

Identification of quantitative trait loci for agronomic traits contributed by a barley (*Hordeum vulgare*) Mediterranean landrace

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Abstract. Some Spanish barley (*Hordeum vulgare* L.) landraces perform better than modern cultivars at low-production sites. The objective of this study was to identify favourable quantitative trait loci (QTLs) for interesting agronomic traits contributed by the landrace SBCC073. To achieve this objective, a population of 100 BC₁F₅ lines was derived from the cross between the elite cultivar Orria, with high productivity, and the Spanish landrace SBCC073, which was the best performer in low-production trials. The population was evaluated in field trials for 3 years (2011, 2013, and 2014) in Zaragoza, Spain. The population was genotyped with a DArTseq genotyping-by-sequencing assay. A genetic linkage map was developed by using markers of four flowering-time genes and 1227 single-nucleotide polymorphisms of good quality. The genetic map resulted in 11 linkage groups, covering a total distance of 871.1 cM. Five QTLs for grain yield were detected on 2H.1, 4H, 5H and 6H.2. Alleles from SBCC073 contributed to increased yield in three of them. A region at the end of chromosome 5H contains favourable alleles for early vigour, higher grain yield and earlier flowering, all derived from SBCC073. Alleles from Orria contributed to increasing grain yield and simultaneously to reducing plant height on the same region of 6H.2, and to increasing 1000-kernel weight on chromosomes 3H and 5H.

Additional keywords: adaptation, breeding, drought, genetic resource, introgression, SNPs.

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Introduction

The production of high-yielding varieties with stable performance across years is a challenging task for cereal breeders working within Mediterranean environments. This is partly due to the large variability in timing, duration and severity of drought stress in the Mediterranean region (Baum *et al.* 2003), which ultimately affects yield (Loss and Siddique 1994; Pswarayi *et al.* 2008).

Barley (*Hordeum vulgare* L.) landraces are acknowledged as a valuable resource for breeding in the Mediterranean region (Nevo 1992; Ceccarelli *et al.* 1995); they were selected in these same unfavourable environments, and often grown in stressful environments with nil to limited agronomic input (Ceccarelli *et al.* 2000). For instance, in Syria, landraces yielded 25–61% more than modern cultivars under stress conditions, whereas modern varieties yielded 6–18% more than landraces under optimum conditions (Ceccarelli 1996). For many crops, including barley, the genetic variability present in old landraces has not been fully exploited in modern breeding (Fischbeck 2003).

Barley landraces therefore represent an important genetic resource for barley improvement, especially for biotic stress (Jefferies *et al.* 2003; Chen *et al.* 2013; Hofmann *et al.* 2013) and abiotic stress (Yahiaoui *et al.* 2014). Many studies have

shown that barley landraces present a high level of genetic diversity for morphological traits (Ceccarelli *et al.* 1987; Lakew *et al.* 1997; Hadado *et al.* 2009), molecular markers (Demissie and Bjørnstad 1997; Russell *et al.* 2003; Yahiaoui *et al.* 2008) and agronomic traits (Yahiaoui *et al.* 2014). Research is now focused on harnessing the value of this genetic resource by building and evaluating core collections (Igartua *et al.* 1998; Bowman *et al.* 2001; Lasa *et al.* 2001; Knüpffer and van Hintum 2003). In the past, the introgression of this exotic variation was a long and difficult process because of linkage drag; however, molecular markers now provide a means to overcome this problem (Forster *et al.* 2000).

Spanish barley landraces reveal a wide genetic diversity, possibly a result of their adaptation to diverse environmental conditions. They also are quite different from current European cultivars, making them a potential new resource for barley breeding (Yahiaoui *et al.* 2008). In several studies, barley landraces from Spain have shown potential to contribute useful genes and alleles for barley breeding (Igartua *et al.* 2008; Silvar *et al.* 2010).

Some Spanish barley landraces showed good agronomic characteristics, outyielding elite barley varieties under low-productivity conditions (Yahiaoui *et al.* 2014). Landrace-derived line SBCC073 was the highest yielding in a series of

field trials, when considering only experiments in which average yield was $<3 \text{ t ha}^{-1}$. The objective of this study was to identify the genomic regions (quantitative trait loci, QTLs) for agronomic traits of interest that may be contributed by this line, crossed to an elite barley cultivar.

Material and methods

Plant material

A barley population of 100 BC_1F_5 lines was developed from the barley backcross population ($\text{SBCC073} \times \text{Orria}$) \times Orria in the framework of the Spanish public barley-breeding program, at the Aula Dei Experimental Station, Zaragoza, Spain. SBCC073 is a landrace-derived inbred line included in the Spanish Barley Core-Collection (Igartua *et al.* 1998), which outyielded 26 cultivars in a series of field trials at low-production sites across Spain (Yahiaoui *et al.* 2014). Orria, with pedigree ((($\text{Api} \times \text{Kristina}$) \times M66.85) \times Sigfrido's) \times 79W40762, is a semi-dwarf cultivar selected in Spain from a CIMMYT nursery, and is highly productive across most Spanish regions. This cultivar is an outstanding parent of the Spanish national barley-breeding program (Igartua *et al.* 2015).

Genotyping and map construction

The BC_1F_5 population was genotyped using DArTseq™ (Ren *et al.* 2015), a genotyping-by-sequencing platform provided by Diversity Arrays Technology, Canberra, ACT. This assay generated 2483 polymorphic single nucleotide polymorphism (SNP) markers. These were filtered according to different quality criteria: 177 SNP markers having $>10\%$ heterozygotes were deleted, 346 SNP markers with $>10\%$ missing data were removed, as well as 13 markers with $>75\%$ of SBCC073 alleles (which were clearly outliers in the distribution of marker allele frequencies). After filtering, 1947 SNP markers were kept. Finally, co-segregating SNPs were removed, leaving a single SNP per genetic position, reducing the number of markers to 1227. This number still ensures excellent coverage for a population of this size and with these characteristics. Flowering time genes *VrnH1*, *PpdH1*, *PpdH2* and *VrnH3* were also placed in the map. They were assayed with perfect markers by using gene-specific primers to genotype the population (see Supplementary Materials table S1 as available at journal's website). Polymerase chain reactions (PCR) were performed on a GeneAmp 2700 thermocycler (Applied Biosystems, Waltham, MA, USA), in a final volume of $15 \mu\text{L}$, containing 50 ng template DNA, $1 \times$ PCR buffer (Biotools, Madrid, Spain), 0.3 mM dNTPs, $0.15 \mu\text{M}$ each primer and 1 U of *Tth* DNA polymerase (Biotools). The amplified products were separated on agarose or acrylamide gels. DArTseq SNP markers and flowering-time genes were used to construct the genetic map and perform the QTL analyses. In a first step, JoinMap 4 (van Ooijen 2006) was used to create linkage groups at LOD (log of odds) score 7. For each linkage group, the maximum likelihood algorithm was used to estimate the best order of markers within it. In a second step, the distance between the markers within each linkage group, was recalculated, based on the Kosambi's mapping function by using MSTMAP (Wu *et al.* 2008). MapChart software (Voorrips 2002) was utilised to draw the genetic map.

