Identification of quantitative trait loci associated with flowering, vegetative traits and photoperiod response in maize (Zea mays L)

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Identification of quantitative trait loci associated with flowering, vegetative traits and photoperiod response in maize (Zea mays L.)

by

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A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Major: Plant Breeding

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This is to certify that the doctoral dissertation of

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has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

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For the Major Program
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I would like to start by thanking my husband, El Hassane Bensellam, and my parents, Fatima and El Houcine Moutiq, for providing me with encouragement and support during my study. I would like also to thank my kids Nora and Sami for being patient when I was busy studying. I will not forget to thank my sisters and brothers for their support.

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In general, maize, especially germplasm from the tropics and subtropics, is sensitive to photoperiod. This sensitivity hindered the exchange of germplasm between latitudes. To identify quantitative trait loci (QTL) associated with the response to photoperiod, a population of 236 F3 lines produced from a cross between a photoperiod-sensitive line CML9 and insensitive inbred A632Ht was used. These F3 lines were evaluated in three long and three short-day environments, in adjacent fields using artificial light, and in fields located in different latitudes, Mexico and Iowa. Days from sowing to anthesis (DTA), final leaf number (FLN) and plant height (PH) were measured. For each of these traits, photoperiod response (PPR) was estimated as the difference between the trait in long- and short-days divided by the trait in short-days. Composite interval mapping was used to detect QTL for each trait and comparison of locations of QTL detected in different daylengths for the same trait and for different traits were examined. A unique set of QTL was detected for each photoperiod and for each trait. One QTL for DTA, three QTL for FLN and four QTL for PH were detected in the same genetic regions in both daylengths. Five QTL for DTA, four QTL for FLN and three QTL for PH were detected only in long-day environments. Nine QTL for DTA, five QTL for FLN and three QTL for PH were detected only in short-day environments. QTL for photoperiod response were detected on four chromosomes for PPR_{DTA}, on three chromosomes for PPR_{FLN} and on three chromosomes for PPR_{PH}. Chromosomes 2, 3, 4, 5, 6, 8, 9, and 10, had a cluster of QTL for different traits. This might suggest a common initial mechanism with subsequent specific pathways that regulate different traits.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Plants, including maize, have improved the chances of survival by their offspring in fluctuating environments by ensuring that their reproductive development is triggered and controlled by specific environmental signals such as photoperiod (Evans, 1987; Roberts and Summerfield, 1987). Photoperiod response of plants, first reported by Garner and Allard in 1923, is generally defined as the response of a plant to the length of the day or the phenomenon whereby daylength regulates flowering (Vince-Prue, 1975).

The center of origin of maize is the tropics where genetic variability is found. With the movement of people after the 16th century, maize was grown in a wider range of climates ranging from tropics and subtropics to temperate areas from 58° N and 40° S (Hallauer and Miranda, 1981). In these environments, plants synchronize flowering with a period of favorable conditions such as availability of rain, in order to assure a good seed production. Maize is generally recognized as a short-day plant but some populations have the ability to grow in long-day environments. Tropical and subtropical maize are grown in short-day environments and are highly sensitive to photoperiod changes in the range of 13 to 16 hours (Russell and Stuber, 1983; Ellis et al., 1992). In long-day environments, this type of maize flowers when daylength begins to shorten (Francis et al., 1969). Materials developed in temperate areas might have been selected naturally or artificially to avoid or reduce the flowering delay in long-days (Hunter et al., 1974). The sensitivity to daylength has generally
hindered the transfer of tropical maize to higher latitudes (Kaan and Derieux, 1986), and slowed the use of temperate maize in the tropics (Salamini, 1985).

Quantitative genetic control of photoperiod sensitivity in maize has been reported but not yet thoroughly investigated. Few genes with major effects seem to control photoperiod sensitivity in maize. The response to selection suggested the presence of few genes with major effects. Classic quantitative genetic methods such as mating designs identified two to nineteen genetic factors controlling flowering. Few genes that affect flowering have been identified in maize such as \textit{Idl} and \textit{D8} while in Arabidopsis 80 genes are known. QTL studies identified at least one QTL for anthesis and for plant height on each of the maize chromosomes especially in long-day environments and QTL for final leaf number on most chromosomes. However these QTL were not related to photoperiod.

Understanding the inheritance of this sensitivity would be of interest for applied and basic research. Identification of genes or loci involved in photoperiod sensitivity might be used in marker-assisted selection programs for the development of maize adapted to a wide range of environments. Identification of candidate genes related to QTL and their cloning can clarify the mechanisms and pathways controlling flowering time and photoperiod sensitivity.

The objectives of this study were to map the genetic factors associated with (i) anthesis (DTA), (ii) plant height (PH), (iii) final leaf number (FLN), in long and short-days and (iv) to map those associated with the photoperiod response related to DTA, FLN, and PH in the \textit{CML9 x A632Ht} \textit{F3} maize population, and (v) to determine the gene effect associated with each these traits.
Dissertation organization

This dissertation includes a general introduction, two manuscripts, and a general conclusion. The first manuscript reports QTL related to anthesis and photoperiod response and will be submitted to Genetics. The second manuscript identified QTL related to plant height and final leaf number and photoperiod responses measured using each of these two vegetative traits. This second manuscript will be submitted to Crop Science. Each manuscript was written in a paper format and has an introduction, materials and methods, results, and discussion. References are listed after each paper and after the literature review.

Literature review

Factors affecting plant growth and development

The seasonal changes are marked by cyclical changes in many components of the environment such as temperature and the length of the day. Generally, variation in temperature and daylength are crucial in controlling development rates and duration in maize.

Light and photoperiod

Light quality and quantity are critical environmental factors affecting plant development. The intensity of illumination as well as its duration have been considered important since both constitute the quantity of light energy (Thomas, 1991). Plants are highly sensitive to very small changes in light intensity and to infrared and ultraviolet. These
changes in light trigger normal plant growth and development, which is termed photomorphogenic response.

The formation of the mature chloroplast in angiosperms requires light. When seedlings are germinated in darkness, the protoplasts develop into etioplasts, which lack most photosynthetic enzymes and they are not photosynthetically competent. However, when exposed to light, etioplasts are rapidly converted into chloroplasts. In light grown seedlings, protoplasts develop directly into chloroplasts. Light might overcome an inhibitory factor that is produced in the dark, blocking leaf and chloroplast development. Chloroplasts are self-replicating organelles that contain their own genome. The chloroplast genome participates in its own development. However, the majority of chloroplast proteins are encoded by nuclear genome. The development of the chloroplast requires the coordinated expression of genes in both compartments (Fosket, 1994).

Organisms are normally subjected to daily cycles of light and dark. Plants possess a mechanism that can determine the time of day at which a particular event occurs. Often, plants exhibit rhythmic behavior in association with light and dark alternation, such as leaf movement (position), stomatal and petal movements, and metabolic processes including photosynthetic capacity and respiration rate (Brady, 1982). Bunning (1973) proposed that the control of flowering by photoperiod is achieved by an oscillation of phases with different sensitivities to light. Light has two distinct roles in the flowering process. Light signals at dawn and dusk set the phase of the photoperiodic rhythm. In addition, the rhythm has a light-sensitive phase called the inducible phase. When light is received during the inducible phase of the rhythm, the effect is to promote or prevent floral induction. The photoperiod response is defined relative to a critical value of daylength which has been defined as the
maximum daylength at which a short-day plant will flower and the minimum daylength at
which a long-day plant will flower (Major, 1980).

The response to daylength is localized in the apical meristem and leaves (Vince-Prue,
1985). Buds were also reported as sites of photoperiod perception (Jacobs and Sutlers,
1974). After inductive daylength for floral initiation occurs, a graft-transmissible substance
is produced and then transported via phloem to terminal and lateral apical meristems that
changes their developmental potential and initiates flowering (Lang et al., 1977). That
transmissible substance was suggested to be a flowering hormone that was named florigen.
Under non-inductive photoperiods, leaves produce substance(s) that inhibit flowering (Lang
et al., 1977), which was suggested to be antiflorigen (Thomas and Vince-Prue, 1997). The
chemical nature and even the existence of florigen and antiflorigen remain speculative
(Fosket, 1994). Failure to identify a single substance responsible for flowering induction or
inhibition, after many years of research, suggest a complex mixture of substances. A
multifactorial model with a number of promoters and inhibitors is more probably controlling
the developmental transition (Bernier, 1988).

In plants, daylength controls numerous traits including flowers initiation and
development, asexual reproduction, storage organs formation, dormancy onset, stem height
and diameter, leaf number and size, and top-root ratio (Vincent-Prue, 1975; Garner and
Allard, 1923; Bonhomme et al, 1991; Warrington and Kanemasu, 1983b; Allison and
Daynard, 1979; Struik, 1982; Russell and Stuber, 1983). The photoperiodic response of final
number of leaves (FLN) in maize has long been recognized (Kiesselbach, 1950). An increase
of FLN from zero (Francis, 1973) to 2.5 (Arnold, 1969), for each hour increase in
photoperiod per day was reported. In some exotic races, up to nine leaves increase was
observed (Stevenson and Goodman, 1972). Alterations in the total number of phytomers could induce nonadditive changes in PH and anthesis time (Vlăduțu et al., 1999).

Temperature

Because daylength is an ambiguous signal that can not distinguish between spring and fall, plants exhibit several strategies to avoid this ambiguity. One is the interaction between photoperiod and temperature (Blondon and Gallais, 1976). The effects of photoperiod and temperature are therefore, not additive but together they affect maize more than either alone (Stevensen and Goodman, 1972).

Contradictory results of the effect of temperature alone on maize growth and development were reported. The rate of reduction in time to anthesis declined continuously as temperature increased from 14 to 28 °C (Warrington and Kanemasu, 1983a). Kiniry (1991) reported that the rate of leaf tip appearance increased linearly with temperature to optimum temperature (To) and declined linearly with temperatures beyond To. Generally, the optimum temperature is estimated to be 30 °C (Bonhomme et al., 1994). Other studies reported an increase in leaf number with temperatures between 15 to 30 °C, resulting in average rates of increase of 0.13 to 0.44 leaves per one degree Celsius increase (Warrington and Kanemasu, 1983b). An increase of 0.04 to 0.26 leaves per degree Celsius increase was observed (Bonhomme et al., 1991). Increasing temperature from 8.6 to 17.2 °C increased FLN by three leaves (Coligado and Brown, 1975). Hesketh et al. (1969) observed an increase in FLN by 2.1 after an increase of the greenhouse temperature by 6 °C and a decrease of temperature by 9 °C caused a decrease in FLN by 1.7. FLN tended to be affected by temperature and daylength for strains with the most leaves (Hesketh et al., 1969). Other
studies found little change in leaf number in response to temperature (Hunter et al., 1974; Aitken, 1977; Manrique and Hodges, 1991). The response to temperature seems therefore cultivar-specific.

FLN indicated that photoperiod sensitivity was greater at higher temperatures. However, the temperature effect is less important on FLN than on other traits such as days to tassel initiation (Russell and Stuber, 1983). Moreover, photoperiod has larger effect than temperature on FLN (Stevensen and Goodman, 1972; Russell and Stuber, 1983). FLN is also much easier to score and it has been suggested as a better trait to measure the sensitivity to photoperiod (Russell and Stuber, 1983).

**Stage of development**

The sensitivity of plants to environmental stimuli, especially daylength, depends on the age of the plant. FLN in maize is affected by cold treatment after the emergence of five to seven leaves and the greatest reduction occurred when the third leave started to emerge from the whorl (Hesketh et al., 1969). Daylength has no effect on floral induction until plants have attained a minimum amount of growth, that is known as the basic vegetative phase (Major, 1980) or juvenile phase (Grant, 1989). Tollenaar and Hunter (1983) found that FLN is determined by photoperiod only between the 5 to 6 leaf stages regardless of temperature and a short photoperiod-insensitive phase just before tassel initiation was observed. Therefore, the timing of flowering is the result of the interaction between environmental factors, which signal the conditions favorable for the success of reproductive development, and the endogenous developmental competence of the plants.
**Photoreceptors**

Physiological, photobiological and molecular genetic studies have demonstrated that plants possess distinct photoreceptors sensing UV-B, UV-A, blue, green, red, and far-red light (Kendrick and Kronenberg, 1994). The known photoreceptors are phytochromes, cryptochromes, phototropin and superchrome. The photoreceptors of UV-B are not yet identified (Casal, 2000).

Phytochromes, the most intensively studied photoreceptors, consist of dimeric chromopeptides that possess two photoconvertible forms: Pr (red light absorbing molecule) and Pfr (far-red absorbing molecule). Synthesis is in the form of Pr (inactive molecule) that is transformed to its active form Pfr upon exposure to red light. Far-red light is able to back-transform Pfr to Pr. In general, phytochromes are responsible for several light mediated responses throughout the plant life cycle. Phytochromes are involved in regulation of internode elongation, inhibition of hypocotyl elongation and stem growth, expansion and positioning of the cotyledons, greening of the plant (initiation of chloroplast development), synthesis of anthocyanins, and induction of seed germination. More importantly, phytochromes control floral induction and circadian rhythm otherwise photoperiod response, most probably through their role in regulating gene expression (Smith, 1995; Fosket, 1994). Phytochromes are involved in various phytochrome signal transduction pathways and can be transcriptional regulators (Briggs and Olney, 2001).

Five different phy gene families (phyA, phyB, phyC, phyD, and phyE) have been identified in *Arabidopsis thaliana*. Sequence comparison of these genes have suggested that divergence of phyA, phyB and phyC genes preceded the divergence of the monocots and
dicots and predicted that all angiosperms have genes in these families (Quail, 1991). In maize, phyA1 is the only cloned photoreceptor gene. That gene had 88% amino-acid identity with the rice (Oriza sativa) type A phytochrome and 65% with the Arabidopsis phyA (Christensen and Quail, 1989). Probed sites phyA2 and phyB1, that might correspond to photoreceptor genes, were mapped in maize using probes produced from oat (Avena) and rice phytochromes (Christensen and Quail, 1989; Dehesh, et al., 1991). In Arabidopsis, phyA is involved in photoperiod measurement. The photoreceptors phyB, phyC, phyD, and phyE control flowering time and are more related to the shade avoidance in response to low-red/far-red ratio than to the measurement of photoperiod (Devlin et al., 1998 and 1999).

Cryptochromes are receptors of blue light and UV-A radiation. Two families (cry1 and cry2) have been identified in Arabidopsis thaliana (Casal, 2000). Mutants lacking cry2 in long-day plants showed reduced or no acceleration of flowering in response to extended photoperiods (Guo et al., 1998). Thus, cry2 was suggested to be involved in photoperiod measurement. Phototropin is a protein that mediates phototropic responses to the direction of blue light, UV-A or even green light. At least two phototropin receptors appear to mediate the primary photoreception of directional blue light cues in dark-grown plants. PhyA and phyB may also be involved in the detection of lateral light to maximize the adaptive advantage of the phototropic response (Liscum and Stowe-Evans, 2000). A photoreceptor, phytochrome 3 (phy3), also named superchrome has been identified that contains both phytochrome and phototropin sequence motifs (Briggs and Olney, 2001). The list of plant photoreceptors is still incomplete and, to our knowledge, both cryptochromes and phototropin are not yet identified in maize.
Epistatic interactions between different photoreceptors were observed. Irradiation with blue or far-red light enhanced the subsequent response to red light (Meijer and Engelsma, 1965). Interactions among photoreceptors in Arabidopsis were observed and resulted in a 70% reduction of the response to photoperiod (Mazzella and Casal, 2001). Five types of interactions between known photoreceptors have been established: 1) antagonism between phyA and phyB under red light, 2) synergism between phyA and phyB under far-red light followed by brief red light), 3) synergism between phyB and cry1, 4) synergism between phototropin and phyA, phyB, cry1 and cry2 and 5) antagonism between phyB and cry2 (Casal, 2000).

A strong association between phytochromes and QTL for plant height was observed, but this association was minimal with QTL for flowering time in sorghum (Sorghum bicolor) (Lin et al., 1995). In maize, phyA1 and phyA2 are both closely associated with height mutants. phyB was associated with height QTL in maize and sorghum (Lin et al., 1995). In rice, phyB was associated with QTL for height and flowering time (Li et al., 1995). The same region (phyB) in rice accounts for one or two genes regulating photoperiodic male sterility (Zhang et al., 1994).

**Genetics of photoperiod response**

**Photoperiod and flowering time**

Photoperiod response is a quantitative trait with preponderance of additive gene action (Russell and Stuber, 1985). The transition from vegetative to reproductive stage is the result of the activation of genes responsible for inflorescence and floral organ formation that
control the identity of the apex and the morphogenesis of the floral organs (Simpson et al., 1999).

In maize, classic quantitative genetic methods, such as mating designs or generation mean analyses, identified 2 to 19 genetic factors controlling flowering with most studies detecting four to six (Giesbrecht, 1960; Hallauer, 1965). The gene effects related to flowering ranged from additive to complete dominance with a prevalence of additive effects (Giesbrecht, 1960; Hallauer, 1965; Russell and Stuber, 1983). The dominance effect, when observed, was towards earliness. Epistatic effects were also detected (Giesbrecht, 1960).

Few genes with major effects seem to control photoperiod sensitivity in maize. Using mass selection, response to selection for earliness in a late-flowering population was 3.8 days per cycle of selection (Hallauer and Sears, 1972). Using DNA marker loci, selective introgressions of alleles for early flowering into late maize lines, were observed on chromosomes 1, 2, 6, 8, 9, and 10 (Koester et al., 1993) and on chromosomes 1, 6, and 8 (Vlăduțu et al., 1999). The response to selection and the selective introgression of DNA fragments supported the presence of few genes with major effects and the prevalence of additive effects.

Quantitative trait loci is a method that relates the phenotypic variation to the allelic variation on the whole genome basis. At least one QTL for anthesis was identified on each of the maize chromosomes especially in long-day environments (Zehr et al., 1992; Koester et al., 1993; Austin and Lee, 1996). Koester et al. (1993) suggested that a QTL on chromosome 8 might be involved in photoperiod response. Two QTL for flowering were detected on the long arm of chromosome 8 and both were involved in controlling the transition of the apical meristem from vegetative to generative structure (Vlăduțu et al.,
A major QTL, named \textit{Vgt1} (\textit{Vegetative to generative transition 1}) and previously mapped to a 5 cM interval on chromosome 8 (Vláduțu et al., 1999) was narrowed to 0.03 cM. Map-based cloning of the gene responsible for that QTL is in process (Salvi et al., 2002). Nourse (2000) detected a QTL on chromosome 10 that is involved in photoperiod-sensitivity.

Few genes that affect flowering have been identified in maize. The gene \textit{indeterminate 1} (\textit{Id1}) delays flowering time and encodes a zinc-finger protein with similarities to animal transcription factors (McSteen et al., 2000). By analogy to the \textit{Arabidopsis} model, the \textit{Id1} gene was classified in the autonomous flower promotion pathway (McSteen et al., 2000). The gene \textit{Dwarf8} (\textit{D8}) produces short and compact plants with short internodes and is not affected by gibberellins. \textit{D8} encodes proteins that resemble nuclear transcription factors and has 62\% amino-acid sequence identity to \textit{GAI} (\textit{Arabidopsis Gibberellin Insensitive}) gene (Peng et al., 1999). The \textit{D8} gene maps to the maize chromosome 1 and has been cloned. Association mapping related \textit{D8} with flowering time in maize inbred lines from stiff-stalk, non stiff-stalk, tropical and semi-tropical groups (Thornsberry et al., 2001). The maize cloned genes \textit{Zag1}, \textit{Zmm2} and \textit{silky1} belong to the MADS-box gene family that is known to have a wide range of functions including the control of flowering time (Ng and Yanofsky, 2001). Moreover, their orthologous genes in \textit{Arabidopsis} are known to control flowering time (Simpson et al., 1999).

In other cereals, three classes of genes controlling flowering time are known: vernalization genes, photoperiod genes and 'earliness per se' genes that control flowering independently from the environment. Other mutations have been reported and have not been related to any of these three classes. In barley, loci for 'earliness per se' (\textit{ea}, \textit{ea}$_\text{sp}$, \textit{ea}$_c$, \textit{ea}$_k$
and ea7) were detected as loci controlling flowering and/or photoperiod sensitivity (Nilan, 1964; Takahashi and Yasuda, 1971; Gallagher et al., 1991; Von wettstein-Knowles, 1992). Two additional genes Ppd-H1 and Ppd-H2 were associated with photoperiod response in barley (Hordeum vulgare L.) (Laurie et al., 1994). In wheat (Triticum aestivum L.), Ppd1, Ppd2 and Ppd3 were identified as major loci for photoperiod sensitivity (Law et al., 1978) and seem to be homeologous to Ppd-H1 loci in barley (Laurie et al., 1994).

In Rice, E1-E3, Se2-Se5, Se-In, Se-1u, I-Se-1, and En-Se-1 were identified as photoperiod-sensitive genes (Okumoto and Tanisaka, 1997). Hdl and Hd6 are two additional photoperiod-sensitive genes that have been identified by QTL mapping and isolated by map-based cloning (Takahashi et al., 2001; Yano et al., 2000). Sel was found to be allelic to Hdl (Yano et al., 2000). The photoperiod response gene, Se5, was also cloned and was suggested to function in the phytochrome-chromophore biosynthesis (Izawa et al., 2000).

