SCREENING THE SPANISH BARLEY CORE COLLECTION FOR DISEASE RESISTANCE

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ABSTRACT

The Spanish Barley Core Collection comprises 159 landrace-derived inbred lines and 16 cultivars adapted to Southern European conditions. The collection was screened for resistance to powdery mildew (*Blumeria graminis*), scald (*Rhynchosporium secalis*), leaf rust (*Puccinia hordei*), net blotch (*Pyrenophora teres* f. *teres*), *Barley yellow dwarf virus* (BYDV) and *Barley mild mosaic virus* (BaMMV). Resistance to powdery mildew was outstanding, with 58 lines presenting mean overall resistance, among them 7 landrace-derived lines resistant to all seven isolates tested. About 26 % of the Spanish lines were resistant to scald. Resistance to leaf rust and to net blotch was scarce, though a few accessions showed resistance levels as good as the checks. Thirteen accessions (12 Spanish) were totally resistant to BaMMV, and ca. 20% of accessions showed moderate tolerance to BYDV. Landrace-derived lines from the Mediterranean Coast and Southern regions of Spain were the most resistant to powdery mildew and leaf rust, but the most susceptible to viruses. Potential sources of resistance might be preserved in some accessions subjected to selective pressure in the region of origin.

Keywords: barley – core collection – disease resistance – powdery mildew – scald – net blotch – viruses

INTRODUCTION

Both, spring and winter barley are susceptible to several pathogens which are responsible for losses not only in yield but also in the quality of the grain harvested. Nowadays, diseases cause considerable economical losses in all continents (Hovmoller et al. 2000, Zhan et al. 2008). Barley diseases are caused by viruses (*Barley yellow dwarf virus, Cereal yellow dwarf virus , Barley mild mosaic virus, Barley yellow mosaic virus, Barley stripe mosaic virus*, etc), bacteria (bacterial blight, bacterial stripe, etc.) and fungi (powdery mildew, scald, leaf rust, net blotch, etc.).

In most cases, the control of diseases involves the use of pesticides. However, environmental and consumer concerns about the excessive use of such chemicals and the persistence of their residues (Gullino and Kuijpers 1994), as well as the risk of insensitivity built up in plant pathogens (Bäumler et al. 2003), recommend the use of resistant cultivars.

Many resistance genes and quantitative trait loci (QTL) against the main diseases affecting barley are already known. Some of them have been successfully introgressed into high yielding cultivars fulfilling commercial requirements (Friedt and Ordon 2007). However, very few resistances combine all the characteristics necessary for a complete solution from a breeding point of view. The use of genetic resistances in breeding is hindered by several causes, especially the rapid breakdown under the pressure of new strains of the pathogens (Hovmoller et al. 2000), but also partial effectiveness, or limited introduction in elite cultivars due to linkage drag. Therefore, breeders need a continuous supply of new sources of disease resistance to keep up with the pace imposed by the challenges of pathogens and the demands of producers.

Wild relatives and landraces represent valuable reservoirs of new interesting traits that were left behind as a consequence of domestication and may be used for crop improvement. In barley, the genetic variability present in old landraces was not fully employed at the beginning of modern breeding (Fischbeck 2003). Breeding for disease resistance of barley is one of the most important

fields where this unexploited variability might still make a useful contribution (Jahoor and Fischbeck 1987, Pickering et al. 1995, Jørgensen and Jensen 1997).

The Spanish Barley Core Collection (SBCC, Igartua et al. 1998) is constituted by a representative sample of the landraces cultivated in Spain before the advent of modern breeding, as well as by a small set of successful old cultivars. The landrace material are inbred lines derived from landraces kept at the Spanish National Germplasm Bank, which holds more than 2000 accessions of cultivated barley. The majority of this collection is constituted by native landraces, collected in Spain predominantly in the first half of the 20th century. Such landraces possess an important history of adaptation and selection under local conditions, which makes them a very attractive resource to explore the natural variation for disease resistance, and other adaptive traits. Previous studies comparing morphological and agronomic traits, and genetic diversity between the SBCC and cultivars from other European countries, indicated that this collection holds distinct and valuable phenotypic and genetic variability, therefore representing a potentially useful breeding resource (Lasa et al. 2001, Yahiaoui et al. 2008). The main goal of the present work is to investigate its potential as source of genetic resistances to a set of the most relevant barley diseases.

MATERIALS AND METHODS

Plant material. The Spanish Barley Core Collection (Igartua et al. 1998) was used in this study. The collection consists of 159 (148 six-rowed and 11 two-rowed) inbred lines derived from local landraces, and 16 commercial cultivars (8 six-rowed and 8 two-rowed) with a long tradition of cultivation in Spanish agriculture (the detailed composition of the collection can be consulted at http://www.eead.csic.es/EEAD/barley/).

