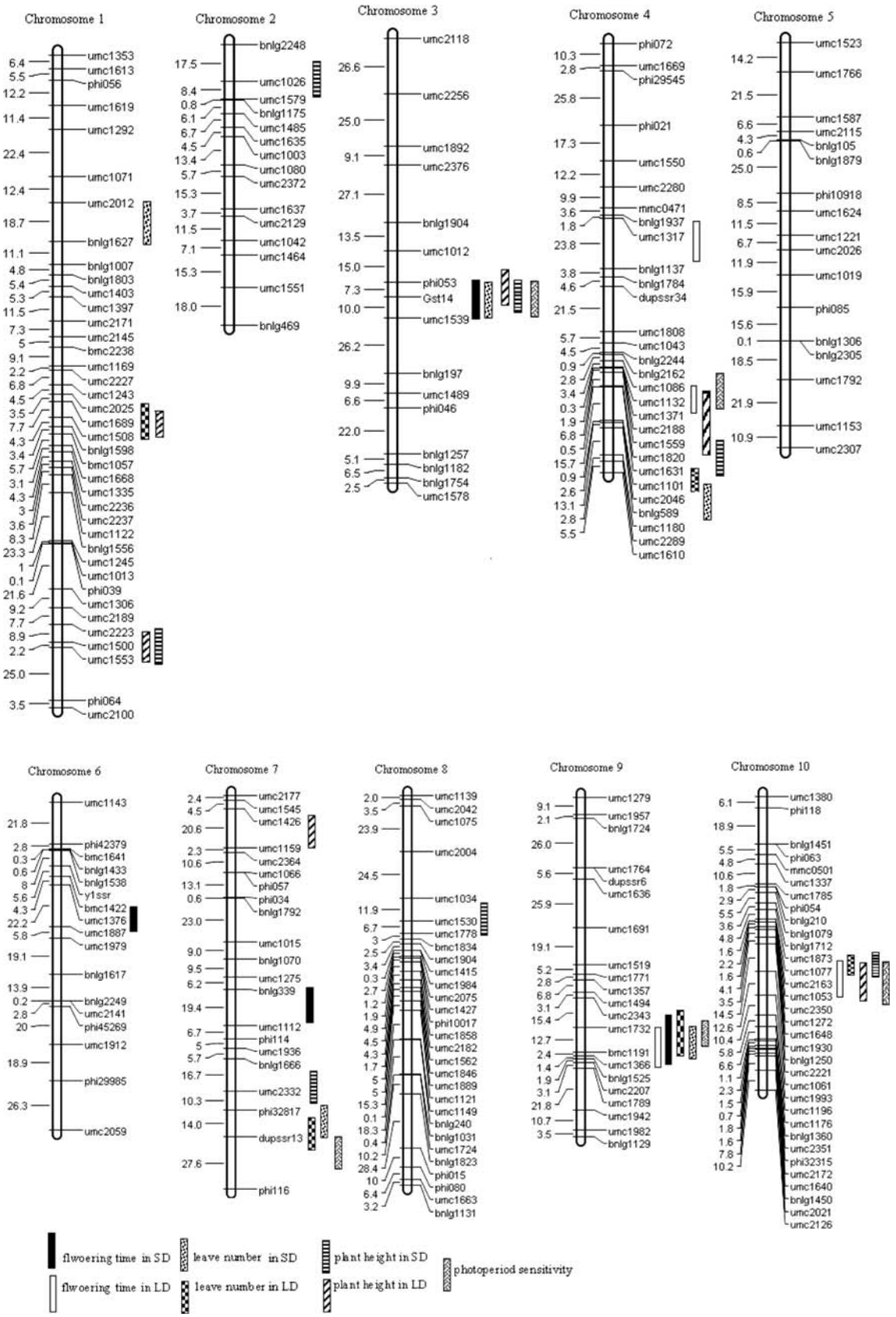


**Fig. 2** The QTL detected for photoperiod sensitivity, and flowering time, leaf number and plant height in the two environments