In sorghum, four genes called maturity genes Mal, Ma2, Ma3 and Ma4 were associated with flowering time (Morgan 1994). Mal and Ma2 were the most photoperiod-sensitive genes. Three classes of genotypes having different combination of alleles from these four genes were identified: strongly, moderately and not delayed flowering. The dominant Mal allele confers increased photoperiod-sensitivity (4.5 leaves per hour increase in daylength) compared with the homozygous mal (1.4 leaves per hour of daylength; Major et al., 1990). The Ma2 gene was implicated in a 'photoperiod x temperature' interaction, although the exact role was not clear. The Ma3 locus seems to regulate gibberellin concentration (Morgan 1994). The Ma3 gene has been cloned and was found to be the phyB
gene (Childs et al., 1997). Two additional photoperiod-sensitive genes were identified: \textit{Ma5} and \textit{Ma6} (Rooney and Aydin, 1999).

In \textit{Arabidopsis}, currently 80 genes are known to be involved in controlling flowering time. These genes could be involved from the perception of the environmental stimulus to the activation of floral meristem identity genes in the apex. The related genes in maize and their effects on phenotypic variation have not been identified. These genes are involved in one or more of the multiple pathways that control flowering time: 1) photoperiod promotion, 2) autonomous promotion, 3) gibberellic acid (GA) promotion, 4) vernalization promotion, and 5) the floral pathway integrators (Simpson et al., 1999; Simpson and Dean, 2002). The photoperiod promotion pathway initiates flowering in response to photoperiod through a number of genes that sense and respond to daylength. The autonomous promotion pathway includes the genes that promote flowering independently from any environmental signals. The autonomous pathway may control the juvenile phase and assure that plants can not flower until they reach the minimum vegetative phase. However, this hypothesis needs to be confirmed (Simpson and Dean, 2002). The gibberellic acid (GA) promotion pathway plays a role in flowering based on signals mediated by GA. The application of GA accelerates the flowering time of wild-type plants under short-days and of the late-flowering mutants under long-days. Under noninductive photoperiods, the \textit{gal} mutant does not flower unless provided with GA. The vernalization pathway accelerates flowering after exposure to cold temperatures. Vernalization was observed in late-flowering ecotypes of \textit{Arabidopsis} and in mutants of the autonomous promotion pathway. Vernalization substitutes for a lack of the autonomous pathway genes. Photoperiod has little effect on vernalization. The floral pathway repressors include genes whose expression or function is regulated by more than
one of the above pathways. A large number of floral repressors was also identified from early flowering mutants (Simpson and Dean, 2002). These genes likely regulate chromatin structure and protein degradation (Simpson and Dean, 2002).

**Photoperiod and vegetative traits**

The interval from planting to anthesis was subdivided to two phases: (i) phytomers initiation and (ii) stem elongation (Vlăduțu et al., 1999). The phytomers initiation corresponds to the interval from planting to the transition of the apical meristem to generative stage. The stem elongation phase corresponds to the interval from tassel initiation to anthesis. The delay in anthesis was primarily due to the delay in the transition of the apical meristem, which enables production of extra phytomers and also to the elongation of the additional internodes produced (Vlăduțu et al., 1999). Genotypes insensitive to photoperiod have approximately the same FLN regardless of photoperiod (Hunter et al., 1974). Based on the apical meristem development, three phases of development have been defined: juvenile, inductive, and reproductive. The number of leaves is determined by the duration of juvenile and inductive phases (Grant, 1989). Embryonic leaves in most mature kernels vary from five to six (Abbe and Stein, 1954; Sass, 1951). Maize is not sensitive to photoperiod until four leaves have emerged and FLN sensitivity to photoperiod was observed during five to six leaf stages regardless of temperature (Tollenaar and Hunter, 1983). A short photoperiod-insensitive phase just before tassel initiation was also observed in maize (Tollenaar and Hunter, 1983). The phases of shoot development might be under the control of different sets of genes (Leng, 1951). Alterations in the total number of phytomers induced non-additive changes in plant height (Vlăduțu et al., 1999).
Plant height (PH) is a quantitative trait with known qualitative mutants (Robertson, 1985, Sheridan, 1988). Mutations were identified because discrete variations with Mendelian classes were easily observed. Continuous variation was also observed and was attributed to the inheritance of several QTL with an array of alleles possible at each QTL (Beavis et al., 1991).

QTL studies have detected genetic factors for FLN and PH on most maize chromosomes but these QTL were usually not related to photoperiod response. In a study conducted in long- and short-days, five QTL for FLN were detected in long-days. QTL were not detected in short-days. One QTL for PH was detected in short-days, one in long-days, and three in long and short-days (Koester et al., 1993). In a study conducted only in short-days, eight QTL for FLN were detected (Jiang et al., 1999). Studies conducted in long-day environments detected at least one QTL for PH on each of the 10 maize chromosomes (Beavis et al., 1994; Schon et al., 1994; Veldboom and Lee, 1996). Two QTL, vgt1 and vgt2, on chromosome 8, were associated with PH and node number (FLN) in long-days (Vlăduțu et al., 1999). These two QTL were suggested to have pleiotropic effects on PH and FLN and to be involved in different pathways (Vlăduțu et al., 1999).

**QTL mapping**

Quantitative traits are controlled by few to many genes, each with minor or major effect. The effect of the environment on these traits is generally important (Falconer and McKay, 1996) which makes the identification of these genes difficult. The development of statistical methods in combination with the availability of an infinite number of DNA markers throughout the genome made marker-based mapping possible. Quantitative trait loci
(QTL) is a region of the genome that is associated with genetic differences for a quantitative trait. A QTL can be a gene or a cluster of linked genes (coding and non-coding regions) that affect gene expression. The resolution of QTL mapping is believed to be better than that of traditional biometrical studies, which assume complete additivity, equal effects and independent segregation of genes (Kuittinen et al., 1997). Violation of the assumptions usually leads to an underestimation of the real number of factors involved (Lande, 1981).

QTL mapping can give information to improve selection efficiency and help understanding the biology and physiology of traits. DNA markers close to QTL might be used in marker-assisted selection. When a genetic region was identified with QTL mapping, further studies might relate a QTL to a specific gene. The map-based cloning is a method that has been used in rice for this purpose (Takahashi et al., 2001; Yano et al., 2000). Identification of candidate genes related to QTL and their cloning can clarify the mechanisms and pathways involved in controlling flowering time and photoperiod sensitivity.

**Single factor analysis**

Individuals are gathered in two or three groups based on the genotypic data at each locus. Contrasts between homozygous classes \[E(MM-mm) = 2(1-2r)a\] (1) and between heterozygotes and mid-parents \[E(Mm-1/2(MM+mm)) = d(1-2r)\] (2) (case of F2 population) are tested for each marker. Significant contrasts indicate linkage between the marker analyzed and a QTL. Because this analysis is based on two equations (1) and (2) with three unknowns, the effect of the QTL can not be distinguished from the recombination frequency \(r\) (Edwards et al., 1987). Consequently, a tight linkage of a marker to a QTL with small effect can not be distinguished from a loose linkage to a QTL with large effect (Lander and Botstein, 1989). Markers with significant effects can be linked to one or more QTL.
**Interval mapping analysis**

To estimate QTL position and effect separately, two flanking markers are used in the interval mapping analysis. A cross between inbred MQN/MQN and mqn/mqn is made to generate F₂ or backcross population. The recombination frequency between the two markers M and N is assumed to be known. However, the recombination frequencies r₁ and r₂ between the QTL and the markers M and N respectively are to be defined as well as the QTL effect. Regression (Haley and Knott, 1992) or maximum likelihood (Lander and Botstein, 1989) approaches have been used to estimate these parameters. Assuming no interference and using Haldane's function, expected QTL effects can be derived for each QTL position in each interval (every 1 or 2 cM along all the interval) and fitted in the model. Using regression method, the position of the QTL in the interval is the one that produces the smallest residual sum of squares. An F-test can be performed to check if the parameters are significantly different from zero. Maximum likelihood method calculates the maximum likelihood estimates and computes the likelihood of the model. The LOD score (Logarithm of the odds ratio) for the putative QTL at a given genetic location is estimated (LOD = \( \log_{10}\frac{\text{likelihood of model with QTL}}{\text{likelihood of model without QTL}} \)). That score indicates how much more probable having a QTL than assuming its absence. The LOD threshold is still not clear. A 1000 permutations of genotypic data on the individuals in the sample or a 2.5 LOD score are mostly used to identify significant QTL. With the interval mapping method, if there are more than one QTL on a chromosome, the estimated position and effect of QTL are more likely to be biased.
Composite interval mapping approach

Composite interval mapping uses multiple regression and maximum likelihood techniques (Zeng, 1994). Flanking markers (i and i+1) of each interval analyzed are used to block the effect of possible linked QTL. The statistical model used based on a backcross population is: \( Y_j = b_0 + b^* x^*_j + \Sigma_{k\neq i, i+1} b_k x_{jk} + e_j \) where \( Y_j \) is the trait value of the \( j^{th} \) individual, \( b_0 \) is the mean of the model, \( b^* \) is the effect of the putative QTL (difference between homozygote and heterozygote), \( x^*_j \) is an indicator variable taking 1 or 0 with probability depending on the genotypes i and (i+1) and the QTL position tested, \( b_k \) is the partial regression coefficient for the \( k^{th} \) marker, \( x_{jk} \) is a known coefficient for the \( k^{th} \) marker in the \( j^{th} \) individual, taking 1 or 0 depending on whether the marker is homozygote or heterozygote, \( e_j \) is a random variable. Assuming identical and independently normally distributed \( e_j \)'s with mean zero and variance \( \sigma^2 \), the likelihood function is defined and differentiated with respect to individual parameters. The derivatives are then equaled to zero and solved in order to estimate the parameters \( b^*, b_k \)'s and \( \sigma^2 \).

Composite interval method has the advantage of defining QTL position and effects more precisely especially when linked QTL are present and when the trait of interest has high heritability. However, this method assumes no epistasis. If epistasis is present, the estimates can be biased. If two QTL are located in adjacent intervals, it is possible to have some interference on testing and estimation between those QTL. Moreover, closely linked QTL are usually difficult to identify especially for QTL with opposite effects that cancel each other (Jansen, 1994).
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CHAPTER 2. GENETIC COMPONENTS OF FLOWERING AND PHOTOPERIOD RESPONSE IN MAIZE

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ABSTRACT

Photoperiod affects the transition from vegetative to reproductive phase in maize (Zea mays L.) and limits germplasm exchange between breeding programs. The number of days from planting to anthesis (DTA) and photoperiod response (PPR), measured as the difference between DTA in long- and short-days divided by DTA in short-days, were studied to assess photoperiod sensitivity in 236 F3 lines. A cross between photoperiod-sensitive inbred CML9 and insensitive line A632Ht produced those F3 lines that were evaluated in long and short-days. A unique set of quantitative trait loci (QTL) was detected for each photoperiod. The QTL on chromosome 2 had similar position in both photoperiods. The QTL on chromosomes 1 (bmc2295), 3 (npi108a), 8, 9 (umc39d), and 10 were associated with DTA in long-days. The QTL on chromosomes 1 (umc23 and umc106), 3 (umc102), 4 (umc353 and

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The QTL for PPR were on chromosomes 8, 9 and 10 in all environments and had similar map positions as QTL for DTA in long-days. The CML9 alleles increased DTA and PPR at most QTL. Dominance deviations were mostly towards earliness and higher PPR. A significant epistatic interaction was observed between chromosomes 3 and 4 for DTA in long-days and between chromosomes 5 and 7 for PPR.

INTRODUCTION

Flowering is a complex trait that is dependent on the genotype and affected by the environmental factors such as the photoperiod and the temperature. Two aspects of flowering have been defined in maize: the base maturity (i.e., basic vegetative phase) and the photoperiod-sensitive phase (Major, 1980; Kiniry et al., 1983). During the base maturity phase, plants are photoperiod-insensitive. Maize is not sensitive to photoperiod until four leaves have emerged (Tollenaar and Hunter, 1983). In wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.), the rate of emergence of the first six to eight leaves was independent of photoperiod whereas the rate of emergence of later leaves varied with daylength (Miralles and Richards, 2000). During the photoperiod-sensitive phase, some maize genotypes are sensitive to photoperiod. The genetic control of flowering and photoperiod-sensitivity has not been identified in maize.

Based on the classic quantitative genetic methods, such as mating designs or generation mean analysis, two to nineteen genetic factors were reported to control flowering in maize with most studies detecting four to six (Giesbrecht, 1960; Hallauer, 1965). The gene effects related to flowering ranged from additive to complete dominance with a
prevalence of additive effects (Giesbrecht, 1960; Hallauer, 1965; Russell and Stuber, 1983). The dominance effect was towards earliness. Epistatic effects were also detected (Giesbrecht, 1960).

Few genes with major effects seem to control photoperiod sensitivity in maize. Using mass selection, response to selection for earliness in the late-flowering population 'Eto Composite' was 3.8 days per cycle of selection (Hallauer and Sears, 1972). This response to selection suggested that variation for flowering was influenced by few genes with major effects and the prevalence of additive effects. Using DNA marker loci, selective introgressions of alleles for early flowering into late maize lines, were observed on chromosomes 1, 2, 6, 8, 9, and 10 (Koester et al., 1993) and on chromosomes 1, 6, and 8 (Vlăduțu et al., 1999).

Mapping quantitative trait loci is a method that relates the phenotypic variation to the allelic variation on the whole genome basis. A QTL study of anthesis in maize was conducted in long- (North Carolina, located at 35°N, 78°W with 14.5 hours daylength) and short-day (Florida, located at 25°N, 20°W with 11 hours daylength) environments but separated in space and time (Koester et al., 1993). Four QTL were detected in long- and short-day environments and four QTL were detected only in long-day environments. The major QTL for anthesis were on chromosomes 1, 8 and 10. A QTL on chromosome 8 was suggested to control photoperiod response (Koester et al., 1993).

Most of the other QTL mapping studies of anthesis in maize were conducted in long-day environments. At least one QTL for anthesis was identified on each of the maize chromosomes. QTL were detected along chromosome 1 in bins 1.02-1.03, bins 1.06-1.08 and bin 1.10 (Koester et al., 1993; Stuber et al., 1992; Zehr et al., 1992; Veldboom et al.,
1994; Cardinal et al., 2001). On chromosome 2, QTL were in bins 2.07-2.08 (Zehr et al., 1992; Cardinal et al., 2001; Koester et al., 1993). On chromosome 3, QTL were along bins 3.04 to 3.08 (Zehr et al., 1992; Austin and Lee, 1996; Cardinal et al., 2001; Stuber et al., 1992). On chromosome 4, bins 4.08-4.09 harbored QTL related to anthesis (Cardinal et al., 2001; Austin and Lee, 1996). On chromosome 5, QTL were in bins 5.04-5.05 (Austin and Lee, 1996; Krakowsky, 2001; Veldboom et al., 1994). On chromosome 6, QTL were in bin 6.02 (Stuber et al., 1992; Zehr et al., 1992) and bins 6.05-6.06 (Veldboom et al., 1994; Koester et al., 1993). On chromosome 7, QTL were in bins 7.03-7.05 (Austin and Lee, 1996; Veldboom et al., 1994; Zehr et al., 1992). On chromosome 8, QTL were in bins 8.03-8.08 (Stuber et al., 1992; Koester et al., 1993; Zehr et al., 1992; Veldboom et al., 1994; Cardinal et al., 2001). On chromosome 8, a major QTL named Vgt1 (Vegetative to generative transition) previously mapped in a 5 cM interval to bin 8.05 (Vlăduţu et al., 1999) was narrowed to 0.03 cM using NILs and AFLP markers (Salvi et al., 2002). On chromosome 9, QTL were in bins 9.05-9.07 (Cardinal et al., 2001; Veldboom et al., 1994; Koester et al., 1993). On chromosome 10, QTL were in bins 10.03 and 10.06 (Koester et al., 1993).

Few genes that affect flowering have been identified in maize. The gene indeterminate 1 (Id1) prolongs vegetative growth and delays flowering time (McSteen et al., 2000). Id1 encodes a zinc-finger protein with similarities to animal transcription factors. By analogy to the Arabidopsis model, the Id1 gene was classified in the autonomous flower promotion pathway (McSteen et al., 2000). The gene Dwarf8 (D8) produces short and compact plants with short internodes but gibberellins do not restore the normal phenotype of that mutant. D8 encodes proteins that resemble nuclear transcription factors. The D8 amino acid sequence has 62% identity to GAI (Arabidopsis Gibberellin Insensitive) gene and both
genes are orthologues (Peng et al., 1999). The D8 gene maps to the maize chromosome 1 and has been cloned. Association mapping related D8 with flowering time in 92 maize inbred lines from stiff stalk, non stiff stalk, tropical and semi-tropical groups (Thornsberry et al., 2001). The maize genes Zag1, Zmm2 and silky1 have been cloned. These genes belong to the MADS-box gene family. The MADS-box genes have a wide range of functions including the control of flowering time (Ng and Yanofsky, 2001). Moreover, their orthologous genes in Arabidopsis are known to control flowering time (Simpson et al., 1999).

Phytochromes (phy) are photoreceptor genes that are responsible for several light-mediated responses throughout the plant life cycle. These genes are also involved in floral induction and circadian rhythm (i.e., photoperiod response; Smith, 1995). Five different phy gene families (phyA, phyB, phyC, phyD, phyE) have been identified in Arabidopsis thaliana. Sequence comparison of these genes have suggested that divergence of phyA, phyB and phyC genes preceded the divergence of the monocots and dicots and predicted that all angiosperms have genes in these families (Quail, 1991). Two more types of photoreceptors, cryptochromes (cry1, cry2) and phototropin have been identified in the model species Arabidopsis thaliana (Casal, 2000). In maize, the only cloned photoreceptor gene, phyA1, had 88% amino-acid identity with rice (Oryza sativa L.) type A phytochromes and 65% with the Arabidopsis phyA (Christensen and Quail, 1989). Probed sites phyA2, and phyB1, that might correspond to photoreceptor genes, were mapped in maize using probes produced from oat (Avena) and rice phytochromes (Christensen and Quail, 1989; Dehesh et al. 1991). In Arabidopsis, the gene phyA is involved in the perception of daylength (Johnson et al., 1994). The photoreceptors phyB, phyC, phyD and phyE control flowering time and are more related to the shade avoidance in response to low-red/far-red ratio than to the measurement of
photoperiod (Devlin et al., 1998 and 1999). Interactions among photoreceptors in
Arabidopsis were observed and resulted in a 70% reduction of the response to photoperiod
(Mazzella and Casal, 2001).

Three classes of genes that control flowering have been identified in the cereals: 1) genes associated with vernalization, 2) genes for photoperiod sensitivity, and 3) genes controlling flowering in both long and short daylengths, usually called 'earliness per se' (Laurie et al., 1995). Other recessive mutations were described in barley (Hordeum vulgare L.) but it is not known if they are the result of mutation of genes at these three preceding classes or if they represent a fourth class of genes (Laurie et al., 1995). In barley, ea, ea_{sp}, ea_{c}, ea_{e}, and ea_{7} were detected as loci controlling flowering and/or photoperiod sensitivity in spring barley (Nilan, 1964; Takahashi and Yasuda, 1971; Gallagher et al., 1991). The genes, ea_{sp}, ea_{c}, and ea_{e} were activated by short-days (Gallagher et al., 1991). Three genes, sh, Sh2, and Sh3 were responsible for winter and spring growth habit (Takahashi and Yasuda, 1970). The Ppd-H1 and Ppd-H2 genes were associated with photoperiod response. Ppd-H1 regulates flowering only in long-days and Ppd-H2 only in short-days (Laurie et al., 1994).

In wheat (Triticum aestivum L.), two major genes and other minor genes affect the photoperiod response (Klaimi and Qualset, 1973). Later, genes Ppd1, Ppd2, and Ppd3 were identified as loci with major effects on photoperiod-sensitivity (Law et al., 1978) and seem to be homeologous to the Ppd-H1 loci in barley (Laurie et al., 1994).

In rice, El-E3, Se2-Se5, Se-In, Se-1u, I-Se-1, and En-Se-1 have been identified as photoperiod-sensitivity genes (Okumoto and Tanisaka, 1997). Hdl and Hd6 are two additional photoperiod-sensitive genes that have been identified by QTL mapping and isolated by map-based cloning (Takahashi et al., 2001; Yano et al., 2000). Hdl was
suggested to promote heading in short-days and to inhibit heading in long-days. That gene is a homologue of the *CONSTANS* (CO) gene in *Arabidopsis* and encodes a protein with a zinc-finger domain (Yano et al., 2000). *Sel* was found to be allelic to *Hdl* (Yano et al., 2000).

*Hd6* encodes the alpha-subunit of protein kinase (*CK2*) that is involved in the phototransduction pathway (Takahashi et al., 2001). The photoperiod response gene, *Se5* was also cloned and was suggested to function in the phytochrome-chromophore biosynthesis (Izawa et al., 2000).

