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QTLs for barley yield adaptation to Mediterranean environments in the 'Nure' × 'Tremois' biparental population

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Abstract Multi-environment trials represent a highly valuable tool for the identification of the genetic bases of crop yield potential and stress adaptation. A Diversity Array Technology®-based barley map has been developed in the 'Nure' × 'Tremois' biparental Doubled Haploid population, harbouring the genomic position of a gene set with a putative role in the regulation of flowering time and abiotic stress response in barley. The population has been evaluated in eighteen location-by-year combinations across the Mediterranean basin. QTL mapping identified several genomic regions responsible for barley adaptation to Mediterranean conditions in terms of phenology, grain yield and yield component traits. The most frequently detected yield QTL had the early flowering *HvCEN_EPS2* locus (chromosome 2H) as peak marker, showing a positive effect from the early winter parent 'Nure' in eight field trials, and explaining up to 45.8 % of the observed variance for grain yield. The *HvBM5A_VRN-H1* locus on chromosome 5H and the genomic region possibly corresponding to *PPD-H2* on chromosome 1H were significantly associated to grain yield in five and three locations, respectively. Environment-specific QTLs for grain yield, and clusters

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of yield component QTLs not related to phenology and or developmental genes (e.g. on chromosome 4H, BIN_09) were observed as well. The results of this work provide a valuable source of knowledge and tools for both explaining the genetic bases of barley yield adaptation across the Mediterranean basin, and using QTL-associated markers for MAS pre-breeding and breeding programmes.

Keywords Barley · QTL mapping · Multi-environment trial · Mediterranean conditions

Introduction

Barley (*Hordeum vulgare* L.) is the predominant crop of the driest Mediterranean areas (<300 mm annual rainfall), where it often represents the main source of livelihood together with durum wheat (*Triticum turgidum* ssp. *durum*). In these environments yield and quality of barley are heavily affected by drought, whose recurrence is even likely to increase in the future, in terms of both frequency and severity (Bolle 2003). Improving crop production under conditions of water scarcity has to be based on both a better exploitation of water in agriculture by using soil moisture conservative cultural practices and breeding for varieties with higher productivity under water limiting conditions.

Even if breeding activities have led to some yield increase in drought-prone environments for barley and other cereals, a gap is still present between yields in optimal and stressed environments. Breeding strategies in drought-prone environments should consider the nature, timing and intensity of the stress events can vary significantly across regions and years, thus cultivars designed to cope with a specific type of drought may underperform when the stress conditions are different or multiple (Ceccarelli 1989; Cattivelli et al. 2008). Because of this strong genotype-by-environment (GE) interaction, selection for yield potential in high yielding conditions has almost always led to some breeding progress under moderate drought conditions (Rizza et al. 2004; Araus et al. 2002, 2008). This implies that traits maximizing productivity normally expressed in the absence of stress, can still sustain a significant yield improvement under mild to moderate drought (Slafer et al. 2005; Tambussi et al. 2005).

In small grain cereals, genetic gain in yield potential has been associated to changes in physiological traits related to time to flowering and plant height, biomass production and partitioning, and yield components such as number of fertile ears per plant and grain number and size (Komatsuda et al. 2007; Ramsay et al. 2011; Araus et al. 2008). In particular, the synchronization of crop cycle with the most favourable environmental conditions is fundamental for maximising yield potential and adaptiveness through the best use of resources (e.g. water and radiant energy) and the avoidance of stress events during growth and grain filling (Slafer et al. 2005; Reynolds et al. 2009). For example, it is well known that a good level of earliness is an effective breeding strategy for enhancing yield in Mediterranean environments where wheat and barley are commonly exposed to terminal drought stress, even if extreme earliness could lead to yield penalty in fertile conditions (Cuesta-Marcos et al. 2008a). In cereals, phenological adjustments are mainly driven by a few well-known photoperiod (*PPD*) and vernalization (*VRN*) responsive genes, as well as earliness per se or early maturity loci (*EPS/EAM*) that affect life-cycle timing independently of these stimuli (Cockram et al. 2007; Distelfeld et al. 2009; Comadran et al. 2012; Higgins et al. 2010). Changes in physiological traits associated with main yield components have been revealed from retrospective studies on wheat. For example, it has been observed that selection for high yield under Mediterranean drought-prone conditions mainly resulted in increasing the number of grains per unit land area rather than mean grain weight (Acreche et al. 2008). This could be related to changes in growth

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partitioning over the phase of stem elongation, immediately before anthesis (Araus et al. 2008).

The advancements in crop physiology and genomics allow nowadays a multidisciplinary approach for the study of cereal adaptation to water-limiting conditions (as reviewed by Tuberosa and Salvi 2006; Cattivelli et al. 2008; Reynolds et al. 2009). In particular, multi-environment trials (METs) conducted with populations of genetically related individuals (i.e. mapping populations), or wide germplasm collections, can help in understanding the genetic basis of grain yield, as well as the morpho-physiological and phenological traits determining yield potential and stability in dry and wet conditions, while dissecting the genetic basis of the GE interaction. Agronomic evaluation of experimental populations under Mediterranean environments resulted in the identification of genomic regions underlying QTL with major and stable effects (Teulat et al. 2001; Baum et al. 2003; Talamé et al. 2004; Comadran et al. 2011; Cuesta-Marcos et al. 2008a; von Korff et al. 2008). Such loci provide the breeders with new knowledge and molecular tools for improving small-grain cereals in terms of yield potential and broad adaptation to the environment. Moreover, the generation of molecular-linkage maps based on candidate genes (molecular-function maps) can shorten the way toward the identification of the genetic determinants of QTLs (Tondelli et al. 2006; Stein et al. 2007), and this can take advantage by the recent release of a sequence assembly of the barley genome gene space (Mayer et al. 2012).

