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RESEARCH

Allelic Effect Variation at Key Photoperiod Response Quantitative Trait Loci in Maize

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ABSTRACT

Tropical maize (*Zea mays* L.) represents a valuable genetic resource containing unique alleles not present in elite temperate maize. The strong delay in flowering in response to long daylength photoperiods exhibited by most tropical maize hinders its incorporation into temperate maize breeding programs. We tested the hypothesis that diverse tropical inbreds carry alleles with similar effects at four key photoperiod response quantitative trait loci (QTL) previously identified in maize. Four tropical maize inbreds were each crossed and backcrossed twice to the temperate recurrent parent B73 to establish four sets of introgression lines. Evaluation of these lines under long daylengths demonstrated that all four QTL have significant effects on flowering time or height in these lines, but the functional allelic effects varied substantially across the tropical donor lines. At the most important photoperiod response QTL on chromosome 10, one tropical line allele even promoted earlier flowering relative to the B73 allele. Significant allelic effect differences among tropical founders were also demonstrated directly in an F_2 population derived from the cross of Ki14 and CML254. The chromosome 10 photoperiod response QTL position was validated in a set of heterogeneous inbred families evaluated in field tests and in controlled environments.

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Abbreviations: ASI, anthesis-silk interval; DTA, days to anthesis; DTS, days to silking; EH, ear height; GDD, growing degree days; GDDTA, growing degree days to anthesis; GDDASI, growing degree days anthesis-silking interval; GDDTS, growing degree days to silking; HIF, heterogeneous inbred family; IBM, intermated B73 × Mo17 population ; NAM, nested association mapping; PH, plant height; SSR, simple sequence repeat; QTL, quantitative trait locus/loci; RIL, recombinant inbred line.

The genetic diversity of elite temperate maize (*Zea mays* L.) germplasm is reduced relative to global intraspecific variation, resulting from intense phenotypic selection for increased yield and crop uniformity over the past century (Goodman, 2004; Tenaillon et al., 2001). The relatively narrow genetic base of temperate maize renders it more vulnerable to evolving pathogen biotypes and may limit future gains in productivity (Smith, 2007). Genetically more diverse tropical maize represents a valuable genetic resource that could be used to enhance the diversity and productivity of temperate maize (Goodman, 2004; Gouesnard et al., 1996). Unfortunately, most tropical germplasm is poorly adapted to temperate growing environments. A major component of the poor adaptation of tropical maize to temperate environments is the response of tropical germplasm to long-day photoperiods. At daylengths greater than 13 h, photoperiod-sensitive maize exhibits delayed flowering, increased plant height (PH), and a greater

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total number of leaves (Allison and Daynard, 1979; Kiniry et al., 1983; Warrington and Kanemasu, 1983).

In the tropical regions of Mexico where maize was first domesticated, precipitation rates and daylengths cycle annually. Specifically, the dry season in Central Mexico occurs from December to April when daylengths are increasing, and the wet season occurs from June to September when daylengths are decreasing (Bullock, 1986; Medina et al., 1998; Ruiz C. et al., 2008). Because water stress during flowering can reduce fertilization and seed set, maize and its predecessor teosinte [*Zea mays* L. subsp. *parviglumis* H. H. Iltis & Doebley] likely evolved photoperiod sensitivity to synchronize their reproductive phases to the wetter, short-day growing season (Campos et al., 2006; Ribaut et al., 1996). A similar evolution of photoperiod sensitivity is believed to have occurred in sorghum [*Sorghum bicolor* (L.) Moench] (Craufurd et al., 1999). Latitude 20° N passes through central Mexico, and at this latitude the daylength shifts from 13.3 h on 16 June to 12.23 h on 16 September (United States Naval Observatory, 2010). Thus, maize adapted to this region is quite sensitive to a 1-h shift in photoperiod. In contrast, temperate maize populations that were introduced to long-day environments during the spread of maize growing culture throughout North and South America were selected to be photoperiod insensitive. The major U.S. corn producing region is centered north of 40° N, where daylength is almost 14 h on 1 May, increasing to 15 h on 16 June and not decreasing below 13 h until 3 September (United States Naval Observatory, 2010). The timing of flowering under these much longer daylengths is greatly delayed in tropical maize, reducing the grain filling period (which must be completed before the first frost) and yield and increasing grain moisture at harvest. These responses can mask the expression of favorable alleles carried by tropical germplasm, resulting in a major barrier to the introgression of tropical germplasm into temperate maize.

Photoperiod response can be eliminated in both temperate × tropical and tropical × tropical populations through phenotypic selection over several generations, resulting in agronomically superior temperate inbred and hybrid lines (Goodman, 1999; Hallauer, 1994; Hallauer and Sears, 1972; Holland and Goodman, 1995; Lewis and Goodman, 2003; Nelson and Goodman, 2008). Methods to efficiently and accurately select against photoperiod sensitivity across a wide variety of tropical germplasm could accelerate the introgression of valuable alleles from tropical maize into temperate maize.

Marker-assisted selection can facilitate plant breeding by determining which members of a segregating population carry detrimental or unfavorable alleles, even when the effects of such alleles are masked by epistasis or incomplete penetrance (Xu and Crouch, 2008). A comprehensive study of the quantitative trait loci (QTL) governing maize photoperiod sensitivity would determine which photoperiodic alleles are common to certain tropical maize populations, and the results of such a study could be used to select among tropical photoperiod sensitive parental lines to produce completely tropical populations segregating for photoperiod insensitivity. Indeed, it is possible to produce photoperiod insensitive progeny from the cross of two photoperiod sensitive parents (Goodman, 1999; Holley and Goodman, 1988).

A small number of photoperiodic response QTL have been identified in common across distinct temperate × tropical maize populations and independent environments (Coles et al., 2010; Ducrocq et al., 2009; Moutiq et al., 2002; Wang et al., 2008), raising the possibility that a substantial proportion of the photoperiodic flowering time variation may be controlled by a few loci. Meta-analysis of numerous maize studies indicated that six flowering time QTL were detected regularly across diverse mapping populations (Chardon et al., 2004). Four of these six QTL are located in the same genomic regions as major photoperiodic QTL identified by Coles et al. (2010) in a joint QTL analysis of four temperate × tropical populations. In contrast, however, analysis of flowering time in the maize nested association mapping (NAM) population under long daylengths indicated that many genes, each with relatively small effects, contribute to flowering time genetic variation across diverse maize germplasm (Buckler et al., 2009). Furthermore, variation among allelic effects from different tropical founder lines were observed commonly for QTL affecting long daylength flowering and photoperiod response (Buckler et al., 2009; Coles et al., 2010), suggesting that tropical maize is not homogeneous for allelic function at photoperiod QTL. Thus, the extent to which a relatively small number of loci control most of the photoperiod response in maize is uncertain.

The first objective of this study was to test if markers flanking the four major photoperiod response QTL previously identified by Coles et al. (2010) were associated with later flowering in backcross-derived families segregating for tropical donor line introgressions in a predominantly temperate genetic background. One tropical donor line, CML254, was a parent of the original mapping study, whereas three other donor lines (CML247, Ki3, and Ki11) represent a previously untested sample of tropical germplasm, permitting evaluation of allelic series at these four QTL. The second objective was to test for allelic effect differences between two different tropical lines at these QTL in progeny from a cross between the tropical mapping line parents used by Coles et al. (2010). The third objective was to validate and refine the position of the major chromosome 10 photoperiod response QTL in heterogeneous inbred families segregating for alleles only in the region of its initial map position.

MATERIALS AND METHODS

Founder Inbred Lines

We studied progenies derived from crosses including maize inbred lines B73, CML247, CML254, Ki3, Ki11, and Ki14. These lines were chosen based both on their photoperiod sensitivity and their

geographic diversity. Some of these lines are founding parents of the NAM population (Buckler et al., 2009), and others were used in a photoperiod mapping study by Coles et al. (2010). The inbred line B73 was released from Iowa State University (Ames, IA) in the United States (Russell, 1972), the CML lines from CIMMYT in El Batán, Edo. de México, México (Srinivasan, 2010), and the Ki lines from Kasetsart University in Bangkok, Thailand (Chutkaew et al., 2010). B73 is a temperate stiff-stalk maize line, and the other lines from this study are genetically diverse inbreds from the tropical heterotic group of maize (Liu et al., 2003). Coles et al. (2010) demonstrated under controlled environment conditions that these tropical lines are more photoperiod-sensitive than B73.

