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Quantitative trait loci for agronomic traits in an elite barley population for Mediterranean conditions

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Abstract Advances in plant breeding through marker-assisted selection (MAS) are only possible when genes or quantitative trait loci (QTLs) can contribute to the improvement of elite germplasm. A population of recombinant inbred lines (RILs) was developed for one of the best crosses of the Spanish National Barley Breeding Program, between two six-row winter barley cultivars Orria and Plaisant. The objective of this study was to identify favourable QTLs for agronomic traits in this population, which may help to optimise breeding strategies for these and other elite materials for the Mediterranean region. A genetic linkage map was developed for 217 RILs, using 382 single nucleotide polymorphism markers, selected from the barley oligonucleotide pool assay BOPA1 and two genes. A subset of 112 RILs was evaluated for several agronomic traits over a period of 2 years at three locations, Lleida and Zaragoza (Spain) and Fiorenzuola d'Arda (Italy), for a total of five field trials. An important segregation distortion occurred during population development in the region surrounding the *VrnH1* locus. A QTL for grain yield and length of growth cycle was also found at this locus, apparently linked to a differential response of the *VrnH1* alleles to temperature. A total of 33 QTLs was detected, most of them for important breeding targets such as plant height and thousand-grain weight. QTL × environment interactions were prevalent for most of the QTLs detected, although most interactions were of a quantitative nature. Therefore, QTLs suitable for MAS for most traits were identified.

Keywords Barley · Breeding · QTL · Agronomic adaptation · Vernalisation · *VrnH1*

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Introduction

Breeding for yield stability in Mediterranean environments has been slow due to wide variability in timing, duration and the severity of a number of climatic stresses (Baum et al. 2003). Consequently, the most

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difficult task for cereal breeders in Mediterranean countries is to develop varieties able to tolerate drought stress fluctuating across years and environments, by improving yield stability. In the Mediterranean area, crop performance is usually related to the response to abiotic stresses (Teulat et al. 2001). Although there have been a number of studies dealing with barley breeding issues for such environments (Ceccarelli et al. 2007, and references therein), barley breeding has made little progress in stress-prone areas (Pswarayi et al. 2008). Therefore, there is still a need for studies addressing barley productivity in Mediterranean conditions. The intrinsic interest of this area of research is enhanced by the current and future effects of climate change on agricultural production which, in a number of Mediterranean countries, are already causing farmers to change cropping from wheat to barley due to the latter's greater abiotic stress tolerance (Comadran et al. 2008).

Studies aiming at the identification of quantitative trait loci (QTLs) for yield and its components in barley are quite abundant in the literature. However, QTLs for grain yield in barley are an elusive target, as many are affected by large QTL × environment interaction (Romagosa et al. 1999), and thus are not suitable targets for marker-assisted selection (MAS). Given the difficulty of finding stable QTLs for yield, some authors claim that the improvement of yield in Mediterranean conditions will probably come through a combination of stable QTLs involved in the expression of traits significantly correlated with yield (Teulat et al. 2001). It has also been suggested that yield QTLs in cereals are not easily transferable between regions and also between plant materials. For this reason, the search for QTLs with immediate potential for application should be carried out as close as possible to the target environments, and with plant materials closely related to the germplasm used in the breeding programs.

We developed a population of recombinant inbred lines (RILs) from a cross between two elite barley cultivars, Orria and Plaisant. This cross has resulted in a large number of lines reaching the final stages of the Spanish National Barley Breeding Program, and has been a source of successful new cultivars in recent years characterised by a wide range of adaptation across Spanish environments. The objective of this study is to investigate the genetic factors that underlie the advantageous traits found in this cross, in order to facilitate the design of new breeding strategies and the implementation of MAS for Mediterranean conditions.

Materials and methods

Plant materials

The cross between two six-row parents, Orria and Plaisant, has proved to be one of the best crosses of the Spanish National Barley Breeding Program. Orria [(((Api × Kristina) × M66.85) × Sigfrido's) × 79W40762], a semi-dwarf cultivar selected in Spain from a CIMMYT nursery, is a facultative cultivar which is highly productive across most regions in Spain and has a very mild vernalisation requirement. Plaisant (Ager × Nymphe) is a French cultivar with strict winter growth habit; whilst it is less productive in Spain, it is one of the few European six-row winter cultivars with acceptable malting quality and consequently was a popular cultivar in Spain. We derived a total of 232 F2:7 RILs from the Orria × Plaisant cross by selfing a single plant for each segregating generation up to and including F7. The seed of these lines was then multiplied, and a subset of 120 RILs was randomly chosen for phenotyping. The number of families chosen for the field trial was considered sufficient to detect QTLs while maintaining an adequate level of replication (given extensive prior experience in the field sites) and still keeping the experiments a manageable size.

Field trials

Five trials were carried out at four locations: Sádaba (Zaragoza, Spain) during the 2008–2009 and 2009–2010 seasons, Gimenells (Lleida, Spain) in 2008–2009, Bell-lloc (Lleida, Spain) in 2009–2010, and Fiorenzuola d'Arda (Piacenza, Italy) in 2010 (Table 1). Due to unfavourable weather conditions during the 2009 fall at Fiorenzuola d'Arda, this trial was sown very late, on March 1. The two Lleida locations are less than 50 km apart and climatically very similar and can therefore be considered as the same location. The experimental design at each trial was an alpha lattice with three replicates, each arranged in eight incomplete blocks of 15 entries per incomplete block. Plots at Sádaba consisted of four rows, 2.7 m long, and 20 cm between rows. At

Table 1 Description of the field trials

Location	Province, Country	Code	Latitude	Longitude	Season	Sowing date	Seasonal precipitation (mm)
Gimenells	Lleida, Spain	L09	41°39′N	0°23′E	2008/2009	01/12/2008	203
Bell-lloc	Lleida, Spain	L10	41°37′N	0°46′E	2009/2010	02/11/2009	276
Sádaba	Zaragoza, Spain	Z09	42°17′N	1°16′W	2008/2009	22/11/2008	318
Sádaba	Zaragoza, Spain	Z10	42°17′N	1°16′W	2009/2010	26/11/2009	322
Fiorenzuola d'Arda	Piacenza, Italy	F10	44°56′N	9°54′E	2009/2010	01/03/2010	434

Gimenells and Bell-lloc, each plot consisted of eight rows 2.5 m long with a spacing of 15 cm between rows. In Fiorenzuola, the single plot consisted of eight rows, 15 cm apart and 3 m long. In all trials, the sowing density was set to 1,050 seeds per plot. Crop management followed local practices at each location. Climatic conditions, monthly average minimum and maximum temperatures for the test locations are shown in Supplementary Fig. 1.

