GENETIC CONTROL OF PRE-HEADING PHASES AND OTHER TRAITS RELATED TO DEVELOPMENT IN A DOUBLE HAPLOID BARLEY (*Hordeum vulgare* L.) POPULATION

Gisela Borràs-Gelonch[1], Gustavo A. Slafer[1,2], Ana M. Casas[3], Fred van Eeuwijk[4] and Ignacio Romagosa[1]

[1] Department of Crop and Forest Sciences, University of Lleida, Centre UdL-IRTA, Av. Rovira Roure 191, 25198, Lleida (Catalonia), Spain;
[2]ICREA (Catalonian Institution for Research and Advanced Studies, www.icrea.es);
[3] Department of Genetics and Plant Production, Aula Dei Experimental Station, CSIC, Zaragoza, Spain;
[4]Wageningen University, Biometris, Department of Plant Sciences, P.O. Box 100, 6700 AC Wageningen The Netherlands.

ABSTRACT

Extending the phase of stem elongation (SE) has been proposed as a tool to further improve yield potential in small-grain cereals. The genetic control of pre-heading phases may also contribute to a better understanding of phenological traits conferring adaptability. Given that an optimized total time to heading is one of the most important traits in a breeding program, a prerequisite for lengthening SE would be that this and the previous phase (leaf and spikelet initiation, LS) should be under different genetic control. We studied the genetic control of these two pre-anthesis sub-phases (from sowing to the onset of jointing, LS, and from then to heading, SE) in terms of Quantitative Trait Loci (QTL) in a barley double-haploid population derived from the cross Henni x Meltan, both two-rowed spring North European barley cultivars. DH lines (118) and their parents were studied in four field trials in Northeast Spain. Genetic control of a number of traits related to leaf appearance and tillering dynamics, which could be important for an early crop canopy structure, were also studied. LS and SE are, at least partially, under a different genetic control in the Henni x Meltan population, mainly due to a QTL on chromosome 2HS. The QTLs responsible for a different control

of LS and SE did not seem to correspond with any major gene reported in the literature. Moreover shortening LS, so as to lengthen SE without modifying heading date, would not necessarily imply a negative drawback on traits that could be important for early vigour, such as phyllochron and the onset of tillering.

Key words: barley, development, QTL, leaf appearance, tillering, double-haploids.

INTRODUCTION

Crop phenology, which allows matching crop development with availability of resources (water, radiation, etc.), is the most important single factor influencing yield and crop adaptation to particular environments (Richards, 1991). This is especially relevant in Mediterranean conditions, where water is the main limiting factor and the occurrence of terminal drought and possible late spring frosts defines an optimal window for time to anthesis in order to maximise yield (Richards, 1991; Loss and Siddique, 1994; Cuesta-Marcos et al., 2009). Therefore keeping or achieving an optimised time to flowering is still an important goal in any breeding programme. In some Mediterranean environments, where intensive breeding has been carried out for centuries, either through farmer's selection or breeding programmes, there could be little scope for improving barley adaptability and yield by further adjustments in time to heading (Martiniello et al., 1987; Muñoz et al., 1998; Slafer et al., 2005). However, knowing the genetic control of different pre-anthesis phases may contribute to a better understanding of phenological traits conferring adaptability (e.g. Limin et al., 2007).

Fine adjustment of phenology could be also important for yield improvement through increasing yield potential. Lengthening duration of the stem elongation phase has been associated to increases in the number of grains/m^2 (Slafer et al., 2001; 2005) which in turn could increase yield potential of small-grain cereals (Fischer, 2007; Miralles and Slafer, 2007; Fischer, 2008). This should be achieved without modifying total time to anthesis, whose optimisation as shown above, is an important objective providing adaptability in breeding programmes (Slafer, 2003). To attain this goal, a prerequisite would be that the phases before and after the onset of stem elongation

should be under different genetic control, as earlier suggested by some authors (Halloran and Pennell, 1982; Slafer and Rawson, 1994; Kernich et al., 1997).

Given the importance of time to anthesis, the genetic control of this trait has been the focus of many studies. Several genes or loci related to the response to photoperiod or vernalisation, or to earliness per se (the three main factors determining heading time, Slafer and Rawson, 1994) have been found on the seven chromosomes of barley. The most widely known genes related to photoperiod are: *Ppd-H1* on 2HS, expressed under long days (Laurie et al., 1994; Laurie et al., 1995) and recently identified as a *PRR*-like (Pseudo-Response Regulator) gene by positional cloning (Turner et al., 2005); and *Ppd-H2* on 1HL, expressed under short days (Laurie et al., 1995) and for which *HvFT3*, a *FT*-like (Flowering locus T) gene, could be a candidate gene (Faure et al., 2007). Other reported genes that determine differences in heading time under short photoperiodic conditions are: *Eam7* on 6HS (Stracke and Börner, 1998), *Eam8* on 1HL (Franckowiak, 1997), *Eam9* on 4HL (Franckowiak, 1997; Lundqvist et al.,, 1997) and *Eam10* on 3HL (Börner et al. 2002). The three most known genes governing the response to vernalization are: *Vrn-H1* (*Sh2, HvVRN1* or *HvBM5A*) on 5HL and *Vrn-H2* (*Sh1*, *HvVRN2* or HvZCCT) on 4HL (Takahashi and Yasuda, 1971; Laurie et al., 1995) for which gene sequences have been also identified (Trevaskis et al., 2003; Yan et al., 2003, 2004), and *Vrn-H3* (*Sh3* or *HvFT1*) (Takahashi and Yasuda, 1971) which has been recently mapped on 7HS and identified as an orthologue of the *Arabidopsis FT* gene (Yan et al., 2006; Faure et al., 2007). Some loci whose effect could not be related to photoperiod or vernalization response are considered *earliness per se* genes (a group much less studied): *eps2S* on 2HS (or *Eam*6), *eps3L* on 3HL, *eps4L* on 4HL, *eps5L* on 5HL, *eps6L.1* and *eps6L.2*, both on 6HL, *eps7S* on 7HS and *eps7L* on 7HL (Laurie et al., 1995). Other homologues of some of the most important genes controlling flowering time in *Arabidopsis* have been found in barley (*HvCO1* to *HvCO8*, Griffiths et al., 2003; *HvGI*, Dunford et al., 2005; *HvFT1* to *HvFT5*, Faure et al., 2007) although most of them do not correspond to any of the above genes and their effect on heading is unclear. Moreover other QTL for heading date, whose position do not seem to coincide with these genes or loci, have been found in other barley populations and their effect on the response to the three main factors exposed above is unknown (e.g. Hayes et al., 1993; Bezant et al., 1996; Tinker et al., 1996; Baum et al., 2003; Li et al., 2006). QTL for heading date usually have an effect on other important

agronomic characters (yield, height, resistance to diseases, quality traits, etc.) (e.g. Hayes et al., 1993; Bezant et al., 1996; Tinker et al., 1996; Baum et al., 2003; Li et al., 2006) in accordance with the fact that heading date is a key trait for adaptability.

Several authors have shown variability in the duration of pre-anthesis phases, comparing different sets of cultivars, and even variability in the late reproductive phase (SE) in varieties with similar time to anthesis, both in wheat (Halloran and Pennell, 1982; Whitechurch et al., 2007) and barley (Appleyard et al., 1982; Kitchen and Rasmusson, 1983; Kernich et al., 1995; Kernich et al., 1997; Borràs et al., 2009). However we are not aware of any study in barley nor in wheat providing evidences of the genetic control of different pre-anthesis (sub)phases. The few studies comparing wheat substitution lines, single chromosome recombinant lines or near isogenic lines (differing in Ppd alleles) are inconclusive as differences in pre-anthesis phases lengths or in responses to photoperiod in each (sub)phase could not be attributed to particular major Ppd genes (see results and comparative review by González et al., 2005). Zhou et al. (2001), using a QTL approach to identify genetic controls of particular phenophases in rice, found some independent QTL for the duration of the vegetative and reproductive phases, either by different magnitude of QTL effects or by opposite allele effects on both phases. Whitechurch et al. (2007) identified variability in the stem elongation phase (SE) independent from the variability in the previous phases of leaf and spikelet initiation (LS) in a rather large set of cultivars, while Borràs et al. (2009) did not find major genetic correlations between both phases (with large genotypic effects and heritabilities for both traits) in the Henni x Meltan barley DH-population. Both results would lead to the suggestion that LS and SE could be under different genetic control also in wheat and barley. However it would be necessary to identify the particular genetic factors responsible for the genetic variability and for this lack of correlation, so as to explore avenues for manipulating LS and SE without modifying total time to heading. Thus, and following the work by Borràs et al. (2009), the first objective of the present study was identifying main QTL for the LS and SE phases in the Henni x Meltan population and comparing them with other genes or loci for developmental time (heading time) reported in the literature.

In addition, leaf appearance and tillering are important processes that determine the crop canopy structure mainly during phases before the onset of jointing. Although

possible drawbacks on the crop canopy formation when shortening LS (so as to lengthen SE without modifying total time to heading) could be agronomically solved through, for example, increased sowing (plant) density, traits related to early vigour could also compensate genetically for shorter LS. Moreover early vigour has been shown as a beneficial trait in temperate cereals breeding under some Mediterranean conditions (Richards et al., 2002). Borràs et al. (2009) showed, through genetic correlations, that shortening LS would not bring negative implications in traits that could be important for early vigour (i.e. phyllochron and the onset of tillering, or early vigour itself) in the Henni x Meltan population. Given also that little is known on the genetic control of leaf appearance and tillering parameters, key traits on growth and development, the second objective of this study was identifying the genetic control of traits related to these processes and compare them with the genetic control for pre-anthesis phases.

MATERIALS AND METHODS

Population and trials

A population of 118 doubled haploid (DH) lines from the cross Henni x Meltan and both parents were studied in four field trials, two locations by two growing seasons. Henni and Meltan are two-rowed spring barley cultivars from Northern Europe, released in 1995 and 1991 respectively and mainly used for feed. Details on the development of the DH-lines are given in Kraakman (2005). Although there could be a narrow genetic base in terms of phenology, the main advantage of this population is that it represents an actual breeding program, as both parents are modern cultivars within the elite European germplasm, in contrast to other studies that use populations derived from parents with phenology patterns extremely different but without a likely application in a realistic breeding program.