Disease assessment. All barley accessions were evaluated for resistance to powdery mildew (*Blumeria graminis* f.sp. *hordei*), leaf rust (*Puccinia hordei*), net blotch (*Pyrenophora teres* f.sp.

teres), *Barley mild mosaic virus* (BaMMV) and *Barley yellow dwarf virus* (BYDV) at the former Institute of Epidemiology and Resistance Resources of the Federal Research Centre for Cultivated Plants (BAZ) in Aschersleben (currently, the Institute for Resistance Research and Stress Tolerance of the Julius Kühn-Institute in Quedlinburg, Germany). Tests for disease resistance against scald (*Rhynchosporium secalis*) were carried out at the Institute for Crop Science and Plant Breeding in Freising, Bavaria (Germany). Infection conditions and disease assessment for the different pathogens are detailed below.

For powdery mildew resistance, plants in the first leaf stage were artificially inoculated in the greenhouse with seven isolates of *B. graminis* (R19, R30, R78, R79, R9, R117 and R126). Ten plants per line and isolate were used. Isolates were chosen according to their virulence spectra observed on the Pallas isogenic lines differential set (Kolster et al. 1986). Disease severity was recorded 8 days after inoculation on the primary leaf at the seedling stage on a scale of 0-4 for all 10 plants, following the procedure of Torp et al. (1978) and Jensen et al. (1992). Plants were classified as resistant if the disease score was 2 or lower and susceptible if higher than 2. Cultivars ‘Alexis’ (resistant, mlo_9), ‘Barke’ (resistant, mlo_9), ‘Pasadena’ (susceptible) and ‘Pongo’ (susceptible) were used as controls.

Experiments for scald were also carried out in the greenhouse. Four seedlings per accession were infected with about 150 - 200,000 spores/ml of the *R. secalis* isolate Sachs 147–1. Disease scores were recorded on the four plants according to a 0-4 scale (0-1=resistant, 1.1-2=moderately resistant, 2.1-3=moderately susceptible and 3.1-4=susceptible). Disease was first evaluated 2 weeks after infection and then every 2-3 days until four scoring dates were obtained. Data shown are the mean values for four dates and four examined plants on each date. Cultivars ‘Steffi’ (susceptible), and ‘Camelot’ (moderately resistant) were included as controls.

Evaluation of resistance to net blotch was performed in the greenhouse on twelve plants per accession. Tests were performed both on detached leaves (Hartleb and Meyer, 1988) and whole

plants. When plants reached the 4-5 leaf stage, they were inoculated with three different isolates (codes 97:1 (Sweden), Am (Germany), NZ (New Zealand)). These isolates were chosen because they had demonstrated high aggressiveness and diverse virulence patterns on a differential set of cultivars. For the detached leaves test, three plants per accession were inoculated with each isolate by spraying the second and third leaves with a freshly prepared spore suspension of 3,000 conidia/ml. The three remaining whole plants per accession were inoculated only with isolate Am, also at the 4-5 leaf stage. Disease symptoms were rated at 7 days (detached leaves) or 14 days (whole plants) after inoculation by calculating the leaf area showing lesions (% showing chlorosis and necrosis) and lesion type according to a 1–10 scale (Tekauz 1985), in which scores of 1-3 were considered resistant, 4 as moderately resistant, 5-6 moderately susceptible, and over 6 as susceptible. Data presented are the mean for each isolate on the detached leaves assay. Cultivars 'Compana' and 'Femina' were included as susceptible standards and 'Zenit' as resistant check.

Resistance to leaf rust was tested in field trials under natural infection. Disease severity was assessed visually as the percentage of infected leaf area in the flag leaves. Plants with a percentage of infection between 0 and 10% were considered as resistant, plants with 10 - 30% of infected leaf area were recorded as intermediate, and plants with over 50% were rated as susceptible. Cultivar 'Vada' was used as a resistant control and 'L94' as the standard for susceptibility. Resistance to leaf rust was also tested in the greenhouse, on five plants per line, using isolate I-80, which is virulent to all known major resistance genes present in European barley, except for *Rph7* (Ivandic et al. 1998). Infection types were scored at 12 days after inoculation following a 0-4 scale (Levine and Cherewick 1952). Infection types 0, 1 and 2 indicate host resistance and types 2-3, 3 and 4, host susceptibility.

