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

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Abstract Grain yield and plant height of 80 recombinant chromosome substitution lines (RCSLs) of barley were measured in six environments with contrasting available moisture profiles. Two environments were in OR, USA (Moro and Pendleton) during one growing season (2004), and four in Chile (Cauquenes and Santa Rosa) during two growing seasons (2004/2005 and 2007/2008). From the yield data obtained in the different environments, yield adaptability (Finlay–Wilkinson slope) and stability (deviations from regression) were calculated. Two commercial cultivars (Harrington and Baronesse) were used as checks in all environments. Marker-quantitative trait associations were identified using 47 simple sequence repeats (SSRs) and the general linear model (GLM) implemented in TASSEL. The mean plant height and grain yield of the 80 RCSLs differed greatly across environments, reflecting differences in water availability. In all environments, there were significant differences ($P < 0.05$) in grain yield among RCSLs. There was also abundant variation in yield adaptability, indicating a differential response of the RCSLs to environmental conditions across environments. Using principal component analysis, it was possible to identify genotypes with better agronomic performance than the recurrent parent cv. Harrington. The association analysis revealed 21 chromosomal regions that were highly correlated with differences in grain yield, plant height and/or yield adaptability (Finlay–Wilkinson slope). In approximately one-fourth of the cases, the *H. spontaneum* donor contributed favorable alleles. The associations were referenced to the quantitative trait loci (QTL) for the same traits reported in the literature.

Keywords Barley · Association mapping · Grain yield · Plant height · SSR markers · QTL

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Introduction

Barley (*Hordeum vulgare* subsp. *vulgare*) is considered a model species for physiological and genetic

studies (Forster et al. 2004) because (a) it is an important crop, particularly in Mediterranean regions; (b) it is diploid ($2n = 2x = 14$), with a low number of relatively large chromosomes; (c) it presents a high degree of self-fertility and it has cross compatibility with species within the primary gene pool; (d) it is an annual species with a relatively short life cycle; (e) it has a high genetic and morpho-physiological diversity; and (f) well documented genetic maps are available.

Crop improvement in barley results from crosses between two parents with desirable characteristics, after which breeders make a careful selection of the best recombinants among the progenies (Thomas 2002). Therefore, a wide and representative collection of germplasm is needed in order to supply genetic diversity. However, the level of diversity in many breeding programs is limited as a consequence of the selection processes (Matus and Hayes 2002).

Since wild (*Hordeum vulgare* subsp. s*pontaneum*, hereafter *H. spontaneum*) and cultivated barley are interfertile, it is possible to increase the genetic diversity of barley using the wild ancestor as a progenitor in crosses with the cultivated barley (Ellis et al. 2000; Matus and Hayes 2002; Baum et al. 2003; Forster et al. 2004; Talamè et al. 2004; Schmalenbach et al. 2008a, b). On average, only 40% of wild barley alleles are represented in cultivated barley (Ellis et al. 2000). There is evidence that the wild ancestor is a source of favourable alleles for a number of agronomic and physiological traits such as plant height, root morphology, precocity, grain yield, salinity tolerance, and drought tolerance (Ellis et al. 2000; Baum et al. 2003; Forster et al. 2004; Talamè et al. 2004; Schmalenbach et al. 2008a).

In the interest of broadening the genetic base of cultivated barley, Matus et al. (2003) developed a population of recombinant chromosome substitution lines (RCSLs) using *H. spontaneum* (Caeserea 26-24 from Israel) as a donor and *H. vulgare* subsp. v*ulgare* cv. Harrington (a North American malting quality standard) as the recurrent parent. These RCSLs represent a rich source of genetic diversity to be used as a model in genetic and physiological studies. Furthermore, *H. spontaneum* (Caeserea 26-24) was collected in a dry and saline environment in Israel, which suggests that this accession could contribute useful genes, particularly for drought tolerance (Ellis et al. 2000; Baum et al. 2003).

The RCSLs were genetically characterized using 47 simple sequence repeat (SSR) markers and a preliminary association analysis revealed that *H. spontaneum* contributed favorable alleles for some agronomic and malting quality traits (Matus et al. 2003). In this report we present analyses of data on quantitative traits (plant height, grain yield, adaptability and stability) measured on a subset of 80 RCSLs with desirable agronomic characteristics in multi-location tests in the USA and Chile. Available moisture contrasted across environments. Marker-quantitative trait associations were identified using the general linear model (GLM) of the TASSEL (Trait Analysis by Association, Evolution and Linkage) software. Association analysis methods identify phenotypic differences associated with differences in allele frequency and have been used for rice (Virk et al. 1996), oats (Beer et al. 1997), potatoes (Simko et al. 2004) and barley (Matus et al. 2003; Ivandic et al. 2003; Pillen et al. 2003; Talamè et al. 2004; Kraakman et al. 2004, 2006). A principal advantage of association analysis is that it does not require assessment of progeny derived from crosses between inbred lines.

The objectives of this research were to: (a) to determine the yield potential of RCSLs in diverse environments; and (b) to identify chromosome regions that are highly correlated with plant height, grain yield, and yield adaptability and stability. Yield adaptability was defined as the slope of the regression between the yield of an individual RCSL and the mean yield (of all RCSLs) across environments (Finlay and Wilkinson 1963), whereas yield stability was estimated from mean squared deviations from regression (Calderini and Dreccer 2002; Kraakman et al. 2004).

