Resistance to powdery mildew in one Spanish barley landrace hardly resembles other previously identified wild barley resistances

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Abstract

Two major quantitative trait loci (QTLs) associated with resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*) were previously identified on chromosome 7H of the Spanish barley line SBCC097. The two QTLs seemed to share the same chromosomal position as the major genes *mlt* and *Mlf*, which were formerly described in *Hordeum vulgare* ssp. *spontaneum*-derived lines. In the present work, different lines that carry *mlt* (RS42-6*O), *Mlf* (RS137-28*E), or a combination of both (SI-4 and SI-6) were compared with SBCC097 to evaluate their relatedness at the phenotypic, cellular, and genetic levels. The resistance of the lines was characterised by inoculating them with a set of 27 isolates of *B. graminis*, which displayed a wide range of virulence. It was revealed that SBCC097 possessed a distinctive resistance spectrum. Microscopic assessment of the cytological development of the resistance response showed that SBCC097 clearly formed fewer well-established colonies and secondary hyphae than the other lines. This was confirmed by the infection type recorded after visual inspection. Genetic analyses of all five lines, based on markers flanking the QTLs derived from SBCC097 both supported the macroscopic and microscopic data and pointed to the presence of a combination of novel genes or alleles in SBCC097, which may be included in the category of "intermediate-acting" genes, governing resistance mainly at the post-penetration stage.

Keywords: Wild barley; landrace; Spanish Core Collection; Disease resistance; *Blumeria graminis*; quantitative trait loci; *mlt*; *Mlf*

Introduction

After wheat, barley is the second most important crop species in Europe in terms of the amount produced and the area of cultivation. Its production is concentrated in Germany, France and Spain, which together account for over 20% of global production (Faostat 2007; http://faostat.fao.org). However, like most crops, barley is plagued by numerous fungal and viral diseases that cause large economic losses. In this respect, the promotion of natural sources of disease resistance is considered as the most cost-effective, consumer-friendly, and environmentally sounds way to protect crop yields.

Powdery mildew, which is caused by the biotrophic fungus *Blumeria graminis* f. sp. *hordei*, is one of the most destructive and widespread diseases of barley; it causes marked reductions in the yield and quality of harvested grain (Zhang et al. 2005). Many resistance genes and quantitative trait loci (QTLs) that act against this pathogen have been identified in barley (Friedt and Ordon 2007). Among them, the *mlo* resistance gene has been widely introgressed into two-rowed spring barley cultivars, which are currently grown throughout Europe; this has continued to be highly effective over the last 30 years. However, *mlo* is not used in six-rowed winter barleys, which are mostly grown in Southern Europe (Panstruga et al. 2005; Dreiseitl 2007).

Broad-spectrum resistance to powdery mildew in this type of barley was found in the Spanish Barley Core Collection (SBCC), a representative sample of mainly six-rowed native landraces with a history of valuable adaptation and selection under Mediterranean conditions (Igartua et al. 1998; Yahiaoui et al. 2008; Silvar et al. 2010a). An exhaustive analysis, based on the SBCC itself and on derived mapping populations (SBCC097×Plaisant and SBCC145×Beatrix), revealed that some lines might possess novel genes or alleles that have not been identified in other genetic barley resources (Silvar et al. 2011a; 2011b). Two major QTLs with large effects were identified on chromosome 7H of the recombinant inbred line (RIL) population SBCC097×Plaisant. The alleles for resistance at each quantitative trait locus were contributed by the Spanish parent SBCC097 (Silvar et al. 2010b).

In a recent study, closely linked flanking markers for both QTLs were identified (Silvar et al. 2012). The positions of these quantitative resistances seem to correspond to the same chromosomal region where Schönfeld et al. (1996) found two race-specific resistance genes from the two *Hordeum vulgare* ssp. *spontaneum*-derived lines RS42-6*O (*mlt*) and RS137-28*E (*Mlf*).