Field experiments

Field experiments were carried out at the El Vedado farm, Zaragoza, Spain, during three seasons (harvests of 2011, 2013 and 2014). In 2011, the population was at the BC_1F_4 generation, whereas BC_1F_5 seed was used for the other two seasons. The experimental design was a check plot, an unreplicated design in which two checks were repeated every five test plots to provide an estimate of the error variance. Individual plots comprised four rows, each 2.5 m long, with 20 cm between rows. Two cultivars well adapted to the region, Barberousse and Cierzo, were used as checks. Sowing dates were 10 November 2010, 15 November 2012 and 11 November 2013. Yield was assessed in all three seasons, flowering time and plant height were evaluated in 2013 and 2014, and early vigour and 1000-kernel weight (TKW) were assessed in 2014.

During the three seasons, rainfall (October–June) was quite variable: 285 and 286 mm in 2010–11 and 2013–14, respectively, and 428 mm in 2012–13 (Fig. 1). Autumn and spring rainfall patterns also varied notably. Autumn 2012 was wetter than autumn 2010 or 2013. On the other hand, spring 2013 was more humid than spring 2011 or 2014, which were similar. In particular, March 2014 was much drier than the same month in 2011 or 2013 (Fig. 1). Winter in the first and third seasons was colder than in the second season. Spring (April–June) was also much warmer in the first and third seasons than in the second season (Fig. 1).

Phenotypic evaluation

Early vigour was scored after a visual assessment on 06 February 2014. Growth aspect and densities of plants were scored from 1 (poor) to 3 (excellent). Flowering time was expressed as the number of days between 1 January and the date when 50% of tillers had 2 cm of visible awns. Plant height was measured in cm for a single, representative plant per plot, from the ground to the base of the spike. Grain yield (kg ha^{-1}) was weight of grain after combine-harvesting. To account for soil irregularities among the plots, and to ensure crossability of the data across years, grain yield of each test plot was transformed to a percentage of the six closest check plots. These were the two closest flanking ones in the same tier, and the corresponding ones in the previous and the next tiers, which consisted in each case of three plots of cv. Cierzo and three plots of cv. Barberousse. TKW (g) was measured as the weight of 1000 clean grains sampled from the combine-harvested seed.

Data analyses

The QTL analyses were performed by using the procedure single-trait linkage analysis (multiple environments) implemented in GENSTAT 16 (Payne *et al.* 2009). First, genetic predictors were constructed at 2 cM. In the first round, simple interval mapping (SIM) was run. Then, QTLs detected in the SIM analysis were used as cofactors to perform composite interval mapping (CIM). CIM was run iteratively until a stable solution was found for every analysis. For all traits, the method of Li and Ji (2005), as implemented in GENSTAT, was used to estimate the significance threshold of the appropriate test statistic ($-\log_{10}P$) to declare putative QTLs. The minimum distance between cofactors and the minimum distance to declare an independent QTL were set

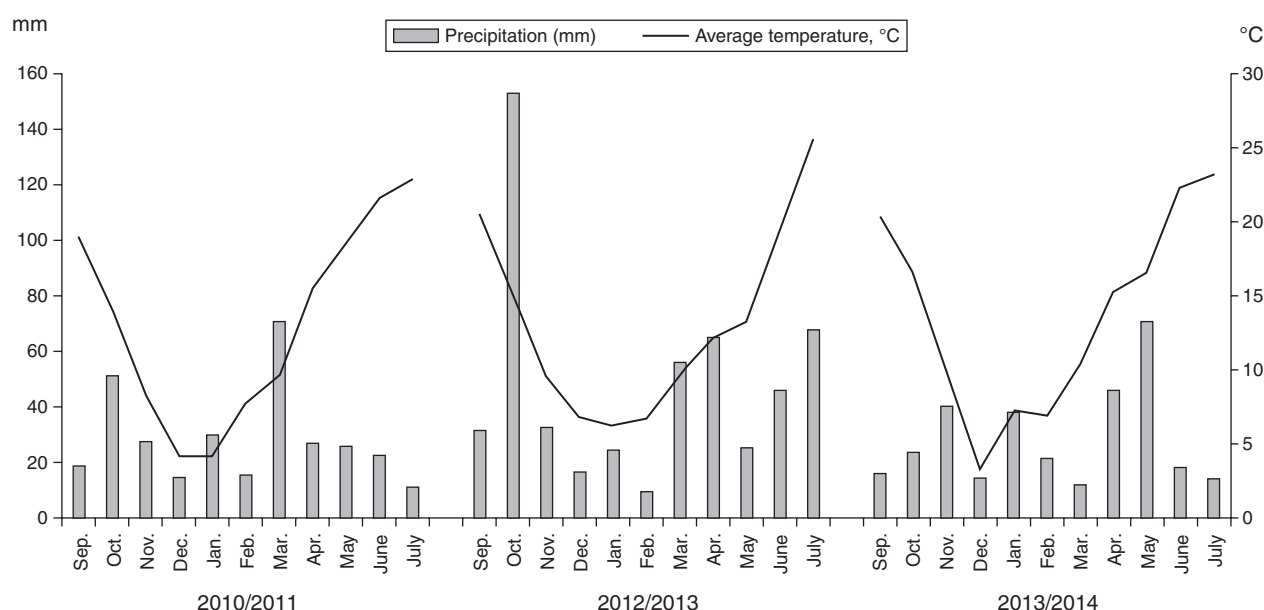


Fig. 1. Monthly average temperature and cumulative precipitations at the Zuera meteorological station, close to the location of the experiment (El Vedado, Zaragoza) during the three cropping seasons studied.

at 20 and 10 cM, respectively. In the last step, the final QTL model was built. GENSTAT 16 was also used to perform all statistical analyses for phenotypic data. Data of the check plots were analysed as a randomised complete block design, and used to calculate an approximate experimental error in order to produce a least significant difference (l.s.d.) to serve as an approximate indication of differences between test lines.

Results

Map construction

The linkage map generated with 1227 SNPs and four flowering-time genes covered a total distance of 871.1 cM. In total, 11 linkage groups were identified (Table 1), representing four whole chromosomes (1H, 3H, 4H and 5H) and three fragmented ones (chromosome 2H in 3 groups, chromosomes 6H and 7H in 2 groups each) (see Supplementary Materials fig. S1). The number of markers per chromosome ranged from 121 on 4H to 221 on 7H.

In a BC₁F₅ population, the expected ratio of allelic frequencies of SBCC073 and Orria should vary around a ratio 25 : 75. Of 1231 markers used, 60 markers displayed significant distortion from this ratio ($P < 0.05$, with a Bonferroni correction for the number of markers), 33 markers with higher than expected SBCC073 allele frequencies and 27 with an excess of the Orria allele. The distortion was in favour of SBCC073 alleles mainly on 1H and 5H, and at the end of chromosome 3H. Most of the markers of linkage group 6H.1 were distorted in favour of the Orria allele (Table 1).