Materials and methods

Germplasm and phenotypes

Eighty of the 140 RCSLs described by Matus et al. (2003) were used for these studies. These RCSLs were selected for their agronomic type and potential. Briefly, these lines were derived from the cross of an accession of *H. spontaneum* (Caeserea 26-24, from Israel) and *H. vulgare* subsp. *vulgare* cv. Harrington (a North American malting quality standard). *H. spontaneum* was the donor parent and *H. vulgare*

was the recurrent parent. The lines were obtained using two backcrosses with the recurrent parent and six generations of self pollination (BC_2F_6). Two check cultivars were used: the recurrent parent (cv. Harrington) and cv. Baronesse. The wild barley parent was not included in these field trials due to the fact that its growth and development traits are not suitable for production conditions.

The 80 RCSLs were grown in field trials in six environments (location/year combinations): one year (2004) at Moro and Pendleton in Oregon, USA, and two years (harvests in January 2005 and 2008) at Cauquenes and Santa Rosa in central Chile. The locations differ greatly in annual rainfall (Table 1). Plots consisted of three 1.5 m-long rows, separated by 0.2 m and were sown at a seeding rate of 120 kg ha^{-1}. The experimental design at all sites was alpha-lattice with two replicates and seven incomplete blocks per replicate; each block contained 12 RCSLs. Each replicate contained 84 genotypes: the two check cultivars (Harrington and Baronesse) were included twice in each replicate.

In Oregon, sowing dates were March 20th and 27th (2004) at Pendleton and Moro, respectively. Plots received 112 kg ha^{-1} of a NPK mixture (16–20–14%) plus 170 kg ha^{-1} of urea. Weeds were controlled before sowing using glyphosate and MCPA. In Chile, sowing dates were May 27, 2004 and June 01, 2007 at Cauquenes, and August 30, 2004 and August 24, 2007 at Santa Rosa. Plots were fertilized with 260 kg ha^{-1} of ammonium phosphate (46% P_2O_5 and 18% N), 90 kg ha^{-1} of potassium chloride (60% K_2O), 200 kg ha^{-1} of sulpomag (22% K_2O, 18% MgO and 22% S), 10 kg ha^{-1} of boronatrocalcita (11% B) and 3 kg ha^{-1} of zinc sulphate (35% Zn). Fertilizers were incorporated with a cultivator before sowing. During

tilling an extra 80 kg ha^{-1} of N was applied. Weeds were controlled with MCPA at 750 g a.i. ha^{-1} + Metsulfuron Metil 8 g a.i. ha^{-1}.

Plant height was assessed at maturity. Grain yield was measured on a plot basis. Regression analyses were performed between the grain yield of each RCSL and the mean yield of all genotypes in each environment (the environmental index; Finlay and Wilkinson 1963). The regression coefficient (Finlay–Wilkinson slope) is a measure of yield adaptability and the mean squared (MS) deviations from regression is an index for grain yield stability (Calderini and Dreccer 2002; Kraakman et al. 2004).

Statistical analysis

ANOVAs of grain yield and plant height data were performed using PROC MIXED (SAS Institute Inc 1999). The RCSLs and environment were considered fixed effects, whereas blocks and incomplete blocks within each replicate were considered random effects. Least square means were calculated for the RCSLs and a significant deviation from the recurrent parent (cv. Harrington) was obtained with the *diff* command in SAS.

Principal component analysis (PCA) was carried out for the 80 RCSLs using the mean values of plant height and grain yield for the six environments, and the traits yield adaptability and stability. Only those components with an eigenvalue >0.9 were selected. The PCA was performed using Microsoft Excel.

Association analysis

DNA marker-quantitative trait (SSR-trait) associations were identified using the general linear model

Table 1 Description of experimental sites and precipitation from sowing to maturity in central Chile (Cauquenes and Santa Rosa) and in OR, USA (Moro and Pendleton) that were used for assessment of 80 barley RCSLs, the recurrent parent (Harrington) and a yield check (Baronesse)

Site	Latitude	Longitude	Altitude (m)	Soil	Precipitation (mm)
Cauquenes	35°58′S	72°17′W	177	Sandy clay loam	2004/2005: 509
					2007/2008: 209
Santa Rosa	36°32′S	71°55′W	220	Clay loam	2004/2005: 351.8*
					2007/2008: 182.7*
Moro	45°29′N	120°41′W	510	Silt loam	2004: 303
Pendlenton	45°39′N	118°46′W	411	Silt loam	2004: 508

* Plus 3 (2004/2005) and 4 (2007/2008) irrigations from heading to maturity

(GLM) in TASSEL (http://www2.maizegenetics.net/). The model used to detect SSR-trait associations considers the effect of the genetic marker (M), the environment (E) and the interaction (MxE). The mean squares of MxE were used as an error term for the estimation of the F-statistic for each marker main effect. The mean squares of the residuals were used to calculate the F-statistic for the MxE effect. An SSR-trait association was considered real when the marker main effect was significant at $P < 0.01$ (Pillen et al. 2003). The presence of an SSR-trait association depending on the environment was identified when the MxE was significant at $P < 0.01$.