In recent years the EU INCO-MED funded project MABDE ('Mapping Adaptation of Barley to Drought-ted Environments') has brought significant advancements in understanding the processes underlying barley adaptation to Mediterranean environments and the consequences of barley breeding carried out in the last century, through the accumulation of exhaustive agronomic, physiologic and molecular marker datasets (Pswarayi et al. 2008a, b; Comadran et al. 2008, 2009, 2011; Francia et al. 2011, 2013). Within this frame, Francia et al. (2011) described the relationships among a series of characters defining grain yield as a function of the length of the different barley developmental phases. Data were collected on a barley segregating population derived from the cross between two elite cultivars, representative of the Mediterranean winter and central European spring barley germplasm-pools, namely 'Nure' and 'Tremois' (Francia et al. 2004). In this biparental cross are segregating at least two loci for vernalization requirement (*VRN-H1* and *VRN-H2*), one locus for response to photoperiod (*PPD-H2*) and one locus for earliness per se (*EPS2*), while *VRN-H3* and *PPD-H1* are genetically fixed. In the present study, we aim to improve our understanding about barley grain yield adaptation to Mediterranean environments through: (1) increasing the marker density of the 'Nure' × 'Tremois' molecular linkage map; (2) identifying, from METs, the QTLs responsible for the adaptation of barley crop to a wide range of Mediterranean environments, in terms of phenology, grain yield and yield components.

Materials and methods

Plant material

Barley Doubled Haploid (DH) lines were derived by anther culture from the F1 of the 'Nure' × 'Tremois' cross (Francia et al. 2004). 'Nure'—[('Fior 40' × 'Alpha'^{2}) × 'Baraka']—is a winter, two-rowed variety, adapted to South European environments, showing high yield potential and yield stability in irrigated as well as in moderately stressed conditions (400 mm rainfall; Rizza et al. 2004). 'Tremois'—[('Dram' × 'Aramir') × 'Berac']—is a spring, high yielding two-rowed malting cultivar, adapted to high-input conditions. In the frame of the MABDE project, a subset of 118 lines from the 'Nure' × 'Tremois' (hereafter designed NT) population was randomly chosen. Pure stock seed of the NT-DH lines was multiplied at ICARDA in the harvest year 2003 to allow for METs in the subsequent harvest seasons 2004 and 2005. Genomic DNAs extracted from the same population were used for molecular marker analyses in the present study.

Genotyping

A 'Nure' × 'Tremois' low resolution linkage map was previously described by Francia et al. (2004). Here, Diversity Array Technology® (DArT) marker assays were performed by Triticarte Pty Ltd (Australia) to enhance map coverage. The NTs were genotyped with an identical set of DArT markers from a *Pst*I/*Bst*NI genomic representation ('*bPb*' markers), as described by Wenzl et al. (2004).

DArT genetic map has been complemented with 35 informative molecular markers on candidate genes known to be involved in the regulation of barley phenology and stress response. We expanded the 20 candidate genes NT's map location reported in previous publications (Tondelli et al. 2006; Francia et al. 2007) with 15 additional candidate genes mapped in the present work. Candidate gene fragment amplification and sequencing were performed by using specific PCR primer pairs (Table S1). Sequence assembly in the software package Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) assisted in the identification of Single Nucleotide Polymorphisms (SNPs) and INsertion/DELetions (INDELs). Based on the polymorphism type, new CAPS markers were developed for mapping 13 candidate genes (Table S1). 5 µl of PCR product were incubated for 1.5 h with 1 U of restriction enzyme, 1× reaction buffer and 0.1 mg/ml of bovine serum albumin, and then separated on a standard 2 % agarose gel. The remaining *TC-MYB1* and *HvBPBF* candidate genes were genotyped by single strand conformation polymorphism acrilamyde gel as described by Tondelli et al. (2006).

Protocols described above were also adopted for SNP detection and mapping of three 'scsnp' markers (scsnp02737, scsnp00177, scsnp15296; Rostoks et al. 2005), after amplification with primers downloaded from the Germinate database (http://germinate.scri.ac.uk/barley_snpdb/index.html). In addition, fluorescently labelled primer pairs for PCR amplification of nine 'scssr' and one 'scind' markers (Rostoks et al. 2005) were obtained from the Germinate database. Reactions were performed following standard protocols and fragment length polymorphisms were checked on an ABI PRISM 3130xl Genetic Analyzer.

Phenotyping

In the frame of the MABDE project, 18 multi-environment field trials were conducted in six countries of the Mediterranean basin: Algeria (DZA), Italy (ITA), Jordan (JOR), Spain (ESP), Syria (SYR) and Turkey (TUR) for two harvest seasons (2004 and 2005; Table 1). In each country, "wet" (W) and "dry" (D) trials were either grown at sites contrasting for natural rainfall (high vs. low, based on past meteorological data), or sown at the same site with one trial being supplied with supplementary irrigation and the other rainfed (Table 1, see also Francia et al. 2011, 2013). Field experimental designs consisted of a replicated trial with two replicates for 'Nure', 'Tremois' and the 118 NTs, augmented by four check entries repeated 15 times in a systematic diagonal fashion to adjust for spatial variation. The first check (cv 'Harmal') was common to all sites and the other three checks were a local landrace, a local old and a local new cultivar relevant to each country. At each site, trials were sown in a rectangular grid of 15 rows and 20 columns, with 6 m^2 plots, and were grown according to local practice for sowing rate and other inputs.

The following traits defining grain yield, yield components and plant phenology were recorded on a plot basis for each trial: grain yield (Yld) in t ha^{-1}, number of spikes per square meter (Ssm), number of grains per spike (Gps), 1,000 grain weight (Tgw) in gram, harvest index (Hi), early growth vigour (Ev) as a visual score from 0 = poor vigour to 5 = good vigour, frost resistance (Fr) as a visual score from 0 = no damage to 5 = all plants killed, plant height (Ht) in cm to the bottom of the spike, days from sowing to heading (Hd), days from sowing to physiological maturity (Md), spike length (Sl) in cm, peduncle length (Pl) in cm from the last node to the bottom of the spike, peduncle extrusion (Pe) in cm from the ligule of the flag leaf to the bottom of the spike, reaction to powdery mildew (Pm) as a visual score from 0 = free to 9 = severe attack. For each trait, the number of analysed field trials depends on the availability of suitable data (Table S2).