**Table 3** QTL detected for photoperiod sensitivity, and flowering time, leaf number and plant height in long-day and short-day environments

| Trait | Chromosome | QTL | Closest marker | Position (cM) | Support interval (cM) | LOD | Effect[a] | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| LDTT | 4 | *qDPS4-1* | bnlg1937 | 82.9 | 80.3–86.7 | 3.50 | 19.86 | 3.4 |
| | 4 | *qDPS4-2* | umc1086 | 152.3 | 149.5–153.3 | 4.10 | 29.30 | 6.7 |
| | 9 | *qDP9* | umc1732 | 130.1 | 123.1–134.8 | 5.33 | 35.02 | 10.0 |
| | 10 | *qDPS10* | umc1873 | 62.7 | 60.7–66.5 | 20.38 | 72.30 | 37.3 |
| SDTT | 3 | *qDPS3* | phi053 | 128.3 | 123.3–131.6 | 4.28 | 18.05 | 15.5 |
| | 6 | *qDPS6* | umc1376 | 47.4 | 36.5–55.4 | 2.95 | 14.02 | 7.6 |
| | 7 | *qDPS7* | bnlg339 | 104.8 | 98.6–116.8 | 3.31 | 14.34 | 8.3 |
| | 9 | *qDPS9* | umc1732 | 126.1 | 108.7–134.8 | 2.91 | 13.69 | 6.8 |
| LDLN | 1 | *qLN1-1* | umc1689 | 165.5 | 160.0–168.5 | 4.64 | −0.44 | 4 |
| | 4 | *qLN4* | umc1631 | 174.2 | 171.2–178.2 | 3.42 | 0.66 | 6.4 |
| | 7 | *qLN7* | dupssr13 | 196.6 | 185.6–201.6 | 9.69 | −0.89 | 8.7 |
| | 9 | *qLN9* | umc2343 | 108.7 | 104.6–130.6 | 10.22 | 0.66 | 7.4 |
| | 10 | *qLN10* | umc1873 | 61.7 | 60.7–64.5 | 36.06 | 1.72 | 38.5 |
| SDLN | 1 | *qLN1-2* | umc2012 | 91.3 | 72.3–91.0 | 2.40 | 0.24 | 7.1 |
| | 3 | *qLN3* | phi053 | 128.3 | 124.3–130.6 | 12.57 | 0.59 | 10.5 |
| | 4 | *qLN4* | umc1631 | 175.2 | 169.2–181.8 | 4.28 | 0.37 | 9.0 |
| | 7 | *qLN7* | dupssr13 | 159.7 | 157.6–169.6 | 2.26 | −0.24 | 8.3 |
| | 9 | *qLN9* | umc1732 | 128.1 | 126.1–134.8 | 4.29 | 0.42 | 7.3 |
| LDPH | 1 | *qPH1-1* | umc1689 | 163.5 | 161.0–165.5 | 7.57 | −8.44 | 9.3 |
| | 1 | *qPH1-2* | umc2223 | 273.8 | 271.8–280.9 | 3.83 | −6.67 | 6.9 |
| | 3 | *qPH3* | phi053 | 126.3 | 109.3–135.9 | 4.60 | 6.29 | 3.9 |
| | 4 | *qPH4* | umc1559 | 152.3 | 148.5–160.9 | 8.19 | 8.37 | 12.6 |
| | 7 | *qPH7-1* | umc1426 | 10.9 | 4.4–21.9 | 3.81 | 5.57 | 3.9 |
| | 10 | *qPH10* | umc1077 | 65.5 | 61.7–67.1 | 16.25 | 11.18 | 20.1 |
| SDPH | 1 | *qPH1-2* | umc1500 | 278.8 | 274.8–280.9 | 7.43 | −5.3 | 8.9 |
| | 2 | *qPH2* | bnlg2248 | 17 | 8.01–22.5 | 4.82 | 4.31 | 4.7 |
| | 3 | *qPH3* | Gst14 | 131.6 | 127.3–133.6 | 18.04 | 9.32 | 28.9 |
| | 4 | *qPH4* | umc1559 | 158.9 | 156.9–175.2 | 5.01 | 4.32 | 6.8 |
| | 7 | *qPH7-2* | umc2332 | 151.3 | 141.6–155.6 | 4.93 | 4.12 | 3.6 |
| | 8 | *qPH8* | umc1530 | 71.8 | 64.9–73.5 | 2.93 | −3.18 | 2.7 |
| | 10 | *qPH10* | umc1873 | 61.7 | 58.1–64.5 | 3.02 | 3.22 | 2.4 |
| PS | 3 | *qPS3* | Gst14 | 131.6 | 121.3–142.6 | 3.54 | −9.47 | 3.4 |
| | 4 | *qPS4* | umc1086 | 153.3 | 150.5–154.7 | 4.25 | 16.39 | 6.0 |
| | 7 | *qPS7* | dupssr13 | 206.6 | 194.6–207.2 | 5.33 | −15.69 | 2.0 |
| | 9 | *qPS9* | umc2343 | 121.1 | 111.7–130.1 | 5.07 | 18.42 | 6.9 |
| | 10 | *qPS10* | umc1873 | 61.7 | 59.7–66.5 | 29.97 | 40.84 | 32.8 |