For the BaMMV test, 20 to 30 seedlings at the 3- to 5-leaf stage were mechanically inoculated twice at an interval of 5 to 7 days with the isolate BaMMV-ASL, which was propagated on the susceptible barley cultivar 'Maris Otter'. Inoculated plants were cultivated in a growth chamber at

12 °C with a photoperiod of 16 h day (nearly 10 klx) and 8 h night. Four to five weeks after the first inoculation, the number of plants with mosaic symptoms was recorded and expressed as Infection Rate (IR, percentage of plants with symptoms). DAS-ELISA was done according to Clark and Adams (1977), to confirm resistance only on those accessions with no symptoms or unclear symptoms.

For testing the reaction to BYDV, all accessions were sown in the field for two variants (virus inoculation, and healthy control). Fifteen seeds per accession and variant (or treatment) were used. The plants were grown until the 1-2 leaf stage. Then, the plots were covered with a fleece and the plants of the 'infected variant' were infested by viruliferous aphids (isolate BYDV-PAV ASL1) of the species *Rhopalosiphum padi* for about 2 weeks. After removal of the cotton covers, the aphids were killed by insecticide treatment. Spraying was repeated regularly to keep the plants without aphids. Disease symptoms were evaluated at heading time following a scale from "no symptoms", scored as 1, to "dead plant", scored as 9, and the degree of attack (DA) was calculated as

$$DA = \frac{\sum_{b=1}^{9}(n_b * (b-1)) * 100}{N * (B-1)}$$

n_b=number of plants per scoring class

b=scoring class

N=total number of tested plants

B=highest scoring class

At harvest, additional measurements of morphological characters (plant height, thousand kernel weight, kernel weight per plant and ears per plant) were recorded in inoculated and non-inoculated plants. The performance of each line was calculated as a relative percentage of infected plants to non-infected ones of the same line [i.e. (infected variant/healthy control) ×100]. Spring barley cv.

'Coracle' and winter barley cv. 'Vixen', both with the resistance gene *Ryd2*, were used as tolerant checks and cv. 'Femina' as susceptible control.

Statistical analysis. In order to establish possible correlations between the degree of resistance of different landraces and its genetic basis, the landrace-based accessions were clustered into four populations (I-IV), according to their genetic similarity (Yahiaoui et al. 2008). Two additional groups were created to include the eight two-rowed (Group V) and the eight six-rowed cultivars (Group VI). Comparisons between groups and mean disease scores were performed by analysis of variance using the general linear model procedure (GLM) in SAS (SAS 1988).

Correlation coefficients were used to analyze the relationship between geographic distribution and disease response. Additionally, landrace-derived inbreds were grouped into agro-climatic regions according to Papadakis classification (Papadakis 1975). The relationship of climate with tolerance to diseases was analyzed with proc ANOVA (SAS 1988).

RESULTS

High levels of resistance were found in the SBCC for powdery mildew and scald, whereas overall resistance levels for leaf rust, BYDV, BaMMV and net blotch were moderate or low. A broad spectrum of disease responses was observed for all pathogens, except for BYDV, with lines going from totally resistant to completely susceptible.

Barley accessions differed in overall resistance to the seven isolates of powdery mildew (Table 1). A majority of lines showed resistance to isolates R9, R117 and R126, whereas a great number of them (79.6%) developed severe symptoms after infection with isolate R19. Severity of infection with isolates R30, R78 and R79 was intermediate. When considering the mean of the disease score for all the isolates, a remarkable proportion of lines (31.6%) had an average score of 2 or lower, i.e., were resistant. About 4.5% of the landraces showed resistance (disease score between 0 and 2)

against all seven isolates, whereas only one line was susceptible to all the strains. The behaviour of cultivars was similar to that of landraces, but none of them was resistant to all seven strains (two out of sixteen were resistant to six strains). The disease scores observed for susceptible and resistant checks were as expected. According to the German descriptive variety list of the Federal Office of Plant Varieties, cv. 'Pasadena' is not classified as highly susceptible to powdery mildew; therefore it can show some resistance depending on the *B. graminis* isolates (Table 1, bottom). A cluster analysis of the seven isolates, based on the resistance scores of the accessions, suggested the presence of three different types of reactions: one to isolates R19, R78, and R79; another one to isolates R9, R117, and R126; and the more distinct one, to isolate R30 (Fig. S1).

The overall resistance against net blotch in the Spanish landraces was quite low (Table 2). Most of the accessions (between 90% and 98%) were classified as susceptible or moderately susceptible for each of the different isolates. Only one accession was found resistant to all three isolates, and another one was classified as moderately resistant to isolate 97:1 and resistant to isolates Am and NZ. The cultivars displayed low resistance levels as well (Table 2). High correlation coefficients (0.68 for 2^{nd} leaves and 0.79 for 3^{rd} leaves) were found between detached leaves and whole plants for isolate Am, and thus only the more complete set of results for the detached leaves test is presented. Check cultivars behaved as expected, 'Compana' and 'Femina' showed susceptibility to all three isolates, whereas cv. 'Zenit' was moderately resistant to isolates Am and NZ, and moderately susceptible to the most aggressive isolate, 97:1.