Statistical and QTL analyses

Genotyping information was recorded for each marker and segregation data entered into a population file (available from GrainGenes at http://wheat.pw.usda.gov/GG2/index.shtml) that also included previously published marker data. JoinMap 4 (Van Ooijen 2006) was used for grouping of markers (LOD score = 4.0) and subsequent determination of marker order (minimum LOD score = 1.0, recombination threshold = 0.4, ripple value = 1, jump threshold = 5). The Kosambi mapping function was applied for converting recombination units into genetic distances through the regression mapping algorithm. In order to avoid a contradictory placement of loci that occurred occasionally, individual maps were recalculated by setting individual loci at 'fixed order'.

Table 1 Trial sites of the 'Nure' × 'Tremois' mapping population for harvest years 2004 and 2005

#	Code	Location—watering[a]	Water input[b] (mm)	Sowing date	Heading date[c]	Yield (t ha^{-1})[c]
1	JOR_4W	Rabba—Wet/Rainfed	194	23/12/2003	n/a	0.07
2	ESP_5D	Foradada—Dry/Rainfed	167	11/11/2004	08/05/2005	0.48
3	JOR_5D	Ramtha—Dry/Rainfed	140	28/12/2004	15/04/2005	0.51
4	JOR_5W	Rabba—Wet/Rainfed	217	28/12/2004	03/04/2005	0.81
5	JOR_4D	Ramtha—Dry/Rainfed	151	16/12/2003	n/a	1.32
6	SYR_4D	Breda—Dry/Rainfed	204	07/12/2003	n/a	1.36
7	SYR_5D	Breda—Dry/Rainfed	143	02/01/2005	n/a	2.35
8	ITA_4D	Foggia—Dry/Rainfed	258	13/01/2004	05/05/2004	3.19
9	TUR_4D	Haymana—Dry/Rainfed	232	03/11/2003	24/05/2004	3.28
10	DZA_5W	El Khroub—Wet/Rainfed	130	19/02/2005	26/05/2005	3.50
11	ITA_4W	Foggia—Wet/Irrigated	327	08/01/2004	06/05/2004	3.77
12	ITA_5D	Foggia—Dry/Rainfed	268	16/12/2004	01/05/2005	3.81
13	TUR_5	Haymana—Dry/Rainfed	174	21/03/2004	31/05/2004	3.86
14	SYR_4W	Tel Hadya—Wet/Rainfed	290	11/12/2003	14/04/2004	4.13
15	TUR_4W	Haymana—Wet/Irrigated	282	03/11/2003	n/a	4.45
16	ITA_5F	Fiorenzuola—Wet/Rainfed	292	17/11/2004	13/05/2005	4.56
17	ITA_5W	Foggia—Wet/Irrigated	362	16/12/2004	30/04/2005	4.86
18	SYR_5W	Tel Hadya—Wet/Rainfed	192	13/12/2004	14/04/2005	5.43

DZA Algeria, *ESP* Spain, *ITA* Italy, *JOR* Jordan, *SYR* Syria, *TUR* Turkey

[a] Sites are classified according to previous meteorological data; in some case the Wet site was created artificially by supplementary irrigation supplied during the growing season

[b] Total rainfall plus irrigation (mm) from sowing to harvest

[c] Mean value across each environment; values <2.5 t ha^{-1} and >2.5 t ha^{-1} represent low and high yielding environments, respectively

The phenotypic data collected in each environment were analysed by mixed models in GenStat version 11 (Payne et al. 2008). Genotypes were first considered random to estimate genotypic and phenotypic variance components and broad sense heritability (h^2). Then, Best Linear Unbiased Estimates (BLUEs) were generated for each NT line by considering entries and repeated checks as fixed effects and rows and columns as random effects; genotypic BLUEs were used for all the subsequent analyses. Main Genotypic and Environmental effects, GE interaction and correlations for any pair of characters of the same dataset have been already described in Francia et al. (2011). In the present paper we focused on the QTL analyses in the NT population, by using the software MapQTL 5 (Van Ooijen 2004). For any trait/environment combination, a LOD threshold value defining the genome-wide significance ($P < 0.05$) of a putative QTL was obtained by permutation tests (1,000 replications). Simple interval mapping analysis was performed at a 1 cM interval and the marker closest to each LOD peak was selected as cofactor in a MQM mapping analysis (namely composite interval mapping, CIM).

Results

A DArT-based linkage map for the 'Nure' × 'Tremois' mapping population

A total of 396 DArT, 18 STS-SNP and 10 SSR loci have been added to the 'Nure' × 'Tremois' molecular linkage map already available. The NT map is now composed of 543 markers, spanning a total length of 1,114 cM, with an average resolution of one marker every 2.8 cM (Fig. 1; http://wheat.pw.usda.gov). Individual linkage groups length ranges from 117.7 cM (1H) to 203.3 cM (5H), and alignment with

the barley consensus map built by Wenzl et al. (2006) showed a high level of conservation of DArT locus order (data not shown). The same genotypic dataset has also been recently used for the construction of a barley high resolution consensus map (Aghnoum et al. 2010). However, six map coverage gaps larger than 20 cM are still present in the NT linkage map and a lower marker density can be noticed in particular for the chromosome 4H. Segregation distortion was observed in several genomic regions, especially on chromosomes 1HL and 6H (http://wheat.pw.usda.gov). However, this should not significantly affect QTL analyses, as described by Xu (2008).

