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Genetic control of pre-heading phases in the Steptoe × Morex barley population under different conditions of photoperiod and temperature

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Abstract Although there are numerous studies on the genetic control of flowering time in barley, little is known on the genetic control of duration of different particular pre-heading phases. Extending the stem elongation phase (SE), without modifying total time to heading, has been proposed as a trait to raise yield potential. Moreover, studying the genetic control of pre-heading phases would be of interest for a better understanding of crop phenology which is crucial for adaptation. We studied the genetic control of the leaf and spikelet intitiation phase (LS), the stem elongation phase, and within this, from the onset of jointing to flag leaf (J-FL), and from then to heading (FL-HD), in the Steptoe × Morex population, which is known to segregate for some major developmental genes, under different environmental conditions. After a preliminary greenhouse study in which the appropriateness of the population was tested, 130 double haploid lines and the two parents were grown under four field environments that differed in photoperiod and temperature conditions. Amongst all QTLs detected (13), only three were significant for HD and for both LS and SE and with the same allele direction (although with greater effects in one phase than the other in some cases). Genotype by environment interactions for LS and HD were due to both photoperiod and other factors as temperature or its interaction with photoperiod, while for SE responses to only photoperiod were negligible. QTL × E interactions were important for some QTLs, and either cross-over (e.g. *Ppd-H1*) or quantitative (e.g. *Ppd-H2*). However, heritability across field environments for the ratio SE/LS was high (0.8) and several of the QTL effects that were significant for only LS or SE, were conserved across different environments, that is, they were significant with the increasing allele derived from the same parent in all or most environments.

Keywords Barley · Development · Flowering · Stem elongation · Genetic control · QTL · QTL × Environment interaction

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Introduction

Flowering time is under a strong, although complex, genetic control that determines responses to vernalisation, photoperiod and earliness per se, the three main

factors that affect phenological traits (Laurie et al. 2004). The wide variation in responses to these factors is responsible for the wide geographical distribution of barley, as shown for example by the distribution of *Ppd-H1*, *Vrn-H1* and *Vrn-H2* alleles among cultivars grown at different regions/latitudes (Cockram et al. 2007a, b). Therefore, heading time is a critical adaptative trait in any breeding program (Richards 1991), and there are numerous studies on its genetic control.

In most areas where cereals have been traditionally grown, there might be little scope for improving yield by further optimising time to heading under current climatic conditions (e.g. in the Mediterranean basin, Martiniello et al. 1987; Muñoz et al. 1998; Slafer et al. 2005). For breeding progress, however, knowledge of the genetic factors (vernalisation, photoperiod and earliness per se pathways) determining adaptability to the prevailing environment is required to understand their modes of action better. On the other hand, most studies on the genetic control of development are mainly focused on total time to heading, because it is easier to assess than particular pre-heading phases. However, some adaptative characters, such as the avoidance of cold temperatures in late spring, could be better assessed by knowing the time of the transition of the apex from vegetative to reproductive rather than the whole time to flowering (Szűcs et al. 2006; Limin et al. 2007).

The genetic control of duration of pre-heading phases is also of interest for improving yield potential. Yield is determined throughout the growing season, but each of the yield components are more determined by particular crop growth phases (Slafer and Rawson 1994). The stem elongation phase is critical as the number of fertile florets at anthesis, which determines the final number of grains, is set during this phase (Fischer 2007; Miralles and Slafer 2007). Lengthening the duration of stem elongation, without modifying total time to heading, which is a key trait in any breeding program, has been proposed as a way to increase yield potential of small-grain cereals (Slafer et al. 2005).

There are some evidences for a different genetic control of duration of pre-heading phases. Several authors have demonstrated phenotypic variability in pre-heading phases even in varieties with similar total time to heading (Appleyard et al. 1982; Kitchen and Rasmusson 1983; Kernich et al. 1995, 1997; Whitechurch et al. 2007). Other authors have reported that responses to photoperiod and temperature may differ between pre-heading phases (Roberts et al. 1988; Slafer and Rawson 1994; Slafer 1996; Miralles and Richards 2000; González et al. 2002). However, little is known on the particular genetic factors responsible for a different genetic control between pre-heading phases. Studies comparing wheat substitution lines, single chromosome recombinant lines or near isogenic lines (differing in *Ppd* alleles) are inconclusive as differences in pre-heading phase durations or in responses to photoperiod in each (sub)phase could not be attributed to particular major *Ppd* genes (González et al. 2005). Zhou et al. (2001) found several independent QTL effects for the duration of the vegetative and reproductive phases in rice. In wheat, Lewis et al. (2008) found that the gene *Eps-A^m* had an effect on the duration of the leaf and spikelet initiation phase (LS), but not on the stem elongation phase. Borràs et al. (2009) found no genetic correlations between the duration of the leaf and spikelet initiation phase (LS) and that of the stem elongation phase (SE) in the Henni × Meltan barley population. Both Henni and Meltan were spring cultivars from Northern Europe, with an expected narrow genetic basis for phenological traits. Nevertheless, considerable genetic variability, transgressive segregation and high heritability were found for both phases and other phenological traits (Borràs et al. 2009), and in Borràs-Gelonch et al. (2010) some QTLs were identified as responsible for the different genetic control between the two pre-heading phases. It is not known, however, if the same QTLs could also cause similar effects in other genetic backgrounds or how major genes for heading affect durations of different pre-heading phases. It is unknown either if differences in the genetic control between these phases are maintained across different photoperiod and temperature conditions.

Unlike the Henni × Meltan population, the Steptoe × Morex population is known to segregate for the major photoperiod genes *Ppd-H1*(*HvPRR7*) (Hayes et al. 1993; Turner et al. 2005) and *Ppd-H2* (*HvFT3*) (Boyd et al. 2003; Kikuchi et al. 2009). The population also segregates for the vernalization gene *Vrn-H1* (*HvBM5*, Close et al. 2009), where Steptoe has a facultative allele and Morex has a spring allele (A. Casas, personal communication, 2009), and for *HvFT1* (Kikuchi et al. 2009). Given the increasing interest in

knowing the genetic control of different pre-heading phases and the scarce number of studies devoted to this issue, the objectives of this work were (i) studying the genetic control of different pre-heading phases in a population segregating for some major developmental genes and (ii) assessing to what extent genetic factors responsible for a different genetic control are maintained across different photoperiod and temperature conditions.

Materials and methods

Preliminary experiment

In a preliminary study a hundred thirty double-haploid lines from the Steptoe × Morex population and both parents were grown in a greenhouse experiment in 2006 located in the campus of the University of Lleida (Catalonia, NE Spain, 41°37′N 0°36′E, 181 m). Plants were firstly vernalized and then transplanted to the greenhouse in spring following a row and column latinized design, with 2 plants per pot for each line and replicate (two replicates for half of the DH-lines, one for the other half). Maximum temperatures were kept below 30°C during all the growing period. Other experimental conditions are detailed in Table 1. The aim of this preliminary study was determining the adequacy of the chosen population, that is, exploring if there was some degree of independence in the genetic control of LS and SE.

Field experiments

The same 130 DH-lines and both parents were grown in four field experiments conducted in 2009, two in Lleida (41°37′N 0°36′E, 181 m) and two in Gimenells (41°38′N 0°22′E, 252 m) (Table 1). The experimental design for the four trials was a row and column latinized design augmented with parents and checks, with two complete replicates for each DH-line at the two trials in Gimenells and, due to insufficient seed of some lines and limited space, two replicates for half of the DH-lines and one for the other half at the experiments in Lleida.