Regarding scald, landraces were distributed uniformly among the four disease resistance classes, with percentages around 25% for all of them (Table 2). Cultivars from the SBCC were classified mainly as either susceptible (37.5%) or moderately resistant (43.8%). Check cultivars 'Steffi' and 'Camelot', were properly classified as susceptible and moderately resistant, respectively.

Most of the landrace-derived inbred lines displayed intermediate levels of resistance to leaf rust under natural infection conditions. Seven of these lines (4.5%) showed a resistance score lower or

equal to ‘Vada’, whereas eighteen (11.6%) showed an infection score equal to or higher than the susceptible check ‘L94’ (Table 3). However, after inoculation under controlled conditions with the isolate I-80, the majority of accessions apparently resistant turned out to be susceptible. Eighty two percent of landrace-derived lines presented a disease score of 3 in this test (Table 3). Regarding cultivars, the majority showed a result of 10% of infected leaf area in the field test, with 2 of them presenting lower scores than the resistant check ‘Vada’. In contrast, no cultivar showed resistance to *P. hordei* isolate I-80. A low correlation was found between results from field and greenhouse experiments, i.e., some accessions showing field resistance were susceptible to isolate I-80, although most lines resistant to I-80 in the greenhouse also displayed field resistance.

High levels of susceptibility were found for BYDV and BaMMV (Table 4). For BYDV, most landraces (81.5%) had a degree of attack similar or higher than the one recorded for the susceptible check ‘Femina’. Only about 20% showed a moderate tolerance (DA between 10 and 30%) and no line displayed a tolerance level as high as the resistant check ‘Coracle’. Variations in the value of morphological characters were especially observed for kernel weight per plant (TKW) and plant height, and more moderately for TKW and number of ears per plant (Table S1). Most cultivars were quite susceptible to BYDV and only 2 of them showed a DA similar to the moderately tolerant cv. ‘Vixen’. After inoculation with BaMMV in the growth chamber, the great majority of lines (69%) displayed infection rates (IR) ranging from 90 to 100%, and only 33 lines out of 168 expressed IR lower than 10%. The results for the test DAS-ELISA confirmed a good level of resistance in 18 landrace-derived lines and in one cultivar (Table 4), among them 13 with total resistance (IR=0%).

The results of the evaluation for all these diseases were combined to find out accessions in which tolerance for several diseases may concur (Table 5). For this purpose, we followed strict criteria to define resistance. Only those accessions with scores similar to or better than resistant checks were considered. No accession presented resistance to all diseases, and 67 turned out to be susceptible to all six diseases tested. A majority was resistant to only one pathogen. Interestingly, 3 landrace-

derived lines were resistant to three pathogens (*B. graminis*, *P. hordei* and *R. secalis* or *P. teres*), and 11 landrace-derived lines and 2 cultivars (one of them Spanish) showed resistance to both powdery mildew and scald (Table 5).

Significant differences were found among the populations defined by Yahiaoui et al (2008) and among the groups of checks in response to powdery mildew, scald, leaf rust and BaMMV (Table 6). In general, lines clustered into population IV showed higher levels of resistance to powdery mildew, leaf rust and scald, although they were highly susceptible to BaMMV. Two-row cultivars (group V) were significantly more resistant to powdery mildew and leaf rust than population IV but they were more susceptible to scald. Population III had a slightly better resistance to scald than others, at the same level as group IV, and lines in this group were the most resistant to the *Barley Mild Mosaic Virus*.

When considering the agro-climatic distribution of landrace-derived inbred lines according to Papadakis index, some differences in disease resistance were also apparent. Accessions belonging to areas with the Mediterranean Maritime (MM) climate showed a higher degree of resistance to powdery mildew and BYDV than those in other groups, but they were the most susceptible to BaMMV. Accessions coming from Temperate Mediterranean and Fresh Temperate Mediterranean were the relatively least resistant to leaf rust and BYDV, respectively, but they showed high resistance against BaMMV (Table 6). Regarding scald, the level of resistance was equally distributed over all climates.