*LDTT, SDTT* flowering time in long-day and short-day environments, respectively (estimated as the sum of effective temperature from sowing to days to pollen shed); *LDLN, SDLN* leaf number in long-day and short-day environments, respectively; *LDPH, SDPH* plant height in long-day and short-day environments, respectively; *PS* photoperiod sensitivity (estimated as $PS = (TT_{LD} - TT_{SD})/(D_{LD} - D_{SD})$); $LOD_{0.05}$ logarithm of odds at $P < 0.05$ significance level; $R^2$ contribution rate

[a] Additive effect: positive values indicated that CML288 carries the allele for an increase in the traits, while negative values indicate that Huangzao4 contributed the allele for an increase in the trait value

among traits was observed in both photoperiods. The major QTL controlling flowering time, leaf number and plant height were mapped in a similar position, consistent with observations of related traits. However, we observed that different sets of QTL were detected in different photoperiod environments. For example, the QTL for DPS thermal time, leaf number and plant height in the long-day environment clustered on chromosome 10, flanked by umc1873–umc1053, while the QTL for these traits under short-day conditions clustered on chromosome 3 between markers phi053 and umc1539. This result was not surprising given the fact that flowering time, plant height and

total leaf number were dictated mainly by the timing transition from vegetative to reproductive development, determined by photoperiod (Irish and Nelson 1991). These results indicated that mechanisms governing flowering time and related traits in maize differed substantially in different photoperiod environments.

### The major QTL associated with photoperiod sensitivity were detected on chromosome 10

In the present study, QTL associated with PS were detected in the 10.04-region between marker umc1873 and umc1053. Moutiq et al. (2002) reported that flowering time QTL on chromosome 10 was linked to marker npi264 (10.04) and had the greatest additive effect exclusively in the long-day environment. Furthermore, QTL for leaf number and plant height in under long days were both mapped to the same chromosome 10 region. These results suggested that this region might contain important photoperiod response elements. In addition, this region likely determined the transition timing from vegetative to reproductive development, controlled by the shoot apical meristem in long-day conditions. Other studies similarly report QTL for flowering time in maize mapped to this region (Ribaut et al. 1996, 2007; Khairallah et al. 1998; Jiang et al. 1999; Bouchez et al. 2002). A synteny conservation approach based on comparative mapping between a maize genetic map and japonica rice physical map showed *osCCA1* associated with QTL for flowering time in bin 10.04 of maize chromosome 10 (Chardon et al. 2004). The *CCA1* gene in rice was reported to exhibit circadian rhythms with a phase similar to that of *CCA1* in *Arabidopsis* (Izawa et al. 2002, 2003). *CCA1/LHY* and *TOC1* comprised the central oscillator of the *Arabidopsis thaliana* circadian clock and formed a negative and positive transcriptional feedback loop that generated fundamental circadian rhythms (Alabadi et al. 2001, 2002). Recent studies showed that *CCA1/LHY* modulated a photoperiodic flowering pathway by negative transcription regulation of *GI*, *CDF1* and *FKF1* (Mizoguchi et al. 2005; Niwa et al. 2007). This coincidence in map position suggested that the maize ortholog to *osCCA1* might be a candidate gene of a QTL detected here in the bin 10.04 region of chromosome 10. However, finer mapping and a gene-specific marker are required to determine if this QTL is in fact orthologous to *osCCA1*.