The two locations were Gimenells (41º37´N, 0º22´E, 248m) and Foradada (41º51´N, 1º0´E, 407m), both in the province of Lleida (Catalonia, North-Eastern Spain). Gimenells is situated in the middle of an irrigated basin, while Foradada is rainfed. The

two growing seasons were 2003/04 and 2005/06. Sowing dates were on 18 December in Gimenells and on 29 December in Foradada in 2003. In 2005 sowing dates were on 19 November and 21 November in Gimenells and Foradada, respectively. The experimental design for the four trials consisted of a latinized row and column design with two complete replicates per DH line and the two parents augmented with four commercial cultivars used as checks (a total of 300 plots per trial arranged in 15 rows and 20 columns). Each plot consisted of 8 rows 0.15 m apart and 4 m long. A sowing rate of 350 seeds m^{-2} was used in all cases. Further details on the trials are given in Borràs et al. (2009).

Phenotyping

Considering their relative importance in the context of the present study, the phases studied were: i) from sowing to the onset of jointing (about stage 30 in the scale of Zadoks et al., 1974), which coincides with the end of spikelet initiation (Kirby et al., 1994), namely the leaf and spikelet initiation phase (LS); ii) the stem elongation phase (SE), from the onset of jointing to heading (Zadoks' stage 55); iii) total time to heading (HD); and iv) the grain filling period (GF, from heading to physiological maturity (Zadoks' stage 92). In order to test more objectively differences in the genetic control of SE and LS that produced substantial changes in the partitioning of total time to heading (and independently of this), we also studied the SE/LS ratio. Duration of the phases was assessed in thermal time (ºC d, using a base temperature of 0ºC).

To study dynamics of leaf and tiller appearance, 2 plants per plot (all in all 600 plants per trial) were tagged in the two trials in 2004. Number of emerged leaves, following the Haun scale (Haun, 1973), and number of live tillers in each plant were recorded weekly from the stage of 2-3 leaves until flag leaf for leaves, and for tillers until its number stabilised. Phyllochron (ºC d per leaf) was estimated for each plant as the reciprocal of the regression coefficient of the relationship between the number of emerged leaves (Haun stages) and thermal time. Number of leaves appeared during LS (LN_{LS}) was estimated from the equation for rate of leaf appearance and the duration of

LS in thermal time. Number of leaves appeared during SE (LN_{SE}) was estimated as final number of leaves (FLN) minus LN_{LS}.

Tillering dynamics was studied as the relationship between number of live tillers and the predicted number of leaves at which they appeared (from the equation of rate of leaf appearance), in order to identify variability in tillering traits independent of that in phyllochron, which is known to have important effects on the tillering capacity (Kirby et al., 1985). Some traits were estimated directly from observed data: Haun stage at the onset of tillering (Ho), Haun at which maximum number of tillers is produced (Hmax), maximum number of tillers appeared (Tillmax), final number of tillers at harvest (FinalTill) and tiller mortality (Tillmort). The rest of traits were parameters derived from a lineal model (with three pieces and two knots) which is described in detail in Borràs et al. (2009). Briefly, in the first section of the model number of tillers increased rapidly following a linear trend. The slope (B) was an estimation of the rate of tillering. Then, at the first breakpoint (C) tiller production stopped and number of tillers stabilised or continued with a considerably slower rate (D) for some time. Thus, C might be considered as the timing of tillering cessation; the 'departure point' in Kirby et al. (1985). Finally, at the second breakpoint (E) tillers started to die rapidly until number of tillers stabilised (data after the end of tiller mortality were removed for fitting the model). F represents the rate of tiller mortality. Table 1 summarizes all traits and abbreviations used to designate them in the text.

Statistical analyses

Phenotypic data was analysed in two steps. In a first step Best Linear Unbiased Estimators (BLUEs) were estimated for each DH line both within individual trials and across all trials to remove spatial (local, within trials) and environment (across all environment) effects. In a second step, BLUEs were used for QTL analyses. BLUEs estimated from individual trials were used to study differences in QTL effects between environments (as a preliminary analysis of QTL x E interaction), while BLUEs estimated across all environments available were used to estimate main QTL effects. Details on the models used to estimate BLUEs are given in Borràs et al. (2009). The use of BLUEs for the QTL analyses was preferred, instead of BLUPs, because we had two

complete replicates for each DH-line and in order to avoid the differential shrinking between trials derived from the use of BLUPs (Möhring and Piepho, 2009).

QTL analyses were carried out using a linkage map with a total of 269 polymorphic markers (AFLPs) (Kraakman, 2005). The final map was 1056 cM long (Kosambi function) and had chromosomes lengths between 104 cM (chr. 4H) and 178 cM (5H). QTL analyses were performed using the restricted MQM mapping procedure (MAPQTL 5.0; van Ooijen, 2004). After initial interval mapping, the markers with the highest LOD values ('peak markers') were taken as co-factors. When new significant LOD peaks appeared, new peak markers were added to the co-factor set until a stable LOD profile was reached (Jansen, 1993). A LOD significance threshold of 2.9 (rounded upwards in the conservative direction) was chosen after permutation tests for each trait with a significance level of $p<0.05$, which was in agreement with thresholds estimated following van Ooijen (1999).

Epistasis and QTL x E interactions for the main detected QTL were studied using linear mixed models, which were performed on BLUEs estimated from individual trials. Each model included as fixed factors the environment, the significant markers for each trait (closest markers to the significant LOD-peaks chosen from previous QTL analysis on main effects) and all 2-level interactions, while the random factor was the remaining genotypic variance. Significant markers were added to the model ordered by their LOD significance (from the highest to the lowest). All linear mixed models were performed with Genstat (Payne 2006).

Candidate genes

As some of the QTLs were found not far from well known characterized genes, we analysed the genetic constitution of both parents and a subset of 16 double haploid lines with differential phenotypic and genetic constitution for the flanking markers of key QTL. Polymorphisms within vernalization and photoperiod response loci were screened with allele-specific primers of candidate genes, i.e. *HvBM5A* for *Vrn-H1* (Yan et al., 2003), *ZCCT-H* for *Vrn-H2* (Karsai et al., 2005), *HvFT1* for *Vrn-H3* (Yan et al., 2006; Faure et al., 2007), *HvPRR7* for *Ppd-H1* (Turner et al., 2005; Jones et al., 2008) and *HvFT3* for *Ppd-H2* (Faure et al., 2007). *Vrn-H1* was tested as size of the first intron

of *HvBM5A* (Zitzewitz et al., 2005); *Vrn-H2* was evaluated as presence of *ZCCT-H* (Karsai et al., 2005). The *Vrn-H3* locus was evaluated for two SNPs in the first intron of *HvFT1* and for a microsatellite in the second intron. Primers HvFT1.1F (5'-acgtacgtccctttcgatg-3') and HvFT1.2R (5'-atctgtcaccaacctgcaca-3') amplified a 506 bp fragment of the *Vrn-H3* gene. To differentiate the two polymorphic sites in the first intron of the gene, digestion of the amplified DNA was carried out with *Tsp509* I (A/T) and/or *Bcl* I (G/C). For testing *Ppd-H1*, exons 2-3 and 6 within *HvPRR7* (the regions with most presence of polymorphisms associated to differences in phenotype in Jones et al., 2008) were sequenced. The polymorphism proposed by Turner et al. (2005), SNP22 within the CCT domain of this gene, was also evaluated after amplification and digestion with *Bst*U I (G/T). Finally, *Ppd-H2* was tested scoring the presence of the candidate gene *HvFT3* using primers HvFT3.1F (5'-atccattggttgtgtggctca-3') and HvFT3.2R (5'-atctgtcaccaacctgcaca-3'), that generated a 431 bp fragment. The subset of DH lines was also tested for polymorphisms in several microsatellite markers (WMC1E8, HvM36, Bmac132, EBmac640, GBM1523, scssr03381, GBM5230, GBM1309, EBmac415, and GBM5060).

RESULTS

QTLs for the duration of developmental phases

About 5 QTLs were found for HD across environments and 6 for LS and SE (some were coincident, others not, and 3 were significant for the ratio SE/LS) (Figures 1 and 2, and Table 2). The QTL with the largest effect on HD (7HS) was also the most significant for LS and SE, with a similar part of variability explained and additive effect on both phases (in all cases the positive additive effect coming from the Henni allele) and thus, it had no effect on the ratio SE/LS. The effect of this QTL on heading seem the sum of the effects of the two component phases, as LOD values, variability explained and additive effects for HD were about twice the values for LS and SE. While for HD the QTL on 7HS explains much more variability than the other significant QTL, for LS and SE the relative weight of all the significant QTLs was more similar (Table 2). The second most important QTL for HD was found on the distal part of 1HL, but the positive allele effect came from Meltan (Figure 1 and Table 2). Although it was more

significant for LS than for SE, this QTL on 1HL it had no significant effect on the ratio SE/LS.

On the contrary the QTL on the short arm of 2H was highly significant and the most important for the SE/LS ratio while it had a small effect on total time to heading (Figure 1). Actually the position of this QTL for HD is unclear as shown by the large confidence interval (Figure 2). This was probably due to the opposite allele effects that this QTL had on LS and SE. The positive effect for LS came from Henni while for SE from Meltan. It was more significant and consistently found for SE than for LS, so the resulting positive allele effect for HD came also from Meltan.

Other two QTLs, one on 2HL and another on the distal part of 3HS were significant for LS but not for SE main effects. Both QTLs were about the significance threshold for the ratio SE/LS (Figure 1). A QTL on 3HS was also found to be significant for SE in one environment (data not shown) and a LOD peak is also detected for main effects (Figure 1), but it seems a different QTL, as the LOD peak mapped quite apart (>30 cM) from the QTL for LS, and the confidence interval of the QTL for LS did not coincide with those for SE and HD (which do overlap) (Figure 2).

The two most significant QTL for GF were also the two most significant for HD (QTL on 7HS and on 1HL) but with an allele effect opposite to the QTL for LS, SE and HD. The QTL on 2H for GF does not seem to coincide with the QTL for previous phases (Figures 1 and 2, and Table 2). Another minor QTL for HD was found distal on 5HL.

No epistatic interactions were found between the QTL significant for each phase. On the other hand the only significant QTL x E interaction was detected for the QTL on 7HS ($p<0.001$) for SE and HD (not for LS neither for GF). This interaction was quantitative (different allelic sensitivity between environments). Significance, additive effect and percentage of variability explained for this QTL was almost identical in 3 of the 4 environments (for both SE and HD). Only in Foradada 2005/06 the effect on both SE and HD was about half the effect in the other environments (data not shown). Thus, and considering also the little overall magnitude of the GxE compared to main

genotypic effects (Borràs et al., 2009), we simplified the presentation of results focusing on the QTL analyses for main effects.