Plots were scored for grain yield, days to heading, plant height, maturity time, thousand-grain weight, hectolitre weight, grain length, grain width, grain area, early vigour, growth habit, and susceptibility to powdery mildew and spot blotch, but not all traits were recorded in all five trials (Supplementary Table 1). Grain yield was measured as the weight of grain combine-harvested per plot and converted to kilograms per hectare by taking the harvested plot area into account. Days to heading were recorded as the number of days between January 1 and the date when approximately 2 cm of awns were visible on 50 % of the stems in each plot (Decimal Growth Stage 49). Plant height was measured in centimetres from the ground to the top of the stalk (excluding the spike). Maturity time was defined as the number of days between January 1 and the day when approximately 50 % of spikes had ripened (turned to yellow, Decimal Growth Stage 91). Thousand-grain weight was estimated from the weight of a sample of 1,000 grains. Hectolitre weight was calculated with a Dickey–John analyser model GAC-II. A Marvin Digital Seed Analyzer (GTA Sensorik GmbH) was used to estimate the average grain length, width and area from a 22 cm^3 sample of seed. Growth habit and early vigour were visually scored, using a scale from 1 (prostrate or poor vigour, respectively) to 3 (erect growth or excellent vigour, respectively). Powdery mildew (*Blumeria graminis* f.sp. *hordei*) and spot blotch (*Cochliobolus sativus*) were rated using a 0–9 scale in which 0 represented no disease symptoms and 9 was more than 90 % of leaf tissue diseased.

Statistical analysis of field trials

The alpha-lattice design was used to produce adjusted means for all traits scored on each individual trial by using the linear mixed model analysis implemented in the REML directive in Genstat 14 (VSN International 2011) to account for spatial differences detected by the incomplete blocks. Genotypes were fitted as a fixed factor and all other effects were considered random. The joint analysis across environments was done on these REML averages. The overall error mean square was calculated as the average of the error mean squares at each individual trial, and added as the residual term to the joint analysis. To account for the loss of the replicates in this analysis, the sums of squares for genotypes, environments and genotype × environment were multiplied by 3. This analysis was done for grain yield, days to heading and plant height for the five trials; for maturity time for trials L09, L10 and F10; and for thousand-grain weight and hectolitre weight only at the two Lleida trials L09 and L10.

Genotyping

Genomic DNA was extracted from leaf samples obtained from 14-day-old individual seedlings of the 232 RILs and the two parents. Genotyping was carried out at the Southern California Genotyping Consortium, using the Illumina GoldenGate Bead array platform Barley Oligo Pooled Array 1, which analyses 1,536 genome-wide single nucleotide polymorphisms (SNPs) (Close et al. 2009). PCR-specific markers for genes *VrnH1* (von Zitzewitz et al. 2005) and *PpdH1* (Turner et al. 2005) were also assayed in the 232 RILs using the primers and protocols described by the authors.

Map construction and QTL mapping

JoinMap 4 (van Ooijen 2006) was used for map construction. As the map locations of most of the 1,536 SNPs were known, we chose a LOD grouping threshold that divided the markers into the appropriate chromosomal groups, although we could not avoid some chromosomes being fragmented into two or more groups. For each linkage group so formed, the maximum likelihood mapping algorithm was used, in a first step, to estimate the best marker order within it. The distances between markers, using Kosambi's mapping function, were then recalculated using the regression mapping algorithm in a second step, but markers that were discarded after the second round of Joinmap 4 were excluded from the final map.

QTL × environment analysis was performed with the multi-environment routine for linkage mapping implemented in Genstat 14. The genotypic data and maps produced by Joinmap 4 were used to estimate genetic predictors for each marker locus and at 2 cM intervals where gaps between adjacent markers were greater than 2 cM. After choosing the best variance–covariance model for each trait, we used simple interval mapping scan to identify an initial set of cofactors for use in iterative rounds of composite interval mapping until there was no change in the cofactors. The final set of cofactors was used in a multi-environment mixed model to test whether each represented a QTL main effect or a QTL × environment effect and to estimate allelic effects at each environment. In all QTL analyses, we used the Li and Ji method to estimate a 5 % genome-wide significance threshold for the $-\log_{10}P$ values. The minimum cofactor distance was set to 30 cM, and the minimum distance to declare independent QTLs was set to 20 cM. Possible interactions between pairs of QTLs for each trait were analyzed using the unbalanced analysis of variance option implemented in Genstat 14, following a factorial model with the two markers closest to the QTLs and "environment" as factors.

Results

Field experiments

Despite the phenotypic similarity of the parents, considerable transgressive segregation was observed at all sites at which variates were scored and the population extremes were generally significantly better or worse than the higher or lower scoring parent, respectively (Table 2). Orria generally had a higher yield and a lower plant height and hectolitre weight than Plaisant. The differences between the parents for heading date were significant only at the Lleida and Fiorenzuola locations, with Plaisant later than Orria. L09 and L10 had greater overall growth (as suggested by larger plant height) and yield potential than the other three trials, together with earlier heading but lower thousand-grain weight. The over-sites analysis revealed not only that there were significant main effects of genotype and site for the six traits measured at all five trials but also that there were significant genotype × site interactions for all, although the mean square for the latter was much less than that for genotype (Supplementary Table 2).

We utilised the linear correlation coefficients between grain yield, days to heading, plant height and thousand-grain weight of the RILs within each trial to interpret the dynamics of grain yield variation across environments (Supplementary Table 3). The nature and magnitude of the correlations between traits varied according to trial location. Some notable contrasts between the coefficients were observed between the Zaragoza trials on one hand and the Lleida and Fiorenzuola trials on the other. The correlation between days to heading and yield was not significant in Z09 and Z10 (i.e., production was independent of cycle length) but was significant and negative in L09, L10 and F10, meaning that later lines produced lower yields. The correlation between thousand-grain weight and grain yield was not significant in Z09 and Z10 but was significant and positive in L09 and L10. The correlation between plant height and yield was significant in four of the five trials, but with opposite signs: negative in Z09 and Z10, and positive in L10 and F10. Other coefficients were more conserved across trials, such as the correlation between thousand-grain weight and both days to heading (negative) and plant height (positive).

A principal component analysis of these variables, based on the correlations between them, offers a better insight into the relationships within and between traits. Days to heading, plant height and thousand-grain weight were rather closely correlated across the trials (Fig. 1). All the points corresponding to each trait were placed in the same quadrant of the graph of the

Table 2 Descriptive statistics (mean, minimum, maximum, standard deviation and coefficient of variation) for the agronomic traits observed in the parents (Orria, Plaisant) and in the population of 112 RILs

