

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

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14

15

16 ABSTRACT

17

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

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

5  
6 Key words: barley, development, QTL, leaf appearance, tillering, double-haploids.

7  
8  
9 INTRODUCTION

10  
11 Crop phenology, which allows matching crop development with availability of  
12 resources (water, radiation, etc.), is the most important single factor influencing yield  
13 and crop adaptation to particular environments (Richards, 1991). This is especially  
14 relevant in Mediterranean conditions, where water is the main limiting factor and the  
15 occurrence of terminal drought and possible late spring frosts defines an optimal  
16 window for time to anthesis in order to maximise yield (Richards, 1991; Loss and  
17 Siddique, 1994; Cuesta-Marcos et al., 2009). Therefore keeping or achieving an  
18 optimised time to flowering is still an important goal in any breeding programme. In  
19 some Mediterranean environments, where intensive breeding has been carried out for  
20 centuries, either through farmer's selection or breeding programmes, there could be  
21 little scope for improving barley adaptability and yield by further adjustments in time to  
22 heading (Martiniello et al., 1987; Muñoz et al., 1998; Slafer et al., 2005). However,  
23 knowing the genetic control of different pre-anthesis phases may contribute to a better  
24 understanding of phenological traits conferring adaptability (e.g. Limin et al., 2007).

25  
26 Fine adjustment of phenology could be also important for yield improvement  
27 through increasing yield potential. Lengthening duration of the stem elongation phase  
28 has been associated to increases in the number of grains/m<sup>2</sup> (Slafer et al., 2001; 2005)  
29 which in turn could increase yield potential of small-grain cereals (Fischer, 2007;  
30 Miralles and Slafer, 2007; Fischer, 2008). This should be achieved without modifying  
31 total time to anthesis, whose optimisation as shown above, is an important objective  
32 providing adaptability in breeding programmes (Slafer, 2003). To attain this goal, a  
33 prerequisite would be that the phases before and after the onset of stem elongation

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

3  
4 Given the importance of time to anthesis, the genetic control of this trait has  
5 been the focus of many studies. Several genes or loci related to the response to  
6 photoperiod or vernalisation, or to earliness per se (the three main factors determining  
7 heading time, Slafer and Rawson, 1994) have been found on the seven chromosomes of  
8 barley. The most widely known genes related to photoperiod are: *Ppd-H1* on 2HS,  
9 expressed under long days (Laurie et al., 1994; Laurie et al., 1995) and recently  
10 identified as a *PRR*-like (Pseudo-Response Regulator) gene by positional cloning  
11 (Turner et al., 2005); and *Ppd-H2* on 1HL, expressed under short days (Laurie et al.,  
12 1995) and for which *HvFT3*, a *FT*-like (Flowering locus T) gene, could be a candidate  
13 gene (Faure et al., 2007). Other reported genes that determine differences in heading  
14 time under short photoperiodic conditions are: *Eam7* on 6HS (Stracke and Börner,  
15 1998), *Eam8* on 1HL (Franckowiak, 1997), *Eam9* on 4HL (Franckowiak, 1997;  
16 Lundqvist et al., 1997) and *Eam10* on 3HL (Börner et al. 2002). The three most known  
17 genes governing the response to vernalization are: *Vrn-H1* (*Sh2*, *HvVRN1* or *HvBM5A*)  
18 on 5HL and *Vrn-H2* (*Sh1*, *HvVRN2* or *HvZCCT*) on 4HL (Takahashi and Yasuda, 1971;  
19 Laurie et al., 1995) for which gene sequences have been also identified (Trevaskis et al.,  
20 2003; Yan et al., 2003, 2004), and *Vrn-H3* (*Sh3* or *HvFT1*) (Takahashi and Yasuda,  
21 1971) which has been recently mapped on 7HS and identified as an orthologue of the  
22 *Arabidopsis FT* gene (Yan et al., 2006; Faure et al., 2007). Some loci whose effect  
23 could not be related to photoperiod or vernalization response are considered *earliness*  
24 *per se* genes (a group much less studied): *eps2S* on 2HS (or *Eam6*), *eps3L* on 3HL,  
25 *eps4L* on 4HL, *eps5L* on 5HL, *eps6L.1* and *eps6L.2*, both on 6HL, *eps7S* on 7HS and  
26 *eps7L* on 7HL (Laurie et al., 1995). Other homologues of some of the most important  
27 genes controlling flowering time in *Arabidopsis* have been found in barley (*HvCO1* to  
28 *HvCO8*, Griffiths et al., 2003; *HvGI*, Dunford et al., 2005; *HvFT1* to *HvFT5*, Faure et  
29 al., 2007) although most of them do not correspond to any of the above genes and their  
30 effect on heading is unclear. Moreover other QTL for heading date, whose position do  
31 not seem to coincide with these genes or loci, have been found in other barley  
32 populations and their effect on the response to the three main factors exposed above is  
33 unknown (e.g. Hayes et al., 1993; Bezant et al., 1996; Tinker et al., 1996; Baum et al.,  
34 2003; Li et al., 2006). QTL for heading date usually have an effect on other important

1 agronomic characters (yield, height, resistance to diseases, quality traits, etc.) (e.g.  
2 Hayes et al., 1993; Bezzant et al., 1996; Tinker et al., 1996; Baum et al., 2003; Li et al.,  
3 2006) in accordance with the fact that heading date is a key trait for adaptability.

4  
5 Several authors have shown variability in the duration of pre-anthesis phases,  
6 comparing different sets of cultivars, and even variability in the late reproductive phase  
7 (SE) in varieties with similar time to anthesis, both in wheat (Halloran and Pennell,  
8 1982; Whitechurch et al., 2007) and barley (Appleyard et al., 1982; Kitchen and  
9 Rasmusson, 1983; Kernich et al., 1995; Kernich et al., 1997; Borràs et al., 2009).  
10 However we are not aware of any study in barley nor in wheat providing evidences of  
11 the genetic control of different pre-anthesis (sub)phases. The few studies comparing  
12 wheat substitution lines, single chromosome recombinant lines or near isogenic lines  
13 (differing in Ppd alleles) are inconclusive as differences in pre-anthesis phases lengths  
14 or in responses to photoperiod in each (sub)phase could not be attributed to particular  
15 major Ppd genes (see results and comparative review by González et al., 2005). Zhou et  
16 al. (2001), using a QTL approach to identify genetic controls of particular phenophases  
17 in rice, found some independent QTL for the duration of the vegetative and reproductive  
18 phases, either by different magnitude of QTL effects or by opposite allele effects on  
19 both phases. Whitechurch et al. (2007) identified variability in the stem elongation  
20 phase (SE) independent from the variability in the previous phases of leaf and spikelet  
21 initiation (LS) in a rather large set of cultivars, while Borràs et al. (2009) did not find  
22 major genetic correlations between both phases (with large genotypic effects and  
23 heritabilities for both traits) in the Henni x Meltan barley DH-population. Both results  
24 would lead to the suggestion that LS and SE could be under different genetic control  
25 also in wheat and barley. However it would be necessary to identify the particular  
26 genetic factors responsible for the genetic variability and for this lack of correlation, so  
27 as to explore avenues for manipulating LS and SE without modifying total time to  
28 heading. Thus, and following the work by Borràs et al. (2009), the first objective of the  
29 present study was identifying main QTL for the LS and SE phases in the Henni x  
30 Meltan population and comparing them with other genes or loci for developmental time  
31 (heading time) reported in the literature.