Field trials

Descriptive statistics for agronomic traits

Over the 3 years of field trials, the population displayed a wide range of variation in terms of average grain yield, ranging from 1.24 t ha⁻¹ in 2014 to 5.49 t ha⁻¹ in 2013 (Table 2). Within years,

2014 presented the smallest variation and 2013 the largest. The performance of the two checks, Cierzo and Barberousse, presented the same pattern as the population (2013 > 2011 > 2014). Cierzo outyielded Barberousse and the average of the population in all 3 years. The grain yield of Barberousse was closer to the population average yield over the 3 years (Table 2). After grain yield correction with respect to the checks (Table 2), 2011 displayed the highest percentage (103%), followed by 2013 (92%), and 2014 displayed the lowest (68%), suggesting that the agronomic conditions of the 3 years were quite different. In 2014, in particular, the average yields were much lower than in previous years, indicating an important effect of drought stress (Fig. 1, Supplementary fig. S2). In this year, the population also presented a much lower average than the checks, compared with the two previous years. This

Table 1. Number of markers, length of each linkage group, and number of markers with distorted frequencies (at $P=0.05$) of the SBCC073 × Orria genetic map

Allele with higher than expected frequency is in parentheses			
Linkage group	No. of markers	Length (cM)	No. of markers with distorted frequencies
1H	132	144.3	14 (SBCC073)
2H.1	121	105.8	0
2H.2	71	52.1	0
2H.3	10	16.8	0
3H	205	144.7	5 (SBCC073)
4H	121	109.8	4 (Orria)
5H	188	169.7	10 (SBCC073)
6H.1	28	12.3	20 (Orria)
6H.2	134	44.1	2 (Orria)
7H.1	63	39.1	1 (Orria)
7H.2	158	138.2	4 (SBCC073)
Total	1231	871.1	60

Table 2. Descriptive statistics for agronomic traits measured in the SBCC073 × Orria population and checks over three years
GY, Grain yield; FT, flowering time; PH, plant height; EV, early vigour; TKW, 1000-kernel weight. For each trait, check means followed by the same letter are not significantly different at $P=0.05$ (population not included)

Trait	Year	Population			Checks		l.s.d. ($P=0.05$)
		Mean	Min.	Max.	Cierzo	Barberousse	
GY (t ha^{-1})	2011	3.96	2.50	6.91	4.15a	3.60b	0.38
	2013	5.49	2.16	8.58	6.56a	5.39b	0.46
	2014	1.24	0.16	2.41	2.12a	1.56b	0.21
GY (% of checks)	2011	102.8	66.2	162.9			
	2013	92.0	37.4	140.6			
	2014	68.1	9.2	144.5			
FT (days)	2013	120	108	125	107b	108a	0.93
	2014	111	105	121	106a	104b	0.64
PH (cm)	2013	64.1	51.0	83.0	57.3a	59.8a	2.57
	2014	36.1	22.0	49.0	35.2a	36.2a	2.23
EV	2014	1.8	1	3	2.6b	2.9a	0.25
TKW (g)	2014	36.7	27.0	44.0	33.5a	27.7b	1.02

fact indicates that, overall, the population was less stress-tolerant than the checks. An analysis of variance for corrected yield between lines, where the residual included the genotype × environment interaction, showed significant differences between lines (Supplementary table S2).

Regarding flowering time, the range of heading dates for the population was quite wide (18 and 17 days in 2013 and 2014, respectively). The two checks flowered at similar dates, and earlier than the population average (Table 2). Plant height of the population was slightly higher than of the checks, and was much greater in 2013 (64.05 cm) than in 2014 (36.12 cm), reflecting the dryness of this last season.

Grain yield was negatively correlated with flowering time in both years in which both traits were recorded (2013 and 2014), indicating that in both seasons, earlier genotypes had higher yields. The correlation between yield and plant height was low in both years (2013 and 2014). Grain yield, on the other hand, was positively correlated with early vigour (0.67) in 2014. Flowering time had negligible correlation with plant height in both years (2013 and 2014). However, it had a negative correlation with

early vigour. No correlation was found between early vigour and plant height (Supplementary table S3, fig. S3).

QTL analyses

For all traits, QTLs were found. Five QTLs were associated with grain yield, on linkage groups 2H.1, 4H (two QTLs), 5H and 6H.2 (Table 3). For the QTL on 6H.2, Orria contributed the favourable allele. For three QTL, SBCC073 contributed the yield enhancing allele, with varying effect sizes. The sign of the allelic effect for the remaining yield QTL, in linkage group 2H.1, varied between environments. There were no major QTLs across all environments, but in single environments, some of the QTLs explained large proportions of variance, for instance, 42.7% by the QTL on 6H.2 in 2013, or 32.1% by the QTL on 5H in 2014 (Table 3).

Three QTLs were found for flowering time; Orria alleles contributed to delaying flowering time on two of them (2H.1 and 5H). The SBCC073 allele on 3H was associated with later heading. The 2H.1 QTL, explaining 11.9% of the phenotypic

Table 3. QTLs for agronomic traits detected by composite interval mapping in SBCC073 × Orria population during 3 years
Additive effect is for substitution of the SBCC073 allele by the Orria allele. * $P<0.05$; n.s., not significant

Traits	Closest SNP	Chr	Position	Confidence interval	Test statistic ($-\log_{10}P$)	Additive effect			% Explained variance			QTL × year
						2011	2013	2014	2011	2013	2014	
Grain yield	3260084	2H.1	60.69	52.18–70.44	4.19	6.20*	−1.38	−9.73*	11.4	0.5	9.2	*
	3263178	4H	44.70	43.50–46.04	4.22	−4.33	−3.42	−14.56*	5.6	2.9	20.6	*
	3257648	4H	96.61	87.55–101.85	7.21	−2.12	−0.14	−12.42*	1.3	0.0	15.0	*
	3258987	5H	169.65	166.79–169.65	11.34	1.24	−7.10*	−18.16*	0.5	12.5	32.1	*
	3258547	6H.2	1.55	0.51–5.72	5.86	3.67	13.13*	2.87	4.0	42.7	0.8	*
Flowering time	3255272	2H.1	27.41	22.85–38.71	4.59		0.16	1.49*		0.1	11.9	*
	3259503	3H	56.75	55.11–60.50	3.90		−2.80*	−1.34*		25.0	9.7	*
	3257463	5H	164.96	163.08–169.65	12.43		2.44*	2.44*		19.0	32.1	n.s.
Plant height	3262819	6H.2	3.62	2.06–5.21	8.60		−5.56*	−1.08		74.3	4.8	*
Early vigour	3258022	5H	166.79	162.68–169.65	9.47			−0.5			40.1	
1000-kernel weight	3259820	3H	43.29	36.95–47.56	6.86			2.07			35.9	
	3257369	5H	122.53	112.1–132.8	4.56			1.68			23.6	

variation in 2014, is very close to *PpdH1*. A major QTL identified on 5H explained 19–32.1% of variance in 2013 and 2014, respectively. The Orria allele at this QTL delayed flowering almost 5 days in both years (Table 3). Only one major QTL for plant height was found on 6H.2, with the SBCC073 allele increasing plant height by 11.1 cm (Table 3). This QTL explained 74.3% of variance in 2013, whereas in 2014 it had a moderate effect. This QTL is within the same confidence interval as the grain yield QTL on the same chromosome (2.06–5.21 cM). The SBCC073 allele at this location is associated with taller plants and lower grain yield (Table 3). One QTL for early vigour was found, explaining a large percentage of variance (40%). It was located at the distal end of 5HL, near a yield QTL, and the allele from SBCC073 conferred increased plant vigour (Fig. 2, Table 3). Finally, two QTLs for TKW were found on 3H and 5H in

2014 (Table 3), and in both, Orria alleles contributed to increased TKW.