Results

Phenotypes

Plant height and grain yield differed greatly across environments reflecting, in part, differences in water availability (Table 2). The ANOVA for the six environments revealed highly significant genotype × environment interaction (Table 3). Separate analyses for each environment revealed significant differences ($P < 0.05$) in grain yield between RCSLs. At the most favourable site in Chile (Santa Rosa) three RCSLs and Baronesse were significantly higher yielding than Harrington (the recurrent parent) in 2004/2005, but not in 2007/2008. At Cauquenes, Moro and Pendleton none of the RCSLs were significantly higher yielding than the recurrent parent.

There were important differences between RCSLs in terms of yield adaptability, as shown in Figs. 1 and 2a. There was also abundant variation in yield stability, with values ranging from 0.01 to 1.48 (Fig. 2b). For example, Harrington had a slope (b) value of 1.0 and a MS of 0.14, which indicates the high capacity of this variety to respond to favourable (wetter) conditions as well as high yield stability (Fig. 1). Two contrasting RCSLs are RCSL-89 ($b = 0.60$; MS = 0.26), which showed a very low

Table 2 Means ± standard deviations and range of values for plant height and grain yield for 80 barley RCSLs, the recurrent parent Harrington, and the yield check (Baronesse) evaluated in six environments—Moro and Pendleton (USA) in one growing season (2004), and Cauquenes and Santa Rosa (Chile) in two growing seasons (2004/2005 and 2007/2008)

Location	Year	Plant height (cm)		Grain yield (mg ha^{-1})	
		Mean	Range	Mean	Range
Moro	2004	63.1 ± 6.1	46.3–77.5	2.1 ± 0.3	1.3–2.6
Pendleton	2004	109.7 ± 7.6	91.5–137.8	4.4 ± 0.4	2.9–5.0
Cauquenes	2004/2005	80.0 ± 7.6	58.8–108.7	4.4 ± 0.9	2.0–6.5
	2007/2008	62.8 ± 7.1	41.0–75.6	1.7 ± 0.4	1.0–2.6
Santa Rosa	2004/2005	94.9 ± 7.6	76.0–122.6	8.0 ± 1.2	5.0–10.6
	2007/2008	87.3 ± 6.8	69.1–108.2	5.6 ± 0.9	3.1–7.2

Table 3 Degree of freedom (DF), mean square (MS) and F value of the analysis of variance for plant height (cm) and grain yield (mg ha^{-1}) for 80 RCSLs and two check cultivars, evaluated in six environments—Moro and Pendleton (USA) in one growing season (2004), and Cauquenes and Santa Rosa (Chile) in two growing seasons (2004/2005 and 2007/2008)

	Plan height			Grain yield		
	DF	MS	F	DF	MS	F
Environment (E)	5	56584.5	1734.6**	5	935.2	1313.9**
Genotype (G)	81	369.4	11.3**	81	2.7	3.77**
G × E	405	54.7	1.68**	402	0.9	1.24*
Error		32.6			0.7	

* $P < 0.01$; ** $P < 0.0001$

Fig. 1 Relationships between grain yield and environmental index in two contrasting lines, RCSL-89 (■, *dotted line*) and RCSL-88 (●, *continuous line*), and the cv. Harrington (Δ, *dashed line*). The regression coefficients (*b* values) were 0.60, 1.0 and 1.33 in lines 89, Harrington and 88, respectively
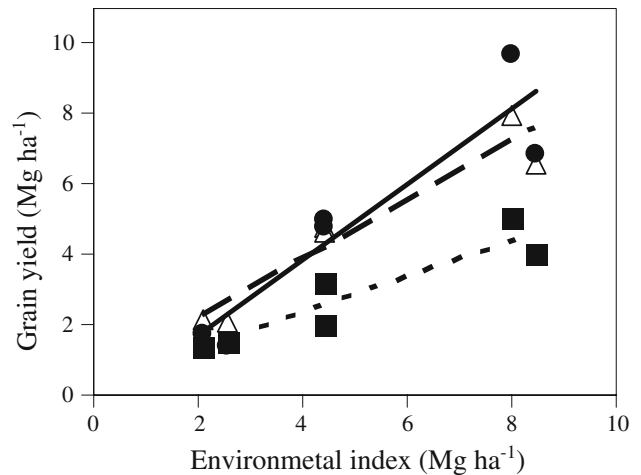
Fig. 2 Frequency distributions for **a** Finlay–Wilkinson slope (*b*), and **b** mean squared (MS) deviation from regression, derived from the relationship between the grain yield of each genotype and environmental index of 80 RCSLs of barley. The *arrows* indicate the values for Harrington (HA) and Baronesse (BA)
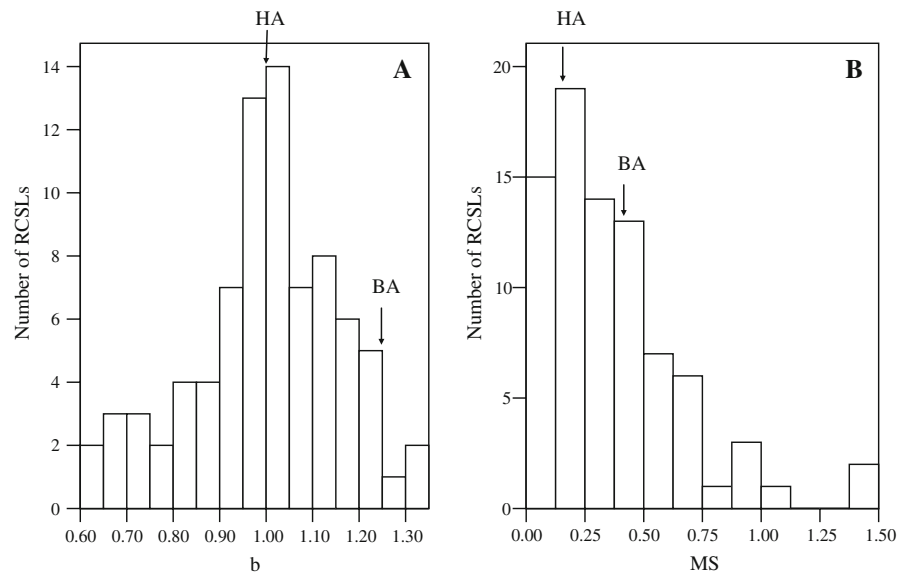
level of response to improving production conditions and RCSL-88 ($b = 1.3$; MS = 0.03), which showed a high response coupled with high yield stability.