Backcross Population Experiments

We developed four $BC_2F_{3:4}$ mapping populations by crossing B73 × CML247, B73 × CML254, B73 × Ki3, and B73 × Ki11. The F_1 of each cross was backcrossed to B73 for two generations to form (B73*3)CML247, (B73*3)CML254, (B73*3)Ki3, and (B73*3)Ki11 populations. In the four backcross populations, we maintained between 26 and 37 unique lineages, each descended from a single BC_1F_1 plant. From these we maintained between 4 and 10 unique BC_2F_1–derived lineages from within each BC_1F_1 family (Supplemental Table S1). A single $BC_2F_{3:4}$ line was then created from each unique BC_2F_1 lineage by two generations of single seed descent, followed by one generation of self-fertilization and harvesting in bulk to create a total of 154 to 219 $BC_2F_{3:4}$ lines from each population. We expect to recover 87.5% of recurrent parent genome on average across BC_2–derived lines.

Experiments were performed in both long daylength (Clayton, NC, in summer 2007) and short daylength (Homestead, FL, in winter 2007–2008) environments. In each environment, lines derived from different founders were evaluated in separate experiments planted in common fields. The experimental design for each population set was an augmented α lattice design with B73 included as a check plot within every incomplete block. In the long-day environment, the total number of entries per experiment was 224 (14 × 16 α design) for the CML247 population, 176 (11 × 16 α design) in the CML254 population, 228 (12 × 19 α design) for the Ki3 population, and 242 (11 × 22 α design) in the Ki11 population. Under short daylengths, photoperiod-sensitive tropical alleles exhibit greatly reduced effects. Therefore, we compared flowering time of B73 under short daylengths to only a subset of lines from the B73 × CML254 (24 lines) and B73 × Ki11 populations (31 lines) that were homozygous for tropical alleles in the QTL region on chromosome 10 to validate its photoperiod response. In the short daylength environment, the total number of entries per experiment was 27 (3 × 9 α design) in the CML254 population and 36 (4 × 9) in the Ki11 population. A single environment evaluation was sufficient to accurately score flowering time for each photoperiod because previous results with related germplasm indicate that genotype × environment interaction variation is very small relative to genetic variation for flowering time within a photoperiod regime (Buckler et al., 2009; Coles et al., 2010).

In both environments, we measured growing degree days to anthesis (GDDTA), growing degree days to silking (GDDTS), PH, and ear height (EH) in each plot. We defined GDDTA and GDDTS as the number of growing degree days between planting and when 50% of the plants in a plot were shedding pollen or showing silks, respectively, using the formula of McMaster and Wilhelm (1997). Growing degree days anthesis-silking interval (GDDASI) was defined as the difference in growing degree days (GDD) between anthesis and silking. We measured PH and EH as the distance between the ground and the last leaf node or the primary ear branch, respectively, on three plants per plot.

Genomic DNA was extracted from the bulked leaf or seed tissue of eight plants or eight seeds representing each of the backcross lines utilizing the protocol of Mogg and Bond (2003). We genotyped each of these populations with simple sequence repeat (SSR) markers (Senior et al., 1998) flanking or within the four *Zea mays Photoperiodic Response* (*ZmPR*) QTL regions (Fig. 1; Tables 1, 2, 3, and 4) originally defined by Coles et al. (2010). Twelve, 23, 12, and 16 SSR markers were genotyped on the families derived from crosses between B73 and CML247, CML254, Ki3, and Ki11, respectively.

Phenotype data from the backcross populations were analyzed using Proc Mixed in SAS Version 9.1.3 (SAS Institute, 2002). Incomplete blocks of the experimental designs were not entirely confounded with the rows and columns of the field plot grids and could account for extraneous spatial variation. Therefore, we tested the effects of complete and incomplete blocks as well as row and column designations from the field layouts and maintained significant terms in the final analysis models. For each trait in each data set (population and environment combination), we tested a full model including random effects due to complete replications, incomplete blocks, and column and row designations from the field layout and fixed effects due to lines. Each random effect was dropped from the full model one at a time to compute likelihood ratio tests for the null hypothesis that the variance component due to the deleted effect equals zero (Littell et al., 1996). The final model selected for each combination of population and trait included only those random effects significant at $p < 0.05$.

Marker-trait associations were tested separately for each population by adding marker effects to the final selected model for each trait–population combination. Marker genotype class was considered a fixed effect, with check lines considered unique marker classes, and line within marker class considered as a random effect. Additive and dominant effects associated with each marker were estimated from comparisons between the means of backcross lines with different marker genotypes (so that check lines such as B73 did not contribute to the estimates). The additive effect associated with each marker was estimated as half the difference between the mean of introgression lines homozygous for the CML254 allele and the mean of introgression lines homozygous for the B73 introgression allele. Dominance associated with markers was estimated as the difference between the mean of segregating introgression lines and the mean of the two homozygous introgression line groups. The phenotypic values were derived from plots containing BC_2F_4 plants derived from common BC_2F_3 ancestors. Thus, only half of the plants within such plots are expected to be heterozygous, and we consequently estimated half of the dominance effect at each marker locus.

Trait heritability on an entry mean basis was estimated by considering genotypes random effects and using the formula $h^2 = \sigma^2_g/[\sigma^2_g + (\sigma^2_e/m)]$, in which σ^2_g is the estimate of genetic variance, σ^2_e is the residual error variance, and m is the harmonic mean of total number of plots observed per entry (Holland et al., 2003).