In sorghum (*Sorghum bicolor* L.), several genes with major effects on flowering time and photoperiod sensitivity have been identified, *Ma1, Ma2, Ma3,* and *Ma4* (Morgan, 1994). The most photoperiod-sensitive genes were *Ma1* and *Ma2*. The dominant *Ma1* allele confers increased photoperiod-sensitivity (4.5 leaves per hour increase in daylength) compared with the homozygous *mal* (1.4 leaves per hour of daylength; Major et al., 1990). The *Ma2* gene was implicated in a ‘photoperiod x temperature’ interaction, although the exact role was not clear. The *Ma3* locus seems to regulate gibberellin concentration (Morgan, 1994). The recessive *ma3R* allele eliminated photoperiod sensitivity (Major et al., 1990). The *Ma3* gene has been cloned and was found to be the *phyB* gene (Childs et al., 1997). Two additional photoperiod-sensitive genes *Ma5* and *Ma6* were identified (Rooney and Aydin, 1999).

In *Arabidopsis*, five pathways that control flowering time have been identified: 1) photoperiod promotion, 2) autonomous promotion, 3) gibberellic acid (GA) promotion, 4) vernalization promotion, and 5) the floral pathway integrators (Simpson et al., 1999; Simpson and Dean, 2002). The photoperiod promotion pathway initiates flowering in response to photoperiod through a number of genes that sense and respond to daylength. Phytochromes and cryptochromes are involved in the detection and transduction of the light
signal. The duration of the day and night is measured by a circadian clock (oscillator). The
gene \textit{CONSTANS} (CO) links the oscillator and flowering time (Simpson and Dean, 2002).
That gene is suggested to function in an output pathway that integrates the perception of
daylight and time keeping mechanisms to promote flowering (Simpson and Dean, 2002).
The autonomous promotion pathway includes the genes that promote flowering
independently from any environmental signal. These genes limit the accumulation of
flowering locus C (\textit{FLC}) mRNA. The low level of this mRNA accounts for the late
flowering of mutants (Simpson and Dean, 2002). The autonomous pathway may monitor
developmental age; plants must have a juvenile phase and can not flower until they reach the
vegetative phase. However, this hypothesis needs to be confirmed (Simpson and Dean,
2002). The gibberellic acid (GA) promotion pathway plays a promotive role in flowering
based on signals mediated by GA. The application of GA accelerates the flowering time of
wild-type plants in short-days and of the late-flowering mutants in long-days. The
vernalization pathway accelerates flowering after exposure to cold temperatures.
Vernalization was observed in late-flowering ecotypes of \textit{Arabidopsis} and in mutants of the
autonomous promotion pathway. Vernalization substitutes for a lack of the autonomous
pathway genes. Photoperiod has little effect on vernalization. The floral pathway repressors
includes genes whose expressions or functions are regulated by more than one of the above
pathways. A large number of floral repressors was also identified from early flowering
mutants (Simpson and Dean, 2002). These genes likely regulate chromatin structure and
protein degradation (Simpson and Dean, 2002). In \textit{Arabidopsis}, about 80 genes involved in
flowering are known (Simpson et al., 1999). However, the related genes in maize and their
effects on phenotypic variation have not been identified.
Location of genetic factors controlling flowering and photoperiod response in maize and identification of marker loci closely associated with QTL would be of interest for applied and basic research. These loci could be used to screen germplasm for their photoperiod-sensitivity and reduce the cost of the phenotypic screening in multiple locations. Breeders can use these loci to introgress photoperiod-sensitive germplasm in their populations and in converting or developing lines adapted to a wider range of environments.

QTL mapping relates variation in the phenotype to specific genetic regions. This method is 'an open system' since it is not limited to known and cloned genes as is the case of association mapping. After identifying a genetic region with QTL mapping, further studies are needed to relate a QTL to a specific gene. The map-based cloning is a method that has been used in rice for this purpose (Takahashi et al., 2001; Yano et al., 2000). Identification of candidate genes related to QTL and their cloning can clarify the mechanisms and pathways involved in controlling flowering time and photoperiod sensitivity. Cloning of genes related to QTL can also help identify new alleles or new genes. As an example, a major QTL for photoperiod response in Arabidopsis was related to a photoreceptor gene CRY2 (El-Assal et al., 2001). After cloning and sequencing that gene, a new allele with a single amino-acid substitution was associated with the differential response to daylength. Relating QTL to candidate genes is also a mean of determining more accurately the effect of the QTL (Mackay, 1995). Putterill et al. (1995) showed that increasing the copy number of co of Arabidopsis led to earlier flowering.

The objectives of the present study are 1) to locate quantitative trait loci (QTL) related to anthesis in long-day and in short-day environments in the CML9 x A632Ht maize
population, 2) to detect QTL related to the photoperiod response for anthesis, and 3) to
determine the gene action of those traits.

MATERIALS AND METHODS

Plant material and field experiments

A photoperiod-sensitive inbred \textit{CML9} was crossed to an insensitive line \textit{A632Ht}. The
\(F_1\) generation plant was self-pollinated to produce the \(F_2\) generation. The \(F_2\) plants were
grown in short daylength (11.5 hours) and were self-pollinated to produce 236 \(F_3\) generation
lines at the CIMMYT research station, Tlaltizapan, Mexico (18°N, 99°W).

The 236 \(F_3\) lines and both parents were evaluated in three short-day and three long-
day environments. The lines and parents were planted in single-row plots 0.75 m apart and
2.5 m long. Ten to thirteen plants were maintained per plot. The plots were arranged in a 24
x 10 alpha (0,1) lattice (Patterson and Williams, 1976) with two replications per
environment. The environments and dates of planting were 1) Tlaltizapan (TL), Mexico, 26
June 1995, under 17 hours daylength, 2) Tlaltizapan, 6 December 1996, under 17 hours
daylength, 3) Ames at the ISU Agronomy and Agricultural Engineering Research Center
(42° N, 93° W), 17 May 1997, with a daylength of 15.5 hours, 4) Tlaltizapan, 26 June 1995,
with normal daylength of 13 hours, 5) Tlaltizapan, 6 December 1996, with a daylength of
11.5 hours, and 6) Tlaltizapan, 11 June 1997, with a daylength of 13 hours. Environments 1,
2, and 3 were considered long-days and environments 4, 5, and 6 were considered short-days.
During 1995 and 1996, the long-day and short-day experiments were planted at the same
time in adjacent fields (experiment 1 adjacent to experiment 4 and experiment 2 adjacent to
experiment 5). Artificial light was used to extend the daylength to 17 hours in environments
1 and 2. Light used had an action spectrum between 400 and 1100 nm with a peak at 950 nm. The distance of the 150-watt lamps from the soil surface was adjusted throughout the season to follow the increased height of the plants. The light intensity was between 0.5 and 1% of full sunlight in the center of the field. The critical full spectrum illumination was 500 mWm$^{-2}$.

**Analyses of phenotypic data**

The number of days from sowing to anthesis (DTA) was recorded when 50% of the plants in a plot were shedding pollen. The photoperiod response (PPR) of DTA of each F$_3$ line and parent was calculated as the difference between the least square means (lsmeans) of DTA in long and short-day environments divided by the lsmean of DTA in short-day environment of the same year. The DTA of the combined environment were estimated from three individual environments in each daylength. Therefore, DTA data were available for three individual and one combined environment in long-days and for three individual and one combined environment in short-days. Each pair of environments (one in long-days and one in short-days) from individual years and one pair from the combined environments were used to estimate PPR. Therefore, four sets of PPR data were available.

Lsmeans of DTA were used to reduce the effect of the environment on the phenotypic data (inter- and intra-block effects). Lsmeans minimize the sum of squares of the residuals (Cochran and Cox, 1992), so that the means approach the genotypic values. The lsmeans were calculated using SAS (SAS Institute Inc., 1999) with F$_3$ lines considered fixed effects, while complete and incomplete blocks for individual environments were random effects (Cardinal et al., 2001). For the combined environment of each daylength, blocks and individual environments were considered random effects. Lsmeans of DTA were used to
calculate PPR and for QTL analyses. Separate analyses for each environment were conducted because Box's tests of the homogeneity of variances were significant for DTA and PPR between individual environments of the same daylength at $P < 0.05$ (Milliken and Johnson, 1992). QTL analyses in the combined environment were conducted because multiple environments allow better estimation of the environment effects and therefore a better estimation of the genetic effects of QTL across environments. Similarly, QTL analyses of PPR were conducted for each year and for the combined years. Blocks and lines were considered random effects when calculating variances (Cardinal et al., 2001). Broad-sense heritabilities on an entry-mean basis and their exact confidence intervals were calculated as described (Knapp et al., 1985).

**Genotypic analyses**

Leaf samples were harvested from individual $F_2$ plants, frozen in liquid nitrogen, lyophilized, ground and stored at $-18 \, ^\circ\mathrm{C}$. DNA was extracted from the parental lines and the 236 $F_2$ plants and digested with EcoRI or HindIII. DNA fragments were separated in agarose gels and transferred to nylon membrane by southern blot. Hybridization with DNA probes was used to detect restriction fragment length polymorphism (Hoisington et al., 1994). Additional loci were detected by Simple Sequence Repeats (SSR) as described by Ribaut et al., (1997). The chi-square test of the ratio of the genotypic classes was significant ($P<0.05$) for 14 markers (11%). Ratios of genotypic classes at six loci ($npi203$, $npi444$, $umc10$, $npi451$, $umc102$, $umc50$) had an excess of the photoperiod-insensitive parent's genotype $A632Ht/A632Ht$. Ratios of two loci ($umc23$, $bnl3.04$) had an excess of the photoperiod-sensitive parent's genotype $CML9/CML9$. Six loci ($npi264$, $bnl5.09$, $O2$, $bnl128$, $bnl13.05$, $bnl8.39$) had an excess of the heterozygous class. Segregation distortion of this nature,
reported in other maize populations (Abler et al., 1991; Edwards et al., 1987; Koester et al., 1993), should not affect the detection of association between marker loci and phenotypic variation (Koester et al., 1993). but, the precision of QTL position may be reduced (Lorieux et al., 1995).

The linkage map was constructed using MAPMAKER Version 3.0 (Lander et al., 1987). Loci were assigned to linkage groups with a minimum log_{10} of the likelihood odds ratio (LOD) of 3.0 and a maximum distance of 50 centimorgans between loci (Haldane cM). For chromosome 4, the minimum distance was extended to 54 (cM) because loci umc123 and umc31 were separated by 53.8 cM. The “Three-point” command was used for each group to estimate the likelihoods of all three-point crosses and choose the best three-point order. The “order” command for multipoint analysis was conducted and the best order has been selected for each linkage group. The command “Try” was used to place the remaining loci in their appropriate linkage group. Finally, 128 loci mapped to unique positions and 10 linkage groups were obtained. The total length of the map was 1658 cM with an average distance between loci of 14 cM. The order of loci was mostly in agreement with previously published maps (Davis et al., 1999).

QTL Analysis

QTL were detected using composite interval mapping (Jansen, 1993; Jansen and Stam, 1994; Zeng, 1994) facilitated by PLABQTL version 1.1 (Utz and Melchinger, 1996). Cofactors were selected based on stepwise regression using ‘cov select’ command (Melchinger et al., 1998). The markers close to QTL were chosen as final cofactors. The threshold for the LOD score was estimated from 1000 permutations of the phenotypic data using PLABQTL (Churchill and Doerge, 1994). The LOD threshold ranged from 3.1 to 3.7
depending on the environment and the number of the final cofactors. Then, all QTL were evaluated in forward and backward regression facilitated by the 'seq/s' statement of PLABQTL (Melchinger et al., 1998). The selection of the final model was based on the Akaike's Information Criterion (AIC). These AIC choose the genetic model with the largest value of the log-likelihood, minus the penalty for the number of free parameters in the model. When comparing two models, the difference between AIC larger than two is considered significant (Jansen, 1993).

Digenic epistatic interactions between all pairs of DNA loci were tested using Epistacy (Holland, 1998). Loci with significant interactions (P<0.00026; Holland et al., 1997) were added to a multiple regression model as well as loci close to QTL. Interactions were accepted as significant and maintained in the final model when the effects of the individual loci near QTL and the effect of the interaction between loci were significant (P<0.05) (Cardinal et al., 2001). The gene effect was determined based on the ratio dominance by additive effects (d/a): additive (0<d/a<0.2), partial dominance (0.2<d/a<0.8), dominance (0.8<d/a<1.2), and overdominance (d/a>1.2) (Stuber et al., 1987).

RESULTS

Phenotypic variation

The photoperiod response of the F3 population and CML9 was evident since their DTA was higher in long-day environments. The difference in flowering time of A632Ht between both daylengths was not significant while CML9 shed pollen 32 and 27 days later in long-day environments in 1995 and 1996, respectively. During these two years, the long-day and short-day experiments were planted at the same time in adjacent fields with or without
artificial light. The PPR was 0.47 and 0.27, which means that anthesis of CML9 was 47% later in long-days in 1995 and 27% later in 1996 (Table 1). The same response was observed in 1997 and in the combined environments.

The distribution of DTA values of the population in short and long-day environments was unimodal and clearly separated (Figure 1). Similar distributions were observed for each pair of environments in each year (data not shown). The DTA of the F3 lines had a greater range and higher mean in long-day environments. In 1995, DTA values ranged between 68 and 95 days and averaged 83 days in long-days. In short-days of the same year, DTA ranged between 56 and 71 days and averaged 62 days. In 1996, DTA values ranged between 99 and 120 days with an average of 109 days in long-days. In short-days of the same year, DTA was between 82 and 99 days with an average of 90 days (Table 1). The average DTA was therefore 33% (PPR=0.33) later in long-days in 1995 and 20% later in long-days in 1996. The same response was observed in the other environments (Table 1).

In the combined long-day environment, the variance components of DTA for experimental error and genotype by environment interaction were smaller than the genotype component (Table 2). Therefore, similar performance of genotypes was observed among the individual long-day environments. The genotypic variance showed significant differences among lines (Table 2). The broad-sense heritability on an entry-mean basis was 0.88 (0.85-0.90 95% CI) (Table 2) indicating the stability and the reproducibility of the data. Heritability estimates of DTA reported in previous studies conducted in long-day environments with photoperiod-insensitive populations were 0.88 (0.84-0.90 95%CI) (Austin and Lee, 1996), 0.92 (0.88-0.95 95%CI) (Vlăduţu et al., 1999), and 0.66 to 0.87 (Austin et
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al., 2001). The heritability of heat units to anthesis for similar combinations of populations and environments was 0.73 (Cardinal et al., 2001) and 0.68 (Krakowsky, 2001).

In short-day environments, the variance components for DTA were lower than in long-day environments (Table 2). The broad-sense heritability on an entry-mean basis was 0.85 (0.82-0.88 95% CI) (Table 2). The heritability of DTA in a previous study conducted in a short-day environment was 0.86 (Ribaut et al., 1996).

The variance components for PPR were based on the PPR in 1995, 1996 and 1997. In the combined pair of environments, the genotypic variance of PPR was significant (Table 2). The heritability on an entry-mean basis was 0.81 (0.77-0.84 CI) and in the same range as the heritabilities of DTA in both daylengths (Table 2).

QTL mapping

Different sets of QTL for DTA were detected in the combined long- and short-day environments. Five QTL were detected on chromosomes 2, 3, 8, 9, and 10 in the combined and all individual long-day environments (Table 3). An additional QTL was observed on chromosome 1 (Bmc2295) only in the 1995 environment. The QTL on chromosomes 2, 3, and 9 were detected in intervals of 12, 20, and 12 cM respectively in all long-day environments. These intervals represent the length of the genetic regions where the QTL were detected in all long-day environments. The QTL on chromosomes 8 and 10 had the largest effects and were detected in an interval of eight and two centimorgans, respectively, in all environments. All these QTL combined explained 50 to 60% of the total phenotypic variation (Table 3).

QTL detection can be influenced by different factors, such as the method used for QTL detection, the sample size and the trait studied. Composite interval mapping method as
expected by theory and simulation studies, allows a better detection of QTL (number, position and effect) for traits with higher heritability and with larger sample size (Zeng, 1994; Melchinger et al., 1998). The power of QTL detection and consequently the number of common QTL over environments is expected to be smaller for traits controlled by a large number of minor QTL than for traits controlled by a small number of major QTL (Melchinger et al., 1998). Likewise, the proportion of the phenotypic variation explained by QTL is expected to be less biased for a trait with a small number of major QTL than with a large number of minor QTL (Melchinger et al., 1998).

In this study, the consistency of the position of QTL detected in different long-day environments, their LOD scores, and the magnitude of the phenotypic variation associated with the marker loci suggest that the QTL detected are not false positives. Moreover, QTL detected in the combined long-days were in the same genetic region as QTL identified previously in long-day environments (Krakowsky, 2001; Cardinal et al., 2001 and Koester et al., 1993). The comparison of QTL position over different studies was based on common markers. If common markers were not available, the Pioneer Composite map 1999 was used (www.agron.missouri.edu).

QTL effects can be inflated especially when a small population size is used and for traits with complex inheritance such as yield (Melchinger et al., 1998). Cross validation tests and validation with independent samples revealed a large bias in the proportion of phenotypic variation ($R^2$) explained by QTL when estimated from the same data set used for QTL detection (Utz et al., 2000). Therefore, the QTL effects and $R^2$ estimated in this study may be inflated because the same sample was used for QTL detection and estimation of their effects.
At all QTL for DTA in long-day environments, the CML9 alleles were associated with later flowering (Table 3). The magnitude of the additive effects ranged from 1 to 6 days and explained from 3 to 46% of the total phenotypic variation. Dominance effects were significant on chromosomes 2, 3 and 10 in the combined long-day environment. The level of dominance was partial for QTL on chromosomes 3 and 10 in three of the four long-day environments. The direction of dominance was towards earlier flowering on chromosome 3 and towards later flowering on chromosome 10. The QTL on chromosome 2 exhibited overdominance in three long-day environments while complete dominance was observed in the 1997 environment. The dominance deviation was towards earlier flowering at that QTL in all environments (Table 3). A significant digenic epistatic effect between chromosomes 3 (umc96) and 4 (npi203) in the combined long-day environment explained 9% of the total phenotypic variation in the model with the main effects. The main effects were represented by markers listed in Table 3. The DTA of the nine genotypic classes defined by these two loci ranged from 87 to 97 days. The lower DTA value was for the F3 lines heterozygous at locus umc96 and homozygous for A632Ht alleles at locus npi203. The highest DTA value was for F3 lines homozygous for CML9 alleles at both loci. The F3 lines homozygous for A632Ht alleles at both loci had a DTA of 92 days. Epistatic effects between QTL for flowering genes were previously detected in maize (Rebai et al., 1997), wheat (Klaimi and Qualset, 1973; Pan et al., 1994), barley (Gallagher et al., 1991), soybean (Cober et al., 1996), rice (Yamamoto et al., 2000) and Arabidopsis (Kuittinen et al., 1997).

In short-day environments, a different set of QTL for DTA was detected. Ten QTL were located on chromosomes 1 (umc23 and umc106), 2 (umc38a), 3 (umc102), 4 (umc353 and npi444), 5 (umc49c and npi409), 6 (umc85), and 9 (umc81) (Table 4). The QTL on
chromosomes 2, 3 and 9 were detected in all short-day environments and their positions were consistent in all environments. The QTL on chromosome 1 (umc23) had the same position in all short-day environments except in 1996 when a QTL (umc106) 72 cM apart was observed. The QTL on chromosome 5 (umc49c) was detected in the same position in 1995 and 1997 but in the combined short-day environment, a QTL 120 cM apart (npi409) was detected (Table 4). The QTL on chromosome 4 (umc353) was detected in three of four short-day environments and with a difference of 2 to 4 cM in map positions among environments. These ten QTL explained 32 to 50% of the total phenotypic variation.

*CML9* parent contributed most alleles for lateness in the combined short-day environments, but *A632Ht* alleles were associated with later flowering at two QTL on chromosomes 5 (npi409) and 9 (umc81). The additive effects ranged from 0.5 to 1.5 days. The dominance effect was significant only on chromosome 2 in all short-day environments. Overdominance was observed at that QTL in the 1995 and 1997 short-day environments while complete and partial dominance was observed in the other environments. The dominance deviation was towards earlier flowering in all environments (Table 4). Epistatic effects were not detected in short-day environments.