Correlation coefficients between altitude, latitude, rainfall and disease score were generally low and only significant for powdery mildew, leaf rust, net blotch and BaMMV (Table 6, bottom). These correlations were performed for the landrace-derived inbred lines only. Powdery mildew susceptibility appeared to be positively correlated with altitude and latitude but negatively correlated with rainfall. A positive correlation exists for leaf rust only with altitude. On the contrary,

accessions from higher altitudes and latitudes were more resistant to BaMMV. Those accessions coming from the rainiest regions resulted more resistant to net blotch.

DISCUSSION

In the present work, evaluation for disease resistance of the 175 accessions in the SBCC has revealed the presence of a large diversity of responses, and important levels of resistance to powdery mildew and scald, moderate resistance to leaf rust, and low levels of resistance to net blotch, BaMMV and BYDV. The web site http://www.eead.csic.es/EEAD/barley/ presents detailed information on each accession. The present study confirmed the value of barley landraces as sources of disease resistance, as found previously in other landrace collections from East Mediterranean, Near East, Nepal, Morocco, Tunisia, Ethiopia, China and the Czech Republic (Jørgensen and Jensen 1997, Sun et al. 1999, Czembor and Johnston 1999, Dreiseitl and Steffenson 2000, Czembor 2002, Shtaya et al. 2006a).

The reactions to isolates of *B. graminis* could be ascribed to three different virulence spectra, with isolate R30 presenting a quite different profile compared to the other isolates. R19 (the most virulent), R126 (the least virulent), and R30 are representative of these three classes. The differential genotypic responses to these groups could be indicative of the presence of race-specific resistances. On the other hand, some accessions displayed resistance against all isolates. This broad-spectrum resistance could involve either a resistance of quantitative nature, or the accumulation of several race-specific genes.

Landraces in the SBCC exhibited intermediate levels of resistance to *P. hordei* when they were tested in field trials. However, most of them showed high infection types (3 score on a 0-4 scale) when infected with the pathotype I-80 at the seedling stage in the greenhouse. This pattern is consistent with the widespread presence of partial resistance, defined as a reduced rate of epidemic

development despite susceptible infection types (Parlevliet 1975). Other surveys in landrace germplasm of the Fertile Crescent (Shtaya et al. 2006a), Ethiopia (Woldeab et al. 2007), and Spain (Shtaya et al. 2006b) found mostly good levels of partial resistance, with a few accessions showing hypersensitive, possibly race-specific, reactions. Leaf rust resistance is more needed in winter barley, where sources are limited (Walther et al. 2000, Mammadov et al. 2007). The resistance levels found in this study, however, may not be sufficient to contribute to improve the already remarkable partial resistance exhibited by current barley cultivars (Niks et al. 2000). A more detailed study of the Spanish resistant accessions will be needed to find out the type and effectiveness of the resistances found.

A remarkable number of lines resistant to *R. secalis* were detected in the SBCC. Similarly, high levels of resistance to scald were found in Ethiopian and Syrian landraces (van Leur et al. 1989; Yitbarek et al. 1998). Recent works in the UK suggested that winter barley cultivars have much better partial resistance to scald than spring barleys, because they have been more exposed to the pathogen (Zhan et al. 2008). This could also be the case for Spanish landraces, a majority of which are winter and facultative types, although high levels of scald resistance were also found in some spring barleys distributed over all 5 climatic zones. This resistance, especially when compared to the results of the checks used, seems extremely interesting for direct use in plant breeding programs.

A modest level of resistance to viruses (BYDV and BaMMV) has been found in the SBCC with most lines showing a degree of attack higher than 40% and an infection rate of 100%. It is likely that the tolerance to BYDV observed in some lines is not originated by the *Ryd2* gene, whose effect on resistance is very clear, as in the check cultivar 'Coracle'. The genes responsible for this moderate resistance might be combined with *Ryd2* in a strategy proposed by Sip et al. (2004), to obtain a more durable resistance, effective against the different viruses and strains of the BYDV-complex. The resistances found in the SBCC could be of this kind, but further work with the most resistant SBCC lines would be needed to confirm that their resistance level is useful for plant

breeding, and that the genes that they may contribute are different from the genes underlying other resistances of similar nature found in modern cultivars (Ovesná et al. 2000).

Resistance to net blotch in the landrace-derived inbreds was not outstanding, and there were very few lines which were as resistant as the most resistant cultivars tested. For eleven of the cultivars included in the SBCC or used as checks, there were net blotch field evaluations registered at the GRIN database of the USDA (http://www.ars-grin.gov/npgs/acc/acc_queries.html). The scores for these cultivars varied between moderately resistant to susceptible (the majority). As the landrace materials barely surpassed these cultivars in resistance, it seems unlikely that there are good sources of net blotch resistance in the SBCC.