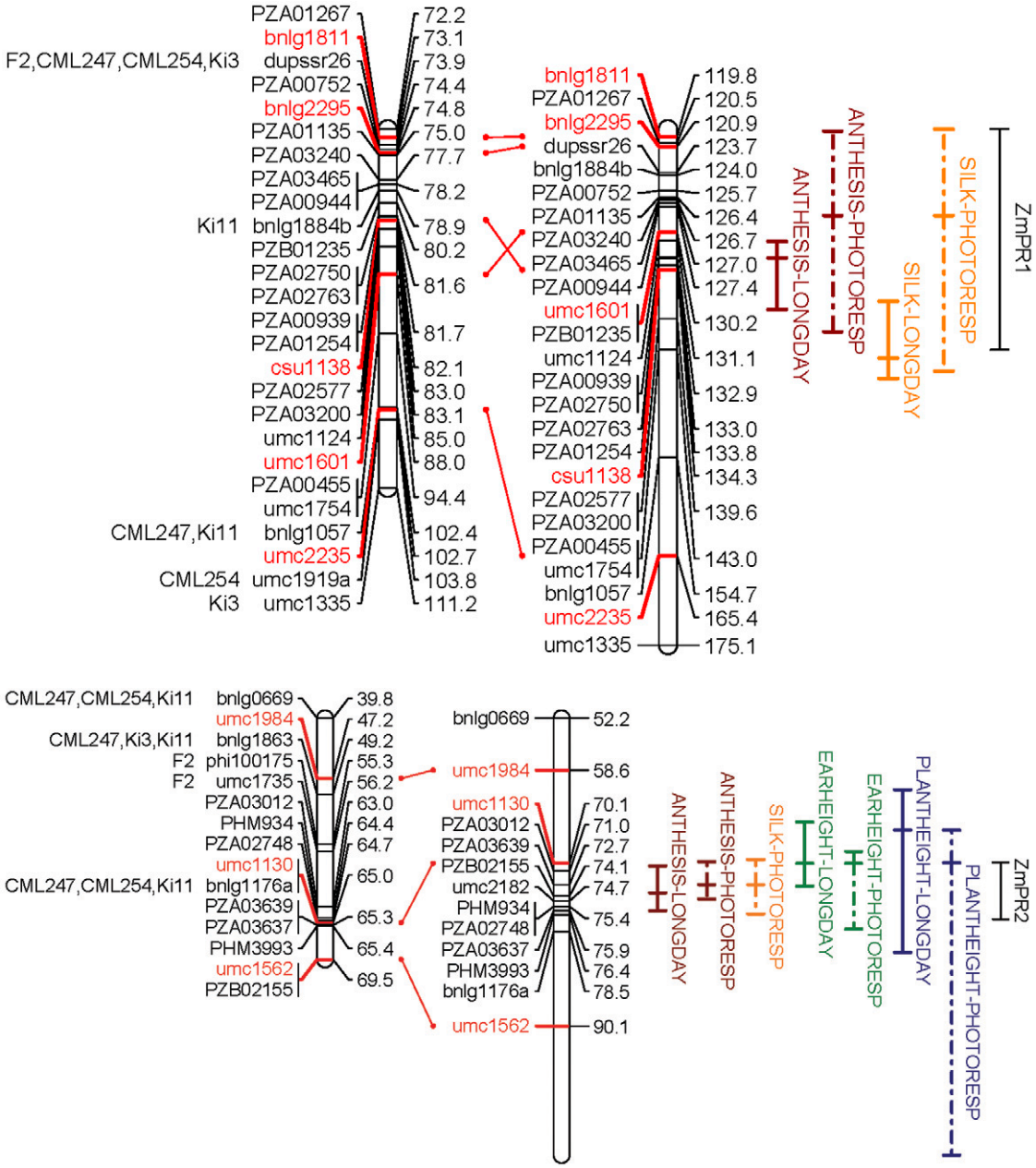

Figure 1. (continued on next page) Linkage map of segments of chromosomes 1, 8, 9, and 10 containing the four key photoperiod response quantitative trait loci (QTL) regions *ZmPR1* through *ZmPR4* as described by Coles et al. (2010). Each segment is shown two different ways; the segment on the right of each pair contains markers ordered according to the analysis of Coles et al. (2010), while the segment on the left contains markers ordered according to the IBM2 Neighbors 2008 map (http://www.maizegdb.org/ [verified 11 Mar. 2011]; Sen et al., 2010) as a reference. Markers that were scored on one or more of the populations in this study are indicated to the left of each IBM2 segment. "F2" indicates that the marker was scored on the Ki14 × CML254 F_2 population. "CML247," CML254," "Ki3," or "Ki11" indicate that the marker was scored on the backcross population derived from that donor parent, respectively. In addition to markers scored on the populations in this study, other markers are shown to provide a framework for each region in question. Markers highlighted in red are part of the backbone of the IBM2 Neighbors 2008 map, meaning that these markers are well-ordered on that map. Quantitative trait loci bars represent the 2-LOD (likelihood of odds ratio) support interval of the QTL position as shown by Coles et al. (2010). The middle hash mark of each bar represents the maximum likelihood position of the QTL. "LONGDAY" refers to QTL that were identified under long daylength environments, while "PHOTORESP" refers to QTL associated with the photoperiod response.

F_2 Population Experiment

To directly test for allelic variation at the four key photoperiod QTL identified in crosses between Ki14 or CML254 and temperate lines (Coles et al., 2010), we crossed these two tropical lines and selfed several F_1 plants to create a random F_2 mapping population. Six consecutive rows of 25 seeds each were planted in Clayton, NC, in the summer of 2007. Plants were then thinned within each row so that there was sufficient space between plants for full development. Growing degree days to anthesis, GDDTS, GDDASI, EH, and PH were measured on a sample of 96 individual plants in these six rows. Only plants that appeared healthy were phenotyped. Leaf tissue was sampled from

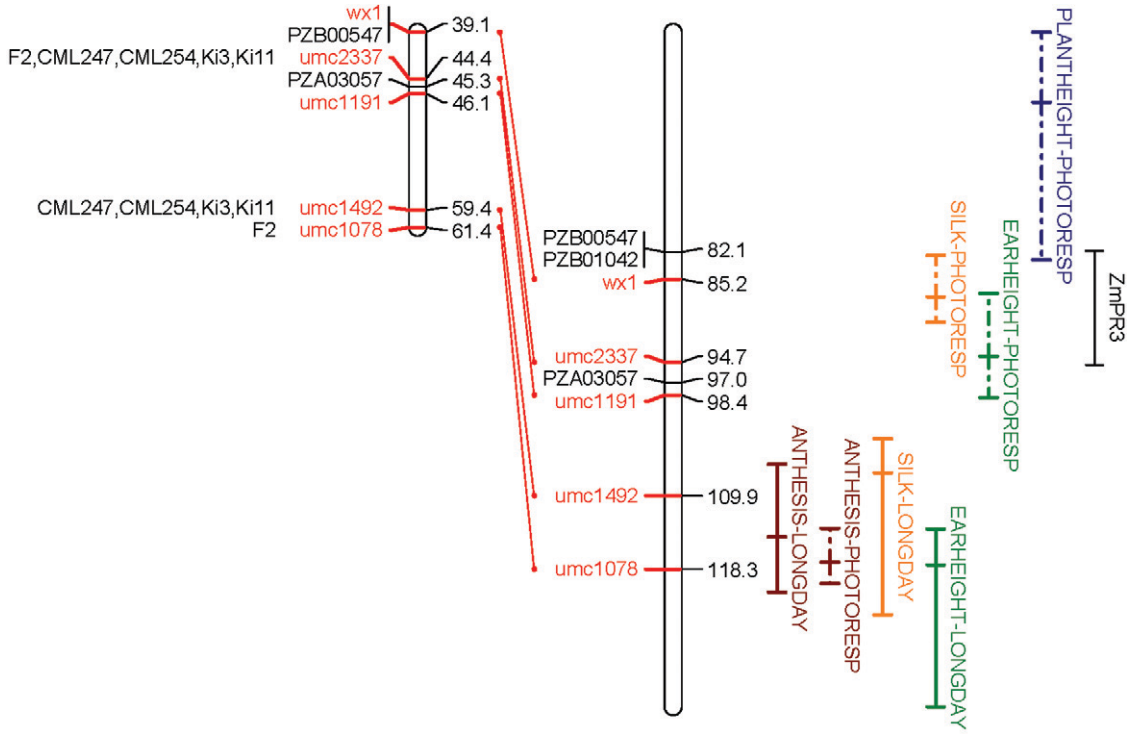

Figure 1. Continued.

each plant and DNA extracted as above. One to three SSR markers linked to each of the four QTL regions were used to genotype each plant. One-way ANOVA were performed for each marker in the Ki14 × CML254 F_2 population. The null hypothesis of no variation among the three genotypic class means was tested using the pooled variation among plants within marker classes as the error term using SAS Proc GLM (SAS Institute, 2002). Additive and dominance effects associated with each marker locus were estimated following Edwards et al. (1987).

Heterogeneous Inbred Families Experiments

Heterogeneous inbred families (HIFs) were derived from two recombinant inbred lines (RILs) from the B73 × CML254 RIL population described by Coles et al. (2010). One $F_{5:7}$ RIL was chosen to derive HIFs because it was segregating at markers flanking the *ZmPR4* region on chromosome 10 but homozygous elsewhere in the genome. Several plants of the RIL were self-pollinated and harvested individually to form $F_{7:8}$ lines. Up to 72 seedlings of each $F_{7:8}$ line were genotyped at 19 SSR markers in the interval from bnlg210 to umc1115 (Fig. 1). A sample of F_8 plants was self-pollinated to form $F_{8:9}$ lines. This sample included plants homozygous for parental haplotypes across the entire interval, plants homozygous for different recombinant haplotypes, and partly heterozygous recombinant progenies from each line. $F_{8:9}$ lines were increased by self-fertilization; one set of plants within each line was harvested individually to form $F_{9:10}$ lines, and a second set of plants from each line was harvested in bulk to form $F_{8:10}$ lines. Thirteen $F_{9:10}$ lines (coded as 1304-1-1-1 to 1304-1-1-13) derived from a single $F_{8:9}$ line that was heterozygous in two regions near the QTL were genotyped individually at four SSR markers in the heterozygous regions, and genotypes at surrounding loci were interpolated based on grandparental F_8 genotype data and the four directly genotyped loci (Fig. 2).

Heterogeneous inbred families representing contrasting parental haplotypes and several recombination events within the *ZmPR4* region were chosen for photoperiod sensitivity study evaluations under controlled conditions in the North Carolina State University Phytotron (Raleigh, NC; http://www.ncsu.edu/phytotron/ [verified 4 Mar. 2011]). Experimental entries included 23 $F_{8:10}$ generation lines, 13 $F_{9:10}$ generation lines, and two instances each of B73 and CML254, resulting in a total of 40 entries per replication.

The experimental design was a randomized complete block design with six replications repeated across two planting dates 1 mo apart. Experimental units were 15-cm diam. plastic pots sown with several seeds of a single line. Seedlings were thinned after emergence to a single plant per pot. The growing medium was a mixture of peat moss and vermiculite, and pots were watered twice daily with a nutrient solution. Within a planting date, pots were arranged in a randomized complete block design within a single growth chamber and subjected to a photoperiod regime of 18/6 h day/night with temperatures set to 30/26°C day/night. Illumination was provided by a mixture of 6020 W cool-white fluorescent and 2400 W incandescent lamps. Lamps were separated from the chamber by a Plexiglas barrier. The light levels measured about one m above the chamber floor were 500 (±15) μmol m^{-2} s^{-1}. Relative humidities in the chambers were typically above 70%. More detailed environmental specifications are available at Saravitz et al. (2009); see description of "B chambers."