	Parents		Recombinant inbred lines				
	Orria*	Plaisant	Mean	Min	Max	SD	CV
L09							
YLD (kg ha^{-1})	5,848[a]	5,879[a]	5,543	3,390	7,619	620	11.2
DHE (days)	105.7[a]	107.7[b]	107.7	99.0	113.0	2.3	2.1
PHE (cm)	108.3[a]	118.3[b]	113.4	95.0	135.0	7.5	6.6
MAT (days)	143.0[a]	145.0[b]	144.9	140.0	149.0	1.6	1.1
TGW (g)	30.4[a]	34.4[b]	33.3	23.3	45.2	4.2	12.6
HEC (kg hl^{-1})	67.7[a]	72.4[b]	70.5	59.9	78.1	3.0	4.3
L10							
YLD (kg ha^{-1})	7,143[a]	6,095[b]	6,329	4,343	7,867	607	9.6
DHE (days)	111.3[a]	114.0[b]	112.9	106.0	119.0	2.2	2.0
PHE (cm)	97.0[a]	101.3[a]	99.2	64.0	118.0	8.5	8.5
MAT (days)	148.0[a]	149.7[a]	149.8	147.0	156.0	2.4	1.6
TGW (g)	36.4[a]	34.6[a]	38.4	23.2	48.5	4.7	12.3
HEC (kg hl^{-1})	69.4[a]	72.2[b]	70.5	59.7	75.6	2.8	3.9
VIG (scale 1–3)	3.0[a]	2.0[b]	2.4	1.0	3.0	0.6	24.1
GRW (scale 1–3)	1.7[a]	3.0[b]	2.3	1.0	3.0	0.8	33.8
Z09							
YLD (kg ha^{-1})	3,964[a]	2,631[b]	3,302	1,982	4,360	371	11.3
DHE (days)	122.3[a]	121.7[a]	122.3	116.0	129.0	2.6	2.1
PHE (cm)	70.7[a]	85.7[b]	73.6	61.0	94.0	5.7	7.8
TGW (g)	38.5	42.4	41.2	33.1	47.9	3.1	7.6
HEC (kg hl^{-1})	69.4	73.6	71.6	67.2	75.8	1.8	2.5
LEN (mm)	8.6	8.1	8.3	7.3	9.2	0.4	4.7
WID (mm)	3.1	3.2	3.1	2.9	3.5	0.1	3.2
ARE (mm^2)	20.8	20.1	20.4	17.3	23.0	1.1	5.5
Z10							
YLD (kg ha^{-1})	4,174[a]	3,015[b]	3,641	2,306	4,613	375	10.3
DHE (days)	116.7[a]	116.0[a]	116.5	112.0	123.0	1.8	1.6
PHE (cm)	71.7[a]	87.7[b]	78.5	61.0	95.0	6.0	7.7
TGW (g)	39.9	37.1	39.7	30.0	52.0	3.9	9.8
HEC (kg hl^{-1})	65.2	69.7	66.8	59.2	71.9	2.9	4.4
LEN (mm)	8.5	7.9	8.6	7.4	10.1	0.7	7.8
WID (mm)	3.0	3.1	3.1	2.8	3.4	0.1	3.5
ARE (mm^2)	19.6	18.9	20.2	16.9	23.5	1.4	6.9
VIG (scale 1–3)	2.3[a]	2.7[a]	2.3	1.0	3.0	0.5	23.4
F10							
YLD (kg ha^{-1})	5,517[a]	3,433[b]	3,775	360	5,540	885	23.4
DHE (days)	144.3[a]	147.3[b]	144.9	135.0	165.0	5.3	3.6
PHE (cm)	70.0[a]	66.7[a]	65.9	50.0	80.0	5.6	8.4
MAT (days)	169.3[a]	170.3[a]	169.7	163.0	185.0	4.7	2.8
POW (scale 0–9)	3.7[a]	6.3[b]	5.7	1.0	8.0	1.4	23.7

Table 2 continued

	Parents		Recombinant inbred lines				
	Orria*	Plaisant	Mean	Min	Max	SD	CV
SPO (scale 0–9)	0.7[a]	6.0[b]	2.0	0.0	8.0	2.1	104.1

* Values followed by the *same letter* are not significantly different from 0 according to a LSD ($P < 0.05$)

YLD grain yield, *DHE* days to heading, *PHE* plant height, *MAT* maturity time, *TGW* thousand grain weight, *HEC* hectoliter weight, *LEN* grain length, *WID* grain width, *ARE* grain area, *VIG* early vigor, *GRW* growth habit, *POW* reaction to powdery mildew, *SPO* reaction to spot blotch

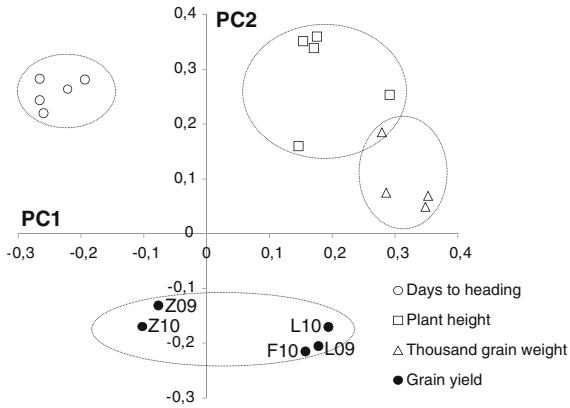

Fig. 1 Plot of the first two axes of a principal component analysis carried out with the variables days to heading, plant height, thousand-grain weight and grain yield, measured at five field trials

loadings on the first two principal components. These two components together explained 54 % of the total variance. Grain yield data points, however, were distributed over two quadrants, indicating changes in the direction of correlations within this trait and between traits.

Genetic map

Fifteen RILs were discarded on the basis of high percentages of missing data. The final mapping population therefore included 217 RILs. Out of the 1,536 SNPs assayed, monomorphic markers, markers with more than 10 % missing data and those with low quality scores (GenTrain score below 0.45) were removed from the data set. Excessive marker redundancy was reduced in a second round, resulting in a total of 384 high-quality markers being used for map construction. These markers formed 13 linkage groups at a LOD score of 7 with chromosomes 1H, 3H, 6H and 7H represented by one group and chromosomes 2H, 4H and 5H fragmented into three, two and four groups, respectively (Supplementary Fig. 2). After ordering the markers, comparison of our map with other consensus maps (Close et al. 2009; Muñoz-Amatriaín et al. 2011) showed good correspondence of marker order in all linkage groups. *PpdH1* was the most distal marker on the short arm of 2H with 11_21015 being the closest SNP to it. 11_21015 maps close but proximal to the BOPA2 markers 12_30871 and 12_30872 (Muñoz-Amatriaín et al. 2011), which are SNPs in *PpdH1*, so the position of *PpdH1* is consistent with previous reports. The *PpdH1* SNPs are located at 25.3 cM on the consensus map of Muñoz-Amatriaín et al. (2011) but the distal region of 2HS is not polymorphic in Orria × Plaisant. *VrnH1* was mapped on the long arm of 5H between SNPs 11_21247 and 11_11080, which is precisely where SNP 12_30883, a SNP in *VrnH1*, maps on the consensus map of Muñoz-Amatriaín et al. (2011), indicating that this developmental gene is also correctly located.

Of the 384 mapped markers, 288 segregated close to the expected 1:1 ratio. However, 55 markers in 1H, 2H.1, 3H, 4H.1 and 6H presented higher than expected Plaisant frequencies (based on a Chi squared test for $P < 0.01$). On the other hand, 19 markers scattered over 2H.3, 3H, 4H.1 and 7H, and all 23 markers on 5H.3, showed higher than expected Orria allele frequencies (Supplementary Fig. 3).