In a recent work, Yahiaoui at al. (2008) reported high genetic divergence in the SBCC compared to reference European cultivars, and a remarkable internal diversity in the collection. The SBCC accessions clustered in four populations (coded I-IV) based on genetic similarity. The geographic distribution of these populations followed climatic patterns. Analysis of variance for disease scores taking into account these groups as sources of variation showed some significant differences in mean disease scores. Group V, composed of two-row cultivars, displayed the highest resistance to both powdery mildew and leaf rust, but they were the most susceptible to scald and BaMMV. Similar results were observed in field tests carried out in Spain, where cultivars in group V were the least susceptible to powdery mildew (Yahiaoui 2006). Accessions in this group are old cultivars of diverse European origins, with a long tradition of successful cultivation in Spain, and their higher resistance to some diseases may be due to the incorporation, through breeding, of some sources of resistance.

Populations III and IV, the most abundant by far in the SBCC, presented contrasting results to three diseases, powdery mildew, leaf rust, and BaMMV. These differences in disease resistance between populations III and IV may be related to their preferential distribution over distinct ecogeographical areas (Yahiaoui et al. 2008), where they may have been subjected to distinct selective pressures for

prevalent diseases. Population III comprises landrace-derived inbreds originating mainly in the central part of the Iberian Peninsula, featuring higher altitudes and latitudes, and cooler climates than the area corresponding to population IV, which comprises mainly accessions from the lower and warmer lands of the Mediterranean Coast and the Southern part of Spain. The geographical distribution of populations I-IV is presented in detail in Yahiaoui et al. (2008). Therefore, the relationships observed between the degree of tolerance to some diseases, ecogeographic factors (altitude, latitude, climate type), and population may be the consequence of a process of adaptation and genetic differentiation of barley populations. This process would have been stimulated by adaptation to environmental conditions, where prevalent diseases may have played some role, and by the inbreeding system of barley. This relationship of disease tolerance with ecogeographic factors was also found in Ethiopia (Yitbarek et al. 1998, Woldeab et al. 2007), which is not surprising as this country features even larger ecological diversity than Spain. There has been enough time for a process like this to occur in Spain, where cultivation of barley dates back to 5000 years BC (Buxó et al. 1997).

Additionally, the distribution of the resistant accessions over the Iberian Peninsula showed some interesting patterns. For most isolates of powdery mildew (R9, R19, R78, R79, R117, R126), the majority of resistant accessions come from the South or the lower lands of the Eastern Mediterranean coast, which is coherent with the significant correlation coefficients of powdery mildew resistance with latitude and altitude. The most resistant accessions overall come from the South and Eastern Mediterranean coast, which coincide with the SM and MM climates. For the most distinct powdery mildew isolate, R30, there was a high frequency of resistant accessions in the Northern half of the country, something that was occasional for the other six isolates (data not shown). Regarding scald, there seems to be resistant accessions scattered all over the country. Only two clusters of remarkable concentration of resistance seem to be in Toledo-Madrid and in the contiguous provinces of Sevilla, Málaga, and Córdoba (data not shown). Two out of the three

landrace-derived inbreds that were resistant to net blotch originated in the Canary Islands. Although a small number of resistant accessions against BaMMV was found, it is remarkable that a significant correlation exists between ecogeographical parameters and resistance. Landrace-derived lines coming from higher and cooler places, therefore with better conditions for the virus, presented higher resistance, which may be a result of a history of co-evolution with the pathogen.

The combination of the distinct genetic diversity found in general in the SBCC (Yahiaoui et al. 2008) with the existence of remarkable levels of resistance with adaptive value, points to a high probability of finding novel sources of disease resistance in the SBCC. The novelty of these sources of resistance must be investigated further and, for that purpose, specific mapping populations have already been developed in collaboration by several of the authoring groups, and are currently under study. The joint occurrence of outstanding resistance to several diseases in some landrace-derived lines will facilitate the transference of resistance genes in breeding programs, as a single cross may serve several purposes.

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SUPPORTING INFORMATION

Figure S1. Dendrogram of the seven isolates of powdery mildew, based on a cluster analysis of the Euclidean distances between the accessions, calculated on the standardized disease scores over isolates, using the Ward method.

Table S1. Plant height and yield components of BYDV infected plants, relative to non-inoculated plants, of accessions in the SBCC. Numbers in columns indicate the number of landraces (Lr) or cultivars (Cv) in each percentage class, for each trait. The bottom of the table indicates the percentages obtained for cultivars used as checks.

Table 1. Disease assessment against seven *B. graminis* isolates in accessions from the SBCC. Numbers in columns below the isolates indicate the number of lines (Lr=landrace-derived inbred lines, Cv=cultivar) with different disease scores. The bottom of the table indicates the disease scores of checks for each isolate.