Plants were grown under long daylength photoperiod growth chamber conditions for 30 d, which Coles et al. (2010) demonstrated was sufficient to induce a very strong delayed flowering photoperiod response in CML254. After 30 d, the plants were moved to a greenhouse with 12 h of artificial lighting and temperatures set at 25°C. In the greenhouse, plants were watered once daily and fertilized with slow-release fertilizer. Days to tassel emergence, days to anthesis (DTA), and PH measurements

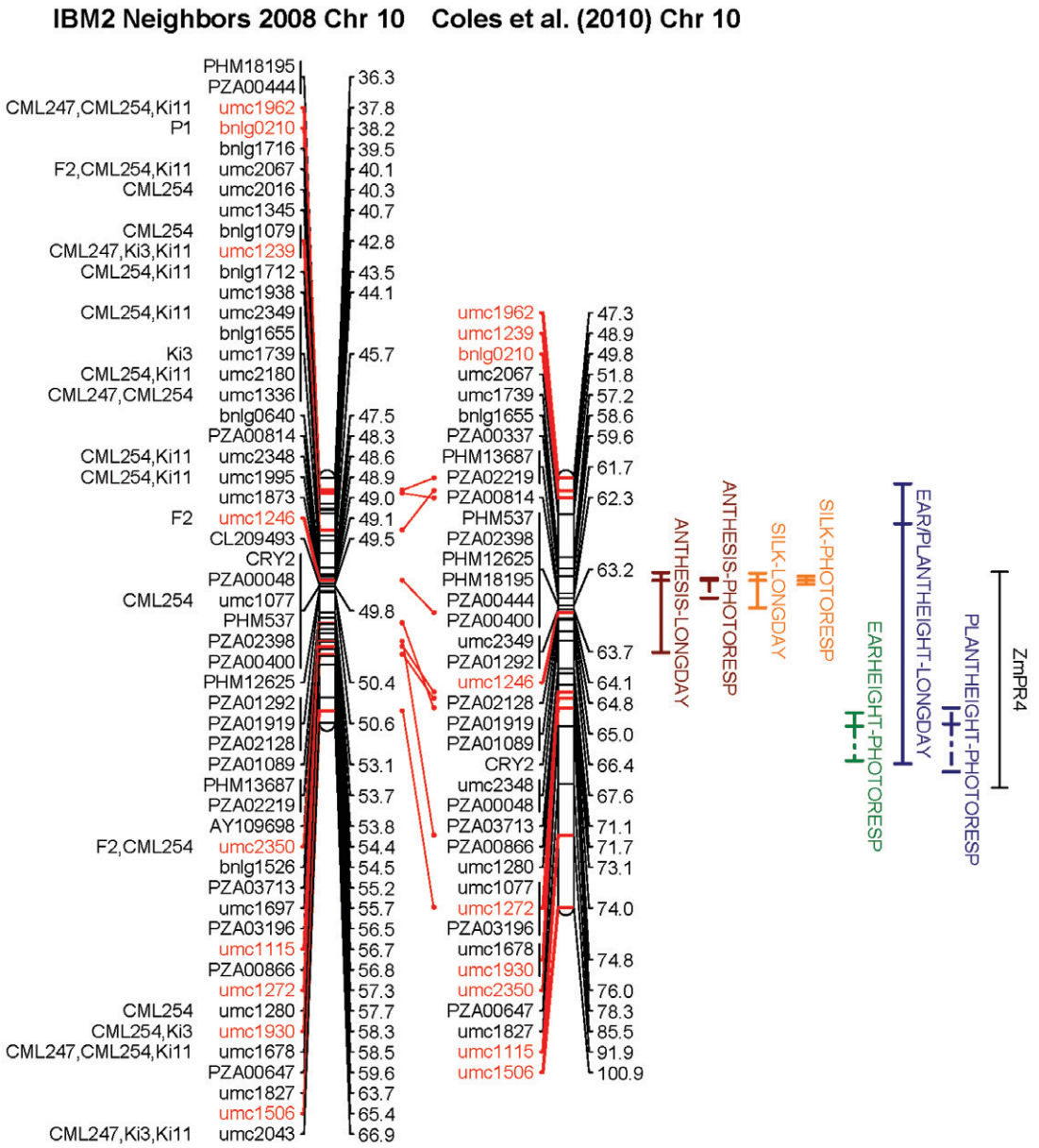

Figure 1. Continued.

were recorded for each individual. Least square means were calculated for each trait and line combination using SAS Proc Mixed (SAS Institute, 2002). Genotypes were considered fixed effects, whereas planting date, replication (nested within planting date), and genotype × planting date interaction were considered random effects. Analysis of variance was conducted on the line least square means to test the null hypothesis of no marker effect for each SSR locus by testing the variation among marker class means using the pooled variation within marker class means.

Five HIF lines from the phytotron study with sufficient seed quantities were chosen for a field evaluation under long daylengths in the summer of 2009. The experimental design was a randomized complete block arrangement of the five HIF lines plus B73 with four replications each at four locations: Colombia, MO, Clayton, NC, Ithaca, NY, and Madison, WI. Measurements were collected for DTA, days to silking (DTS), PH, EH, and total leaf number. Anthesis–silk interval (ASI) was calculated as the difference between DTS and DTA.

Locations were first analyzed separately using SAS Proc GLM; linear combinations were used to estimate the effect of the testing region between the two subsets of HIFs. Locations were then combined and analyzed using SAS Proc Mixed. Genotypes were treated as fixed effects, and location, replication (nested within location), and genotype × location interaction were included as random effects. All pairs of genotypes were compared using the pdiff option of the lsmeans statement in Proc Mixed.

RESULTS AND DISCUSSION

Backcross Populations

We expected 25% of BC_2F_1 plants to carry tropical alleles at a QTL and 9.4% of $BC_2F_{3:4}$ lines to be homogeneous homozygous for the tropical allele at a QTL. On average, across the four backcross populations we identified 9% of lines homozygous at *ZmPR1*, 6% homozygous at *ZmPR2*, 8%

Table 1. Additive (*a*)† and dominant (*d*)‡ effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)CML254 $BC_2F_{3:4}$ lines evaluated in long daylength conditions in summer 2007.

Chromosome	Marker	IBM2§ position	GDDTA¶		GDDTS		GDDASI		EH		PH	
			a	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*
		IcM#	growing degree days						cm			
ZmPR1												
1	dupssr26	391.1	–0.4	1.4	3.0	–4.0	4.0	–6.7	–2.3	8.6	–3.4	9.1
1	umc1919a	555.8	10.1	–9.3	11.6	–11.5	2.2	–2.4	1.6	–7.7	0.4	–4.0
ZmPR2												
8	bnlg0669	191.0	6.6	15.2*	11.2*	11.3	4.5	–3.4*	2.3	7.6***	1.0	6.9
8	umc2182	unknown	10.9*	7.5	13.7**	7.9	2.8	0.7	1.3	10.2***	–1.4	13.3***
8	bnlg1176a	330.4	3.6	12.9	7.2	16.1	3.7	3.4	0.6	5.8	–2.2	5.8
ZmPR3												
9	umc2337	220.1	2.8	–12.8	5.8	–18.5	2.6	–5.2	3.5	2.9	6.0*	10.1
9	umc1492	308	10.4*	5.4	8.4	10.2	–2.2	4.7	1.7	5.4	1.5	7.9
ZmPR4												
10	umc1962	180.7	17.1***	–9.6	14.9***	–3.3	–2.5	6.5	0.9	3.4	2.5	5.5
10	bnlg0210	183.4	16.2***	–11.4	14.8***	–3.0	–1.6	8.4	0.6	3.8	2.7	7.5
10	umc2067	194.5	19.8***	–5.9	19.7***	–2.4	0.0	3.4	1.3	6.2	2.9	5.8
10	umc2016	195.4	15.2***	–8.0	14.0***	–2.3	–1.6	5.6	0.5	4.4	2.4	7.1
10	bnlg1079	213.1	17.6***	–5.6	16.9***	0.4	–0.9	5.9	1.0	7.9*	3.0	8.3
10	bnlg1712	217.8	20.6***	–10.9	19.2***	–7.2	–1.8	4.2	2.2	1.7	4.4*	2.2
10	umc2349	227.9	21.1***	–10.9	19.6***	–6.7	–1.9	4.5	2.4	2.1	4.6*	3.7
10	umc2180	228.3	21.9***	–12.5	21.0***	–6.7	–1.2	6.1	2.6	3.8	4.7*	6.8
10	umc1336	228.3	17.5***	–7.3	15.7***	–1.7	–2.2	5.7	0.5	6.5*	2.1	9.3*
10	umc2348	244.6	21.8***	–8.3	21.6***	–7.0	–0.6	1.5	1.3	3.2	3.5	4.2
10	umc1995	245.9	18.3***	–8.7	17.2***	–6.7	–1.6	2.2	–0.3	3.4	3.2	4.7
10	umc1077	253.5	18.1***	–7.9	15.7***	–4.5	–2.9	3.6	0.1	3.6	3.4	4.2
10	umc2350	283.5	12.3***	3.6	11.7*	7.3	–0.9	4.1	–0.3	6.1*	2.5	8.0*
10	umc1280	303.3	11.9***	–1.3	8.0	11.5	–3.9	13.2	–1.2	5.8	1.0	6.0
10	umc1930	306.9	13.6***	–3.8	10.2*	3.9	–3.7	8.0	–0.9	5.2	0.1	8.3*
10	umc1678	308.7	16.9***	–6.3	15.7***	0.2	–1.2	6.7	1.2	4.0	2.9	5.9

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

†Additive effect estimated as half the difference between homozygous CML254 class and homozygous B73 class means.