The two trials in Lleida were sown simultaneously in autumn (the optimal sowing date for barley in that region). While one was grown with natural day length (Ll09-Au-PhN), the other was grown with the natural day length plus an artificially extended photoperiod of 3 h (Ll09-Au-PhE). This was achieved with low-intensity incandescent lamps, installed above the plots, that were switched on at sunset (regulated automatically by a photoelectric cell) and off at the end of the photoperiod treatment (regulated automatically by a timer). Photoperiod treatments were separated by curtains that lowered at dusk and lifted up after the photoperiod treatment (regulated also by a timer). For each genotype and replicate, a row of 1.7 and 0.20 m apart was grown, with each seed uniformly distributed every 2 cm. To achieve this, seeds were previously distributed on a degradable adhesive strip for each row, and then sown by hand on the same date after soil preparation.The two trials in Gimenells were sown on different sowing dates in adjacent plots, one in autumn (Gi09-Au) and the other in winter (Gi09-Wi; see Table 1 for details in the experimental conditions). For each genotype in each replicate 2 rows of 1.5 m, 0.15 m apart, were sown with a seed-drill at a density of c. 300 seeds/m^{-2}.

Traits

The phenological phases considered were: (i) from sowing to heading (HD); within that, (ii) from sowing

Table 1 Summary of growing conditions and treatments in each trial

Condition	Location	Season	Sowing date	Vernalization	Photoperiod	Code
Greenhouse (preliminary)	Lleida	2006	23 March[a]	42 days at 4°C	Natural[b]	Gr06-Sp
Field	Lleida	2008/2009	21 November	Natural	Natural	Ll09-Au-PhN
Field	Lleida	2008/2009	21 November	Natural	Extended (Natural + 3 h)	Ll09-Au-PhE
Field	Gimenells	2008/2009	30 November	Natural	Natural	Gi09-Au
Field	Gimenells	2008/2009	26 February	Natural	Natural	Gi09-Wi

[a] Date of transplanting after the vernalization treatment

[b] Except during the vernalization treatment when the light period was 8 h

to the onset of jointing (leaf and spikelet initiation phase, LS), (iii) from then to heading (the spike differentiation phase or stem elongation, SE); and within SE (in the four field environments) (iv) the phase from the onset of jointing to flag leaf (J-FL) and (v) from then to heading (FL-HD). The onset of stem elongation was estimated as the date when the first internode had elongated about 0.5 cm (approximately Zadoks stage 30, which is closely correlated with the end of floral primordia initiation; Kirby et al. 1994) through destructive samplings of c. 3 plants in each plot every 4 or 5 days, since the most advanced genotypes in the trial started jointing until most plants in the plot reached that stage. In the preliminary experiment (Gr06-Sp), as destructive samplings were not possible, the onset of stem elongation was assessed as the appearance of the first detectable node above the soil surface. Flag leaf was recorded when it was completely unfolded in half of the plants (Zadoks 39). Heading date was recorded when at least half of the spike had emerged from the flag leaf sheath of the main stem in c. 50% of the plants (Zadoks 55). All durations of phases were estimated as accumulated thermal time (°C days, with a base temperature of 0°C). Additionally the QTL analysis was also performed on the ratio SE/LS to test more objectively differences in QTL effects between pre-heading phases.

Data analyses

Best linear unbiased predictors (BLUPs) were estimated for DH-lines in each trial using a mixed model in which row, column and DH-line were the random factors, and replicate was the fixed factor. Narrow-sense heritabilites (h^2) across environments were estimated as

$$h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge}/e + \sigma^2_{res}/\mathrm{re}),$$

where σ^2_g is the variance component of genotypes (DH lines); σ^2_{ge}, the variance component of the interaction genotype × environment; σ^2_{res}, the error; e, the number of environments, and r, the number of replicates per environment, which here would be (2 * 2 + 2 * 1.5)/4. The same model was applied for analyses with the two sowings and with the two photoperiod treatments. Heritabilities within each environment (or repeatabilities) were also estimated (following the same model but without σ^2_{ge}).

For the QTL analyses, we utilised a linkage map derived from genotypic information for the 130 DH-lines from Steptoe × Morex. The map contained 794 of the 884 polymorphic SNP markers from both the Barley Oligo Pooled Array 1 and 2 (BOPA1 and BOPA2). All these markers are also represented in the individual S×M maps and in the consensus map by Close et al. (2009). As expected, the map was very similar to the individual S×M map derived from BOPA1 and BOPA2 in Close et al. (2009), with the same marker order (also in the consensus map) and with only small differences in the total length of each chromosome (5 cM as much), representing all barley genome regions, with a total map length of 952 cM.

Using BLUPS for each DH line in each environment, we conducted QTL × Environment analysis accounting for the appropriate variance–covariance relationships between lines and environments (Piepho and Pillen 2004) with the procedures implemented in Genstat 12 (Payne et al. 2009). Despite BLUEs are preferable to BLUPs for a QTL × E analyses in a second step (Smith et al. 2001), we used BLUPs given the high heritabilities for duration of phenological phases (thus results should not differ much from BLUEs) and that in the two environments in Lleida only half of the 130 DH-lines were included in the second repetition. After estimating genetic predictors and choosing the best variance–covariance model, simple and composite interval mapping were run with a multi-environment mixed model to estimate both QTL and QTL × E effects that exceeded the estimated genome wide significance −log10(P-value) threshold of 3.4. Additive × additive epistatic interactions and the significance of the QTL × E interactions were studied with linear mixed models, using BLUPs estimated from each individual trial. Each model included as fixed factors the environment, the significant QTLs for each trait (closest markers to the significant −log(P-value)-peaks chosen from previous CIM analysis), all 2-factor interactions and E × QTL × QTL interactions, while the random factor was the remaining genotypic variance. Significant markers were added to the model ordered by their −log(P-value) significance (from the highest to the lowest). All linear mixed models were analysed with Genstat 12 (Payne et al. 2009).

Results

Environmental conditions

Average daily mean temperatures, minimum temperatures and photoperiod during LS and SE (mean of the population) in each environment are given in Table 2. Environments are ordered from the least to the most inductive (considering photoperiod and temperature conditions), until the preliminary greenhouse study in which plants were grown out of the season, where temperatures were much higher than in most common growing conditions (although the recorded daily maximum temperatures were always below 30°C). We therefore considered the results of the preliminary experiment separately. Temperatures were almost identical in Ll09-Au-PhN and Ll09-Au-PhE. Thus, these two environments only differed in photoperiod conditions, while the two sowings in Gimenells differed in both temperature and photoperiod.

Preliminary study

In the preliminary study a wide range of variability was observed for both LS and SE (Fig. 1), particularly for SE and HD. Heritabilities were high for the three phases. The genetic correlation between LS and SE was significant and positive but not strong ($r = 0.31$).The most important QTL for both LS and SE was that on 2H, close to *Ppd-H1* locus, but it had a much greater effect on SE than on LS and therefore it was also the most significant for the ratio SE/LS (Fig. 2a; Table 3). Two other QTLs, which were significant for only LS or SE, were also significant for the ratio SE/LS.

Field experiments

The magnitude of genotypic variability for DH-lines differed widely among the four field environments (Fig. 1). Heritabilities (or repeatabilities) within each environment were high in most cases. The lowest heritabilities were for the phase FL-HD, with the exception of experiment Gi09-Wi. Differences between parents also varied between phases and environments. Steptoe was later than Morex for most phases, but differences between both parents were greater for LS than for SE, and for all phases, except FL-HD at Gi09-Wi, the general trend was that the more inductive the environment (photoperiod and/or temperature) the smaller the difference between parents. Distributions were approximately normal for all phases and environments (Fig. 1).

Genetic correlations between LS and SE were only significant in Ll09-Au-PhE (Table 5). However, the correlation was not very strong and thus, even in this environment, there was a wide range of values for SE within any LS value (data not shown). Genetic correlations between J-FL and FL-HD were not significant (Gi09-Au and Ll09-Au-PhN) or weak (Gi09-Sp and Ll09-Au-PhE; Table 5).

Overall environmental effects and G × E interactions

Considering the four field environments, environmental main effects were highly significant for all phases.