32  
33 In addition, leaf appearance and tillering are important processes that determine  
34 the crop canopy structure mainly during phases before the onset of jointing. Although

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

## 14 15 16 MATERIALS AND METHODS

### 17 18 *Population and trials*

19  
20 A population of 118 doubled haploid (DH) lines from the cross Henni x Meltan and  
21 both parents were studied in four field trials, two locations by two growing seasons.  
22 Henni and Meltan are two-rowed spring barley cultivars from Northern Europe, released  
23 in 1995 and 1991 respectively and mainly used for feed. Details on the development of  
24 the DH-lines are given in Kraakman (2005). Although there could be a narrow genetic  
25 base in terms of phenology, the main advantage of this population is that it represents an  
26 actual breeding program, as both parents are modern cultivars within the elite European  
27 germplasm, in contrast to other studies that use populations derived from parents with  
28 phenology patterns extremely different but without a likely application in a realistic  
29 breeding program.

30  
31 The two locations were Gimenells (41°37'N, 0°22'E, 248m) and Foradada (41°51'N,  
32 1°0'E, 407m), both in the province of Lleida (Catalonia, North-Eastern Spain).  
33 Gimenells is situated in the middle of an irrigated basin, while Foradada is rainfed. The

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

10

### 11 *Phenotyping*

12

13 Considering their relative importance in the context of the present study, the phases  
14 studied were: i) from sowing to the onset of jointing (about stage 30 in the scale of  
15 Zadoks et al., 1974), which coincides with the end of spikelet initiation (Kirby et al.,  
16 1994), namely the leaf and spikelet initiation phase (LS); ii) the stem elongation phase  
17 (SE), from the onset of jointing to heading (Zadoks' stage 55); iii) total time to heading  
18 (HD); and iv) the grain filling period (GF, from heading to physiological maturity  
19 (Zadoks' stage 92). In order to test more objectively differences in the genetic control of  
20 SE and LS that produced substantial changes in the partitioning of total time to heading  
21 (and independently of this), we also studied the SE/LS ratio. Duration of the phases was  
22 assessed in thermal time (°C d, using a base temperature of 0°C).

23

24 To study dynamics of leaf and tiller appearance, 2 plants per plot (all in all 600  
25 plants per trial) were tagged in the two trials in 2004. Number of emerged leaves,  
26 following the Haun scale (Haun, 1973), and number of live tillers in each plant were  
27 recorded weekly from the stage of 2-3 leaves until flag leaf for leaves, and for tillers  
28 until its number stabilised. Phyllochron (°C d per leaf) was estimated for each plant as  
29 the reciprocal of the regression coefficient of the relationship between the number of  
30 emerged leaves (Haun stages) and thermal time. Number of leaves appeared during LS  
31 (LN<sub>LS</sub>) was estimated from the equation for rate of leaf appearance and the duration of

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

3 Tillering dynamics was studied as the relationship between number of live tillers  
4 and the predicted number of leaves at which they appeared (from the equation of rate of  
5 leaf appearance), in order to identify variability in tillering traits independent of that in  
6 phyllochron, which is known to have important effects on the tillering capacity (Kirby  
7 et al., 1985). Some traits were estimated directly from observed data: Haun stage at the  
8 onset of tillering ( $H_0$ ), Haun at which maximum number of tillers is produced ( $H_{max}$ ),  
9 maximum number of tillers appeared (Tillmax), final number of tillers at harvest  
10 (FinalTill) and tiller mortality (Tillmort). The rest of traits were parameters derived  
11 from a lineal model (with three pieces and two knots) which is described in detail in  
12 Borràs et al. (2009). Briefly, in the first section of the model number of tillers increased  
13 rapidly following a linear trend. The slope (B) was an estimation of the rate of tillering.  
14 Then, at the first breakpoint (C) tiller production stopped and number of tillers  
15 stabilised or continued with a considerably slower rate (D) for some time. Thus, C  
16 might be considered as the timing of tillering cessation; the ‘departure point’ in Kirby et  
17 al. (1985). Finally, at the second breakpoint (E) tillers started to die rapidly until number  
18 of tillers stabilised (data after the end of tiller mortality were removed for fitting the  
19 model). F represents the rate of tiller mortality. Table 1 summarizes all traits and  
20 abbreviations used to designate them in the text.

21

## 22 *Statistical analyses*

23

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



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

3  
4 QTL analyses were carried out using a linkage map with a total of 269  
5 polymorphic markers (AFLPs) (Kraakman, 2005). The final map was 1056 cM long  
6 (Kosambi function) and had chromosomes lengths between 104 cM (chr. 4H) and 178  
7 cM (5H). QTL analyses were performed using the restricted MQM mapping procedure  
8 (MAPQTL 5.0; van Ooijen, 2004). After initial interval mapping, the markers with the  
9 highest LOD values ('peak markers') were taken as co-factors. When new significant  
10 LOD peaks appeared, new peak markers were added to the co-factor set until a stable  
11 LOD profile was reached (Jansen, 1993). A LOD significance threshold of 2.9 (rounded  
12 upwards in the conservative direction) was chosen after permutation tests for each trait  
13 with a significance level of  $p < 0.05$ , which was in agreement with thresholds estimated  
14 following van Ooijen (1999).

15  
16 Epistasis and QTL x E interactions for the main detected QTL were studied  
17 using linear mixed models, which were performed on BLUEs estimated from individual  
18 trials. Each model included as fixed factors the environment, the significant markers for  
19 each trait (closest markers to the significant LOD-peaks chosen from previous QTL  
20 analysis on main effects) and all 2-level interactions, while the random factor was the  
21 remaining genotypic variance. Significant markers were added to the model ordered by  
22 their LOD significance (from the highest to the lowest). All linear mixed models were  
23 performed with Genstat (Payne 2006).

#### 24 25 *Candidate genes*

26  
27 As some of the QTLs were found not far from well known characterized genes,  
28 we analysed the genetic constitution of both parents and a subset of 16 double haploid  
29 lines with differential phenotypic and genetic constitution for the flanking markers of  
30 key QTL. Polymorphisms within vernalization and photoperiod response loci were  
31 screened with allele-specific primers of candidate genes, i.e. *HvBM5A* for *Vrn-H1* (Yan  
32 et al., 2003), *ZCCT-H* for *Vrn-H2* (Karsai et al., 2005), *HvFT1* for *Vrn-H3* (Yan et al.,  
33 2006; Faure et al., 2007), *HvPRR7* for *Ppd-H1* (Turner et al., 2005; Jones et al., 2008)  
34 and *HvFT3* for *Ppd-H2* (Faure et al., 2007). *Vrn-H1* was tested as size of the first intron



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

17

18

## 19 RESULTS

20

### 21 *QTLs for the duration of developmental phases*

22

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

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

3  
4 On the contrary the QTL on the short arm of 2H was highly significant and the  
5 most important for the SE/LS ratio while it had a small effect on total time to heading  
6 (Figure 1). Actually the position of this QTL for HD is unclear as shown by the large  
7 confidence interval (Figure 2). This was probably due to the opposite allele effects that  
8 this QTL had on LS and SE. The positive effect for LS came from Henni while for SE  
9 from Meltan. It was more significant and consistently found for SE than for LS, so the  
10 resulting positive allele effect for HD came also from Meltan.

