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The resistance to leaf rust and powdery mildew of recombinant lines of barley (*Hordeum vulgare* L.) derived from *H. vulgare* × *H. bulbosum* crosses

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Abstract

A set of 23 recombinant lines (RLs) of barley (*Hordeum vulgare* L.) derived from *H. vulgare* × *H. bulbosum* L. crosses was inoculated with barley leaf rust (*Puccinia hordei*) and powdery mildew (*Blumeria graminis* f.sp. *hordei*) at the seedling stage to identify their levels and mechanisms of resistance. Eight RLs were studied further in glasshouse and field tests. All three barley parents ('Emir', 'Golden Promise' and 'Vada') were highly susceptible to powdery mildew and leaf rust isolates. Several RLs showed partial resistance expressed as high relative latency periods and low relative infection frequencies against leaf rust. This high level of partial resistance was due to a very high level of early aborting colonies without host cell necrosis. Several RLs showed hypersensitive resistance to some or all isolates. For powdery mildew, one RL was completely resistant to the CC1 isolate and had a hypersensitive resistance to the CO-02 isolate. Three RLs derived from 'Emir' were completely resistant to both powdery mildew isolates, and three more RLs tested in the field had higher levels of partial resistance than their parents. The results indicate that *H. bulbosum* contains major and minor gene(s) for resistance to leaf rust and powdery mildew that can be transferred to cultivated barley.

Key words: *Hordeum vulgare* — *Hordeum bulbosum* — *Blumeria graminis* f. sp. *hordei* — *Puccinia hordei* — hypersensitive resistance — partial resistance

Leaf rust (*Puccinia hordei* Otth.) and powdery mildew (*Blumeria graminis* (DC). f. sp. *hordei* Em.), are two of the most important foliar diseases on barley and cause significant economic losses. Wild barley relatives such as *Hordeum vulgare* L. ssp. *spontaneum* (C. Coch.) have been widely used in barley-breeding programmes for disease resistance. *Hordeum vulgare* ssp. *spontaneum* (C. Coch.) has broad resistance to leaf rust and powdery mildew, and many genes have been identified and transferred to cultivated barley (Jahoor and Fischbeck 1993, Kintzios et al. 1995, Backes et al. 2003). However, most sources of powdery mildew and leaf rust resistance have been overcome by corresponding virulence within the pathogen. New sources of resistance should, therefore, be identified for breeding programmes.

The wild barley species *Hordeum bulbosum* L., the only species in the secondary genepool of barley, is interesting to plant breeders for two reasons. First, its chromosomes are eliminated in crosses with barley to produce doubled haploids. Secondly, it has some desirable agronomic characters such as disease resistance (Thomas and Pickering 1983, Xu and Snape 1989, Walther et al. 2000) and has shown resistance for many years to many powdery mildew and leaf rust isolates. This resistance can be transferred to cultivated barley. Xu and Snape (1989) reported resistance to powdery mildew and rusts in *H. vulgare* × *H. bulbosum* hybrids. Xu and Kasha (1992) and Pickering et al. (1995) reported the transfer of powdery mildew resistance gene(s) from *H. bulbosum* to *H. vulgare*. Pickering (1992) identified chromosome substitution lines developed from *H. vulgare* × *H. bulbosum* hybrids that were more resistant to powdery mildew and other foliar diseases than their *H. vulgare* parents. Pickering et al. (2006) reported the transfer of a dominant gene for scald resistance from *H. bulbosum* to barley.

The objectives of the present study were: (i) to record the level of resistance and characterize the mechanisms of resistance to powdery mildew and leaf rust in recombinant lines (RLs) derived from *H. vulgare* × *H. bulbosum* crosses; (ii) to identify race-specific resistance genes in the RLs and to determine their novelty by comparing their infection types (ITs) with the ITs on a differential set; (iii) to evaluate the partial resistance of several of the RLs in the field by comparing mean disease severities (MDS) with their parents. Disease resistant RLs were selected in New Zealand and subsequent tests carried out in Spain and Germany.

Materials and Methods

Plant materials: Twenty-three recombinant lines (RLs) of barley were used in the Spanish experiments, eight of which were also tested in Germany for powdery mildew (Table 1). The RLs contain introgressed DNA from *Hordeum bulbosum* and were derived from hybrids between *H. vulgare* × *H. bulbosum* (Pickering et al. 1998 and 2000, Pickering and Johnston 2005). These plants, together with the three recurrent barley parents 'Vada', 'Emir' and 'Golden Promise', were studied for resistance. Three resistance alleles in two RLs have already been assigned gene symbols: 38P18 with resistance to leaf rust (*Rph18.ag)* and 81882 with resistance to both powdery mildew (*Mlhb1.a*) and leaf rust (*Rph17.af*) (Pickering et al. 1995, 1998, 2000).

The Pallas isolines differential set for powdery mildew of barley (Kølster et al. 1986) and a differential set for leaf rust (Steffenson et al. 1993) were used to determine the virulence spectrum of all isolates used.