Table 2 Mean of daily photoperiod and daily minimum and mean temperatures in each environment during the phases LS and SE (average of all the population)

Phase	Environment	Photop. (h)	Mean T (°C)	Min T (°C)
LS	Ll09-Au-PhN	10.0	5.5	0.9
	Gi09-Au	10.0	5.5	1.3
	Ll09-Au-PhE	13.0	5.4	0.9
	Gi09-Wi	12.0	9.9	3.7
	Gr06-Sp	12.9	19.5	12.4
SE	Ll09-Au-PhN	12.6	10.8	4.3
	Gi09-Au	12.6	11.0	4.6
	Ll09-Au-PhE	15.5	10.3	4.1
	Gi09-Wi	13.8	14.1	7.6
	Gr06-Sp	14.2	22.9	16.0

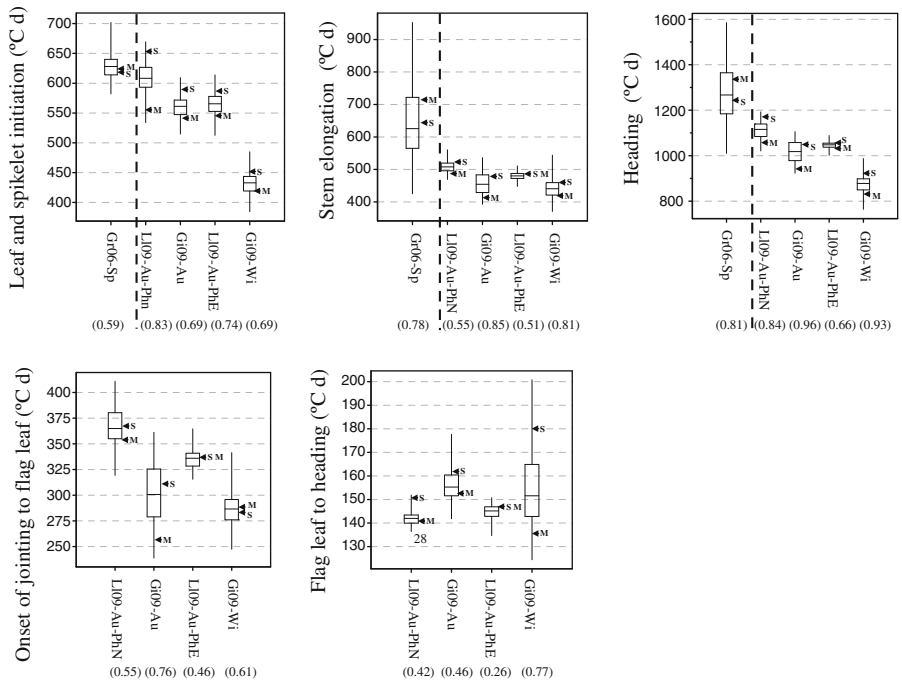


Fig. 1 Boxplots of *DH-lines* (BLUPs) for pre-anthesis phases in each environment. *Initials* indicate values for parents: *S* Steptoe, *M* Morex. *Values between brackets* are heritabilities (or repeatabilities) estimated in each environment

Significance was much greater for LS than for SE, so environmental effects were also highly significant for the ratio SE/LS. Despite the great differences in photoperiod and temperature conditions between the four field environments, the genotype variance component was at least twice the genotype × environment variance component for all phases (except FL-HD), and particularly very low for the ratio SE/LS. Thus, heritabilities across all environments were high (Table 4). Even if the preliminary study (where conditions were completely different, particularly temperature) was included in the analysis, the heritability for LS was only slightly lower (0.75), although G × E variance components for SE and HD were much higher and heritabilities for both much lower (0.45 and 0.40 respectively) than in the analysis with the four field environments.

Environmental main effects were also highly significant for the ratio SE/LS, comparing the two sowings, but not when comparing the two photoperiod treatments, where the significance was more similar between LS and SE (Table 4). Genotype × environment interactions were more important for all phases between the two sowings than between the two photoperiod treatments (comparing genotype with genotype × environment variance components for each trait). In fact, genotype × environment variance components were very small for SE and the two subphases within SE (lower than their respective standard errors) between the two photoperiod treatments. Interestingly, the genotype × environment interaction for the ratio SE/LS was also very small in both analyses comparing photoperiod treatments and sowings.

If differences between sowing dates were only or mainly due to responses to photoperiod (which was almost identical between Ll09-Au-PhN and Gi09-Au and only about 1 h greater in Ll09-Au-PhE than in Gi09-Wi), a relationship with differences between photoperiod treatments would be expected. Figure 3 shows these relationships, indicating genotypes carrying the Steptoe or Morex alleles at the QTL that reflects the segregation of *Ppd-H1* (this QTL is closely linked to the *PRR*-like gene in barley, 0.7 cM, in the barley integrated map of SNPs of Close et al. 2009, so we refer it to *Ppd-H1* allele classes from now onwards). A clear lineal relationship ($R^2 = 0.44$) was observed for HD, mainly due to differences in responses (differences between photoperiod treatments and sowing dates) between the Steptoe and Morex alleles at *Ppd-H1*. However, within each allele class at this locus there was a wide dispersion of responses ($R^2 < 0.05$ within each *Ppd-H1* allele class). For LS there was also a significant lineal

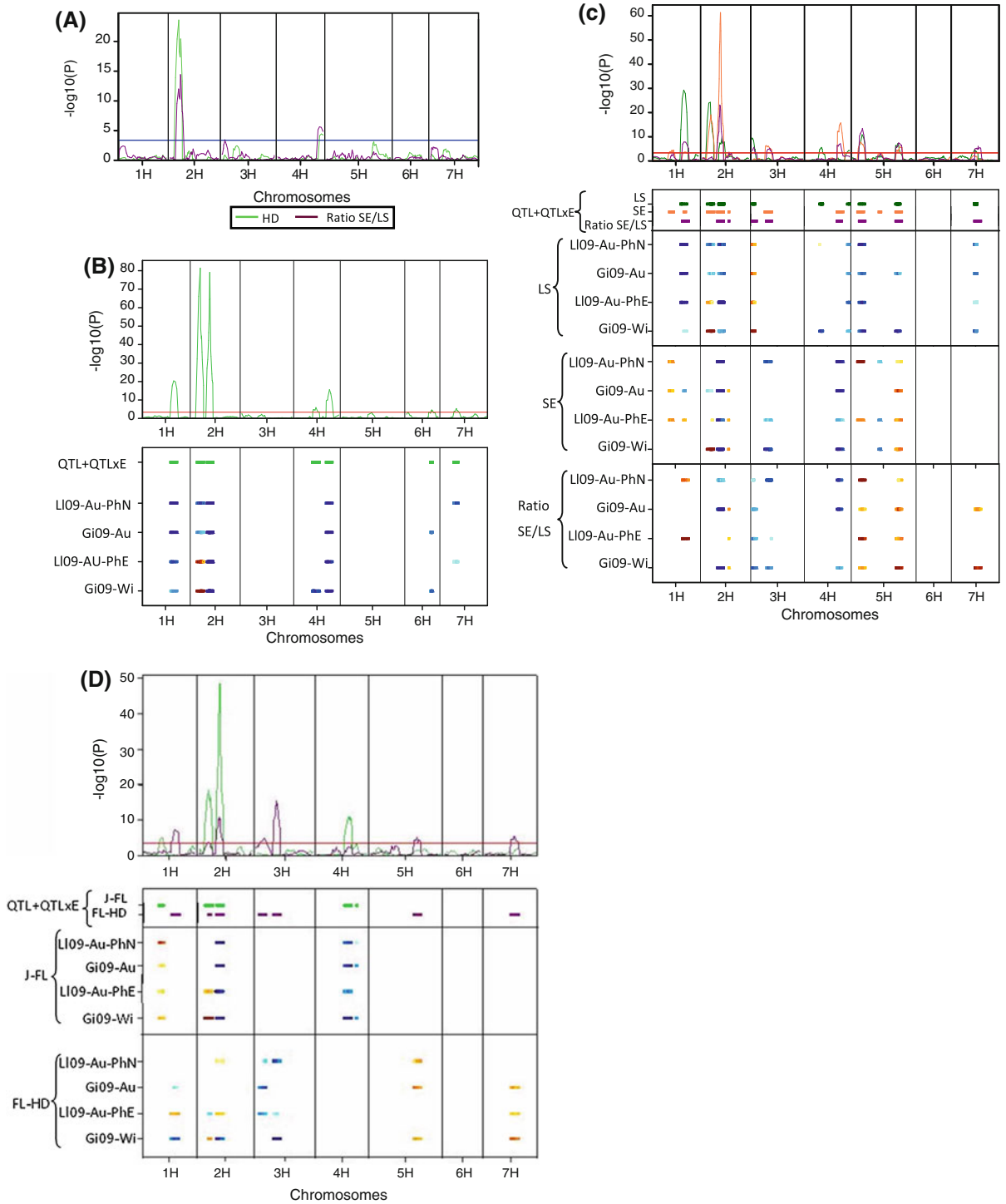


Fig. 2 QTL analyses for all phases (°Cd) and the ratio SE/LS. **a** QTL analysis in the preliminary study for HD and the ratio SE/LS. **b** Multi-environment QTL analysis (field conditions) for HD. QTL main effects plus QTL × E LOD profile (*above panel*) and significance and direction of QTLs in each environment (*below panel*). Bars: blue, additive effect from Steptoe; yellow–red, from Morex. The *darker the bar*, the greater the significance of the QTL. **c** LS, SE and the ratio SE/LS and **d** J-FL and FL-HD