11  
12 Other two QTLs, one on 2HL and another on the distal part of 3HS were  
13 significant for LS but not for SE main effects. Both QTLs were about the significance  
14 threshold for the ratio SE/LS (Figure 1). A QTL on 3HS was also found to be  
15 significant for SE in one environment (data not shown) and a LOD peak is also detected  
16 for main effects (Figure 1), but it seems a different QTL, as the LOD peak mapped quite  
17 apart (>30 cM) from the QTL for LS, and the confidence interval of the QTL for LS did  
18 not coincide with those for SE and HD (which do overlap) (Figure 2).

19  
20 The two most significant QTL for GF were also the two most significant for HD  
21 (QTL on 7HS and on 1HL) but with an allele effect opposite to the QTL for LS, SE and  
22 HD. The QTL on 2H for GF does not seem to coincide with the QTL for previous  
23 phases (Figures 1 and 2, and Table 2). Another minor QTL for HD was found distal on  
24 5HL.

25  
26 No epistatic interactions were found between the QTL significant for each  
27 phase. On the other hand the only significant QTL x E interaction was detected for the  
28 QTL on 7HS ( $p < 0.001$ ) for SE and HD (not for LS neither for GF). This interaction was  
29 quantitative (different allelic sensitivity between environments). Significance, additive  
30 effect and percentage of variability explained for this QTL was almost identical in 3 of  
31 the 4 environments (for both SE and HD). Only in Foradada 2005/06 the effect on both  
32 SE and HD was about half the effect in the other environments (data not shown). Thus,  
33 and considering also the little overall magnitude of the GxE compared to main

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

### 3 4 5 *Candidate genes*

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

18  
19 No polymorphism was either detected for Bmac132, scssr03381 or GBM5230,  
20 all three closely linked to *Eam6*. EBmac640 (also linked to *Eam6*) was polymorphic,  
21 but a number of recombinants were found between this marker and the most significant  
22 markers for durations of phases on 2HS and 2HL among the DHLs genotyped. There  
23 was also polymorphism for the microsatellite WMC1E8, very close to *Eam8* (and no  
24 recombinants in the subset of DH-lines were found between WMC1E8 and the most  
25 significant marker for HD in 1HL).

26  
27 There was a deletion in the first intron of *HvBM5A* (*Vrn-H1*) in both parents, and  
28 a polymorphism was found between them, although several recombinants were found in  
29 the subset of DH-lines between *HvBM5A* and the most significant marker for HD on  
30 5HL. As expected, given that both parents are spring types, both carried the recessive  
31 allele of *Vrn-H2*, insensitive to vernalization. Finally the highest QTL for LS, SE and  
32 HD on 7HS lied in the vicinity of *HvFT1* and *eps7S*. Both parents carried the same  
33 diagnostic SNPs in the first intron of *HvFT1*, but they can be distinguished by a  
34 microsatellite in the second intron of this gene.

## 1 *QTLs for leaf appearance and tillering parameters*

2  
3       Phyllochron and number of leaves (FLN) were controlled by different QTL and  
4 the most important coincided with QTL for duration of phenological phases. The most  
5 significant QTL for phyllochron was also that for phenological phases on 7HS and the  
6 second significant QTL was found in the same position than the QTL for phases distal  
7 on 1HL. The direction of allele effects were the same than for the duration of phases.  
8 On the other hand for FLN the most important QTL coincided with the QTL for LS on  
9 2HL, with less effect from the other two significant QTL on 3HS and on 4HL. The QTL  
10 on 2HS was also significant for  $LN_{LS}$  and  $LN_{SE}$  but with an opposite allelic effect as  
11 their respective phases. For early vigour the only significant QTL was also detected on  
12 2HL, but its confidence interval did not overlap with the QTL for LS, FLN and  $LN_{LS}$   
13 (Figures 1 and 2, and Table 2).

14  
15       The most significant QTL for tillering parameters coincided with QTL for  
16 number of leaves (QTL on 2HS, 2HL and 4HL), although with differences between  
17 traits (Figure 2 and Table 2). The QTL on 7HS, significant for phases and phyllochron,  
18 had no effect on any trait related to tillering. The QTL on 2HS significant for LS, SE,  
19 HD,  $LN_{LS}$  and  $LN_{SE}$  was also significant for Tillmax, Tillmort, B, F and FinalTill. The  
20 sign of the additive effect for all these traits related to tillering was the same than for LS  
21 and  $LN_{LS}$  (positive effect from Henni) except for F, which is expressed in negative  
22 values. The QTL on 2HL, significant for LS, FLN and  $LN_{LS}$ , was also detected for Ho  
23 and interestingly with the same allele effect (for all these traits the positive effect came  
24 from Meltan). The QTL on 4HL, which was only significant for FLN, was the most  
25 significant QTL for FinalTill, and the second most significant QTL for Ho (but with  
26 opposite allele effects between both traits). The QTL on 1HL for phases was also  
27 significant for B. Finally a slightly significant QTL on 5HL was detected for Ho and B,  
28 which was also significant for Tillmax, FinalTill and early vigour, although for these  
29 last three traits it was significant in only one environment (data not shown). No QTL  
30 were found for the other tillering parameters (Hmax, C, D and E), which showed very  
31 low heritability (Borràs et al., 2009).

32  
33       No significant epistasis was found for any of the traits related to leaf appearance  
34 and tillering. However, some QTL interacted significantly with the environment: for

1 instance the QTL on 7HS for FLN ( $p=0.004$ ), which was only slightly significant in 1 of  
2 2 environments but not for main effects (data not shown); the QTL for FinalTill on 2HS  
3 (only slightly significant for main effects and in 1 out of the 4 environments of the  
4 study), and on 4HL, which was highly significant with similar effects in all  
5 environments except Gimenells 2003/04 ( $p=0.02$  and  $p<0.001$  for QTLx $E$  on 2HS and  
6 4HL respectively); and the QTL on 4HL for Ho ( $p=0.002$ ; significant in Foradada  
7 2003/04 but not in Gimenells 2003/04). In all cases the interactions were non-crossover  
8 or quantitative.