Inoculum: In Spain, plants were inoculated with five isolates of barley leaf rust, representing a wide virulence range, and two isolates of barley powdery mildew. In Germany, inoculations were carried out

Line code	*H. vulgare* parent	*H. bulbosum* parent	Introgression location
81882[1]	'Vada'	S1	2HS
38U4/1/3/8	'Golden Promise'	2920/4	5HL, 6HS
38U4/1/3/9	'Golden Promise'	2920/4	6HS
38U4/1/3/10	'Golden Promise'	2920/4	6HS, 7HL
38U16	'Golden Promise'	2920/4	5HL
53A8[1]	'Golden Promise'	2920/4	4HL
182Q20[1]	'Golden Promise'	A17/1	2HL
212Y1[1]	'Golden Promise'	2920/4	6HS, 7HS
102C2/14	'Emir'	2032	2HL
119Y4	'Emir'	2920/4 × 2929/1	6HS, 7HS, 7HL
171J1	'Emir'	2920/4 × 2929/1	6HS, 7HS
177L20[1]	'Emir'	A17/1	7HL
181P158	'Emir'	A17/1	4HL
200A3[1]	'Emir'	A17/1	2HS
120G4	'Emir'	2920/4 × 2929/1	6HS, 7HS
129F2	'Emir'	2920/4	4HL
169P15	'Emir'	A17/1	4HL
170R1	'Emir'	2920/4 × 2929/1	6HS
36L36	'Emir'	2920/4	2HS
38P18	'Emir'	2032	2HL
203S1	'Emir'	A17/1	5HL
216U3[1]	'Emir'	A17/1	7HL
219W4[1]	'Emir'	A17/1	7HL

[1]Tested in Germany with 24 isolates of powdery mildew.

Table 1: Barley recombinant lines (RLs) with introgressed DNA fragment from *Hordeum bulbosum* used in the study

with 24 isolates of powdery mildew on eight RLs and the Pallas isolines (Kølster et al. 1986). The virulence/avirulence factors and the origin of the isolates used in the experiment are shown in Table 2.

Inoculation: For leaf rust, three to four seeds per RL were sown in 35 × 20 × 8 cm trays in three replicates. Each tray contained eight accessions. The susceptible line L94 and the partially resistant cv. 'Vada' were added to each box as references. Eleven days after sowing, when the primary leaf was fully expanded and the second leaf was emerging, first leaves were placed in a horizontal position, adaxial surface up, with the help of metal staples, and inoculated with *P. hordei* in a settling tower by dusting a mixture of freshly collected spores with talcum powder (1 : 10, v/v). Each box was inoculated with 3 mg of spores of the appropriate isolate (Niks and Rubiales 1994). The inoculated plants were kept in an inoculation chamber in darkness for about 11 h at 20°C with a relative humidity of about 100%. Plants were then transferred to a growth chamber at 20°C and white fluorescent light (12 h light/12 h dark). To reduce the risk of cross-contamination of the isolates, inoculation with each isolate was carried out on different days.

