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Small cross mapping of flowering time determinants

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Introduction

Flowering time plays a major role in barley adaptation, particularly in dry areas. Vernalization requirement and photoperiod response are the primary determinants of flowering time, and genes controlling these processes have been well characterized by means of allele-specific or linked markers in biparental populations. However, the allelic effects and interactions must be validated at the germplasm-pool level, in order to know whether they are useful or not in breeding programs involving a wide genetic base.

Materials and methods

Plant material: Seventeen small populations of doubled haploid lines, from representative crosses of the Spanish public barley breeding program were used (Table 1). A maximum of twenty lines per population were selected. Winter, Spring and Facultative growth types were represented.

Table 1. Populations used in this study. GH: Growth Habit; N: Number of doubled haploid lines

Parents		GH	N	Parents		GH	N	Parents		GH	N
Seira	Orria	S-F	20	Angora	Clarine	W-W	20	Clarine	Plaisant	W-W	20
Seira	Tipper	S-W	20	Barberousse	Albacete	W-F	10	Gaelic	Tipper	F-W	8
Seira	Alexis	S-S	20	Barberousse	Monlon	W-F	12	Nevada	Beka	S-S	20
Albacete	Monlon	F-F	7	Barberousse	Plaisant	W-W	20	Pané	Plaisant	W-W	20
Albacete	Plaisant	F-W	20	Barberousse	Tipper	W-W	8	Plaisant	Orria	W-F	20
Alexis	Pane	S-W	20	Beka	Monlon	S-F	20				

Field trials: Heading dates were recorded at four field trials, two autumn sowings in mid November 2002 and two winter sowings in late January 2004, at two locations in the provinces of Huesca and Zaragoza (North-Eastern Spain, latitude around 41.5º). The field trials consisted of three replications of two-row plots, 1m long, following a randomized complete block design. Heading date was recorded as the moment when 50% of the tillers exhibited 2 cm of protruding awns.

Genotyping: We used 72 markers covering all the genome, specially the areas involved in flowering time, according to previous studies. These included allele-specific markers for vernalization genes *Vrn-H1* and *Vrn-H2* (von Zitzewitz *et al*., 2005), and for the photoperiod gene *Ppd-H1* (Turner *et al.,* 2005). Linked markers were used for the photoperiod gene *Ppd-H2* and some earliness *per se* genes. Some spring x spring and winter x winter populations, for which no polymorphic markers were found in these regions, were genotyped with 8 more markers in chromosome 6H. A consensus map of the 17 populations was constructed using Joinmap 3.0 (Van Ooijen and Voorrips, 2001) (Fig. 1).

Marker analysis: In a first step, individual markers were analyzed, with the marker main effect as a fixed term and the marker by population interaction as a random term. In the next step, multiple marker models were used, where all significant markers, but the marker that is being specifically analyzed, were included as co-factors. Rare alleles (relative frequency below 1%) were not included in the analyses.

Results and discussion

Marker positions in the consensus map agree with other previously published barley linkage maps (Karakousis *et al*., 2003; Francia *et al.,* 2004). Some markers showed a significant main effect, consistent across populations, and therefore independent of the genetic background. Most of them have been previously described as closely linked to genes determining flowering time (Fig. 2; Table 2).