The main goal of the present work was to obtain information on the relatedness between the resistance against powdery mildew found in SBCC097 and that conferred by previously identified major genes derived from *H. vulgare* ssp. *spontaneum* (subsequently *H. spontaneum*). To this end, four wild barley-derived lines were compared, using a three-pronged strategy with the following steps: i) macroscopic evaluation of disease resistance against a wide range of isolates with different patterns of virulence, ii) microscopic assessment of the infection process at the cellular level, and iii) genetic analysis of the haplotypes obtained with the molecular markers flanking each QTL in SBCC097.

Materials and Methods

Plant and fungal materials: The Spanish landrace-derived barley line SBCC097, which carries two major QTLs for powdery mildew resistance (Silvar et al. 2010b), and four *H. spontaneum*-derived lines (F_7 generation), carrying the major resistance genes; RS137-28*E (*Mlf*), RS42-6*O (*mlt*), SI-4 (derived from 1B-87 (*Mlf*+*mlt*)), and SI-6 (derived from RS164-6, (*Mlf*+*mlt*)) (Jahoor and Fischbeck 1987; Schönfeld et al. 1996; Backes and Jahoor 2001), were used for this study. Plants were infected with 27 isolates of *B. graminis* f. sp. *hordei* held at the collection of the Julius Kühn Institute in Kleinmachnow (Germany). Information about the geographical origins and complexities of the isolates can be found elsewhere (Silvar et al. 2011a). The susceptible variety Haisa (spring, two-rowed cultivar) was included as a control for susceptibility in the different experiments.

Macroscopic evaluation of the disease reaction: Experiments involving detached leaves were carried out at the Institute for Plant Protection in Field Crops and Grassland, Kleinmachnow, Germany, according to the methodology previously described by Silvar et al. (2011a). Briefly, leaf segments derived from primary, fully expanded leaves were excised from each line and placed with the adaxial surface up in a square Petri dish filled with 0.6% agar and 30 ppm benzimidazole. In each Petri dish, two to four leaf segments per line were randomly fixed (three replicates). A settling tower was used to blow spores over the leaf segments. To reduce the risk of cross-contamination of the isolates, inoculation with each isolate was carried out in different rooms and on different days. Petri dishes were firstly incubated in a growth chamber at 16 °C in the dark for 12 h. They were then transferred to a growth chamber with continuous fluorescent light at the same temperature as before. About 12 days after inoculation, the infection type (IT) was recorded on a scale of 0–4 (including intertypes), in accordance with the procedure of Jahoor (1986) and Jensen et al. (1992). This scale was extended by the inclusion of an additional symbol, 0(P), for IT characterised by only a few pustules on otherwise mildew-free leaf segments. Plants with an IT of 0 (no symptoms) or 1 (presence of chlorosis and/or necrosis, but absence of mycelium) were classified as highly resistant, those with an IT of 1–2 or 2 were classified as moderately resistant (weak to moderate mycelial growth), and those with an IT greater than 2 (moderate to strong mycelial growth and sporulation) were classified as susceptible. All lines giving the same avirulence profile with all tested isolates were grouped into the same resistance spectra (RS) according to a previous work (Silvar et al. 2011a).

Microscopic assessment of the resistance response: Experiments with whole plants were performed at the Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany. Isolate 82 was propagated on plants of the susceptible cv. Haisa. Seedlings of SBCC097, RS137-28*E, RS42-6*O, SI-4, SI-6, and Haisa were grown under mildew-free conditions at 20 °C with 60% to 70% relative humidity and a 16 h light/8 h dark photoperiod. Ten days after sowing, when

the primary leaf was fully expanded, ten plants per line were inoculated with isolate 82 by brushing them with powdery mildew spores collected from infected leaves. Inoculated plants were maintained in a growth chamber with the same conditions as those described above. At 60 and 96 hours post-inoculation (hpi), leaf segments of ca. 2 cm in length were excised from the middle part of the infected primary leaves and immersed in ethanol:chloroform:trichloroacetic acid (75:25:1 v/v) for 24 h to bleach the tissue. Next, these leaf segments were stained with 0.2% Calcofluor White (Uvitex 2B) for five minutes (Darken 1962). Three plants per line were collected at each time point, and the experiments were repeated twice.