Principal component analysis

The first two principal components (PCs) gave eigenvalues > 0.9 and explained 70.8% of observed variability. The first PC explained 47.8% of the variability and was associated with grain yield (42.3%) and yield adaptability (38.6%). The second PC explained 23.0% of the variability and was associated with yield stability (90.1%). Grain yield was positively correlated with yield adaptability ($r = 0.72$; $P < 0.0001$) and negatively with plant height ($r = -0.24$; $P < 0.05$). As shown in Fig. 3, the first two PCs provided clear discrimination between RCSLs; for e.g., RCSL-88 located at the right extreme of the diagram, was one of top yielding lines (4.9 mg ha^{-1}; mean of six environments), it had the shortest plant height (66.8 cm), and a it had a high value of yield stability. In contrast, RCSL-89 had the lowest yield (2.9 mg ha^{-1}) and the greatest plant height (99.5 cm).

Association analysis

Association analyses were performed using four traits assessed in six environments (Table 4). Twenty-one chromosomal regions, as defined by SSRs, had

Fig. 3 Biplot of the first two principal components (PC1 and PC2) for the principal component analysis of four traits evaluated in 80 RCSLs of barley. Correlations for grain yield (GY), plant height (PH), yield adaptability (Finlay–Wilkinson slope, *b*) and yield stability (Y_{STAB}) are indicated. The positions of the RCSLs 88 and 89, and the recurrent parent cv. Harrington (HA) are also shown
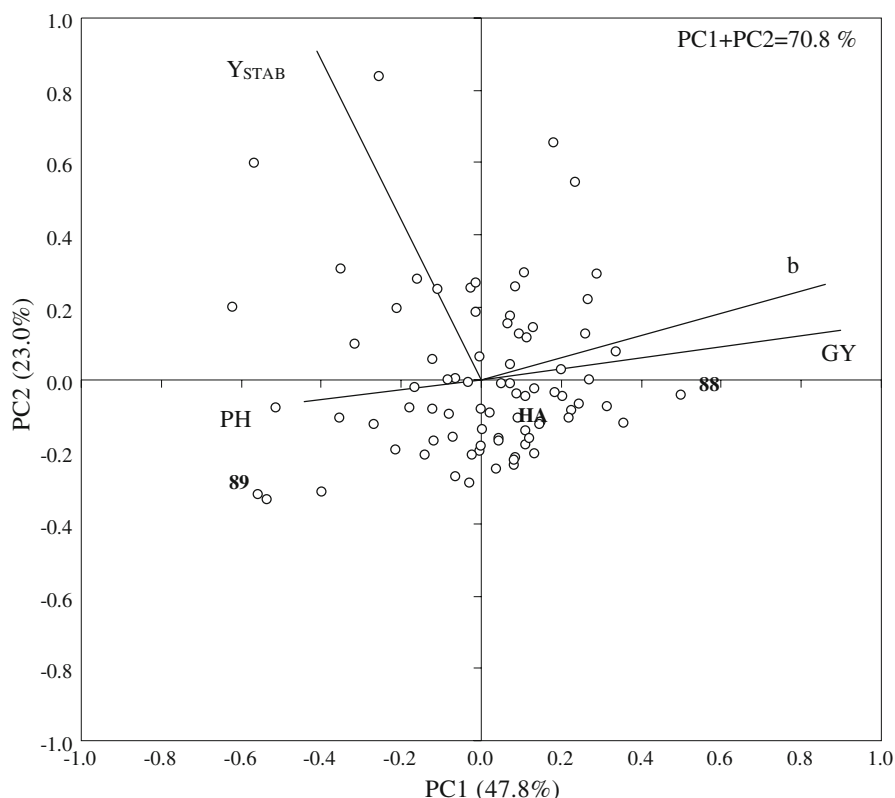
significant trait associations. Twelve markers showed significant associations with grain yield. Of these, seven were significant for the marker main effect and five associations were environment-dependent. The yield QTL are distributed throughout the genome: one in chromosome 1H, one in 2H, two in 3H, one in 5H, four in 6H, and one in 7H. In all cases of a significant main effect, the *H. spontaneum* allele had a negative contribution to grain yield (Table 4). For the environment-dependent associations, further GLM analyses were performed for each environment. These analyses revealed that the *H. spontaneum* alleles at Ebmac415 (2H) and GMS21 (1H) increased grain yield by 19 and 13%, respectively in the most favourable environment (Santa Rosa 2004/2005). But, the *H. spontaneum* alleles at GMS001 (5H), Bmag173 (6H) and Bmac18 (6H) decreased grain yield by 16–40% (in Moro and Santa Rosa), 7.5% (in Santa Rosa) and 12–37% (in Moro and Santa Rosa), respectively.