	***B. graminis* isolates**															
Disease Score	**R19[a]**		**R30[b]**		**R78[c]**		**R79[d]**		**R9[e]**		**R117[f]**		**R126[g]**		**Mean***	
	Lr	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**
0	3	1	1	2	3	0	4	0	7	2	6	6	4	1	0	0
1	2	0	22	3	12	0	5	1	52	7	54	3	75	12	8	1
2	8	0	36	6	41	5	30	4	77	5	74	7	74	3	41	8
3	42	7	63	4	82	9	104	11	19	2	21	0	2	0	106	7
4	99	8	32	1	16	2	12	0	0	0	0	0	0	0	0	0
Alexis	0.5		0		0.5		0		0.5		0		0		0.2	
Pongo	3.5		3.5		3		-		0		0		0		-	
Pasadena	3.5		2		2		3		-		0		0.5		-	
Barke	1.5		0		0		1		0		0		0		0.4	

*Mean of disease score for the seven isolates

[a] Virulent/avirulent on *Mla7, a10, a12, a13, ra, k, nn, p, at, g, La, h/ a1, a3, a6, a9, a22, a23, o5*

[b] Virulent/avirulent on *Mla1 a12, a22, ra, nn, p, at, La, h/ a3, a6, a7, a9, a10, a13, a23, k, g, o5*

[c] Virulent/avirulent on *Mla6, a7, a9, a10, a12, a13, ra, k, nn, p, g, La, h/ a1, a3, a22, a23, at, o5*

[d] Virulent/avirulent on *Mla6, a7, a9, a10, a12, a13, ra, k, nn, p, at g,, La, h/ a1, a3, a22, a23, o5*

[e] Virulent/avirulent on *Mla10, a23, ra, k, nn, p, at, g, La, h/ a1, a3, a6, a7, a9, a12, a13, a22, o5*

[f] Virulent/avirulent on *Mla3, a6, a12, a22, ra, nn, p, at, La, h/ a1, a7, a9, a10, a13, a23, k, g, o5*

[g] Virulent/avirulent on *Mla3, a6, a7, a22, ra, nn, p, g, La, h/ a1, a9, a10, a12, a13, a23, k, at, o5*

Table 2. Disease assessment of SBCC accessions against three *P. teres* isolates (detached leaves test), and one isolate of *R. secalis*. Numbers in columns below the isolates indicate the number of lines (Lr=landrace-derived inbred lines, Cv=cultivar) with different disease scores (S=susceptible; MS=moderately susceptible; MR=moderately resistant; and R=resistant). The bottom of the table indicates the disease scores of checks for each isolate.

	***P. teres* isolates[1]**								***R. secalis***	
Disease	**97:1**		**Am**		**NZ**		**Mean[2]**		**Sachs 147–1**	
score	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**
R	1	0	2	1	12	1	0	1	40	1
MR	2	1	2	0	4	0	3	0	37	7
MS	42	4	42	2	30	5	38	5	44	2
S	110	11	109	13	109	10	114	10	34	6
Compana	7 (S)		7 (S)		8 (S)		-		-	
Femina	7 (S)		8 (S)		8 (S)		-		-	
Zenit	7 (S)		4 (MR)		3 (R)		-		-	
Steffi	-						4 (S)			
Camelot	-						1.9 (MR)			

[1] Data from the detached leaves assay

[2]Mean of disease score for the three isolates

Table 3. Disease assessment against *P. hordei* in accessions from the SBCC. Data are from a field trial under natural infection (expressed as percentage of infected leaf area in the flag leaf), and from a greenhouse test with isolate I-80, based on a 0-4 scale (Lr=landrace-derived inbred lines, Cv=cultivar). The bottom of the table indicates the disease scores of checks.

Percentage	*P. hordei*				
	Field test		Isolate I-80		
	Lr	Cv	Score	Lr	Cv
0	1	2	**0**	0	0
1	6	0			
3	24	0	**1**	5	0
5	28	0			
10	35	7	**2**	24	0
15	18	3			
20	25	3	**3**	130	16
30	14	1			
40	1	0	**4**	0	0
50	3	0			
L94	30		-		
Vada	1		-		

Table 4. Disease assessment against BYDV and BaMMV in accessions from the SBCC (Lr=landrace-derived inbred lines, Cv=cultivar). Percentage score for BYDV represents the variable Degree of Attack (DA), whereas data for BaMMV are based on percentage of infection rates in a growth chamber test, and further determination of resistant accessions with DAS-ELISA (for further explanation, see text). The bottom of the table indicates the disease scores of checks.