Pathogen	Isolate	Country of origin	Virulence/avirulence factors
Leaf rust	CO-01	Spain	*Rph1,2,4,6,8,12/3,5,7*
	AL-02	Spain	*Rph1,2,3,4,5,6,8,9,12/7*
	1.2.1	Holland	*Rph1,2,4,5,6,8,9/3,7,12*
	IVP200	Holland	*Rph1,2,4,5?,6,8,9,12/3,7*
	TU-03	Tunisia	*Rph1,2,3,4,5,6,7,8,9,12/7*
Powdery mildew	CO-02	Spain	*Mla1,a7,a8,a9,a10,a12,a22,a23,k,p,g,La,h/a3,a6,a13,a14,t,o5*
	CC1	UK	*Mla7,a8,a9,a10,a12,a13,k,p,t,g,La,h/a1,a3,a6,a14,a22,a23,o5*
	1	Denmark	*Mla22,ra,k,nn,p,La/a1,a3,a6,a14,a7,a9,a10,a12,a13,at,g,h,o5*
	2	Denmark	*Mla12,a22,nn,p,La,h/a1,a3,a6,a14,a7,a9,a10,a13,ra,k,at,g,o5*
	3	Denmark	*Mla1,a22,nn,p,at,La,h/a3,a6,a14,a7,a9,a10,a12,a13,ra,k,g,o5*
	4	Germany	*Mla6,a14,a22,ra,nn,p,La,h/a1,a3,a7,a9,a10,a12,a13,k,at,g,o5*
	5	Denmark	*Mla6,a14,a22,ra,nn,p,g,h/a1,a3,a7,a9,a10,a12,a13,k,at,La,o5*
	6	Denmark	*Mla9,a10,a22,k,nn,p,La,h/a1,a3,a6,a14,a7,a12,a13,ra,at,g,o5*
	7	Denmark	*Mla6,a14,a22,ra,nn,p,g,La,h/a1,a3,a7,a9,a10,a12,a13,k,at,o5*
	8	Germany	*Mla6,a14,a7,a12,a22,ra,nn,p,La,h/a1,a3,a9,a10,a13,k,at,g,o5*
	9	Germany	*Mla6,a14,a7,a22,ra,k,nn,p,g,La,h/a1,a3,a9,a10,a12,a13,at,o5*
	10	Germany	*Mla6,a14,a7,a12,a22,ra,nn,p,g,La,h/a1,a3,a9,a10,a13,k,at,o5*
	11	Denmark	*Mla3,a14,a6,a7,a22,ra,nn,p,g,La,h/a1,a9,a10,a12,a13,k,at,o5*
	12	Denmark	*Mla7,a9,a10,a13,ra,k,nn,p,g,La,h/a1,a3,a6,a14,a12,a22,at,o5*
	13	Germany	*Mla1,a7,a10,a12,ra,nn,p,g,La,h/a3,a6,a14,a9,a13,a22,k,at,o5*
	14	Germany	*Mla6,a14,a7,a10,a13,ra,k,nn,p,at,La,h/a1,a3,a9,a12,a22,g,o5*
	15	Germany	*Mla3,a6,a14,a7,a22,ra,nn,p,g,La,h/a1,a9,a10,a12,a13,k,at,o5*
	16	Germany	*Mla6,a14,a7,a13,a22,ra,k,nn,p,at,g,La,h/a1,a3,a9,a10,a12,o5*
	17	Denmark	*Mla6,a14,a7,a9,a12,a22,ra,k,nn,p,g,La,h/a1,a3,a10,a13,at,o5*
	18	Denmark	*Mla3,a7,a9,a10,a12,ra,k,nn,p,at,g,La,h/a1,a6,a14,a13,a22,o5*
	19	Germany	*Mla6,a14,a7,a10,a12,a13,ra,k,nn,p,at,g,La,h/a1,a3,a9,a22,o5*
	20	Denmark	*Mla6,a14,a7,a9,a10,a12,a13,ra,k,nn,p,g,La,h/a1,a3,a22,at,o5*
	21	Germany	*Mla3,a6,a14,a7,a12,a13,a22,ra,nn,p,at,g,h/a1,a9,a10,k,La,o5*
	22	Germany	*Mla3,a6,a14,a7,a9,a10,a12,ra,k,nn,p,g,La,h/a1,a13,a22,at,o5*
	23	Austria	*Mla6,a14,a7,a9,a10,a12,a13,ra,k,nn,p,at,g,La,h/a1,a3,a22,o5*
	24	Germany	*Mla6,a14,a7,a9,a10,a12,a13,a22,ra,k,nn,p,g,La,h/a1,a3,at,o5*

Table 2: Virulence/avirulence factors of the leaf rust and powdery mildew isolate

For powdery mildew, seedlings of all RLs were grown under mildew-free conditions at 16°C and 10 000 lx continuous light. Eleven days after sowing when the primary leaf was fully expanded, 50 mm (Spain) or 30 mm (Germany) of a central leaf segment was excised from each seedling and placed adaxial surface up in a square Petri dish filled with 0.6% agar and 125 ppm (Spain) or 30 ppm (Germany) Benzimidazole. In each Petri dish, two to four leaf segments per line were randomly fixed in three replicates. One day before inoculum was required, heavily infected plants were shaken to remove ageing conidia, to ensure a supply of vigorous young spores. Inoculation was carried out by blowing spores from the infected plants over the leaf segments using a settling tower. A glass slide was placed in the settling tower to monitor inoculum density, which was adjusted to give approximately 20 conidia/mm^2 (Spain) or 2–4 conidia/mm^2 (Germany; Haugaard et al. 2002). After inoculation, Petri dishes were transferred to a growth chamber at 18–20°C (Spain) or 16°C (Germany) and incubated in darkness for 12 h. They were then transferred to a growth chamber with fluorescent lighting (12 h light/12 h dark – Spain, or continuous light – Germany) with temperatures as before (Edwards 1993).

Field tests: RLs were grown in the field during 2003, Germany, with resistant and susceptible controls in a randomized block with two replicates (Moll et al. 2000). Strips of mildew-susceptible cultivars were grown between each block, and these were artificially inoculated with a mixture of 11 isolates at growth stage Zadoks 21–23 (Zadoks et al. 1974).

Preparation of leaves for microscopy: *Leaf rust:* Five days after inoculation, a central leaf segment of nearly 2 cm^2 was collected from each plant in Spain. Leaves were fixed and cleared by boiling for 1.5 min in lactophenol/ethanol (1 : 2, v/v) and stored overnight in this mixture at room temperature. Segments were then washed once with 50% ethanol for 30 min, once with 0.05 M NaOH for 30 min, rinsed three times in water (10 min each), and soaked in 0.1 M Tris/HCl buffer (pH 8.5) for 30 min. They were then stained with a 0.1% solution of Uvitex 2B in the same buffer. This was followed by rinsing four times with water before washing in a solution of 25% glycerol for 30 min. A few drops of lactophenol were added to the solution to prevent deterioration by fungi. Leaf segments were examined at 100× with Leica epifluorescence equipment (DM LB, 330–380 nm wavelength transmission).