In 1995 and 1996, the long- and short-day environments were in adjacent fields and the population was planted on the same date. Comparison of QTL detected under such conditions should minimize the influence of factors such as temperature, soil type and water on the assessment of DTA and PPR. Within those two years, daylength was the main effective environmental variable. The QTL on chromosome 2 was detected in the same genetic region in both daylengths. The other QTL detected in the 1995 and 1996 environments were observed in either long- or short-days. The difference between the
positions of the QTL on chromosome 2 in both daylengths of the same year was 2 to 12 cM. In both daylengths, overdominance towards earlier flowering was observed for that QTL except in short-days of 1996 when dominance was partial. CML9 alleles contributed to increase DTA in all environments (Figure 2, Tables 3 and 4). Therefore, one QTL might be located on chromosome 2 and might be controlling DTA in both long- and short-day environments and thus acting independently from photoperiod. The positions of the QTL on chromosome 9 were 10 to 16 cM apart in both daylengths of the same year. The CML9 alleles increased DTA in all long-day environments while A632Ht alleles increased DTA in the short-day environments. That might indicate the presence of two different QTL on chromosome 9, one acting only in long-days and the other acting only in short-days. Both QTL were therefore photoperiod dependent. Another possibility is that one QTL is located at that region with one allele functional in one daylength and the other allele active at the second daylength. On chromosomes 1 and 3, QTL were detected in both daylengths. However, their positions were 66 cM and 24 to 54 cM apart, respectively, within the same year which suggested the presence of two different QTL that are photoperiod dependent on each of these two chromosomes. Therefore, in adjacent fields in the same year and environments, QTL on chromosomes 1 (bmc2295), 3 (npi108a), 8, 10, and possibly 9 (umc39d) were associated with DTA only in long-days. QTL on chromosomes 1 (umc23 and umc106), 3 (umc102), 4 (umc353 and npi444), 5 (umc49c and npi409), 6, and possibly 9 (umc81) were associated with DTA only in short-days (Tables 3 and 4, Figure 2).

QTL for PPR were detected on chromosomes 8, 9, and 10 in all pairs of environments within a given year and the pair of the combined environments and explained 58% of the total phenotypic variation in the combined environment. The positions of these three QTL
were consistent over environments. The difference in their map position between pairs of environments was 2 to 14 cM (Table 5). Additional QTL were identified on chromosomes 1 and 3 in the pair of the 1995 environments and on chromosome 4 in 1996 (Table 5). The QTL on chromosome 4 (npi444) was in the same genetic region as a QTL for DTA (6 cM apart) in the combined short-day environment. The QTL on chromosomes 1, 3, 8, 9, and 10 were located in the same regions as QTL for DTA in the combined long-day environments.

Additive, dominance, and epistatic effects were observed for PPR. All QTL had highly significant additive effects in all pairs of environments with alleles from CML9 associated with higher PPR (Figure 2, Tables 3 and 5). The dominance effect was significant only on chromosome 10 in the combined and 1995 environments and on chromosome 8 in the 1995 environments. The dominance deviation was towards higher PPR value, which means that the dominance effect contributed to a stronger photoperiod response. An epistatic interaction was significant between chromosomes 5 (umc90) and 7 (O2) in the combined environment (Figure 2). This interaction, when added to the model with the main effects explained 2% of the total phenotypic variation. The PPR of the nine genotypic classes defined by these two loci ranged from 0.23 to 0.33. The lower PPR value was for F3 lines homozygous for A632Ht alleles at both loci. The highest DTA value was for F3 lines homozygous for A632Ht alleles at umc90 and homozygous for CML9 alleles at O2. F3 lines homozygous for CML9 alleles at both loci had a PPR of 0.32. In addition to having similar map positions as QTL for DTA in long-days, the QTL for PPR had also similar gene effects, highly significant additive effect, dominance significant on chromosome 10, and high phenotypic variation explained by each QTL.
The experiments of 1996 were subjected to lower temperatures than the experiments of 1995 and 1997. In 1996, the experiments were planted in the 'cycle A' growing season, November to April, in Tlaltizapan. The experiments of 1995 and 1997 were planted in the 'cycle B' growing season, June to November, at the same location. 'Cycle A' had lower temperatures. The average low temperatures were 11°C in 'cycle A' of 1996, 17°C in 'cycle B' of 1995 and 13°C in 'cycle B' of 1997 at Tlaltizapan. Previous studies showed that lower temperatures delayed flowering and reduced photoperiod sensitivity (Alison and Daynard, 1979; Hesketh et al., 1969; Edmeades et al., 1992). 'Cycle A' is a dry season and irrigation was regularly applied. 'Cycle B' is a wet season and occasional irrigation was provided.

In 1996, both parents and the F3 population had higher DTA compared with 1995 and 1997 in the same daylength. Plants likely grew faster with higher temperature and regular irrigation. A decrease of one degree Celsius caused four days delay to silking (Jong, 1980). The interaction between photoperiod and temperature was also previously detected (Bloc et al., 1983; Russell and Stuber, 1985). Comparing QTL in 'cycle A' of 1996 and 'cycle B' of 1995 and 1997 of the same daylength, three different QTL for DTA were observed. On chromosome 1, in 1996, the position of QTL for DTA in short-day environments was 72 cM away from the position of the QTL detected in 1995 and 1997. QTL on chromosomes 3 (umc18) and 4 (npi444) were detected only in the 1996 short-day environment (Table 4). In long-day environments, the number and position of QTL for DTA was similar in both growing seasons except the QTL on chromosome 1 (bmc2295) detected only in 'cycle B' of 1995.

PPR of A632Ht parent was higher in the 1996 environments while CML9 inbred and the F3 lines had lower PPR in 1996 than in 1995 and 1997 (Table 1). Three different QTL
for PPR were observed in 'cycle A' of 1996 and 'cycle B' of 1995 and 1997. QTL for PPR on chromosome 4 (npi444) was detected only in 'cycle A' of 1996 while QTL on chromosomes 1 and 3 were detected only in 'cycle B' of 1995. The QTL for PPR on chromosome 4 was in the same region (6 cM apart) as QTL for DTA in short-days. Both of these QTL were detected in 'cycle A' growing season (Table 4 and 5).

The differences in phenotypic data and QTL number and position of DTA and PPR between 'cycle A' and cycle B might be attributed to the difference in temperature in both environments. Therefore, the lower temperatures in 'cycle A' might explain the higher DTA in 1996 than 1995 and 1997 experiments and the lower PPR of CML9 and the F3 lines in 1996. QTL for DTA on chromosomes 1 (umc106), 3 (umc18), and 4 (npi444) were associated with DTA only in the cooler short-day environments of 1996, suggesting that these QTL might be influenced by daylength and temperature. Since QTL for DTA in short-days and QTL for PPR on chromosome 4, were detected only in 'cycle A' and in the same region (npi444), one QTL might be controlling both traits only in cooler environments. Another explanation might be that different QTL for DTA and PPR that are sensitive to daylength and temperature were clustered in the region of npi444. However, since other factors, such as irrigation, can be other sources of variation, and further experiments are needed to test these hypotheses.

DISCUSSION

QTL for DTA and PPR in CML9/A632Ht maize population

Different sets of QTL for DTA were observed in long and short-day environments. The QTL on chromosome 2 is more likely involved in controlling DTA in both photoperiods
and seems to be photoperiod independent. That QTL could be classified in the ‘autonomous promotion pathway’, as described in *Arabidopisis* (Simpson et al., 1999), or ‘earliness per se’, as defined in *Hordeum vulgare* L. (Laurie et al., 1995). The QTL on chromosomes 1 (*bmc2295*), 3 (*npi108a*), 8, 9 (*umc39d*), and 10 were associated with DTA only in long-days. These five QTL might be active and initiate anthesis only when daylength is above the critical value for maize (ca. 14.5 hours of daylength; Francis, 1972) and seem therefore photoperiod sensitive. The QTL on chromosomes 1 (*umc23* and *umc106*), 3 (*umc102*), 4 (*umc353* and *npi444*), 5 (*umc49c* and *npi409*), 6, and 9 (*umc81*) were related to DTA only in short-day environments and might promote flowering when the daylength is below the critical value for maize. These nine QTL also seem to be photoperiod sensitive. Therefore, photoperiod-sensitive genes related to anthesis might be subdivided in two subclasses: a group of genes active in long-days and a group of genes functional in short-days. Different sets of QTL were also detected in rice (*Oryza Sativa* L.) (Maheswaran et al., 2000) and soybean (*Glycine max* L.) (Tasma, 2001) populations grown in long and short daylengths.

The QTL related to PPR were detected on chromosomes 8, 9 (*umc39d*), and 10 with same gene effects in all environments. The locations of these QTL were also consistent and were the same as QTL associated with DTA in long-days. A QTL on chromosome 3 (*npi108*) was also associated with PPR in only one environment (1995) and slightly below the threshold in the other environments. That QTL was detected in the same region as the QTL on chromosome 3 associated with DTA in long-days. That might suggest that each of these QTL on chromosomes 8, 9, and 10 and probably the QTL on chromosome 3, have a pleiotropic effect on photoperiod response and DTA in long-days. Also, these QTL related
to PPR and DTA in long-days could be linked genes. The coincident location of QTL for those traits may also be determined by the “numerical relationship” between them.

Because all the phenotypic variation was not explained by QTL for DTA in both daylengths as well as by QTL for PPR, there are certainly more QTL than the ones detected in this study. That might be explained by many factors, such as the sample size, the environments of evaluation, linked QTL, thresholds used and interactive gene effects. Use of larger populations (e.g., 976 progeny) and larger number of environments for evaluation (e.g., 19 environments) for QTL mapping allowed detection of larger numbers of QTL for grain yield and plant height (Openshaw and Frascaroli, 1997). Closely linked QTL are usually difficult to identify especially for QTL with opposite effects that cancel each other (Jansen, 1994): The thresholds used in this study were defined by permutations and were relatively high to reduce type I errors (false positive) (Churchill and Doerge, 1994). However, type II errors (missed QTL) might have been increased and some QTL with minor effects might have been missed.

Epistatic interactions are rarely detected for quantitative traits in studies of this type. The lack of epistatic QTL may suggest the lower power of the methods used for detecting these interactions. Herein, digenic epistatic effects were detected between chromosomes 3 and 4 for DTA in long-days and between chromosomes 5 and 7 for PPR. One of the DNA markers flanking QTL, not the most likely position of the QTL as defined by composite interval mapping, was used in the analyses for epistatic effects. Interactive effects might be underestimated with simple analysis of variance because of possible recombination between the QTL and the linked marker used in the analysis. The QTL for DTA detected herein in long-days on chromosome 9 was 10 cM away from the closest marker and the QTL for PPR
on chromosome 8 was 12 cM away from the closest marker. Larger populations derived from divergent parents, more closely spaced markers, especially in the regions where QTL were found in this population, and development of homozygous lines such as NILs (Yamamoto et al., 2000) carrying different combinations of QTL and markers might help to identify more epistatic interactions and get a better estimation of their effects.

DTA and PPR were mostly controlled by additive effects but dominance and epistatic effects were also involved. The CML9 parent contributed all the alleles for lateness in long-days. In short-days, A632Ht also contributed one (1995 and 1997) to two (1996 and combined environments) alleles for lateness but most of the alleles for late flowering were from CML9. The combination of earliness alleles from both parents could explain the lower DTA values than A632Ht in short-days and higher values of PPR than CML9 in some of the transgressive F3 lines. The accumulation of complementary alleles at multiple loci in some progeny has been used as an explanation of transgressive segregants (Tanksley, 1993; Veldboom et al., 1994). Dominance, when significant, was mostly towards earliness for DTA in both photoperiods. Similar results were observed in different maize populations at the whole genome level (Yang, 1949; Giesbrecht, 1960) and at QTL (Cardinal et al., 2001; Bubeck et al., 1993). The same tendency, dominance towards earliness, was observed in other cereals such as wheat (Triticum aestivum L.) where earlier heading was partially dominant to later heading (Klaimi and Qualset, 1973). In contrast, dominance towards lateness was prevalent in a population derived from an interspecific cross in sorghum (Sorghum bicolor L. x S. propinquum L.; Lin et al., 1995).
Comparison of QTL positions with other studies in maize

Comparison among QTL map positions in different maize populations may help to confirm and complement the results obtained from one population. One population can not detect all genes controlling a trait because it is difficult to have all loci segregating in one population. However, comparing results from different studies can be limited by many factors, such as the method of QTL detection (simple, interval and composite interval mapping), absence of common markers, threshold used, sample size, type of population, and different environments. Comparison of QTL position to other maize studies was based on common DNA loci. When no common markers were available, the Pioneer Composite 1999 map (www.agron.missouri.edu) was used to compare QTL locations on the basis of the position of marker loci adjacent to detected QTL. For comparisons, only QTL detected in the combined environments herein were used. Except in one study (Nourse, 2000), where both experiments were conducted in adjacent fields under both daylengths, the studies used for comparison were conducted in one daylength or in different daylengths but experiments were separated in time and space. The confounding of flowering effect with other environmental factors, such as temperature, might be involved in these studies. In the present study, four of six environments were at the same location and season. Only photoperiod was different which minimized the confounding effect.

On chromosome 1 (umc23), the QTL for DTA detected in short-days was in the same genetic region as QTL identified in other maize populations in long-day environments (Veldboom et al., 1994; Cardinal et al., 2001. Koester et al., 1993) and in short-day environments (Koester et al., 1993). Introgression of a DNA segment from early germplasm (Gaspé flint) to a late inbred line (recurrent parent) in a backcrossing program was found in
the region of umc58 (Koester et al., 1993), which might confirm that this site is involved in controlling flowering time. In this study, the only QTL found in long-day environments on chromosome 1 was 66 cM from umc23. Therefore, two linked QTL controlling flowering might be located on chromosome 1 near umc23. One QTL might be functional only in short-day environments. The second QTL might be active only in long-day environments and the CML9/A632Ht population may be monomorphic at that second locus. These QTL near umc23 seem then to be photoperiod sensitive because they were detected either in long- or short-days. However, they were not involved in the photoperiod response since QTL for PPR were not detected in that location.

The QTL on chromosome 2 was linked to QTL for anthesis detected in other maize populations in long-day environments (Cardinal et al., 2001; Krakowsky, 2001) and to a QTL associated with silking time in short-days near umc5 (Khairallah et al., 1998). Since the QTL was found herein within a 12cM interval in both photoperiods, that might suggest that one QTL on chromosome 2 controls flowering independently from photoperiod.

On chromosome 3, the QTL detected in short-days near umc102 was identified in the same region in short (CIMMYT, 1994) and in long-days (Zehr et al., 1992; Cardinal, 2001; Krakowsky, 2001). Thus, this region of chromosome 3 (umc102) might harbor at least two QTL; one QTL active in short-days and seems photoperiod-sensitive and a second QTL that promotes flowering in long-days. The photoperiod sensitivity of the second QTL found in long-days in the other studies at umc102 can not be assessed, because the experiments in which it was detected were conducted only in long-days. The QTL found on chromosome 3 (npi108) in long-days in this study was detected in the same genetic region as QTL detected
in previous studies conducted in long-days (Stuber et al., 1992; Cardinal et al., 2001; Krakowsky, 2001). That QTL \((npi108)\) seems, therefore, photoperiod-sensitive.

On chromosome 4, a QTL \((npi444)\) was detected in short-days. A QTL in that region was found in other populations in long-days (Cardinal et al., 2001) and in short-days (CIMMYT, 1994). In this study, a QTL was not detected in this region in long-days. Consequently, this region might have at least two different QTL, one active in short-days and the other functional in long-days. The QTL identified in CML9/A632Ht population seems therefore, photoperiod-sensitive.

On chromosome 5, the QTL detected in short-day environments \((npi409)\) was linked to a QTL detected in long-days near umc90 (Krakowsky, 2001). Therefore, two different QTL might affect flowering time on chromosome 5 in the region \(npi409-umc90\), one active in long-days and the other active in short-days. The QTL detected in short-days seems to be photoperiod-sensitive.

On chromosome 8 \((umc138b)\), a QTL for anthesis was detected in long-days in this study and in other populations evaluated in long-days (Koester et al., 1993; Stuber et al., 1992; Zehr et al., 1992; Vlăduţu et al., 1999). Alleles in that region were introgressed from early germplasm (Gaspé flint) into a late inbred line in a backcrossing program (Koester et al., 1993; Vlăduţu et al., 1999). Moreover, a QTL \(vgtl\) was mapped to 0.3 cM interval with fine mapping (Salvi et al., 2002) which is only around 3 cM away from the position of the QTL detected in this study. Therefore, the QTL for DTA on chromosome 8 seems to be photoperiod-sensitive. A QTL for PPR was closely linked (8 cM) to the QTL for DTA in long-days on that chromosome. That genetic region is, therefore, also involved in the
photoperiod response and was suggested to control photoperiod response in another population (Koester et al., 1993).

On chromosome 9, QTL for DTA (umc39d-umc81) found in the CML9/A632Ht population in long- and short-days were in the same regions as QTL detected in other populations in long-days (Cardinal et al., 2001; Veldboom et al., 1994; Koester et al., 1993) and short-days (Koester et al., 1993). In other experiments conducted under both long- and short-days in adjacent fields, a QTL in that region was found only in long-days (Nourse, 2000). Therefore, at least two QTL controlling flowering may be present in the region umc39d-umc81. The first QTL (umc39d) is active in long-days and the other QTL (umc81) is functional in short-days. That region of chromosome 9 (umc39d-umc81) is involved in the photoperiod response as well as DTA since a QTL for PPR was also identified in that location.

On chromosome 10 (npi264), a QTL for DTA was found in this study only in long-days. A previous study detected QTL in the same region only in long-days (Nourse, 2000) while another study found a QTL in that region in both long and short-day environments (Koester et al., 1993). Therefore, that region (npi264) might contain at least two different QTL, one expressed in long-days and the other detected in short-day environments. Because a QTL for PPR was identified in that region, chromosome 10 seems to be involved in the photoperiod response.

In summary, QTL for DTA detected in short-days on chromosomes 1, 3 (umc102), and 4 in the CML9/A632Ht population had similar positions as QTL identified in long and short-days in other maize populations. The QTL on chromosome 5 detected herein in short-days had similar position as QTL detected previously in long-days. Therefore, on
chromosomes 1, 3 (umc102), 4, and 5, there are at least two different QTL for DTA, one QTL functional in long-days and the other QTL active in short-days. Similarly, the QTL for DTA detected herein in long-days on chromosome 10 was in the same genetic region as QTL detected in long and short-days in other studies suggesting two different QTL for DTA that are photoperiod dependent. QTL detected in this population on chromosomes 3 (npi108) and 8 in long-days were in similar region as QTL identified only in long-days in other populations. That might suggest that on each of chromosomes 3 (npi108) and 8, one QTL is present and controls flowering only in long-days. On chromosome 9, comparison with other studies supported the hypothesis of two different QTL for DTA in the CML9/A632Ht population, one QTL functional in long-days and the other QTL active in short-days even though they had close position herein. On chromosome 2, the comparison supported the hypothesis of one QTL that is photoperiod independent and controls flowering in both daylengths.

Comparison of QTL positions in maize and other grass species

Grass species have maintained gene content and order of loci on large chromosomal segments even after 65 million years of divergence (Hulbert et al., 1990). Comparison of QTL positions over different taxa might help elucidate the process of adaptation and evolution. QTL for flowering time in different grass species were identified in the same region (same species) or corresponding regions (different species) as QTL detected herein. Common markers were used to identify similar or corresponding regions. In rice, a QTL controlling flowering (Li et al., 1995) was detected in the corresponding region of maize chromosome 1 (umc83) where a QTL was detected in short-days in CML9/A632Ht maize population. A QTL controlling flowering in sorghum was detected in long-days in linkage
group (LG) B near umc5 (Lin et al., 1995). That region corresponds to the maize chromosome 2 where the QTL for DTA was detected in long and short daylengths in this study. The sorghum LG F, where umc156 and umc126 were mapped, corresponds to maize chromosome 5 where QTL for DTA was detected herein in short-days. In that region, a DNA segment was introgressed from early germplasm into a late inbred after backcrossing. Another introgression was observed in sorghum LG D, the corresponding region to maize chromosome 10 (npi264). In that same region in sorghum (npi264), a QTL for flowering was observed (Lin et al., 1995). The paralogous region to maize chromosome 10 in rice (Paterson et al., 1995) also contained a QTL related to heading date in long-days (Li et al., 1995). Moreover, the proportion of the phenotypic variation explained by the QTL found herein on chromosome 10 was the largest ($R^2 = 35$ to 46\%) as similarly found in the corresponding region in sorghum (Lin et al., 1995) and barley (Laurie et al., 1994). Perhaps, variation in genes conferring adaptation to long-day environments (maize chromosomes 1, 2, 5 and 10) occurred at the same ancestral loci of many cereals as described previously (Paterson et al., 1995). This might support the hypothesis that adaptation to higher latitudes preceded speciation.