Thirty-five candidate genes encoding mainly barley transcription factors have been mapped up to now on the NT linkage function map (in bold italic in Fig. 1). It is noteworthy that the asset of the current map includes flowering time genes. Sequencing in both 'Nure' and 'Tremois' of the genomic region spanning *HvPRR*, the genetic determinant of *PPD-H1* on chromosome 2H (Turner et al. 2005), did not reveal any polymorphism. In fact, the same recessive, late-flowering *ppd-H1* allele is present in both parental genotypes. In order to determine the position of this major developmental gene on our map the first useful, tightly associated polymorphism was identified 7.5 Kb upstream the *HvPRR* start codon, based on the sequence of the 'Morex' BAC clone Hv673I14 (that contains *HvPRR*; Turner et al. 2005). Similarly, no sequence polymorphism has been detected within the coding region of *HvFT1*, the gene underlying *VRN-H3* on barley chromosome 7H (Yan et al. 2006). Both 'Nure' and 'Tremois' carry the recessive *vrn-H3* allele, more frequent in barley varieties, and due to this it was impossible to map it. Wang et al. (2010) mapped *VRN-H3* between the Bmag0007 and EBmac0603 SSR markers on the BC_2DH advanced backcross population S42. The same microsatellite markers are present on chromosome 7H in the NT map (BIN_01 and BIN_04, respectively; Fig. 1), thus defining a relative position of the locus. A CAPS marker has been developed for the mapping of the 'Morex' BAC clone Hv347D22, that harbours *HvFT3*, the candidate gene for *PPD-H2* on barley chromosome 1H (Faure et al. 2007), and a third gene belonging to the *FT* gene family (*HvFT4*; Faure et al. 2007) has been positioned at the peri-centromeric region of chromosome 2H (Fig. 1). As reported by Comadran et al. (2012), we previously identified the phosphatidylethanolamine-binding protein gene *HvCEN* as the determinant of the *EPS2* locus on chromosome 2H (78.7 cM). The major loci for vernalization requirement (*VRN-H1* and *VRN-H2*) have been previously mapped in the NT linkage function map (Francia et al. 2004; von Zitzewitz et al. 2005). The *VRN-H1* candidate gene *HvBM5A* is a member of the AP1/SQUA subfamily of MADS box genes; other members of the same subfamily, *HvBM8* and *HvBM3* (Schmitz et al. 2000), segregate along with *HvCEN* on 2H, *HvBM8* cosegregating with it, and *HvBM3* a few cM away (Fig. 1; Table S1).

Grain yield across the Mediterranean basin

In the different Mediterranean environments, average grain yield ranged between 0.07 t ha^{-1} (#1-JOR_4W) and 5.43 t ha^{-1} (#18-SYR_5W) (Table S3). In seven trials this value was below 2.5 t ha^{-1}, previously suggested as the crossover point at which cultivars with high yield potential could produce less than cultivars with lower yield potential, but better adapted to stress (Ceccarelli and Grando 1991; von Korff et al. 2008). With the exception of #6-SYR_4D, #10-DZA_5W and #17-ITA_5W, the 'Nure' parent always outperformed for grain yield the 'Tremois' parent in the different field trials. Transgressive segregation has been observed for yield and flowering time in all trials (Table S3), probably due to the very different genetic background of the two parental varieties. Broad sense heritability (h^2) ranged from 16 % (#14-SYR_4W) to 77 % (#7-SYR_5D) for Yld, and from 42 % (#10-DZA_5W) to 97 % (#18-SYR_5W) for Hd (Table S3). A more detailed description of the environments, including meteorological data collected over the two-year experiment, and their impact on the IBMP performance has already been reported elsewhere (Francia et al. 2011).

QTLs for yield adaptation to Mediterranean conditions

Composite interval mapping (CIM) analyses for yield revealed eight QTL on four barley chromosomes (Table 2; Fig. 1). Among them, five loci were identified in single field trials, while three in multiple environments. The most frequently detected QTL maps on chromosome 2H, BIN_07.2 (8 trials), followed by a locus on chromosome 5H, BIN_10.5 (5 trials), and a region corresponding to chromosome 1H, BIN_11.3 (3