Table 3 Most significant SNPs from the QTLs detected by CIM for each phase and the ratio SE/LS, their chromosome, position (cM, from the map used in the present study, which does not coincide with the individual S × M maps in Close et al. 2009) and bin (Marcel et al. 2007), confidence interval of the QTL (2- −log(*P*-value)), significance or −log(*P*-value), additive effect in each environment with its mean standard error (se) (negative, additive effect from Steptoe; positive, from Morex; if not included, no significant), and significance of the QTL*Environment interaction (ns, not significant). Additionally, QTL effects from the individual analysis for the preliminary study (Gr06-Sp, column 8) are included

Trait	SNP	Chr.	Pos.	bin	Confidence interval	$-\log_{10}$ (*P*-value)	Ll09- Au-PhN Add. Eff.	Gi09-Au Add. Eff.	Ll09-Au-PhE Add. Eff.	Gi09-Wi Add. Eff.	QTL*E	Gr06-Sp Add. Eff.
HD (°Cd)	11_11189	1H	96.6	11	87.9–105.3	19.8	−15.1	−18.7	−5.0	−6.9	<0.001	–
	11_21015	2H	24.5	4.2	17.6–25.8	81.4	−8.9	−6.6	6.1	34.2	<0.001	83.9
	12_10035	2H	52.7	8.1	51.4–53.4	79.0	−23.4	−32.0	−13.2	−35.4	<0.001	–
	11_20580	4H	60.6	7	51.9–66.0	5.9	–	–	–	−9.6	<0.001	–
	11_10387	4H	126.6	12.2	108.4–127.2	15.6	−11.2	−15.0	−5.9	−11.1	<0.001	−31.0
	11_20892	6H	72.3	7	64.3–80.3	4.7	–	−6.6	–	−7.2	<0.001	–
	11_10772	7H	43.2	5.1	37.1–45.2	5.4	−8.1	–	−2.3	–	<0.001	–
Mean standard error							2.0	2.0	1.2	2.1		8.2
LS (°Cd)	11_11189	1H	96.6	11	87.9–105.3	27.8	−20.5	−11.2	−11.3	−3.0	<0.001	–
	11_21015	2H	24.5	4.2	17.6–25.8	24.2	−6.6	−3.2	5.1	10.7	<0.001	14.2
	12_10035	2H	52.7	8.1	37.2–59.4	7.3	−8.0	−2.9	−5.8	−2.3	0.011	−8.5
	11_20797	3H	0.0	1.1	0–4.7	9.4	5.7	4.5	4.9	6.2	ns	–
	11_11180	4H	45.8	5.1	30.9–49.2	5.4	–	–	–	−4.6	<0.001	–
	11_10387	4H	126.6	12.2	112.9–127.2	4.4	−4.9	−3.6	−4.6	−2.9	ns	–
	12_10499	5H	27.6	2.3/2.4	22.2–35.7	10.8	−9.6	−4.6	−6.7	−4.8	0.002	–
	11_20375	5H	133.3	11.2	129.2–137.3	7.1	–	−3.7	–	−5.9	0.002	–
	11_20060	7H	64.7	7.1	57.3–74.07	4.7	−5.5	−3.7	−2.2	−4.1	ns	−5.5
Mean standard error							1.7	1.2	1.3	1.1		1.8
SE (°Cd)	11_10798	1H	60.3	9.1	49.6–63.7	4.7	3.9	4.8	3.0	–	0.031	–
	11_11189	1H	96.6	11	83.8–105.3	3.8	–	−6.1	2.8	–	ns	–
	11_21015	2H	24.5	4.2	17.6–25.8	19.2	–	−4.1	2.0	16.6	<0.001	67.1
	12_10035	2H	52.7	8.1	51.4–53.4	61.4	−10.6	−24.3	−5.3	−29.3	<0.001	–
	11_10214	2H	78.3	10	74.3–89.4	3.7	–	6.0	3.1	6.5	ns	–
	11_20666	3H	42.5	5.2	29.1–51.9	6.5	−4.3	–	−2.5	−7.6	0.043	−21.5
	11_20974	4H	100.2	11.1	94.9–108.4	15.9	−6.9	−11.4	−2.4	−9.4	<0.001	−34.2
	11_11048	5H	22.2	2.3/2.4	16.1–35.7	7.9	6.3	–	2.8	–	ns	–
	12_21497	5H	81.4	8.2/9.1	69.3–88.1	3.6	–	−3.0	−2.8	−6.1	ns	–
	11_20388	5H	137.3	12	130.6–148.1	6.1	2.1	7.3	2.6	6.1	0.018	–
Mean standard error							1.1	1.8	0.9	1.7		8.4

Table 3 continued

Trait	SNP	Chr.	Pos.	bin	Confidence interval	$-\log_{10}$ (*P*-value)	Ll09- Au-PhN Add. Eff.	Gi09-Au Add. Eff.	Ll09-Au-PhE Add. Eff.	Gi09-Wi Add. Eff.	QTL*E	Gr06-Sp Add. Eff.
Ratio SE/LS (1/1000)	11_11189	1H	96.6	11	83.8–105.3	7.8	12.7	–	19.5	–	0.01	–
	11_21015[a]	2H	24.5	4.2	17.6–25.8	–	–	–	–	–	–	85.0
	12_10035	2H	52.7	8.1	50.7–55.4	23.1	−9.7	−29.4	–	−47.3	<0.001	–
	11_21027	3H	4.7	1	0–15.5	5.6	−6.3	−10.2	−10.9	−17.7	ns	−21.7
	12_11387	3H	51.3	5.2	37.1–62.0	5.2	−11.1	–	−4.8	−15.2	0.01	−24.6
	11_20974	4H	100.2	11.1	94.9–108.4	7.1	−11.9	−16.4	–	−13.8	ns	−56.3
	11_11048	5H	22.2	2.3/2.4	16.1–35.7	13.4	18.9	10.3	15.8	14.6	ns	–
	11_20375	5H	133.3	11.2	129.2–138.6	7.2	5.5	13.3	8.3	22.1	<0.001	–
	12_11529	7H	74.1	7.2	71.4–90.2	6.0	–	7.8	–	15.3	<0.001	–
Mean standard error							2.8	3.6	3.1	4.6		12.4