For microscopic analysis, leaf segments were placed with the adaxial side up on a microscope slide on a drop of glycerin:water (1:1 v/v). Microscopic observation was carried out at ×200 or ×400 magnification with a Nikon Eclipse 90i microscope fitted with epifluorescence equipment, using the filter combination UV-2A (330±380 nm excitation filter, 400 nm dichroic mirror, and 420 nm barrier filter). Photos were taken with an Olympus DP71 camera fitted to the microscope. Evaluation of the interaction between the barley lines and the respective *B. graminis* isolate was based on the number of germling-forming appressorial germ tubes (AGT), appressoria, and haustoria that generated well-established colonies. The number of elongated secondary hyphae (ESH) on those colonies and the development of secondary penetration sites along the ESH were also considered. Note was taken of whether failed germlings that stopped growth at the penetration stage were associated with a papilla beneath the primary germ tube (PGT), the AGT, or the presence of secondary appressorial lobes. The hypersensitive response (HR) and subsequent cell death were scored for epidermal and mesophyll cells by the direct visualisation of whole-cell autofluorescence under ultraviolet light (Clark et al. 1995). Cell death was expressed as the percentage of germlings or colonies that caused HR in at least one surrounding epidermal cell.

Genotypic analysis: Closely linked flanking markers of the two QTL regions were employed to genotype the lines that carry *mlt*, *Mlf*, or both (see above). For the QTL on chromosome 7HS, the

SNP markers QBS23 and k08921/QBS30 (co-segregating markers) and the simple sequence repeat (SSR) GBM1060, located 0.9 and 0.6 cM distal and 0.9 cM proximal, respectively, were used. On the other hand, SNP markers QBS52 and QBS44, positioned at 0.5 cM distal and 0.7 cM proximal of the QTL, respectively, and QBS46, which apparently co-segregated with the QTL, were tested on the long arm of chromosome 7H. Restriction analysis, PCR amplification, and genotyping of these markers were performed as described previously (Silvar et al. 2010b; 2012).

Data analysis: Statistical analyses were performed with the software SPSS Statistics 17.0 and GenStat 15. Spearman's rank correlation coefficient was conducted to analyse the correlation among the IT from different lines. Comparisons among lines at the microscopic level were carried out using the Kruskal-Wallis test ($P \leq 0.001$) followed by the multiple comparison procedure described by Conover (1980).

Results

Table 1 shows the visual observations of IT of all the tested lines (SBCC097, RS137-28*E, RS42-6*O, SI-4, and SI-6) using 27 isolates of *B. graminis* with a broad range of virulence. The *H. spontaneum* line SI-4 was highly resistant (scores up to 1) to 26 isolates, followed by SI-6 (18 isolates), SBCC097 (16 isolates), RS137-28*E (14 isolates), and RS42-6*O (13 isolates). Moderate resistance (scores of either 1–2 or 2) was found in SI-4 (1 isolate), SBCC097 (7 isolates), RS42-6*O (6 isolates), RS137-28*E (5 isolates) and SI-6 (4 isolates). Almost no lines exhibited a score of 4, but the high frequency with which an IT of either 2–3 or 3 was recorded suggested some level of residual resistance against several isolates. Four RS were identified within the five lines. The lines RS137-28*E (*Mlf*) and RS42-6*O (*mlt*) had similar RS against the 27 isolates, whereas SI-4, SI-6 and SBCC097 each showed distinct RS (Table 1). Results obtained using Spearman's rank coefficient supported the inferences on RS made on the basis of the single IT. The most significant

coefficient value (0.940, $P < 0.001$) was found between RS137-28*E and RS42-6*O. No significant correlation was detected among resistances from wild barley lines and SBCC097 (Table 1).