**Candidate genes**

Many genes controlling flowering time and photoperiod response were identified in maize and other cereals. Association between loci with quantitative and qualitative effects was previously suggested by Robertson (1985). Relating QTL to known genes can help in the assessment of the biology of flowering and photoperiod response. The candidate genes discussed below have genetic positions linked to the QTL detected herein according to the Composite Pioneer 1999 map (www.agron.missouri.edu). *Idl* (Indeterminate growth1), *phyl*
(Phytochrome1), and D8 (Dwarf8) genes involved in flowering time are linked to the regions of chromosome 1 (umc23 and umc106) where two QTL for DTA were detected in the short-day environments in the CML9/A632Ht population. The QTL near umc106 was detected in only one short-day environment (1996) and was associated with only 7% of the total phenotypic variation. On chromosome 3 (umc102), where a QTL for DTA was found in short-days, zag2 (Zea AGAMOUS homolog2; Schmidt et al., 1993) was mapped. Based on its polypeptide sequence, that gene has 49% identity with Agamous Gene (AG) of Arabidopsis and might be involved in flowering time in short-days as in Arabidopsis. In Arabidopsis, the mutant ag restricts flowering to short-day environments and the overexpression of AG alleles results in early flowering (Simpson et al., 1999). A locus phyA2 (Wright et al., 1987), identified by a probe derived from rice phytochrome, was linked to the QTL for DTA on chromosome 5 in short-days detected herein. The phybl locus was linked to QTL for DTA detected in long and short-days on chromosome 9 and linked to a QTL for PPR in the same region. On chromosome 10, a homologous gene to zag2 (zmml) in maize (Theissen et al. 1995) was mapped in less than 30 cM from the QTL for DTA in long-days and a QTL for PPR. The zag2 and zmml genes belong to the MADS-box gene family. Their contribution in controlling flowering time is suggested but not yet proven. In other cereals, in the corresponding region to maize chromosome 10, photoperiod response and flowering genes were mapped: sorghum gene Mal (Lin et al., 1995), rice genes Sel and Se3 (Paterson et al., 1995), wheat photoperiod response genes ppd1, ppd2, and ppd3 (Hart et al., 1993), and barley photoperiod response gene pPD-H1 (Laurie et al., 1994). Thus, that region of chromosome 10 is involved in flowering time and photoperiod response of maize and
other cereals. Therefore, the hypothesis of same ancestral loci before divergence of these cereals might be supported.

QTL studies are important for gene mapping and suggest links between quantitative and qualitative traits. This method of mapping is based on relating variation in phenotype with allelic variation at DNA marker loci. Association mapping is another approach to mapping that starts with variation in genotype using cloned genes and relates allelic variation of those genes with variation in phenotype. Association mapping is limited to cloned genes and can be useful when a gene is suspected to control a trait. QTL mapping is a much broader method since it is not limited to specific genes and it may detect any region of the genome that may have an association with a given phenotype. These two methods can be complementary in relating genes to traits. On chromosome 1, association mapping and this present study related flowering time to the region of umc106 and to the D8 gene. However, that location does not seem to be a major region in controlling DTA in the CML9/A632Ht population and other maize populations. Association mapping could be used especially to confirm the relation between candidate genes with major QTL found herein on chromosomes 8, 9, and 10. Map-based cloning can be another alternative for identifying genes responsible for these major QTL and confirming if the candidate genes reported above are effectively involved in controlling DTA and PPR, as have been done in rice (Yano et al., 2000; Takahashi et al., 2001).

Implications for basic and applied research

QTL mapping studies are population specific. In this study, QTL detection was based on the polymorphism between CML9 and A632Ht. Genomic regions other than the ones found in this study, might contribute to control flowering and photoperiod response. Because
of lack of polymorphism, these regions might not be associated with flowering in this sample of maize germplasm. Moreover, QTL with minor effects are more difficult to identify with QTL mapping especially with small sample sizes (Beavis et al., 1994). QTL could be mapped to 10 to 30 cM (Remington et al., 2001) and the effect of each QTL and the proportion of genotypic variance they explain can be inflated (Melchinger et al., 1998). Further studies, such as map-based cloning, are needed to relate a QTL to a specific chromosomal region and to a specific gene. Moreover, a QTL can be a gene or a cluster of linked genes (coding and non-coding regions) that affect gene expression. Relating a QTL for flowering and for PPR to genes can help elucidate the process of adaptation of plants to their environments. Geneticists studying evolution of plants can also use QTL mapping to make a more advanced comparative analyses between species. That might help resolve some questions in the evolution process such as whether the adaptation to the environment of cereals started before or after divergence between cereals. Identifying and sequencing the genomic regions responsible for a QTL for DTA and PPR can provide more precise clues on how plants started to adapt to their environments. For example, transposable elements could be the origin of a mutation and after their insertion, these elements might have lost their ability to transpose producing a stable mutation responsible for major differences between tropical and temperate maize. The cause can also be a point mutation as has been found in Arabidopsis (El-Assal et al., 2001).

Identification of genes controlling DTA and PPR could be useful to breeders. Identification of QTL related to DTA and PPR can be a starting point for isolation and cloning of genes controlling both traits. Genes responsible for the major QTL could be used in transformation studies that could allow faster conversion of lines. DNA marker loci
closely linked to QTL for DTA and PPR could be used for a quick screening in the laboratory of maize seeds before sending them to winter nurseries to check for their sensitivity to photoperiod. These markers can also be used in marker-assisted selection in breeding programs incorporating tropical material in temperate maize or vice-versa. A marker locus close to the QTL or both markers flanking the QTL can be used to assist selection programs, especially for QTL with major effects such as QTL for DTA and PPR on chromosomes 8, 9, and 10 identified herein. The DNA markers can assist breeders in converting late flowering maize line to early photoperiod-insensitive flowering. The 15% earliest F₃ lines (36 lines) in the combined long-day environment in the CML9/A632Ht population had a DTA of 86 days. The population average in the combined long-day environment was 92 days. A632Ht parent had a DTA of 78 days in the combined long-day environment. One of those F₃ lines (line 208) had high percentage of the CML9 parent alleles (64%) and DTA of 87 days. Another line (line 217) had only 30% of the CML9 alleles and DTA of 83 days. Therefore, the F₃ line 208 with 64% of CML9 alleles could be selected and backcrossed to CML9 parent, in order to recover as many CML9 alleles as possible, while maintaining its earliness. Moreover, all the 15% earliest lines except 3 or 4, had at least one A632Ht allele at all DNA markers flanking the three major QTL for DTA in long-days on chromosomes 8, 9, and 10. That might confirm that the loci flanking QTL for DTA on chromosomes 8, 9, and 10 could be converted to A632Ht alleles and would be enough to convert a late line to early flowering.
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28:286-289.


Figure 1. Distribution of days to flowering (DTA) of F₃ lines of the CML9 x A632Ht maize population in the combined long (■) and short-day (□) environments.
Figure 2. Genetic map of chromosomes 1 through 10 of the CML9 x A632Ht F3 maize population and position of QTL for photoperiod response (PPR) and for days from sowing to anthesis (DTA) in the combined analyses of long and short-day environments. QTL are indicated by boxes at the left of each chromosome. Locus names with asterisks *, **, *** indicates loci with distorted genotypic ratios at P < 0.05, 0.01 and 0.001 respectively. Chromosome number is at the top of each linkage group.
Table 1. Phenotypic values of flowering time (DTA) and photoperiod response (PPR) of F3 lines of the *CML9 x A632Ht* maize population and the parent inbred lines *CML9* and *A632Ht*

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Genotypes:</td>
<td>LD§</td>
<td>SD§</td>
<td>LSD‡</td>
<td>LD SD LSD‡</td>
</tr>
<tr>
<td>DTA (in days)</td>
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</tr>
<tr>
<td>- <em>CML9</em></td>
<td>101</td>
<td>69</td>
<td>19</td>
<td>127 100 19</td>
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<tr>
<td>- <em>A632Ht</em></td>
<td>68</td>
<td>71</td>
<td>12</td>
<td>97   88 6</td>
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<td>- LSD‡</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>5    2</td>
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<tr>
<td>- F3 lines (mean)</td>
<td>83 62 1</td>
<td>109 90 1</td>
<td>84 60 1</td>
<td>92 71 1</td>
</tr>
<tr>
<td>- Range</td>
<td>68-95</td>
<td>56-71</td>
<td>99-120</td>
<td>82-99</td>
</tr>
<tr>
<td>- LSD‡</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>3    8</td>
</tr>
<tr>
<td>PPR‡</td>
<td></td>
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<tr>
<td>- <em>CML9</em></td>
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<td>0.59</td>
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<td>0.11</td>
<td>0.09</td>
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<td>- LSD‡</td>
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<td>- F3 lines (mean)</td>
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<tr>
<td>- Range</td>
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<td>0.23-0.60</td>
<td>0.15-0.52</td>
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<td>- LSD‡</td>
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<td>---</td>
<td>---</td>
<td>0.01</td>
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</table>

† The Least Significant Difference (α<0.05) for comparing parents in the same daylength
‡ The Least Significant Difference (α<0.05) for comparing the same parent in different daylengths
§ LD = long-days and SD = short-days as defined in Materials and Methods
¥ Combined analysis of each daylength as defined in Materials and Methods
Table 2. Variance components of photoperiod response (PPR) and days to anthesis (DTA) of F3 lines of the *CML9 x A632Ht* maize population in long- and short-day environments

<table>
<thead>
<tr>
<th>Variance</th>
<th>Trait</th>
<th>Genotypic</th>
<th>Error</th>
<th>Genotype x environment</th>
<th>Heritability §</th>
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<tr>
<td></td>
<td>DTA†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Short-day</td>
<td>5 (4-6)</td>
<td>2 (1-2)</td>
<td>2 (2-4)</td>
<td>0.85 (0.82-0.88)</td>
</tr>
<tr>
<td></td>
<td>- Long-day</td>
<td>18 (15-22)</td>
<td>10 (9-12)</td>
<td>2 (1-2)</td>
<td>0.88 (0.85-0.90)</td>
</tr>
<tr>
<td></td>
<td>PPR‡</td>
<td>0.004 (0.000-0.007)</td>
<td>0.003 (-0.001-0.006)</td>
<td>----</td>
<td>0.81 (0.77-0.84)</td>
</tr>
</tbody>
</table>

† DTA measured in three environments with two replications in each daylength
‡ PPR estimated in three pair of environments as described in Material and Methods
§ Broad-sense heritability on an entry-mean basis
Values in parentheses represent the 95% confidence interval
Table 3. Summary of QTL for flowering time (DTA) in long-day environments in the F$_3$ lines of the *CML9 x A632Ht* maize population

<table>
<thead>
<tr>
<th>Environment</th>
<th>Chromosome</th>
<th>DNA Loci</th>
<th>LOD$\varepsilon$</th>
<th>Additive Effect†(days)</th>
<th>Partial R$^2$§ Additive</th>
<th>Dominance Effect‡(days)</th>
<th>Partial R$^2$§ Dominance</th>
</tr>
</thead>
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<td>10.5</td>
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<td></td>
<td>2</td>
<td><em>umc5</em></td>
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<td>1.2*</td>
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<td>-2.1**</td>
<td>6.2</td>
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<tr>
<td></td>
<td>3</td>
<td><em>npi108a</em></td>
<td>3.2</td>
<td>1.7**</td>
<td>8.2</td>
<td>-1.0</td>
<td>1.3</td>
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<tr>
<td></td>
<td>8</td>
<td><em>umc138b</em></td>
<td>8.1</td>
<td>2.4**</td>
<td>15.4</td>
<td>0.0</td>
<td>0.0</td>
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<td><em>umc81</em></td>
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<td>12.2</td>
<td>0.6</td>
<td>0.4</td>
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<tr>
<td></td>
<td>10</td>
<td><em>npi264</em></td>
<td>25.0</td>
<td>5.6**</td>
<td>44.5</td>
<td>1.7**</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Total Adjusted R$^2$§: 57

† DNA locus closest to the most likely position of the QTL
‡ CML9 alleles increase (positive effect) or decrease (negative effect) the value of the trait
£ CIMMYT experimental station at Tlaltizapan (TL), Mexico. Artificial light was used to extend daylength
§ Partial R$^2$ = Percentage of phenotypic variation explained by QTL when all other QTL effects were unchanged
§ Total adjusted R$^2$ = Percentage of phenotypic variation explained by the model including all QTL
$\varepsilon$ LOD (log$_{10}$ of likelihood odds ratio) (Lander and Botstein, 1989). These values were obtained using the chi-square approximation (Zeng, 1994) in PLABQTL before QTL were used in a simultaneous multiple regression to estimate the gene effects and partial R$^2$ listed in this table
¥ ISU Agronomy and Agricultural Engineering Research Center, Iowa
*, ** Significant effect at p < 0.05 and 0.01 respectively
Table 3. Continued

<table>
<thead>
<tr>
<th>Environment</th>
<th>Chromosome</th>
<th>DNA</th>
<th>LOD€ Effect‡(days)</th>
<th>Partial R²§</th>
<th>Additive Effect‡(days)</th>
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<td></td>
<td>3 npi108a</td>
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<tr>
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<td>11.1</td>
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<td>8.3</td>
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Table 4. Summary of QTL for flowering time (DTA) in short-days in the F₃ lines of the CML9 x A632Ht maize population

<table>
<thead>
<tr>
<th>Environment</th>
<th>Chromosome</th>
<th>DNA locus †</th>
<th>LOD</th>
<th>Environment</th>
<th>DNA locus †</th>
<th>LOD</th>
<th>Environment</th>
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† DNA locus closest to the most likely position of the QTL
‡ CML9 alleles increase (positive effect) or decrease (negative effect) the value of the trait
£ CIMMYT experimental station at Tlaltizapan (TL), Mexico. Artificial light was used to extend daylength
§ Partial R² = Percentage of phenotypic variation explained by QTL when all other QTL effects were unchanged
§ Total adjusted R² = Percentage of phenotypic variation explained by the model including all QTL
€ LOD (log₁₀ of likelihood odds ratio) (Lander and Botstein, 1989). These values were obtained using the chi-square approximation (Zeng, 1994) in PLABQTL before QTL were used in a simultaneous multiple regression to estimate the gene effects and partial R² listed in this table
*, ** Significant effect at p < 0.05 and 0.01 respectively
Table 4. Continued

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<td>0.0</td>
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<tr>
<td></td>
<td>umc85</td>
<td>6.4</td>
<td>0.7**</td>
<td>5.7</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>umc81</td>
<td>3.2</td>
<td>-0.5**</td>
<td>4.8</td>
<td>-0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Total Adjusted $R^2$: 34</td>
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<tr>
<td>Combined</td>
<td>umc23</td>
<td>6.0</td>
<td>0.6**</td>
<td>4.9</td>
<td>-0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Short-days</td>
<td>umc38a</td>
<td>7.0</td>
<td>0.8**</td>
<td>8.9</td>
<td>-0.8**</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>umc102</td>
<td>3.4</td>
<td>1.2**</td>
<td>15.0</td>
<td>-0.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>umc353</td>
<td>6.8</td>
<td>1.0**</td>
<td>9.5</td>
<td>-0.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>npi444</td>
<td>5.8</td>
<td>0.5**</td>
<td>3.0</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>npi409</td>
<td>4.2</td>
<td>-0.6**</td>
<td>4.8</td>
<td>-0.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>umc81</td>
<td>12.3</td>
<td>-1.0**</td>
<td>12.6</td>
<td>-0.3</td>
<td>0.7</td>
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<tr>
<td>Total Adjusted $R^2$: 40</td>
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</table>
Table 5. Summary of QTL for photoperiod response (PPR) of flowering time (DTA) in the F3 lines of the CML9xA632Ht maize population

<table>
<thead>
<tr>
<th>Environment</th>
<th>Chromosome</th>
<th>DNA Loci†</th>
<th>LOD€</th>
<th>Additive Effect‡(days)</th>
<th>Partial R²§</th>
<th>Dominance Effect‡(days)</th>
<th>Partial R²§</th>
</tr>
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<tbody>
<tr>
<td>1995</td>
<td>1</td>
<td>umc185</td>
<td>4.3</td>
<td>0.03**</td>
<td>6.3</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>bnl15.20</td>
<td>5.3</td>
<td>0.04**</td>
<td>11.0</td>
<td>0.02</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>umc152</td>
<td>6.4</td>
<td>0.03**</td>
<td>6.1</td>
<td>0.04**</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>umc81</td>
<td>12.6</td>
<td>0.06**</td>
<td>27.9</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>npi264</td>
<td>26.8</td>
<td>0.10**</td>
<td>42.4</td>
<td>0.04**</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Total Adjusted R² §: 57

† DNA locus closest to the most likely position of the QTL
‡ CML9 alleles increase (positive effect) or decrease (negative effect) the value of the trait
§ Partial R² = Percentage of phenotypic variation explained by QTL when all other QTL effects were unchanged
§ Total adjusted R² = Percentage of phenotypic variation explained by the model including all QTL
€ LOD (log₁₀ of likelihood odds ratio) (Lander and Botstein, 1989). These values were obtained using the chi-square approximation (Zeng, 1994) in PLABQTL before QTL were used in a simultaneous multiple regression to estimate the gene effects and partial R² listed in this table
¥ PPR was calculated as ((DTA in long-days - DTA in short-days) / DTA in short-days) for each year in for the combined years
*, ** Significant effect at p < 0.05 and 0.01 respectively
Table 5. Continued

<table>
<thead>
<tr>
<th>Environment</th>
<th>Chromosome</th>
<th>DNA loci†</th>
<th>LOD£</th>
<th>Additive Effect‡(days)</th>
<th>Partial $R^2$</th>
<th>Dominance Effect‡(days)</th>
<th>Partial $R^2$</th>
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<tr>
<td>1996</td>
<td>4</td>
<td>npi444</td>
<td>3.6</td>
<td>-0.02**</td>
<td>12.1</td>
<td>-0.01</td>
<td>0.5</td>
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<tr>
<td></td>
<td>8</td>
<td>umc138b</td>
<td>7.6</td>
<td>0.02**</td>
<td>13.9</td>
<td>0.00</td>
<td>0.1</td>
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<tr>
<td></td>
<td>9</td>
<td>umc39d</td>
<td>11.8</td>
<td>0.03**</td>
<td>18.8</td>
<td>0.00</td>
<td>0.1</td>
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<td>npi264</td>
<td>15.7</td>
<td>0.04**</td>
<td>28.5</td>
<td>0.01</td>
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<td><strong>Total Adjusted $R^2$: 42</strong></td>
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<tr>
<td>1997</td>
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<td>umc152</td>
<td>9.4</td>
<td>0.03**</td>
<td>15.7</td>
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<tr>
<td></td>
<td>9</td>
<td>umc81</td>
<td>18.7</td>
<td>0.05**</td>
<td>34.5</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>npi264</td>
<td>24.2</td>
<td>0.06**</td>
<td>36.7</td>
<td>0.01</td>
<td>0.6</td>
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<td></td>
<td><strong>Total Adjusted $R^2$: 52</strong></td>
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<tr>
<td>Combined</td>
<td>8</td>
<td>umc152</td>
<td>9.7</td>
<td>0.03**</td>
<td>17.7</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Years</td>
<td>9</td>
<td>umc81</td>
<td>18.8</td>
<td>0.04**</td>
<td>34.6</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>npi264</td>
<td>24.4</td>
<td>0.06**</td>
<td>45.6</td>
<td>0.02**</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Total Adjusted $R^2$: 58</strong></td>
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</table>
CHAPTER 3. QUANTITATIVE TRAIT LOCI OF FINAL LEAF NUMBER, PLANT HEIGHT, AND THEIR RESPONSE TO PHOTOPERIOD IN THE CML9 X A632Ht MAIZE POPULATION

A paper to be submitted to Crop Science

R. Moutiq*, M. Lee*, G. Edmeades†, J.M. Ribaut†, and M.D. Krakowsky*‡

ABSTRACT

Photoperiod affects different aspects of maize (Zea mays L.) growth and development, which hinders the evaluation of germplasm. Little is known about the genetic control of sensitivity to photoperiod. Final leaf number (FLN) and plant height (PH) are sensitive to daylength and easily measured. In this study, both traits were used to assess photoperiod response in a population of 236 F3 lines of a cross between photoperiod-sensitive inbred CML9 and photoperiod-insensitive inbred A632Ht. The F3 lines and the parents were evaluated in three long and three short-day environments. A different set of quantitative trait loci (QTL) was detected for each photoperiod and for each trait. Three QTL for FLN and four for PH were detected in the same genetic regions in both daylengths. Four

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QTL for FLN and three for PH were detected only in long-day environments. Five QTL for FLN and three for PH were detected only in short-days. Regarding photoperiod response (PPR), three QTL for PPR of FLN and three for PPR of PH were detected. QTL for FLN, PH, and PPR of one or both traits were located to common regions of chromosomes 2, 3, 6, 8, 9, and 10.

INTRODUCTION

The sensitivity of maize (*Zea mays* L.) to photoperiod has limited the exploitation of the diversity of the germplasm. Photoperiod affects several aspects of maize growth and development. Longer days stimulate vegetative growth, which results in taller plants and greater leaf number, whereas shorter days signal a shift to the reproductive phase (Stevenson and Goodman, 1972; Manrique and Hodges, 1991). Based on apical meristem development, three phases of plant development have been defined: juvenile, inductive, and reproductive. The number of leaves is determined by the duration of the juvenile and inductive phases. Sensitivity to photoperiod begins with the inductive phase, which is terminated by tassel initiation (Grant, 1989). The leaf number in maize was found to be influenced by photoperiod between the five and the six-leaf stages and a brief photoperiod-insensitive phase just before tassel initiation was observed (Tollenaar and Hunter, 1983). The phases of shoot development might be under the control of different sets of genes (Leng, 1951). Based on a genetic study of node number, plant height, and days to anthesis, the interval from planting to anthesis was divided into two phases: (i) phytomer initiation and (ii) stem elongation (Vlăduțu et al., 1999). Phytomers are the basic structural units of the maize shoot, consisting of leaf, internode, prophyll, and bud (Poethig, 1994). The initiation phase
corresponds to the interval from planting to the transition of the apical meristem to the generative stage. The stem elongation phase corresponds to the interval from tassel initiation to anthesis. Alterations in the total number of phytomers could induce non-additive changes in plant height (PH) (Vlăduțu et al., 1999).