Only one significant interaction between grain yield QTLs was detected. In a combined analysis for the three seasons, there was an interaction for grain yield between the QTL on 2H.1 and the first QTL on 4H (96.61 cM). Only when the Orria allele was present at the 2H.1 QTL was there a detectable effect for the QTL on 4H (Table 4).

Discussion

As mentioned by Yahiaoui *et al.* (2014), some Spanish barley landraces showed good agronomic traits resulting in high yield under low-production conditions. This potential can be used in breeding programs to increase production of elite varieties for

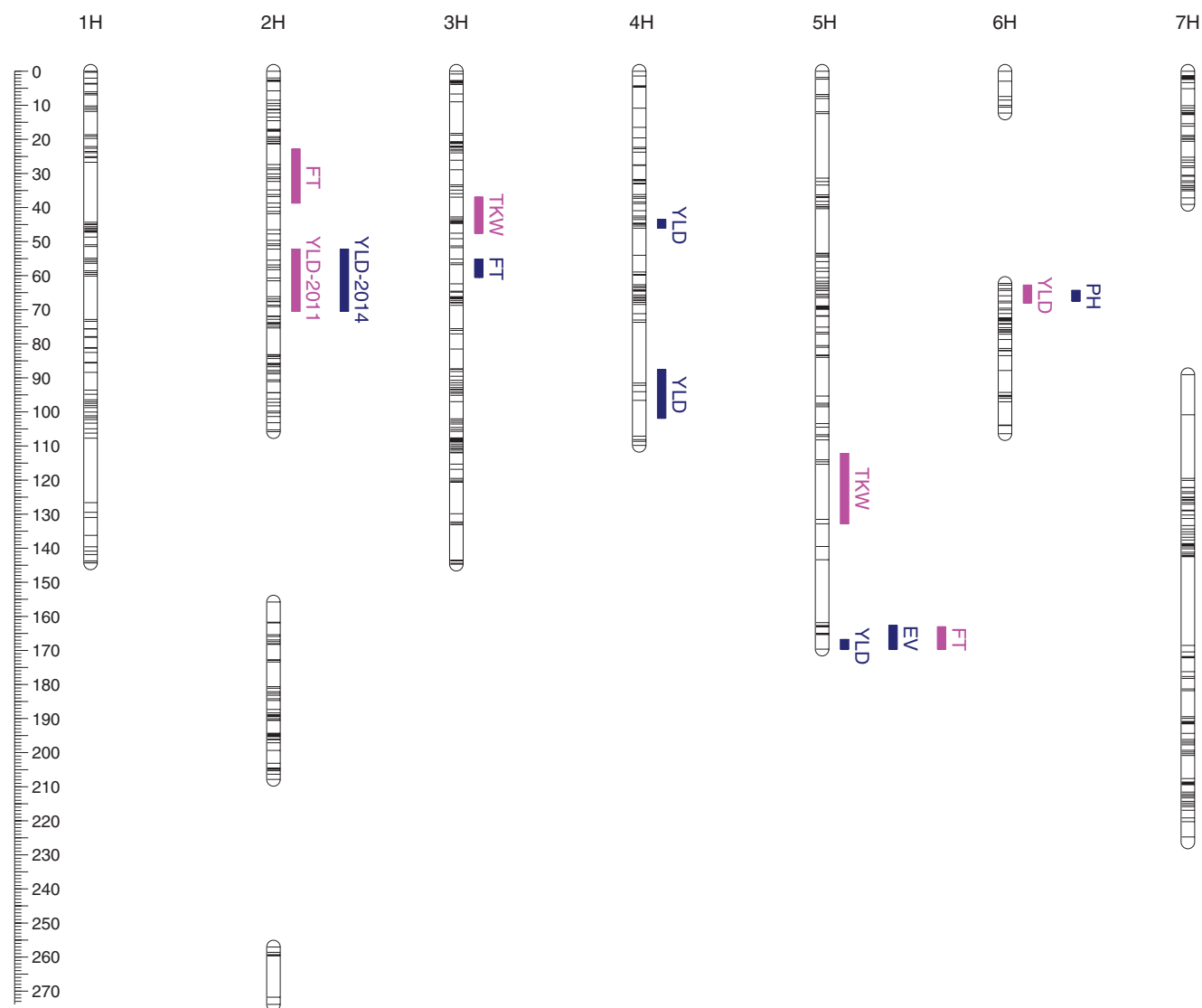


Fig. 2. Representation of QTLs found in the (SBCC073 × Orria) × Orria population on graphical chromosomes. QTL confidence intervals are shown to the right of the chromosomes. Blue (darker) bars, SBCC073 alleles produce an increase in the trait; pink (lighter) bars, Orria alleles produce an increase in the trait. YLD, Yield as percentage of checks; FT, flowering time; PH, plant height; EV, early vigour; TKW, 1000-kernel weight. The codes of the QTLs are associated with years when a qualitative interaction was observed.

Table 4. Significant interactions ($P < 0.05$) between QTLs detected for corrected yield (%)

Values followed by the same letter are not significantly different		
3256587 (2H.1)	257648 (4H)	Yield (average of 3 seasons)
SBCC073	SBCC073	92.8b
SBCC073	Orria	93.7b
Orria	SBCC073	106.6a
Orria	Orria	84.9b

stress-prone areas. In the present study, we used a population derived from the best yielding genotypes reported in that study for good conditions (cv. Orria) and poor conditions (landrace SBCC073), with the objective of localising useful genomic regions that might be related to the agronomic advantage of line SBCC073 in low-production trials.

Although no selection pressure was applied during the construction of the population, and therefore, we did not expect to find regions with allelic frequencies departing significantly from the expected, some regions presented distorted allelic frequencies. This suggests that some selection was suffered by the population, probably in response to uncontrolled environmental traits.

In Mediterranean areas, precipitation and temperature are the main factors associated with grain yield (Yau *et al.* 1991; Royo *et al.* 2014). The present QTL study was carried out over three quite varied cropping seasons. Overall, grain yield was relatively good in 2011 and 2013, and rather poor in 2014. From the climate data (Fig. 1), the two years of good yield had in common high precipitation in March. This is a crucial month for winter cereals in the region, because it is when overall tillering and the production of fertile tillers is determined (García del Moral *et al.* 1984, 1991).