Trait	SNP	Chr.	Pos.	bin	Confidence interval	$-\log_{10}$ (*p-value*)	Ll09-Au-PhN Add. Eff.	Gi09-Au Add. Eff.	Ll09-Au-PhE Add. Eff.	Gi09-Wi Add. Eff.	QTL*E
J-FL (°Cd)	12_10314	1H	49.6	7.1	42.8–54.9	3.7	5.2	3.5	2.1	–	ns
	11_21015	2H	24.5	4.2	19.9–25.8	18.0	–	–	9.1	2.6	<0.001
	12_10035	2H	52.7	8.1	50.7–53.4	52.8	−11.5	−19.4	−13.3	−5.8	<0.001
	11_20974	4H	100.2	11.1	90.2–104.3	13.0	−5.2	−9.9	−5.2	−2.4	<0.001
Mean standard error							1.3	1.7	1.0	0.8	
FL-HD (°Cd)	12_11144	1H	83.2	10	76.4–96.6	7.2	–	–	1.4	−4.0	<0.001
	11_21015	2H	24.5	4.2	15.3–33.9	4.2	–	–	−0.9	4.0	<0.001
	12_10035	2H	52.7	8.1	48.7–55.4	10.8	–	–	1.3	−7.0	<0.001
	11_10559	3H	18.9	3.1	12.1–32.1	5.7	−0.9	−2.8	−1.5	–	ns
	11_20276	3H	52.6	5.2	48.6–58	10.0	−0.9	–	–	−6.2	<0.001
	11_11456	5H	119.8	11.1	112.5–134.6	3.7	0.8	1.7	0.1	2.6	0.039
	11_20485	7H	78.5	7.2	64.7–87.5	6.8	–	2.0	1.3	3.9	0.010
Mean standard error							0.3	0.5	0.4	1.0	

[a] No significant in the multi-environment QTL analysis (only in the preliminary experiment)

Table 4 Significance of the factor Environment (fixed), Genotype and Genotype×Environment variance components and residual, with their standard errors, and heritability (h^2) from A: the multi-environment analysis (field conditions), B: the analysis comparing photoperiod treatments in Lleida (Ll09-Au-PhN and Ll-Au-PhE) and C: the analysis comparing the two sowings in Gimenells (Gi09-Au and Gi09-Wi)

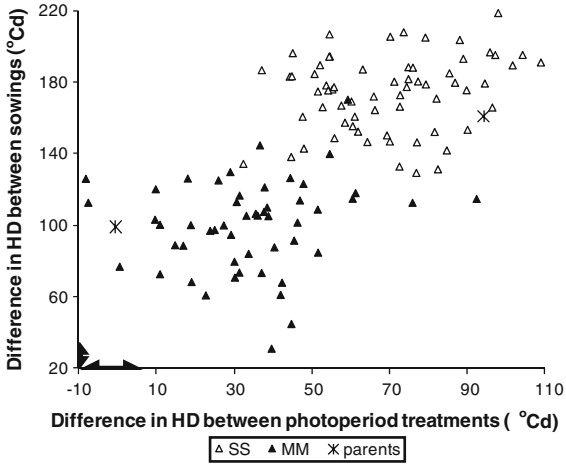
	Env $-\log_{10}$ (P-value)	Genotype	se	Geno × Env	se	Residual	se	h^2
A: All Env.								
HD (°C days)	56	1,044	155	585	59	303	24	0.84
LS (°C days)	35	489	73	134	33	400	30	0.84
SE (°C days)	15	667	101	238	46	525	39	0.83
Ratio SE/LS (1/1,000)	17	41.1	6.3	5.9	3.4	51.5	3.8	0.82
J-FL (°C days)	17	510	79	116	42	591	44	0.82
FL-HD (°C days)	5	69.6	15.3	53.3	17	227	17.2	0.60
B: Photoperiod								
HD (°C days)	6	761	144	368	94	431	54	0.70
LS (°C days)	5	670	106	112	49	320	38	0.80
SE (°C days)	3	490	96	22	70	627	71	0.69
Ratio SE/LS (1/1,000)	ns	40.5	6.3	0	0	38.8	3.9	0.76
J-FL (°C days)	4	459	90	10	70	615	71	0.69
FL-HD (°C days)	ns	73.2	22.4	0	0	282	31	0.44
C: Sowings								
HD (°C days)	51	1,002	216	1,051	153	232	24	0.63
LS (°C days)	22	348	77	179	59	456	46	0.63
SE (°C days)	4	877	155	358	82	473	48	0.74
Ratio SE/LS (1/1,000)	12	54.8	9.6	5.0	5.7	62.9	6.1	0.75
J-FL (°C days)	3	543	109	218	74	604	60	0.68
FL-HD (°C days)	ns	83	27	100	28	190	19	0.46

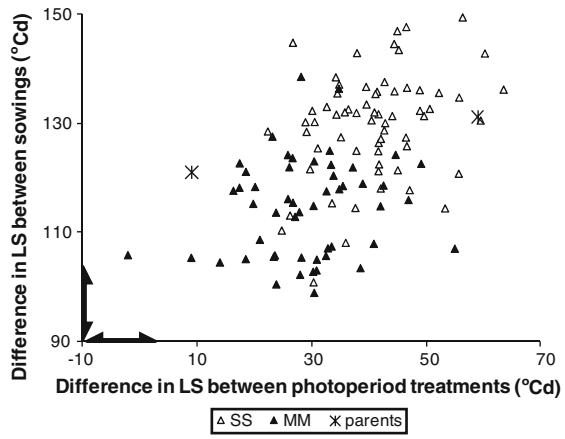
relationship between the two kind of responses, although the distinction between *Ppd-H1* alleles was less clear and R^2 for all genotypes lower (0.23) than for HD. For SE the relationship was very small (although still significant, with a R^2 of 0.10) as expected, given that G × E between photoperiod treatments was much smaller than for LS and HD (Table 4).

QTL and QTL × E effects

QTL + QTL × E for heading date could be explained mainly by the two QTLs on 2H (Fig. 2b; Table 3). Two other QTLs (one on 1HL and another distal on 4HL) were also highly significant in all the four environments. QTLs with much smaller effects were found less consistently across environments and were located on 4H, 6HL and 7HS. Steptoe × Morex is known to segregate for *Ppd-H1*, *Ppd-H2*, *HvFT1* (Hayes et al. 1993; Turner et al. 2005; Kikuchi et al. 2009) and *Vrn-H1* (*HvBM5*) (Close et al. 2009). By comparing maps we concluded that the QTLs on 1HL corresponded to the location of *Ppd-H2* and that on 2HS, to *Ppd-H1* as shown above. QTL × E interaction was highly significant for all QTLs for HD, while for LS and SE several interactions were not significant. Interactions were either quantitative (e.g. *Ppd-H2*) or cross-over (e.g. *Ppd-H1*) (Fig. 2b; Table 3). Few and only slightly significant QTL × QTL and QTL × QTL × environment interactions were detected (with small effects and all of them were quantitative interactions; data not shown).

Several other QTLs were found when analysing LS and SE, apart of those significant for HD, and most had different effects between both phases (Fig. 2c; Table 3). For LS the most significant QTLs (QTL + QTL × E) were *Ppd-H1* and *Ppd-H2*, while for SE, the other major QTL on 2H was by far the most important, followed by *Ppd-H1*. Among all QTLs significant for LS and SE only four were significant for both phases: *Ppd-H1*, *Ppd-H2*, the other QTL on 2H





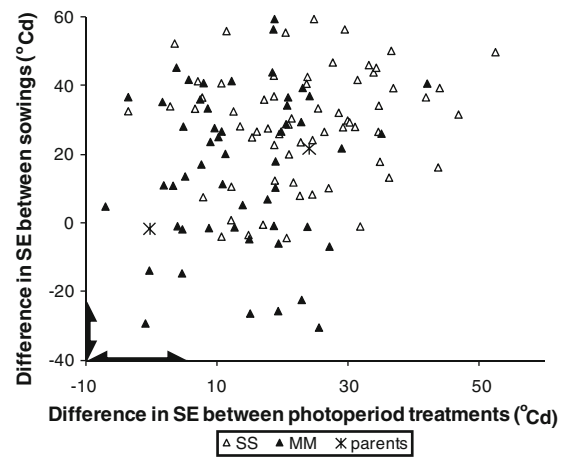


Fig. 3 Relationships between responses to photoperiod treatments (difference between Ll09-Au-PhN and Ll09-Au-PhE) and to sowing dates (difference between Gi09-Au and Gi09-Wi) for HD, LS and SE. Responses are estimated as the G × E effect for each genotype (effect from the least inductive environment minus effect from the most inductive environment) plus the environmental effect (constant for all genotypes), from the analyses with the two environments in Lleida and with the two environments in Gimenells. *Symbols* indicate *Ppd-H1* alleles for each genotype. The length of the *bars* with *arrows* indicates the mean standard error of differences when comparing the same genotype between the two environments

and a QTL distal on 4HL. The other major QTL on 2H and that distal on 4HL were the only QTLs significant for both phases and in all environments, although in most of them, they had a greater effect on SE than on LS, so they were also significant for the ratio SE/LS in three field environments (also in the preliminary study in the case of the QTL on 4HL). On the contrary *Ppd-H1* effects differed widely depending on the phase and the environment and had no effect on the ratio in any field environment. *Ppd-H2* was the most important QTL for LS in the three autumn sowings (Ll09-Au-PhN, Ll09-Au-PhE, Gi09-Au) but had a small effect in the winter sowing (Gi09-Wi, no significant either in Gr06-Sp) (Fig. 2c; Table 3). Although *Ppd-H2* was found to be slightly significant in two environments for SE, effects on LS were much higher than on SE (also significant for the ratio SE/LS).