Twelve SSR-trait associations were found for plant height; eleven were significant for the marker main effect and one was environment-dependent (Table 4). The QTL showed genome-wide distribution: one in

chromosome 1H, three in 2H, two in 4H, two in 5H, one in 6H, and three in 7H. At six QTL (located on chromosomes 1H, 2H, and 4H), *H. spontaneum* alleles reduced plant height (5–24%). The GLM analysis for the environment-dependent association revealed that the *H. spontaneum* allele at Bmag173 (6H) reduced plant height by 14% in the most favourable environment (Santa Rosa 2004/2005).

For yield adaptability, one chromosome region was identified in chromosome in 5H, and two in 6H. In chromosomes 6H, the *H. spontaneum* alleles caused an increase (20%) in the value of the slope. In chromosomes 5H and 6H the *H. spontaneum* alleles decreased the slope by 30 and 23%, respectively. For yield stability, none of the SSR-trait associations were significant.

Discussion

The optimum plant height for modern barley cultivars ranges from 70 to 90 cm (Abeledo et al. 2002). Greater height increases the risk of crop lodging and lower height complicates harvest and reduces the

Table 4 Chromosome regions associated with grain yield (GY), plant height (PH) and the Finlay–Wilkinson slope (*b*) in 80 RCSLs evaluated in six environments

Locus	Ch	cM	BIN	GY		PH		*b*	
				Ass.	RP[Hsp] (%)	Ass.	RP[Hsp] (%)	Ass.	RP[Hsp] (%)
GMS21	1H (5)	17	2	M × E***					
Bmag382	1H (5)	97	12			M**	−24.0		
Bmac0134	2H (2)	5	5			M**	−7.1		
Bmac0093	2H (2)	50	9			M***	−5.0		
Bmag378	2H (2)	44	9			M**	−5.4		
Ebmac415	2H (2)	105	13	M × E**					
Bmag606	3H (3)	125	13	M***	−11.6				
Bmag0013	3H (3)	141	14	M**	−12.7				
Bmag353	4H (4)	48	7						
Ebmac701	4H (4)	78	10			M**	−4.5		
HVM67	4H (4)	118	13			M**	−8.1		
Bmag223	5H (7)	66	7			M***	+5.9		
GMS061	5H (7)	126	11			M**	+9.0		
GMS001	5H (7)	187	14	M × E***				M***	−30.0
Bmag500	6H (6)	38	3	M**	−8.2				
Bmag173	6H (6)	79	7	M × E***		M × E***		M***	−23.0
Bmac18	6H (6)	102	8	M × E***				M***	+20.2
Bmac40	6H (6)	143	13	M**	−3.4				
Bmag273	7H (1)	80	4	M**	−10.3	M***	+5.3		
HvCMA	7H (1)	85	6	M**	−11.8	M**	+5.5		
Bmag507	7H (1)	107	8	M**	−13.6	M**	+5.5		

BIN refers to chromosome bins based on the QTL summary of Hayes et al. (2000)

Ass. significant association; *M* Main effect; *M* × *E* Main × Environment effect

, * significant at *P* < .01 and *P* < .001, respectively

RP[Hsp]: The relative performance of homozygous Hsp genotypes compared to homozygous Hv genotypes at the given marker locus

ability of the crop to compete with weeds. Height is highly influenced by the environment, and particularly by drought (Baum et al. 2003). In this study, we observed reductions in plant height due to drought of 42% in the USA (Moro vs. Pendleton) and 16% in Chile (Cauquenes vs. Santa Rosa).

The range of Finlay–Wilkinson slopes (0.60–1.33) for the relationship between the environmental index and observed yield we obtained for these RCSLs agrees with that reported by Kraakman et al. (2004) for 146 modern European 2-row spring barley cultivars. The choice of genotype based on its slope value will depend on the yield potential of the environment. In high yielding environments the selection of genotypes with slope values >1 is recommended, but for dryland Mediterranean environments genotypes with values close to one appears optimum (Calderini and Dreccer 2002). Genotypes with low *b* values (low adaptability) and high MS values (low yield stability) are genotypes that yield more under drought conditions, but are not able to respond to higher levels of moisture. RCSL-5 is an example of such a genotype.