On the basis of the markers most tightly linked to both 7HS and 7HL QTL, the detected haplotypes differed across all lines (Table 2). Regarding the 7HS interval, SBCC097 showed a different allele for all tested markers. Only the allele at marker QBS30 was shared with SI-6, but the two sources of resistance could be distinguished by using the SSR marker GBM1060, which exhibited distinct alleles for each line. The marker pattern at 7HL did not generate such a clear outcome, although the data pointed to a closer relationship between SBCC097 and SI-6 than between SBCC097 and the other lines.

The *B. graminis* isolate 82, which was avirulent on all of the lines tested, was selected to follow the cytological development of the resistance response. The Haisa line was employed as a positive control in order to evaluate the success of infection. Visually, the six barley lines gave different disease phenotypes and infection scores at 10 days after inoculation. Whereas SI-4 was the only one with a score of 0, the other lines ranged from 1 (evident necrosis and/or chlorosis) to 1–2 (necrosis and/or chlorosis accompanied by weak development of mycelium) (Fig. 1). At 60 hpi, differences in the development of isolate 82 on the six lines were detected at the cytological level (Fig. 2). The most consistent contrast was identified between SI-4 and the other lines. Only a few conidia could germinate on the leaf surface of SI-4 and the resulting appressoria did not penetrate the epidermal layer or form a colony (Fig. 2a). The most common plant response to the germlings was the formation of papillae (Fig. 3a). The appearance of second appressorial lobes was occasionally recorded (Fig. 3a). Regarding the other lines, the majority of germlings successfully penetrated the cell. However, marked differences were observed in the percentage of germlings that formed well-established colonies, with this number being significantly lower in SBCC097 and RS137-28*E than in the susceptible control Haisa (Fig. 2a). Mature haustoria were clearly detected in Haisa, but not in the other lines, for which observed haustoria were small, immature, and did not fully develop

digitate processes (Fig. 3b). A HR was unusually observed in SI-4 (Fig. 2b). The occurrence of germling-associated cell death, both in the epidermal layer and in a few mesophyll cells, was recorded for the landrace-derived line SBCC097 and occasionally for SI-6, whereas only epidermal cell death was found in all lines, being significantly different from that in the control line Haisa (Fig. 2b; Fig. 3c).

Although isolate 82 formed colonies on all barley lines, significantly more ESH developed on RS42-6*O and SI-6 than on RS137-28*E and SBCC097 (Fig. 2c; Fig. 3d). However, the hyphal growth was entirely superficial, and the infection was restricted to a few cells around the germling. The formation of secondary penetration sites and secondary haustoria derived from ESH was clearly observed only on the susceptible control at 96 hpi. At this time, the colonies produced on the resistant lines were much smaller than those formed on Haisa, particularly for SBCC097 and RS137-28*E (Fig. 3e).

Discussion

The reaction patterns observed between known and novel types of resistance against a wide range of *B. graminis* isolates enabled indirect assessment of the similarities between the underlying bases of different types of resistance. The reaction patterns also suggested which potential genes or alleles might be involved in the novel forms of resistance. In the present work, 27 isolates of *B. graminis* were used to compare the responses to infection in four *H. spontaneum*-derived lines with the resistance previously found in the Spanish barley line SBCC097 (Silvar et al. 2010b). All lines turned out to be highly or moderately resistant to the large majority (from 70.4% to 100%) of isolates. This high level of resistance found in the wild barley-derived lines might be influenced by the natures of the pathogen isolates. The set of *B. graminis* isolates was chosen because they exhibit virulence to most major resistance genes used in Europe in both two- and six-rowed barley. Indeed, Jahoor and Fischbeck (1987) reported that lines RS137-28*E and RS42-6*O (included in the