In 2014, under less favourable climatic conditions, the population yielded, on average, much less than the two checks (Table 2), suggesting that the recurrent parent Orria (whose alleles constitute 75% of the genome of the lines of the population overall) was more drought-sensitive than the checks. This assumption was supported by the finding of more grain-yield QTLs for which the favourable allele came from SBCC073 in 2014 than in the other two years (2011, 2013). Apparently, the positive effects of SBCC073 were more conspicuous under low-production conditions, in agreement with its purported agronomic behaviour, as explained in the *Introduction*. Grain yield QTLs detected on 5H and 6H.2, although not always significant, at least presented effects with consistent sign across very different years, and seem the most promising QTLs for breeding. The QTLs on 4H were detected only in the year of lowest yield but, at least, do not reduce yield in the two more favourable seasons. Therefore, they do not have detrimental effects on the genotypes and so they may be considered favourable alleles for stressful conditions.

The grain-yield QTL on 5H is particularly interesting for its large effect, and because it co-locates with QTLs for early vigour and flowering time, with the favourable allele always contributed by SBCC073. This QTL on 5H shows an apparently constitutive effect of SBCC073 alleles with positive effect on yield across a range of levels of production. It is located at the distal end of the long arm, distal to all QTLs of several agronomic traits found by Tondelli *et al.* (2014), co-locating with the region of a yield QTL reported in an association panel tested under drought (Comadran

et al. 2008). In addition, von Korff *et al.* (2008) found a grain-yield QTL in this region for the Tadmor \times ER/Apm population tested under Mediterranean environments. They reported a QTL for grain yield with qualitative marker \times environment interaction. Cuesta-Marcos *et al.* (2009), working in environments similar to ours, also found two QTLs for grain yield on 5H in a doubled haploid population, but they are both proximal to our QTL. Inostroza *et al.* (2009), testing a population of introgression lines derived from wild barley, found a marker \times environment interaction for grain yield on 5HL in a similar position to the one described for our population. In that study, the *H. spontaneum* allele at the QTL decreased grain yield by 16–40%. We do not know whether this is the same QTL as the one found in our study, although the results do not suggest that. In another study, Naz *et al.* (2012) found a QTL derived from wild barley on 5HL that, when introgressed in a drought-susceptible cultivar (Scarlett), increased the root volume. In a greenhouse experiment carried out with the parents of this population (Boudiar 2014), root dry weight of SBCC073 was higher than of Orria under drought and control conditions. We do not have data to prove that the distal end of 5HL of SBCC073 is responsible for favourable root traits, but it is a hypothesis that we look to addressing in the future. The coincidence of a QTL for grain yield and early vigour on 5HL adds interest to this hypothesis. Ceccarelli *et al.* (1991) reported that early vigour is one of the traits leading to adaptation in marginal environments. Early vigour has been exploited to improve water-use efficiency and yield in wheat (Rebetzke *et al.* 2007; Richards *et al.* 2007). This relationship could be due to a more profuse root growth, which may arise from several factors, among them a more efficient nitrogen uptake (Pang *et al.* 2014). In wheat, a QTL associated with early germination was located on the long arm of chromosome 5D (Landjeva *et al.* 2010), in a syntenic region to that found in our study. These pieces of evidence suggest that a gene(s) on the distal part of chromosome 5H could underlie the relationship between early vigour and root growth. In wheat breeding, vigour of early seedling growth receives considerable attention because of its role in improving wheat performance. Researchers are currently breeding for this characteristic by using indirect traits, such as width of seedling leaves (Zhang *et al.* 2015) or seedling root growth (Atkinson *et al.* 2015). In the latter study, two QTLs for root seedling traits co-localised with QTLs for grain yield and nitrogen uptake. Interestingly, George *et al.* (2014) identified a QTL for rhizosheath weight in a barley population, in the same region as our grain yield QTL on 5HL. An alternative hypothesis compatible with the pattern of traits related with this QTL is differential response to frost damage. No direct evaluation of frost damage was done, and therefore, this hypothesis cannot be discarded. It is unlikely that frost had much effect in 2013 (absolute minimum temperature of -5.0°C). In 2011, the absolute minimum temperature was -8.8°C , but no QTL effects were detected in that year. In 2014, the years in which QTLs for grain yield and early vigour were detected concurrently, the absolute minimum temperature of -6.9°C occurred just 12 days after sowing, and may have affected seedling emergence.

Two QTLs for grain yield were found on 4H, with the SBCC073 alleles conferring higher yield. Grain-yield QTLs on that chromosome were found in linkage mapping populations

(Marquez-Cedillo *et al.* 2001; Baum *et al.* 2003) and in an association panel (Locatelli *et al.* 2013). QTLs for several grain-yield components were also found on 4H under Mediterranean conditions (Tondelli *et al.* 2014); although the position of those QTLs is not very precise, they do not seem to coincide with ours.

The grain yield QTL found on linkage group 2H.1 presented qualitative interaction depending on the year (Fig. 2). It is located distal to well-known earliness gene *EAM6*, but it falls within the confidence interval. Mansour *et al.* (2014) found a grain-yield QTL at a similar position in another population that also had Orria as one of the parents, whereas Francia *et al.* (2011) found a large effect of *EAM6* on grain yield. In these two studies, as in our case, the QTL also exhibited a strong crossover interaction between locations. In other association studies, a grain-yield QTL has also been reported in that position (Comadran *et al.* 2011; Locatelli *et al.* 2013).

In general, grain yield presented a clear negative correlation with flowering date for the 2 years with available data. Earliness has been repeatedly associated with higher yields in Mediterranean environments (Ceccarelli *et al.* 1998; Francia *et al.* 2011; Varshney *et al.* 2012; Tondelli *et al.* 2014). The apparent relationship of early vigour with grain yield observed in this population fits well with prior hypothesis linking fast early development with grain yield. The duration of growth phases of winter cereals to optimise yield in Mediterranean environments has been discussed for a long time (Ceccarelli *et al.* 1991; Slafer *et al.* 2001). In this respect, similar hypotheses were put forward for wheat (Miralles and Slafer 2007) and barley (Francia *et al.* 2011), proposing fast early development ensued by a relatively longer duration phase between jointing and (early) flowering as the best combination to attain high grain yields in Mediterranean conditions.

Three QTLs for flowering time were found on 2H.1, 3H and 5H. The QTL located on 5H is in the same region as the QTL for grain yield, and coincides with a QTL reported in a mapping population (Borràs-Gelonch *et al.* 2010). Orria alleles on 2H.1 and 5H contributed to later flowering time. Cuesta-Marcos *et al.* (2008a, 2008b) and Tondelli *et al.* (2014) detected many QTLs for flowering time under similar conditions to ours, but surprisingly, we have found no QTLs in common with theirs for this trait, except for a small QTL found on apparently the same region of 3H, only in autumn sowings (Cuesta-Marcos *et al.* 2008b). The well-known long-photoperiod-response gene *PpdH1* falls within the confidence interval for the 2H.1 QTL. The Orria allele, which carries the recessive allele at this gene, is associated with later heading. This same QTL was detected in the Orria × Plaisant population, showing a QTL × environment interaction (Mansour *et al.* 2014). It was also detected in a wide-cross population between SBCC145 and Beatrix (Ponce-Molina *et al.* 2012).

