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Quantitative trait loci for growing degree days to flowering and photoperiod response in Sunflower (*Helianthus annuus* L.)

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Abstract The number of days from seedling emergence to flowering (DTF) is a major consideration in sunflower breeding programs. This is a complex trait determined by the genotype, environmental conditions and interactions. Photoperiod and temperature have major effects on DTF and could be important sources of genotype× environment interaction. The objectives of this study were to locate quantitative trait loci (QTLs) associated with growing degree days (GDD) to flowering and photoperiod (PP) response in an elite sunflower population. Two hundred and thirty five F_2-generation plants and their $F_{2:3}$ and $F_{2:4}$ progenies of a single-cross population of two divergent inbred lines were evaluated in six environments (locations, years and sowing dates) with photoperiods known to elicit a PP response between the inbred lines. Detection of QTLs was facilitated with a genetic linkage map of 205 RFLP loci and composite interval mapping. The 205 restriction fragment length polymorphism (RFLP) loci covered 1380 cM and were arranged in 17 linkage groups, which is the haploid number of chromosomes in this species. The average interval size was 5.9 cM. Six QTLs in linkage groups A, B, F, I, J and L were associated with GDD to flowering and accounted for 76% of the genotypic variation in the mean environment. QTLs in linkage groups A and B accounted for 72% of the genetic variation. QTL×environment (QTL×E) interactions were highly significant for linkage groups A, B, F and J (P<0.01). QTLs in linkage groups A and B were highly dependent on PP. Also, QTL mapping of the ratio of the GDD required by a progeny to flower at a PP of 12.1 and 15.0 h, defined as the photoperiod response (PPR), suggested that alleles at QTLs in linkage groups A and B were responsive to PP. QTLs in linkage groups F and J showed QTL×E interaction but the LOD values were not associated with PP. QTL×E interactions for additive effects were highly significant (P<0.01) for linkage groups A, B and F. QTL×E interactions for QTLs with dominant effects were significant (P<0.01) for linkage groups A, B and J. The dominant effect of QTLs in linkage group B increased in environments with a longer PP. The knowledge of how these QTLs influence the GDD for flowering and how they interact with the environment will facilitate marker- assisted selection and backcross conversion of photoperiod-sensitive germplasm.

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Introduction

The principal goal of sunflower breeding programs is the development of cultivars with a high oil yield. Progress towards that goal is dependent upon selection for several traits related to adaptation, productivity and stress tolerance. Days to flowering (DTF) is an important trait in sunflower breeding programs because cultivars with certain ranges of cycle length provide optimum yield in specific environments. The potential yield of a genotype can only be achieved if it is phenologically adapted to the target environment (Kinet et al. 1985). Oil yield in sunflower is sensitive to the environmental conditions during the grain-filling period (Andrade and Ferreiro 1996; Connor and Hall 1997). Therefore, selection of genotypes with the most-appropriate cycle length is critical.

In sunflower, DTF is controlled primarily by the genotype, photoperiod (PP) and temperature (Goyne et al. 1977; Marc and Palmer 1981; Goyne and Hammer 1982). Although the inductive phase (the phase in which the PP length influences DTF) has not been clearly established in sunflower, researchers have used the PP at

seedling emergence (the VE stage) to predict DTF and classify genotypes regarding their PP response (Hammer et al. 1982; Goyne and Schneiter 1987).

Studying sunflower genotypes at a PP between 9 and 16 h at VE, Goyne and Schneiter (1987) observed PP responses that included day neutral (PP insensitive), short-day, long-day and ambiphotoperiodic (short- or long-day response depending on PP). At a PP between 14.5 and 16 h, DTF did not vary appreciably. Therefore, temperature can be used in models to predict DTF within that range of PP (Goyne et al. 1989a).

Genetic investigations for DTF in sunflower have utilized conventional breeding and biometrics procedures and have usually reported polygenic inheritance patterns (Stoenescu 1974; Machacek 1979). However, there is some evidence of genetic factors with major, qualitative effects on flowering (Jan 1986). Gene action is usually additive (Miller et. al. 1980; Roath et al. 1982; Alvarez et al. 1992; El-Hity 1992; Reid 1992) but dominant effects have been observed less frequently (Jan 1986). DTF has been found to be of high broad-sense heritability, with values of 0.95 (Alvarez et al. 1992), over 0.90 (Shabana 1974), and of 0.62 to 0.95 (Berretta de Berger and Miller 1984).

