Marker-trait association for disease resistance in the Spanish barley core collection

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There are abundant examples of the utility of landraces and wild relatives as potential sources of new genes and alleles for crop breeding. In crops like barley, the genetic variability of landraces was not fully exploited at the beginning of modern breeding (Fischbeck, 1992). Linkage drag is one major issue when considering introgression from exotic sources. Introgression of new alleles into elite cultivars is done more effectively from landraces than from wild relatives, as they are genetically closer. For these reasons, barley landraces should be thoroughly mined for new genes or alleles. The Spanish Barley Core Collection (SBCC) was conceived as a resource for research, and was assembled as a representative sample of the genetic variability present in the collection of over 2000 accessions held at the National repository for plant genetic resources (CRF-INIA). The objective of this study was to carry out a preliminary survey of the potential of the SBCC as a source of disease resistance genes or alleles, and to assess the possibility of detecting disease resistance QTLs via association mapping. In a companion paper (Yahiaoui *et al*., this volume), we tested the feasibility of carrying out association analysis for agronomic and morphological data.

Material and methods

Plant material: The SBCC is constituted by 159 (148 six-row, 11 two-row) inbred lines derived from local landraces, and 16 cultivars (8 six-row, 8 two-row) commonly grown in Spain for long periods during the 20th century.

Phenotypic evaluation: The accessions of the Spanish Barley Core Collection were examined for the presence of disease resistance traits against several fungal and viral diseases, at the Institute of Epidemiology and Resistance Resources in Aschersleben (*Drechslera teres, Puccinia hordei, Blumeria graminis*, Barley yellow dwarf virus), and at the Institute for Crop Production and Plant Breeding from Bavaria (*Rhynchosporium secalis*). The methods followed for each pathogen were:

(i) Powdery mildew (*Blumeria graminis*): Barley seedlings were artificially inoculated with seven different isolates of the fungus in the greenhouse. Disease symptoms were assessed on the primary leaf of the seedlings, following a scoring scale of 0 to 4 (0, 1, 2, resistant; 3, 4 susceptible). Results were expressed individually for each pathogen isolate, and as an overall mean for all seven isolates.

(ii) Scald (*Rhynchosporium secalis*): Seedlings were infected with one isolate of pathogen in the greenhouse. Disease visual scores (from 0, resistant, to 4, susceptible), were given for four leaves of each plant, every 2-3 days, up to four scoring dates. Finally, scores were converted into four categories (0, resistant; 1, moderately resistant; 2 moderately susceptible; 3 susceptible).

(iii) Leaf rust (*Puccinia hordei*): Field test. Naturally occurring symptoms were assessed visually (0, no symptoms, 4 corresponds to 50% of leaf surface covered).

(iv) Net blotch (*Dreschlera teres*): Seedlings were artificially infected in the greenhouse with three different isolates. Percentage of leaf surface affected, and a visual score (1 through 8, Tekauz, 1985) were recorded at several leaves for each plant. An average score with four categories (0, resistant; 1, moderately resistant; 2 moderately susceptible; 3 susceptible) was calculated, taking into account the results for the three isolates. Only this average score is reported.

(v) Barley yellow dwarf virus: Up to 15 plants per accession were infected, and visually scored in 9 different dates (from 0, resistant, to 9, susceptible). The same number of uninfected plants was kept as control. The degree of attack based on these scores was calculated as in Schmidt *et al*. (1980). Grain weight, plant height, and number of ears were recorded for each single plant (infected and uninfected). Thousand kernel weight was also calculated on the pooled kernels for each accession and treatment. For the last four variables, the degree of resistance was calculated as the percentage of the value for each trait in the infected plants over the uninfected ones.

Molecular markers: In a previous study, 225 barley accessions (including the 175 SBCC accessions) were genotyped with 73 markers, distributed along the barley genome (see Fig. 1 in Yahiaoui *et al*., *Genome wide…*, this volume). Population structure was evaluated with the software STRUCTURE (Falush *et al*., 2003), using data on 64 SSR. For association analysis, only markers with band frequencies in between 5% and 95% were used. To assess the association of each maker locus with traits, we fitted a multiple regression model of the trait response on the set of allele indicator variables, as reported by Kraakman *et al*. (2006), also taking into account population structure. Multiple testing was addressed with a Bonferroni correction for 73 independent tests (P threshold of 0.0014, equivalent to a genome-wise P=0.10). When several markers showed apparent association for the same trait, a combined analysis of variance including all significant markers (as well as a variable summarizing the population structure), was used to check independence of their effects.