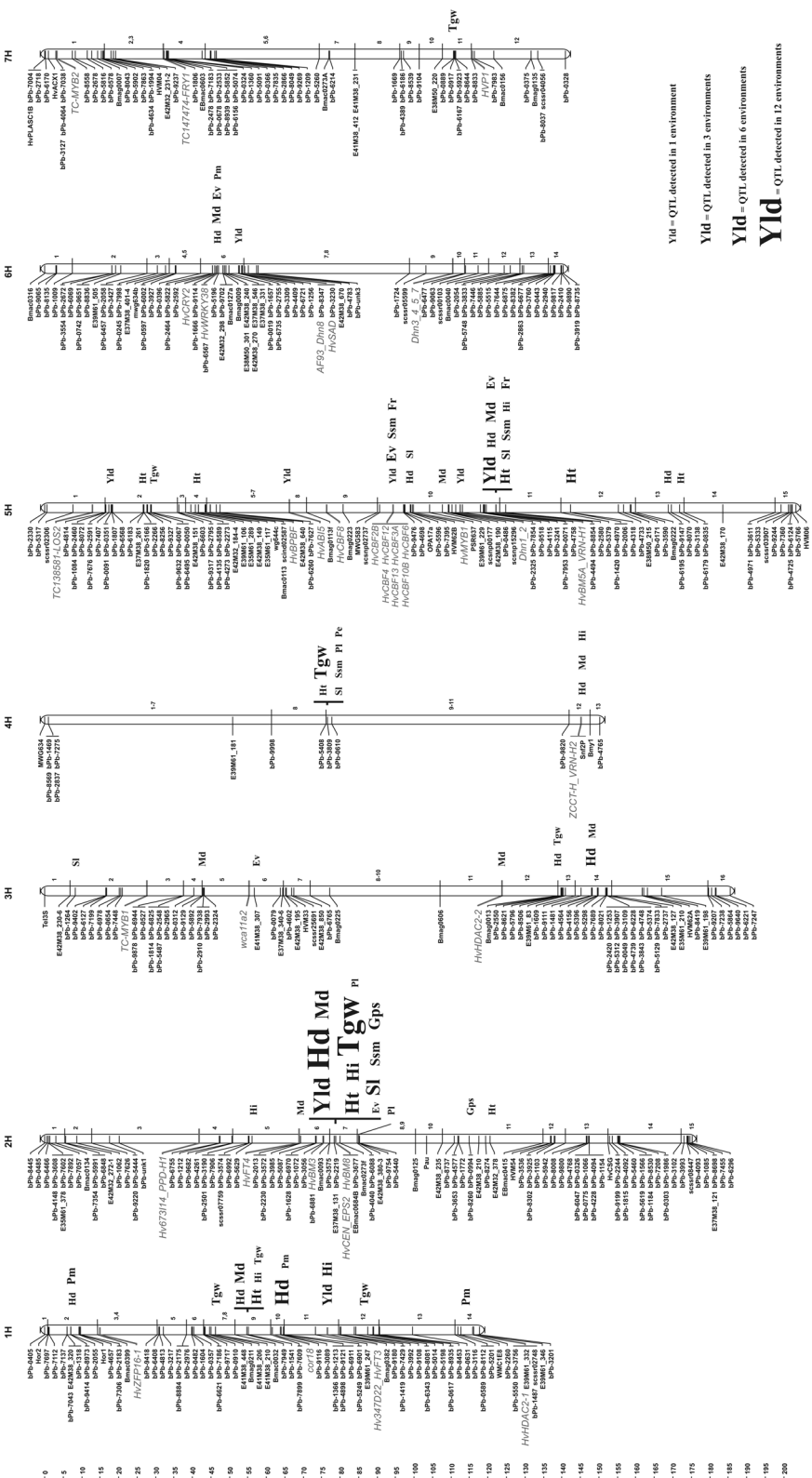

Fig. 1 The 'Nure' × 'Tremois' genetic linkage map. Chromosomes are oriented with short arms on the *top* and distances on the *left ruler* are in Kosambi cM. Chromosome BIN assignment derives from Aghnoum et al. (2010). Candidate genes are in *grey*, ***bold italic***; QT loci nomenclature follows abbreviations reported in the "Materials and methods" section and in Table S4. The font size for QTL names is proportional to the number of occurrences of the QTL across the 18 environments

trials). The *HvCEN* gene was the peak marker of the most significant QTL on chromosome 2H (Fig. 1), with the 'Nure' allele showing a positive effect on grain yield in both low and high yielding environments (Table 2). The QTL was responsible for 13.7–45.8 % of the observed phenotypic variance and overlapped with the *EPS2* locus, which confers early maturity under both long and short day conditions (Laurie et al. 1995; Horsley et al. 2006; Comadran et al. 2012). Highly significant days to heading QTL (up to LOD 37.0 and 72.6 % of explained phenotypic variance) were detected at the same genomic region in 12 out of 13 sites evaluated for the trait (Table 2), with NT lines carrying the 'Nure' allele at *HvCEN_EPS2* flowering on average 5.7 days before NT lines carrying the 'Tremois' allele. Moreover, a significant plant height decrease was associated with the same allele in 6 environments (Table S4). The second most frequently detected QTL for grain yield on the long arm of chromosome 5H, BIN_10.5, was co-segregating with *HvBM5A_VRN-H1* (Fig. 1). Inversion in the additive effect indicated a QTL-by-environment interaction (Table 2): in three autumn sowing trials (#2-ESP_5D, #9-TUR_4D and #15-TUR_4W) a positive contribution on grain yield was reported for the recessive (vernalization responsive) 'Nure' allele, opposite to the results from the two late sowing sites #10-DZA_5W and #13-TUR_5. The same *HvBM5A* gene represented the peak marker for days to heading QTL in #8-ITA_4D and #13-TUR_5 environments (Table 2). Finally, a significant grain yield increase was always associated with the 'Tremois' allele at the Hv347D22_*HvFT3* marker on chromosome 1H, BIN_11.3. This QTL was detected in two low-yielding environments, #5-JOR_4D and #7-SYR_5D, as well as in the high-yielding trial #12-ITA_5D, and explained from 8.4 to 11.9 % of the observed phenotypic variance.

Five environment-specific QTL for grain yield were positioned on the NT map (Table 2). Four of them were located on different regions of chromosome 5H, with a positive contribution from the 'Nure' parent, whereas a positive effect on the trait from the 'Tremois' parent was observed at the *bPb-6735* locus on chromosome 6H.

We decided to further investigate the yield and days to heading behaviour of the eight haplotype classes in which the NT population could be partitioned on the basis of the allelic state at the three QTLs most frequently detected for grain yield: *HvCEN_EPS2* on chromosome 2H, Hv347D22_*HvFT3* on 1H and *HvBM5A_VRN-H1* on 5H (Fig. S1). A mixed model was fitted on all the available grain yield and days to heading data, considering both haplotypes and environments fixed effects and entries within haplotype random (Lacaze et al. 2009). A highly significant effect ($P < 0.0001$) of the haplotype on grain yield was observed, with a predominant role of *HvCEN_EPS2* in determining higher grain yield in contrasting Mediterranean environments. Genotypes carrying the 'Nure' allele at this locus outperform the other haplotypes, independently of the allelic state at Hv347D22_*HvFT3* and *HvBM5A_VRN-H1*. Moreover, no epistatic interactions were detected between the three loci (data not shown). As suggested by the co-location of the major 2H chromosome QTL, these effects are mainly related to differences in number of days from sowing to heading, with the earliest flowering haplotypes showing the highest grain yield (Fig. S1).