Powdery mildew: Half of each previously inoculated leaf segment (about 25 mm) was excised 48 h after inoculation. These leaf segments were placed, with the adaxial (inoculated) surface up, on filter paper moistened with ethanol: acetic acid (3 : 1, v/v) for fixation. The fixative was changed every day until the leaves were free from chlorophyll. Leaves were then transferred on to filter paper moistened with water for 24 h, and finally stored on filter paper moistened with lacto glycerol (lactic acid : glycerol : water, 1 : 1 : 1 v/v) for microscopic observation (Rubiales and Carver 2000).

Macroscopic observation: Latency period (LP) of leaf rust was determined daily by counting the number of uredia visible in a marked area (2–3 cm^2) on each seedling, using a 6× lens. The LP was calculated as the time from the beginning of the inoculation to the time at which 50% of the uredia had appeared (Parlevliet 1975). The final number of uredia was used to determine the infection frequency (IF). The actual LP and IF were converted into relative latency period (RLP) and relative infection frequency (RIF), taking the LP and IF of L94 as 100%. The infection type (IT) was recorded 12 days after inoculation using the 0–9 scale of McNeal et al. (1971) where: 0, no uredinia or other macroscopic sign of infection; 1, few faint hypersensitive flecks; 2, no uredinia, but clear hypersensitive necrotic flecks present; 3, flecks with small uredinia surrounded by necrosis; 4, small to medium uredinia often surrounded by necrosis and chlorosis, small sporulation; 5, medium uredinia often surrounded by necrosis and chlorosis, reasonable sporulation; 6, medium-sized to large uredinia surrounded by necrosis and chlorosis, reasonable sporulation; 7, medium-sized to large uredinia surrounded by chlorosis but not necrosis, good sporulation; 8, medium-sized to large uredinia surrounded by a little chlorosis but not necrosis, good sporulation; and 9, large uredinia without chlorosis or necrosis, very good sporulation.

Infection type (IT) of powdery mildew was recorded 5 days (Spain) or 12 days (Germany) after inoculation, following the 0–4 scale of Moseman (1965) where: 0, no visible signs of infection; 1, brown necrotic lesions with little or no mycelial development; 2, some necrosis and chlorosis with slight to moderate mycelial development; 3, chlorosis with moderate mycelial development; and 4, abundant mycelial development with little or no necrosis or chlorosis. In addition to this, in Spain the disease severity (DS) was estimated for each leaf segment as the percentage of the leaf surface covered by powdery mildew colonies. Infection frequency was calculated by counting the number of mildew colonies using a 6× lens and converting to colonies/cm^2. In Germany, in a glasshouse, ITs of 0–2 were considered resistant and 3–4 susceptible. In the field trials in Germany, disease development was assessed by recording the percentage leaf area infected on three dates and converting to mean disease severity – MDS (Moll et al. 2000).

Microscopic observations: Accessions of leaf rust showing high levels of partial resistance or hypersensitive reaction, their recurrent parents and the two control lines were selected for microscopic observation. One hundred infection units were studied per leaf segment at 100× magnification, and classified according to their stage of development (Niks 1982). Early aborting colonies (EA) were defined as individuals that formed a primary infection hypha and no more than six haustorial mother cells. Those colonies that formed more than six haustorial mother cells were classified as established colonies (EST). Colony size (CS) was estimated by calculating the length (L) and the width (W) of 20 randomly chosen established colonies and CS was calculated using the formula:

$$\mathrm{CS} = \frac{\pi \mathrm{LW}}{4}.$$

Accessions of powdery mildew showing resistance reactions (low IT) were selected for microscopic observation. To stain fungal structures and facilitate microscopy, a drop of Trypan blue in lactoglycerol (0.1%) was placed on a coverslip and a clear leaf segment was lowered on to the coverslip, so that the inoculated surface of the leaf segment contacted the stain. The coverslip was then inverted on to a microscope slide smeared with lactoglycerol to complete the mount (Rubiales and Carver 2000). Observations were made with Leica epifluorescence equipment (DM LB, 330–380 nm wavelength transmissions).

To determine the success of attempted plant epidermal cell penetration by fully developed germlings, 50–100 mature appressoria were examined on each leaf. If more than one fungal germ tube was in contact with a single epidermal cell, the germlings were disregarded, thus avoiding possible interactive effects between multiple attacks on the same cell. Some host epidermal cells survived attack, producing a papilla beneath the appressorium of the fungus and resisting penetration (EA−); other epidermal cells died in response to attack and whole-cell autofluorescence was evident (EA+). Other cells that survived attack were penetrated by the fungus that formed a haustorium within the epidermal cells (EST−) with subsequent mycelial ramification.

Data analysis: Analysis of variance (ANOVA) was calculated by using PROC GLM in the SAS programme (SAS Institute 1988) or with SAS-Application RESI (Moll et al. 2000). Comparisons between lines were made by the Duncan test (Spain) or the Dunnett test (Germany).