‡Half of the dominant effect estimate as the difference between segregating line means and the average of the two homozygous parental class means. Segregating lines were expected to be composed of 50% heterozygous plants, such that this estimates (1/2)*d*.

§IBM map unit (Balint-Kurti et al., 2007).

¶GDDTA, growing degree days to anthesis; GDDTS, growing degree days to silking; GDDASI, growing degree days anthesis-silking interval; EH, ear height; PH, plant height.

#IcM, intermated centiMorgan.

homozygous for *ZmPR3*, and 9% homozygous for *ZmPR4*, slightly below the expectation (Supplemental Table S1). Coles et al. (2010) observed significant and much stronger segregation distortion favoring temperate alleles at each of these regions in RIL populations, presumably due to unavoidable selection for adaptation to long daylengths. The closer agreement between expected and observed homozygous tropical allele genotypes in the backcross-derived lines suggests that the tropical alleles can be effectively transferred to a B73 background without substantially reducing fitness.

The primary objectives of this study were to validate the four major QTL detected in the RIL populations of Coles et al. (2010) and to test the effects of a more diverse sample of tropical line donor alleles at these QTL in an otherwise primarily B73 genetic background. The four QTL that we tested in this study were named *Zea mays Photoperiodic Response1* (*ZmPR1*, on chromosome 1 between markers bnlg1811 and umc1754), *Zea mays Photoperiodic Response2* (*ZmPR2*, on chromosome 8 between markers umc1130 and PHM3993), *Zea mays Photoperiodic Response3* (*ZmPR3*, on chromosome 9 between markers PZB01042 and umc2337), and *Zea mays Photoperiodic Response4* (*ZmPR4*, on chromosome 10 between markers PZA00337 and umc1827) (Fig. 1).

We first tested the hypothesis that CML254 alleles at these four QTL were significantly associated with later flowering (as measured by GDDTA and GDDTS) in the (B73★3)CML254 introgression lines, as predicted from the results from the original B73 × CML254 RIL population (Coles et al., 2010). We observed significant associations between flowering time phenotypes under long daylength conditions and marker loci near three of the

Table 2. Additive (*a*)† and dominant (*d*)‡ effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)CML247 $BC_2F_{3:4}$ lines evaluated in long daylength conditions in summer 2007.

Chromosome	Marker	IBM2§ position	GDDTA¶		GDDTS		GDDASI		EH		PH	
			a	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*
		IcM#	growing degree days						cm			
ZmPR1												
1	dupssr26	391.1	0.0	–8.3	3.6	–8.9	3.4*	–0.4	–2.0	–4.1	–4.7**	–5.2
1	bnlg1057	548.3	–1.0	–8.8	3.2	–22.2*	3.6	–12.5*	–2.8*	12.2**	–6.1***	13.4**
ZmPR2												
8	bnlg1863	245.7	5.7	–1.1	5.2	–2.3	–1.4	2.5	0.3	–1.8	1.2	–0.1
8	bnlg1176a	330.4	7.6*	–11.9	5.5	–8.0	–2.2	4.4	–1.7	2.3	–0.6	3.6
ZmPR3												
9	umc2337	220.1	–0.2	–7.3	–3.6	–8.6	–3.0	–2.9	1.5	3.9	–0.5	1.8
9	umc1492	308.0	1.3	–0.9	3.8	–6.9	1.1	–5.8	6.2***	1.5	7.2***	–6.5
ZmPR4												
10	umc1962	180.7	2.1	–1.5	2.8	–1.7	–0.7	–1.1	0.2	0.8	–0.9	1.7
10	umc2016	195.4	–1.6	5.0	0.0	1.5	0.0	–5.2	1.5	3.3	0.2	5.8
10	umc1239	213.3	0.1	–1.9	1.5	–10.7	0.5	–8.1*	1.5	3.5	1.5	5.8
10	umc1336	228.3	2.5	–2.1	5.2	–13.6	1.6	–11.9**	2.1	6.7*	2.5	9.3*
10	umc1678	308.7	–1.2	–4.4	–0.8	–8.2	–0.6	–3.5	1.5	0.6	2.9	1.9
10	umc2043	352.9	–4.2	–0.4	–4.9	–6.0	–1.8	–3.8	1.2	2.0	0.5	3.4

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

†Additive effect estimated as half the difference between homozygous CML247 class and homozygous B73 class means.

‡Half of the dominant effect estimate as the difference between segregating line means and the average of the two homozygous parental class means. Segregating lines were expected to be composed of 50% heterozygous plants, such that this estimates (1/2)*d*.

§IBM map unit (Balint-Kurti et al., 2007).

¶GDDTA, growing degree days to anthesis; GDDTS, growing degree days to silking; GDDASI, growing degree days anthesis-silking interval; EH, ear height; PH, plant height.

#IcM, intermated centiMorgan.

Table 3. Additive (*a*)† and dominant (*d*)‡ effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)Ki3 $BC_2F_{3:4}$ lines evaluated in long daylength conditions in summer 2007.

Chromosome	Marker	IBM2§ position	GDDTA¶		GDDTS		GDDASI		EH		PH	
			a	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*
		IcM#	growing degree days						cm			
ZmPR1												
1	dupssr26	391.1	6.0**	–11.3*	6.1*	–9.4	0.4	1.2	1.5	–1.2	3.6*	–2.9
1	umc1335	593.8	1.1	2.9	1.5	–3.1	0.3	–4.5	–4.0***	–3.6	–5.1**	–6.7
ZmPR2												
8	bnlg669	191.0	0.8	–3.7	4.7	–1.7	3.6	1.5	–2.5*	1.0	–2.7	–2.9
8	bnlg2082	200.3	14.5*	–15.9	18.3*	–15.6	2.3	2.3	–2.3	1.1	–0.5	–4.6
8	bnlg1863	245.7	0.1	3.1	–0.7	6.2	–0.5	2.2	–5.1*	1.8	–7.2*	–0.3
8	bnlg1176a	330.4	6.6	–4.1	5.6	–4.6	–1.3	–1.4	–4.8**	7.3	–7.2**	11.4*
ZmPR3												
9	umc2337	220.1	2.4	–4.2	2.4	–3.2	0.0	1.4	3.8***	–1.1	2.8	1.0
9	umc1492	308.0	2.8	–0.9	5.5	–3.5	2.2	–1.8	4.2**	–1.7	5.0*	–3.2
ZmPR4												
10	umc1239	213.3	0.6	–10.4	–1.3	–9.3	–1.2	0.7	2.3	–5.1	4.5	–6.5
10	umc1739	228.0	–1.3	3.7	–2.8	–3.2	–1.2	–7.0*	2.2*	–1.3	2.3	0.4
10	umc1930	306.9	–6.7*	2.7	–8.4*	1.0	–2.2	–0.8	1.8	–1.3	4.3	–2.9
10	umc2043	352.9	–2.6	–3.8	–1.5	–12.8*	0.6	–8.0*	2.2	1.1	2.6	2.6

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

†Additive effect estimated as half the difference between homozygous Ki3 class and homozygous B73 class means.

‡Half of the dominant effect estimate as the difference between segregating line means and the average of the two homozygous parental class means. Segregating lines were expected to be composed of 50% heterozygous plants, such that this estimates (1/2)*d*.

§IBM map unit (Balint-Kurti et al., 2007).

¶GDDTA, growing degree days to anthesis; GDDTS, growing degree days to silking; GDDASI, growing degree days anthesis-silking interval; EH, ear height; PH, plant height.

#IcM, intermated centiMorgan.