## 12 DISCUSSION

### 14 *Genetic control for duration of phenological phases*

16 Although both Henni and Meltan are modern spring cultivars from Northern  
17 Europe, with an expected narrow genetic basis for phenological traits, considerable  
18 genetic variability, transgressive segregation and high heritabilities were found for  
19 duration of phases and other traits related to leaf and tiller appearance (Borràs et al.,  
20 2009). Despite the most significant QTL on 7HS for HD was also the most significant  
21 for LS and SE, there were differences in QTL effects between both (sub)phases, in  
22 agreement with the high genetic correlation between HD and both LS and SE but with  
23 lack of major correlation between the duration of these two component phases (Borràs  
24 et al., 2009). This could be explained (i) mainly by the most significant QTL for the  
25 ratio SE/LS, that on 2HS, with opposite effects on both phases and with a greater  
26 significance on SE than on LS, and (ii) to a lesser extent, by differences in the effect of  
27 minor QTLs on LS and SE (i.e. QTLs on 2HL and 3HS). The effect from other minor  
28 QTL would be supported by the fact that Henni had a longer SE and higher ratio SE/LS  
29 than Meltan (Borràs et al., 2009). Thus, in line with the lack of major genetic correlation  
30 between LS and SE and given the differences in QTL effects on both phases found in  
31 the present study, it can be concluded that LS and SE are, at least partially, under  
32 different genetic control in the Henni x Meltan DH-population. These results were in  
33 agreement with Zhou et al. (2001) in rice, who found that the vegetative and  
34 reproductive phases were independent in terms of QTLs effects, either by different

1 magnitude of QTLs effects or also by opposite effects on both phases, and in line with  
2 other works supporting the hypothesis of an independent genetic control (Appleyard et  
3 al., 1982; Kitchen and Rasmusson, 1983; Slafer and Rawson, 1994; Kernich et al.,  
4 1995; Kernich et al., 1997; Whitechurch et al., 2007). Thus, it would be possible  
5 modifying the duration of different pre-heading phases without modifying total time to  
6 heading.

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

22  
23 The two most important QTL for HD (on 7HS and 1HL) were also the most  
24 significant for GF but with opposite allele effects in agreement with the strong negative  
25 genetic correlation between both traits (Borràs et al., 2009). These results are common  
26 under Mediterranean conditions, in which barley crops are usually exposed to high  
27 temperatures and dry conditions during grain filling, and might be due (as other studies  
28 reporting negative correlations between HD and GF) to environmental effects given that  
29 later heading exposes GF to harsher conditions (Richards, 1991; Loss and Siddique,  
30 1994; Cuesta-Marcos et al., 2009), so they are much less relevant in the context of the  
31 present study.

32  
33  
34

## 1 *Candidate genes*

2

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

26

27       The QTL on 7HS lies in bin 4, very close either to *eps7S* (Laurie et al., 1995; bin  
28 3.2) or to *HvFT1* (Yan et al., 2006; Faure et al., 2007, in bin 4.1). Although it is not  
29 clear the causal polymorphism in *HvFT1*, candidate gene for *Vrn-H3* (Yan et al., 2006),  
30 there was no polymorphism for the diagnostic SNPs proposed by Yan et al. (2006).  
31 Another possibility is that our QTL could correspond to *eps7S*, which could be a  
32 different gene than *HvFT1* since either Igri or Triumph (Laurie et al., 1995) carry the  
33 same recessive allele *vrn-H3* (Yan et al., 2006), as the parents used in the present study.



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

3  
4 The most significant markers on 1HL were situated in bins 13-14, although the  
5 confidence interval for most traits spans over a wider region (probably due to the large  
6 gap between 77 and 127 cM). There was no polymorphism for *HvFT3*, the candidate  
7 gene for *Ppd-H2* (bin 11.2), but we found that WMC1E8 (*Eam8*, bin 14.3) was  
8 polymorphic and no recombinants were found in the subset of 16 lines between  
9 WMC1E8 and the most significant marker for HD (E42M32-272). Moreover in a DArT  
10 map for Henni x Meltan no polymorphic marker was found either in that region  
11 between bins 10-12, around *Ppd-H2* (data not published). Therefore our QTL on 1HL  
12 could correspond to *Eam8* rather than *Ppd-H2*. While QTL reported in the same region  
13 than *Ppd-H2* (bin 11.2) are found only under short photoperiod conditions, QTL  
14 reported at a position closer to *Eam8* (bin 14) are reported either under short or long  
15 photoperiod conditions (see references in Figure 2).

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

### 28 29 *Genetic control of traits related to leaf and tiller appearance*

30  
31 The most important QTL for leaf number and phyllochron coincided with QTL  
32 for phases and with the same direction of allele effects than those, which could be  
33 expected as both traits largely determine total time to heading (García del Moral et al.,  
34 2002). However both traits were controlled mainly by different QTLs. While

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

12

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

25

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

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

## 7 8 *Conclusions*

9  
10 Summarizing, the main conclusions that arise from the present study are: i) LS  
11 and SE are, at least partially, under different genetic control in the Henni x Meltan  
12 population, mainly due to a QTL on 2HS which had different effects (both in the  
13 direction of allele effects and in the magnitude) between both phases; ii) the QTLs  
14 responsible for a different genetic control of LS and SE do not seem to correspond to  
15 major genes or QTLs reported in the literature; iii) shortening LS so as to lengthen SE  
16 without modifying HD would not imply a negative drawback on traits that could be  
17 important for early vigour, as phyllochron and the onset of tillering. Given that the  
18 range of variability in the Henni x Meltan population was quite limited, it would be  
19 interesting to explore other populations in order to identify QTLs with potentially larger  
20 effects. Further studies (combining genetics and physiology) are also required to  
21 understand better the way (photoperiod sensitivity during SE, e.g. Miralles and  
22 Richards, 2000; or differences in earliness per se for this phase, e.g. Slafer, 1996) in  
23 which QTLs can promote substantial differences on lengths of pre-anthesis phases.

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

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Abbreviation	Trait
LS	Leaf and spikelet initiation phase (°C d)
SE	Stem elongation phase (°C d)
HD	Total duration from sowing to heading (°C d)
GF	Grain filling period (°C d)
LN <sub>LS</sub>	Number of leaves appeared during the leaf and spikelet initiation phase
LN <sub>SE</sub>	Number of leaves appeared during the stem elongation phase
FLN	Final leaf number
-	Phyllochron; inverse of the rate of leaf appearance (°C 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

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

Chromosomal region	Trait (main effects)	Marker	Position (cM)	LOD	% Expl.	Add.Eff.
1HL bin 13-14	LS (°C days)	E39M55-165	127.3	3.7	7.2	-6
	SE (°C days)	E40M38-118	146.1	2.6 †	4.9	-4
	HD (°C days)	E42M32-178	135.3	8.1	11.1	-11
	GF (°C days)	E40M38-118	146.1	5.6	8.5	7
	Phyllochron (°C 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 (°C days)	E42M48-308	20.6	3.4	6.6	6
	SE (°C days)	E42M32-272	7.8	5.4	11.9	-7
	Ratio SE/LS	E42M32-272	7.8	8.1	23.8	-0.019
	HD (°C days)	E42M32-272	7.8	3.0	4.1	-6
		E35M48-185	58.1(bin 4)	3.0	5.0	-6
	LN <sub>LS</sub>	E42M48-308	20.6	3.0	8.6	0.1
	LN <sub>SE</sub>	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 (°C 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 (°C days)	E33M55-592	121.8	2.9	5.1	-5
	Ratio SE/LS	E33M55-592	121.8	2.3 †	7.4	0.011
	LN <sub>LS</sub>	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 (°C 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 (°C 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 (°C 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 (°C days)	E37M38-291	52.3	7.9	16.8	10
	SE (°C days)	E37M38-291	52.3	9.0	20.5	9
	HD (°C days)	E37M38-291	52.3	26.0	46.6	23
	GF (°C days)	E37M38-291	52.3	7.7	12.6	-8
	Phyllochron (°C days/leaf)	E37M38-291	52.3	7.2	17.7	1.2