Many QTLs for plant height have been reported on all seven barley chromosomes (Backes *et al.* 1995; Sameri *et al.* 2006). The use of semi-dwarf genes has greatly improved barley yield, with controlled plant height being used to reduce yield loss arising from lodging and to increase the harvest index (Bezant *et al.* 1996; Chloupek *et al.* 2006). One of the problems associated with the use of landraces for breeding, which is also true for the Spanish landraces, is excessive plant height

(Yahiaoui *et al.* 2014). Only one QTL increasing plant height derived from SBCC073 alleles was found on 6H.2, but with a very large effect. It is near the position of the grain-yield QTL, with both favourable alleles contributed by Orria, although it is not possible to know whether this is due to linkage or pleiotropy. Mansour *et al.* (2014) found a QTL for plant height in the same region of 6H, also with the Orria allele reducing plant height. Markers from this region would be useful in breeding to select simultaneously for both traits. This finding contributes greatly to fulfil our objective for the focused introgression of favourable landrace traits in the background of Orria.

Future work with this population will address the combination of all favourable alleles in a single genotype, with the help of marker-assisted selection (MAS). BC₁F₅ lines with the highest number of favourable alleles have been selected, and crosses are planned to achieve an optimum genotype, with optimised grain yield, and keeping a maximum of other desirable agronomic traits of parent Orria.

It is possible that other Mediterranean landraces carry the same plant-height allele on chromosome 6H, and therefore, it may be of general use to introgress landrace material in current cultivars.

Conclusion

The objective of this study was to detect the QTLs responsible for the good performance of landrace line SBCC073 under low-yielding conditions, possibly related to drought stress, and to locate QTLs for undesirable traits, common to barley landraces, such as excessive plant height and low TKW. This objective was achieved, with several grain-yield QTLs being found, some of them particularly effective under low-yield conditions (year 2014) in which the Orria background showed poor adaptation. These QTLs, together with the plant height, TKW and flowering time QTLs, will allow a targeted introgression of SBCC073 favourable traits (and, possibly, from other landraces) into the Orria background through backcross MAS.

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References

- Atkinson JA, Wingen LU, Griffiths M, Pound MP, Gaju O, Foulkes MJ, Gouis JL, Griffiths S, Bennett MJ, King J, Wells DM (2015) Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *Journal of Experimental Botany* **66**, 2283–2292. doi:10.1093/jxb/erv006
- Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* **90**, 294–302. doi:10.1007/BF00222217
- Baum M, Grando S, Backes G, Jahoor A, Sabbagh A, Ceccarelli S (2003) QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' × *H. spontaneum* 41-1.

- Theoretical and Applied Genetics* **107**, 1215–1225. doi:10.1007/s00122-003-1357-2
- Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1996) Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross. *Heredity* **77**, 64–73. doi:10.1038/hdy.1996.109
- Borrás-Gelonch G, Slafer GA, Casas AM, van Eeuwijk F, Romagosa I (2010) Control of pre-heading phases and other traits related to development in a double-haploid barley (*Hordeum vulgare* L.) population. *Field Crops Research* **119**, 36–47. doi:10.1016/j.fcr.2010.06.013
- Boudiar R (2014) Identification of QTL for agronomic traits in a backcross involving a barley landrace. MSc Thesis, IAMZ-CIHEAM, Zaragoza, Spain.
- Bowman JGP, Blake TK, Surber LMM, Habernicht DK, Bockelman H (2001) Feed-quality variation in the barley core collection of the USDA National Small Grains Collection. *Crop Science* **41**, 863–870. doi:10.2135/cropsci2001.413863x
- Ceccarelli S (1996) Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity. In 'Plant adaptation and crop improvement'. (Eds M Cooper, GL Hammer) pp. 467–486. (CABI Publishing: Wallingford, UK)
- Ceccarelli S, Grando S, Van Leur JAG (1987) Genetic diversity in barley landraces from Syria and Jordan. *Euphytica* **36**, 389–405. doi:10.1007/BF00041482
- Ceccarelli S, Acevedo E, Grando S (1991) Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. *Euphytica* **56**, 169–185. doi:10.1007/BF00042061
- Ceccarelli S, Grando S, Van Leur JAG (1995) Barley landraces in the Fertile Crescent offer new breeding options for stress environments. *Diversity* **11**, 112–113.
- Ceccarelli S, Grando S, Impiglia A (1998) Choice of selection strategy in breeding barley for stress environments. *Euphytica* **103**, 307–318. doi:10.1023/A:1018647001429
- Ceccarelli S, Grando S, Tutwiler R, Baha J, Martini AM, Salahieh H, Goodchild A, Michael M (2000) A methodological study on participatory barley breeding. I. Selection phase. *Euphytica* **111**, 91–104. doi:10.1023/A:1003717303869
- Chen GD, Liu YX, Wei YM, McIntyre CL, Zhou MX, Zheng YL, Liu CJ (2013) Major QTL for Fusarium crown rot resistance in a barley landrace. *Theoretical and Applied Genetics* **126**, 2511–2520. doi:10.1007/s00122-013-2151-4
- Chloupek O, Forster BP, Thomas WTB (2006) The effect of semi-dwarf genes on root system size in field-grown barley. *Theoretical and Applied Genetics* **112**, 779–786. doi:10.1007/s00122-005-0147-4
- Comadran J, Russell J, Eeuwijk FA, Ceccarelli S, Grando S, Baum M, Stanca AM, Pecchioni N, Mastrangelo AM, Akar T, Al-Yassin A, Benbelkacem A, Choumane W, Ouabbou H, Dahan R, Bort J, Araus JL, Pswarayi A, Romagosa I, Hackett CA, Thomas WTB (2008) Mapping adaptation of barley to droughted environments. *Euphytica* **161**, 35–45. doi:10.1007/s10681-007-9508-1
- Comadran J, Russell JR, Booth A, Pswarayi A, Ceccarelli S, Grando S, Stanca AM, Pecchioni N, Akar T, Al-Yassin A, Benbelkacem A, Ouabbou H, Bort J, van Eeuwijk FA, Thomas WTB, Romagosa I (2011) Mixed model association scans of multi-environmental trial data reveal major loci controlling yield and yield related traits in *Hordeum vulgare* in Mediterranean environments. *Theoretical and Applied Genetics* **122**, 1363–1373. doi:10.1007/s00122-011-1537-4
- Cuesta-Marcos A, Casas AM, Yahiaoui S, Gracia MP, Lasa JM, Igartua E (2008a) Joint analysis for heading date QTL in small interconnected barley populations. *Molecular Breeding* **21**, 383–399. doi:10.1007/s11032-007-9139-1
- Cuesta-Marcos A, Igartua E, Ciudad FJ, Codesal P, Russell JR, Molina-Cano JL, Moralejo M, Szűcs P, Gracia MP, Lasa JM, Casas AM (2008b) Heading date QTL in a spring × winter barley cross evaluated in Mediterranean environments. *Molecular Breeding* **21**, 455–471. doi:10.1007/s11032-007-9145-3
- Cuesta-Marcos A, Casas AM, Hayes PM, Gracia MP, Lasa JM, Ciudad F, Codesal P, Molina-Cano JL, Igartua E (2009) Yield QTL affected by heading date in Mediterranean grown barley. *Plant Breeding* **128**, 46–53. doi:10.1111/j.1439-0523.2008.01510.x
- Demissie A, Bjørnstad A (1997) Geographical, altitude and agro-ecological differentiation of isozyme and hordein genotypes of landrace barleys from Ethiopia: implications to germplasm conservation. *Genetic Resources and Crop Evolution* **44**, 43–55. doi:10.1023/A:1008686009866
- Fischbeck G (2003) Diversification through breeding. In 'Diversity in barley'. (Eds R von Bothmer, T van Hintum, H Knüpfner, K Sato) pp. 147–169. (Elsevier: New York)
- Forster BP, Ellis RP, Thomas WTB, Newton AC, Tuberosa R, This D, El-Enein RA, Bahri MH, Ben Salem M (2000) The development and application of molecular markers for abiotic stress tolerance in barley. *Journal of Experimental Botany* **51**, 19–27. doi:10.1093/jexbot/51.342.19
- Francia E, Tondelli A, Rizza F, Badeck FW, Nicosia OLD, Akar T, Grando S, Al-Yassin A, Benbelkacem A, Thomas WTB, van Eeuwijk F, Romagosa I, Stanca AM, Pecchioni N (2011) Determinants of barley grain yield in a wide range of Mediterranean environments. *Field Crops Research* **120**, 169–178. doi:10.1016/j.fcr.2010.09.010
- García del Moral LF, Ramos JM, Recalde L (1984) Tillering dynamics of winter barley as influenced by cultivar and nitrogen fertilizer: A field study. *Crop Science* **24**, 179–181. doi:10.2135/cropsci1984.0011183X002400010042x
- García del Moral LF, Ramos JM, García del Moral MB, Jimenez-Tejada MP (1991) Ontogenetic approach to grain production in spring barley based on path-coefficient analysis. *Crop Science* **31**, 1179–1185. doi:10.2135/cropsci1991.0011183X003100050021x
- George TS, Brown LK, Ramsay L, White PJ, Newton AC, Bengough AG, Russell J, Thomas WTB (2014) Understanding the genetic control and physiological traits associated with rhizosheath production by barley (*Hordeum vulgare*). *New Phytologist* **203**, 195–205. doi:10.1111/nph.12786
- Hadado TT, Rau D, Bitocchi E, Papa R (2009) Genetic diversity of barley (*Hordeum vulgare* L.) landraces from the central highlands of Ethiopia: comparison between the 'Belg' and 'Meher' growing seasons using morphological traits. *Genetic Resources and Crop Evolution* **56**, 1131–1148. doi:10.1007/s10722-009-9437-z
- Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Gracia MP, Bruno Contreras MB, Wallwork H, Igartua E, Schweizer G (2013) Fine mapping of the *Rrs1* resistance locus against scald in two large populations derived from Spanish barley landraces. *Theoretical and Applied Genetics* **126**, 3091–3102. doi:10.1007/s00122-013-2196-4
- Igartua E, Gracia MP, Lasa JM, Medina B, Molina-Cano JL, Montoya JL, Romagosa I (1998) The Spanish barley core collection. *Genetic Resources and Crop Evolution* **45**, 475–481. doi:10.1023/A:1008662515059
- Igartua E, Gracia MP, Lasa JM, Yahiaoui S, Casao C, Molina-Cano JL, Moralejo M, Montoya JL, Ciudad FJ, Kopahnke D, Ordon F, Karsai I, Szucs P, Casas AM (2008) Barley adaptation to Mediterranean conditions: Lessons learned from the Spanish landraces. In 'Proceedings 10th International Barley Genetics Symposium'. Alexandria, Egypt. (Eds S Ceccarelli, S Grando) pp. 205–214. (ICARDA Publishing: Aleppo, Syria)
- Igartua E, Mansour E, Cantalapiedra CP, Contreras-Moreira B, Gracia MP, López-Fuster P, Escribano J, Molina-Cano JL, Moralejo M, Ciudad FJ, Thomas WTB, Karsai I, Casas AM (2015) Selection footprints in barley breeding lines detected by combining genotyping-by-sequencing with reference genome information. *Molecular Breeding* **35**, doi:10.1007/s11032-015-0194-8