Few genetic factors for DTF in sunflower have been reported. Mestries et al. (1998) identified two QTLs that accounted for 30% of the phenotypic variation at one field location. Leon et al. 2000 detected five QTLs that accounted for 73% and 89% of the phenotypic and genotypic variations across four locations with a limited range of PP (15 to 16.4 h). The genetic positions and parental effects of the QTLs were stable across environments and generations, with the exception of minor QTLs in linkage groups H and I. With the exception of the dominant effects at the QTLs in linkage group B, gene action was additive. To our knowledge, the effects of PP on QTLs for GDD to flowering and DTF have not been investigated in the sunflower by using a comprehensive genetic map based on DNA marker loci.

The sunflower inbred lines ZENB8 and HA89, the parents of the mapping population of this and previous studies (Leon et al. 1995, 1996, 2000), exhibit a differential response to photoperiods ranging from 12.1 to 16.4 h (present report). When seedlings emerge during a PP of 12 or 15 h, ZENB8 flowers 10-days earlier or later than HA89, respectively. Therefore, replicated evaluation of their mapping population under that range of PP could identify genetic factors associated with the PP response. To conduct a comprehensive study of the effect of PP on GDD to flowering, environments and planting dates were selected to include a wide range of PP (12.1–16.4). The objectives of this study were: (1) to locate QTLs affecting GDD to flowering using replicated progeny evaluated in environments witha different PP, and (2) to identify QTLs associated with a response to PP.

Materials and methods

Germplasm and field design

A cross between non-restorer inbred lines (B lines) ZENB8 (female) and HA89 (male) was made to create the F_2 population and their respective $F_{2:3}$ and $F_{2:4}$ progenies. The seed of the F_2 generation was created by self-pollinating a single plant of the F_1 generation. Seed for the $F_{2:3}$ progenies was produced by self-pollinating individual F_2 plants. Seed for $F_{2:4}$ progeny was produced by self-pollinating ten F_3 plants per $F_{2:3}$ progeny and then combining ("bulking") an equal quantity of seed from each F_3 plant in a given family. ZENB8, a proprietary inbred line, was known to flower earlier (10 days) than the HA89 line when planted at a short PP (12 h) and later (10 days) than HA89 when planted at a long PP (15 h). Within this range of PP, the responses of ZENB8 and HA89 were classified as short-day and long-day, respectively. When studied in a wider range of PP (9 to 16 h) HA89 has been classified as ambiphotoperiodic (Goyne and Schneiter 1987). Therein, the response of HA89 changed from a short-day response to a long-day response at a PP of 12 to12.5 h.

Six environments with a PP ranging from 12.1 to 16.4 h were used to evaluate the parents and their progeny. The six environments consisted of Fargo, N.D.; Balcarce, Argentina and Venado Tuerto, Argentina (one planting date in 1992 and three planting dates in 1997 at Venado Tuerto). On May 14th 1992, the F_2 generation was planted in rows at Fargo, N.D. (46.8° latitude north; PP=16.4 h). Two or three seeds per hill were sown with a hand planter and thinned to one plant per hill. The rows were 3-m long, the space between them was 70–75 cm, and the distance between hills was 30 cm. Five rows of each parent and the F_1 were planted to estimate the within-row error variance (Allard 1966; Leon et al. 1995). Before anthesis, individual heads were covered with pollination bags to ensure self-pollination and the production of F_3 -generation seed. In 1992, 235 $F_{2:3}$ families were planted on November 20th at Balcarce (37.4° latitude south; PP=15.5 h), and on November 18th at Venado Tuerto (33.2° latitude south; PP=15 h), Argentina. In 1997, 235 $F_{2:4}$ families were planted at Venado Tuerto at three different sowing dates (August 26th, September 27th and October 20th; which corresponded to a PP of 12.1, 13.1 and 14.0 h, respectively). One row per family was planted in each environment. Fifteen (1992 trials) and five (1997 trials) replicates of each parent and the F_1 hybrid were included to provide an estimate of the error variance within and across locations, respectively. Families, parents and the F_1 genotype were randomly assigned to plots in each environment.