In classical QTL analysis, phenotype-marker associations can be analyzed using one, two, or multiple genetic markers. TASSEL utilizes a fixed effects linear model to test for association between segregating loci (SSR alleles in this case) and quantitative traits. Each of the phenotype-marker association tests is conducted individually: e.g., one chromosome with "*n*" markers, would have "*n*" single-marker tests. This method is a good choice when the purpose is to detect a QTL tied to a single marker (Doerge 2002).

However, when two or more adjacent markers simultaneously identify a QTL, it is not possible to determine whether each marker represents an independent effect or if all adjacent significant markers represent a single genetic effect (Pillen et al. 2003; Talamè et al. 2004).

There are many QTL reports in barley, the majority of which focus on grain yield and quality phenotypes (Pillen et al. 2003). Most of these QTL were detected in balanced populations derived from single crosses, e.g., F2 or doubled haploid (DH) (Doerge 2002). In this association study, a BC2F6 population was used. This population type was necessary since the donor parent of the RCSLs was an accession of *H. spontaneum*. This ancestral germplasm was used in order to determine if it would serve a reservoir of favorable alleles or to detect favorable alleles in the recurrent parent via negative allele substitution, or "knockout" (Matus et al. 2003). Backcrosses were used in order to segment the donor genome sufficiently to allow for selection of RCSLs with agronomic potential and substantial introgressions of the ancestral genome (Matus et al. 2003; Pillen et al. 2003; Talamè et al. 2004; Schmalenbach et al. 2008a). Thus, two back-crosses to the cultivated recurrent parent were sufficient to recover a large number of progeny with potential for direct assessment in contemporary agricultural systems.

Our results confirm the validity of this strategy in that 21 associations (QTLs) were identified. At seven of these (24%), the *H. spontaneum* accession contributed favorable alleles. Pillen et al. (2003) used a BC2F2 population from the cross of Apex (*H. vulgare* ssp. *vulgare*) and ISR101-23 (*H. vulgare* ssp. *spontaneum*) and found that the *H. spontaneum* accession contributed favorable alleles at 34% of the QTLs. Talamè et al. (2004), using 123 doubled haploid lines from a BC1F2 population derived from the cross of Barke (*H. vulgare* ssp. *vulgare*) and HOR11508 (*H. vulgare* ssp. *spontaneum*), reported that 52% of the positive QTL alleles traced to the wild ancestor. More recently Schmalenbach et al. (2008a), using 39 introgression lines (S42ILs) derived from the cross of cv. Scarlett (*H. vulgare* ssp. *vulgare*) and the accession ISR 42-8 (*H. vulgare* ssp. *spontaneum*), reported that on 40.9% of the 44 QTLs detected (for six agronomic traits),

the wild barley had favorable effects on trait performance.

Studies with *Oryza* and *Lycopersicon* showed that the majority of the genetic diversity in these taxa was found in the genetic pool of the wild species (Tanksley and McCouch 1997). Similar results have been reported for *Hordeum* (Ellis et al. 2000). Therefore, it is probable that at least a fraction of the QTLs (associations) identified in this study, where the *H. spontaneum* donor contributed favorable alleles, may be novel alleles absent from the elite barley germplasm pool.

Many factors influence QTL detection, including the type of cross, the size and structure of the population, the number of environments sampled, analysis method, and the number of molecular markers. In this preliminary study, we used a very limited number (47) of SSRs. We recognize this is limited genome coverage; fortunately, this germplasm will be saturated with single nucleotide polymorphisms (SNPs) in a recently-funded Generation Challenge (GCP) Project. Via that project we will be able to empirically assess the effect of marker density, number of environments sampled, and population size using the full set of RCSLs. The analysis method has the greatest impact on QTL results (Pillen et al. 2003). The methods most frequently applied in classic QTLs analysis are simple interval mapping (SIM), composite interval mapping (CIM) and simplified composite interval mapping (sCIM) (Pillen et al. 2003).

In this study, we used TASSEL and identified 21 associations (QTLs) for four traits. This number of QTLs is within the range (11–159) of QTLs identified by the classic analyses in barley (Pillen et al. 2003). The advantages of association mapping include flexibility in terms of the type of population to which it is applied (Simko et al. 2004). In order to assess the robustness of the associations revealed by TASSEL, we used two comparative approaches. One approach was based on the association maps reported by Pillen et al. (2003), (2004), Talamè et al. (2004), and Von Korff et al. (2006). The other approach was based on the QTL summary developed by Hayes et al. (2000). This comparative approach is complicated by the evolution in marker technology, since many of the early QTL analyses were based on RFLP and AFLP markers and we used only SSRs. We overcame this limitation, insofar as possible, by reference to the

(Steptoe × Morex, map) (http://barleygenomics.wsu.edu/databases/databases.html), which includes multiple marker types assigned to BINs (Kleinhofs and Han 2002). We assumed that any two QTLs found in different studies were the same when their location corresponded to the same BIN. We limited our comparative analysis to grain yield and plant height, since there is abundant prior data on these traits.