The single trait-single environment QTL analysis reported in this study suggests a strong relationship between loci responsible for yield components traits and the major developmental genes described above (Fig. 1; Table S4). In fact, the increase in grain yield associated to the 'Nure' allele at *HvCEN_EPS2* is also associated to: a higher Tgw, in 12 locations out of 14; an increased number of Ssm, in three environments out of six; a higher Hi, in five sites out of seven. Notably, in the highest yielding environment #18-SYR_5W, the locus explained 71.5 % of the variability recorded for grain weight. On the contrary, a negative effect of the same allele was observed for the traits Sl (6 trials) and Gps (4 trials). On chromosome 1H, Hv347D22_*HvFT3* was the peak marker of a Hi QTL detected at three locations, with a positive contribution of the 'Tremois' allele, as already observed for grain yield (Table S4). On the contrary, 'Nure' allele at three distinct regions of chromosome 1H (from 46 cM to 87 cM) was revealed by CIM analysis as associated to higher grain weight, with a maximum additive effect of 1.49 g. Overlapping of grain yield and yield component QT loci was also observed at *HvBM5_VRN-H1* (Fig. 1). For this QTL, 'Nure' allele increased the number of Ssm in the TUR_4 dry and wet experiments (trials #9-TUR_4D and #15-TUR_4W), while having a negative effect on spike length (#4-JOR_5W and #15-TUR_4W) and harvest index (#13-TUR_5). A further QTL for grain yield was mapped on chromosome 5H, at the *HvCBF_FR-H2* frost resistance locus (Francia et al. 2004); it accounted

Table 2 Grain yield and heading date QTL detected in the 'Nure' × 'Tremois' mapping population

QTL	Field trial (#)[a]	Chr_BIN[b]	Peak marker (cM)	Peak position (cM)[c]	LOD[c]	R^2 (%)[c]	Additive[c,d]
Grain Yield							
QYld.NuTr-1H.1	5, 7,12	1H_11.3	Hv347D22_*HvFT3* (76.6)	74.2, 76.2, 79.6	3.5, 4.5, 3.4	11.9, 8.8, 8.4	−0.14, −0.27, −0.16
QYld.NuTr-2H	1, 3, 5, 7, 11, 12, 16, 18	2H_07.2	*HvCEN_EPS2* (78.7)	78.7, 78.3, 78.7, 78.7, 78.7, 78.7, 78.3, 78.7	3.3, 9.8, 5.1, 17.3, 8.1, 11.2, 4.6, 5.3	13.7, 35.5, 17.1, 45.8, 27.9, 32.6, 13.9, 21.0	0.03, 0.16, 0.17, 0.59, 0.23, 0.30, 0.20, 0.24
QYld.NuTr-5H.1	16	5H_02.2	*bPb-0351* (17.9)	17.9	3.9	11.7	0.18
QYld.NuTr-5H.2	8	5H_08.2	*HvABI5* (66.3)	62.4	4.0	18.2	0.15
QYld.NuTr-5H.3	15	5H_09.1	*HvCBF_FR-H2* (95.0)	95	5.2	13.4	0.45
QYld.NuTr-5H.4	16	5H_10.4	*bPb-2325* (113.1)	112.5	6.5	19.4	0.23
QYld.NuTr-5H.5	2, 9, 10, 13, 15	5H_10.5	*HvBM5A_VRN-H1* (122.2)	119.6, 122.2, 122.2, 119.6, 122.2	5.8, 7.2, 6.3, 3.0, 5.9	22.9, 27.5, 24.5, 12.4, 15.4	0.06, 0.52, −0.56, −0.29, 0.48
QYld.NuTr-6H	11	6H_06.2	*bPb-6735* (52.5)	52.5	2.9	9.0	−0.17
Heading date							
QHd.NuTr-1H.1	8	1H_02.1	*bPb-9414* (7.0)	7.0	5.8	5.7	0.82
QHd.NuTr-1H.2	2, 16	1H_09.2	*bPb-7949* (54.6)	54.6, 54.6	5.2, 7.8	9.0, 7.2	0.98, 1.13
QHd.NuTr-1H.3	8, 11, 12, 14, 17, 18	1H_11.1	*bPb-5249* (64.7)	64.7, 64.7, 64.7, 64.7, 64.7, 64.7	10.1, 5.1, 6.2, 5.4, 4.0, 5.2	11.0, 6.8, 5.7, 8.8, 6.4, 5.9	1.15, 0.72, 0.62, 1.13, 0.48, 0.78
QHd.NuTr-2H	2, 3, 4, 8, 9, 11, 12, 13, 14, 16, 17, 18	2H_07.2	*HvCEN_EPS2* (78.7)	78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7, 78.7	21.1, 11.7, 11.3, 26.9, 8.4, 25.1, 33.3, 22.0, 20.8, 37.0, 22.8, 30.7	53.7, 37.6, 39.6, 45.0, 22.8, 54.8, 61.6, 35.8, 49.5, 72.6, 57.0, 66.7	−2.36, −3.10, −1.62, −2.26 −2.66, −2.05, −2.03, −2.69, −2.70, −3.60, −1.44, −2.63
QHd.NuTr-3H.1	16	3H_13.1	*Bmag0013* (138.9)	133.7	5.2	5.3	−0.80
QHd.NuTr-3H.2	8, 11, 12	3H_14.2	*bPb-1481* (148.0)	148.0, 148.0, 147.2	6.3, 6.2, 4.5	6.3, 8.5, 4.2	−0.84, −0.80, −0.51
QHd.NuTr-4H	13	4H_12.3	*ZCCT-H_VRN-H2* (145.2)	145.2	6.4	7.1	1.18
QHd.NuTr-5H.1	9	5H_10.2	*bPb-5596* (98.9)	98.9	7.9	21.2	−2.53
QHd.NuTr-5H.2	8, 13	5H_10.5	*HvBM5A_VRN-H1* (122.2)	122.2, 121.6	7.3, 18.2	7.4, 26.8	0.93, 2.27
QHd.NuTr-5H.3	14	5H_13.3	*bPb-6195* (168.7)	168.7	3.4	5.3	0.86
QHd.NuTr-6H	12	6H_06.1	*HvCRY2* (46.5)	46.5	3.1	2.7	−0.56