Definition of phenotypic data

Herein, sunflower growth stages are defined according to Schneiter and Miller (1981). The number of days from VE to flowering (R5.5; when 50% of the flowers of a capitula are open), DTF, was recorded for F_2 plants and their corresponding progenies in each environment. The DTF of an $F_{2:3}$ and $F_{2:4}$ progeny was the day when 50% of the plants in a row reached the R5.5 stage. Growing degree days (GDD) were used to quantify the cycle of the parents, the F1 genotype, and corresponding progenies for the period of time between growth stages VE and R5.5. GDD to flowering were calculated as the sum of the mean daily temperature minus a base temperature of 6°C, per day. ZENB8 and HA89 have a similar base temperature of 6°C (Leon, unpublished). This base temperature has also been used in a previous investigation of flowering response in sunflower genotypes (Goyne et. al. 1989b). Temperature and PP are the most -important environmental factors that control DTF. The influence of temperature differences across environments can be reduced if DTF is expressed as growing degree days (GDD) to flowering. Values for the traits were used as phenotypes for QTL mapping: (1) GDD to flowering and (2) photoperiod response (PPR), as the ratio of the GDD required by a genotype to flower at 12.1 h (Venado Tuerto, August 26th, 1997 planting) and at 15.0 h (Venado Tuerto, November 18th, 1992 planting) of PP. Values low-

er and higher than 1 suggest that the GDD to flowering of a genotype is influenced by the photoperiod. QTLs associated with PPR should be those GDD QTLs that are responding to changes in PP.

Genetic mapping

The genetic map and segregation data used have been described previously (Berry et al. 1995; Leon et al. 1995, 1996). The RFLP data were obtained with DNA samples isolated from leaf tissue of the self-pollinated F_2-generation plants. The 205 RFLP loci covered 1380 cM and were arranged in 17 linkage groups, which is the haploid number of chromosomes in this species. The average interval size was 5.9 cM. The genetic map was constructed using MAPMAKER version 3.0 (Lander et al. 1987). Genotypic classes at 23 loci deviated significantly from the expected ratios. Those loci exhibited a deficiency in the ZENB8 homozygous class. The majority (18) of the loci with deviant ratios were located in four regions, representing linkage groups G, L and P (Berry et al. 1995).

Statistical analysis

In the F_2 generation, the total phenotypic variability due to genetic effects was estimated by calculating the broad-sense heritability for GDD to flowering, according to Allard (1966), for individual plants. The within-row F_2 variance was estimated by pooling the within-row variances of rows containing the parents and F_1 genotype. The error variance among rows was estimated in the F_2 generation from the variance among mean row values. Genetic variation was estimated by subtracting the within- and among-row variances from the phenotypic variance (Leon et al. 1995). In the $F_{2:3}$ and $F_{2:4}$ progenies, broad-sense heritabilities were estimated using variance components according to Fehr (1987); for heritability on a plot basis (for each environment) $h^2 = \frac{\sigma_g^2}{\sigma_e^2 + \sigma_g^2}$ and for herita-bility on an entry mean basis (across locations) $h^2 = \frac{\sigma_g^2}{\sigma_e^2/rt + \sigma_{ge}^2/t + \sigma_g^2}$, with t and r the number of environments and replications within environments, σ^2_e the experimental error variance, σ^2_g the genotypic variance, and $\sigma^2_{g\times e}$ the genotype×environment interaction variance. Estimates of σ^2_e within and across locations were obtained from the parents and F_1, according to Hallauer and Miranda (1988). The significance of the genotype by environment (G×E) interaction was tested according to Hallauer and Miranda (1988) using the σ^2_e estimated from the parents and F_1 across locations.