The rest of the QTLs (9) were significant for only LS or SE, or if significant for both phases (two QTLs on 5H), with opposite effects between them (Fig. 2c; Table 3). These QTLs had smaller effects than the four above, and were not significant for HD (with the exception of the QTL near 4HC which was only significant in Gi09-Wi). QTLs on the distal part of 3HS, on 5HS and on the proximal region of 7HL were significant for LS in the four field environments. On the contrary a QTL proximal on 3HS and another on 5HL were significant for SE in almost all environments. Another three QTLs were found to have small effects on SE, but not on LS, under different conditions (near 1HC, 2HC and 5HC). There was little evidence of strong QTL × E effects for all the minor QTLs noted above and three, those on 3HS, 5HS and 5HL, were significant for the ratio SE/LS in the four field environments. Moreover, some of these QTLs for LS, SE and the ratio were also significant (and in the same direction) in the preliminary study (Fig. 2a; Table 3).

There was even a different genetic control between the two SE sub-phases (Fig. 2d; Table 3). For J-FL the second QTL on 2H and the QTL on 4HL were consistently found in the four field environments,

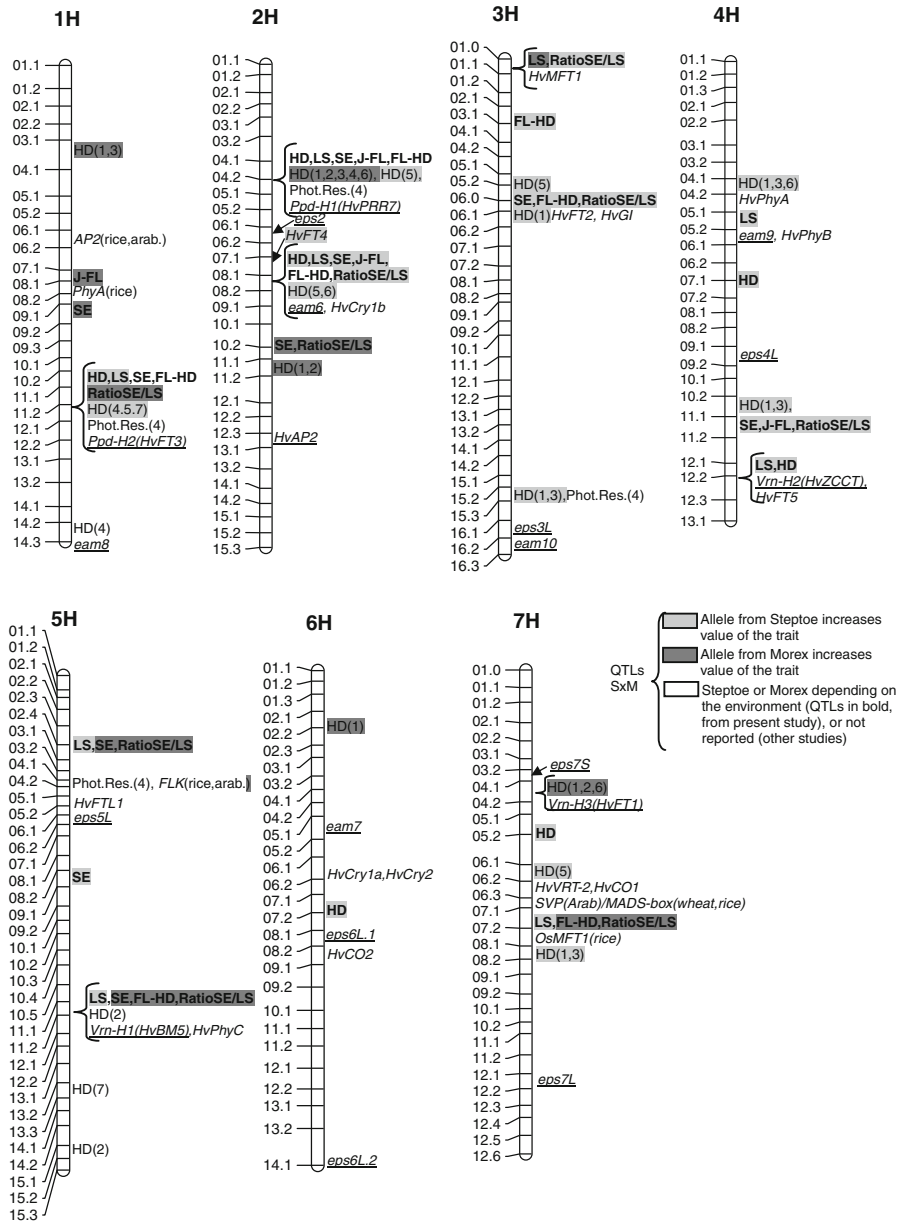


Fig. 4 Approximate location of QTLs (most significant markers) from the present study and from other studies for heading and response to photoperiod, also in Steptoe × Morex, following bin notation in Marcel et al. (2007). QTLs in the present study are highlighted in bold, while for QTLs from other studies, the *reference* is indicated between *brackets*: *1* Hayes et al. 1993 (Northern USA and Southern Canada, spring sowings); *2* Borem et al. 1999 (Northern USA and Southern Canada, spring sowings); *3* Márquez-Cedillo et al. 2001 (Northern USA and Southern Canada, spring sowings); *4* Boyd et al. 2003 (Australia, different photoperiod conditions); *5* Rao et al. 2007 (India, autumn sowing); *6* Ramsay et al. 2008 (Scotland, spring sowings); *7* Kikuchi et al. 2009 (12 h photoperiod, controlled conditions). In italics, genes found in barley related to flowering time (including *eps* loci from Laurie et al. 1995): *underlined*, genes in barley that have been found to have an effect on heading in at least one population; other genes found in barley but without known effects on flowering time (whose name start with '*Hv*') are *not underlined*; position of SNPs which have some degree of similarity (best BLASTX at HarvEST) to some genes related to flowering in other species (wheat, rice and *Arabidopsis*), and which map (in the consensus SNPs map) close to the QTLs from the present study, are also indicated with the name of these genes and its species between *brackets* (HarvEST, Close et al. 2009)

Table 5 Genetic correlations between the two phases within heading (LS and SE) and between the two phases within SE (J-FL and FL-HD; the value above is the coefficient of correlation and the value below, the significance level; ns, no significant)

	Ll09-Au-PhN	Gi09-Au	Ll09-Au-PhE	Gi09-Wi
LS vs. SE	−0.099	−0.055	−0.496	−0.047
	ns	ns	<0.001	ns
J-FL vs. FL-HD	−0.096	−0.086	−0.277	0.2643
	ns	ns	0.002	0.004

while *Ppd-H1* was only significant in the two environments with longer photoperiod (Gi09-Wi and Ll09-Au-PhE). QTL effects for FL-HD depended more on the environment, consistent with the lower h^2 than previous phases. The QTL on 4HL significant for J-FL was not significant for FL-HD, and the other three QTLs for FL-HD, which were also significant for J-FL, interacted highly (crossover interactions) with the environment. Another four QTLs (2 on 3H, 1 each on 5H and 7H) were significant for FL-HD but not for J-FL with the magnitude of their effects depending also on the environment (non-crossover interactions).