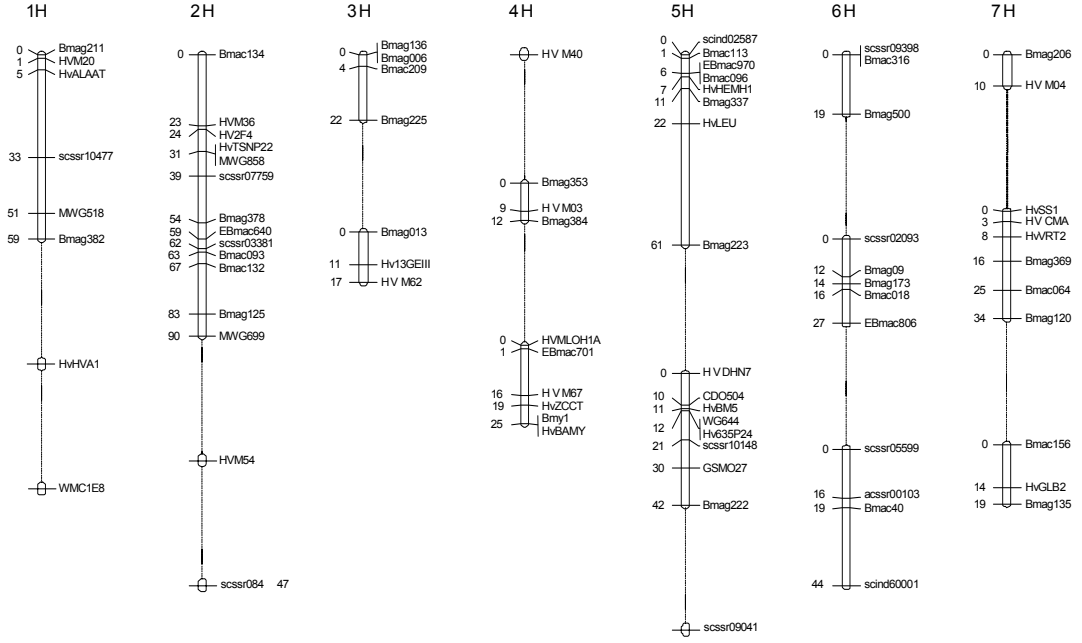

Fig. 1. Consensus map of the 17 populations. Linkage groups were set at LOD 3. In case of insufficient linkage, groups and/or markers within the same chromosome are linked with dotted lines. Their relative position in the figure was estimated according to other published barley linkage maps.

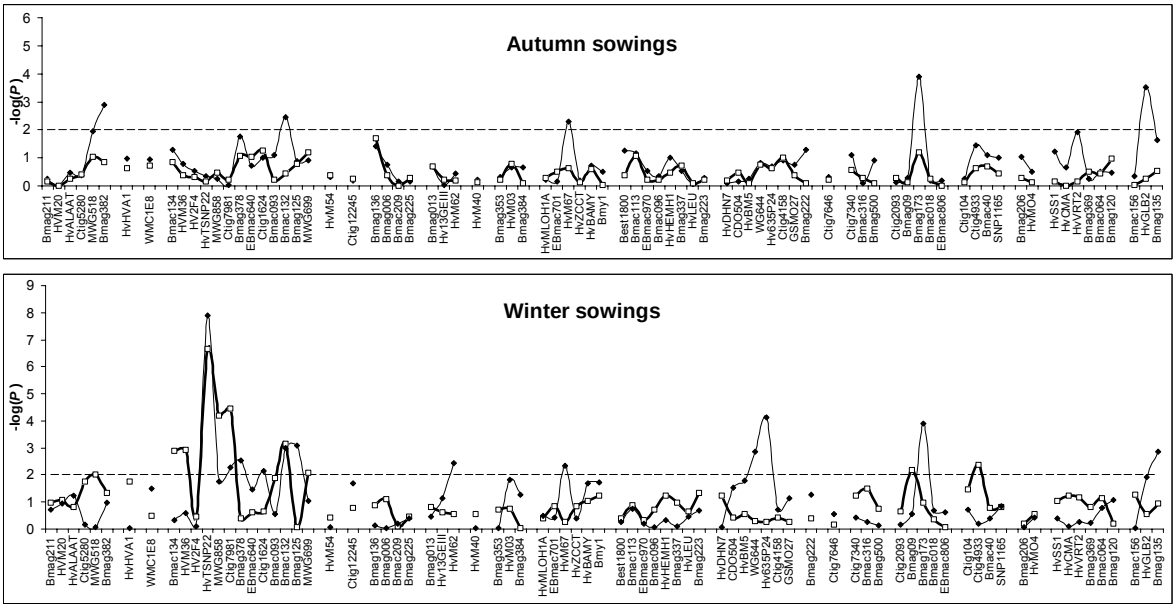

Figure 2. Marker main effect with the marker by population interaction as a random term.
Marker main effect in the multilocus model that includes all the other significant markers as co-factors.