[a] Field trial number, according to Table 1

[b] Chromosome BINs follow Aghnoum et al. (2010)

[c] Data refer to the order reported in the corresponding 'Field trial' column

[d] Positive values indicate that the 'Nure' allele increases the trait value

for 13.4 % of the phenotypic variance observed in #15-TUR_4W environment, with the 'Nure' allele increasing grain yield of 0.9 t ha^{-1}. Co-location of QTL for frost resistance, early vigour, heading date, spike length and number of Ssm was detected at the same genomic region (Fig. 1; Table S4). Other three genomic regions on chromosome 5H resulted significantly associated with grain yield in single environments, #8-ITA_4D and #16-ITA_5F, with a positive allelic effect from 'Nure' (Table 2). In addition, a low significance QTL was mapped on chromosome 6H, with the 'Tremois' allele at the *bPb-6735* DArT marker increasing Yld in #11-ITA_4W site. All of them have been detected in higher (i.e. >2.5) yielding environments, and could represent interesting yield-per se loci. As a general trend, we were not able to define QTL sets specific of a group of low- or high-yielding environments.

A cluster of QTLs determining yield components and not related to phenology/developmental genes was observed on chromosome 4H, BIN_09 (Fig. 1). We observed, in five environments, a positive contribution on 1,000 grain weight from the 'Nure' allele at the *bPb-3809* DArT marker. The same locus was associated to QTL for plant height, peduncle length and peduncle extrusion, which have been previously considered as adaptive traits under drought conditions. A reduction in plant height was observed in DHs plants carrying the 'Nure' allele at *bPb-4318* on chromosome 5H-BIN_12 in three low (i.e. <2.5 t ha^{-1}) yielding environments #1-JOR_4D, #4-JOR_5W and #7-SYR_5D, and not associated to major developmental genes (Table S4).

Finally, heading date QTL with no significant effects on grain yield have been detected on chromosomes 3H-BIN_14.2 in three different field trials (Table 1). Based on a comparison with previously published maps, it is unlikely this QTL coincides with the early maturity gene *eam10* (Borràs-Gelonch et al. 2010). Interesting, a slight reduction in the number of days to heading and maturity was associated with the 'Nure' allele at the cryptochrome gene *HvCRY2*, mapping at the centromeric region of chromosome 6H (Fig. 1; Table S4).

Discussion

The study aimed to obtain a better understanding of barley grain yield adaptation to Mediterranean environments by using the 'Nure' × 'Tremois' biparental population. To reach the goal, the first objective was to improve the marker coverage and density of the NT linkage map by means of the DArT marker platform, contemporarily increasing the number of mapped gene-derived functional markers. Even if a sequence assembly of the barley genome gene space has been recently delivered (Mayer et al. 2012), dense transcript and gene-based maps (Rostoks et al. 2005; Stein et al. 2007; Sato et al. 2009; Szűcs et al. 2009; Comadran et al. 2012) still represent useful tools for the identification of the genetic bases of complex traits through genetical proofs (Ramsay et al. 2011; Comadran et al. 2012). In this study we followed the same approach by mapping transcription factors that may be involved in the regulation of flowering time, plant development and adaptation to the environment, including abiotic stress tolerance (Table S1). One of the positive characteristics of the NT population is the contemporary segregation of major developmental loci (*VRN-H1* and *VRN-H2*, *PPD-H2* and *EPS2*), differently regulated by environmental stimuli, that can allow their impact on yield in Mediterranean environments to be assessed, as well as in more fertile environments. In a previous report on the same experimental dataset, Francia et al. (2011) interpreted the variation observed in the NT population for grain yield as function of the length of the different developmental phases. In environments characterized by terminal drought events the best performing (adapted) NT lines were those capable of rapidly reaching the most critical stage in grain yield determination for barley, i.e. the period prior to anthesis. Nevertheless, the available data did not allow the complete dissection of the so-called stem elongation phase, that some authors described as of special relevance for grain yield determination (Araus et al. 2008). In the present work a classical quantitative genetic approach has been pursued to find the genetic basis (QTLs) for yield, yield components and developmental traits. Then, it has been our aim to evaluate the QTLs detected for consistency vs. specificity over environments, for association with the candidate genes positioned in the map, as well as for possible pleiotropic/associated effects upon other important traits. The QTL mapping results obtained for grain yield and other agronomical traits in this biparental study have the advantage of being one of the widest examples of METs across the Mediterranean cereal growing areas. Overall, in the different environments

we have observed from one to three loci explaining together from 12.4 to 56.0 % of the observed phenotypic variation for yield (Table 2). Environment-specific QTLs, generally with minor impact on the trait, account for an additional 9.0–19.4 % of the variation for grain yield. Due to the relatively small population size and thus limited statistical power of the NT population, it was difficult to detect yield QTLs with smaller effects.