present study), and 1B-87 and RS164-6 (resistance donors for SI-4 and SI-6, respectively) ranged from highly to moderately resistant to 11 European isolates of *B. graminis*. Unfortunately, common isolates were not shared between this previous study and the present work, although some of them likely possess similar geographical origin and patterns of virulence. The line SBCC097 displayed a spectrum of resistance almost as wide as that of the wild barley-derived lines, but it differed markedly, i.e. SBCC097 is infected by distinctive isolates, as confirmed by Spearman's test. This broad RS is not surprising in view of the landrace-derived origin of this line. Landraces have been used extensively within the primary gene pool of barley to mine both genes and alleles associated with disease resistance (Jørgensen and Jensen 1997; Czembor 1999; 2001). Indeed, several powdery mildew resistance genes used commercially are derived from barley landraces (Fischbeck and Jahoor 1991). Along with its wideness, the characteristic RS observed in SBCC097 preliminarily suggests that the resistance found in this line is different from that of the *mlt* and *Mlf* alleles identified in *H. spontaneum*, despite their apparent co-localization on chromosome 7H.

Genotypic analysis of all five lines based on molecular markers flanking the 7H QTLs in SBCC097 did not provide much insight into the relationship between the resistance genes, although it also pointed to the presence of specific loci in SBCC097, based on the analysis of haplotypes. We have to take into account that these flanking markers arose from results obtained with *B. graminis* isolates other than isolate 82. However, given that we seem to be dealing with non-race-specific resistance, the presence of common IT among all those isolates and the results of QTL analysis obtained with additional isolates (unpublished data) suggest that the selected markers suitably match the resistance in SBCC097, independently of the pathogen isolate tested. Still, the presence of other very minor QTLs that contribute to the resistance cannot be completely ruled out. A combination of the SSR GBM1060 and the SNP markers QBS52 and QBS46 or QBS44 might be useful to distinguish between candidate resistance loci that map to chromosome 7H in other germplasm collections, as well as for the management of these resistance sources in breeding

programs, provided that greater accuracy can be achieved through the development of markers spaced over shorter intervals.

Understanding the mechanism of resistance at the cellular level should complement genetic and phenotypic approaches to improve breeding strategies. Histological analysis of tissues of infected genotypes allowed investigation of whether the differences in the reactions observed in the resistance test correspond with different plant strategies to protect against powdery mildew infection. Microscopic assessment revealed that resistance was the result of either divergent mechanisms or similar strategies implemented at different times that impaired fungal growth. Evidence of consistent penetration failure of *B. graminis* isolate 82 was only found in SI-4, in which the production of papillae beneath the apressorium and the development of secondary appressorial lobes were observed. Such characteristics are considered to indicate impaired penetration and thus, initial penetration resistance in the barley line SI-4 (Zeyen et al. 2002; Eichmann and Hückelhoven 2008; Sugai et al. 2010). The earlier inhibition of the development of isolate 82 on SI-4 compared with that in the other lines corresponded to the macroscopic resistance phenotype seen in this line (IT 0), and indicates a higher level of resistance of SI-4 towards this isolate. No formation of papillae was detected for the other lines. Mature haustoria were easily recognized only for the susceptible control. One plausible explanation for this observation is that haustoria that formed from the tip of the penetration peg on RS137-28*E, RS42-6*O, SI-6, and SBCC097 were not sufficiently developed to be unmistakably detected at the level of microscopic resolution employed in this study. Hypersensitive epidermal cell death in those lines might also hamper the formation of visible fungal haustoria (Koga et al. 1990). Given these uncertainties, it was not possible to assess penetration resistance based on a haustorial index, which constitutes unambiguous evidence of fungal penetration.