Results

Reaction to leaf rust

Table 3 shows the macroscopic observations (IT, RLP and RIF) of the RLs and their recurrent parents with five isolates of leaf rust. The RLP of the partially resistant check 'Vada'

Table 3: Infection type (IT), relative latency period (RLP), and relative infection frequency (RIF) of five isolates of *Puccinia hordei* on barley recombinant lines with DNA segments introgressed from *Hordeum bulbosum*

		Isolates														
		CO-01			AL-02			TU-03			1.2.1			IVP2000		
Barley line	Genetic background[1]	IT[2]	RLP[3]	RIF[3]	IT	RLP	RIF	IT	RLP	RIF	IT	RLP	RIF	IT	RLP	RIF
'Emir'	—	9	102 de[4]	97 a	9	108 c	105 abc	9	107 ef	131 a	9	104 cde	107 ab	9	96 e	60 efg
102C2/14	'Emir'	1	—[5]	—	4	—	—	1	—	—	1	—	—	1	—	—
119Y4	'Emir'	3	—	—	4	—	—	3	—	—	8	104 cde	84 bcd	4	—	—
171J1	'Emir'	9	103 cd	50 de	9	108 c	98 abc	9	115 bc	45 f	9	112 b	74 cde	9	104 cde	94 abcde
177L20	'Emir'	9	102 de	88 ab	9	111 abc	105 abc	9	106 f	79 bcde	9	103 cde	80 cd	9	105 cde	68 def
181P158	'Emir'	9	102 de	75 bc	9	114 a	96 abc	9	112 cde	67 cdef	8	110 bc	70 de	9	104 cde	63 efg
200A3	'Emir'	9	106 bc	61 cd	9	112 abc	130 a	9	109 def	56 def	7	110 bc	55 ef	9	114 bc	115 ab
120G4	'Emir'	7	107 b	66 c	9	110 bc	121 ab	9	116 bc	56 def	9	110 bc	75 cde	9	107 cd	114 ab
129F2	'Emir'	9	106 bc	75 bc	9	112 ab	109 abc	9	113 cd	89 bc	9	107 bcd	64 def	9	107 cd	90 abcde
169P15	'Emir'	1	—	—	1	—	—	1	—	—	1	—	—	0	—	—
170R1	'Emir'	9	107 b	47 e	9	111 abc	114 abc	9	119 b	39 f	9	110 bc	50 ef	9	108 cd	122 a
36L36	'Emir'	6	—	—	9	115 a	82 bcde	9	103 fg	64 cdef	6	—	—	9	114 bc	66 def
38P18	'Emir'	1	—	—	4	—	—	1	—	—	1	—	—	1	—	—
203S1	'Emir'	9	102 de	64 cd	9	110 bc	91 abcd	9	108 def	53 def	9	103 cde	84 bcd	9	104 cde	93 abcde
216U3	'Emir'	9	101 de	71 c	9	112 abc	83 abcde	9	103 fg	80 bcd	9	103 de	111 a	9	104 cde	110 abc
219W4	'Emir'	9	106 b	47 e	9	114 a	62 e	6	—	—	9	107 bcde	97 abc	9	107 cde	81 bcde
'Vada'	—	9	115 a	29 f	9	116 a	81 abcde	9	127 a	51 ef	9	138 a	44 f	9	119 ab	70 def
L94	—	9	100 e	100 a	9	100 d	100 abc	9	100 g	100 b	9	100 e	100 abc	9	100 de	100 abcd
'Vada'	—	9	115 a[4]	29 c	9	116 a	81 ab	9	127 a	51 b	9	138 a	44 c	9	119 a	70 b
81882	'Vada'	8	101 b	50 b	9	116 a	82 ab	9	111 b	71 b	8	124 b	32 c	9	115 b	71 b
L94	—	9	100 b	100 a	9	100 b	100 a	9	100 c	100 a	9	100 c	100 a	9	100 d	100 a
'Golden Promise'	—	9	102 de	77 b	9	107 b	81 ab	9	105 cd	127 a	9	106 cde	81 bc	9	107 d	60 b
38U16	'Golden Promise'	8	110 bc	71 b	9	103 de	82 ab	9	110 c	61 b	9	105 cde	86 ab	9	116 c	57 bc
53A8	'Golden Promise'	9	110 bc	75 b	9	108 cd	80 ab	5	—	—	9	103 de	67 cd	9	122 ab	51 bc
182Q20	'Golden Promise'	9	112 ab	27 d	9	121 a	41 d	0 (9)	—	—	9	140 a	7 g	9	119 bc	37 c
38U4/1/3/8	'Golden Promise'	9	103 de	24 d	8	124 a	66 bc	9	121 ab	24 b	8	123 b	25 f	7	120 bc	61 b
38U4/1/3/9	'Golden Promise'	9	107 c	48 c	9	118 a	67 bc	9	114 bc	44 b	9	114 bcd	59 de	9	122 ab	58 bc
38U4/1/3/10	'Golden Promise'	9	113 ab	47 c	9	116 a	78 abc	9	109 cd	51 b	9	117 bc	48 e	9	117 bc	54 bc
212Y1	'Golden Promise'	9	104 d	73 b	9	118 a	57 cd	9	109 cd	58 b	9	111 cde	66 cd	9	126 a	64 b
'Vada'	—	9	115 a	29 cd	9	116 a	81 ab	9	127 a	51 b	9	138 a	44 e	9	119 bc	70 b
L94	—	9	100 e (138 h)	100 a (54)	9	100 c (140 h)	100 a (55)	9	100 d (180 h)	100 a (18)	9	100 e (169 h)	100 a (69)	9	100 e (135 h)	100 a (69)