Candidate genes

Some of the QTLs for duration of phases lied in the vicinity of well known genes controlling time to heading (Figure 2). Table 3 summarizes the polymorphisms found for the tested genes or markers. The QTL on 2HS is close to *Ppd-H1*, while the QTL on 1HL is close to *Ppd-H2* and *Eam8*. Both Henni and Meltan had identical sequences in exons 2-3 and 6 of *HvPRR7* (*Ppd-H1*), and presented the same polymorphisms (4 SNPs in each region) respect to Igri (which carries the dominant allele Ppd-H1). The two parents carried the same nucleotides for the functional polymorphisms SNP22 (T) and SNP48 (T), which correspond (both) to recessive alleles, insensitive to photoperiod, ppd-H1 (Turner et al., 2005; Jones et al., 2008, respectively). Both parents were neither polymorphic for *HvFT3* (*Ppd-H2*), and they carry the active allele (expressed under short photoperiods).

No polymorphism was either detected for Bmac132, scssr03381 or GBM5230, all three closely linked to *Eam6.* EBmac640 (also linked to *Eam6*) was polymorphic, but a number of recombinants were found between this marker and the most significant markers for durations of phases on 2HS and 2HL among the DHLs genotyped. There was also polymorphism for the microsatellite WMC1E8, very close to *Eam8* (and no recombinants in the subset of DH-lines were found between WMC1E8 and the most significant marker for HD in 1HL).

There was a deletion in the first intron of *HvBM5A* (*Vrn-H1*) in both parents, and a polymorphism was found between them, although several recombinants were found in the subset of DH-lines between *HvBM5A* and the most significant marker for HD on 5HL. As expected, given that both parents are spring types, both carried the recessive allele of *Vrn-H2*, insensitive to vernalization. Finally the highest QTL for LS, SE and HD on 7HS lied in the vicinity of *HvFT1* and *eps7S*. Both parents carried the same diagnostic SNPs in the first intron of *HvFT1*, but they can be distinguished by a microsatellite in the second intron of this gene.

QTLs for leaf appearance and tillering parameters

Phyllochron and number of leaves (FLN) were controlled by different QTL and the most important coincided with QTL for duration of phenological phases. The most significant QTL for phyllochron was also that for phenological phases on 7HS and the second significant QTL was found in the same position than the QTL for phases distal on 1HL. The direction of allele effects were the same than for the duration of phases. On the other hand for FLN the most important QTL coincided with the QTL for LS on 2HL, with less effect from the other two significant QTL on 3HS and on 4HL. The QTL on 2HS was also significant for LN_{LS} and LN_{SE} but with an opposite allelic effect as their respective phases. For early vigour the only significant QTL was also detected on 2HL, but its confidence interval did not overlap with the QTL for LS, FLN and LN_{LS} (Figures 1 and 2, and Table 2).

The most significant QTL for tillering parameters coincided with QTL for number of leaves (QTL on 2HS, 2HL and 4HL), although with differences between traits (Figure 2 and Table 2). The QTL on 7HS, significant for phases and phyllochron, had no effect on any trait related to tillering. The QTL on 2HS significant for LS, SE, HD, LN_{LS} and LN_{SE} was also significant for Tillmax, Tillmort, B, F and FinalTill. The sign of the additive effect for all these traits related to tillering was the same than for LS and LN_{LS} (positive effect from Henni) except for F, which is expressed in negative values. The QTL on 2HL, significant for LS, FLN and LN_{LS}, was also detected for Ho and interestingly with the same allele effect (for all these traits the positive effect came from Meltan). The QTL on 4HL, which was only significant for FLN, was the most significant QTL for FinalTill, and the second most significant QTL for Ho (but with opposite allele effects between both traits). The QTL on 1HL for phases was also significant for B. Finally a slightly significant QTL on 5HL was detected for Ho and B, which was also significant for Tillmax, FinalTill and early vigour, although for these last three traits it was significant in only one environment (data not shown). No QTL were found for the other tillering parameters (Hmax, C, D and E), which showed very low heritability (Borràs et al., 2009).

No significant epistasis was found for any of the traits related to leaf appearance and tillering. However, some QTL interacted significantly with the environment: for

instance the QTL on 7HS for FLN (p=0.004), which was only slightly significant in 1 of 2 environments but not for main effects (data not shown); the QTL for FinalTill on 2HS (only slightly significant for main effects and in 1 out of the 4 environments of the study), and on 4HL, which was highly significant with similar effects in all environments except Gimenells 2003/04 (p=0.02 and p<0.001for QTLxE on 2HS and 4HL respectively); and the QTL on 4HL for Ho (p=0.002; significant in Foradada 2003/04 but not in Gimenells 2003/04). In all cases the interactions were non-crossover or quantitative.

DISCUSSION

Genetic control for duration of phenological phases

Although both Henni and Meltan are modern spring cultivars from Northern Europe, with an expected narrow genetic basis for phenological traits, considerable genetic variability, transgressive segregation and high heritabilities were found for duration of phases and other traits related to leaf and tiller appearance (Borràs et al., 2009). Despite the most significant QTL on 7HS for HD was also the most significant for LS and SE, there were differences in QTL effects between both (sub)phases, in agreement with the high genetic correlation between HD and both LS and SE but with lack of major correlation between the duration of these two component phases (Borràs et al., 2009). This could be explained (i) mainly by the most significant QTL for the ratio SE/LS, that on 2HS, with opposite effects on both phases and with a greater significance on SE than on LS, and (ii) to a lesser extent, by differences in the effect of minor QTLs on LS and SE (i.e. QTLs on 2HL and 3HS). The effect from other minor QTL would be supported by the fact that Henni had a longer SE and higher ratio SE/LS than Meltan (Borràs et al., 2009). Thus, in line with the lack of major genetic correlation between LS and SE and given the differences in QTL effects on both phases found in the present study, it can be concluded that LS and SE are, at least partially, under different genetic control in the Henni x Meltan DH-population. These results were in agreement with Zhou et al. (2001) in rice, who found that the vegetative and reproductive phases were independent in terms of QTLs effects, either by different

magnitude of QTLs effects or also by opposite effects on both phases, and in line with other works supporting the hypothesis of an independent genetic control (Appleyard et al., 1982; Kitchen and Rasmusson, 1983; Slafer and Rawson, 1994; Kernich et al., 1995; Kernich et al., 1997; Whitechurch et al., 2007). Thus, it would be possible modifying the duration of different pre-heading phases without modifying total time to heading.

Finding an independent genetic control for LS and SE in the Henni x Meltan population would be a relevant result considering that variability in this population was quite limited (100 ºC d for both LS and SE; Borràs et al., 2009) (as expected given that both parents were spring varieties from Northern Europe), and therefore QTL effects were rather small. However these relatively small effects are interesting in the context of this study as the objective would be fine-tuning rates of the development before and after the onset of jointing (rather than looking for major differences in overall phenology). Moreover the population may well represent the situation of most advanced breeding programs in which variability for time to heading within the elite material would not be large. As expected by the little overall GxE compared to main genotypic effects, (particularly for duration of phases) previously found (Borràs et al., 2009), and the similarity between the four trials in photoperiod and temperature conditions, only one QTL (7HS) interacted significantly with the environment (non-crossover interaction).

The two most important QTL for HD (on 7HS and 1HL) were also the most significant for GF but with opposite allele effects in agreement with the strong negative genetic correlation between both traits (Borràs et al., 2009). These results are common under Mediterranean conditions, in which barley crops are usually exposed to high temperatures and dry conditions during grain filling, and might be due (as other studies reporting negative correlations between HD and GF) to environmental effects given that later heading exposes GF to harsher conditions (Richards, 1991; Loss and Siddique, 1994; Cuesta-Marcos et al., 2009), so they are much less relevant in the context of the present study.

Candidate genes

The most significant QTL for the ratio SE/LS is located on 2HS in the vicinity of *Ppd-H1*. The functional polymorphisms SNP22 (Turner et al., 2005) and SNP48 (Jones et al., 2008) were not polymorphic, and both parents had identical sequences in the exons 2-3 and 6 (those with most variability associated to differences in phenotype in Jones et al., 2008). Therefore there is strong evidence that both parents carry the same recessive allele ppd-H1, insensitive to photoperiod, which is typical in cultivars from Northern Europe (Cockram et al., 2007a). The most significant markers for SE and the SE/LS ratio (for which there was the highest effects from this QTL) were located between bins 2 and 3 in 2H (Marcel et al., 2007). We tried to locate the most significant QTL for heading date reported in the literature in this region (with consensus maps as in Wenzl et al., 2006; Marcel et al., 2007; the bin map published in 2005 at http://barleygenomics.wsu.edu/ and others available at Graingenes http://wheat.pw.usda.gov). It is also interesting to note that most of the highest significant markers in these studies (without considering those closer to *eps2* or *Eam6*) are located in bin 4, very close to *Ppd-H1*, not in more distal bins on 2HS (and all them were found under long photoperiod conditions; see references in Figure 2). An exception could be a QTL, reported by Castro et al. (2008), for difference in heading time between two extreme sowing dates and for grain filling, between bins 2 and 3 (between Bmac134 and HvM36), which had no effect on time to heading or to physiological maturity in any trial. Therefore this QTL could be associated to another gene, placed in a more distal position than *Ppd-H1*. On the other hand the large confidence interval for HD on 2H seems rather an imprecision in the position due to the small effect on HD.

The QTL on 7HS lies in bin 4, very close either to *eps7S* (Laurie et al., 1995; bin 3.2) or to *HvFT1* (Yan et al., 2006; Faure et al., 2007, in bin 4.1). Although it is not clear the causal polymorphism in *HvFT1*, candidate gene for *Vrn-H3* (Yan et al., 2006), there was no polymorphism for the diagnostic SNPs proposed by Yan et al. (2006). Another possibility is that our QTL could correspond to *eps7S*, which could be a different gene than *HvFT1* since either Igri or Triumph (Laurie et al., 1995) carry the same recessive allele *vrn-H3* (Yan et al., 2006), as the parents used in the present study.

QTL for heading reported in the literature in the same region were found either under long or short photoperiod conditions (see references in Figure 2).

The most significant markers on 1HL were situated in bins 13-14, although the confidence interval for most traits spans over a wider region (probably due to the large gap between 77 and 127 cM). There was no polymorphism for *HvFT3*, the candidate gene for *Ppd-H2* (bin 11.2), but we found that WMC1E8 (*Eam8*, bin 14.3) was polymorphic and no recombinants were found in the subset of 16 lines between WMC1E8 and the most significant marker for HD (E42M32-272). Moreover in a DArT map for Henni x Meltan no polymorphic marker was found either in that region between bins10-12, around *Ppd-H2* (data not published). Therefore our QTL on 1HL could correspond to *Eam8* rather than *Ppd-H2*. While QTL reported in the same region than *Ppd-H2* (bin 11.2) are found only under short photoperiod conditions, QTL reported at a position closer to *Eam8* (bin 14) are reported either under short or long photoperiod conditions (see references in Figure 2).