QTL analyses

QTLs were found for all traits, except for grain length, early vigour, growth habit and spot blotch tolerance. A total of 33 QTLs was detected for the traits under study but 23 were not consistent across locations as they were detected as interactions with the environment,

Table 3 QTLs for agronomic traits detected by composite interval mapping in the RILs of 'Orria' × 'Plaisant' cross in the five trials

Trait	SNP	Chr.	Pos.	CI.	Additive effect					% Explained variance[¥]						
					$-\text{Log}_{10}\ P$	L09	L10	Z09	Z10	F10	L09	L10	Z09	Z10	F10	QTL × environment
YLD	11_10275	1H	44.6	37.5–46.6	4.7	121.9	−40.0	26.7	−79.6	−3.0	9.1	0.7	1.0	7.4	0.0	<0.001
	11_11430	2H.1	54.1	48.4–61.8	5.7	80.8	126.9	−90.0	−71.8	−148.3	4.0	6.6	10.9	6.0	3.1	<0.001
	VrnH1	5H.3	14.8	11.1–18.1	6.9	−100.9	−138.2	−68.4	30.4	−591.8	6.2	7.9	6.3	1.1	49.8	<0.001
	11_10327	7H	58.2	51.5–67.5	5.4	−173.3	−99.9	34.3	−1.1	−205.7	18.4	4.1	1.6	0.0	6.0	<0.001
DHE	*PpdH1*	2H.1	5.9	0.0–9.8	24.7	0.3	0.4	−1.3	−0.5	−2.7	2.1	3.2	26.4	8.6	26.4	<0.001
	VrnH1	5H.3	14.8	11.1–18.1	12.0	1.0	1.2	1.1	0.8	3.7	20.9	28.2	18.7	17.8	49.8	<0.001
	11_10327	7H	58.2	52.6–67.5	5.4	0.7	0.7	0.7	0.7	0.7	11.7	11.4	9.3	17.2	2.0	ns
PHE	*PpdH1*	2H.1	3.9	0–13.5	4.2	0.6	0.2	−0.4	−0.3	−1.7	1.0	0.1	0.7	0.4	13.3	<0.001
	11_11505	2H.1	33.0	24.8–35.2	3.8	−0.1	0.7	1.3	0.5	−0.4	0.0	1.1	7.8	0.8	0.8	<0.001
	11_10379	4H.1	62.5	61.0–62.7	5.6	−0.4	−1.6	−0.2	−0.7	−1.7	0.4	5.4	0.1	1.7	13.6	<0.001
	11_10954	6H	25.2	19.4–28.2	6.8	2.9	2.5	1.8	2.5	0.8	23.2	13.6	13.3	22.5	2.8	<0.001
	11_20200	7H	87.1	82.3–93.2	4.1	1.1	1.1	1.1	1.1	1.1	3.6	3.0	5.7	4.7	6.4	ns
MAT	11_21015	2H.1	13.5	5.9–18.6	10.6	−0.1	−0.1	–	–	−2.3	0.2	0.1	–	–	24.9	<0.001
	11_20850	5H.2	35.4	30.5–42.6	3.8	0.5	0.5	–	–	0.5	12.9	5.5	–	–	1.2	ns
	VrnH1	5H.3	14.7	12.9–16.5	16.9	0.7	1.1	–	–	3.4	27.9	25.9	–	–	57.1	<0.001
	11_10327	7H	58.2	46.7–67.5	4.7	0.5	0.5	–	–	0.5	13.2	5.6	–	–	1.2	ns
TGW	11_10379	4H.1	62.5	61.0–62.7	6.0	−1.3	−1.3	−1.3	−1.3	–	11.4	8.3	16.6	10.8	–	ns
	11_10610	4H.2	21.3	14.1–23.0	5.2	1.0	0.5	0.9	−0.1	–	7.4	1.4	7.3	0.1	–	<0.001
	11_20892	6H	40.8	37.2–41.1	3.9	0.9	1.3	0.1	0.8	–	5.7	9.2	0.2	4.7	–	<0.001
HEC	11_20267	1H	112.0	104.6–113.5	14.5	0.4	1.0	0.8	1.5	–	2.3	15.7	21.4	26.2	–	<0.001
	11_10818	2H.1	57.6	50.6–61.8	7.7	0.9	0.9	0.9	0.9	–	11.1	11.8	23.9	9.3	–	ns
	11_21440	2H.2	11.8	3.9–14.4	4.0	0.4	−0.4	−0.1	−0.1	–	2.1	1.8	0.4	0.2	–	<0.001
	11_21362	3H	212.7	202.5–212.7	3.8	0.7	0.1	0.4	0.6	–	6.9	0.1	5.8	3.6	–	<0.001
	11_20010	5H.1	18.1	8.3–25.4	5.6	0.7	0.6	0.1	0.8	–	6.9	5.5	0.1	7.4	–	<0.001
	11_20074	7H	63.8	58.2–67.5	11.1	−0.8	−0.8	−0.2	−1.2	–	9.9	10.3	0.8	17.5	–	<0.001
WID	*PpdH1*	2H.1	3.9	0–11.7	5.3	–	–	0.04	0.04	–	–	–	13.3	11.8	–	ns
	11_10379	4H.1	62.5	60.5–62.7	5.5	–	–	−0.04	−0.04	–	–	–	12.6	11.2	–	ns
	11_20441	5H.2	0	0–5.1	5.4	–	–	0.04	0.002	–	–	–	11.9	0	–	<0.001
ARE	11_20267	1H	104.6	94.7–124.7	5.2	–	–	−0.5	−0.5	–	–	–	20.1	13.2	–	ns
	11_10379	4H.1	62.5	60.5–62.7	3.9	–	–	−0.4	−0.4	–	–	–	11.7	7.6	–	ns

Table 3 continued

Trait	SNP	Chr.	Pos.	CI.	Additive effect					% Explained variance[¥]					
					$-\text{Log}_{10}\,P$	L09	L10	Z09	Z10	F10	L09	L10	Z09	Z10	F10	QTL × environment
POW	11_10383	2H.2	17.6	7.0–17.8	4.0	–	–	–	–	0.3	–	–	–	–	9.5	–
	11_20924	4H.1	70.5	67.1–77.1	6.3	–	–	–	–	–0.4	–	–	–	–	16.1	–
	11_10576	7H	50.4	44.8–58.2	4.4	–	–	–	–	0.3	–	–	–	–	11.0	–

Chr. Chromosome, *Pos.* position, *CI* confidence interval, *ns* not significant

YLD grain yield, *DHE* days to heading, *PHE* plant height, *MAT* maturity time, *TGW* thousand grain weight, *HEC* hectoliter weight, *LEN* grain length, *WID* grain width, *ARE* grain area, *VIG* early vigor, *GRW* growth habit, *POW* reaction to powdery mildew, *SPO* reaction to spot blotch

¥ % Phenotypic variance explained by detected QTLs

* Indicates significance ($P < 0.05$)

although significant cross-over interactions were only detected for three of them.