[1]RLs are separated into groups according to their genetic background.
[2]IT on a scale of 0–9 (McNeal et al. 1971).
[3]Relative latency period (RLP) and relative infection frequency (RIF) referred to L94 = 100 %. The actual values for L94 with each isolate are indicated in the table between brackets.
[4]Data with the same letter per column per group, according to their genetic background, do not differ significantly (Duncan, $P = 0.05$).
[5]Could not be determined because of low number of uredia due to low IT.

varied from 115% to 138% of L94, depending on the isolate. 'Golden Promise' and 'Emir' showed moderate levels of partial resistance.

Many RLs showed RLPs higher than their recurrent parents and as high as the partially resistant check 'Vada' (Table 3). In the 'Golden Promise' RLs, 182Q20 showed a higher RLP and a lower RIF than 'Golden Promise' and was similar to 'Vada' with all isolates used. Two lines (38U4/1/3/10 and 38U4/1/3/8) showed high RLPs to various isolates. Their partial resistance was higher than 'Golden Promise' and as high as 'Vada'. 53A8 was resistant (IT = 5–6) to one isolate, but susceptible to the other four isolates (Table 3).

Six 'Emir' RLs showed hypersensitive resistance to all or some of the isolates used. 102C2/14, 169P15 and 38P18 showed strong hypersensitive resistance (IT = 0–4) to all the isolates. 119Y4 was resistant (IT = 3–4) to four isolates (CO-01, Al-02, TU-03 and IVP2000), but susceptible (IT = 8) to 1.2.1 isolate. 36L36 was resistant (IT = 6) to CO-01 and 1.2.1 isolates, but susceptible to the other three isolates. 219W4 was resistant (IT = 5–6) to one isolate, but susceptible to the other four isolates (Table 3).

The results of the microscopic observations are shown in Table 4. The high level of partial resistance in 182Q20, and to some extent in 38U4/1/3/8 and 38U4/1/3/10, was due to a high percentage of early aborting colonies without host cell necrosis. The resistance of 102C2/14, 169P15 and 38P18 was due to a high percentage of early aborting colonies associated with host cell necrosis. This result accords with the lower IT observed macroscopically (Table 3).

Reaction to powdery mildew

Table 5 shows the macroscopic observations on infection type (IT), disease severity (DS) and infection frequency (IF) of all the RLs and their parents using two isolates of powdery mildew in Spain. All three barley parents were highly susceptible to powdery mildew isolates (IT = 4), but they showed different levels of severity. 'Vada' was the most susceptible parental line to the CO-02 isolate, but it was only moderately susceptible to the CC1 isolate. 81882 was completely resistant to the CC1 isolate (IT = 0) and had a hypersensitive resistance (IT = 2) to the CO-02 isolate. 'Golden Promise' and 'Emir' gave similar susceptible reactions to both isolates. None of the 'Golden Promise' RLs was more resistant than 'Golden Promise'. Of the 'Emir' RLs, 200A3 and 169P15 had lower DS and IF than 'Emir' with the CO-02 isolate, and with the CC1 isolate they were moderately susceptible. 102C2/14 showed a DS and IF lower than 'Emir' with the CC1 isolate, but with the CO-02 isolate it was moderately susceptible. 216U3 and 219W4 were completely resistant (IT = 0) to both isolates. 177L20 was fully resistant to the CC1 isolate, and only a few colonies were observed with the CO-02 isolate [IT = 0(4)].

All the eight tested RLs gave similar ITs to the 26 powdery mildew isolates used, with one or two exceptions (Tables 5 and 6). 212Y1 was not fully susceptible to some isolates and although 177L20, 216U3 and 219W4 were fully resistant to the CC1 and CO-02 isolates, in Germany there was slight susceptibility of 177L20 and 219W4 to two isolates and of 216U3 to five isolates. More striking were the results from 200A3: in Spain, ITs of 4 were recorded whereas in Germany it showed strong resistance to all but one of the 24 isolates.