\*Expressed in negative values

† No significant with rMQM

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

Chrom.	Gene or marker tested	Polymorphic	N° of recombinants	Most significant marker
1H	<i>HvFT3</i> (11.2)	No	-	
	WMC1E8 (14.2)	Yes	0	E42M32-178 (13-14)
2H	<i>Ppd-H1</i> SNP22 (4.2)	No	-	
	<i>Ppd-H1</i> SNP48 (4.2)	No	-	
	<i>Ppd-H1</i> (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	<i>HvBM5A</i> (11.1)	Yes	6	E33M61-144 (15.2)
7H	<i>HvFT1</i> SNP1(1rst intron) (4.2)	No	-	
	<i>HvFT1</i> SNP2 (1rst intron) (4.2)	No	-	
	<i>HvFT1</i> SSR (2nd intron) (4.2)	Yes	1	E37M38-291 (4.2)
	GBM5060 (3.2)	No	-	

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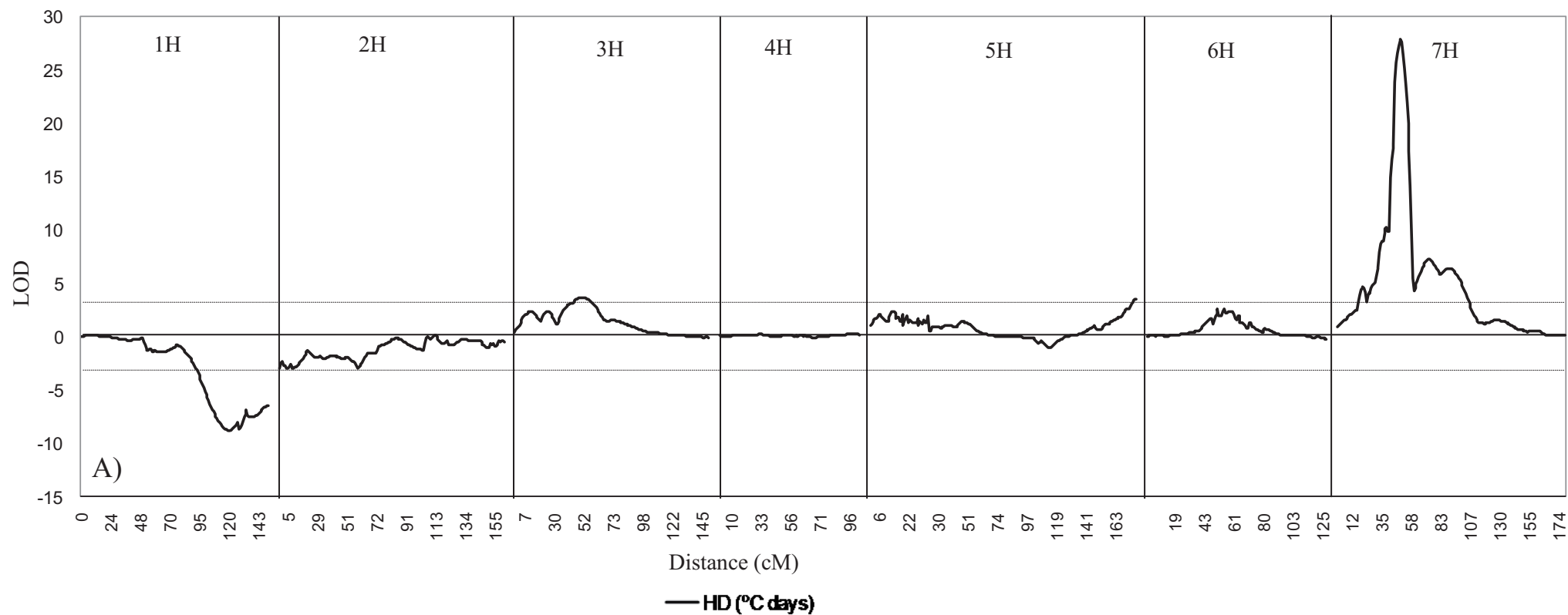
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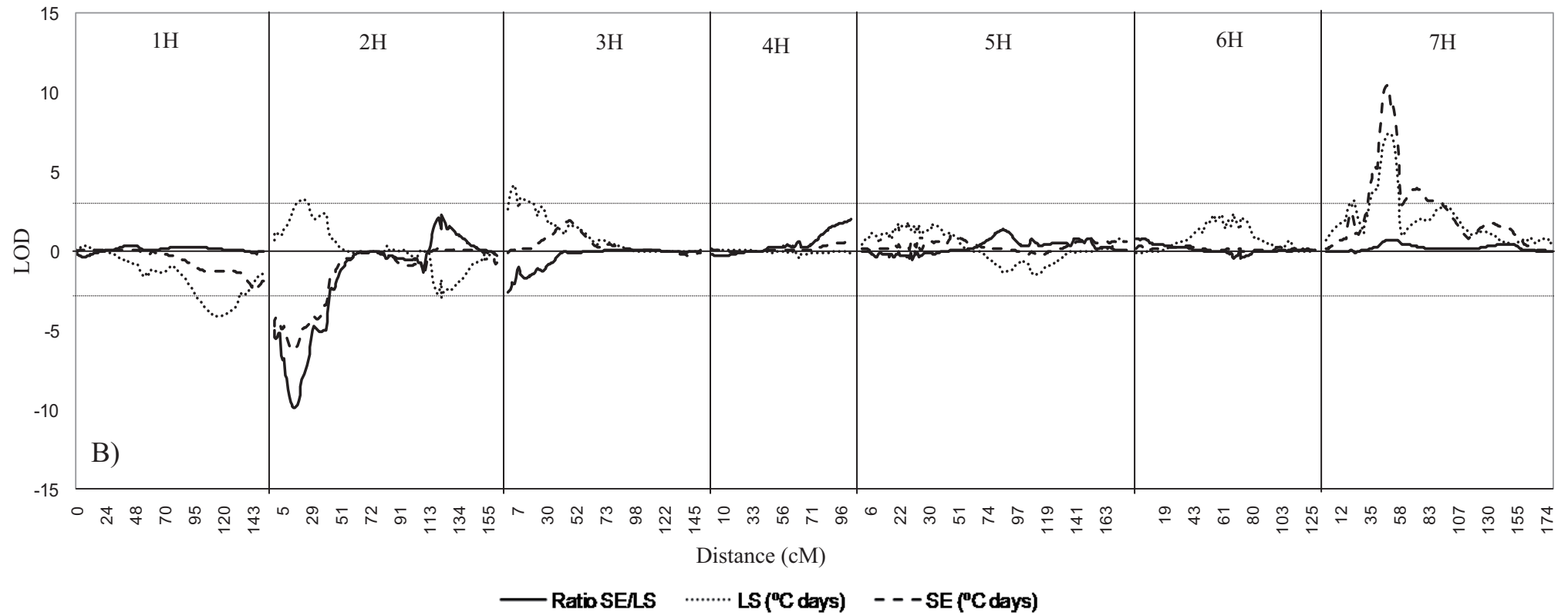