- Inostroza L, del Pozo A, Matus I, Castillo D, Hayes P, Machado S, Corey A (2009) Association mapping of plant height, yield, and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. *Molecular Breeding* **23**, 365–376. doi:10.1007/s11032-008-9239-6
- Jefferies SP, King BJ, Barr AR, Warner P, Logue SJ, Langridge P (2003) Marker-assisted backcross introgression of the *Yd2* gene conferring resistance to barley yellow dwarf virus in barley. *Plant Breeding* **122**, 52–56. doi:10.1046/j.1439-0523.2003.00752.x
- Knüpfner H, van Hintum T (2003) Summarised diversity—the barley core collection. In 'Diversity in barley'. (Eds R von Bothmer, T van Hintum, H Knüpfner, K Sato) pp. 259–267. (Elsevier: New York)
- Lakew B, Semeane Y, Alemayehu F, Gebre H, Grando S, van Leur JA, Ceccarelli S (1997) Exploiting the diversity of barley landraces in Ethiopia. *Genetic Resources and Crop Evolution* **44**, 109–116. doi:10.1023/A:1008644901982
- Landjeva S, Lohwasser U, Börner A (2010) Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth. *Euphytica* **171**, 129–143. doi:10.1007/s10681-009-0016-3
- Lasa JM, Igartua E, Ciudad FJ, Codesal P, Garcia EV, Gracia MP, Medina B, Romagosa I, Molina-Cano JL, Montoya JL (2001) Morphological and agronomical diversity patterns in the Spanish barley core collection. *Hereditas* **135**, 217–225. doi:10.1111/j.1601-5223.2001.00217.x
- Li J, Ji L (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* **95**, 221–227. doi:10.1038/sj.hdy.6800717
- Locatelli A, Cuesta-Marcos A, Gutiérrez L, Hayes PM, Smith KP, Castro AJ (2013) Genome-wide association mapping of agronomic traits in relevant barley germplasm in Uruguay. *Molecular Breeding* **31**, 631–654. doi:10.1007/s11032-012-9820-x
- Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* **52**, 229–276. doi:10.1016/S0065-2113(08)60625-2
- Mansour E, Casas AM, Gracia MP, Molina-Cano JL, Moralejo M, Cattivelli L, Thomas WTB, Igartua E (2014) Quantitative trait loci for agronomic traits in an elite barley population for Mediterranean conditions. *Molecular Breeding* **33**, 249–265. doi:10.1007/s11032-013-9946-5
- Marquez-Cedillo LA, Hayes PM, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2001) QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. *Theoretical and Applied Genetics* **103**, 625–637. doi:10.1007/PL00002919
- Miralles DJ, Slafer GA (2007) Sink limitations to yield in wheat: how could it be reduced? *The Journal of Agricultural Science* **145**, 139–149. doi:10.1017/S0021859607006752
- Naz AA, Ehl A, Pillen K, Léon J (2012) Validation for root-related quantitative trait locus effect of wild origin in the cultivated background of barley (*Hordeum vulgare* L.). *Plant Breeding* **131**, 392–398. doi:10.1111/j.1439-0523.2012.01972.x
- Nevo E (1992) Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in Fertile Crescent. In 'Barley genetics, biochemistry, molecular biology and biotechnology'. (Ed. PR Sherwy) pp. 19–43. (CAB International: Wallingford, UK)
- Pang J, Palta JA, Rebetzke GJ, Milroy SP (2014) Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. *Functional Plant Biology* **41**, 215–222. doi:10.1071/FP13143
- Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM (2009) 'GENSTAT for Windows. Introduction.' 12th edn (VSN International: Hemel Hempstead, UK)
- Ponce-Molina LJ, Casas AM, Gracia PM, Silvar C, Mansour E, Thomas WB, Schweizer G, Herz M, Igartua E (2012) Quantitative trait loci and candidate loci for heading date in a large population of wide barley cross. *Crop Science* **52**, 2469–2480. doi:10.2135/cropsci2012.01.0029
- Pswarayi A, Van Eeuwijk FA, Ceccarelli S, Grando S, Comadran J, Russell JR, Francia E, Pecchioni N, Li Destri O, Akar T, Al-Yassin A, Benbelkacem A, Choumane W, Karrou M, Ouabbou H, Bort J, Araus JL, Molina-Cano JL, Thomas WTB, Romagosa I (2008) Barley adaptation and improvement in the Mediterranean basin. *Plant Breeding* **127**, 554–560. doi:10.1111/j.1439-0523.2008.01522.x
- Rebetzke GJ, Ellis MH, Bonnett DG, Richards RA (2007) Molecular mapping of genes for coleoptiles growth in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **114**, 1173–1183. doi:10.1007/s00122-007-0509-1
- Ren R, Ray R, Li P, Xu J, Zhang M, Liu G, Yao X, Kilian A, Yang X (2015) Construction of a high-density DArTseq SNP-based genetic map and identification of genomic regions with segregation distortion in a genetic population derived from a cross between feral and cultivated-type watermelon. *Molecular Genetics and Genomics* doi:10.1007/s00438-015-0997-7
- Richards RA, Watt M, Rebetzke GJ (2007) Physiological traits and cereal germplasm for sustainable agricultural systems. *Euphytica* **154**, 409–425. doi:10.1007/s10681-006-9286-1
- Royo C, Nazco R, Villegas D (2014) The climate of the zone of origin of Mediterranean durum wheat (*Triticum durum* Desf.) landraces affects their agronomic performance. *Genetic Resources and Crop Evolution* **61**, 1345–1358. doi:10.1007/s10722-014-0116-3
- Russell JR, Booth A, Fuller JD, Baum M, Ceccarelli S, Grando S, Powell W (2003) Patterns of polymorphism detected in the chloroplast and nuclear genomes of barley landraces sampled from Syria and Jordan. *Theoretical and Applied Genetics* **107**, 413–421. doi:10.1007/s00122-003-1261-9
- Sameri M, Takeda K, Komatsuda T (2006) Quantitative trait loci controlling agronomic traits in recombinant inbred lines from a cross of oriental- and occidental-type barley cultivars. *Breeding Science* **56**, 243–252. doi:10.1270/jsbbs.56.243
- Silvar C, Casas AM, Kopahnke D, Habekus A, Schweizer G, Gracia MP, Lasa JM, Ciudad FJ, Molina-Cano JL, Igartua E, Ordon F (2010) Screening the Spanish barley core collection for disease resistance. *Plant Breeding* **129**, 45–52. doi:10.1111/j.1439-0523.2009.01700.x
- Slafer GA, Abeledo LG, Miralles DJ, Gonzalez FG, Whitechurch EM (2001) Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. In 'Wheat in a global environment'. pp. 487–496. (Springer: Dordrecht, The Netherlands)
- Tondelli A, Francia E, Visioni A, Comadran J, Mastrangelo AM, Akar T, Al-Yassin A, Ceccarelli S, Grando S, Benbelkacem A, van Eeuwijk FA, Thomas WTB, Stanca AM, Romagosa I, Pecchioni N (2014) QTLs for barley yield adaptation to Mediterranean environments in the 'Nure' × 'Tremois' biparental population. *Euphytica* **197**, 73–86. doi:10.1007/s10681-013-1053-5
- van Ooijen JW (2006) 'JoinMap 4, software for the calculation of genetics linkage maps in experimental populations.' (Kyazma B.V.: Wageningen, The Netherlands)
- Varshney RK, Paulo MJ, Grando S, Van Eeuwijk FA, Keizer LCP, Guo P, Ceccarelli S, Kilian A, Baum M, Graner A (2012) Genome wide association analyses for drought tolerance related traits in barley (*Hordeum vulgare* L.). *Field Crops Research* **126**, 171–180. doi:10.1016/j.fcr.2011.10.008
- von Korff M, Grando S, Del Greco A, This D, Baum M, Ceccarelli S (2008) Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley. *Theoretical and Applied Genetics* **117**, 653–669. doi:10.1007/s00122-008-0787-2

- Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity* **93**, 77–78. doi:[10.1093/jhered/93.1.77](https://doi.org/10.1093/jhered/93.1.77)
- Wu Y, Bhat PR, Close TJ, Lonardi S (2008) Efficient and accurate construction of genetic linkage maps from the Minimum Spanning Tree of a graph. *PLOS Genetics* **4**, e1000212. doi:[10.1371/journal.pgen.1000212](https://doi.org/10.1371/journal.pgen.1000212)
- Yahiaoui S, Igartua E, Moralejo M, Ramsay L, Molina-Cano JL, Ciudad FJ, Lasa JM, Gracia MP, Casas AM (2008) Patterns of genetic and eco-geographical diversity in Spanish barleys. *Theoretical and Applied Genetics* **116**, 271–282. doi:[10.1007/s00122-007-0665-3](https://doi.org/10.1007/s00122-007-0665-3)
- Yahiaoui S, Cuesta-Marcos A, Gracia MP, Medina B, Lasa JM, Casas AM, Ciudad FJ, Montoya JL, Moralejo M, Molina-Cano JL, Igartua E (2014) Spanish barley landraces outperform modern cultivars at low productivity sites. *Plant Breeding* **133**, 218–226. doi:[10.1111/pbr.12148](https://doi.org/10.1111/pbr.12148)
- Yau SK, Ortiz-Ferrara G, Srivastava JP (1991) Classification of bread wheat-growing environments based on differential yield responses. *Crop Science* **31**, 571–576. doi:[10.2135/cropsci1991.0011183X003100030004x](https://doi.org/10.2135/cropsci1991.0011183X003100030004x)
- Zhang L, Richards RA, Condon AG, Liu DC, Rebetzke GJ (2015) Recurrent selection for wider seedling leaves increases early biomass and leaf area in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **66**, 1215–1226. doi:[10.1093/jxb/eru468](https://doi.org/10.1093/jxb/eru468)