81882 was more resistant than its parent, 'Vada', in seedling tests to all isolates tested (Tables 5 and 6) and field tests (81882 MDS = 5.6, 'Vada' MDS = 40.6). 'Golden Promise' and two of its RLs, 53A8 and 182Q20, were fully susceptible to all 26 test isolates. As 182Q20 was also susceptible in the field, it probably does not have any mildew resistance genes. Although 53A8 appeared to show higher partial resistance than 'Golden Promise' in the field (MDS = 14.6 vs. 40.7, respectively) the difference was not significant. In contrast, the third 'Golden Promise' RL, 212Y1, was resistant to six of the isolates at the seedling stage and showed a significantly higher partial resistance (MDS = 13.8) than 'Golden Promise' (MDS = 40.7). The RLs in an 'Emir' background, 177L20, 200A3, 216U3, and 219W4, showed different reaction patterns from 'Emir', which only has *Mla12* resistance (Torp et al. 1978). Hence, they must contain other resistance genes or gene combinations. The genes of 177L20 and 219W4 are probably identical because of their similar reaction patterns to all isolates. In the field, only the RL 200A3 (MDS = 3.5) had a significantly higher resistance than 'Emir' (MDS = 46.1).

Table 7 shows the microscopic observations of the resistant RLs and their parents with the two isolates. The resistant RLs generally showed high percentages of early aborting colonies not associated with host cell necrosis. 219W4 and 81882 showed a higher percentage of early aborting colonies associated with host cell necrosis with the isolates CC1 and CO-02, respectively.

Discussion

Hordeum bulbosum as a source of resistance

The present study clearly indicates that *H. bulbosum* is an important and useful source of partial and hypersensitive resistance to barley leaf rust and powdery mildew, confirming the observations of Thomas and Pickering (1983), Xu and Snape (1989), Pickering (1992), Xu and Kasha (1992), Pickering et al. (1995) and Pickering et al. (2006).

Different resistance reactions were observed among the RLs and their barley parents. Many RLs showed low ITs and/or longer LPs to one or more isolates used in the study. It seems that the introgressed DNA segments from *H. bulbosum* contain minor and major gene(s) for partial and hypersensitive resistance to leaf rust and powdery mildew.

Resistance to leaf rust

Several RLs showed high RLPs and low RIFs against leaf rust. The high level of RLP in 182Q20 against all isolates of leaf rust used was remarkable, as it was higher than its recurrent parent ('Golden Promise') and as high as the partially resistant check 'Vada'. 182Q20 contains a DNA fragment from *H. bulbosum* located distally on chromosome 2HL and this fragment may contain some minor genes that confer the high level of partial resistance present in 182Q20. The high level of PR to all isolates used was due to a very high level of early aborting colonies without host cell necrosis, and may indicate a durable form of resistance. 53A8 'Golden Promise' RL showed hypersensitive resistance to one isolate of leaf rust (TU-02) indicating that this DNA fragment on chromosome 4HL may harbour some specific major gene(s) for leaf rust resistance. The hypersensitive resistance of 53A8 to isolate TU-02 was due to a high level of early aborting colonies with host cell necrosis.

Although three 'Emir' and 'Golden Promise' RLs carry a distal *H. bulbosum* DNA fragment on chromosome 2HL conferring leaf rust resistance, 182Q20 showed a different

Table 4: Microscopic components of resistance to five isolates of *Puccinia hordei* in barley recombinant lines (RLs) with DNA segments introgressed from *Hordeum bulbosum*