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5 **Figure 1. LOD profile from rMQM for A) Heading (HD), and B) leaf and spikelet initiation phase (LS), stem elongation phase (SE) and the ratio**  
 6 **SE/LS (main effects from the four trials). Horizontal dashed lines indicate the significance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

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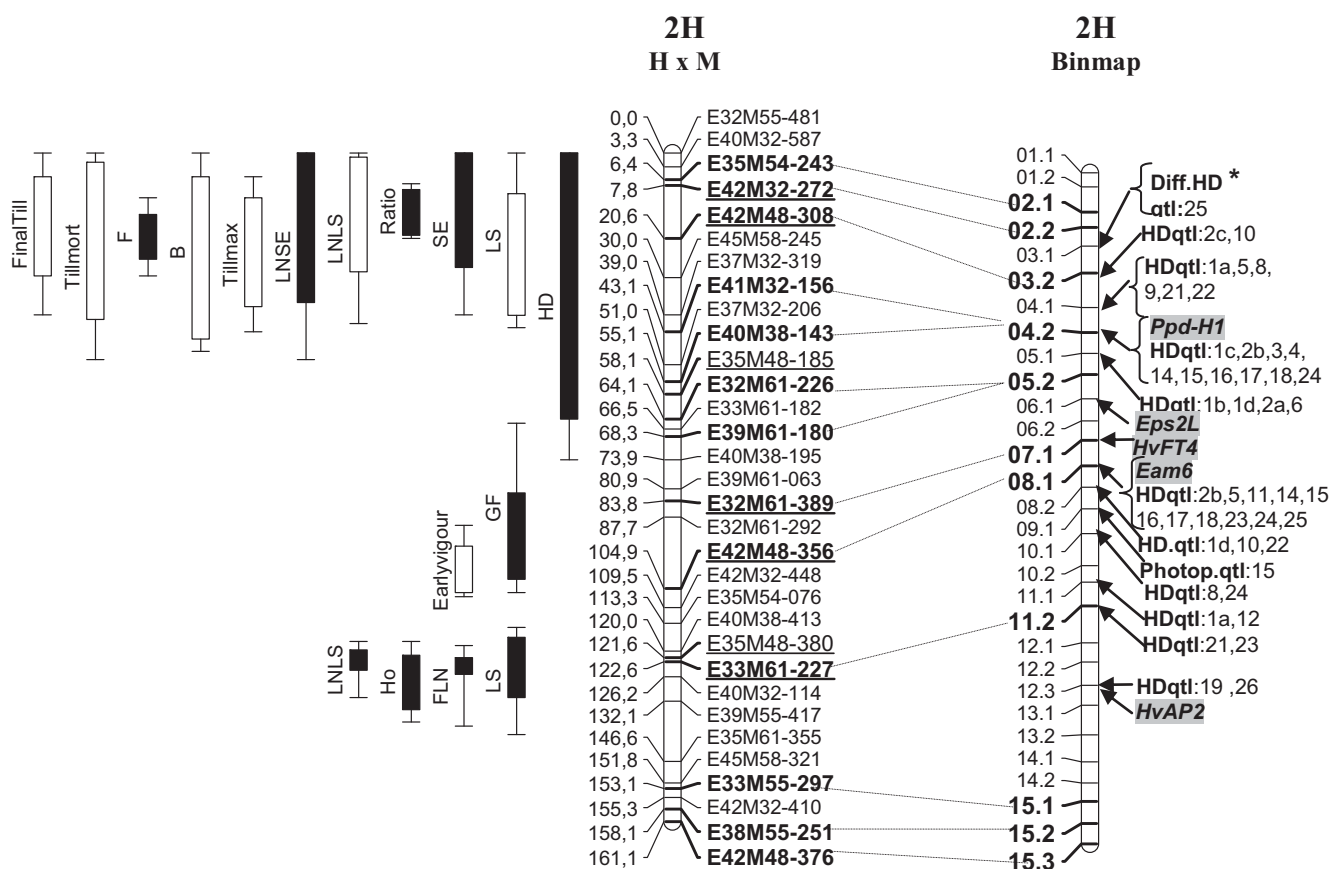
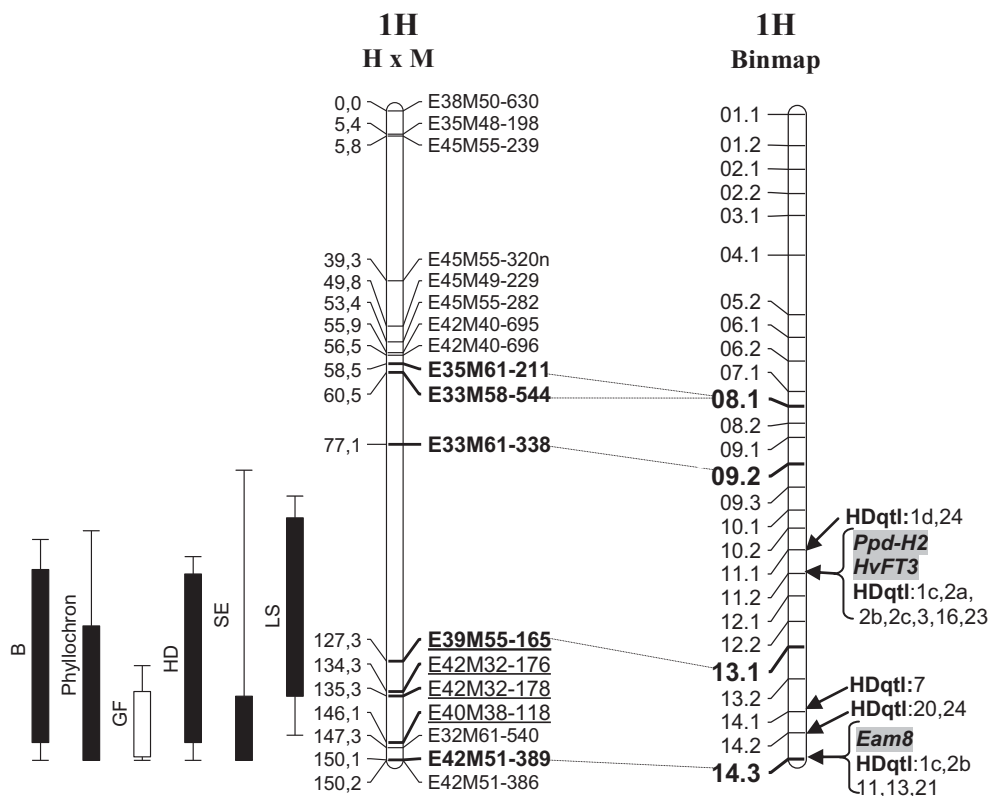
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3 **Figure 1. (continued)**