Results and discussion

The results of the phenotypic evaluation are presented in Table 1. In several cases, cultivars with well-known reactions were used as checks. Their results are also included in Table 1.

Apparently, there were a number of accessions resistant to BYDV, though this fact needs further discussion, after the association analysis. Leaf rust and net blotch resistance levels recorded were quite low. For *D. teres*, even the accessions recorded as resistant (4) were on the lower margin of this category, though the evaluations very consistent across the three isolates. There were rather high numbers of resistant accessions to scald and powdery mildew. Scald testing was repeated later with the same isolate and the results were consistent (not shown). The most resistant accessions were tested further with a second isolate, and a good number of them were still resistant (not shown). Powdery mildew resistant accessions came all from low coastal regions.

After the molecular marker analysis and the search for population structure, the accessions were assigned to four genetically distinct populations, clearly separating the Spanish landraces from other European materials. The inclusion of population structure in the analyses reduced the number of significant associations. Even though the number of markers was low, still some associations were strong enough to point out possible QTLs. Table 2 presents the models including all significant markers for each trait, and the coefficient of determination accounted for by these markers.

There were no significant associations with net blotch and leaf rust at the significance level used in this study.

For BYDV, there was an apparent QTL of large effect at HvBM5. This result might be an artefact, as this locus corresponds to the vernalization gene *VRN-H1*. The allele presenting high susceptibility (4850 bp) is typical of Spanish winter genotypes, and confers prostrate growth habit. The infection by the virus may have been affected by differences in plant development between the different growth classes (winter, facultative, spring). No associations were found at the previously reported regions: *Yd2* in chromosome 3H (Collins *et al*., 1996), QTLS in 2H and 3H (Scheurer *et al*., 2001), and in 4H, 5H and 7H (Toojinda *et al*., 2000). The apparent QTL at Bmag013 seems new, but the level of resistance associated is quite low.

Table 1. A, BYDV; B, fungal diseases. In each sub table, top: number of accessions from the SBCC distributed according to the resistance scale used for each disease; bottom: scores of check cultivars

A	Barley Yellow Dwarf Virus				
Score	Degree of attack	Thousand kernel weight	Grain weight per plant	Plant height	Ears per plant
	---------- Number of accessions, SBCC ----------				
0	0	37	4	16	23
1	0	65	5	49	16
2	5	46	8	33	17
3	29	13	7	28	31
4	41	1	25	22	28
5	33	0	28	10	20
6	23	0	24	6	12
7	19	0	27	3	10
8	10	0	24	1	8
9	8	6	15		3
Cultivars	---------- Scores ----------				
Femina	4	2	5	1	4
Coracle	0	0	0	0	0
Vixen	0	0	0	0	0

B	Powdery mildew (*Blumeria graminis*)								Scald	Net blotch	Leaf rust
Score†	R19	R30	R78	R79	R9	R117	R126	Mean	*R. secalis*	*D. teres*	*P. hordei*
	---------- Number of accessions, SBCC ----------										
0	6	19	9	5	22	26	24	7	40 (resistant)	4 (resistant)	7
1	8	25	18	21	85	94	134	45	37	11	52
2	24	61	74	75	62	42	8	119	43	44	53
3	84	56	64	70	2	9	0	0	33 (suscep.)	97 (suscep.)	40
4	53	9	5	0	0	0	0	0			4
Cultivars	---------- Scores ----------										
Alexis	0.5	0	0.5	0	0.5	0	0				
Ponto	3.5	3.5	3		0	0	0				
Pasadena	3.5	2	3.5	3		0	0.5				
Barke	1.5	0	1.5	1	0	0	0				
Steffi									3		
Camelot									1		
Alexis									3		

†0, resistant; 4, susceptible. Inverse of percentages of infected over uninfected plants, converted into scores by decades (0%-10% = 0; 11%-20% = 1, etc)