For these two traits, 17 of the QTL we detected corresponded to those in the QTL summary and 13 corresponded with those reported from association mapping. For grain yield, six QTLs were verified in the QTL summary. Two associations found in 7H (BIN 4 and 6) coincide with QTLs reported by Hayes et al. (1993) and Tinker et al. (1996). One association identified in 3H (BIN 13) coincides with QTLs reported by Thomas et al. (1995) and Powell et al. (1997). Two associations identified in 6H (BIN 7 and 8) coincide with QTLs reported by Hayes et al. (1993) and Backes et al. (1995). Finally, one association in 1H (BIN 2) corresponds to Hayes et al. (1996). When the SSR-grain yield associations were compared with those identified in association maps, eight associations were coincident. Two associations detected in 2H (BIN 13) and 1H (BIN 2) corresponds to those reported by Pillen et al. (2003), and one association identified in 5H (BIN 14) corresponds with that reported by Pillen et al. (2004). Six associations—7H (BIN 8), 2H (BIN 13), 3H (BIN 13 and 14) and 6H (BIN 7 and 8)—correspond to the associations reported by Von Korff et al. (2006).

For plant height, eleven associations were verified in the QTL summary. Three associations in 7H (BIN 4, 6 and 8) coincide with QTLs reported by Thomas et al. (1995); Tinker et al. (1996); De la Peña et al. (1999)and Marquez-Cedillo et al. (2001). Three associations in 2H (BIN 5 and 9) agree with Borem et al. (1999) and Marquez-Cedillo et al. (2001). In 4H (BIN 10 and 13), two associations correspond to those reported by Backes et al. (1995); Marquez-Cedillo et al. (2001) and Hackett et al. (1992). One association identified in 6H (BIN 7) coincides with QTLs reported by Hayes et al. (1993). Two associations identified in 5H (BIN 7 and 11) coincide with QTLs reported by De la Peña et al. (1999) and Marquez-Cedillo et al. (2001). Five QTL were coincident with those reported in prior association mapping studies. One association identified in 7H (BIN 107) corresponds to that reported by Pillen et al.

(2003). Two associations in 2H (BIN 9) coincide with Pillen et al. (2004), and finally, two associations identified in 4H (BIN 10 and 13) agree with Von Korff et al. (2006).

When plants are exposed to abiotic stresses such as drought, salinity or low temperature, they undergo physiological and biochemical responses. These responses can be quantified using a single metric: yield adaptability (Finlay–Wilkinson slope). One of the biochemical responses to drought and other stresses is the induction of dehydrin (genes encoding proteins involved in plant protective reactions against dehydration) genes. These *Dhn* genes are present in chromosomes 3H, 4H, 5H and 6H of barley (Choi et al. 2000). In this study we report three associations with yield adaptability (b), which are located in chromosome 5H and 6H. Of these, the *H. spontaneum* allele at Bmac18 located in 6H (BIN 8) had a positive effect on the Finlay–Wilkinson slope (*b*). Confirmation of a causative and functional role of *Dhn* alleles will require additional work. Ivandic et al. (2003) using 52 genotypes of *H. spontaneum* and 33 SSRs also reported that Bmac18 was related with adaptability, total dry matter, and grain yield.

The PCA (Fig. 3) shows that RCSL-88 was higher yielding than the recurrent parent cv. Harrington. RCSL-88 has introgressions of *H. spontaneum* in chromosomes 1H (Bmag382), 2H (Bmag378 and Bmac415), 4H (HVM67) and 6H (Bmac40). At all of these regions, *H. spontaneum* alleles reduced plant height (Table 4). This reduction in plant height can be considered a favourable effect of *H. spontaneum* because in the environments sampled, plant height was negatively correlated with grain yield. According to Teulat et al. (2003) the chromosome 2H region close to Bmag378 is also associated with relative water content and osmotic adjustment. RCSL-88 has an *H. spontaneum* introgression in the distal region of chromosome 6H (Bmag40) (Fig. 4). Harrington contributes the favourable allele associated with Bmag40 (Table 4). Teulat et al. (2003) reported QTLs in this region for relative water content, osmotic adjustment, osmotic potential, osmotic potential at full turgor, and thousand kernel weight. Also Schmalenbach et al. (2008a) reported a line with introgression of *H. spontaneum* in chromosome 2H which exhibited a favourable effect on days until heading, plant height, thousand grain weights. The higher resolution mapping and scoring of drought tolerance phenotypes,

which are underway, will resolve this apparent contradiction. It is possible, e.g., that there are multiple QTL in this region in repulsion linkage.

RCSLs are useful for identifying favourable alleles in exotic germplasm, as in the case of the 1H, 2H, and 4H introgressions in RCSL-88. RCSLs are also useful for identifying regions of the recurrent parent genome which should be retained. For example, RCSL-89 had the lowest agronomic performance and in this line introgressions of *H. spontaneum* in chromosome 3H