Figure 4 shows the approximate location of QTLs found in the present study together with QTLs for heading in Steptoe × Morex found in other studies, and genes in barley with known or unknown effects on flowering time. More detailed comparisons between the location of QTLs in the present study and several of these genes can be easily made with the SNPs names in Table 3 and the consensus map by Close et al. (2009).

Discussion

Overall genotype × environment interactions

A wide range of variability was found for all developmental phases studied as expected in a population segregating for major developmental genes, although the range of variability depended upon the environment (Fig. 1). Steptoe and Morex are known to differ widely in photoperiod response and in intrinsic earliness, and in a lesser extent by their vernalisation response (Karsai et al. 2001). The temperatures encountered in all trials in the present study should have been enough to satisfy the vernalisation requirement for a population derived from a cross between a facultative and a spring parent, and actually both Morex and Steptoe have a very low response to vernalization (Karsai et al. 2001).

The overall genotype × environment interaction was much more important between sowing dates than between photoperiod treatments, particularly for SE (between photoperiod treatments the G × E interaction was inappreciable, Table 4). Therefore, different sensitivities amongst genotypes to temperature or to the interaction photoperiod × temperature during SE would explain most of the observed genotype × environment interaction for this phase in the field experiments. Genotypic differences in photoperiod responses were relevant when explaining G × E for LS and HD, as shown in Table 4 and Fig. 3, but for these two phases different sensitivities to temperature or its interaction with photoperiod might have been also important (Fig. 3). QTL and QTL × E analyses reinforced these results, as shown for example by the relevance of *PpdH1*, *PpdH2* and *Eam6* depending on the phase and the environment (see Fig. 2c and next discussion about QTL × E). On the other hand, although *Ppd-H1* explained an important part of photoperiod responses, particularly for HD, there could be more genetic factors involved, as there were still significant differences in the response to photoperiod between genotypes within each *Ppd-H1* allele class, for both HD and LS (Fig. 3).

These results may not be comparable under higher photoperiods than in the present study, since responses may be greater in Steptoe under more contrasting photoperiod conditions (Karsai et al. 2001), but they may well represent a wide range of photoperiod and temperature conditions in which barley is grown. On the other hand, an interesting finding for breeding purposes was that the genotype × environment interaction for the ratio SE/LS was very low or inappreciable when comparing either photoperiod treatments or sowings, or the four field conditions (Table 3).

Differences in the genetic control of pre-heading phases

Genetic correlations between LS and SE were not significant or weak (Table 5). Previous studies had found genetic variation for the pre-heading phases and no relationships between them, or where correlations

were significant they were not consistent between studies (Appleyard et al. 1982; Kitchen and Rasmusson 1983; Kernich et al. 1995, 1997; Whitechurch et al. 2007; Borràs et al. 2009). The lack of highly significant genetic correlations (Table 5) and the different QTLs detected for LS and SE (Fig. 2; Table 3) in the present study confirmed a different genetic control between these two pre-heading phases in the Steptoe × Morex population, as found by Zhou et al. (2001) in rice and Borràs-Gelonch et al. (2010) in barley. Several QTLs that were significant just for LS or SE (or for both but with opposite effects, on 5H) were not significant for HD. Thus, more QTLs can be detected when dividing total time to heading into different sub-phases further reinforcing the results by Zhou et al. (2001) and Borràs-Gelonch et al. (2010), and showing the relevance of studying different pre-heading phases. The different genetic control between LS and SE would allow extending duration of SE for improving yield potential, without modifying heading, as proposed by e.g. Slafer et al. (2005), or for fine-tunning crop development in particular environmental conditions for breeding adaptability. Moreover, the high heritability of the ratio SE/LS and the consistency of several QTL effects across different photoperiod and temperature conditions make this independence in the genetic control of LS and SE more interesting for breeding purposes.

There were also large differences in the genetic control of the two sub-phases within SE (J-FL and FL-HD), as shown by genetic correlations (Table 5) and the QTL analysis (Fig. 2d). This could be relevant to yield formation since most florets die during the last part of stem elongation when the peduncle is elongating (Kirby 1988; Arisnabarreta and Miralles 2006). However, QTL effects for FL-HD depended more on environmental conditions, and some of the QTLs for FL-HD were co-located with significant QTLs for previous phases but with an opposite effect. Therefore, some of the QTL effects on FL-HD could be the result of effects during previous phases that would expose FL-HD to different conditions of photoperiod and temperature.

QTL × E interactions

QTLs significant for both LS and SE

Ppd-H1 had significant crossover interactions with the environment with the later allele being derived from Morex in the three environments with longer photoperiod (Gr06-Sp, Ll09-Au-PhE and Gi09-Wi), while it was derived from Steptoe for the two shortest photoperiod conditions. Usually, *Ppd-H1* only has significant effects on flowering under long photoperiod conditions of 13 h or more (Laurie et al. 1994; Pan et al. 1994; Boyd et al. 2003; Cuesta-Marcos et al. 2008a). In the Steptoe × Morex population the *Ppd-H1* locus has been found to have highly significant effects in high latitude and/or long photoperiod environments with the late allele always derived from Morex (Hayes et al. 1993; Borem et al. 1999; Boyd et al. 2003). However, Rao et al. (2007) found *Ppd-H1* had a great effect ($r^2 = 30\%$) under a winter sowing in India (29°N) with the late allele derived from Steptoe, as detected in the present study under the two environments with the shortest photoperiod conditions (Table 3). This is an interesting finding as *Ppd-H1* had not been reported before to produce differences under short (less than 13 h) photoperiod or in low latitude conditions. There could be also an interaction with temperature in the present study, as differences in effects from *Ppd-H1* between the three environments with longest photoperiod corresponded to differences in temperature (highest effects by far in Gr06-Sp, followed by Gi09-Wi and with the lowest effects in Ll09-Au-PhE). Previous reports have suggested that responses to photoperiod may change between phases and with temperature (Slafer and Rawson 1994, 1996).

The second most significant QTL for heading, which was also significant for both LS and SE, was located in bin 8.1 on 2H (Marcel et al. 2007), where *Eam6* is also likely to be located (GrainGenes). This locus had a greater effect on SE than LS, and it interacted with the environment less than *Ppd-H1*, with the late allele from Steptoe. Large effects from QTLs in the same bin for heading, which have been associated to *Eam6*, have been also reported in other populations in a wide range of conditions, both in low and high latitudes (Márquez-Cedillo et al. 2001; Moralejo et al. 2004; Castro et al. 2008) and in autumn, winter and spring sowings, with or without vernalisation, and under short or long photoperiods (Cuesta-Marcos et al. 2008a, b). Therefore, it seems highly probable that the QTLs that we detected reflect the segregation of *Eam6* in the Steptoe × Morex population. This QTL had greater effects on SE than

on LS, and except in Ll09-Au-PhE, it was significant for the ratio SE/LS, with its greatest effects in Gi09-Wi. This might be due to temperature effects (others than vernalizing temperatures), since it has been found that *Eam6* effects vary with mean temperature (Visioni and Romagosa, unpublished), but its effects might depend also on photoperiod conditions (Cuesta-Marcos et al. 2008a).

The significant QTL distal on 4HL maps in the vicinity of *Vrn-H2* (*HvZCCT*) but both Steptoe and Morex carry spring alleles at *HvZCCT* (A. Casas, personal communication, 2009). The QTL could therefore reflect the segregation of another gene, for example *HvFT5* (Faure et al. 2007) which also maps on bin 12.2 (Graingenes; Marcel et al. 2007), or another unknown linked gene. Like *Eam6*, this QTL was also significant for the ratio SE/LS except in Ll09-Au-PhE, due to its greater effects on SE than on LS (even in the preliminary study, Fig. 2a). Therefore, both also contributed to differences in the genetic control between LS and SE.

Two minor QTLs were detected for HD, but not for any of its sub-phases. The one on 6HL mapped close to *eps6L.1* (Laurie et al. 1995) and *HvCO2* (Griffiths et al. 2003) whilst the one on 7HS mapped in the region of bins 4.2–5.1, close to *HvFT1* (which is known to segregate in Steptoe × Morex; Kikuchi et al. 2009) and *eps7S* (Graingenes; Marcel et al. 2007; Fig. 4). Hayes et al. (1993) also found a heading QTL that was located in the same region of 7HS in the S × M population for heading. Our results suggest that it is different to QTLs for LS and the ratio SE/LS since these mapped in a different region of 7H (bins 6.2–7.2).