Quantitative trait loci analysis is a method that relates the variation of the phenotype with allelic variation in the genome. Studies of QTL have detected genetic factors for FLN and PH on most maize chromosomes but these QTL were usually not related to photoperiod response. In a study conducted in long (North Carolina, 35°N, 78°W with 14.5 hours daylength) and short-days (Florida, 25°N, 20°W with 11 hours daylength), QTL for FLN were detected on chromosomes 1, 2, 6, 8, and 10 in long-days. QTL for FLN were not detected in short-days. QTL for PH were detected on chromosome 6 only in short-days, on chromosome 9 only in long-days, and on chromosomes 1, 8, and 10 in long and short-days (Koester et al., 1993). In a study conducted only in short-days, QTL for FLN were detected on chromosomes 2, 3, 4, 6, 7, 8, 9, and 10 (Jiang et al., 1999). At least one QTL for PH was detected on each of the 10 maize chromosomes in long-day environments (Beavis et al., 1994; Schon et al., 1994; Veldboom and Lee, 1996). Two QTL, vgt1 and vgt2, on chromosome 8, were associated with PH and node number (FLN) in long-days (Vlăduțu et al., 1999). These two QTL were suggested to have pleiotropic effects on PH and FLN and to be involved in different pathways (Vlăduțu et al., 1999). Later, vgt1 was more precisely mapped to a 0.3 cM interval and exhibited single-factor inheritance with large effects on node numbers and days to anthesis (Salvi et al., 2002).

Qualitative mutants affecting PH have been identified on all maize chromosomes. However, the involvement of those genes in response to different photoperiods has not been
reported to our knowledge. Phytochromes, the most studied photoreceptors genes, are known to be involved in floral induction and photoperiod response. In a comparative study across Poaceae, phyA1 and phyA2 were closely associated with height mutants in maize, while phyB was associated with height QTL in maize, sorghum (Sorghum bicolor L.), and rice (Oryza sativa L.) (Lin et al., 1995). The association was declared when the genetic distance between genes and QTL was no more than 30 centimorgans (cM).

A maize population of F3 lines derived from a cross between a photoperiod-sensitive inbred CML9 and a photoperiod-insensitive inbred A632Ht was evaluated in long and short daylengths. A subset of the experiments were conducted in adjacent fields with artificial light to extend daylength in short-day environments to allow the study of photoperiod response associated with FLN and PH, while minimizing the confounding effects of other environmental factors. The objectives of this study were: (i) to map QTL for PH in long- and short-days; (ii) to map QTL for FLN in both daylengths; (iii) to map QTL for photoperiod response (PPR) of FLN and PH; and (iv) to estimate the gene action of those traits.

MATERIAL AND METHODS

Plant material and field experiments

The photoperiod-sensitive inbred, CML9, was crossed to an insensitive line, A632Ht. The F1 plants were self-pollinated to produce the F2 generation. The F2 plants were grown at short daylength (11.5 hours) and were self-pollinated to produce the F3 generation lines at the CIMMYT research station, Tlaltizapan, Mexico. The parents and 236 F3 lines were evaluated in three short-day and three long-day environments. The lines were planted in single-row plots 0.75 m apart and 2.5 m long and arranged in a 24 x 10 alpha (0,1) lattice
with two replications per environment. Ten to 13 plants were maintained per plot. The environments and dates of planting were 1) Tlaltizapan (TL) (18°N, 99°W), Mexico, 26 June 1995 (17 hours daylength), 2) Tlaltizapan, 6 December 1996 (17 hours daylength). 3) Ames (42° N 93° W), Iowa, at the Iowa State University Agronomy and Agricultural Engineering Research Center, in 17 May 1997 (15.5 hours daylength), 4) Tlaltizapan, 26 June 1995 (13 hours daylength), 5) Tlaltizapan, 6 December 1996 (11.5 hours daylength), and 6) Tlaltizapan, 11 June 1997 (13 hours daylength). Environments 1, 2, and 3 were considered long-day and environments 4, 5, and 6 were considered short-day. Artificial light was used to extend the daylength to 17 hours in environments 1 and 2. The distance of the 150-watt lamps from the soil surface was adjusted throughout the season to follow the increased height of the plants. The supplemental light had an action spectrum between 400 nm and 1.1 μm with a peak at 950 nm. The light intensity was between 0.5 and 1% of full sunlight in the center of the field. The critical full spectrum illumination was 500 mWm$^{-2}$.

**Analyses of phenotypic data**

Plant height was measured in centimeters from the ground to the base of the tassel and averaged over ten plants per plot. The final leaf number was counted two weeks after flowering and averaged over ten plants per plot. The fifth and tenth leaves on each plant were identified to assist counting the FLN. Combined analyses for each daylength were conducted because Box's tests of the homogeneity of variances were not significant at 5% level for either FLN or PH within both daylengths (Milliken and Johnson, 1992). The least square means (lsmeans) for FLN and PH in each daylength for the combined environments were calculated by using SAS (SAS Institute Inc., 1999). The F$_3$ lines were considered fixed effects, whereas environments and complete and incomplete blocks were random effects. To
calculate variances, lines were also considered random effects (Cardinal et al., 2001). Lsmeans were used to calculate photoperiod response (PPR) and for QTL analyses of each trait. PPR of each F3 line and parents were calculated as the difference between lsmeans of FLN or PH in long-days and in short-days divided by lsmean of the respective trait in short-days. Broad-sense heritabilities on an entry-mean basis and their exact confidence intervals were calculated (Knapp et al., 1985). Genetic correlations were calculated using PROC GLM (SAS Institute, Inc, 1999).

Genotypic analyses

Leaf samples were harvested and DNA was extracted from the parental lines and the 236 F2 plants. Enzymes EcoRI or HindIII digested the DNA and fragments were separated in agarose gels then transferred to nylon membranes. Hybridization with probes was used to detect restriction fragment length polymorphism (Hoisington et al., 1994). Additional loci were detected by Simple Sequence Repeats (SSR). The linkage map was constructed by using MAPMAKER Version 3.0 (Lander et al., 1987). Loci were assigned to linkage groups with a minimum log10 of the likelihood odds ratio (LOD) of 3.0 and a maximum distance of 50 centimorgans between loci (Haldane). For chromosome 4, the minimum distance was extended to 54 cM because loci umc123 and umc31 were separated by 53.8 cM. The “Three-point” and “Order” commands were used sequentially, and the best order was selected for each linkage group. The command “Try” was used to place the remaining loci in their appropriate linkage group.

The total length of the genetic map was 1658 cM and the average distance between loci was 14 cM. Segregation ratios of genotypic classes at 14 (11%) loci deviated at P = 0.05 from the expected ratios for a F2 population. Six loci (npi203, npi444, umc10, npi451).
umc102, and umc50) had an excess of the genotype A632Ht/A632Ht. Two loci (umc23 and bnl3.04) had an excess of the genotype CML9/CML9, and six loci (npi264, bnl5.09, O2, bnl128, bnl13.05, and bnl8.39) had an excess of the heterozygous class. Ten linkage groups were obtained (Fig. 1).

QTL mapping

QTL were detected by composite interval mapping (Jansen, 1993; Zeng, 1994) facilitated by PLABQTL version 1.1 (Utz and Melchinger, 1996). The selection of cofactors was based on stepwise regression using the ‘cov select’ command. The DNA loci closest to QTL were chosen as final cofactors. The threshold for the LOD score ranged between 3.6 and 3.8 and was estimated from 1000 permutations of the phenotypic data by using PLABQTL (Churchill and Doerge, 1994). Then, all QTL were evaluated in forward and backward regression facilitated by the ‘seq/s’ statement of PLABQTL. The selection of the final model was based on the Akaike’s Information Criterion (Jansen, 1993). For comparing positions of QTL in different daylengths or for different traits, QTL less than 30 cM apart were considered to be in the same genetic region (Koester et al., 1993; Hyne et al., 1995). To estimate the distance between QTL found herein with QTL in other studies, pioneer composite map 1999 (www.agron.missouri.edu) was used when common DNA markers in both studies were not available. The distance between DNA markers close to QTL was estimated with the pioneer map. Therefore, the distance between QTL could be estimated.

The level of dominance at each QTL was based on the ratio, dominance effect divided by additive effect (d/a): additive (0 < d/a < 0.2), partial dominance (0.2 < d/a < 0.8), dominance (0.8 < d/a < 1.2), and overdominance (d/a > 1.2) (Stuber et al., 1987). Digenic epistatic interactions between all pairs of DNA loci were tested by using Epistacy (Holland,
Loci with significant interactions ($P < 0.00026$; Holland et al., 1997) were added to a multiple regression model. The main terms of that regression model were loci close to QTL. Interactions were significant and maintained in the final model when the effects of the individual loci near QTL and the effect of the interaction between loci were significant ($P < 0.05$) (Cardinal et al., 2001).

RESULTS

Phenotypic traits

The final leaf number (FLN) and plant height (PH) were affected by photoperiod. The $CML9$ inbred and $F_3$ lines had greater FLN and PH in long-days than in short-days. The inbred $CML9$ exhibited a greater photoperiod response (PPR) of PH and FLN than $A632Ht$. $CML9$ had six more leaves and was 75cm taller in the combined long-day environment (Table 1). Because a subset of the experiments (1995 and 1996) were planted at the same time in adjacent fields, with or without artificial light, confounding of photoperiod with other environmental factors such as temperature was minimized. $CML9$ had eight and five more leaves in 1995 and 1996 respectively and was 108cm taller in 1995.

The distributions of the $F_3$ lines for FLN and PH were unimodal and close to normality. The FLN and PH of the $F_3$ lines had a greater range and higher mean in long-day environments. The $F_3$ lines had four more leaves and were 57cm taller in the combined long-day environment (Table 1). In long-day environments of 1995 and 1996, $F_3$ lines had five and two more leaves respectively and were 66cm taller in long-days of 1995. The genotypic variance among lines was significant (Table 2). Compared with PH and $PPR_{PH}$, FLN and $PPR_{FLN}$ were less affected by the environmental factors within a given daylength. The error
variances were lower for FLN and PPR_{FLN} than for PH and PPR_{PH}. The broad-sense heritabilities were 0.84 and 0.87 for FLN in long- and short-days, respectively, and 0.74 for PPR_{FLN} (Table 1). These heritabilities indicated the stability and the reproducibility of the data. PH was more affected by the environment in long-days than in short-days. The heritability of PH was lower in long-days and the heritability of PPR_{PH} was only 0.33 (Table 1). The genetic correlation between FLN and PH were 0.55 in short-days and 0.59 in long-days (both P < 0.001). The genetic correlations between FLN in long- and short-days was 0.56 (P < 0.001) and between PH in long and short-days was 0.88 (P < 0.001).

QTL mapping

Different sets of QTL were detected in long- and short-day environments for FLN. In the combined long-day environment, seven QTL were detected and explained 59% of the total phenotypic variation. In the combined short-days, eight QTL were detected and explained 52% of the total phenotypic variation (Table 2). The QTL on chromosomes 2 (umc49b), 5, and 8 were detected in the same region with the same parental effect in both photoperiods. That might explain the genetic correlation of 0.56 between FLN in long and short-days. The QTL on chromosome 9 was located in the same region but with contrasting parental effects which suggested the presence of two different QTL in that region. The QTL on chromosomes 3 (umc39a), 6 (umc62), 9 (umc39d), and 10 were identified only in the long-day environment. The QTL on chromosomes 2 (umc34), 3 (umc102), 4, 7, and 9 (umc386) were detected only in the short-day environment (Tables 2). The gene effect was additive in both daylengths. CML9 alleles increased FLN values at all QTL detected in long and short-days, except for QTL on chromosomes 7 and 9 in short-days. Epistatic interactions were between chromosomes 5 (umc104) and 10 (umc130) in short-days and between
chromosomes 3 (umc63) and 5 (umc126) and between two loci (npi264 and umc44) on chromosome 10 in long-days (Table 3).

The QTL for PPRFLN were detected on chromosomes 8, 9, and 10 and explained 56% of the total phenotypic variation (Table 2). The QTL on chromosome 10 was in the same region as the QTL for FLN in long-days, whereas the QTL on chromosomes 8 and 9 were in the region of QTL for FLN detected in both daylengths. The gene effect was mostly additive. CML9 alleles increased PPRFLN at all QTL. The dominance effect was significant only for the QTL on chromosome 10 with dominance of CML9 alleles. The epistatic interaction between loci npi264 and umc44 on chromosome 10 observed for FLN in long-days, was significant also for PPRFLN (Table 3).

Different sets of QTL for PH were also detected in long and short-day environments. In long-days, eight QTL, explaining 43% of the total phenotypic variation were detected. In short-days, seven QTL were identified that explained 47% of the total phenotypic variation (Table 4). The QTL on chromosomes 2 (umc6), 4, 5 (npi409), and 8 were located in the same region in both photoperiods and might explain the genetic correlation of 0.88 between PH in long and short-days. The QTL on chromosomes 6 (umc205 and umc62), 7, and 10 were identified only in long-days. The QTL on chromosomes 3, 5 (umc39b), and 9 were detected only in short-day environments (Tables 4). CML9 alleles increased PH for five of eight QTL in long-days and for four of seven QTL in short-days. The dominance effect was significant for one QTL (chromosome 6) in long-days and two QTL (chromosomes 5 and 9) in short-days, with CML9 alleles dominant to A632Ht alleles (Table 4). The QTL on chromosomes 6 and 9 presented partial dominance and the QTL on chromosome 5 had complete dominance. Epistatic effects were detected between chromosomes 5 (umc83) and 6
(npi208) in short-days and between chromosomes 3 (umc18) and 10 (umc98B) and between chromosomes 4 (umc353) and 5 (umc83) (Table 5).

The QTL for PPR\textsubscript{PH} were detected on chromosomes 3, 9, and 10 and explained 22% of the total phenotypic variation (Table 4). On chromosomes 3 and 9, the QTL for PPR\textsubscript{PH} and the QTL for PH in short-days were in the same region but with contrasting parental effects. On chromosome 3, CML9 alleles increased PH in short-days, while A632Ht alleles increased PPR\textsubscript{PH}. On chromosome 9, A632Ht alleles increased PH in short-days, whereas CML9 alleles increased PPR\textsubscript{PH}. The QTL for PPR\textsubscript{PH} on chromosome 10 was in the same region and had similar parental effect as QTL for PH in long-days (Table 4). CML9 alleles increased PPR\textsubscript{PH} for two of three QTL (Table 4). Epistasis was not identified for PPR\textsubscript{PH}.

**Correspondence of the position of QTL for different traits**

Coincidence of the positions of QTL for PH and FLN was observed in long- and short-days. In short-days, chromosomes 3 (umc102), 8 (npi108) and 9 (umc386) harbored QTL for PH and FLN in the same region. In long-days, QTL for FLN and PH were mapped to the same region on chromosomes 6 (umc62), 8 (umc138b), and 10 (npi445). This coincidence of QTL position for FLN and PH might explain the genetic correlation between FLN and PH, 0.55 in short-days and 0.59 in long-days.

Six genetic regions harbored QTL for FLN and PH and/or QTL for PPR\textsubscript{FLN} or PH. On chromosome 2, the region between umc6 and umc38b had QTL for PH in both long- and short-days and a QTL for FLN in short-days. On chromosome 3, the region of umc102 had QTL for FLN and PH in short-days and QTL for PPR\textsubscript{PH}. On chromosome 6, the region of umc62 had QTL for FLN and PH in long-days. On chromosome 8, the region between umc138b and npi108c had QTL for FLN and PH in both daylengths and QTL for PPR\textsubscript{FLN}. 
On chromosome 9, the region between umc386 and umc39d had QTL for FLN in both daylengths, QTL for PH in short-days, QTL for PPRFLN, and QTL for PPRPH. On chromosome 10, QTL for FLN and PH in long-days and QTL for PPRFLN and of PH were clustered in the region between npi445 and npi264.

**DISCUSSION**

**QTL mapping**

For PH and FLN, three sets of QTL were detected: (i) QTL with similar map position in long and short-days, (ii) QTL detected only in long-days, and (iii) QTL identified only in short-days. The first set was detected in the same genetic region (less than 30 cM interval) with same parental effect in long- and short-days and included QTL for FLN on chromosomes 2 (umc49b), 5 (umc1-bnl7.7l), and 8 and QTL for PH on chromosomes 2 (umc6), 4, 5 (npi409), and 8. Therefore, for this set and for each of FLN and PH, one QTL on each of the listed chromosomes may control each trait independently from photoperiod. Two linked QTL may also be possible, one active in long-days and the other active in short-days. If one QTL is controlling FLN and PH independently from photoperiod, then these QTL might be involved in controlling the juvenile phase during which FLN and PH are not affected by photoperiod. A QTL on chromosome 8 in the same region as the QTL herein was suggested to belong to the ‘earliness per se’ or the autonomous class of genes insensitive to photoperiod (Salvi et al., 2002). The QTL for FLN on chromosome 9 was in the same genetic region in both photoperiods but with contrasting parental effects. CML9 alleles increased FLN in long-days, whereas in short-days, A632Ht alleles increased the trait. QTL
for days to anthesis detected in long-days and short-days were in the same region and also had contrasting parental effects (data not shown). Thus, two linked QTL are possibly present on chromosome 9, one active in long-days and the other functional in short-days. QTL for FLN with similar map position as QTL herein on chromosomes 2 and 8 were identified previously in short-days (Jiang et al., 1999) and in long-days (Koester et al., 1993, Vlăduțu et al., 1999; Salvi et al., 2002). QTL for PH on chromosomes 2, 4, and 8 were previously detected in short-days (CIMMYT, 1994; Jiang et al., 1999) and in long-days (Beavis et al., 1994; Schon et al., 1994; Vlăduțu et al., 1999).

The second set of QTL was identified only in long-days and included QTL for FLN on chromosomes 3 (umc39a), 6 (umc62), 9 (umc39d), and 10 and QTL for PH on chromosomes 6 (umc205 and umc62), 7, and 10. This set of QTL seems therefore to be photoperiod dependent, which means these QTL were active only when daylength is above the critical value for maize (ca. 14.5 hours of daylength; Francis, 1972). QTL were identified previously in long-days on chromosomes 6 and 10 in the same regions as QTL found herein for FLN and for PH (Koester et al., 1993; Schon et al., 1994; Veldboom and Lee, 1996). However, QTL on chromosomes 6 and 10 for PH and on chromosomes 6, 9, and 10 for FLN were identified also in short-days (Koester et al., 1993; Jiang et al., 1999).

The third set of QTL identified in the CML9 x A632Ht population only in short-days included QTL for FLN on chromosomes 2 (umc34), 3 (umc102), 4, 7, and 9 (umc368), and QTL for PH on chromosomes 3, 5 (umc39b), and 9. This set also seems photoperiod dependent, which means these QTL were active only when daylength is below the critical value for maize. QTL were identified previously in short-days on chromosomes 3, 4, 7, and 9 for FLN and on chromosomes 3, 5 (umc39b), and 9 for PH in the same regions as herein
However, the QTL for FLN on chromosome 2 (Koester et al., 1993) and QTL for PH on chromosomes 3 and 9 (Koester et al., 1993; Schon et al., 1994) were detected also in long-days. Therefore, two linked QTL are probably located on each of chromosomes 3, 6, 9, and 10 for PH and on each of chromosomes 2, 6 and 10 for FLN. The first QTL of each pair is functional in long-days, and the second is functional in short-days. The QTL of sets two and three might be involved in controlling the inductive phase, which is photoperiod-sensitive.

The QTL for PPR of either FLN or PH were mapped to the same genetic region as QTL for the corresponding trait, FLN or PH, in one or both daylengths. QTL for PPRFLN and QTL for FLN in long-days were mapped to the same region on chromosomes 9 and 10. The QTL for PPRFLN and the QTL for FLN detected in both photoperiods on chromosome 8 were mapped to the same region. The QTL for PPRPH were detected on chromosomes 3, 9, and 10. On chromosome 3, the QTL had contrasting parental effects but similar map position as QTL for PH detected in short-days. The QTL on chromosome 9 had similar position and parental effects as QTL for PH in short-days while the one on chromosome 10 had similar map position and parental effects as QTL for PH in long-days. QTL for days to anthesis (DTA) and QTL PPRDTA were detected on chromosomes 8, 9, and 10 (data not shown) in the same regions as herein, which confirms the implication of these three chromosomes in photoperiod response. QTL for PPRFLN and PPRDTA (data not shown) were mostly located in the same region of QTL of the corresponding trait detected in long-days. This suggested that these QTL, except the one on chromosome 8, are having pleiotropic effects rather than the presence of different and linked QTL. On chromosome 8, the QTL for FLN suggested above to be photoperiod-insensitive and the QTL for PPRFLN were detected in the same region.
Two different QTL are then more likely on that chromosome and one of them is involved in controlling photoperiod-sensitivity.