### Another important QTL associated with photoperiod sensitivity and flowering time were detected on chromosome 9

In this study, the major QTL for DPS thermal time, qDPS9, located in the bin of 9.05–9.06 on the chromosome 9 were detected under both photoperiod environments. Moreover, QTL for leaf number under both photoperiod environments and QTL associated with PS were also detected in this region; so, we presume that there is a specific photoperiod response gene in this region. Various authors also found QTL for DPS (Ribaut 1996; Bohn et al. 1997; Kozumplik et al. 1996) or QTL for heat units to pollen (Veldboom 1994, 1996) in the bin 9.05–9.06 region of chromosome 9. It is worthwhile considering the association between the identified QTL and genes involved in photoperiod pathway. Sheehan et al. (2004) located phytochrome B2 (*Phy B2*) in the region of 9.05–9.06 in maize chromosome 9. Phytochrome B2 was one of the primary photoreceptors mediating photoperiod-dependent floral transition and was necessary to repress flowering under long day photoperiods (Sheehan et al. 2007). Much finer mapping and a gene-specific marker are needed to prove if this QTL actually is *PhyB2*.

### Tropical germplasm use in temperate zones

The narrow genetic base of temperate maize germplasm continues to be a widespread concern. To increase temperate germplasm variability, the incorporation of tropical germplasm has been advocated to assist in temperate maize breeding programs (Goodman et al. 2000; Goodman 2004, 2005; Nelson et al. 2006). However, to date, limited tropical germplasm has been successfully integrated into temperate accessions, because most tropical maize cannot withstand the photoperiod conditions of temperate maize. Consequently, a major problem confronting breeders is how to improve the ability of maize adapted to tropical environments to succeed under temperate environmental parameters. Extensive mass selection and backcrossing methods have been practiced to favor specific adaptations in exotic germplasm, but these approaches are time-consuming and require many generations of selective regimes. What more, it is difficult to select tropical maize germplasm based on its performance in temperate, because the PS usually conceals most of valuable genetic variation. Therefore, conventional mass selection and screening techniques to evaluate tropical and subtropical maize germplasm are laborious and time-consuming. On the other hand, marker-assisted selection (MAS) is superior to conventional selection when alleles are not expressed in the selection environments (Holland et al. 2004). Markers associated with photoperiod insensitivity can be screened for evaluation and selection of photoperiod insensitive traits in tropical environments. Furthermore, introgression of targeted genomic regions into tropical germplasm that significantly improves photoperiod insensitivity can be achieved without adversely affecting other important agronomic traits. In this study, QTL for PS, and flowering

time, plant height and leaf number under long-day conditions were located in clusters in the chromosome 10.04 region within a genetic distance of 10.1 cM between markers umc1873 and umc1053. Alleles contributed by Huangzao4 (at the abovedescribed QTL) were responsible for reductions of 32.8°C/h in PS, as well as a 72.30°C reduction in the thermal time for DPS, 1.72 decrease in leaf number and an 11.18 cm loss in plant height during long days. Therefore, the results of this study, and the resolution of several notable QTL, may provide valuable information for MAS. This methodology can be used to evaluate and apply tropical maize germplasm in temperate germplasm enhancement and constitutes a significant step towards the identification of genes responsible for PS.

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