Four QTLs for grain yield were identified on 1H, 2H.1, 5H.3 and 7H (Table 3). Whilst all considerably exceeded the significance threshold, all showed significant interactions with the environment. The most significant was the QTL located on linkage group 5H.3, at the *VrnH1* locus, where Plaisant alleles reduced grain yield significantly at three trials, but not significantly at Z09 or Z10. This cross-over interaction QTL had a strong additive effect of -591.8 kg ha^{-1} at F10 and explained 49.8 % of the phenotypic grain yield variation at this trial. Similarly, the Plaisant allele at the QTL located between SNPs 11_10327 and 11_20074 on chromosome 7H significantly reduced grain yield at the same three trials with the greatest effect again at F10, but was also not significant at the two Zaragoza trials. The second most significant QTL was detected on linkage group 2H.1 between SNPs 11_11430 and 11_10818 and was significant at all four Spanish sites but exhibited a strong cross-over interaction between Zaragoza, where the Plaisant allele reduced yield, and Lleida, where the same allele increased yield. The fourth QTL was located at SNPs 11_10275 and 11_10597, which are co-located on chromosome 1H. Whilst it was only significant at two sites, it was again a cross-over interaction with the Plaisant allele decreasing yield at one Zaragoza site but increasing yield at one Lleida site.

Three QTLs for days to heading (DHE) located on 2H.1, 5H.3 and 7H were detected, explaining rather large percentages of days to heading variation at the five trials (Table 3). The QTL located on 2H.1, between *PpdH1* and SNP 11_21015, was significant at three sites (Z09, Z10 and F10) but not at the two Lleida trials. This QTL explained 26.4, 8.6 and 26.4 % of days to heading variation at Z09, Z10 and F10, respectively, with the Plaisant allele associated with earlier heading. At the two QTLs located on 5H.3 (at *VrnH1*) and 7H (between SNPs 11_10327 and 11_20074), the Plaisant allele was consistently associated with later heading at all trials, although only the latter was a main effect as the larger effect at F10 resulted in the former being detected as a scaling effect QTL × environment interaction. Three of the four QTLs detected for time to maturity were in the same regions as the three DHE QTLs, with an additional QTL detected on 5H.2. As for DHE, the Plaisant allele at the locus in the region of *PpdH1* was associated with

earliness at F10 but the characteristic was not measured at the Zaragoza sites so it was only significant at one out of three sites and its lack of effect at the Lleida sites may have affected its exact positioning on 2H.1. The QTL at *VrnH1* was the most significant for maturity, accounting for over 25 % of the phenotypic variation at each site. Whilst the Plaisant allele increased maturity, as would be expected from its effect on DHE, the effect at F10 was much greater than at the Lleida sites so, like the DHE QTL, it was detected as a scaling effect QTL × environment interaction. The QTL on 5H.2 was located between SNPs 11_10578 and 11_20850, with the Plaisant allele increasing maturity as a consistent main effect across all three sites. As for DHE, the QTL on 7H was a main effect, with the Plaisant allele increasing maturity.

Five QTLs were detected for plant height, between *PpdH1* and SNP 11_21015 and at SNP 11_11505 on 2H.1, at SNP 11_10379 on 4H.1, between SNPs 11_20936 and 11_10954 on 6H, and at SNP 11_20200 on 7H. The QTL on 7H was a main effect, with the Plaisant allele contributing a consistent increase in plant height at all trials. The QTL on 6H was the most significant, with the Plaisant allele increasing height at all four Spanish sites and accounting for over 13 % of the phenotypic variation at any one, but no significant effects were found at F10. The Plaisant allele at the second QTL on 2H.1 was also associated with a significant increase in height, but only at Z09. In contrast, the Orria allele significantly increased plant height at the other two QTL, being significant at F10 for the first QTL on 2H.1 and at L10 and F10 for the QTL on 4H.1.

Three QTLs were detected for thousand-grain weight (TGW), located on 4H.1, 4H.2 and 6H, and explaining 25, 19, 24 and 16 % of the phenotypic variance for the characteristic at L09, L10, Z09 and Z10, respectively. The QTL on 4H.1 was co-located with the plant height QTL at SNP 11_10379 and was a main effect with a consistent reduction associated with the Plaisant allele. Plaisant alleles at the other two QTLs, at SNP 11_10610 on 4H.2 and at SNPs 11_20892 and 11_21469 on 6H, were associated with significant increases in TGW in 2009 for the former and at all sites except Z09 for the latter. Six QTLs were detected for hectolitre weight (HEC). The one located on 2H.1 between SNPs 11_11430 and 11_10818, the same interval in which we found a yield QTL, was a main effect with the Plaisant allele increasing the characteristic. The other five QTL were all QTL × environment interactions and significant at three of the four sites. They were located on 1H between SNPs 11_20267 and 11_20921, 2H.2 at SNP 11_21440, 3H at SNP 11_21362, 5H.1 between SNPs 11_20010 and 11_21065, and 7H between SNPs 11_20074 and 11_11014. The Plaisant alleles at all but the QTL on 2H.2 and 7H were associated with increases in the characteristic. The 2H.2 QTL indicated a cross-over interaction with the Plaisant allele increasing HEC at L09 but decreasing it at L10. Although neither of the effects was significantly different from zero, the difference between the extreme effects was indeed significant. At the 7H QTL, Plaisant alleles significantly decreased the characteristic at all sites except Z09. Grain width and area were only estimated at two trials, Z09 and Z10, with three and two QTLs detected, respectively. All but a grain width QTL on 5H.2 at SNP 11_20441 were detected as consistent main effects at the two sites. A QTL for both characters was detected at SNP 11_10379 on 4H.1, where we also detected a QTL for TGW, and, as for TGW, the Plaisant allele decreased each characteristic. The other QTL for grain width was located between *PpdH1* and SNP 11_20105 on 2H.1, and the other grain area QTL was located on 1H between SNPs 11_20550 and 11_20267. The Plaisant allele was associated with an increase for the former but a decrease for the latter.

Powdery mildew infection was estimated at Fiorenzuola d'Arda (F10), as there was an attack severe enough to reveal genotypic differences. The most significant QTL was located at SNP 11_10924 on 4H.1, where the Plaisant allele was the more resistant. The Orria allele was the more resistant at the other two QTLs, which were located at SNP 11_10383 on 2H.2 and between SNPs 11_10056 and 11_10576 on 7H.

Interactions between pair of QTLs were found for grain yield, plant height and HEC (Table 4). These interactions are presented in detail in Supplementary Table 5. Four of the seven interactions involved one of the flowering time genes *VrnH1* or *PpdH1*. For grain yield, all the interactions detected were significant when the interaction with environment was also included. They were caused in all cases by differential responses of some classes at the F10 and both Lleida trials (Supplementary Table 4).