**Clustered QTL for FLN and PH**

Six genetic regions harbored QTL associated with more than one trait (PH, FLN, PPR<sub>PH</sub> or PPR<sub>FLN</sub>): chromosomes 2 (npi287), 3 (umc102), 6 (umc62), 8, 9, and 10. That could explain the genetic correlation observed between FLN and PH: 0.59 in long-days and 0.55 in short-days. Each of these regions on chromosomes 2, 3, 6, 8, 9, and 10 may have only one QTL with a pleiotropic effect. Those QTL could, for example, code for a transcription factor that is common to different genes downstream in the pathway controlling these traits. Linked QTL might also be present at each of these regions. Moreover, chromosomes 8, 9, and 10 harbored QTL for PPR<sub>DTA</sub> and QTL for DTA in long-days (chromosomes 8 and 10) or in both daylengths (chromosome 9) (data not shown). Clustering of QTL controlling different traits was previously reported in maize and rice and a pleiotropic effect was suggested to be the major reason for this phenomenon (Xiao et al., 1996; Jiang et al., 1999). Thus, the pleiotropic effect might be the more likely hypothesis. If the pleiotropic effect hypothesis is true, a common initial mechanism controlling PH, FLN, PPR<sub>PH</sub>, and PPR<sub>FLN</sub> can be suggested. Because other QTL were associated with only one or two of these traits, subsequent specific pathways regulating different traits may exist downstream. Further studies are needed to confirm these hypotheses.

**Gene action**

The gene action was additive for QTL for FLN in both photoperiods and for PPR<sub>FLN</sub>. A similar result for FLN was observed by generation mean analysis involving the whole
maize genome (Russell and Stuber, 1983). In addition to additive effect, PH and PPRPH presented mostly partial dominance. Tallness was dominant to shortness as reported previously (Jiang et al., 1999; Khairallah et al., 1998). Epistatic effects also were involved in controlling both PH and FLN in both photoperiods and PPRFLN. In previous studies in maize, epistatic interactions in long-days were related to PH but not to node number (Vlăduţu et al., 1999).

Comparison of QTL position between cereals and candidate genes

Comparison of QTL position between different cereals revealed similarities in QTL locations across cereal species. QTL for PH in sorghum were located in the corresponding region of maize chromosomes 3, 6, 8, and 9 where QTL for PH were identified herein (Lin et al., 1995; Periera et al., 1994). A QTL for PH in rice also was identified in the corresponding region of maize chromosome 9 (Li et al., 1995). Using molecular marker loci, conversion to early alleles in a backcrossing program for earliness was observed in sorghum in the corresponding region of maize chromosomes 8, 9, and 10 where QTL for PH were identified in this study (Lin et al., 1995). Correspondence of QTL position with same function across different cereals might confirm that ancestral mutations occurred before species started to diverge. More advanced comparative studies across cereals in long and short daylength and use of more common markers between species could allow a better understanding of the genetic control of photoperiod response and the identification of pathways involved.

QTL for PH and FLN found herein on chromosomes 2, 3, 5, 6, 8, 9, and 10 were located in the same region as some known genes with qualitative effects in maize or related cereals (chromosome 2: d5 and d10; chromosome 3: rd4, sdw2, and d*N282; chromosome 5: d*-6, phyA2 and tdl, bvl, and pbs2; chromosome 6: pyl; chromosome 8: lhcb3, sdw1, and
petl; chromosome 9: d3010 and d3; and chromosome 10: Ppd). These candidate genes are maize plant height mutations except phyA2 and Ppd. PhyA2 is a locus identified by a probe from a cDNA of the rice type A photoreceptor gene. Ppd, is a barley gene that had a pleiotropic effect on plant height and photoperiod response (Karsai et al., 1997).

Implications

This study has several implications. FLN and PPRFLN were less affected than PH by environmental factors other than photoperiod since they had high and stable heritabilities over different daylengths. Therefore, FLN appears to be a trait of choice to assist studying photoperiod sensitivity in maize and selecting for early and photoperiod-insensitive maize. QTL detected herein might be used for a detailed comparison between different species to elucidate the process of evolution and adaptation. Starting with the QTL detected herein, map-based cloning can be attempted to identify and sequence the genes responsible for the QTL which might help detect the pathways involved in controlling FLN, PH and PPR of each of these traits. Map-based cloning can also help test the hypotheses suggested herein regarding the pleiotropic effect of some QTL on different traits. Since chromosomes 8, 9, and 10 had a cluster of QTL with major effects for most traits analyzed, cloning and sequencing of the genes responsible for these QTL would be worthwhile. A QTL on chromosome 8, vgt1, that was mapped to the same region as the QTL detected in this study, is in its way to be cloned (Salvi et al., 2002). The loci close to the QTL on chromosomes 8, 9, and 10 can be used for marker-assisted selection for photoperiod-insensitive maize and the production of lines and hybrids with greater adaptation.
LITERATURE CITED


controlling the transition from the vegetative to the reproductive phase in maize. Plant Mol. Biol. 48:601-613.


Fig. 1. Genetic map of chromosomes 1 through 10 of the CML9 x A632Ht F<sub>3</sub> maize population. Chromosome number is at the top of each linkage group. Horizontal bars on each chromosome indicate restriction fragment length polymorphism (RFLP) or simple sequence repeat (SSR) DNA loci. Locus names with asterisks *, **, *** indicate loci with distorted genotypic ratios at P < 0.05, 0.01, and 0.001, respectively. The centromere is indicated by a solid dot on each chromosome.
Table 1. Phenotypic values of the parents and the F₃ lines of the *CML9 x A632Ht* maize population in long- and short-day environments and the heritabilities of final leaf number (FLN) and plant height (PH) and their photoperiod response (PPR).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Long-days</th>
<th>Short-days</th>
<th>LSD *</th>
<th>PPR y</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLN (leaves)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>CML9</em></td>
<td>28</td>
<td>22</td>
<td>2.5</td>
<td>0.30</td>
</tr>
<tr>
<td><em>A632</em></td>
<td>20</td>
<td>18</td>
<td>1.8</td>
<td>0.10</td>
</tr>
<tr>
<td>LSD *</td>
<td>2.7</td>
<td>1.0</td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>F₃ lines (mean)</td>
<td>24</td>
<td>20</td>
<td>0.2</td>
<td>0.19</td>
</tr>
<tr>
<td>Range of F₃ lines</td>
<td>21-28</td>
<td>19-22</td>
<td>0.06-0.34</td>
<td></td>
</tr>
<tr>
<td>LSD w</td>
<td>0.9</td>
<td>0.4</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Genotypic variance</td>
<td>1.6***</td>
<td>0.57***</td>
<td>0.003***</td>
<td></td>
</tr>
<tr>
<td>G x E variance</td>
<td>0.26**</td>
<td>0.17**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error variance</td>
<td>0.70***</td>
<td>0.16***</td>
<td>0.002***</td>
<td></td>
</tr>
<tr>
<td>Heritability</td>
<td>0.84</td>
<td>0.87</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>95% CI v</td>
<td>0.81-0.87</td>
<td>0.84-0.89</td>
<td>0.68-0.79</td>
<td></td>
</tr>
</tbody>
</table>

* Least significant difference (LSD) at P < 0.05 for comparison in different daylengths.

* Photoperiod response = (trait in long-days - trait in short-days) / trait in short-days.

* Least significant difference (LSD) at P < 0.05 for comparison in the same daylength.

* 95% confidence interval of the heritability.

*** Significant at P<0.001.

v Genotype by environment variance

u The broad-sense heritability on an entry-mean basis
<table>
<thead>
<tr>
<th>Trait</th>
<th>Long-days</th>
<th>Short-days</th>
<th>LSD</th>
<th>PPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH (cm)</td>
<td>CML9</td>
<td>223</td>
<td>147</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>A632Ht</td>
<td>162</td>
<td>123</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>LSD x</td>
<td>115</td>
<td>55</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>F3 lines (mean)</td>
<td>204</td>
<td>147</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Range of F3 lines</td>
<td>165-244</td>
<td>116-179</td>
<td>0.18-0.68</td>
</tr>
<tr>
<td></td>
<td>LSD x</td>
<td>16</td>
<td>8</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Genotypic variance</td>
<td>242.6***</td>
<td>145.6***</td>
<td>0.004***</td>
</tr>
<tr>
<td></td>
<td>G x E variance</td>
<td>267.89***</td>
<td>69.22***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error variance</td>
<td>52.16***</td>
<td>33.4***</td>
<td>0.002***</td>
</tr>
<tr>
<td></td>
<td>Heritability</td>
<td>0.72</td>
<td>0.87</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.65-0.77</td>
<td>0.84-0.89</td>
<td>0.17-0.46</td>
</tr>
</tbody>
</table>
Table 2. Position and gene effects of quantitative trait loci (QTL) for final leaf number (FLN) in long and short-days and for photoperiod response (PPR) of FLN in the F$_3$ lines of the CML9 x A632Ht maize population.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome</th>
<th>DNA loci</th>
<th>LOD score</th>
<th>Additive (leaves)</th>
<th>Dominance (leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DNA loci</td>
<td>LOD score</td>
<td>Effect $^y$</td>
<td>Partial $R^2^x$</td>
</tr>
<tr>
<td>Long-days</td>
<td>FLN</td>
<td>2</td>
<td>umc49b</td>
<td>6.9</td>
<td>0.5**</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>umc39a</td>
<td>3.8</td>
<td>0.4**</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>umcl</td>
<td>5.0</td>
<td>0.3**</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>umc62</td>
<td>6.9</td>
<td>0.4**</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>umc138b</td>
<td>16.7</td>
<td>0.8**</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>umc39d</td>
<td>8.9</td>
<td>0.5**</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>npi264</td>
<td>23.3</td>
<td>1.1**</td>
<td>39.5</td>
</tr>
</tbody>
</table>

Total adjusted $R^2^w$: 59

$^z$ DNA loci closest to the most likely position of the QTL.

$^y$ CML9 alleles increase (when positive effect) or decrease (when negative effect) the value of the trait.

$^x$ Percentage of phenotypic variation explained by QTL when all other QTL effects were unchanged.

$^w$ Percentage of phenotypic variation explained by all QTL integrated in the final model.

*, ** Significant effect at $P < 0.05$ or $0.01$ respectively.

PPR = (FLN in long-days - FLN in short-days) / FLN in short-days.
<table>
<thead>
<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome</th>
<th>DNA $^z$ loci</th>
<th>LOD score</th>
<th>Additive (arbitrary unit) Effect $^y$ Partial $R^2^x$</th>
<th>Dominance (arbitrary unit) Effect $^y$ Partial $R^2^x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-days</td>
<td>FLN</td>
<td>2</td>
<td>umc49b</td>
<td>7.7</td>
<td>0.3** 12.4</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>umc34</td>
<td>4.3</td>
<td>0.2** 7.6</td>
<td>-0.1 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>umc102</td>
<td>10.8</td>
<td>0.4** 21.6</td>
<td>0.0 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>umc311</td>
<td>4.3</td>
<td>0.2** 7.9</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>bnl7.71</td>
<td>4.3</td>
<td>0.2** 7.7</td>
<td>0.0 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>npi108b</td>
<td>5.3</td>
<td>-0.2** 10.8</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>npi108c</td>
<td>3.7</td>
<td>0.2** 8.7</td>
<td>0.0 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>umc386</td>
<td>6.2</td>
<td>-0.3** 13.6</td>
<td>0.0 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total adjusted $R^2$: 52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| PPR of FLN |       | 8           | umc12b        | 7.2       | 0.02** 15.9                                         | -0.01 2.2                                          |
|            |       | 9           | umc81         | 18.0      | 0.04** 32.4                                         | 0.00 0.3                                           |
|            |       | 10          | npi445        | 23.7      | 0.05** 46.1                                         | 0.01* 2.8                                          |
|            |       |             | Total adjusted $R^2^w$: 56 |
Table 3. Quantitative trait loci for final leaf number (FLN) in long and short-days and for photoperiod response of FLN in the CML9 x A632Ht F<sub>3</sub> maize population and epistatic effects.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chr&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Type III SS&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Pr&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Partial R&lt;sup&gt;2w&lt;/sup&gt;</th>
<th>Type III SS&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Pr&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Partial R&lt;sup&gt;2w&lt;/sup&gt;</th>
<th>Type III SS&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Pr&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Partial R&lt;sup&gt;2w&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>umc49b</td>
<td>2</td>
<td>4.09</td>
<td>0.0004</td>
<td>3.6</td>
<td>5.72</td>
<td>0.0055</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>umc34</td>
<td>2</td>
<td>4.71</td>
<td>0.001</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>umc102</td>
<td>3</td>
<td>10.96</td>
<td>&lt;0.0001</td>
<td>9.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>umc39</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20.66</td>
<td>&lt;0.0001</td>
<td>8.2</td>
</tr>
<tr>
<td>umc111</td>
<td>4</td>
<td>2.97</td>
<td>0.0024</td>
<td>2.6</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>bnl7.71</td>
<td>5</td>
<td>4.00</td>
<td>0.0022</td>
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<td></td>
</tr>
<tr>
<td>umc1</td>
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<td></td>
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<td>10.39</td>
<td>0.0011</td>
<td>4.1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>umc62</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>19.63</td>
<td>&lt;0.0000</td>
<td>7.8</td>
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<td>1.3</td>
<td>0.011</td>
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<sup>w</sup> Percentage of the total phenotypic variation explained by each component.

<sup>x</sup> Probability or level of significance.

<sup>y</sup> Sum of squares type III.

<sup>z</sup> Chromosome number.
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<th>Pr$^x$</th>
<th>Partial R$^2w$</th>
<th>Type III SS$^y$</th>
<th>Pr$^x$</th>
<th>Partial R$^2w$</th>
<th>Type III SS$^y$</th>
<th>Pr$^x$</th>
<th>Partial R$^2w$</th>
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<td>npi264</td>
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<td>0.4</td>
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<td>0.6959</td>
<td>0.2</td>
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<td>0.006</td>
<td>0.0244</td>
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<td>0.0294</td>
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</tr>
<tr>
<td>umc63*umc126</td>
<td>3*5</td>
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<td>0.0240</td>
<td>5.0</td>
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<td>npi264*umc44</td>
<td>10*10</td>
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<td>8.38</td>
<td>0.0107</td>
<td>3.3</td>
<td>0.016</td>
<td>0.0004</td>
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Table 4. Position and gene effects of quantitative trait loci (QTL) for plant height (PH) in long and short-days and for photoperiod response (PPR) of PH in the F₃ lines of *CML9 x A632Ht* maize population

<table>
<thead>
<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome DNA loci closest to the most likely position of the QTL</th>
<th>DNA LOD score</th>
<th>Additive (cm) Effect</th>
<th>Partial R² Effect</th>
<th>Dominance (cm) Effect</th>
<th>Partial R² Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-days</td>
<td>PH</td>
<td>umc6</td>
<td>3.8</td>
<td>4.6**</td>
<td>8.3</td>
<td>-0.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>umc66</td>
<td>5.6</td>
<td>4.4**</td>
<td>5.9</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>npi409</td>
<td>4.4</td>
<td>-4.8**</td>
<td>8.5</td>
<td>-1.3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>umc205</td>
<td>4.3</td>
<td>-3.2**</td>
<td>3.7</td>
<td>3.3</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>umc62</td>
<td>5.2</td>
<td>4.4**</td>
<td>6.8</td>
<td>3.0*</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>umc116</td>
<td>4.5</td>
<td>-4.0**</td>
<td>5.3</td>
<td>2.9</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>umc138b</td>
<td>17.6</td>
<td>7.9**</td>
<td>18.2</td>
<td>-2.2</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>npi445</td>
<td>7.6</td>
<td>7.4**</td>
<td>15.9</td>
<td>2.4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total adjusted R²w: 43</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*z DNA loci closest to the most likely position of the QTL.

*y CML9 alleles increase (when positive effect) or decrease (when negative effect) the value of the trait.

*w Percentage of phenotypic variation explained by all QTL integrated in the final model.

** Significant effect at P < 0.05 or 0.01 respectively.

PPR = (PH in long-days - PH in short-days) / PH in short-days.
<table>
<thead>
<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome</th>
<th>DNA (^z) loci score</th>
<th>LOD</th>
<th>Additive (arbitrary unit) Effect (^y)</th>
<th>Partial R(^2)</th>
<th>Dominance (arbitrary unit) Effect (^y)</th>
<th>Partial R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-days</td>
<td>PH</td>
<td>2</td>
<td>umc6 (5.5)</td>
<td>5.5</td>
<td>4.0**</td>
<td>9.9</td>
<td>-0.7</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>umc102 (15.4)</td>
<td>5.1**</td>
<td>14.0</td>
<td>2.3</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>npi444 (6.8)</td>
<td>4.4**</td>
<td>11.8</td>
<td>2.2</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>npi409 (4.1)</td>
<td>-3.3**</td>
<td>6.4</td>
<td>0.5</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>umc39b (3.8)</td>
<td>-2.6**</td>
<td>3.4</td>
<td>2.9*</td>
<td>1.9</td>
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<td></td>
<td>8</td>
<td>npi108c (6.4)</td>
<td>5.7**</td>
<td>18.2</td>
<td>-0.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>umc386 (7.7)</td>
<td>-4.8**</td>
<td>11.7</td>
<td>2.9*</td>
<td>1.9</td>
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<td></td>
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<td>Total adjusted R(^2): 47</td>
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<tr>
<td>PPR of PH</td>
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<td>3</td>
<td>umc18 (5.0)</td>
<td>-0.03**</td>
<td>5.4</td>
<td>-0.02</td>
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<tr>
<td></td>
<td></td>
<td>9</td>
<td>umc81 (5.0)</td>
<td>0.03**</td>
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<td>0.00</td>
<td>0.1</td>
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<tr>
<td></td>
<td></td>
<td>10</td>
<td>npi445 (5.8)</td>
<td>0.04**</td>
<td>11.9</td>
<td>0.02</td>
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<td>Total adjusted R(^2): 22</td>
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Table 5. Quantitative trait loci for plant height (PH) in the combined short and long-days in the CML9 x A632Hr F3 maize population with epistatic effects.

<table>
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<th>Loci</th>
<th>Chr</th>
<th>Type III SS</th>
<th>Pr&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Partial R&lt;sup&gt;2w&lt;/sup&gt;</th>
<th>Type III SS</th>
<th>Pr&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Partial R&lt;sup&gt;2w&lt;/sup&gt;</th>
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<tr>
<td>umc6</td>
<td>2</td>
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<td>1039.48</td>
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<tr>
<td>umc66</td>
<td>4</td>
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<td></td>
<td></td>
<td>1432.80</td>
<td>0.015</td>
<td>3.5</td>
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<tr>
<td>npi409</td>
<td>5</td>
<td>903.01</td>
<td>0.001</td>
<td>3.6</td>
<td>851.61</td>
<td>0.080</td>
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<td>umc39</td>
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<td>&lt;0.0001</td>
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<tr>
<td>umc62</td>
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<td>0.000</td>
<td>7.1</td>
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<td>0.100</td>
<td>1.9</td>
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<td>npi108</td>
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<td>&lt;0.0001</td>
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<td>3332.62</td>
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<td>0.001</td>
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<td>umc83</td>
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<td>0.856</td>
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<td>umc83*npi208</td>
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<td>&lt;0.0001</td>
<td>9.4</td>
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<td>umc18*umc98B</td>
<td>3*10</td>
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<td></td>
<td>930.42</td>
<td>0.234</td>
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<td>umc353*umc83</td>
<td>4*5</td>
<td></td>
<td></td>
<td></td>
<td>928.16</td>
<td>0.235</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<sup>w</sup> Percentage of the total phenotypic variation explained by each component.

<sup>x</sup> Probability or level of significance.

<sup>y</sup> Sum of squares type III.

<sup>z</sup> Chromosome number.
CHAPTER 4. GENERAL CONCLUSIONS

To identify QTL associated with the response to photoperiod, a population of 236 $F_3$ lines produced from a cross between a photoperiod-sensitive line $CML9$ and insensitive inbred $A632Ht$ was evaluated in three long- and three short-day environments. Using each of days from sowing to anthesis (DTA), final leaf number (FLN) and plant height (PH), photoperiod response (PPR) was estimated as the difference between the trait in long- and short-days divided by the trait in short-days. Three different sets of QTL were detected for each of DTA, FLN, and PH. The first set had similar effects and positions in both photoperiod environments. The second set of QTL was detected only in long-days and the third set was observed only in short-days.