The method of composite interval mapping (CIM) (Zeng 1994) that combines interval mapping by regression (Haley and Knott 1992) with the use of cofactors, was used for mapping QTLs. The phenotypic data consisted of trait values for each F_2 plant, the $F_{2:3}$ and $F_{2:4}$ families evaluated in each environment, and the average for each $F_{2:3}$ and $F_{2:4}$ based on all environments (herein, 'the mean environment'). The traits used in QTL mapping were GDD to flowering and PPR. The use of single replicates of each family in each environment has been described previously for QTL mapping in sunflower for DTF (Leon et al. 2000), in maize for grain yield (Stuber et al. 1992; Beavis et al. 1994) and plant height (Beavis et al. 1991), and in maize×teosinte crosses for morphological traits (Doebley et al. 1990). Computations were carried out using PLABQTL Version 1.1 (Utz and Melchinger 1996) as described in detail by Bohn et al. (1996) and Austin and Lee (1998). Initially, an analysis was made with the *first* statement to check the database for errors and outliers. A second analysis with the *model D* and *scan* statements with a LOD threshold value of 2.5 was conducted to select cofactors. A third analysis was done adding the pre-selected cofactors in the *cov* statement and the *smodel* statement for the detection of digenic epistatic interactions between QTLs with significant main effects. For GDD, the coefficient of determination (R^2) from the model for the mean environment was compared with the estimated broad-sense heritability to estimate the amount of genetic variation associated with RFLP loci in multiple regression. Epistatic effects among all pairs of loci were assessed with two-way analyses of variance using the program EPISTACY (Holland 1998). A total of 20910 pairwise tests were conducted and the interlocus interaction variance was partitioned into additive×additive, additive×dominance, dominance×additive and dominance×dominance interactions. Due to the high number of comparisons, an epistatic interaction was declared when the *F*-test was significant at the $P<0.0001$ level. Interactions were then added to the multiple regression model in the *seq* statement of the PLABQTL program to estimate the amount of genetic variation associated with the complete model.

QTLs associated with the response to PP were identified by two methods: (1) QTL×E interaction for GDD to flowering estimated using PLABQTL, as described in Utz and Melchinger (1996); and (2) detection of QTLs associated with the PPR values. QTLs associated with PPR should be those GDD QTLs that are associated with the response to PP. Thus, the PPR variable per se indicates the interaction between GDD to flowering and PP.

Estimates of the additive (*a*) and dominant (*d*) effects were obtained by fitting a model including all QTLs as described in Bohn et al. (1996). In $F_{2:3}$ and $F_{2:4}$ families, the method for determining the number of days from seedling emergence to flowering, and therefore the GDD to flowering, establishes the median value of a row (when 50% of the plants in a row have flowered) and not the mean value of the trait. Therefore, dominant effects for GDD to flowering were not multiplied by a factor of 2. Estimates of *d* when using median values in $F_{x(x>2)}$ progenies were calculated as in the F_2 generation according to Leon et al. (2000). The expected median value of a progeny is $E(\tilde{Y}_{Fx,\,H}) = \mu + d\,(1-2p)$; where $E\tilde{Y}_{Fx,\,H}$ is the median value of the *x* progeny, *p* is the probability of having at least 50% of the F*x n* plants, from an F_2 plant heterozygous at a given locus, homozygous (AA) for that locus in a row. For an *n* value of ten plants in a row of $F_{2:3}$ or $F_{2:4}$ progeny *p* is equal to 0.02 and 0.13, respectively. Thus, $E(\tilde{Y}_{F2:3,H}) = \mu + 0.96\,d$ ($\cong \mu + d$) and $E(\tilde{Y}_{F2:3,H}) = \mu + 0.74\,d$ ($\cong \mu + d$) are approximately direct estimates of dominance effects in the F_2. The d/a (dominant/additive) ratio scale described by Edwards et al. (1987) was used to classify gene action. [A=additive or partial dominance ($0<|d/a|<0.55$); D=partial dominance or dominance ($0.55<|d/a|<1.20$), OD=overdominance ($|d/a|>1.20$)].

Results and Discussion

Trait characterization and variance components

Mean values for GDD to flowering and DTF are presented in Table 1. The flowering response of HA89 was more sensitive than that of ZENB8. When the PP at the VE stage was equal to or less than 14 h, HA89 required more GDD to flower than ZENB8. In the three environments with a longer PP, ZENB8 required more GDD to flower than HA89. HA89 exhibited a long-day response in agreement with what was reported by Goyne and Scheniter (1987) for this range of PP; whereas, ZENB8 appeared to have an ambiphotoperiodic response across the range of PP in this study, with a maximum GDD to flowering between 13.5 and 14.5 h

The mean values and the distribution of progeny values for GDD to flowering suggest that HA89 alleles confer dominant gene action for higher or lower days to flowering depending on the PP. The mean values for GDD and DTF of the F_1 genotype were similar to those of HA89 in all environments. Mean values of the F_2, $F_{2:3}$, and $F_{2:4}$ generations were between the average of