Fig. 4 Graphical genotypes of RCSL-88 and RCSL-89 showing segments of *H. spontaneum* (*in blue*) introgressed into an *H. vulgare* cv. Harrington (*in black*) background. Chromosome numbers according to the new barley nomenclature are: 1 (7H), 2 (2H), 3 (3H), 4 (4H), 5 (1H), 6 (6H) and 7 (5H). Source: http://barleyworld.org/northamericanbarley/Germplasm/IvanRCSL/RCSLmain.php
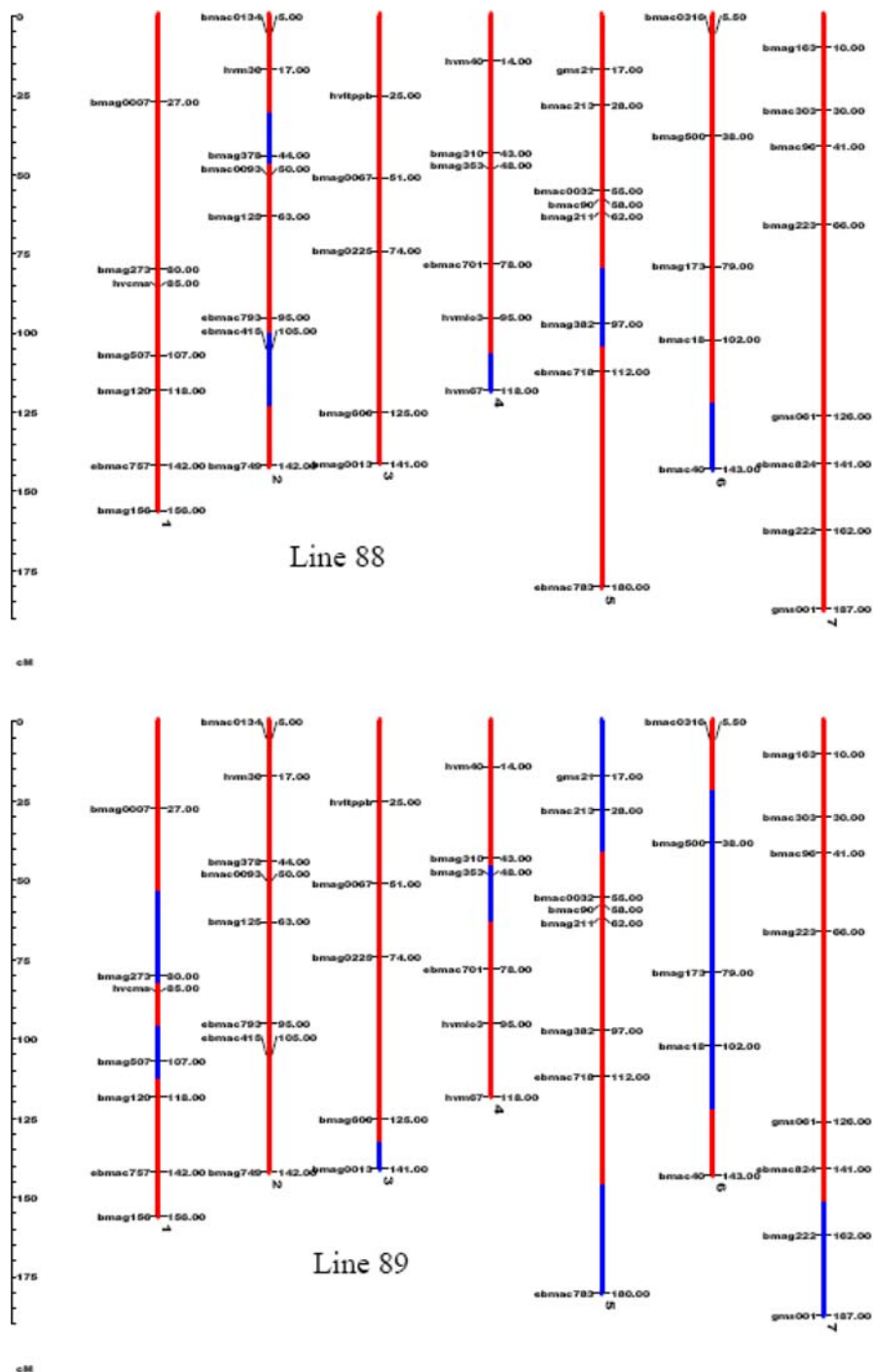
and 7H (Fig. 4) were associated with reductions in yield and increases in plant height. These "negative introgressions" serve as "knock outs" by revealing favourable Harrington alleles that should be retained during further germplasm development efforts.

In summary, the 80 RCSLs resulting from the introgression of *H. spontaneum* genome segments into Harrington allowed us to identify chromosome regions significantly related with plant height, grain yield and adaptability. We show that introgression of wild progenitor chromosome segments can improve the agronomic performance of an elite cultivar. Intogressed regions were associated with phenotypic variation in plant height and grain yield, and with reported QTL for traits related to water status and drought tolerance. The regions that correspond with locations of QTL reported from other barley germ-plasm combinations may represent novel allelic variants at previously-described loci. The associations without precedent in the literature may be false positives or they may represent the effects of novel alleles at loci where alleles were fixed during domestication and subsequent selection. The complete set of RCSLs will provide a valuable resource for association mapping based on a saturated genome map and extensive assessment of a suite of phenotypes. The set will also allow for detailed physiological studies involving specific RCSLs based on novel genome haplotypes and phenotypic attributes.

In a subsequent report we will present associations between molecular makers and physiological traits related with drought tolerance, such as canopy temperature, fraction of intercepted radiation, relative leaf water content, chlorophyll concentration, leaf conductance and ^{13}C discrimination.

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