Table 4 Summary of epistatic interactions between pairs of QTLs, or between pairs of QTLs with the environment (only interactions with probability level $P \leq 0.01$ are shown)

Trait	Marker-1 (M1)	Marker-2 (M2)	M1 × M2	M1 × M2 × environment
Grain yield	*VrnH1*	11_10275	ns	0.010
Grain yield	*VrnH1*	11_11430	0.000	0.001
Grain yield	11_10275	11_10327	ns	0.001
Plant height	*PpdH1*	11_11505	0.000	ns
Plant height	*PpdH1*	11_10954	0.003	ns
Plant height	11_10954	11_10200	0.000	ns
Hectolitre weight	11_10267	11_20074	0.003	ns
Hectolitre weight	11_10818	11_20010	0.000	ns

ns not significant

Discussion

Despite the narrow genetic base, progeny from the cross Orria × Plaisant have proved remarkably high yielding in the Spanish National Barley Breeding Program, with two cultivars, Cierzo and Yuriko, already commercialised, that ranked first in their respective official national register trials. The relevance of this study is that it has been carried out with the best germplasm possible for the region, and its application to barley breeding for Mediterranean conditions is straightforward. This study was therefore carried out to identify the favourable quantitative trait loci from each parent that have been recombined in the successful progeny. Unravelling the genetic factors underlying the agronomic advantages of this material for Mediterranean conditions will help optimise future breeding strategies to improve the chances of producing elite cultivars.

The vernalisation gene *VrnH1* was co-located with QTL on chromosome 5H.3 for grain yield, days to heading, and days to maturity in this population, with the Orria allele conferring earliness and significantly higher yield at three sites. Growing conditions were better at the two Lleida locations (L09 and L10), as manifested by higher grain yields and plant height of the parents and the population. In addition, heading occurred earlier in Lleida than at Zaragoza, especially in 2009, even though the Lleida trial was sown later that season. This was caused by the warmer conditions experienced at the Lleida locations throughout the two seasons (Supplementary Fig. 1). Consequently, the accumulation of growing degree days occurred faster at the Lleida (L) than at the Zaragoza (Z) sites. A significant delay in heading will reduce the grain filling period in Mediterranean environments where summer temperatures become excessive, so the QTL effects detected for grain yield, days to heading and maturity are as we would expect for all sites apart from the Zaragoza ones, especially Z10. The delay in days to heading at Z10 was less marked than at the other sites and that difference coupled with greater late-season moisture availability and/or a delay in the onset of high summer temperatures may have enabled the later heading types with the Plaisant allele to make use of a greater vegetative biomass and produce a higher yield.

Wang et al. (2010) reported an effect of *VrnH1* on grain yield in an advanced backcross study of a *Hordeum spontaneum* × elite spring barley population, although they did not detect an effect upon heading date. Sameri and Komatsuda (2007) also detected an effect in the region of *VrnH1* on grain number per plant and kernel weight with opposing effects of alleles from the parents, Azumamugi and Kanto Nakate Gold, but they did not assess heading date. No effect of *VrnH1* on grain yield was found in a study carried out in similar Mediterranean environments with the spring × winter population Beka × Mogador (Cuesta-Marcos et al. 2009), nor was it found to have any significant effect on days to heading from an autumn sowing (Cuesta-Marcos et al. 2008a). Comadran et al. (2011) found significant QTL × environment interaction for SNPs closely linked to *VrnH1* and *VrnH2* in a genome-wide association study of yield for a diverse panel of barley genotypes that had been trialled over a number of different Mediterranean environments. Furthermore,

Francia et al. (2011) reported significant effects of the developmental genes *VrnH1*, *VrnH2*, *PpdH2* and *Eam6* on grain yield, both as main factors and in interactions with the environment from a study of the Nure × Tremois mapping population trialled at a number of Mediterranean environments. This study found that, whilst *PpdH2* and *Eam6* explained a large proportion of the main genotypic effect, *VrnH1* explained the largest proportion of genotype × environment interaction (17.6 %), so our findings show considerable consistency with previous reports.

The QTL for grain yield on linkage group 2H.1 at SNP 11_11430, unlinked to *PpdH1,* seems to be in the area of a QTL hotspot for barley and is in the same region as SNP 11_10818, which we detected as a main effect QTL for hectolitre weight. Comadran et al. (2011) found a QTL for grain yield and days to heading on this chromosome at SNP 11_10191, less than 1 cM distant (Close et al. 2009; Muñoz-Amatriaín et al. 2011). Both Comadran et al. (2011) and Wang et al. (2012) identified a heading date QTL in this region in two different association panels. This region was reported by Borrás-Gelonch et al. (2012) as having a very large effect on days to heading and on the duration of developmental phases of barley, and was highlighted by Cuesta-Marcos et al. (2008a,b) as the one having the main earliness QTL for Mediterranean environments, co-locating with the gene *Eam6*. The effect of this gene has been linked to variations in the minimum temperatures during the heading phase (van Eeuwijk et al. 2010). As we did not detect any associations with heading date in this region, it is possible that there may be more than one linked locus with differential effects at this region. Indeed, the region is centromeric so we can expect a number of linked genes in the region and the exact balance will largely depend upon parental origins as recombination will be restricted. The grain yield QTL, on 1H in the region of SNP 11_10275, does not appear to have been previously reported in elite barley crosses, although several authors have reported a grain yield QTL on 1H, in the vicinity of Bmac090 (Li et al. 2005; Bauer et al. 2009), from studies of *H. spontaneum* introgressions. Bmac090 is located just 3 cM away from SNP 11_10275 (WTB Thomas, unpublished data) so we could have detected a similar effect. Recently, Fisk et al. (2013) reported a frost tolerance QTL in the same region, in crosses NB3437f/OR71 and NB713/OR71, both involving at least one facultative parent. The closest marker to the QTL was 11_10764, which is located in our map just 0.8 cM away from 11_10275, so they both may be pointing at the same gene. This possibility is confirmed by the fact that there is a good agreement between the average temperatures of the Spanish environments for the first 2 months of the crop and the sign of the effects observed: at the coldest year, 2009 (4.7 °C, average of December and January), the Plaisant allele at 11_10275 offered a yield increase (significant at Z09). In 2010, which was warmer (5.4 °C), there were negative effects from the Plaisant alleles (significant at L10). This pattern, however, was broken by the late sowing at F10 in which, if the yield QTL was actually a frost tolerance QTL, we would expect a positive effect of the Orria allele which did not occur.

QTL for grain yield, flowering time and maturity were identified in a similar position on chromosome 7H. The closest marker to the QTL was SNP 11_10327, with the Orria allele associated with higher yield, earlier flowering and maturity. Notably, the effect for flowering time at this SNP was the only one for this character that we detected as a main effect. The effect for maturity at SNP 11_10327 was also detected as a main effect, although the trait was not measured at Z09 or Z10. The SNP's effect on grain yield was, however, detected as a QTL × environment interaction but, whilst the effects detected at the Zaragoza sites contrasted with those at the other three, neither were significant. SNP 11_10327 is 5 cM proximal to SNPs 12_30983, 12_30894 and 12_30895 (Close et al. 2009), which are all located in the developmental gene *VrnH3*. Wang et al. (2010) identified an effect of *VrnH3* on grain yield, and Ponce-Molina et al. (2012) detected a QTL for flowering date at the locus. Whilst the parents of our population differ for the promoter of *VrnH3* (unpublished data), the confidence intervals for the three QTL that we detected do not extend beyond SNP 11_10838, which is still proximal to the *VrnH3* SNPs (Close et al. 2009). It therefore appears very unlikely that the QTL that we have detected in this region of 7H reflects allelic differences at *VrnH3*. It is, however, noticeable that the confidence interval for the grain yield QTL overlaps with that for the hectolitre weight QTL that we detected in the region of SNP 11_20074. Here, the Orria allele also increases hectolitre weight so there would be considerable agronomic benefit in selecting for Orria alleles in this region for Mediterranean barley.