Regarding powdery mildew, it is interesting to note that the only association found for the average score is different from the associations for individual isolates (which would be consistent with horizontal resistance). This average score correlated significantly with a visual score recorded in field trials in Spain under natural infection conditions (r=0.50). The marker involved (Bmac156) maps in the long arm of chromosome 7H, in the same region as the resistant QTLs from *H. spontaneum* reported by Schönfeld *et al*. (1996), and by von Korff *et al*. (2005). Three of the markers with associations for specific races (HvBKASI, Bmag206 and Bmag013) are located in regions with QTLs for powdery mildew resistance in three different crosses of *H. vulgare* with *H. spontaneum* (Schönfeld *et al*., 1996; Backes *et al*., 2003; von Korff *et al*., 2005).

For scald, there are numerous genes and QTLs described in the literature (Williams *et al*., 2003).

The SBCC seems to be quite rich in scald resistance (Table 1). However, the only marker associated with scald resistance is not located near any of previously described loci and thus could be marking an undescribed locus. The discrimination in scald resistance offered by this marker, however, is low, and its effect could still be a reflection of some other region in LD with the marker.

Table 2. Molecular markers significantly associated with disease resistance traits (A, BYDV; B, fungal diseases). Means followed by the same letter were not significantly different at P<0.05

A. BYDV		Degree of attack		Grain weight per plant		Plant height		Ears per plant	
HvBM5	n	Mean		Mean		Mean		Mean	
4850	93	6.17	a	25.9	b	59.0	b	48.4	b
1200	50	3.63	b	55.4	a	87.7	a	80.1	a
150	9	4.14	b	61.9	a	82.6	a	75.3	a
1900	10	3.29	b	48.1	ab	80.9	a	76.1	a
Bmag013									
149	20			54.3	ab	76.9	abc	76.4	a
153	10			49.3	ab	80.5	ab	78.4	a
155	38			52.9	ab	82.6	a	75.3	a
157	14			41.3	bc	78.1	ab	62.4	ab
159	14			44.0	abc	72.6	bc	62.4	ab
161	18			34.8	c	68.9	c	51.9	b
163	16			56.4	a	82.8	a	72.4	a
165	9			49.7	abc	78.0	ab	80.6	a
Bmag120									
230	12			42.3	ab				
232	9			73.0	a				
234	20			47.8	ab				
236	48			50.5	a				
244	9			32.4	c				
262	9			48.5	ab				
264	21			40.5	bc				
R^2		40.9		54.3		54.1		46.7	

B. *Rhynchosporium secalis*			
HvALAAT	n	Mean	
197	10	1.90	c
198	14	2.01	c
199	29	2.48	bc
200	23	2.36	bc
202	74	2.81	ab
203	18	3.30	a
R^2		8.9	

Blumeria graminis, race 30			
HvBM5	n	Mean	
4850	93	2.10	b
1200	49	1.65	c
150	9	2.99	a
1900	10	2.86	a
R^2		6.9	

Blumeria graminis, race 19			
HvBKASI	n	Mean	
198	24	3.51	a
199	113	3.35	a
200	33	2.72	b
R^2		10.0	

Blumeria graminis, average			
Bmac156	n	Mean	
135	14	1.47	c
137	26	2.15	a
139	19	1.91	ab
141	19	1.76	bc
143	26	2.19	a
R^2		17.7	

Blumeria graminis, race78			
Bmac113	n	Mean	
181	70	2.31	b
191	17	2.94	a
193	22	2.77	a
195	22	2.55	ab
197	10	2.85	a
Bmag206			
245	16	3.01	a
247	36	2.25	c
249	12	2.55	abc
251	15	2.75	ab
253	13	2.37	bc
255	10	3.12	a
257	11	2.74	ab
R^2		31.7	

Blumeria graminis, race 79			
HvBKASI	n	Mean	
198	24	2.64	ab
199	114	2.73	a
200	33	2.30	b
Bmag136			
195	15	2.73	a
197	31	2.50	a
201	89	2.18	b
203	31	2.82	a
Bmag013			
149	21	2.38	bc
153	10	2.25	c
155	38	2.40	c
157	14	2.70	abc
159	14	2.30	c
161	18	3.02	a
163	17	2.79	ab
165	9	2.63	abc
R^2		27.1	

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