Among major QTLs for yield conserved through environments, the largest and most frequent effect reported in this work coincides with the *EPS2* locus on chromosome 2H (Table 2). In a genome-wide association study performed on the MABDE Diverse Barley Germplasm panel assayed over 28 site-by-year combinations, we already observed robust and stable pleiotropic effect for yield, heading date, harvest index and 1,000 grain weight at the same locus (Comadran et al. 2011). Together these two studies represented the starting point for the recent cloning of *HvCEN*, a homolog of Antirrhinum *CENTRODIALIS*, whose allelic variation contributed to the differentiation of winter and spring barley genepools, thus representing a main driver of environmental adaptation for the crop (Comadran et al. 2012). The *HvCEN* early flowering allele from the parent 'Nure' significantly increased grain yield in four stressed environments where the average yield was <2.5 t ha^{-1}, as well as in four higher yielding (>2.5 t ha^{-1}) field trials; notably, no yield penalty due to the early heading was observed in the top three yielding environments where the average yield was >4.5 t ha^{-1} (Table 2). The stable effect of *EPS2* we have observed across very different agro-meteorological conditions reveals its more general role in wide adaptation, and this is further confirmed by the detection of a QTL for yield adaptability at the same genomic locus (Fig. 1). The importance of the locus for the barley crop in the Southern European autumn- or winter-sown environments has also been highlighted in previous studies on biparental populations evaluated in METs (Cuesta-Marcos et al. 2008a; von Korff et al. 2008). However, in the 'Beka' × 'Mogador' barley population evaluated in Northern Spain (Cuesta-Marcos et al. 2008a), individuals with intermediate heading dates showed higher yield potential; while in the present work the highest grain yield was observed for the early flowering NT lines (i.e. lines combining the 'Nure' allele at *HvCEN_EPS2* and the 'Tremois' allele at Hv347D22_*HvFT3*). Hence, there seems to be more than one optimal flowering time associated with higher yield in the different Mediterranean environments.

As already demonstrated in diploid wheat (Lewis et al. 2008), *EPS* genes influence different grain yield components. In the NT population earliness was associated in several environments to a shorter spike and a smaller number of Gps, but a higher 1,000 grain weight and harvest index (Table S4), probably due to a pleiotropic effect of the *HvCEN* mutation. Notably, a higher number of Ssm was observed in barley lines carrying the 'Nure' allele at *HvCEN*; a similar positive relationship between grain yield, the number of grains per unit area and the fast development until anthesis was already observed by Francia et al. (2011).

HvBM5A, the well known vernalization responsive gene for the *VRN-H1* locus on chromosome 5H, accounted for 12.4–27.5 % of the variation for grain yield. Due to its role in conferring winter frost tolerance (Francia et al. 2004), the co-location with QTL for early vigour and number of Ssm is not surprising. In three autumn sowing trials subjected to low-temperature stress (#2-ESP_5D, #9-TUR_4D and #15-TUR_4W), the positive effect of the *VRN-H1* frost-resistance allele from the winter parent 'Nure' resulted in a significant increase in grain yield. No effect was recorded in the same environments at the *EPS2* locus. Finally, Hv347D22_*HvFT3*, was the peak marker of the third yield QTL detected in more environments in a genomic regions of chromosome 1H where QTL clusters for both grain yield and heading date were not overlapping. One possibility is the existence of linked genes in the region, while a second hypothesis is the lack of resolution of the NT map. In particular, the observed marker segregation distortion in the region towards the 'Tremois' parent (genotypic data available at http://wheat.pw.usda.gov) could be a cause of uncertainty towards a better definition of the effect. In fact, the peak marker of the yield QTL has been proposed as the candidate gene for *PPD-H2*, a major determinant of heading date in Mediterranean environments (Igartua et al. 1999; von Korff et al. 2008; Cuesta-Marcos et al. 2008a, b).

As already observed by Araus et al. (2008), Marker assisted selection (MAS) at a few selected key loci may be pertinent for manipulating phenology, and thus yield, when breeding for adaptation to Mediterranean rainfed environments. To this purpose, it is of primary importance to evaluate the rate of fixation of alleles at

such developmental loci in the Southern European germplasm and, in case of fixation of positive alleles, the feasibility of their use in crosses with unadapted, high yielding, Northern European germplasm. Further contribution could come from QTLs mapped in single/few environments, and not obviously related to plant life-cycle. For example, the 'Nure' allele of the HvABI5 bZIP transcription factor significantly increased barley yield in environment #8-ITA_4D (Table S4). The *HvABI5* candidate gene for drought tolerance (Kobayashi et al. 2008) maps to chromosome 5H, BIN_08, a genomic region where multiple loci for abiotic stress tolerance co-locate (Pecchioni et al. 2012). As observed by Araus et al. (2008), selection for yield under drought can result in plants with high dehydration tolerance, but lower yield potential, and for this reason the development of drought-resistant cultivars has benefited more from genes that control constitutive traits than from drought-responsive genes. However, the introduction in high yielding genotypes of traits able to improve drought tolerance could improve yield in specific environments without detrimental effects on yield potential, as suggested by Cattivelli et al. (2008).

The NT population has been widely exploited to study barley tolerance to low temperatures, due to the segregation of two major resistance loci, *FR-H1*, coincident with *VRN-H1*, and *FR-H2*, coincident with a cluster of *CBF* genes (Francia et al. 2004, 2007; Knox et al. 2010). Even if the involvement of CBF/DREB transcription factor in barley response to drought stress has been reported (Skinner et al. 2005) and cannot be excluded, we hypothesize that the *FR-H2* locus could have had a role in the best establishment of plant juvenile phase, as suggested by the co-location of QTLs for frost resistance, early vigour and number of Ssm.

In conclusion, the present work represents the most detailed QT characterization of a barley biparental population grown under different water regimes, in increasingly drought-prone Mediterranean environments, for several agronomic traits. Compared to previous studies (e.g. Cuesta-Marcos et al. 2008a; von Korff et al. 2008), this work covers a much wider range in environments and locations and a larger number of traits. Despite the heterogeneity of the environmental conditions, we have identified genomic regions consistently associated to yield, together with environment-specific QT regions. The results presented thus provide a valuable source of knowledge and tools for both explaining the genetic bases of barley yield adaptation across the Mediterranean basin, and use of QTL-associated markers for MAS pre-breeding and breeding programmes. Further advancements in terms of genomic colinearity of relevant QTLs, and of their relative effects in different backgrounds, could also be reached exploiting complementary approaches of biparental and association mapping in different populations. This could be done through a joint genetic analysis of data coming from other barley populations evaluated in the same environments, namely a second biparental population ('Henni' × 'Meltan', Borràs-Gelonch et al. 2010) and the Diverse Barley Germplasm panel (Comadran et al. 2008, 2009, 2011).

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