Table 1 Growing degree days (GDD) trait means, variance components, and broad-sense heritabilities across environments (mean environment) and in the six individual environments with different photoperiods at emergence

Environment: photoperiod (h):	VT 97 12.1	VT 97 13.1	VT 97 14.0	VT 92 15.0	Balcarce 15.5	Fargo 16.4	Mean environment
	GDD means[a]						
Entries:							
ZENB8	1063±24 (93)[b]	1085±18 (80)	1068±14 (72)	1164±12 (69)	1022±10 (74)	947±24 (86)	1047±8 (78)
HA89	1156±24 (99)	1167±18 (85)	1116±14 (75)	987±12 (59)	861±10 (62)	870±24 (80)	973±8 (74)
F_1	1139±24 (98)	1196±18 (87)	1120±14 (75)	1003±12 (60)	895±10 (64)	833±24 (78)	989±8 (73)
F_2						886±24 (81)	
$F_{2:3}$				1062±12 (63)	933±10 (67)		
$F_{2:4}$	1123±24 (97)	1187±18 (86)	1141±14 (76)				
	Variance components[c]						
σ^2_e	730	401	230	386	229	1847	767
σ^2_g	8194	8374	4873	3663	2822	2232	3072
σ^2_{gxe}							1817
σ^2_{ph}	8924	8775	5103	4049	3051	4079	
H	0.92	0.95	0.95	0.90	0.92	0.55	0.88

[a] Mean±2 standard errors of mean
[b] The mean values for days to flowering (DTF) are in brackets below the corresponding value for GDD
[c] σ^2_e=experimental error variance, σ^2_g=genotypic variance, σ^2_{gxe}=genotype x environment interaction variance, σ^2_{ph}=phenotypic variance, *H*=broad-sense heritability

the parents and the value for HA89. The coefficients of skewness for the distribution of GDD to flowering for all generations of progeny were positive (distribution with an asymmetric tail extending towards higher values of GDD) and significant for the environments with a PP equal to or greater than 14 h. The coefficients were 0.02, 0.12, 0.28* and 0.59**, 0.60**, 1.52** for the environments with a PP of 12.1, 13.1, 14, 15, 15.5 and 16.4, respectively. The coefficient of skewness for the mean environment was also significant (0.31*). The trend of increased coefficients from 12.1 to 16.4 h of PP suggests that the dominant effects for earliness become more pronounced at a longer PP.

Broad-sense heritabilities ranged from 0.55 in the F2 population (Fargo, N.D.) to 0.95 in the $F_{2:4}$ progenies at Venado Tuerto (PP=13.1 and 14 h) (Table 1). The heritability estimated on an entry basis in the mean environment was 0.88. Estimates of heritability values for GDD to flowering have not been reported in the literature. These values of heritability for GDD agree with those reported for DTF with other populations in other environments (Shabana 1974; Berretta de Berger and Miller 1984; Alvarez et al. 1992) and with this population evaluated in a narrower range of PP (15 to 16.5 h; Leon et al. 2000). There were highly significant (P<0.01) differences among environments and genotypes. The term for genotype×environment (G×E) interaction was also highly significant (P<0.01). The lack of G×E interaction in Leon et al. (2000) might be attributed to the similarity in PP among environments in that report (15–16.4 h). Herein, the wider range of PP (12.1–16.4 h) could have provided the conditions for revealing significant G×E interaction and enabling the detection of QTLs that interact with daylength.

Detection of QTLs for GDD to flowering and PPR

In the mean environment, six QTLs on linkage groups A, B, F, I, J and L were associated with GDD to flowering (Table 2). These QTL accounted for 67% and 76% of the phenotypic and genotypic variation in the mean environment. In agreement with this finding polygenic inheritance was reported for DTF by Stoenescu (1974) and Machacek (1979). QTLs in linkage groups A and B had the highest LOD scores in the mean environment (LOD 21.2 and 15.5, respectively) and they accounted for 72% of the genetic variation associated with allelic variation at RFLP loci. Genetic factors with major qualitative effects on flowering were also reported by Jan (1986). Digenic epistatic interactions were not detected between QTLs with significant main effects related to GDD for flowering. However, a highly significant interaction (additive×additive) was found between loci that did not have significant main effects. That interaction between RFLP loci C0062 and C0290 (linkage groups E and G, respectively) explained 13% of the total phenotypic variation in the mean environment. However, this interaction did not increase the R^2 when added to the model. Genetic effects for higher values of GDD to flowering were derived from both parents. QTLs with additive effects for higher GDD to flowering were derived from HA89 in linkage groups A, F and J, and from ZENB8 in linkage groups B, I and L.