A large-effect QTL for days to heading in the region of *PpdH1* on chromosome 2H has been found recurrently in several studies (Li et al. 2005, 2006; von Korff et al. 2006; Bauer et al. 2009; Wang et al. 2010; Pasam et al. 2012). The QTL found in this study reinforces the importance of this locus for the control of flowering time in Mediterranean conditions, although it did not have a noticeable effect on grain yield, contrary to our findings for *VrnH1*. Laurie et al. (1994) reported a pleiotropic effect of *PpdH1* on plant height and yield components. Similar results were reported by other authors (von Korff et al. 2006; Bauer et al. 2009; Wang et al. 2010). In all cases, the later allele was associated with increases in plant height, as we have seen in the present study. The effect of *PpdH1* is, however, more marked under longer day lengths than those experienced in the current study so, whilst we also found that the later allele resulted in an increase in plant height, it is not surprising that we did not find any co-location of yield QTL.

The QTL for plant height on 6H is associated with SNP 11_10954, with the Orria allele reducing plant height. This marker is 1 cM proximal to the SSR marker Bmag0009 (WTB Thomas, unpublished data), which is associated with a plant height QTL in the Tadmor × ER/APM population (Teulat et al. 2001) and also overlaps with a QTL hotspot, including plant height, detected in the Tankard × Livet population (Rajasekaran et al. 2004), so there may be a general growth QTL still segregating in elite gene pools as well as landrace material in this region. The QTL for plant height in the region of SNP 11_20200 on 7H is in a similar position to a QTL for this trait found in the region of Bmag0516 by Rajasekaran et al. (2004) in the Tankard × Livet population. Bmag0516 is located just proximal to SNP 11_11219 (WTB Thomas, unpublished data) and thus is in a similar position to SNP 11_20200. Varshney et al. (2012) identified an association with plant height with the DArT marker bPb-2379, which mapped in the same position as SNP 11_20200 in the OWB mapping population (http://wheat.pw.usda.gov/ggpages/maps/OWB/, as reported by Szűcs et al. 2009). The β-glucan synthesis gene *CslF6* (Burton et al. 2008) is located within 1 cM of SNP 11_11219 and it is possible that the polymorphisms that have been and are being reported in this region are due to the persistence of high β-glucan lines in non-malting barley types, which is linked to other genes of agronomic importance.

The most significant QTL for thousand-grain weight was detected in the region of SNP 11_10379 on 4H.1. The QTL was detected as a main effect with Orria alleles increasing the characteristic, apparently through an increase in grain width and area, as QTL for these characters were also found to be associated with SNP 11_10379. The SNP was also associated with a plant height QTL, with Orria alleles producing a significant increase in the characteristic at L10 and F10. Using the maps of Muñoz-Amatriaín et al. (2011) and Szűcs et al. (2009) and comparing locations of bin markers, we conclude that the QTLs associated with SNP 11_10379 are located in the same region as the thousand-grain weight QTLs detected in Igri × Danilo (Backes et al. 1995) and Vogelsanger Gold × Tystofte Prentice (Kjaer and Jensen 1996). Thousand-grain weight QTLs reported by Li et al. (2006) and Baum et al. (2003) together with a height QTL detected in the region of HVM3 in Derkado × B83-12/21/5 (Chloupek et al. 2006), which is also in the same bin, add further support to our conclusion.

Another QTL for thousand-grain weight was detected in the region of SNP 11_10610, which co-segregates with SNPs in the vernalisation gene *VrnH2* (12_30889 and 12_30892; Muñoz-Amatriaín et al. 2011). This effect most probably reflects minor differences in vernalisation requirement affecting grain fill, although it has not manifested itself in changes in grain width or area. QTLs for thousand-grain weight have also been reported in the area of *VrnH2* (Teulat et al. 2001; Bauer et al. 2009).

It is noticeable that all the powdery mildew QTLs are independent of the agronomic QTLs. The most significant, in the region of SNP 11_20924 on 4H, is in the same region as Bmag0353 and the bin marker bBE54A (Szűcs et al. 2009; Varshney et al. 2007). This would place the powdery mildew resistance QTL in the same region as the major resistance gene *Mlg*. Plaisant carries the resistant allele at the QTL but has only been reported as carrying the *Mlra* resistance gene (www.cprad.scri.ac.uk), so it is more likely that the effect that we have detected is the result of a minor gene rather than *Mlg*. Similarly, SNP 11_10383 maps between cnx1 and Zeo1 on 2H.2, which would place it in the same region as the major resistance gene *MlLa*. Neither Orria nor Plaisant are, however, likely to carry this gene and it is likely that the resistant allele carried by Orria again represents a minor gene. The

confidence interval of the resistant QTL allele carried by Orria at SNP 11_10576 overlaps with those of the heading date and height QTLs detected at the adjacent marker SNP 11_10327 and it is highly likely that shorter and earlier alleles of Orria render it less susceptible to powdery mildew. We therefore conclude that this most probably represents an escape mechanism rather than a true resistance effect.

Our data and those other studies indicate that grain yield under Mediterranean conditions depends to a remarkable extent on phenology, but also that not all phenology genes affect grain yield to the same extent or in the same manner. This effect of phenology on grain yield was already recognised in classical studies, although the genetic underpinnings were not fully understood at the time. For instance, van Oosterom et al. (1993) already stated clearly that "development pattern has a marked effect on yield response across environments". The overall picture given by the grain yield QTLs is better understood after the examination of epistatic interactions. In this population, we have examined two alleles of the allelic series present at *VrnH1* (Hemming et al. 2009; Casao et al. 2011a), which induce a gradient of vernalisation responses in genotypes that carry an active *VrnH2,* i.e., a strict winter haplotype (Plaisant) and an intermediate winter haplotype (Orria). The role of *VrnH1* in the determination of grain yield is intensified by its interaction with other QTLs. It seems clear by now that the Plaisant allele at *VrnH1* is detrimental at the warmer sites (F10, L10 and L09), with yield reductions correlated with temperature during early growth. Moreover, the effect of *VrnH1* has been associated not only with vernalisation response, but also with frost tolerance (Francia et al. 2004). Its interaction with the QTLs on 1H (possibly *FrH3*, as pointed out before) and 2H.1 suggests that the effect of *VrnH1* on yield is complex, and dependent upon the genetic constitution at other loci that may also be related with response to temperature, including frost tolerance. Therefore, the role of phenological traits can be more complex than previously thought, and may extend beyond plant cycle duration. In addition, the fact that some phenological features play a major role in grain yield determination means that even in a highly elite material, there is room for improvement and fine-tuning of some of the main adaptation genes. This was not an expected result. Based on our long experience with this cross in the Spanish barley breeding program, we were expecting to find grain yield QTLs independent of phenological traits, at least for the autumn-sown trials. Very few studies have found grain yield QTLs of such type under Mediterranean conditions and, in general, they are detected in crosses with exotic sources, either *H. spontaneum* or landrace-derived material (von Korff et al. 2006, 2008; Lakew et al. 2011).