The HR to isolate 82 in lines RS137-28*E, RS42-6*O, SI-6, and SBCC097 did not differ significantly at the time points selected. In other studies that dealt with mildew resistance genes,

such cell death-associated resistance varied along the time course of infection among different barley lines (Kruger et al. 2003; Sanchez-Martin et al. 2011). More extensive cell death, involving both epidermal and mesophyll cells, was only recorded in SBCC097 and, to a lesser extent, in SI-6. Considering the superficial hyphal growth, cell death in neighbouring mesophyll cells might be triggered by signalling factors built up within the epidermis, such as those derived from the oxidative burst, more than by intracellular ESH development (Thordal-Christensen et al. 1997; Proels et al. 2010). It was expected that lower HR would allow greater numbers of germlings to develop ESH and would promote mycelial development. No such clear connection could be drawn in our work between the percentage of cell death and the number of secondary hyphae at the time points studied. However, a reduction in colony size was identified at 96 hpi in SBCC097 and RS137-28*E. The extent of fungal development observed by microscopic analysis of those lines was consistent with the visible infection phenotype of 1, which indicates chlorosis and/or necrosis but absence of mycelium, compared with the cases in RS42-6*O and SI-6 (IT 1–2, weak mycelium growth). The much earlier inhibition of pathogen penetration in SI-4 and the distinctive reduction in colony sizes in SBCC097 and other lines suggest clear associations with differences in both the timing and the distribution of the resistance response.

Kruger et al. (2002) classified barley R-genes for resistance to powdery mildew into the groups of "fast-", "intermediate-" and "slow-acting" genes, depending on the reaction speed and the infection phenotype. "Fast-acting" genes, including *Mlg* and *mlo5* (Kruger et al. 2002; 2003), are those that enable the avoidance of fungal penetration at very early stages of infection, giving an IT of 0. In contrast, the "intermediate-" (*Mla12*) and "slow-acting" categories (*Mlk, Mlp*) are characterized by a delayed resistance response, which permits haustorium differentiation and substantial hyphal development, in combination with a slower HR, before the pathogen is arrested (Freialdenhoven et al. 1994; Huckelhoven et al. 2000; Kruger et al. 2003). Whereas the former would correspond to IT 1 or 1–2 (small visible colony development), the latter would match IT 2 or 2–3 (more abundant

visible colony development) (Kruger et al. 2002). Such differences in the behaviour of these resistance genes were also evident at the level of different alleles. Thus, *Mla* alleles were mainly grouped into the "fast-acting" (e.g. *Mla1*, *Mla6* and *Mla13*) and "intermediate-acting" (e.g. *Mla7* and *Mla12*) classes (Boyd et al. 1995; Shen et al. 2003; Caldo et al. 2006; Prats et al. 2010). All of the phenotypic (i.e. IT 1 or 1–2) and histological evidence suggests that those genes or alleles present in the Spanish line SBCC097 might be included in the "intermediate-acting" category, potentially exhibiting quantitative resistance due to genes that stoically support slight fungal development, which limits pathogen growth at post-penetration stages through slower or delayed HR. Such delayed response would stop the flow of nutrients to the pathogen, restricting its growth and frustrating sporulation.

At the selected time points, differences in the onset of resistance were not always found after comparison between SBCC097 and RS137-28*E (*Mlf*), RS42-6*O (*mlt*), and SI-6. Further work with additional avirulent isolates using a time course survey and more exhaustive analysis of the resistance response (e.g. transcript analysis of defence-related genes) will confirm whether the significant decrease in hyphal elongation and therefore the smaller fungal colonies, as well as the localization of cell death to the mesophyll cells, might be attributable to post-haustorial resistance mediated by particular loci or specific alleles in SBCC097. In addition, greenhouse and field experiments with adult plants would provide additional clues on the nature of the resistance, which might show variable magnitude in later-formed leaves or in different environments, as reviewed elsewhere (Develey-Riviere and Galiana 2007). Indeed, former experiments carried out in greenhouse assays on potted plants pointed to slightly higher levels of resistance to powdery mildew (Silvar et al. 2010a). Although the set of isolates used in those previous studies possessed a narrower virulence spectrum than the group of isolates employed in the present work, some level of adult plant resistance cannot be discarded and it should be investigated through different environments.