Table 4. Additive (*a*)† and dominant (*d*)‡ effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)Ki11 $BC_2F_{3:4}$ lines evaluated in long daylength conditions in summer 2007.

Chromosome	Marker	IBM2§ position	GDDTA¶		GDDTS		GDDASI		EH		PH	
			a	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*	*a*	(1/2)*d*
		IcM#	growing degree days						cm			
ZmPR1												
1	bnlg1884b	419.8	11.9**	17.4	16.8***	6.4	4.3**	–5.1	2.6*	0.2	1.4	0.3
1	bnlg1057	548.3	3.1	12.8	1.9	15.5	0.6	2.0	1.6	–2.6	0.1	–10.3*
ZmPR2												
8	bnlg669	191.0	–2.4	–1.7	0.6	–9.7	3.2	–5.0	–2.1	2.7	–0.9	1.3
8	bnlg1863	245.7	3.3	–11.9	3.8	–10.8	1.2	–0.2	–2.0	3.7	–2.5	7.8*
ZmPR3												
9	umc2337	220.1	5.9	2.0	13.1***	6.5	3.1	3.3	1.6	3.2	0.6	5.1
9	umc1492	308.0	18.4***	–4.0	22.2***	14.6	2.3	9.1	3.2**	–3.4	2.8	–2.1
ZmPR4												
10	umc1962	180.7	19.6***	–22.0	13.8***	–23.5	–2.1	–1.0	4.4***	6.4	6.1**	9.2
10	umc2067	194.5	19.2**	–0.2	13.7	4.9	–2.0	1.2	4.2*	3.9	3.8	6.5
10	umc1239	213.3	19.1**	–7.1	17.7*	–10.5	–1.7	0.6	2.8	6.0	1.6	10.7*
10	bnlg1712	217.8	14.0**	–3.3	13.3*	–7.5	–0.5	–1.5	3.0*	2.3	3.1	4.5
10	umc2349	227.9	9.8	0.6	8.1	0.4	–1.6	2.0	1.7	4.4	1.0	7.5
10	umc2180	228.3	11.3*	–1.9	9.3	–1.5	–1.6	1.9	1.7	5.3	1.2	9.1
10	umc2348	244.6	11.6*	1.0	12.0**	1.0	0.0	1.7	1.1	6.6	0.8	9.8*
10	umc1995	245.9	12.2**	3.6	10.5	4.3	–1.5	1.8	1.5	3.7	1.5	6.7
10	umc1678	308.7	16.1**	–17.6	10.5	–9.2	–2.9	5.4	3.7**	1.1	3.9*	0.1
10	umc2043	352.9	13.8*	–8.2	5.3	–3.7	–5.3*	3.9	4.0**	1.5	5.8**	–0.5

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

†Additive effect estimated as half the difference between homozygous Ki14 class and homozygous B73 class means.

‡Half of the dominant effect estimate as the difference between segregating line means and the average of the two homozygous parental class means. Segregating lines were expected to be composed of 50% heterozygous plants, such that this estimates (1/2)*d*.

§IBM map unit (Balint-Kurti et al., 2007).

¶GDDTA, growing degree days to anthesis; GDDTS, growing degree days to silking; GDDASI, growing degree days anthesis-silking interval; EH, ear height; PH, plant height.

#IcM, intermated centiMorgan.

four *ZmPR* regions (Table 1). CML254 alleles at markers linked to *ZmPR3* and *ZmPR4* were significantly associated with later flowering and greater plant height (Table 1). A significant additive effect for later flowering but not for height was also observed at *ZmPR2* (Table 1). Significant dominance effects were observed for flowering time only at one locus near *ZmPR2*, where later flowering was dominant. At *ZmPR2* and *ZmPR4*, significant dominant effects, in the overdominant range ($\left|\hat{d}/\hat{a}\right| > 10$), for greater plant height were observed.

We did not detect significant flowering time or height associations at *ZmPR1* in the (B73⋆3)CML254 backcross population. Coles et al. (2010) reported that the differences between CML254 and B73 alleles at *ZmPR1* for flowering time and height were smaller than those at the other *ZmPR* loci. The relative weakness of the allelic effect along with the looser linkage between the *ZmPR1* QTL peak position and the markers tested in this study compared to the other QTL likely contributed to our inability to validate this QTL.

We next tested for significant marker-trait associations at the four *ZmPR* loci in each of the other three backcross populations (Tables 2, 3, and 4). Each of these populations had at least one significant *ZmPR* locus affecting flowering time and height traits. In the (B73⋆3)CML247 population, a significant additive effect at *ZmPR2* and a significant dominant effect at *ZmPR1* on flowering time were observed (Table 2). Significant additive or dominant effects for EH and PH were observed at *ZmPR1*, *ZmPR2*, and *ZmPR4* (Table 2). CML247 alleles at *ZmPR1* were associated with reduced PH, whereas those at *ZmPR3* were associated with increased PH, and all significant dominant effects contributed to increased height in heterozygotes. We also observed significant negative dominant effects on GDDASI at *ZmPR1* and *ZmPR4*, indicating overdominance ($\left|\hat{d}/\hat{a}\right| > 3$) for reduced ASI at these loci.

In the (B73⋆3)Ki3 population, we detected significant additive effects on flowering time at *ZmPR1*, *ZmPR2*, and *ZmPR4* (Table 3). Ki3 alleles were associated with later flowering time at *ZmPR1* and *ZmPR2* but earlier flowering time at *ZmPR4*. All four loci were associated with significant additive effects on height traits. The Ki3 allelic effects on height were opposite in sign to the effects on flowering time at the three loci that also had flowering time effects (Table 3).

In the (B73⋆3)Ki11 population, we detected significant additive effects on flowering time and height at

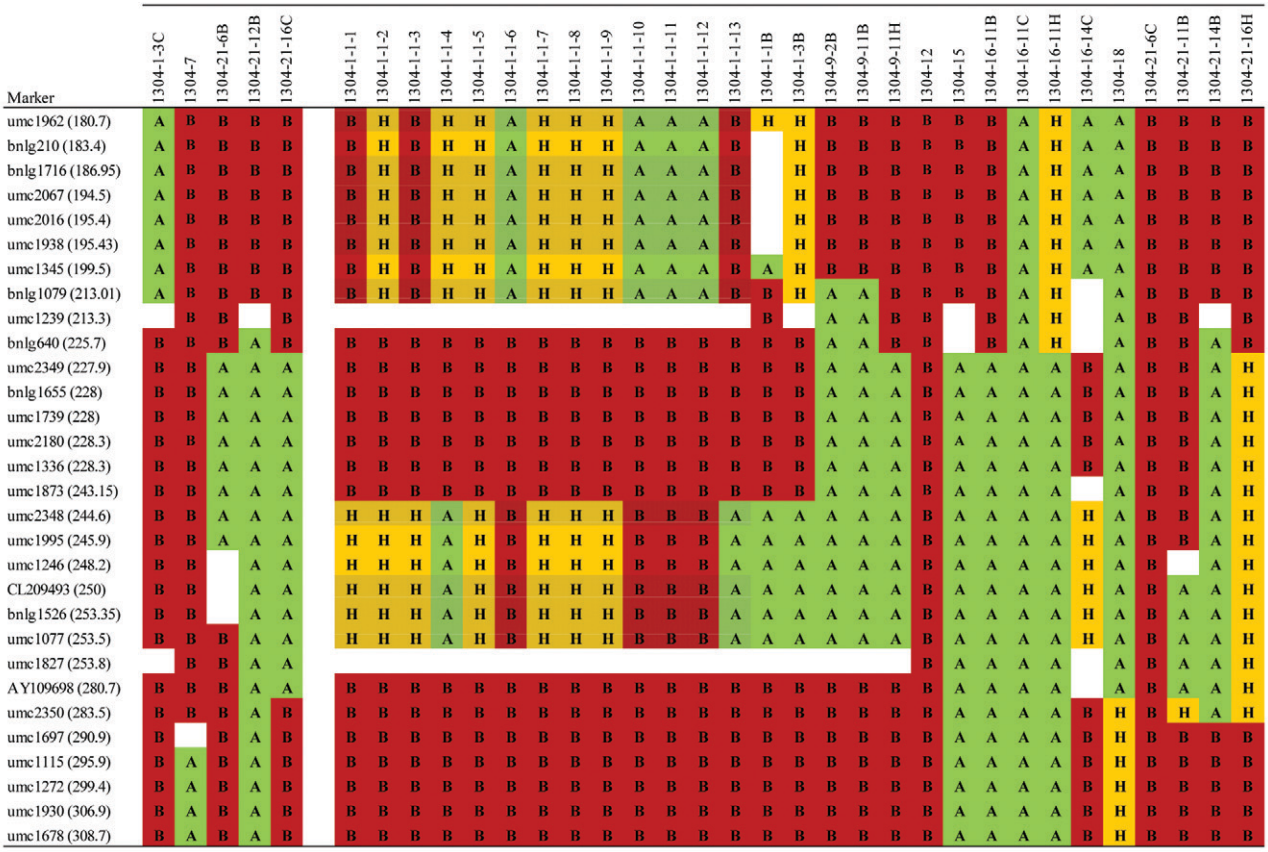

Figure 2. Graphical genotypes for heterogeneous inbred families (HIFs) tested in the 2009 field and phytotron experiments. The five leftmost HIFs displayed were tested in the field experiment; all HIFs shown were tested in the phytotron study. Loci that are homozygous for the B73 allele are denoted by "A," while loci that are homozygous for the CML254 allele are denoted by "B" and those heterozygous are denoted by "H." Shaded loci indicate interpolated marker scores. IBM2 Neighbors 2008 map (http://www.maizegdb.org/ [verified 11 Mar. 2011]; Sen et al., 2010) positions are indicated in parentheses next to each marker.