Gene action was mainly additive (Table 2) in accordance with previous reports for DTF (Miller et al. 1980; Roath et al. 1982; Alvarez et al. 1992; Reid et al. 1992). However, dominant effects were evident in linkage groups B and J. The dominant effects are towards lower values of GDD. Dominant effects for DTF were also reported by Jan (1986). Based on the RFLP loci, four of

Table 2 Parameters of the QTLs for GDD to flowering in the mean environment for the HA89×ZENB8 sunflower population

Linkage group	Position (cM)[a]	Left-right[b] locus	LOD	R^2 [c]	a[d]	d[e]	Gene[f] action
A	38	C0266–C0341	21.23	34.0	–54.3	9.6	A
B	64	C1735–C0741	15.50	26.2	35.2	–25.8	D
F	8	C0865–C1437	2.89	5.5	–15.3	– 5.9	A
I	58	C1891–C0851	6.74	12.6	24.2	– 2.9	A
J	54	C1294–C1610	2.70	5.2	–13.4	–11.4	D
L	64	C0628–C0589	8.91	16.0	26.9	– 7.7	A
Total[g]				67.0			

[a] Position of likelihood peak (highest LOD score)
[b] Loci flanking the likelihood peak for a putative QTL according to the linkage map of Berry et al. (1995)
[c] Coefficient of determination: percentage of phenotypic variance explained by the QTL
[d] Additive (a) value. A negative sign means an increase of the mean value of the trait due to HA89 alleles. A positive sign means an increase of the mean value of the trait due to ZENB8 alleles
[e] Dominant (d) values. A positive sign means dominance for higher value of the trait. A negative value means dominance for lower value of the trait
[f] A=additive or partial dominance ($0<|d/a|<0.55$); D=partial dominance or dominance ($0.55<|d/a|<1.20$), OD=overdominance ($|d/a|>1.20$). Based on the scale of the ratio d/a reported by Edwards et al. (1987)
[g] Estimate of total variance obtained from the simultaneous fit of all putative QTLs associated with growing degree days

Table 3 Analysis of variance of QTL×E interaction for growing degree days (GDD) to flowering for the QTLs in six environments

Source	*df*	MS	*F*
Environments	5		
Genotypes	234	21032	8.13**
QTLs	12	273675	13.63**
Residuals	222	7375	3.63**
Genotype×env	1167	2586	
QTL×env	60	12848	6.33**
Res×env	1107	2030	

** Significant at the 0.01 probability level

Table 4 LOD score for QTLs associated with GDD to flowering at a different PP at emergence for the HA89×ZENB8 sunflower population

Linkage group[a]	Photoperiod (PP) in hours (h)					
	12.1	13.1	14 LOD	15 score[b]	15.5	16.4
A*	**15.2**	**21.4**	**15.1**	**7.8**	**8.7**	**6.0**
B*	NS	NS	**7.8**	**28.1**	**24.8**	**24.5**
F*	**5.2**	NS	NS	**2.6**	NS	NS
I	**4.4**	**3.8**	**3.6**	**5.3**	**5.7**	NS
J*	NS	**3.4**	NS	NS	NS	NS
L	**5.7**	**4.3**	**4.1**	**6.1**	**5.2**	**3.4**

* QTL with significant QTL×E interaction ($P<0.01$)
[a] Letters represents linkage group according to Berry et al. (1995)
[b] LOD scores are higher than the threshold value of 2.5

Table 5 Parameters of the QTLs for photoperiod response (PPR)[a]

Linkage group	Position (cM)[b]	Left-right[c] loci	LOD	R^2 [d]
A	38	C0266–C0341	6.47	12.0
B	64	C1735–C0741	4.72	8.9

[a] Ratio of the GDD required by a genotype to flower at a 12.1 and at a 15.0 h PP
[b] Position of likelihood peak (highest LOD score)
[c] Markers flanking the likelihood peak for a putative QTL according to the linkage map by Berry et al. (1995)
[d] Coefficient of determination: percentage of phenotypic variance explained by the QTL

the QTLs and their genetic effects reported herein for GDD to flowering (linkage groups A, B, I and L) were also observed for DTF (Leon et al. 2000).