Nevertheless, the results reported here indicate that the loci/alleles identified in the Spanish line SBCC097 bear little resemblance to genes previously identified in wild barley at the phenotypic, cytological and genetic levels. This suggests that the newly identified genes might provide novel sources of broad-spectrum, non-race-dependent resistance to powdery mildew.

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Table 1 Infection types of 27 isolates of *B. graminis* f. sp. *hordei* evaluated for four wild barley-derived lines and the Spanish barley landrace-derived line SBCC097. The symbol 0(P) indicates the presence of a few pustules on otherwise mildew-free leaf segments. The bottom of the table shows the Spearman's rank correlation coefficients between lines. For calculation purposes, intermediate scores were converted to averages as follows: 1–2 = 1.5, 2–3 = 2.5, and 3–4 = 3.5; IT = 0(P) was considered as 0.

Isolate	Barley lines				
	SI-4 (*Mlf,mlt*)	SI-6 (*Mlf,mlt*)	RS42-6*O (*mlt*)	RS137-28*E (*Mlf*)	SBCC097
75	0	3	4	3	1
78	1–2	2–3	2–3	3	1–2
79	0	2–3	1–2	1–2	0(P)
82	0	1–2	1–2	1	1
114	0	0	2	1	0(P)
116	0	0	0	0	0
118	0	0	2	1–2	1
120	0	0	1	1–2	2–3
121	0	0	0	0	0
122	0	2	0	1	1–2
125	0	3	0	1	2–3
126	0	0	0	0	3
127	0	2	0	0	2
164	0	1	1	1	0
167	0	0	0	0	0
168	0	0	3	2–3	2
170	0	0	2	1–2	0(P)
176	0	3	0	0	0(P)
178	0	0	3	3	1–2
179	0	0	0	0	2–3
180	0	0	3	2–3	0(P)
199	0	0	4	3–4	0(P)
211	0	1–2	2–3	3	2
212	0	0	3	3	0(P)
221	0	0	2	2	1
224	0	0	0	0	2
225	0	0	0	0	1
SBCC097	0.124	0.113	-0.191	-0.063	
RS137-28*E	0.233	0.056	0.940*		
RS42-6*O	0.183	-0.039			
SI-6	0.274				

*Correlation coefficients are significant at $P < 0.001$

Table 2 Allele patterns detected on four wild barley derived lines and the Spanish barley landrace-derived line SBCC097 with the markers most tightly linked to the resistance QTLs on chromosome 7H (Silvar et al. 2010a; 2012). Different letters indicate different alleles. Chrom, chromosome; NA, no amplification.

Chrom	Marker name	Marker type	Barley lines				
			SI-4 (*mlt*,*Mlf*)	SI-6 (*mlt*,*Mlf*)	RS42-6*O (*mlt*)	RS137-28*E (*Mlf*)	SBCC097
7HS	QBS23	SNP	a	a	a	a	b
	k08921	SNP	a	a	a	a	b
	QBS30	SNP	a	b	NA	NA	b
	GBM1060	SSR	a	b	a	a	c
7HL	QBS52	SNP	a	a	a	b	b
	QBS46	SNP	a	b	a	a	b
	QBS44	SNP	a	b	a	a	b

Fig. 1 Macroscopic reactions of barley lines after inoculation with *B. graminis* isolate 82. (**a**) RS42-6*O (*mlt*), (**b**) RS137-28*E (*Mlf*), (**c**) SBCC097, (**d**) SI-4 (*Mlf, mlt*), (**e**) SI-6 (*Mlf, mlt*), and (**f**) the susceptible cultivar Haisa.