Table 2. Adjusted allelic means for the markers with highest *P* values in the regression analysis, using the other significant markers as cofactors. Letters indicate means separation (alpha=0.05); (1) Allele size in base pairs (2) Days from sowing to heading (3) Allele denomination for a SNP marker (1: same allele as Dicktoo; 2: same allele as Morex; Szücs *et al*., 2006) (4) Allele denomination for a SNP marker (1: same allele as Triumph; 2: same allele as Igri; Turner *et al*., 2005)

Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)	Allele (1)	No.	Mean (2)
Autumn sowings																				
Bmag382 (1H)			Bmag378 (2H)			Bmac132 (2H)			HvM67 (4H)			Bmag173 (6H)			HvVRT2 (7H)[3]			HvGLB2 (7H)		
105	139	165.4a	137	19	167.3a	189	87	166.7a	108	14	165.7a	158	25	167.7a	1	122	165.1a	null	9	167.8a
111	141	163.7b	139	250	164.4b	183	29	164.1ab	116	86	164.7a	122	13	165.6a	2	159	164.1b	210	20	165.5ab
			149	12	163.5b	191	164	162.9b	112	55	164.5a	152	39	165.6a				214	12	165.4abc
										126	163.3b	124	139	164.1a				212	150	163.6bc
												150	25	163.8a				216	67	163.3cd
												148	39	160.7b				218	14	161.8d
Winter sowings																				
Bmag378 (2H)			HvTSNP22 (2H)[4]			Bmac132 (2H)			HvM67 (4H)			Bmag173 (6H)			Bmag135 (7H)			Hv635P2 (5H)		
137	19	118.9a	1	121	119.7a	189	87	119.4a	108	14	117.9a	158	25	118.8a	160	9	119.0a	192	142	118.5a
139	250	116.9b	2	159	114.9b	191	164	116.6b	112	55	117.6a	122	13	118.2a	142	54	116.6a	196	16	118.1ab
149	12	116.8b				183	29	115.9b	116	86	117.6a	152	39	118.1a	null	218	116.4b	198	74	117.1b
									118	126	116.3	124	139	117.2a				194	49	115.6c
												150	25	117.0a						
												148	39	114.6b						

Regarding autumn sowings, the most significant markers were: (i) Bmag382 on chromosome 1HL, linked to the photoperiod response gene *Ppd-H2* (Laurie *et al*., 1995; Boyd *et al*., 2003) which causes late flowering under short photoperiod conditions; and (ii) HvVRT2, with effect on vernalization response (Kane *et al*., 2005) under short-day conditions.

In winter sowings, the most significant marker is HvT_SNP22, which is a diagnostic marker of the photoperiod response gene *Ppd-H1* (Turner *et al*., 2005) with effect under photoperiod of 13 hours or longer (Laurie *et al*., 1995). The effect of Hv635P24, closely linked to the vernalization gene *Vrn-H1* (Francia *et al*., 2004), was also important.

Other markers showed a consistent effect in both sowing types, as is the case of Bmac132, on the centromeric region of chromosome 2H, linked to *Eam6* (Franckowiak and Konishi, 2002), which effect on heading date is evident under both long and short-day conditions; HvM67 on chromosome 4HL coincident with *eps4L*, Bmag173 on 6HL, coincident with *eps6L* and, in the distal region of 7H, Bmag135 and HvGLB2, coincident with the position of *eps7L* (Laurie *et al*., 1995).

For most of the QTL found, there were apparently two alleles, as suggested by means separation. HvGLB2 and Hv635P24 were the only cases where more than two QTL alleles may exist. The main effects of these QTLs were detected regardless the fact that not all populations were polymorphic for each marker and QTL, and thus the effects could be underestimated. But the consistent results found make them a useful tool for breeding programs.

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