In Leon et al. (2000), the genetic positions and parental effects of the QTLs associated with DTF were consistent across environments and generations. With the exception of QTLs on linkage groups H and I, all QTLs were detected in every environment. Also, the G×E and QTL×E interactions were not significant. Herein, the G×E and QTL×E interactions were highly significant (Table 3). Four of the six QTLs for GDD to flowering (linkage groups A, B, F and J) had significant QTL×E interactions ($P<0.01$). The LOD scores for QTLs in linkage groups A and B were highly dependent on PP (Table 4). The LOD scores of QTLs in linkage group A decreased, while the LOD values of QTLs of linkage group B increased, as the PP increased from 12.1 to 16.4 h. Moreover, the LOD scores for QTLs in linkage group B were not significant at a PP of 12.1 and 13.1.

The QTL mapping of PPR agrees with these results. Two QTLs (in linkage groups A and B) were associated with this trait (Table 5). These QTLs were located at the same genetic positions as QTLs associated with GDD to flowering (Table 2) at intervals C0266–C0341 and C1735–C0741 for QTLs in linkage groups A and B, respectively. Together, the analysis of QTL×E interaction for GDD and QTL mapping of PPR indicate that QTLs

Table 6 Additive and dominant effects for GDD to flowering of the QTLs with QTL×E interaction in the HA89×ZENB8 sunflower progenies

Linkage group	Photoperiod (PP) in hours (h)					
	12.1	13.1	14	15	15.5	16.4
Additive effects						
A	–89	–99	–62	–41	–38	–35
B	25	8	37	51	40	49
F	–37	–18	–14	–15	–4	–2
Dominant effects						
A	26	17	28	2	2	19
B	–12	8	–9	–38	–33	–42
J	0	–28	–1	–8	–3	–8

on linkage groups A and B were associated with a response to changes in PP.

The QTLs of linkage groups F and J had significant QTL×E interaction for GDD but LOD values did not appear to change with PP. These QTLs may be associated with other environmental factors that affect cycle length, such as water availability and mineral nutrition. The QTLs in linkage groups I and L were associated with GDD to flowering in all environments and did not exhibit QTL×E interaction, which suggests that they control days to flowering independent from the PP where the progenies are grown.

QTL×E interaction for additive effects was highly significant ($P<0.01$) for QTLs in linkage groups A, B and F. When PP increased, the additive effects of QTLs in linkage group B increased whereas those of QTLs in linkage groups A and F decreased (Table 6). QTL×E interaction for dominant effects was significant ($P<0.01$) for QTLs in linkage groups A, B and J (Table 6). The dominant effect of QTLs in linkage group B increased with a longer PP. This observation might explain the larger positive coefficients of skewness for the GDD to flowering at a longer PP . Dominant effects of QTLs in linkage group J were not associated with PP (Table 6).

Leon et al. (2000) found five QTLs (linkage groups A, B, H, I and L) associated with DTF in the same population used herein. The wider range of PP in this study revealed that the two QTLs with the strongest association with DTF (linkage groups A and B) were responsive to PP. A response of sunflower to PP was reported by Goyne and Scheniter (1987) and Goyne et al. (1989) through phenological studies. Rawson and Hindmarsh (1982) and Connor and Sadras (1992) found that some genotypes have a long-day response in the sowing-floral initiation period or a short/neutral-day response from flower initiation to anthesis. These opposite responses to PP agree with the opposite response of QTLs A and B to that environmental variable. However, QTLs detected in this research can be either acting at the same or different periods from emergence to anthesis.

Mapping studies for photoperiod response have been carried in other species. A photoperiod-sensitive gene (*Se-1*) in rice has been found on chromosome 6 using classical genetic studies (Yokoo et al. 1980; Mackill et al. 1993; Ohshima et al. 1996). Moreover, two photoperiod-responsive genes were reported in barley (Laurie et al. 1995). Ppd-H1 (chromosome 2 S) regulated flowering time under long days, whereas Ppd-H2 (chromosome 5L) was detected only under short days.

The improved understanding of the components of DTF and their interaction with the environment will enable marker-assisted selection for adjusting DTF in a wider range of environmental conditions and for converting photoperiod-sensitive exotic germplasm to photoperiod insensitivity (Lee 1998).

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