One QTL for DTA, three QTL for FLN, and four QTL for PH had similar positions in both photoperiods. These QTL seem, therefore, photoperiod independent and could be classified in the 'autonomous promotion pathway' as described in *Arabidopisis* or 'earliness per se' as defined in *Hordeum vulgare* (L.). Five QTL for DTA, four QTL for FLN, and three QTL for PH were detected only in long-day environments. These QTL might be active only when daylength is above the critical value for maize and seem therefore photoperiod sensitive. Nine QTL for DTA, five QTL for FLN, and three QTL for PH were detected only in short-day environments. These QTL might be active only when daylength is below the critical value for maize and seem therefore also photoperiod sensitive. Therefore, photoperiod-sensitive genes could be subdivided in two subclasses: a group of genes active in long-days and a group of genes functional in short-days. Two different pathways seem to be controlling DTA, FLN, and PH, one functional in short-day environments and the second becomes active in long-day environments.
QTL related to photoperiod response were detected on four chromosomes for PPR_{DTA}, on three chromosomes for PPR_{FLN}, and on three chromosomes for PPR_{PH}. QTL for PPR_{FLN} and for PPR_{DTA} were mostly located in the same region of QTL for the corresponding trait detected in long-days. This suggested that these QTL, except the one on chromosome 8, are having pleiotropic effects rather than the presence of different and linked QTL. On chromosome 8, the QTL for FLN suggested to be photoperiod-insensitive and the QTL for PPR_{FLN} were detected in the same region. Two different QTL might then be present on that chromosome and one of them is involved in controlling photoperiod-sensitivity.

Additive effects were prevalent in controlling all the traits studied herein but dominance and epistatic effects were also identified. DTA, FLN, PH, and their corresponding PPR were mostly controlled by additive effects in both photoperiods. For DTA and FLN in long-day environments, all alleles coding for lateness and for high leaf number were from the $CML9$ parent. In short-day environments, both parents contributed alleles for lateness and for high leaf number but most of these alleles were from $CML9$. For PH, both parents contributed tallness alleles in long and short-day environments. Except FLN and PPR_{PH}, all traits presented significant dominance effects at one to three QTL. Dominance, when significant, was mostly towards earliness for DTA and towards tallness for PH in both photoperiods. Epistatic effects were also detected for all traits except DTA in short-days and PPR_{PH}.

Six chromosomal regions, chromosomes 2 ($npi287$), 3 ($umc102$), 6 ($umc62$), 8 ($umc138b$), 9 ($umc81$), and 10 ($npi445$) had a cluster of QTL for FLN, PH, PPR_{FLN}, and/or PPR_{PH}. Comparing the position of QTL for FLN, PH, their corresponding PPR and also QTL for DTA and PPR_{DTA}, chromosomes 2 ($npi287$), 3 ($umc102$ and $umc39a$), 4 ($umc311$...
and npi444), 5 (npi409), 6 (umc62), 8 (umc138b), 9 (umc81), and 10 (npi445), harbored QTL of at least two of these traits. The cluster of QTL for different traits suggested that these traits share part of their pathways. The subsequent steps of their pathways seem to diverge since each trait had QTL that map to chromosomal regions that did not have QTL for other traits.

Comparison of QTL position with previous maize studies suggested the presence of at least two different QTL, one active in long-days and the other functional in short-days, at most of the genetic regions where QTL were detected. Compared across different grass species, the correspondance of QTL position with same function across different cereales suggested that the variation in genes conferring adaptation to long-day environments occurred at the same ancestral loci of many cereals. This might support the hypothesis that adaptation to higher latitudes preceded speciation.

Chromosomes 8, 9, and 10 harbored QTL with major effects for most of the traits. These QTL for different traits were clustered in the same region. Map-based cloning seems to be worthwhile to be attempted, at these chromosomal regions to identify major genes involved in photoperiod response. Markers close to these QTL could also be used in marker-assisted selection programs.
## APPENDIX 1. EPISTATIC EFFECTS IN INDIVIDUAL AND COMBINED LONG-DAY ENVIRONMENTS FOR DTA IN THE \textit{(CML9XA632HT)} MAIZE POPULATION

<table>
<thead>
<tr>
<th>Source</th>
<th>Chromosome</th>
<th>1995 Partial R²†</th>
<th>1996 Partial R²†</th>
<th>1997 Partial R²†</th>
<th>combined Partial R²†</th>
</tr>
</thead>
<tbody>
<tr>
<td>npi264</td>
<td></td>
<td>10 18.45*** 18.0</td>
<td>17.87*** 11.5</td>
<td>17.45*** 17.1</td>
<td>17.44*** 16.5</td>
</tr>
<tr>
<td>umc138b/umc152</td>
<td>8</td>
<td>6.91*** 6.7</td>
<td>22.73*** 14.7</td>
<td>13.80*** 13.6</td>
<td>16.70*** 15.8</td>
</tr>
<tr>
<td>umc81/umc39d</td>
<td>9</td>
<td>3.62* 3.5</td>
<td>5.66** 3.7</td>
<td>8.52*** 8.4</td>
<td>5.21** 4.9</td>
</tr>
<tr>
<td>npi108a</td>
<td>3</td>
<td>5.62** 5.5</td>
<td>4.88** 3.2</td>
<td>5.55** 3.8</td>
<td>7.02** 6.6</td>
</tr>
<tr>
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<td>8.13*** 5.3</td>
<td>3.89* 5.5</td>
<td>7.73*** 7.3</td>
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</tr>
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<td>0.61NS 0.6</td>
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† (Type III sum of squares divided by total sum of squares) * 100
## APPENDIX 2. EPISTATIC EFFECTS IN INDIVIDUAL AND COMBINED ENVIRONMENTS FOR PPR OF DTA IN THE CML9 X A632HT MAIZE POPULATION

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<th>Source</th>
<th>Chromosome</th>
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<th>Partial $R^2$†</th>
<th>1996 F</th>
<th>Partial $R^2$†</th>
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<td>--</td>
<td>--</td>
<td>7.22**</td>
<td>2.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>umc138/umc152</td>
<td>8</td>
<td>10.26***</td>
<td>7.7</td>
<td>4.74*</td>
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<tr>
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<td>22.61***</td>
<td>16.9</td>
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<td>21.82***</td>
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<td>28.95***</td>
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<td>9.73***</td>
<td>3.8</td>
<td>8.37***</td>
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<td>--</td>
</tr>
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<td>--</td>
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<td>0.0</td>
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<td>3.70**</td>
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† (Type III sum of squares divided by total sum of squares)*100
APPENDIX 3: PERCENT GENOME COMPOSITION OF 15% EARLY F3 LINES IN LONG-DAY (LD) ENVIRONMENTS (DTA IN LONG-DAYS). Only dominant markers were used. AA correspond to number of loci homozygous for A632Ht alleles. BB correspond to number of loci homozygous for CML9 alleles. AB correspond to number of heterozygous loci.

<table>
<thead>
<tr>
<th>Line number</th>
<th>AA</th>
<th>BB</th>
<th>AB</th>
<th>Total allele: % A632Ht allele</th>
<th>% CML9 allele</th>
<th>DTA in LD</th>
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<tbody>
<tr>
<td>233</td>
<td>42</td>
<td>12</td>
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<td>214</td>
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</tr>
<tr>
<td>81</td>
<td>25</td>
<td>27</td>
<td>54</td>
<td>212</td>
<td>49%</td>
<td>51%</td>
</tr>
<tr>
<td>217</td>
<td>51</td>
<td>8</td>
<td>50</td>
<td>218</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>184</td>
<td>37</td>
<td>24</td>
<td>54</td>
<td>230</td>
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<td>44%</td>
</tr>
<tr>
<td>110</td>
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<td>73</td>
<td>234</td>
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<td>45%</td>
</tr>
<tr>
<td>44</td>
<td>15</td>
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<td>218</td>
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<td>54%</td>
</tr>
<tr>
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<td>44%</td>
</tr>
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<td>29</td>
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<td>64</td>
<td>234</td>
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<td>48%</td>
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<td>40%</td>
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<td>7</td>
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<td>56%</td>
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<td>32</td>
<td>37</td>
<td>198</td>
<td>49%</td>
<td>51%</td>
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APPENDIX 4. PHENOTYPIC VALUES OF THE PARENTS AND THE F3 LINES OF *CLM9 x A632Ht* MAIZE POPULATION IN LONG (LD) AND SHORT-DAY ENVIRONMENTS FOR FINAL LEAF NUMBER (FLN) AND PLANT HEIGHT (PH) AND THEIR PHOTOPERIOD RESPONSE (PPR).

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<td>0.8</td>
<td>0.7</td>
<td>0.7</td>
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<td>24</td>
<td>22</td>
<td>0.2</td>
<td>0.12</td>
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<td>17-22</td>
<td>0.07-0.48</td>
<td>21-28</td>
<td>19-25</td>
<td>-0.01-0.31</td>
<td>18-22</td>
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<tr>
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<td>0.9</td>
<td>1.4</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
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<td>1.1</td>
<td>0.6</td>
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<td></td>
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<tr>
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<td>0.4</td>
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<td>0.1</td>
<td>0.1</td>
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<td>158</td>
<td>119</td>
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<td>11</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<tr>
<td>F3 lines (mean)</td>
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<td>132</td>
<td>2.4</td>
<td>0.61</td>
<td>167</td>
<td>210</td>
<td>150</td>
<td>1.5</td>
<td>0.41</td>
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<td>127-203</td>
<td>162-2542</td>
<td>108-191</td>
<td>0.05-0.85</td>
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<td></td>
</tr>
<tr>
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<td>21.6</td>
<td>13.8</td>
<td>14.0</td>
<td>14.0</td>
<td>14.0</td>
<td>14.0</td>
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<td>41.3</td>
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<td>36.8</td>
<td>36.8</td>
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† LSD for comparison in the same photoperiod
‡ LSD for comparison among the same parent in different photoperiods
§ LD = Long-days and SD = Short-days
¶ Photoperiod response defined as: (trait in long days - trait in short days) / trait in short days.
APPENDIX 5. SUMMARY OF QTL FOR FINAL LEAF NUMBER (FLN) AND PLANT HEIGHT (PH) IN INDIVIDUAL LONG-DAY ENVIRONMENTS IN THE $F_3$ OF $CML9 \times A632Ht$ MAIZE POPULATION

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<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome Marker</th>
<th>Additive Effect</th>
<th>Partial $R^2$</th>
<th>Dominance Effect</th>
<th>Partial $R^2$</th>
</tr>
</thead>
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<td>0.2</td>
</tr>
<tr>
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</tr>
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<td>9</td>
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<td>9.7</td>
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<td>umc138b</td>
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<td>-5.1</td>
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<td>npi445</td>
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<td>8.8</td>
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<td>0.0</td>
</tr>
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<td>umc138b</td>
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<td>8.6</td>
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<td>0.7</td>
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<td>1.3</td>
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<td>Total adjusted $R^2$: 51.8</td>
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† Genetic marker closer to the QTL most likely position
‡ $CML9$ alleles increase (when positive effect) or decrease (when negative effect) the value of the trait
£ CIMMYT experimental station at Tlaltizapan, Mexico
¥ ISU Agronomy and Agricultural Engineering Research Center, West Ames, Iowa
§ Percentage of phenotypic variation explained by QTL when all other QTL effects was fixed
* Significant effect at $P < 0.05$  ** Significant effect at $P < 0.01$
### APPENDIX 5. CONTINUED

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<th>Environ- trait</th>
<th>Chromosome Marker †</th>
<th>Additive</th>
<th>Dominance</th>
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<td></td>
<td>loci</td>
<td>Effect ‡</td>
<td>Partial R² §</td>
</tr>
<tr>
<td>Ames ¥ 9 PH</td>
<td></td>
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<tr>
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<td>npi409</td>
<td>-7.6**</td>
<td>12.5</td>
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<td>5.8</td>
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<td>umc152</td>
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<td>11.5</td>
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<td>npi108c</td>
<td>4.2**</td>
<td>3.8</td>
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<tr>
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<td>npi264</td>
<td>9.5**</td>
<td>17.4</td>
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Total adjusted R²: 40.3
### APPENDIX 6. SUMMARY OF QTL FOR FINAL LEAF NUMBER (FLN) AND PLANT HEIGHT (PH) IN INDIVIDUAL SHORT-DAY ENVIRONMENTS IN THE F3 LINES OF CML9 x A632Ht MAIZE POPULATION.

<table>
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<tr>
<th>Environment</th>
<th>Trait</th>
<th>Chromosome Marker</th>
<th>Additive Effect</th>
<th>Partial R² §</th>
<th>Dominance Effect</th>
<th>Partial R² §</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>umc49b</td>
<td>0.3**</td>
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<td>-0.1</td>
<td>0.4</td>
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</tr>
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<td>umc34</td>
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<td>-0.1</td>
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</tr>
<tr>
<td>3</td>
<td>umc102</td>
<td>0.5**</td>
<td>21.4</td>
<td>-0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>umc158</td>
<td>0.3**</td>
<td>6.0</td>
<td>-0.1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
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<td>bnl7.71</td>
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<td>0.1</td>
<td>0.4</td>
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</tr>
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<td>umc117</td>
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<td>umc386</td>
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<td>0.2</td>
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<tr>
<td><strong>Total adjusted R²</strong>: 44.0</td>
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<td></td>
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<td><strong>TL 1995 PH</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>umc6</td>
<td>3.7**</td>
<td>6.8</td>
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<td>0.0</td>
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</tr>
<tr>
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<td>umc102</td>
<td>5.6**</td>
<td>12.5</td>
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<td>1.1</td>
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</tr>
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<td>umc63</td>
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<td>umc39b</td>
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<tr>
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<td>0.3</td>
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</tr>
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<td><strong>Total adjusted R²</strong>: 45.9</td>
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<tr>
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<tr>
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† Genetic marker closer to the QTL most likely position
‡ CML9 alleles increase (positive effect) or decrease (negative effect) the value of the trait
§ CIMMYT experimental station at Tlaltizapan, Mexico
$ Percentage of phenotypic variation explained by QTL when other QTL effects were fixed
* Significant effect at P < 0.05 ** Significant effect at P < 0.01
## APPENDIX 6. CONTINUED

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<th>Environment</th>
<th>Chromosome Marker †</th>
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<th>Dominance Effect ‡</th>
<th>Partial $R^2$ §</th>
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<td>loci</td>
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<td>17.4</td>
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<td>1.5</td>
</tr>
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<td>4.7</td>
<td>2.2</td>
<td>0.9</td>
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<td>0.1</td>
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<td><em>umc102</em></td>
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<td>8.6</td>
<td>0.0</td>
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<td><em>umc6</em></td>
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<td>8.3</td>
<td>-0.1</td>
<td>0.0</td>
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<td><em>umc102</em></td>
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<td>4.1</td>
<td>2.4</td>
<td>0.9</td>
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<td>-0.4</td>
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<td>-5.1**</td>
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<td>2.3</td>
<td>0.9</td>
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<td><strong>Total adjusted $R^2$: 29.7</strong></td>
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APPENDIX 7. SUMMARY OF QTL FOR PHOTOPERIOD RESPONSE (PPR) OF FINAL LEAF NUMBER AND OF PLANT HEIGHT (PH) IN THE F₃ LINES OF CML9xA632Ht MAIZE POPULATION IN INDIVIDUAL ENVIRONMENTS.

| Environment      | Trait  | Chromosome Marker | Loci | Additive Effect | Partial R² $|$ | Dominance Effect | Partial R² $|$ |
|------------------|--------|-------------------|------|----------------|----------------|-----------------|----------------|
| 1995 PPR of FLN  | 8      | umc12b            | 0.031** | 11.2         | -0.02          | 1.5             |
|                  | 9      | umc81             | 0.045** | 21.3         | 0.01           | 0.9             |
|                  | 10     | npi445            | 0.063** | 29.6         | 0.018*         | 1.8             |
|                  |        |                   | Total adjusted R²: 40.3 |
| 1995 PPR of PH   | 3      | umc102            | -0.048** | 3.9         | 0.00           | 0.0             |
|                  | 8      | umc12b            | -0.006  | 0.1          | -0.080**       | 5.3             |
|                  | 10     | npi445            | 0.061** | 6.3          | 0.00           | 0.0             |
|                  |        |                   | Total adjusted R²: 12.2 |
| 1996 PPR of FLN  | 6      | umc36b            | 0.01**  | 4.3          | 0.01           | 0.6             |
|                  | 8      | umc138b           | 0.02**  | 6.7          | -0.01          | 0.3             |
|                  | 9      | umc81             | 0.04**  | 34.0         | 0.00           | 0.0             |
|                  | 10     | npi445            | 0.06**  | 45.1         | 0.01           | 0.9             |
|                  |        |                   | Total adjusted R²: 53.5 |
| 1997 PPR of PH   | 2      | npi298            | 0.012** | 5.3          | 0.01           | 1.0             |
|                  | 3      | umc102            | -0.015**| 7.8          | -0.01          | 1.7             |
|                  | 4      | npi444            | -0.016**| 10.5         | 0.00           | 0.3             |
|                  | 9      | umc386            | 0.016** | 8.5          | 0.00           | 0.0             |
|                  | 10     | npi445            | 0.012** | 4.9          | 0.00           | 0.3             |
|                  |        |                   | Total adjusted R²: 26.3 |

† Genetic marker closer to the QTL most likely position
‡ CML9 alleles increase (when positive effect) or decrease (when negative effect) the value of the trait
§ Percentage of phenotypic variation explained by QTL when all other QTL effects was fixed
* Significant effect at P < 0.05      ** Significant effect at P < 0.01
APPENDIX 8. GENOTYPIC AND PHENOTYPIC CORRELATIONS BETWEEN DAY TO ANTHESIS (DTA), FINAL LEAF NUMBER (FLN), AND PLANT HEIGHT (PH) IN LONG-DAY (LD) AND SHORT-DAY (SD) ENVIRONMENTS.

<table>
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<th>Trait 1</th>
<th>Trait 2</th>
<th>Genotypic correlation</th>
<th>Phenotypic correlation</th>
</tr>
</thead>
<tbody>
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<td>FLN&lt;sub&gt;SD&lt;/sub&gt;</td>
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<td>0.52</td>
</tr>
<tr>
<td>PH&lt;sub&gt;LD&lt;/sub&gt;</td>
<td>PH&lt;sub&gt;SD&lt;/sub&gt;</td>
<td>0.88</td>
<td>0.74</td>
</tr>
<tr>
<td>DTA&lt;sub&gt;LD&lt;/sub&gt;</td>
<td>DTA&lt;sub&gt;SD&lt;/sub&gt;</td>
<td>0.53</td>
<td>0.39</td>
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APPENDIX 9. FORMULAS USED TO ANALYZE PHENOTYPIC DATA

1. Least Square Means (lsmeans):

The model used in the mixed model was: \( Y_{ijk} = \mu + p_i + b_{i(j)} + \tau_k + \epsilon_{ijk} \)

\( Y_{ijk} \): Observation of the ith replication in jth block of the kth treatment

\( \mu \): Overall mean

\( p_i \): Replication effect

\( b_{i(j)} \): Block effect nested within replication

\( \tau_k \): entry effect

\( \epsilon_{ijk} \): Residual

Least square method chooses means that minimize the sum of squares of the residual:

\[ \sum (y_{ijk} - m - p_i - b_{i(j)} - \tau_k)^2 \]

2. Least square difference (LSD):

Standard errors (SE) were first calculated and LSD were derived using the following formula:

\[ \text{LSD} = \frac{t_{a/2} \times SE}{t_{a/2} \times \sqrt{2 \times \sigma^2/n}} \]

\( t_{a/2} \): is the t-student coefficient with \( a/2 \) degree of freedom

\( \sigma^2 \): error variance

\( n \): number of values used to calculate the means

3. Heritability:

Broad-sense heritability on an entry-mean basis was calculated using the following formula:

\[ H^2 = \frac{\sigma^2_g}{\sqrt{((\sigma^2_g + r1) + (\sigma^2_{ge}/1) + \sigma^2_e)}} \]
\( \sigma_g^2 \): genotypic variance
\( \sigma_{ge}^2 \): genotype by environment variance
\( \sigma_e^2 \): error variance

4. Correlations:

The genotypic correlations between traits 1 and 2 were calculated using the following formula:

\[
 r_g = \frac{\text{cov}_g(1,2)}{\sqrt{\sigma_{g1}^2 \times \sigma_{g2}^2}}
\]

\( \text{cov}_g(1,2) \): genotypic covariance between traits 1 and 2
\( \sigma_{g1}^2 \): genotypic variance of the trait 1
\( \sigma_{g2}^2 \): genotypic variance of the trait 2

The phenotypic correlations were calculated using:

\[
 r_p = \frac{\text{cov}_p(1,2)}{\sqrt{\sigma_{p1}^2 \times \sigma_{p2}^2}}
\]

\( \text{cov}_p(1,2) \): phenotypic covariance between traits 1 and 2
\( \sigma_{p1}^2 \): phenotypic variance of the trait 1
\( \sigma_{p2}^2 \): phenotypic variance of the trait 2

5. Box method:

The homogeneity of variances were tested using box method:

The data were subdivided into 3, 4, or 5 groups.

For each group, the variance and then the \( \log_{10} \) of the variance were calculated.

Analysis of variance of \( \log_{10} \) of the variance was conducted.
6. Photoperiod response (PPR):

The photoperiod response for each trait was calculated as:

$$\text{PPR}_{\text{trait}} = \frac{\text{trait in long-days} - \text{trait in short-days}}{\text{trait in short-days}}$$