There are no genes or loci (the most known related to the response to photoperiod and vernalization, or to earliness per se) described on 2HL and on 3HS, although some authors have found minor QTL for heading in these regions (see references in Figure 2) and *HvAP2* has been recently identified as a candidate gene for one of the QTLs on 2HL (bin 12.3; Chen et al., 2009). Finally the QTL on 5HL, which had minor effects on HD, Ho and B, is located in bins 13-15. There was a polymorphism for *Vrn-H1* (*HvBM5A*, bin 11.1), but both parents had deletions within the first intron, typical of spring cultivars (Cockram et al., 2007b), and several recombinants (5 of 16) were found between HvBM5A and E33M61-144 (the most significant marker for HD), so this QTL is probably another gene, in line with the fact that both parents are spring cultivars (both also null for *Vrn-H2*).

Genetic control of traits related to leaf and tiller appearance

The most important QTL for leaf number and phyllochron coincided with QTL for phases and with the same direction of allele effects than those, which could be expected as both traits largely determine total time to heading (García del Moral et al., 2002). However both traits were controlled mainly by different QTLs. While

phyllochron was controlled mainly by the QTL on 7HS and in a lesser extent by the QTL on1HS (the two main QTL for HD), the most important QTL for FLN was on 2HL, with minor effects from other QTL on 3HS and 4H, which is in agreement with the fact that HD in the Henni x Meltan population was more determined by phyllochron than by FLN (Borràs et al., 2009) and with lack of strong genetic correlations between both traits (Dofing, 1999; Borràs et al., 2009). Thus, as the QTL for phyllochron does not coincide with those that could be responsible for a different partitioning of HD in LS and SE (mainly QTL on 2HS, and in a lesser extent QTL on 2HL and 3HS), shortening LS (so as to lengthen SE without modifying HD) genetically would have little or no correlated response to selection for phyllochron, a trait that could be important for early vigour.

QTLs for tillering parameters coincided mainly with those for number of leaves, in agreement with genetic correlations found between these traits and number of leaves (Borràs et al., 2009) and with considerations in the literature about the close link between tillering and leaf appearance dynamics (Kirby et al., 1985; Miralles and Richards, 2000). However there were differences in the significance and effects of these QTLs between traits. The QTL on 2HS seemed related to the capacity of tiller production (the later the onset of stem elongation the longer the tillering period, although no other QTL significant for LS or number of leaves had an effect on Tillmax). It could explain the high genetic correlation between Tillmax and B (Borràs et al., 2009), while it had little effect on FinalTill. For this QTL on 2HS, as well as for the QTL on 1HL, the allele that lengthened LS also increased B, which could have negative implications for early vigour when shortening LS.

On the other hand the allele from Henni for the QTL on 2HL shortened LS while reduced Ho (which would be beneficial for early vigour), and could even increase early vigour itself (closely linked to the QTL for LS, FLN, LN_{LS} and Ho). These QTL effects on 2HL could compensate, together with QTLs for phyllochron, for any negative effects on the crop canopy formation when shortening LS. Moreover, B had a low heritability in this population (Borràs et al., 2009) which would be in agreement with low LOD-values and small additive effects of QTL significant for B. Another QTL on 4HL, which had no effect on length of phases, was also quite significant for Ho and it was the most important for FinalTill. Therefore it seems that manipulating LS and SE could have also

little or no effect on FinalTill (despite FinalTill was slightly positively correlated with Tillmax, it was not correlated with any phase or number of leaves; Borràs et al., 2009). Some significant QTLxE interactions were found for tillering traits as it might be expected as tillering is affected by availability of resources (e.g. in response to water stress; Cone et al., 1995) and the two sites differed in water availability. Nevertheless, all QTLxE interactions were non-crossover or quantitative.

Conclusions

Summarizing, the main conclusions that arise from the present study are: i) LS and SE are, at least partially, under different genetic control in the Henni x Meltan population, mainly due to a QTL on 2HS which had different effects (both in the direction of allele effects and in the magnitude) between both phases; ii) the QTLs responsible for a different genetic control of LS and SE do not seem to correspond to major genes or QTLs reported in the literature; iii) shortening LS so as to lengthen SE without modifying HD would not imply a negative drawback on traits that could be important for early vigour, as phyllochron and the onset of tillering. Given that the range of variability in the Henni x Meltan population was quite limited, it would be interesting to explore other populations in order to identify QTLs with potentially larger effects. Further studies (combining genetics and physiology) are also required to understand better the way (photoperiod sensitivity during SE, e.g. Miralles and Richards, 2000; or differences in earliness per se for this phase, e.g. Slafer, 1996) in which QTLs can promote substantial differences on lengths of pre-anthesis phases.

ACKNOWLEDGEMENTS

We thank Maria Bagà, Josep A. Betbesé and the rest of technicians from the field crops team of the Centre UdL-IRTA for their technical assistance. We are also very grateful to Alex Pswarayi for his help with the data recording in the field, in the experiments in 2004. GB held a pre-doctoral FPU scholarship from the Spanish Ministry of Science and Innovation. This study was partially funded by the European Union – INCO - MED program (ICA3-CT2002-10026) Mapping Adaptation of Barley to Drought Environments (MABDE) and by the Spanish Ministry of Science and

Innovation competitive grants AGL2008-05541/C02 and AGL2006-07814/AGR. The Centre UdL-IRTA forms part of the Centre CONSOLIDER on Agrigenomics.

REFERENCES

Appleyard, M., Kirby, E.J.M., Fellowes, G., 1982. Relationships between the duration of phases in the pre-anthesis life cycle of spring barley. Aust. J. Agric. Res. 33, 917-925

Backes, G., Graner, A., Foroughi-Wehr, B., Fischbeck, G., Wenzel, G., Jahoor, A., 1995. Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L). Theor. Appl. Genet. 90: 294-302.

Baum, M., Grando, S., Backes, G., Jahoor, A., Sabbagh, A., Ceccarelli, S., 2003. QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' x *H spontaneum* 41-1. Theor. Appl. Genet. 107:1215-1225.

Bezant, J., Laurie, D., Pratchett, N., Chojecki, J., Kearsey, M.,1996. Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L) cross. Heredity 77:64-73.

Borràs, G., Romagosa, I., van Eeuwijk, F., Slafer, G., 2009. Genetic variability in the duration of pre-heading phases and relationships with leaf appearance and tillering dynamics in a barley population. Field Crop Res. 113: 95-104.

Borem, A., Mather, D.E., Rasmusson, D.C., Fulcher, R.G., Hayes, P.M., 1999. Mapping quantitative trait loci for starch granule traits in barley. J. Cereal Sci. 29: 153-160.

Börner, A., Buck-Sorlin, G.H., Hayes, P.M., Malyshev, S., Korzun, V., 2002. Molecular mapping of major genes and quantitative trait loci determining flowering time in response to photoperiod in barley. Plant Breed. 121:129-132.

Boyd, W.J.R., Li, C.D., Grime, C.R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Jalal Kamali, M.R., Barr, A.R., Moody, D.B., Lance, R.C.M., Logue, S.J., Raman, H., Read, B.J., 2003. Conventional and molecular analysis of factors contributing to variation in the timing of heading among spring barley (*Hordeum*

vulgare L) genotypes grown over a mild winter growing season. Aust. J. Agric. Res. 54: 1277-1301.

Castro, A.J., Hayes, P., Viega, L., Vales, I., 2008. Transgressive segregation for phenological traits in barley explained by two major QTL alleles with additivity. Plant Breed. 127: 561-568.

Chen, A., Bumann, U., Fincher, G.B., Collins, N.C., 2009. *Flt-2L*, a locus in barley controlling flowering time, spike density, and plant height. Funct. Integr. Genomics 9: 243-254.

Cockram, J., Jones, H., Leigh, F.J., O'Sullivan, D., Powell, W., Laurie, D.A., Greenland, A., 2007a. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. J. Exp. Bot. 58: 1231-1244.

Cockram, J., Chiapparino, E., Taylor, S.A., Stamati, K., Donini, P., Laurie, D.A., O'Sullivan, D., 2007b. Haplotype analysis of vernalization loci in European barley germoplasm reveals novel VRN-H1VRN-H1 alleles and a predominant winter VRN-H1VRN-H1/VRN-H2VRN-H2 multi-locus haplotype. Theor. Appl. Genet. 115: 993–1001.

Cone, A.E., Slafer, G.A., Halloran, G.M., 1995. Effects of moisture stress on leaf appearance, tillering and other aspects of development in *Triticum tauschii*. Euphytica, 86:55-64.

Cuesta-Marcos, A., Igartua, E., Ciudad, F., Codesal, P., Russell, J.R., Molina-Cano, J.L., Moralejo, M., Szucs, P., Gracia, M.P., Lasa, J.M., Casas, A.M., 2008a. Heading date QTL in a spring x winter barley cross evaluated in Mediterranean environments. Mol. Breed. 21: 455-471.

Cuesta-Marcos, A., Casas, A.M., Yahiaoui, S., Gracia, M.P., Lasa, J.M., Igartua, E., 2008b. Joint analyis for heading date QTL in small interconnected barley populations. Mol. Breed. 21: 383-399.

Cuesta-Marcos, A., Casas, A.M., Hayes, P.M., Gracia, M.P., Lasa, J.M., Ciudad, F., Codesal, P., Molina-Cano, J.L., Igartua, E., 2009. Yield QTL affected by heading date in Mediterranean grown barley. Plant Breed. 128: 46-53.

Dofing, S.M., 1999. Inheritance of phyllochron in barley. Crop Sci 39: 334-337.

Dunford, R.P., Griffiths, S., Christodoulou, V., Laurie, D.A., 2005. Characterisation of a barley (*Hordeum vulgare* L) homologue of the *Arabidopsis* flowering time regulator *GIGANTEA*. Theor. Appl. Genet. 110: 925-931.

Emibiri, L.C., Moody, D.B., 2006. Heritable basis for some genotype-environment stability statistics: Inferences from QTL analysis of heading date in two-rowed barley. Field Crop Res. 96: 243-251.

Faure, S., Higgins, J., Turner, A., Laurie, D.A., 2007. The *FLOWERING LOCUS T*-like gene family in barley (*Hordeum vulgare*). Genet. 176: 599-609.