The QTL on 4H proximal to the centromere was significant for HD and LS but only in Gi09-Wi. Hayes et al. (1993), also detected a QTL in a nearby position (bin 4.1) in Steptoe × Morex with small effects under spring growing conditions in North America. Two other studies have found QTLs in this region, both of them in high latitudes and with small effects (Tinker et al. 1996; Li et al. 2006). Yin et al. (2005) reported QTLs for parameters related to photoperiod response and Szűcs et al. (2006) mapped *HvPhyB* in the same region.

Ppd-H2

Ppd-H2 was the third most significant QTL for heading and the second most important for LS. *Ppd-H2* has been found to determine differences in heading only under short photoperiods (from 8 to 12 h; Pan et al. 1994; Laurie et al. 1995; Cuesta-Marcos et al. 2008a, b; Kikuchi et al. 2009) and in autumn or winter growing conditions (Laurie et al. 1995; Cuesta-Marcos et al. 2008a, b). Also in the Steptoe × Morex population *Ppd-H2* was not found by Hayes et al. (1993) and Borem et al. (1999) in high latitudes and spring sowings in North America nor from spring sowings in Scotland (Ramsay et al. 2008), but it had been found in winter sowings by Boyd et al. (2003) in Australia under natural photoperiod and by Rao et al. (2007) in India. Therefore, it seems that differences between environments and phases in the present study could be due to photoperiod conditions as it had little effect under the winter sowing and no effect in the preliminary study, and its effects were much greater on LS than on SE.

We cannot distinguish if the different effects of *Ppd-H2* on LS and SE could be attributed only to the environmental conditions or also to an intrinsic difference between the two phases, since SE experienced more inductive conditions than LS (which also applies to the other QTLs found for only LS or SE). Kikuchi et al. (2009) reported further evidence that *HvFT3* is the candidate gene for *Ppd-H2* (Faure et al. 2007) and reported a great effect from this gene ($r^2 = 30\%$) on heading in the Steptoe × Morex population under short photoperiod. In their study *HvFT3* was highly expressed in Morex (not in Steptoe) under short photoperiod (12 h constant during all the growing period) during the first weeks (until the 7th leaf of a total of 12 leaves) and thereafter the expression dropped dramatically for the rest of the growing period until heading. Under long photoperiods (16 h), *HvFT3* was also expressed in Morex during the first weeks (until the 3rd–4th leaf of a total of 7), although with levels much lower than under 12 h of photoperiod (Kikuchi et al. 2009). These findings would support the idea that little or no effect of *Ppd-H2* on SE found in the present study could be due also to an intrinsic characteristic (differences in expression between phases). Several authors have shown that sensitivity to photoperiod could change between LS and SE, and that this variation between these phases can be largely independent (Slafer and Rawson 1994; Miralles and Richards 2000; González et al. 2002).

QTLs only significant for LS or SE

The consistency of the effects of the remaining QTLs renders them more interesting targets for manipulating the sub-phases LS and SE and the high heritability across environments for the ratio SE/LS, re-inforces this. Interestingly a significant QTL for LS distal on 3HS was also found in Borràs-Gelonch et al. (2010) in Henni × Meltan. This QTL was significant for the ratio SE/LS in the four field conditions and even in the preliminary study. We have not found a previous report of a QTL for heading in bins 1–4 of 3H. However, Yin et al. (2005) reported QTLs for sensitivity to photoperiod and for the onset of the photoperiod sensitive phase (bin 1.1), and more recently Kikuchi et al. (2009) mapped *HvMFT1* in the same region, also using the Steptoe × Morex population. In Kikuchi et al. (2009) *HvMFT1* had no significant effect on total time to heading, neither in an over-expression experiment in rice.

The other QTL on 3HS was significant only for SE and was located in bin 5.2. In Borràs-Gelonch et al. (2010) another peak was found in a similar region, whose confidence interval did not overlap with the other QTL more distal on 3HS, but it was significant for HD, not for LS or SE (main effects). Nevertheless, it was significant for SE in one location, and not for LS (Borràs-Gelonch et al. 2010). In Steptoe × Morex, QTLs in this region have been also reported for HD with small effects from both, spring in North America (Hayes et al. 1993) and winter in India (Rao et al. 2007) sowings, with the later allele derived from Steptoe in both cases, as in the present study for SE. The genes *HvFT2* and *HvGI* (Faure et al. 2007; Dunford et al. 2005; Kikuchi et al. 2009; Fig. 4) are also located in bin 6 of 3H.

The QTL on 5HS maps around bins 2.4–3.1. Again, QTLs for heading in the vicinity (between bins 2–4) have been reported to have small effects (e.g. Thomas et al. 1995, in Steptoe × Morex). Kikuchi et al. (2009) reported another *FT*-like gene on 5H in Steptoe × Morex (*HvTFL1*), with no effect on heading, but it mapped quite apart of our QTL (in bin 5.1). Boyd et al. (2003) found a QTL for photoperiod response in bin 4.1 in the Steptoe × Morex population, but no QTL for heading, in the same region. Opposite QTL effects for the pre-heading phases have been also observed in other studies (Zhou et al. 2001; Borràs-Gelonch et al. 2010).

The QTL on 5HL maps in the same bin than *HvBM5* (*Vrn-H1*), which is known to segregate in Steptoe × Morex. The temperatures encountered in all trials in the present study should have been enough to satisfy the vernalisation requirement for a population from a cross between a facultative and a spring parent (Karsai et al. 2001), as discussed above, so the QTL could be due to a different linked gene. Szűcs et al. (2006) mapped *HvPhyC* in the same bin (11.1), closely linked to *HvBM5*, and a QTL with significant effects on photoperiod response (see also Fig. 4).

The QTL proximal on 7HL was found in all environments for LS, but not for SE, and it was significant for the ratio SE/LS in two environments. It maps in the vicinity of *HvVRT-2*, which had effects on photoperiod response in Szűcs et al. 2006, and *HvCO1* (Griffiths et al. 2003). It could be also another *MFT*-like gene or similar to the SHORT VEGETATIVE PHASE in Arabidopsis (Fig. 4). Rao et al. (2007), found a QTL (on bin 6) with small effects on heading in Steptoe × Morex. Three other minor QTLs were found significant only for SE under different conditions around 1HC, 2HC and 5HC (Fig. 2c), in regions where no known genes related to flowering have been reported (Fig. 4).

Conclusions

In summary the three main conclusions from the present study were: (i) There were large differences in the genetic control of LS and SE in the Steptoe × Morex population, a population that segregates for major developmental genes. The different genetic control between LS and SE was mainly due to the sum of additive effects from minor QTLs (7 or more QTLs) that had little or no effect on total time to heading, but also to *Ppd-H2* (much greater effects on LS than on SE), to *Eam6* and another QTL distal on 4HL (both with greater effects on SE than on LS). (ii) For SE, genotypic differences in the sensitivity to temperature or its interaction with photoperiod would explain most of the observed G × E, as genotypic differences in the response to photoperiod were not significant. On the other hand, for LS and HD both photoperiod and other factors (i.e. temperature or its interaction with photoperiod) were important in explaining G × E. (iii) Despite the G × E observed for both LS and SE, the G × E for the ratio SE/LS was inappreciable, its

heritability was high (0.8) and several of the QTLs only significant for LS or SE had effects well conserved across the different environments. These results are interesting for breeding if the objective were changing duration of phases without modifying total time to heading. The numerous QTLs found for LS and SE but not for total time to heading also show the relevance of studying durations of different pre-heading phases. Further studies should be required to understand better the way in which these QTLs act. For example, in what extent differences in the QTL effects between phases were due to an intrinsic characteristic of the phase (i.e. different sensitivity to environmental factors) or to the increasing inductive conditions during the growing season or to both, as shown for example with the case of *Ppd-H2*.

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