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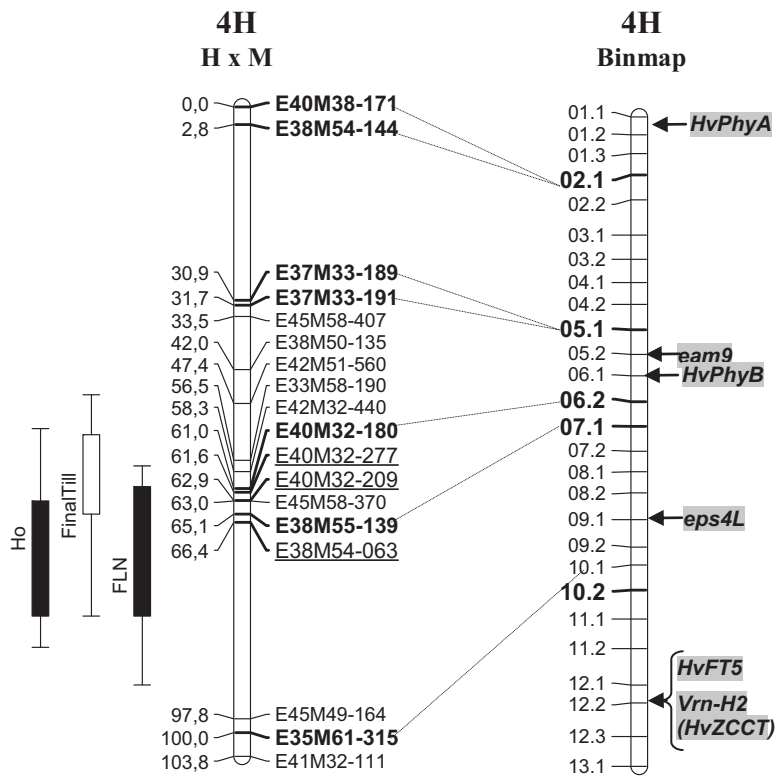
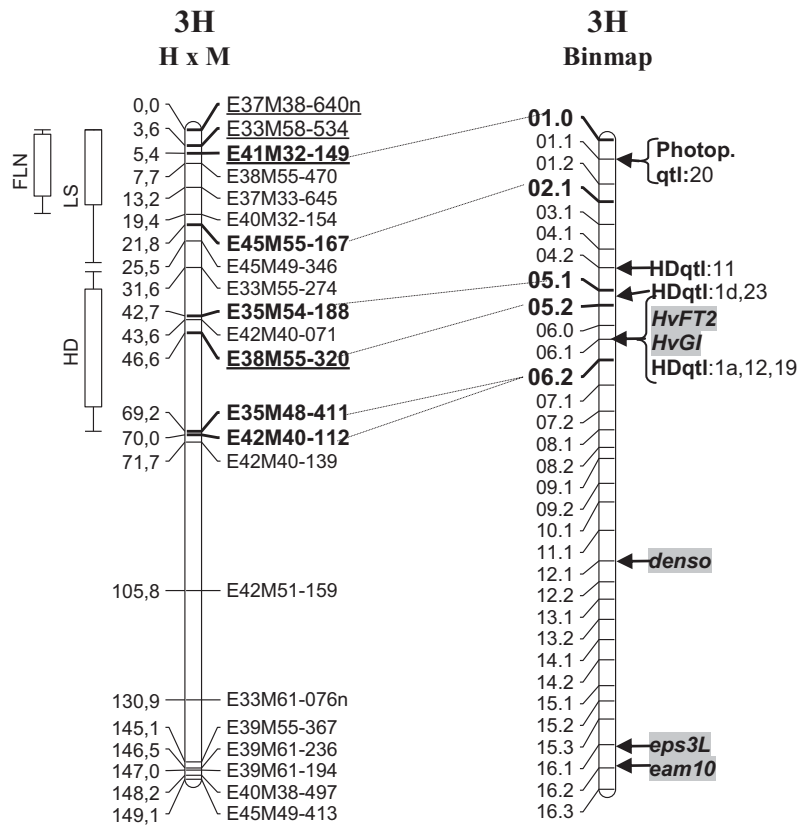


\* QTL for difference in heading time (GDD) between two extreme sowing dates

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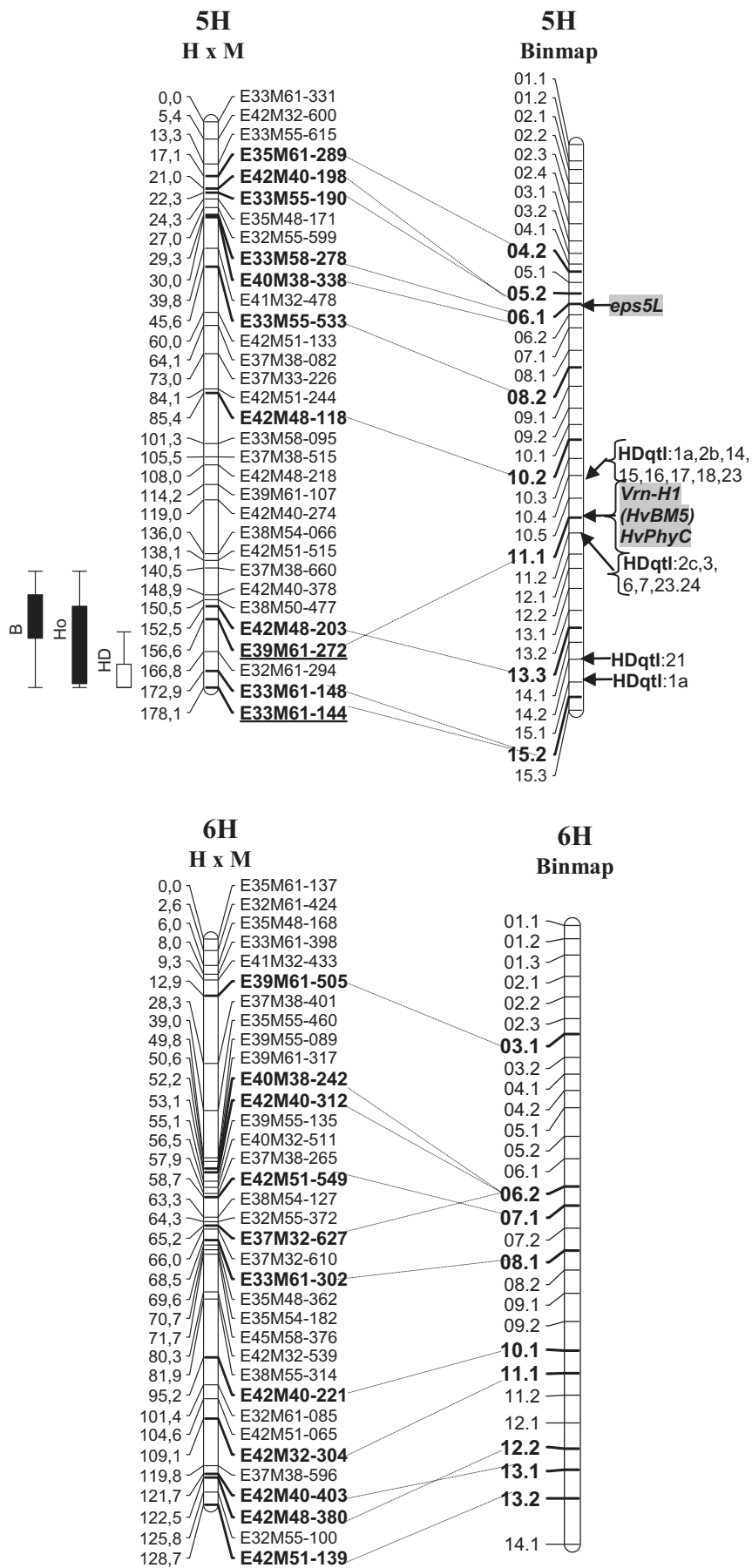
1 **Figure 2.** For each chromosome group, on the left, linkage map for the Henni x Meltan  
2 population and, on the right, binmap from Marcel et al. (2007) (Mapchart, Voorrips 2002). In  
3 the linkage map for Henni x Meltan: boxes indicate the 1-LOD interval and lines the 2-LOD  
4 interval (roughly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