Fischer, R.A., 2007. Understanding the physiological basis of yield potential in wheat. J. Agric. Sci. 145: 99-113.

Fischer, R.A., 2008. The importance of grain or kernel number in wheat: A reply to Sinclair and Jamieson. Field Crop Res. 105: 15-21.

Franckowiak, J.D., 1997. Revised linkage maps for morphological markers in barley, *Hordeum vulgare.* Barley Genet. Newsl. 26: 9-21.

García del Moral, L.F., Miralles, D.J., Slafer, G., 2002. Initiation and appearance of vegetative and reproductive structures throughout barley development In: GA Slafer, JL Molina-Cano, R Savin, JL Araus and I Romagosa (ed) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality, Food Product Press, New York, pp 243-267.

González, F.G., Slafer, G.A., Miralles, D.J., 2005. Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes *Ppd-D1* and *Ppd-B1.* Euphytica 146, 253-269.

Griffiths, S., Dunford, R.P., Coupland, G., Laurie, D.A., 2003. The evolution of *CONSTANS*-like gene families in barley, rice, and Arabidopsis. Plant Physiol. 131: 1-13.

Halloran, G.M., Pennell, A.L., 1982. Duration and rate of development phases in wheat in two environments. Ann. Bot. 49,115-121.

Hayes, P.M., Liu, B.H., Knapp, S.J., Chen, F., Jones, B., Blake, T., Franckowiak, J., Rasmusson, D., Sorrells, M., Ullrich, S.E., Wesenberg, D., Kleinhofs, A., 1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor. Appl. Genet. 87:392-401.

Haun, J.R., 1973. Visual quantification of wheat development. Agron. J. 65,116-119.

Jansen, R.C., 1993. Interval mapping of multiple quantitative trait loci. Genet. 135:205-211.

Jones, H., Leigh, F.J., Mackay, I., Bower, M.A., Smith, L.M.J., Charles, M.P., Jones, G., Jones, M.K., Brown, T.A., Powell, W., 2008. Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the Fertile Crescent. Mol. Biol. Evol. 25 (10):2211-2219.

Karsai, I., Szűcs, P., Mészáros, K., Filichkina, T., Hayes, P.M., Skinner, J.S., Láng, L., Bedó, Z., 2005. The *Vrn-H2* locus is a major determinant of flowering time in a facultative x winter growth habit barley (*Hordeum vulgare* L) mapping population. Theor. Appl. Genet. 110: 1458-1466.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1995. Variation in development patterns of wild barley (*Hordeum spontaneum* L) and cultivated barley (*H vulgare* L). Euphytica 82,105-115.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1997. Variation in duration of pre-anthesis phases of development in barley (*Hordeum vulgare*). Aust. J. Agric. Res. 48, 59-66.

Kicherer, S., Backes, G., Walther, U., Jahoor, A., 2000. Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L). Theor Appl Genet. 100: 881-888.

Kitchen, B.M., Rasmusson, D.C., 1983. Duration and inheritance of leaf initiation, spike initiation and spike growth in barley. Crop Sci. 23, 939-943.

Kirby, E.J.M., Appleyard, M., Fellowes, G., 1985. Leaf emergence and tillering in barley and wheat. Agronomie 3: 193-200.

Kirby, E.J.M., Appleyard, M., Simpson, N.A., 1994. Co-ordination of stem elongation and Zadoks growth stages with leaf emergence in wheat and barley. J. Agric. Sci. 122, 21-29

Kjaer, B., Jensen, J., Giese, H., 1995. Quantitative trait loci for heading date and Straw characters in barley. Genome 38: 1098-1104.

Kraakman, A.T.W., 2005. Mapping of yield, yield stability, yield adaptability and other traits in barley using linkage disequilibrium mapping and linkage analysis PhD thesis, Wageningen University

Laurie D.A., Pratchett, N., Bezant, J.H., Snape, J.W., 1994. Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of *Hordeum vulgare.* Heredity 72:619-627.

Laurie D.A., Pratchett, N., Bezant, J.H., Snape, J.W., 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter x spring barley (*Hordeum vulgare* L) cross. Genome 38: 575-585.

Li, J.Z., Huang, X.Q., Heinrichs, F., Ganal, M.W., Röder, M.S., 2006. Analysis of QTLs for yield components, agronomic traits ans disease resistance in an advanced backcross population of spring barley. Genome 49: 454-466.

Limin, A., Corey, A., Hayes, P., Fowler, D.B., 2007. Low-temperature acclimation of barley cultivars used as parents in mapping populations: response to photoperiod, vernalization and phonological development. Planta 226, 139-146.

Loss, S.P., Siddique, K.H.M., 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Adv. Agron. 52: 229-276.

Lundqvist, U., Franckowiak, J.D., Konishi, T., 1997. New and revised descriptions of barley genes. Barley Genet. Newsl. 26: 22-516.

Marcel, T.C., Varshney, R.K., Barbieri, M., Jafary, H., de Kock, M.J.D., Graner, A., Niks, R.E., 2007. A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor. Appl. Genet. 114: 487-500.

Márquez-Cedillo, L.A., Hayes, P.M., Kleinhofs, A., Legge, W.G., Rossnagel, B.G., Sato, K., Ullrich, S.E., Wesenberg, D.M., 2001. QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theor. Appl. Genet. 103: 625-637.

Martiniello, P., Delogu, G., Oboardi, M., Boggini, G., Stanca, A.M., 1987. Breeding progress in grain yield and selected agronomic characters of winter barley (*Hordeum vulgare* L.) over the last quarter of a century. Plant Breeding 99: 289-294.

Miralles, D.J., Richards, R.A., 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Ann. Bot. 85: 655-663.

Miralles, D.J., Slafer, G.A., 2007. Sink limitations to yield in wheat: how could it be reduced?. J. Agric. Sci. 145: 139-149.

Möhring, J., Piepho, H.P., 2009. Comparisons of weighting in two-stage analysis of plant breeding trials. Crop Sci. 49: 1977-1988.

Muñoz, P., Voltas, J., Araus, J.L., Igartua, E., Romagosa, I., 1998. Changes over time in the adaptation of barley releases in north-eastern Spain. Plant Breeding 117: 531-535.

Pan, A., Hayes, P.M., Chen, F., Blake, T.H.H., Wright, S., Karsai, I., Bedö, Z., 1994. Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L). Theor. Appl. Genet. 89: 900-910.

Payne, R.W., 2006. The Guide to GenStat ® Release 9 VSN International, Hertfordhire.

Powell, W., Thomas, W.T.B., Baird, E., Lawrence, P., Booth, A., Harrower, B., McNicol, J.W., Waugh, R., 1997. Analysis of quantitative traits in barley by the use of amplified fragment length polymorphism. Heredity 79: 48-59.

Qi, X., Nicks, E.E., Stam, P., Lindhourt, P., 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theor. Appl. Genet. 96: 1205-1213.

Rao, H.S., Basha, O.P., Singh, N.K., Sato, K., Dhaliwal, H.S., 2007. Frequency distributions and composite interval mapping for QTL analysis in 'Steptoe' x 'Morex' barley mapping population. Barley Genetics Newsletter 37, 5-20.

Richards, R.A., 1991. Crop improvement for temperate Australia: Future opportunities. Field Crop Res. 26: 141-169.

Richards, R.A., Rebetzke, G.J., Condon, A.G., Herwaarden, A.F., 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci. 42: 111-121.

Slafer, G.A., 1996. Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalization. A viewpoint of the intrinsic earliness hypothesis. J. Agric. Sci. 126: 403-419.

Slafer, G.A., Rawson, H.M., 1994. Sensitivity of wheat phasic development to major environmental factors: A re-examination of some assumptions made by physiologists and modellers. Aust. J. Plant. Phys. 21, 393-426.

Slafer, G.A., Abeledo, L.G., Miralles, D.J., González, F.G., Whitechurch, E.M., 2001. Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. Euphytica 119: 191-197.

Slafer, G.A., 2003. Genetic basis of yield as viewed from a crop physiologist's perspective. Ann. Appl. Biol. 142: 117-128.

Slafer, G.A., Araus, J.L., Royo, C., García del Moral, L.F., 2005. Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Ann. Appl. Biol. 46: 61-70.

Stracke, S., Börner, A., 1998. Molecular mapping of the photoperiod response gene ea7 in barley. Theor. Appl. Genet. 97:797-800 .

Szűcs, P., Karsai, I., von Zitzewitz, J., Mészáros, K., Cooper, L.L.D., Gu, Y.Q., Chen, T.H.H., Hayes, P.M., Skinner, J.S., 2006. Positional relationships between photoperiod response QTL and photoreceptor and vernalization genes in barley.

Takahashi, R., Yasuda, S., 1971. Genetics of earliness and growth habit in barley In: Proceedings of the 2nd International Barley Genetics Symposium Edited by RA Nilan Washington State University Press, Washington, pp 388-408

Teulat, B., Merah, O., Souyris, I., This., D., 2001. QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. Theor. Appl. Genet. 103: 774-787.

Tinker, N.A., Mather, D.E., Blake, T.K., Briggs, K.G., Choo, T.M., Dahleen, L., Dofing, S.M., Falk, D.E., Ferguson, T., Franckowiak, J.D., Graf, R., Hayes, P.M., Hoffman, D., Irvine, R.B., Kleinhofs, A., Legge, W., Rossnagel, B.G., Saghai Maroof, M.A., Scoles, G.J., Shugar, L.P., Steffenson, B., Ullrich, S., Kasha, K.J., 1996. Regions of the genome that affect agronomic performance in two-row barley. Crop Sci. 36:1053-1062.

Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J., Dennis, E.S., 2003. MADS-box genes control vernalization-induced flowering in cereals. Proc. Natl. Acad. Sci. USA 100: 13099-13104.

Turner, A., Beales, J., Faure, S., Dunford, R.P., Laurie, D.A., 2005. The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. Sci. 310: 1031-1034.

van Ooijen, J.W., 1999. LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613-624.

van Ooijen, J.W., 2004. MapQTL 5 Software for the mapping of quantitative trait loci in experimental populations, pp 63 Kyazma BV, Wageningen.

Voorrips, R.E., 2002. Mapchart: Software for the graphical presentation of linkage maps and QTLs. J. Heredity 93 (1): 77-78.

Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K.P., Muehlbauer, G.J., Chalmers, K.J., Kleinhofs, A., Huttner, E., Kilian, A. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7: 206.