ZmPR1, *ZmPR3*, and *ZmPR4* (Table 4). Ki11 alleles were associated with later flowering time and increased heights at all three loci.

In general, fewer significant QTL effects were observed for GDDASI compared to GDDTA or GDDTS, and most of those effects were dominant, rather than additive, effects (Tables 1, 2, 3, and 4). Furthermore, all significant dominant effects on GDDASI were negative, indicating that heterozygosity at these QTL regions tended to be associated with reduced ASIs.

Our results accord well with the effect estimates of CML247, Ki3, and Ki11 QTL alleles from the maize NAM study of flowering under long daylengths (Buckler et al., 2009). *ZmPR4* on chromosome 10 was detected as the strongest flowering time QTL in that study as well as by Coles et al. (2010) and this study. Relative to the reference B73 founder allele, Buckler et al. (2009) estimated a strong effect of delayed flowering due to the Ki11 allele, no effect of the CML247 allele, and an effect of reduced time to flowering due to the Ki3 allele (labeled 10_42.9 in Fig. 4A of Buckler et al., 2009), in excellent agreement with the allele effects observed in the backcross lines in this study (Tables 2, 3, and 4). The allelic effects at all three founders at *ZmPR1* (corresponding to 1_84.6 in Fig. 4A of Buckler et al., 2009) were estimated to be weaker than *ZmPR4*, with Ki11 having the strongest effect, as we observed in the backcross lines (Tables 2, 3, and 4). CML247 and Ki3 had later flowering alleles than B73 but the Ki11 allele had no effect at *ZmPR2* (corresponding to 8_66.9 in Fig. 4A of Buckler et al., 2009) in NAM, and we obtained the same result (Tables 2, 3, and 4). Congruence of our results with those of Buckler et al. (2009) was limited at *ZmPR3* (labeled 9_62.2 in Fig. 4A in Buckler et al., 2009) where Ki3 and Ki11 had relatively strong late flowering alleles, and CML247 had a weaker late flowering allele in NAM, but we only detected the later flowering effect of the Ki11 allele in this study (Tables 2, 3, and 4).

We further showed that the phenotypic effects of tropical alleles at the *ZmPR* QTL are strongly influenced by the photoperiod of the evaluation environment. We tested introgression lines carrying CML254 or Ki11 alleles at the target QTL regions in a short daylength environment and observed no significant additive effects at the four QTL and flowering time in this environment (Supplemental Tables S2 and S3). One marker linked to the CML254 allele at *ZmPR4* was associated with increased EH and PH and one marker linked to the Ki11 marker at *ZmPR3* was associated with reduced PH

(Supplemental Tables S2 and S3), but otherwise these QTL did not affect flowering or height under short daylengths.

Ki14 × CML254 F_2 Population

The phenotypic ranges of traits measured in the Ki14 × CML254 F_2 population were large: 28 d (419 GDD) for DTA, 35 d (544 GDD) for DTS, 90 cm for EH, and 95 cm for PH. The substantial variation observed suggests that distinct allelic combinations in the tropical inbred lines Ki14 and CML254 strongly affect flowering time and PH. These two tropical inbred lines are both extremely photoperiod sensitive but differ for flowering time in both long and short daylength environments (Coles et al., 2010). Furthermore, CML254 and Ki14 allelic effects for flowering and height under long daylengths at some of the *ZmPR* QTL were different in temperate × tropical mapping populations (Coles et al., 2010). Thus, functional allelic differences at least at some *ZmPR* loci are predicted to contribute to flowering time variation in the F_2 population derived from the cross of Ki14 and CML254. The current study provides the first direct comparison of the effects of the Ki14 and CML254 alleles on flowering time and height in a common population.

In the *ZmPR4* region, we found significant additive effects on GDDTS, whereby CML254 decreased time to silking and ASI relative to the Ki14 allele (Table 5). This difference agrees with previous results by Coles et al. (2010), who estimated similar effects for these two *ZmPR4* alleles on GDDTA but a numerically greater increase in GDDTS and a statistically significant increase in GDDASI caused by Ki14 compared to CML254 relative to the temperate founder alleles. Coles et al. (2010) also estimated significantly stronger effects of CML254 than Ki14 alleles on delayed anthesis at *ZmPR1-3*. Those differences were not observed in this study, likely due to low power of detection caused by small sample size, unreplicated single plant phenotyping, and limited marker coverage, but the CML254 allele at umc1735 near *ZmPR2* was associated with longer time to anthesis at $p < 0.07$.

Significant negative dominant effects were observed for both GDDTA and GDDTS at *ZmPR4*, indicating that heterozygotes flowered earlier than either homozygous class and suggesting repulsion phase linkage of later flowering alleles at multiple causal genes in this region. *ZmPR2* was associated with significant additive effects on EH and PH, with CML254 alleles being associated with increased PH (Table 5). This agrees with the estimated allelic effects on height under long daylengths at the chromosome 8 QTL linked to *ZmPR2* in the temperate × tropical mapping populations studied by Coles et al. (2010).

Heterogeneous Inbred Family Experiments

We had sufficient seed of five HIF lines for replicated field evaluations in North Carolina, Missouri, New York, and Wisconsin (Fig. 2). The lines flowered very late in New York, so data were not available from this location. In the combined analysis across the other three locations, all HIFs were significantly later flowering (DTA or DTS) and taller (EH or PH) and had more leaves than B73 (Table 6). The HIF flowering phenotypes fell into two distinct and significantly different groups. Heterogeneous inbred families 1304-1-3C and 1304-7 flowered 4.5 to 7.4 d later than HIFs 1304-21-6B, 1304-21-12B, and 1304-16C (Table 6). These two groups consistently differed for allelic constitution at markers defining an interval on the intermated B73 × Mo17 population (IBM) map from positions 225.7 to 253.5 (equivalent to about 7 cM; Balint-Kurti et al., 2007). The two lines homozygous for CML254 alleles in this region represented the later flowering group, whereas the three lines homozygous for B73 alleles in this region represented the earlier flowering group (Fig. 2). The two later flowering lines differed from each other genotypically at markers flanking this region, providing further evidence that the flowering time QTL is delimited by this 7 cM region (Fig. 2). Some pairs of HIFs in the two groups did not differ for PH and EH, and no variation for leaf number was observed among these HIFs (Table 6).

Sufficient seed was available to conduct a replicated controlled long-daylength environment evaluation of a larger group of HIFs, including the same five used in the field evaluation. Three recombination events within the 7-cM QTL region defined in the field study were represented in this larger group of HIFs (Fig. 2). Tassel emergence, anthesis date, and PH varied significantly among the HIFs evaluated in the growth chamber and greenhouse study. The flowering time QTL region identified in the field study also had highly significant effects on flowering time in the controlled environment study (Fig. 3). The additional recombinations represented by the HIFs in this study further delimited the flowering time QTL region to three consecutive markers (umc2348, umc1995, and umc1246), encompassing IBM positions 244.6 to 248.2, an interval slightly less than 1 cM (Fig. 3). In this region, plants homozygous for the CML254 allele flowered about 6 d later than the plants homozygous for the B73 allele. Two smaller QTL peaks were observed for PH, one at the same position as the flowering time QTL and a second QTL near IBM position 283 (Fig. 3). At both QTL, the homozygotes with CML254 alleles were taller than B73 homozygotes. These results provide further evidence for the presence of two separate QTL in the region, affecting flowering time and PH differently, as suggested by Coles et al. (2010).