10 **In the binmap by Marcel et al. (2007):** in each linkage group, on the left there are the sub-bins  
11 and on the right, positions (sub-bins) of the most known genes controlling responses to  
12 photoperiod, vernalization or earliness per se in barley; some homologues in barley of the most  
13 important genes controlling flowering time in *Arabidopsis* (in bold and grey; see text for  
14 references); and position of most significant markers of QTL for total time to heading (HDqtl)  
15 or for photoperiod response found in the literature (only those in the vicinity of QTLs for  
16 duration of phases in the present study). In some cases genes or marker positions could not be  
17 assigned with so much precision as sub-bins and therefore some positions are an approximation.  
18 Numbers designate populations in which the indicated QTL for heading or for photoperiod  
19 response were found: 1a, Steptoe x Morex (Hayes et al. 1993); 1b, Steptoe x Morex (Borem et al.  
20 1999); 2a, Dicktoo x Morex (Pan et al. 1994); 3, Igri x Triumph (Laurie et al. 1995); 4, Igri x  
21 Danilo (Backes et al. 1995); 5, Tystofte Prentice x Vogelsanger Gold (Kjaer et al. 1995); 6,  
22 Blenheim x Kym (Bezant et al. 1996); 7, Harrington x TR306 (Tinker et al. 1996); 8, Blenheim x  
23 E22/3 (Powell et al. 1997); 9, Vada x L94 (Qi et al. 1998); 10, Krona x HOR1063 (Kicherer et al.  
24 2000); 11, Harrington x Morex (Márquez-Cedillo et al. 2001); 12, Tadmor x Er/Apm (Teulat et  
25 al. 2001); 13, Oregon Wolfe Barley (Börner et al. 2002); 1c, Steptoe x Morex; 2b, Dicktoo x  
26 Morex; 14, Chebec x Harrington; 15, Alexis x Sloop; 16, Halcyon x Sloop; 17, Tallon x Kaputar;  
27 18, Arapiles x Franklin (Boyd et al. 2003); 19, Arta x HS41-1 (Baum et al. 2003); 20, Apex x  
28 Prisma (Yin et al. 2005); 21, VB9524 x ND11231 (Emibiri and Moody 2006); 22, Brenda x  
29 HS584 (Li et al. 2006); 2c, Szucs et al., 2006; 1d, Rao et al., 2007; 23, Beka x Mogador (Cuesta-  
30 Marcos et al. 2008a); 24, small interconnected populations (Cuesta-Marcos et al. 2008b); 25,  
31 BCD47 x Baronesse (Castro et al. 2008); 26, Haruna Nujo x Galleon and Amagi Nijo x WI2585  
32 (Chen et al. 2009).



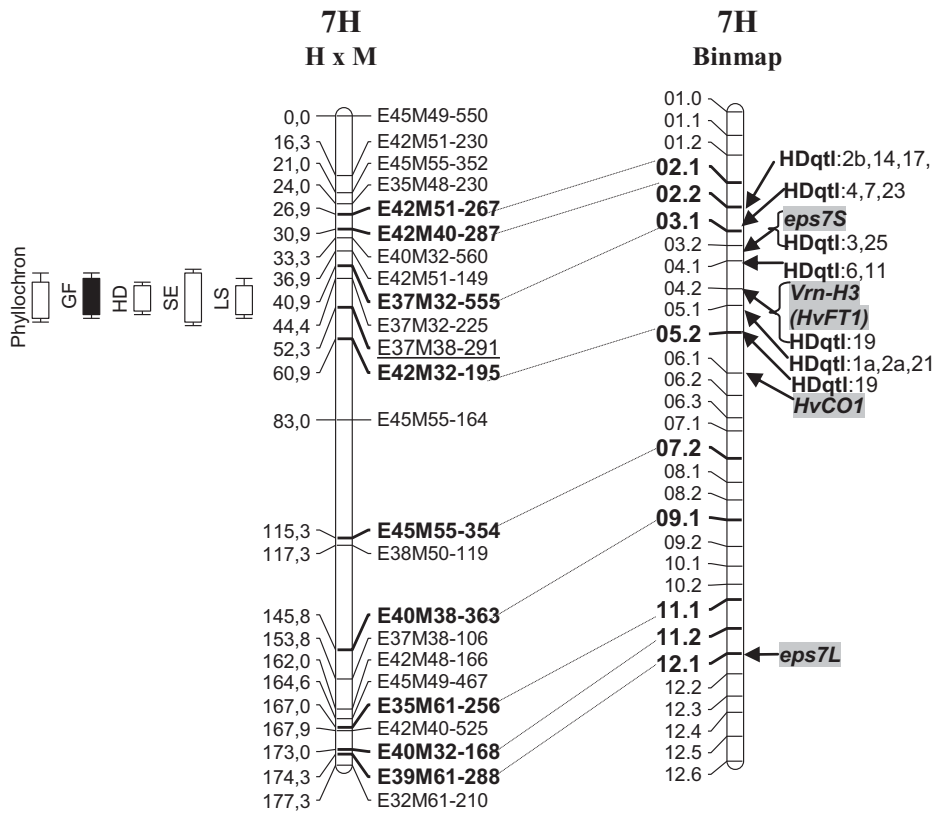
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2 **Figure 2. (Continued).**



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Figure 2. (Continued).



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**Figure 2. (Continued).**