Whitechurch, E.M., Slafer, G.A., Miralles, D.J., 2007. Variability in the duration of stem elongation in wheat and barley genotypes. J Agron Crop Sci 193: 138-145.

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., Dubcovsky, J., 2003. Positional cloning of the wheat vernalization gene *VRN1*. Proc. Natl. Acad. Sci. USA 100: 6263-6268.

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., San Miguel, P., Bennetzen, J.L., Echenique, V., Dubcovsky, J., 2004. The wheat *VRN2* gene, a flowering repressor down-regulated by vernalization. Sci. 303: 1640-1644.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., Dubcovsky, J., 2006. The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc. Natl. Acad. Sci. USA 103: 19581-19586.

Yin, X., Struik, P.C., van Eeuwijk, F.A., Stam, P., Tang, J., 2005. QTL analysis and QTL-based prediction of flowering phenology in recombinant inbred lines of barley. J. Exp. Bot. 56: 967-976.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. Decimal code for the growth stage of cereals. Weed Res., 14: 415-421.

Zhou, Y., Li, W., Wu, W., Chen, Q., Mao, D., Worland, A.J., 2001. Genetic dissection of heading time and its components in rice. Theor. Appl. Genet. 102: 1236-1242.

Zitzewitz, J. von, Szücs, P., Dubcovsky, J., Yan, L., Francia, E., Pecchioni, N., Casas, A.M., Chen, T.H.H., Hayes, P.M., Skinner, J.S., 2005. Molecular and structural characterization of barley vernalization genes. Plant Molecular Biology 59: 449-467.

Table 1. Abbreviations used in the text to designate each of the studied traits.

Abbreviation	Trait
LS	Leaf and spikelet initiation phase (ºC d)
SE	Stem elongation phase (ºC d)
HD	Total duration from sowing to heading (ºC d)
GF	Grain filling period (ºC d)
LN_{LS}	Number of leaves appeared during the leaf and spikelet initiation phase
LN_{SE}	Number of leaves appeared during the stem elongation phase
FLN	Final leaf number
-	Phyllochron; inverse of the rate of leaf appearance (ºC d / leaf)
B	Rate of tillering (tillers / leaf)
C	Number of leaves at tillering cessation (at B, the main rate of tillering)
D	Secondary rate of tillering (tillers / leaf)
E	Number of leaves when tillering mortality started
F	Rate of tillering mortality (tillers / leaf)
Ho	Haun stage when the first tiller tip emerges
Tillmax	Maximum number of tillers per plant produced
Hmax	Haun stage at which maximum number of tillers is reached
Tillmort	Number of died tillers per plant
FinalTill	Final tiller number per plant at harvest
-	Early vigour, visually assessed at c. Haun 5

Table 2. Most significant markers (closest to the LOD peaks) for main effects, by chromosomal regions (bins according to Marcel et al. 2007). Position (in cM), LOD value, percentage of variability explained (% Expl.) and additive effect (Add. Eff.) are given for each marker.

Chromosomal region	Trait (main effects)	Marker	Position (cM)	LOD	% Expl.	Add.Eff.
1HL bin 13-14	LS (ºC days)	E39M55-165	127.3	3.7	7.2	-6
	SE (ºC days)	E40M38-118	146.1	2.6 †	4.9	-4
	HD (ºC days)	E42M32-178	135.3	8.1	11.1	-11
	GF (ºC days)	E40M38-118	146.1	5.6	8.5	7
	Phyllochron (ºC days/leaf)	E42M32-176	134.3	2.9	6.4	-0.7
	B (tillers/leaf)	E39M55-165	127.3	3.0	8.2	-0.11
2HS bin 2-3	LS (ºC days)	E42M48-308	20.6	3.4	6.6	6
	SE (ºC days)	E42M32-272	7.8	5.4	11.9	-7
	Ratio SE/LS	E42M32-272	7.8	8.1	23.8	-0.019
	HD (ºC days)	E42M32-272	7.8	3.0	4.1	-6
		E35M48-185	58.1(bin 4)	3.0	5.0	-6
	LN_{LS}	E42M48-308	20.6	3.0	8.6	0.1
	LN_{SE}	E42M32-272	7.8	2.7	6.3	-0.1
	Tillmax	E42M48-308	20.6	6.4	18.6	0.6
	B (tillers/leaf)	E42M48-308	20.6	3.4	8.7	0.11
	F (tillers/leaf)*	E42M48-308	20.6	5.1	14.9	-0.14
	Tillmort	E42M48-308	20.6	3.1	8.0	0.3
	FinalTill	E42M48-308	20.6	2.7	6.2	0.1
2HL bin 7	GF (ºC days)	E32M61-388	83.2	3.8	5.5	-5
2HL bin 8	Early vigour	E42M48-356	104.9	3.1	8.5	0.2
2HL bin 11	LS (ºC days)	E33M55-592	121.8	2.9	5.1	-5
	Ratio SE/LS	E33M55-592	121.8	2.3 †	7.4	0.011
	LN_{LS}	E33M55-592	121.8	7.2	19.0	-0.2
	FNL	E33M61-227	122.6	9.2	21.3	-0.2
	Ho	E33M61-227	122.6	8.3	19.9	-0.08
3HS bin 1	LS (ºC days)	E41M32-149	5.4	3.8	7.0	6
	Ratio SE/LS	E37M38-640	0.0	2.7 †	5.1	-0.009
	FNL	E33M58-534	3.6	3.5	6.9	0.1
3HS bin 5	HD (ºC days)	E38M55-320	46.6	3.5	2.2	6
4HL bin 6-7	FNL	E40M32-209	62.7	3.4	6.7	-0.1
	Ho	E38M54-063	66.4	4.2	8.7	-0.06
	FinalTill	E40M32-277	61.5	5.4	13.2	0.2
5HL bin 13-15	HD (ºC days)	E33M61-144	178.1	3.4	5.3	7
	Ho	E39M61-271	156.6	2.8	5.5	-0.04
	B (tillers / leaf)	E39M61-271	156.6	2.7	6.0	-0.10
7HS bin 4	LS (ºC days)	E37M38-291	52.3	7.9	16.8	10
	SE (ºC days)	E37M38-291	52.3	9.0	20.5	9
	HD (ºC days)	E37M38-291	52.3	26.0	46.6	23
	GF (ºC days)	E37M38-291	52.3	7.7	12.6	-8
	Phyllochron (ºC days/leaf)	E37M38-291	52.3	7.2	17.7	1.2

*Expressed in negative values

† No significant with rMQM

Table 3. Genes or markers screened for polymorphism between Henni and Meltan and in a subset of 16 DH-lines. Numbers between brackets designate bins in which each gene or marker is located. Where the gene or marker was polymorphic, number of recombinants between them and some of the most significant marker for durations of phases (found in the subset of DH-lines) were indicated.

Chrom.	Gene or marker tested	Polymorphic	Nº of recombinants	Most significant marker
1H	*HvFT3* (11.2)	No	-	
	WMC1E8 (14.2)	Yes	0	E42M32-178 (13-14)
2H	*Ppd-H1* SNP22 (4.2)	No	-	
	Ppd-H1 SNP48 (4.2)	No	-	
	Ppd-H1 (exons 2-3 and 6)	No	-	
	HvM36 (3.2)	No	-	
	GBM1523 (5.2)	No	-	
	Bmac132 (7.1)	No	-	
	EBmac640 (7.1)	Yes	6	E42M32-272 (2.2)
			8	E33M55-592 (11.2)
	scssr03381 (7.1)	No	-	
	GBM5230 (7.1)	No	-	
	GBM1309 (11.2)	Yes	2	E33M55-592 (11.2)
	EBmac415 (12.2)	Yes	1	E33M55-592 (11.2)
5H	*HvBM5A* (11.1)	Yes	6	E33M61-144 (15.2)
7H	*HvFT1* SNP1(1rst intron) (4.2)	No	-	
	HvFT1 SNP2 (1rst intron) (4.2)	No	-	
	HvFT1 SSR (2nd intron) (4.2)	Yes	1	E37M38-291 (4.2)
	GBM5060 (3.2)	No	-	

Figure

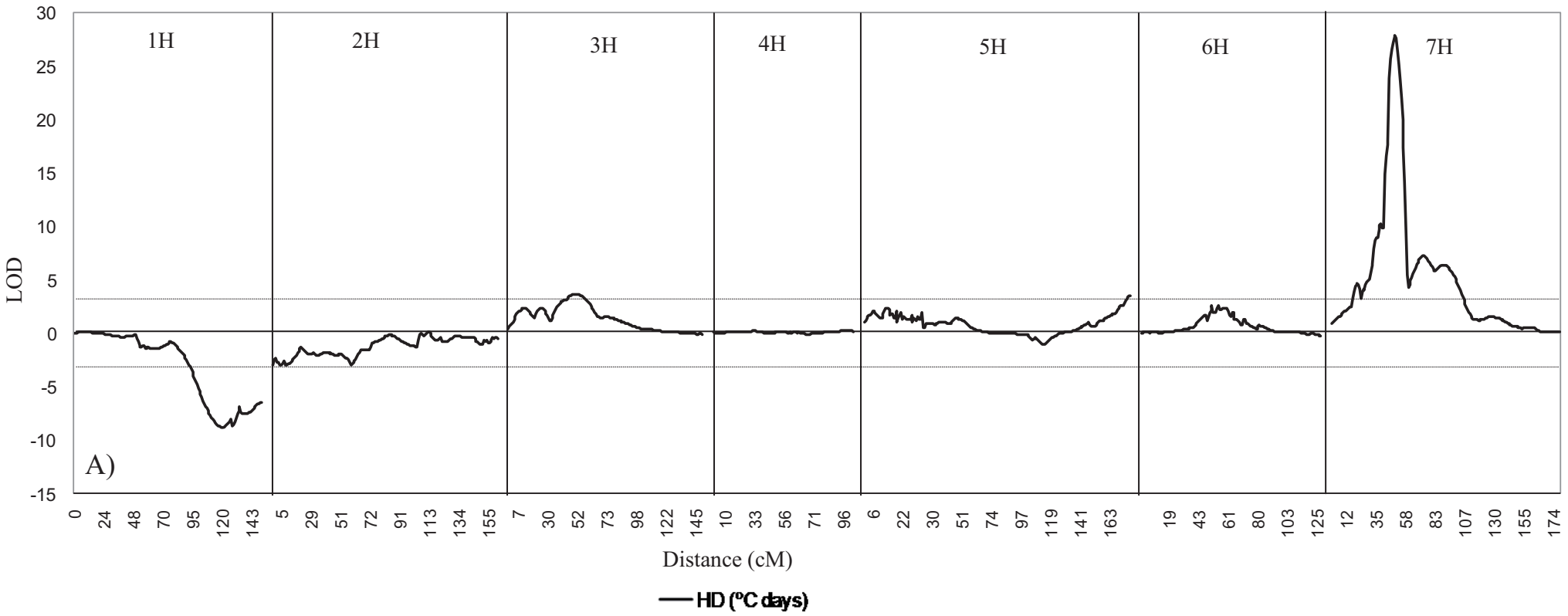


Figure 1. LOD profile from rMQM for A) Heading (HD), and B) leaf and spikelet initiation phase (LS), stem elongation phase (SE) and the ratio SE/LS (main effects from the four trials). Horizontal dashed lines indicate the significance LOD-threshold. Positive and negative LOD values mean that the positive additive effect comes from Henni or Meltan respectively.