	BYDV		**BaMMV**			
	Field test		**Growth chamber**		**Test Elisa**	
Percentage	**Lr**	**Cv**	**Lr**	**Cv**	**Lr**	**Cv**
0-10	0	0	32	1	18	1
10-20	0	0	1	0	4	0
20-30	28	1	3	0	0	0
30-40	36	5	2	0	1	0
40-50	27	6	0	0	1	0
50-60	22	1	3	0	1	0
60-70	18	1	4	0	0	0
70-80	10	0	1	0	3	0
80-90	4	1	3	2	3	0
90-100	3	0	103	13	29	2
Femina	30		-			
Vixen	10		-			
Coracle	4		-			
Maris Otter	-		99			

Table 5. Classification of accessions from the SBCC, according to their reactions against the six diseases tested (Lr=landrace-derived inbred lines, Cv=cultivar). Footnotes indicate the criteria used to classify the accessions ('+' indicates resistance).

Powdery Mildew[1]	Scald[2]	Net Blotch[3]	Leaf Rust[4]	BYDV[5]	BaMMV[6]	Nº of lines	
						Lr	Cv
+	+	-	+	-	-	2	0
+	-	+	+	-	-	1	0
+	-	-	+	-	-	0	1
+	+	-	-	-	-	11	2
+	-	-	-	-	+	2	0
-	+	-	+	-	-	1	0
-	+	-	-	-	+	1	0
+	-	-	-	-	-	33	6
-	+	-	-	-	-	25	0
-	-	-	+	-	-	3	1
-	-	-	-	-	+	9	1
-	-	-	-	-	-	67	5
58	42	1	9	0	13	171	

[1]Mean of seven isolates. Disease score 0-2

[2]Disease score 0-1

[3]Mean of three isolates. Disease score ≤ 3

[4]Percentage of infected leaf area ≤ 1

[5]Degree of attack ≤ 10%

[6]Infection Rate =0 in the DAS-ELISA test

Table 6. Means of disease score for the SBCC lines grouped according to Yahiaoui et al. (2008), or based on the climates in the collection site (Papadakis, 1975). Populations I-IV are landrace-derived accessions while groups V-VI are cultivars. Only landrace-derived inbred lines are classified according to climatic regions (CM=continental Mediterranean, TM=temperate Mediterranean, FTM= Fresh temperate Mediterranean, SM=Subtropical Mediterranean, MM= Mediterranean maritime). The bottom of the table indicates correlation coefficients between latitude, altitude, rainfall and disease score.

Populations or groups	**Powdery Mildew[1]**	**Scald[2]**	**Net blotch[3]**	**Leaf rust[4]**	**BYDV[5]**	**BaMMV[6]**
I	2.25 a	2.08 a	6.51 a	15.77 b	46.00 a	57.64 bc
II	1.91 ab	1.70 abc	5.96 a	26.43 a	35.43 a	98.60 a
III	2.31 a	1.16 c	6.47 a	13.27 bc	46.69 a	54.74 c
IV	1.95 b	1.33 c	6.39 a	9.77 d	46.59 a	84.50 a
V	1.65 b	2.06 ab	6.15 a	8.12 cd	38.75 a	100.00 a
VI	2.05 ab	1.29 bc	6.00 a	17.50 ab	51.25 a	83.20 ab
Papadakis index						
CM	2.24 a	1.53 a	6.73 a	12.33 ab	44.69 bc	65.89 b
TM	2.17 a	1.31 a	6.32 ab	16.73 a	50.09 ab	55.43 b
FTM	2.15 a	1.42 a	6.56 ab	12.67 ab	54.41 a	57.75 b
SM	2.13 a	1.19 a	6.28 ab	8.24 b	42.79 bc	98.09 a
MM	1.68 b	1.54 a	6.10 b	10.53 b	37.86 c	95.51 a
Rainfall	-0.16*	0.13	-0.17*	-0.03	-0.02	0.08
Latitude	0.23*	0.15	0.11	0.08	-0.15	-0.34*
Altitude	0.16*	-0.06	0.14	0.22*	-0.12	-0.27*

Means with different letter within the same column indicate significant differences at P=0.05

[1] Disease score (0 resistant – 4 susceptible), mean for seven isolates

[2] Disease score (0 resistant – 4 susceptible), mean for four dates on four plants

[3] Disease score (1 resistant – 10 susceptible), mean for three isolates on detached leaves assay

[4] Percentage of leaf surface infected in field test

[5] Degree of attack

[6] Incidence (percentage of plants with symptoms)

* Correlation coefficients are significant at P=0.05