These HIF results are consistent with the position of a strong flowering time or photoperiod response QTL in this region (Buckler et al., 2009; Coles et al., 2010; Moutiq et al., 2002; Wang et al., 2008), which Ducrocq et al. (2009) fine-mapped to a 170-kb interval around 94.0 Mbp on the maize AGPV1 physical map (www.maizesequence.org [verified 4 Mar. 2011]; Schnable et al., 2009), including sequences just upstream of a homolog of *Ghd7*, which controls flowering

Table 5. Additive (*a*)† and dominant (*d*)‡ effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in Ki14 × CML254 F_2 plants evaluated in long daylength conditions in summer 2007.

Chromosome	Marker	IBM2§ position	GDDTA¶		GDDTS		GDDASI		EH		PH	
			a	*d*	*a*	*d*	*a*	*d*	*a*	*d*	*a*	*d*
		IcM#	growing degree days						cm			
ZmPR1												
1	dupssr26	391.1	–6.4	12.6	13.2	51.7*	16.3	33.6*	–2.3	1.5	–2.0	2.8
ZmPR2												
8	phi100175	274.9	11.8	14.8	21.2	34.6	13.8	14.2	5.8*	0.6	6.6*	–1.8
8	umc1735	279.9	18.8	–4.8	27.6	–5.2	4.1	–5.1	5.2*	–0.9	8.4**	–3.5
ZmPR3												
9	umc2337	220.1	14.6	2.5	3.6	–6.8	–10.9	–3.6	0.3	–4.3	5.0	–5.3
9	umc1078	322.6	15.6	0.9	2.8	–0.1	–9.2	–2.9	0.3	4.6	3.5	7.8
ZmPR4												
10	umc2067	194.5	3.4	–27.9	–39.6*	–39.8	–36.8**	–18.1	–4.4	3.3	–3.7	4.8
10	umc1246	248.2	10.1	–36.2*	–31.6	–47.6*	–34.8**	–18.3	–2.6	2.5	–1.2	3.9
10	umc2350	283.5	8.7	–32.9*	–37.9*	–39.0	–39.9***	–12.8	–2.0	–2.5	–0.1	4.3

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

†Additive effect estimated as half the difference between homozygous CML254 class and homozygous Ki14 class means.

‡Dominant effect estimated as the difference between heterozygous class mean and the average of the two homozygous parental class means.

§IBM map unit (Balint-Kurti et al., 2007).

¶GDDTA, growing degree days to anthesis; GDDTS, growing degree days to silking; GDDASI, growing degree days anthesis-silking interval; EH, ear height; PH, plant height.

#IcM, intermated centiMorgan.

Table 6. Least square means of heterogeneous inbred family and check entry lines across three environments for days to anthesis (DTA), days to silk (DTS), anthesis-silk interval (ASI), ear and plant heights, and total leaf number.

Line	DTA	DTS	ASI	Ear height	Plant height	Leaf number
	d			m		
1304-1-3C	86.7 a†	87.9 a	1.0 ab	1.31 a	2.32 a	27.0 a
1304-7	86.2 a	88.1 a	1.8 b	1.27 ab	2.31 ab	27.1 a
1304-21-6B	81.5 b	81.0 b	–0.7 c	1.26 ab	2.25 ab	26.5 a
1304-21-12B	81.7 b	81.1 b	–0.6 c	1.23 ab	2.26 ab	26.6 a
1304-21-16C	81.3 b	80.7 b	–0.7 c	1.16 b	2.17 b	26.4 a
B73	69.8 c	69.8 c	–0.1 ac	0.81 c	1.66 c	20.1 b

†Least square means followed by the same letter within a trait are not significantly different at the 0.05 probability level.

time in rice (Xue et al., 2008). All three of these studies involved distinct photoperiod-sensitive founder lines, suggesting that photoperiod-sensitive QTL alleles at this region are common in tropical maize although not necessarily ubiquitous or functionally identical, as demonstrated in this study.

CONCLUSION

The *ZmPR* loci identified by Coles et al. (2010) were found to have significant effects in our B73 × CML254 backcross population, with the exception of *ZmPR1*. These results demonstrate the veracity of these QTL and their strong additive effects in a B73 background. Across a diverse set of temperate × tropical populations, many of the *ZmPR* QTL were also detected, but we observed significant variation among tropical founder alleles for their effects on flowering time and height under long daylength photoperiods, including one tropical-derived allele at the most important photoperiod response QTL (*ZmPR4*) that reduced time to flowering relative to the B73 allele. We directly compared functional effects of alleles of the two photoperiod sensitive tropical lines Ki14 and CML254 and observed significant differences between them for flowering time at *ZmPR4* and for height near *ZmPR2*. These results corroborate the conclusions made by Buckler et al. (2009) and Coles et al. (2010) that allelic series are common among diverse maize lines, although with the current level of QTL resolution we cannot discount the possibility that variation among the effects of a single chromosomal region from different lines is due to variation in the linkage arrangements of two or more tightly linked biallelic causal genes. Nevertheless, these results provide an explanation for how Holley and Goodman (1988) were able to select lines with significantly reduced photoperiod sensitivity from crosses between highly photoperiod-sensitive tropical parents. The substantial functional variation among tropical lines at these key photoperiod response QTL will complicate marker-assisted selection for reduced photoperiod response across very diverse samples of temperate × tropical crosses.

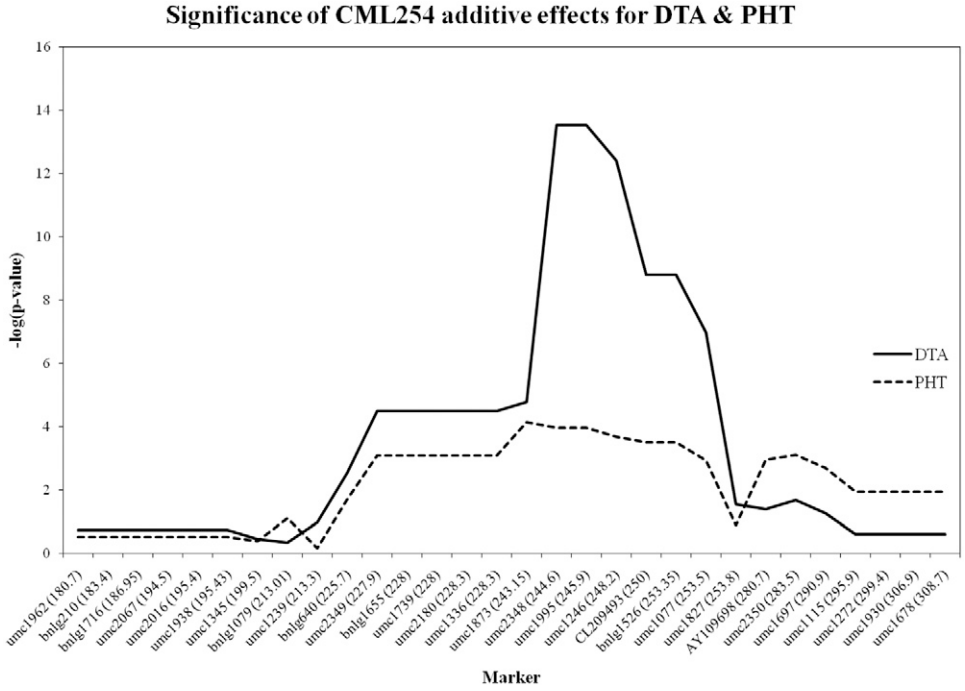

Figure 3. Significance (negative log of *p*-value) of additive effects at simple sequence repeat (SSR) loci in the ZmPR4 quantitative trait loci (QTL) region for days to anthesis (DTA) and plant height (PHT). Markers are ordered according to the IMB2 2008 Neighbors map, and positions are indicated in parentheses to the right of the marker names.

Supplemental Information Available

Supplemental material is available free of charge at http://www.crops.org/publications/cs.

Supplementary Table S1. Distribution of the number of BC_2-derived families within each independent BC_1 lineage from each of four backcross populations used to estimate allelic effects at four major photoperiod quantitative trait loci (QTL).

Supplementary Table S2. Additive and dominant effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)CML254 $BC_2F_{3:4}$ lines evaluated in short daylength conditions in winter 2007.

Supplementary Table S3. Additive and dominant effect estimates associated with markers linked to *ZmPR* quantitative trait loci (QTL) in (B73*3)Ki11 $BC_2F_{3:4}$ lines evaluated in short daylength conditions in winter 2007.

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