Fig 1

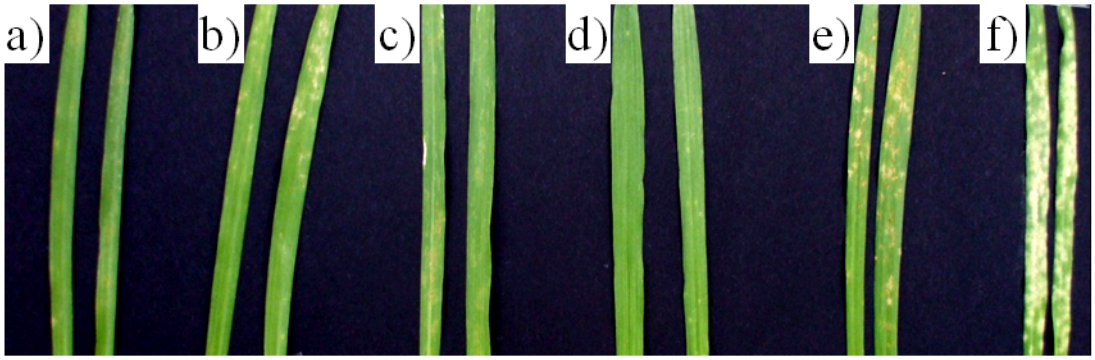

Fig. 2 Microscopic assessment of *B. graminis* infection on different barley lines at 60 hpi. (**a**) Percentage of well-established colonies. (**b**) Percentage of dead epidermal cells. (**c**) Number of secondary hyphae per colony. Different number of asterisks above the bars indicates significant differences among lines at $P < .05$.

Fig. 2

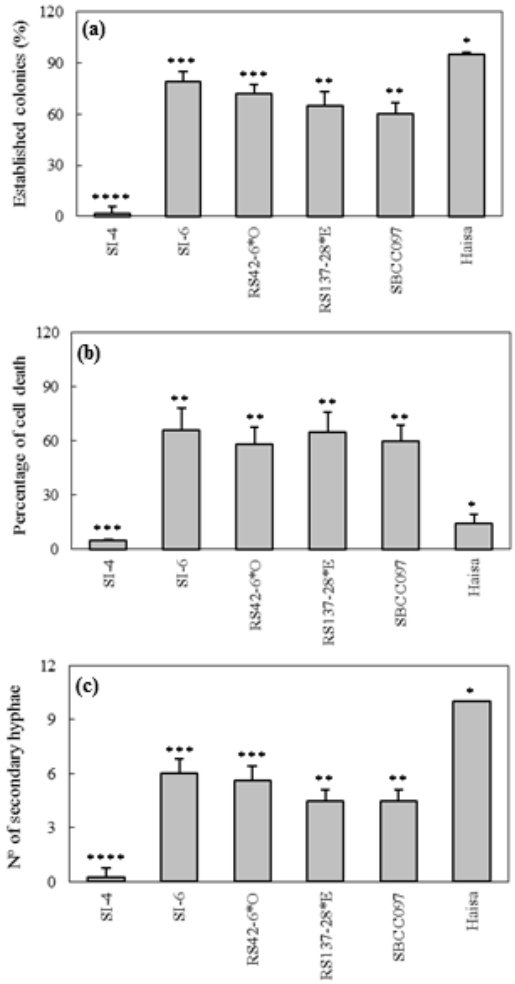

Fig. 3 Micrographs of various stages of *B. graminis* development and plant cell responses in the different barley lines. (**a**) Failed attempt at penetration and formation of a secondary lobe (SL) on SI-4, (**b**) immature haustorium, (**c**) elongated secondary hyphae (ESH) and established colony on SBCC097, (**d**) epidermal cell that responded with a hypersensitive response (HR) to fungal attack, and (**e**) fungal colonies at 96 hpi on SBCC097 (left) and Haisa (right). C: conidium; PGT: primary germ tube; AGT: appressorial germ tube; P: papilla.

Fig. 3