The clustering of traits in the principal component analysis gives an indication of their genetic control. The tighter distribution of points for plant height, days to heading and thousand-grain weight suggests that they have higher heritability and/or are under simpler genetic control. The scattering of grain yield points over two quadrants, on the other hand, suggests a shift in the relationships between traits across trials. Grain yield was influenced by different sets of traits at different trials, probably as a result of a distinct reaction to diverse environmental conditions, reinforcing the view expressed in the previous paragraph about the interaction of temperature with grain yield QTLs. This is not unexpected under our conditions. Varshney et al. (2012) found a similar pattern in a recent association study with barley in the Mediterranean region, and attributed this fact to the differences in environmental conditions across sites triggering different genetic pathways, and to the strong conditioning of yield by earliness. Comadran et al. (2008), in an independent association study, found 43 QTLs for grain yield in 27 field trials across seven Mediterranean countries, but few were detected at several trials, and 22 were detected at only one trial. It is remarkable that the grain yield at autumn-sown trials in Lleida (L09 and L10) cluster close to F10, a March sowing in Italy, for which not much vernalisation potential was expected, and not to the Zaragoza trials, which were located only 140 km apart. This indicates that the range of conditions that may be encountered in autumn sowings in Northern Spain can be remarkably wide in terms of winter temperatures between locations and between years and, therefore, cultivars grown under these conditions should have enough flexibility to respond to these variable conditions.

The regions that contained some QTLs detected in this study presented distorted allelic frequencies, which might have influenced the results. Even though this might be the case, the dense map obtained for our population would make up for the loss of power due to distorted frequencies in the QTL analysis (Xu 2008).

Actually, the examination of the regions with distorted allelic frequencies and the genes that are located in them offers insights into the selective pressures acting on them during development. Allelic frequencies departing from a 1:1 ratio in regions harbouring flowering time genes are commonly observed in populations developed or multiplied under natural conditions. This seems to have occurred in linkage group 5H.3, due to selection at the *VrnH1* region. It may have occurred as well in the development of the population Nure × Tremois (Francia et al. 2011). In that population, two of the QTLs for heading date were located in the regions of *Eam6* and *PpdH2* (Francia et al. 2004), and the frequencies of the markers used to tag these genes indicate a possible selection during the development of the population, with probabilities of 0.003 and 0.00006, respectively, according to a Chi squared test (own calculations based on supplementary data provided by the authors). In another study, Ponce-Molina et al. (2012) detected a strong selection towards the spring *VrnH1* allele, which induced a small vernalisation requirement in the population SBCC145 × Beatrix (versus the alternative allele, which induced a higher vernalisation requirement). The population was multiplied in a greenhouse, without any vernalisation provided. Similarly, in Orria × Plaisant, we observed selection for the *VrnH1* allele inducing a lesser vernalisation requirement. Orria has a unique *VrnH1* allele with reduced vernalisation requirement. The first intron is similar to the *HvVRN1-4* allele of Hemming et al. (2009) but it contains an additional 7-bp deletion (GenBank accession DQ492705). Under controlled conditions it behaves like the Spanish landrace SBCC058 (Casao et al. 2011b). The RIL population was developed in Lleida and, therefore, its rather warm temperatures may have shifted the population towards an over-representation of the Orria allele at *VrnH1*, resulting in the skewed allelic frequencies observed in the linkage group 5H.3. During the advancement of the generations, some lines were occasionally discarded because they produced almost no seed, most probably because they had the Plaisant allele at *VrnH1* and, during warmer seasons, failed to flower normally.

Use of the QTLs for MAS

The use of molecular markers can greatly increase selection efficiency, if the traits targeted are not severely affected by genotype × environment interaction. Three of the four QTLs detected in this study for grain yield show clear cross-over interactions and the remaining one was not clearly a scaling effect. It is therefore not evident which would be the best allele to select and a risk analysis would be necessary to identify the most appropriate allele. For instance, for the effect associated with *VrnH1* on 5H.3, it would be best to select for the Orria allele in environments where significant frost events are unlikely to occur, but it would be preferable to select for the Plaisant allele in environments where frost is more likely. The interactions between QTLs and environment for grain yield actually suggest that a combined selection for Orria alleles at the QTLs on 1H and 2H.1 may actually override the allelic effect at *VrnH1*. This finding should be investigated further, and we plan to go back to the original population to search for lines with specific haplotypes to study these interactions in more depth.

Reducing plant height is one of the goals of the current Spanish barley breeding program. Of the five QTLs found for plant height in this study, four showed interaction with the environment, but they were all scaling effects with no evidence of significant cross-over interactions. Thus, although two of the QTLs were significant in just one environment and might not be such good targets for MAS, consistent selection for the shorter allele at four of them would be feasible, and would even benefit from favorable epistatic interactions (Supplementary Table 5). The second QTL at 2H.1 (11_11505) was only significant at one location, and it showed a qualitative interaction with *PpdH1* which made it useless for MAS (Supplementary Table 5). Favorable (short) alleles were derived from both parents, explaining the large transgressive segregation found for this trait (Table 2). The same can be said for hectolitre weight, which also will benefit from epistatic interactions if favorable alleles are combined at QTLs at 1H, 2H.1, 5H.1 and 7H.

Considering the heading date QTL, appropriate selection strategies for *VrnH1* have been described above and, as Orria contributes the "early" allele consistently for the QTL on 7H, it can be used to adjust the growth cycle as necessary. The QTL at *PpdH1* appeared only at the Z and F trials. This is consistent with the well-proven effect of this gene under long photoperiod. Plaisant contributes the "early" allele at this locus, provided the plants are grown under long

days. At both L trials, heading occurred too soon in the year for *PpdH1* to have any effect, but it occurred later in the other three trials, so that *PpdH1* had an effect on the growth cycle. We consider that the sensitive (Plaisant) allele should always be incorporated into winter cultivars for the Mediterranean area as it provides an insurance mechanism to induce flowering before temperatures rise too much in the season, which should also be built into the risk analysis strategy outlined above. The adaptive mechanism provided by photoperiod response has already been identified as one of the main forces driving the latitudinal spread of barley landraces in Europe, with the sensitive *PpdH1* allele restricted to lower latitudes (Lister et al. 2009).

A possible antagonistic effect exists for the QTL in the region of SNP 11_10379 on 4H.1, as the Plaisant allele decreased plant height but also decreased grain weight and width. Selection for the Orria allele would appear to be the best strategy, as the relative effect on grain weight is greater than that on plant height. Furthermore, the increase in plant height could be offset by selection at other plant height QTLs, although some might be associated with undesirable effects on other characteristics not measured in this study. For instance, selection for the Orria allele at SNP 11_20200 would reduce height but, as noted above, it should be verified that this would not affect grain β-glucan content if breeding for the malting market.

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