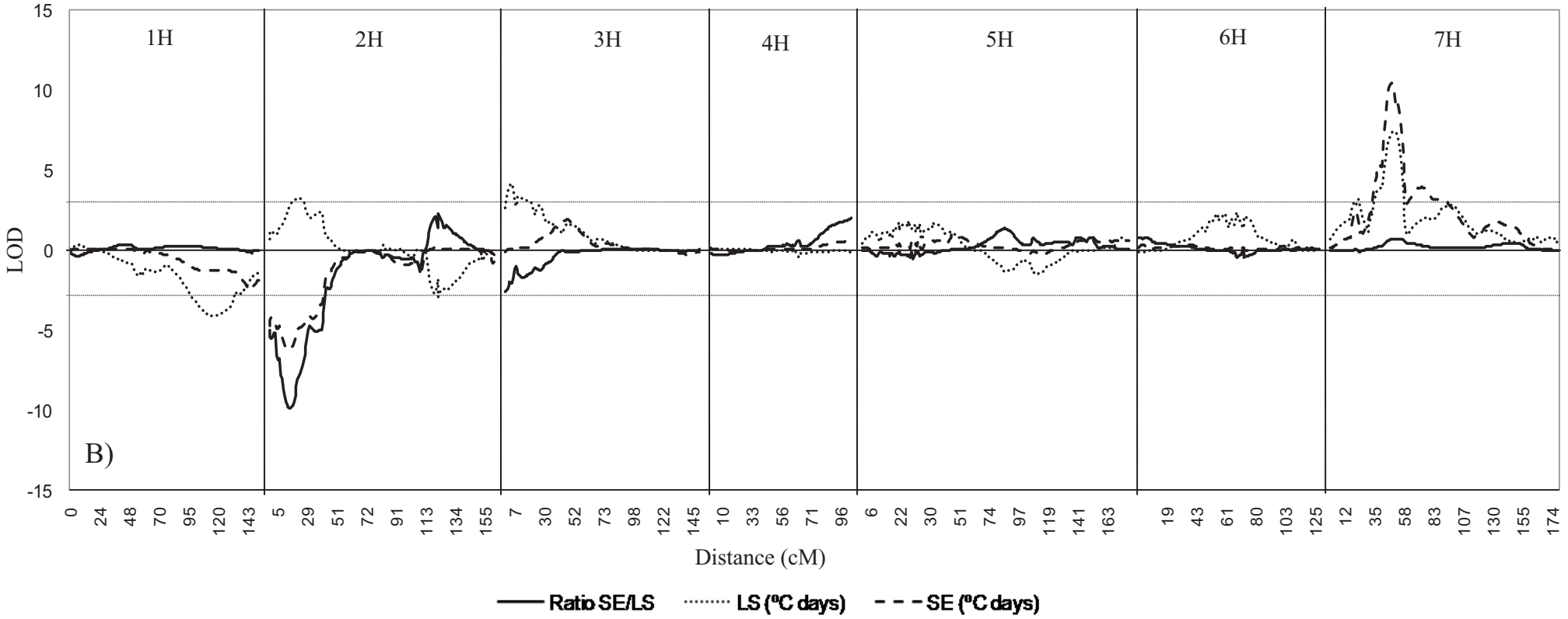
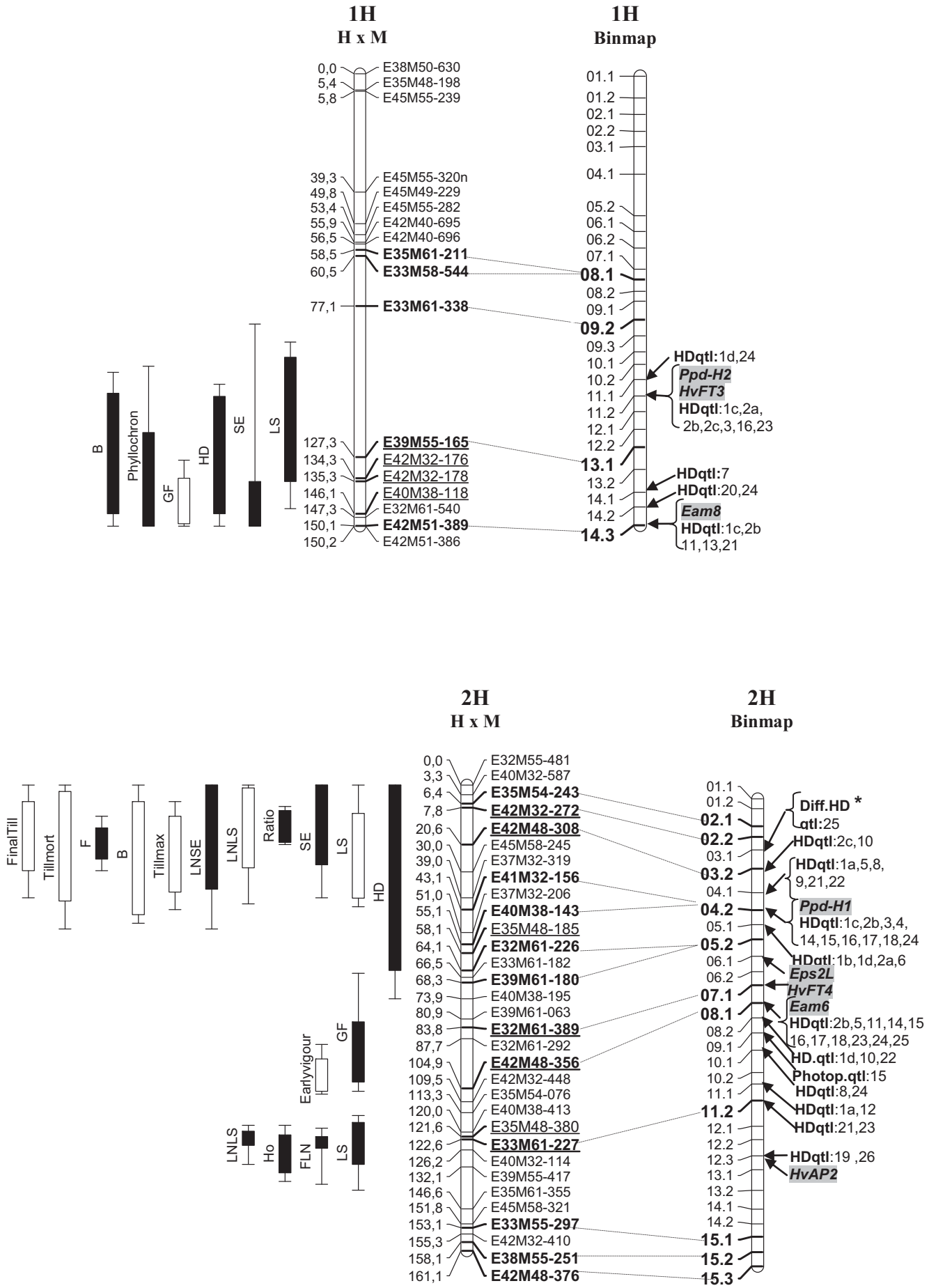


Figure 1. (continued)

Figure



* QTL for difference in heading time (GDD) between two extreme sowing dates

Figure 2. For each chromosome group, on the left, linkage map for the Henni x Meltan population and, on the right, binmap from Marcel et al. (2007) (Mapchart, Voorrips 2002). In the linkage map for Henni x Meltan: boxes indicate the 1-LOD interval and lines the 2-LOD interval (roughly the 90% and 95% confidence interval respectively) of the QTLs detected in the present study (white: positive additive effect from Henni; black: positive additive effect from Meltan.); underlined, most significant markers for the traits studied; in bold, markers that have correspondence with the binmap (lines join these markers with the sub-bin to which they correspond). Some markers are omitted in order to clear up the graphical representation (but distances from original map are kept).

In the binmap by Marcel et al. (2007): in each linkage group, on the left there are the sub-bins and on the right, positions (sub-bins) of the most known genes controlling responses to photoperiod, vernalization or earliness per se in barley; some homologues in barley of the most important genes controlling flowering time in *Arabidopsis* (in bold and grey; see text for references); and position of most significant markers of QTL for total time to heading (HDqtl) or for photoperiod response found in the literature (only those in the vicinity of QTLs for duration of phases in the present study). In some cases genes or marker positions could not be assigned with so much precision as sub-bins and therefore some positions are an approximation. Numbers designate populations in which the indicated QTL for heading or for photoperiod response were found: 1a, Steptoe x Morex (Hayes et al. 1993); 1b, Steptoe x Morex (Borem et al. 1999); 2a, Dicktoo x Morex (Pan et al. 1994); 3, Igri x Triumph (Laurie et al. 1995); 4, Igri x Danilo (Backes et al. 1995); 5, Tystofte Prentice x Vogelsanger Gold (Kjaer et al. 1995); 6, Blenheim x Kym (Bezant et al. 1996); 7, Harrington x TR306 (Tinker et al. 1996); 8, Blenheim x E22/3 (Powell et al. 1997); 9, Vada x L94 (Qi et al. 1998); 10, Krona x HOR1063 (Kicherer et al. 2000); 11, Harrington x Morex (Márquez-Cedillo et al. 2001); 12, Tadmor x Er/Apm (Teulat et al. 2001); 13, Oregon Wolfe Barley (Börner et al. 2002); 1c, Steptoe x Morex; 2b, Dicktoo x Morex; 14, Chebec x Harrington; 15, Alexis x Sloop; 16, Halcyon x Sloop; 17, Tallon x Kaputar; 18, Arapiles x Franklin (Boyd et al. 2003); 19, Arta x HS41-1 (Baum et al. 2003); 20, Apex x Prisma (Yin et al. 2005); 21, VB9524 x ND11231 (Emibiri and Moody 2006); 22, Brenda x HS584 (Li et al. 2006); 2c, Szucs et al., 2006; 1d, Rao et al., 2007; 23, Beka x Mogador (Cuesta-Marcos et al. 2008a); 24, small interconnected populations (Cuesta-Marcos et al. 2008b); 25, BCD47 x Baronesse (Castro et al. 2008); 26, Haruna Nujo x Galleon and Amagi Nijo x WI2585 (Chen et al. 2009).

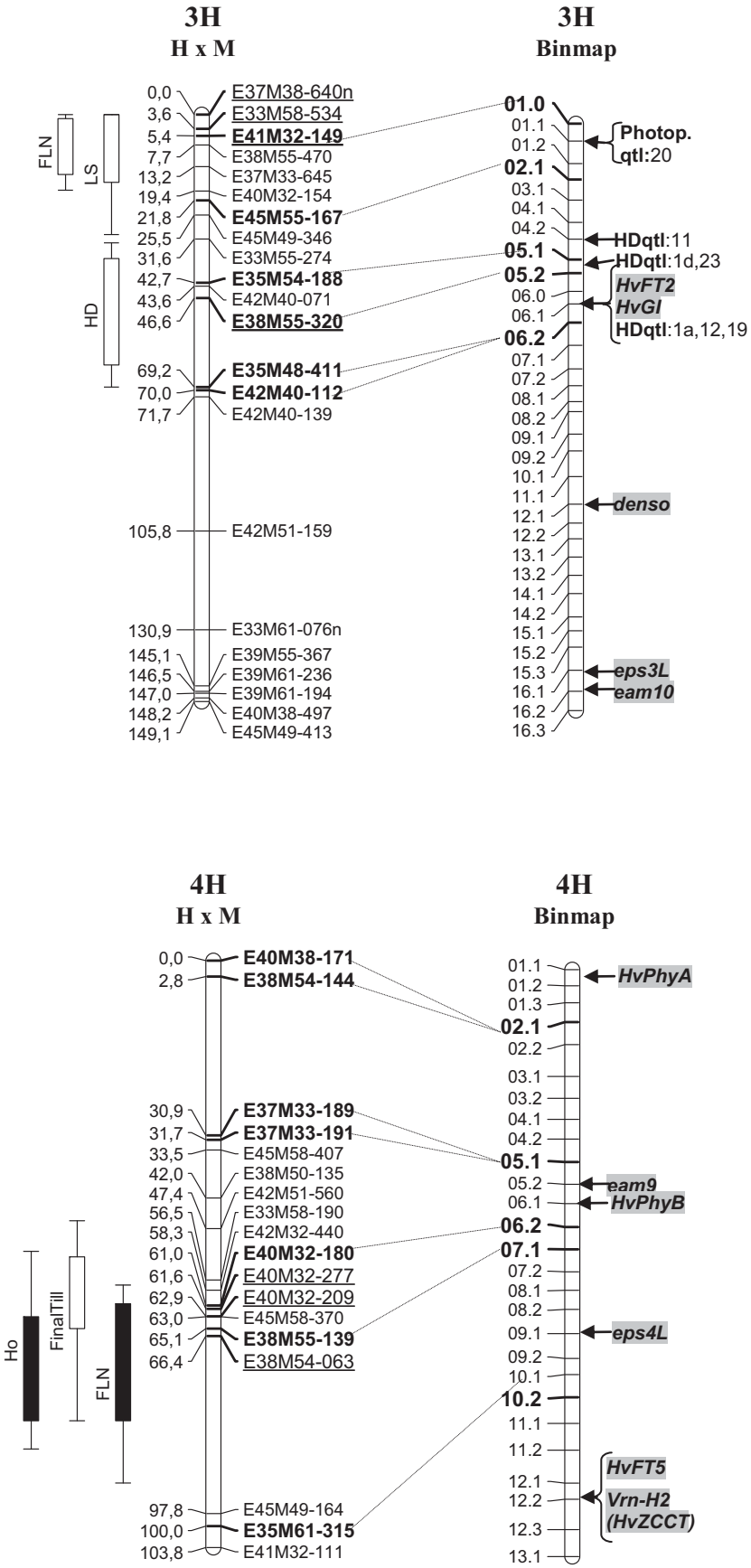


Figure 2. (Continued).

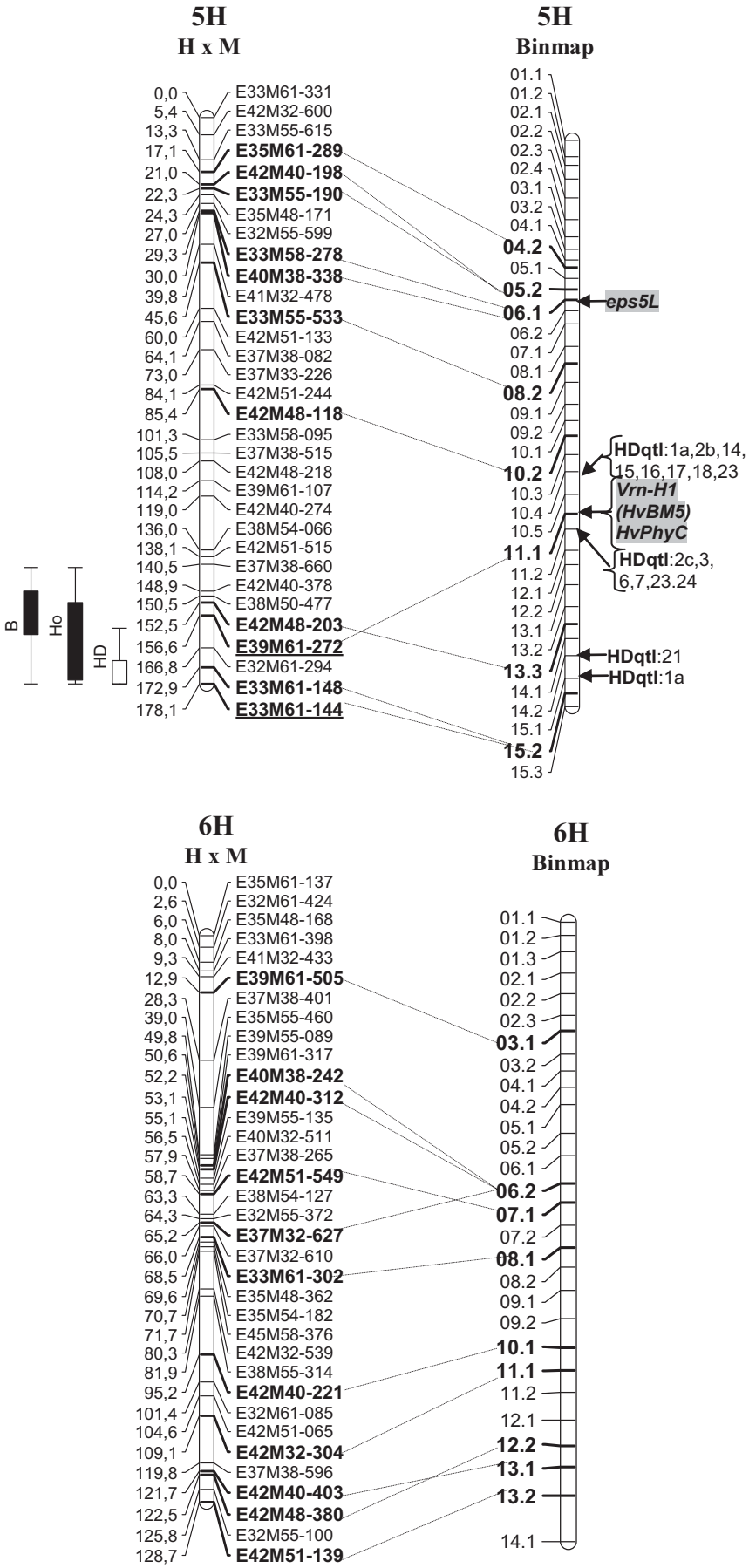


Figure 2. (Continued).

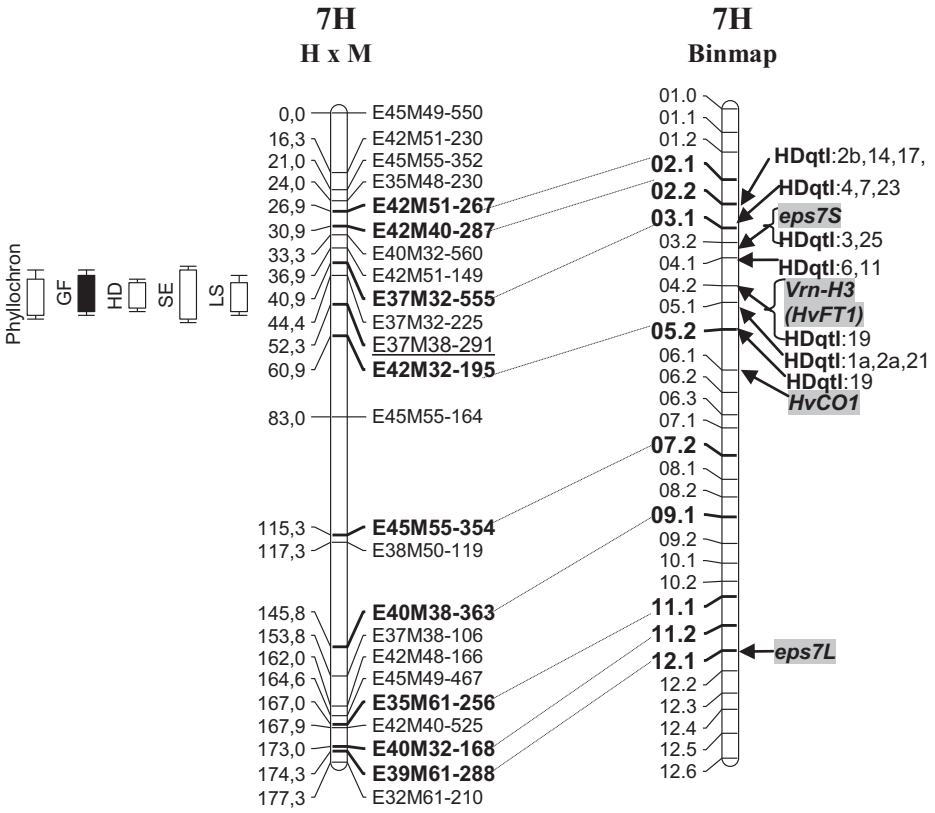


Figure 2. (Continued).