		Isolates														
		CO-01			AL-02			TU-03			1.2.1			IVP2000		
Barley line	Genetic background[1]	EA+[2]	EA–[2]	CS[2]	EA+	EA–	CS	EA+	EA–	CS	EA+	EA–	CS	EA+	EA–	CS
'Vada'	—	0.3 a[3]	40.3 a	0.016 b	0.0 a	4.8 a	0.135 b	0.0 b	28.8 ab	0.019 b	0.3 a	31.3 a	0.035 c	0.7 a	20.0 a	0.115 b
81882	'Vada'	2.2 a	31.3 a	0.014 b	0.0 a	3.6 a	0.113 c	5.2 a	23.6 b	0.016 b	0.0 a	18.8 b	0.064 b	0.0 a	19.5 a	0.142 b
L94	—	0.0 a	0.0 b	0.075 a	0.0 a	0.0 a	0.222 a	1.1 ab	0.0 c	0.054 a	0.0 a	0.0 c	0.184 a	0.0 a	0.0 b	0.284 a
'Golden Promise'	—	0.6 b	2.8 d	0.049 b	0.0 a	0.0 b	0.138 b	0.0 c	24.1 b	0.040 b	0.7 b	0.0 e	0.117 b	2.5 a	9.3 bc	0.159 b
53A8	'Golden Promise'	0.7 b	16.5 c	0.025 c	0.0 a	0.0 b	0.134 c	70.6 a	0.0 c	—[4]	0.0 b	3.5 de	0.099 b	5.8 a	4.4 c	0.103 bc
182Q20	'Golden Promise'	9.9 a	85.1 a	0.011 e	0.0 a	26.1 a	0.075 bc	7.7 b	88.7 a	0.008 c	3.0 a	74.3 a	0.073 c	0.0 a	45.1 a	0.105 bc
38U4/1/3/8	'Golden Promise'	0.6 b	32.3 b	0.013 de	0.0 a	28.2 a	0.065 bc	0.0 c	32.1 b	0.016 c	1.8 ab	30.7 b	0.056 c	2.6 a	34.0 ab	0.105 bc
38U4/1/3/9	'Golden Promise'	2.1 b	9.7 cd	0.016 de	0.0 a	3.7 b	0.103 bc	0.0 c	35.7 b	0.018 c	0.0 b	20.5 c	0.072 c	0.0 a	29.1 ab	0.101 bc
38U4/1/3/10	'Golden Promise'	7.9 a	16.2 c	0.026 c	1.3 a	31.1 a	0.079 bc	0.0 c	16.2 b	0.016 c	0.0 b	16.9 c	0.056 c	0.0 a	16.6 bc	0.093 bc
212Y1	'Golden Promise'	1.2 b	5.4 cd	0.022 cd	0.0 a	6.8 b	0.101 bc	2.5 c	31.0 b	0.019 c	0.0 b	9.0 d	0.075 c	0.7 a	51.1 a	0.084 c
'Vada'	—	0.3 b	40.3 b	0.016 de	0.0 a	4.8 b	0.135 bc	0.0 c	28.8 b	0.019 c	0.3 b	31.3 b	0.035 d	0.7 a	20.0 bc	0.115 bc
L94	—	0.0 b	0.0 d	0.075 a	0.0 a	0.0 b	0.222 a	1.1 c	0.0 c	0.054 a	0.0 b	0.0 e	0.184 a	0.0 a	0.0 c	0.284 a
'Emir'	—	0.7 e[3]	2.7 de	0.041 b	0.0 d	0.0 b	0.228 a	0.8 d	9.1 bc	0.091 a	1.0 d	0.0 c	0.225 a	0.0 c	0.7 d	0.235 ab
102C2	'Emir'	95.5 a	4.1 cde	—[4]	38.4 c	2.1 b	—	95.2 ab	4.8 bc	—	96.5 a	3.5 bc	—	61.2 a	11.0 b	—
119Y4	'Emir'	51.6 c	24.3 b	—	1.9 d	0.0 c	—	34.1 c	4.3 bc	—	0.0 d	0.0 c	0.113 c	2.0 c	1.0 d	—
169P15	'Emir'	67.1 b	7.0 cde	—	100 a	0.0 c	—	85.8 b	12.0 b	—	87.7 b	3.3 bc	—	40.3 b	1.0 d	—
36L36	'Emir'	36.9 d	11.9 c	—	0.0 d	0.0 c	0.140 b	10.0 d	11.3 b	0.049 ab	21.0 c	2.4 bc	—	0.0 c	1.8 cd	0.219 abc
38P18	'Emir'	95.0 a	3.7 cde	—	61.3 b	0.7 c	—	100 a	0.0 c	—	94.0 ab	5.7 bc	—	59.0 a	11.7 b	—
219W4	'Emir'	0.0 e	2.6 de	0.038 b	1.3 d	0.0 c	0.142 b	4.3 d	4.3 bc	—	1.0 d	7.1 b	0.119 c	0.0 c	0.0 d	0.178 bc
'Vada'	—	0.3 e	40.3 a	0.016 c	0.0 d	4.8 a	0.135 b	0.0 d	28.8 a	0.019 b	0.3 d	31.3 a	0.035 d	0.7 c	20.0 a	0.115 d
L94	—	0.0 e	0.0 e	0.075 a	0.0 d	0.0 c	0.222 a	1.1 d	0.0 c	0.054 ab	0.0 d	0.0 c	0.184 b	0.0 c	0.0 d	0.284 a

[1]RLs are separated into groups according to their genetic background.
[2]Expressed are percentage of early aborting colonies associated with plant cell necrosis (EA+), percentage of early aborting colonies without plant cell necrosis (EA–) and colony size in mm^2 (CS).
[3]Data with the same letter per column per group, according to their genetic background, do not differ significantly (Duncan, $P = 0.05$).
[4]CS could not be measured because of plant cell necrosis.

Table 5: Infection type (IT), disease severity (DS), and infection frequency (IF), of two isolates of powdery mildew on barley recombinant lines (RLs) with DNA segments introgressed from *Hordeum bulbosum*

		Isolates					
		CC1			CO-02		
Barley line	Genetic background[1]	IT[2]	DS[3]	IF[3]	IT	DS	IF
'Vada'	—	4	14 a[4]	25 a	4	75 a	91 a
81882	'Vada'	0	0 b	0 b	2	41 b	54 b
'Golden Promise'	—	4	23 ab	35 abc	4	56 ab	64 b
38U4/1/3/10	'Golden Promise'	4	25 ab	35 abc	4	63 a	72 ab
38U16	'Golden Promise'	4	27 ab	31 bc	4	54 ab	64 ab
53A8	'Golden Promise'	4	15 b	22 bc	4	48 ab	53 b
182Q20	'Golden Promise'	4	18 b	15 c	4	51 ab	67 ab
38U4/1/3/8	'Golden Promise'	4	31 a	56 a	4	66 a	82 a
38U4/1/3/9	'Golden Promise'	4	25 ab	38 ab	4	66 a	79 a
212Y1	'Golden Promise'	4	18 b	17 bc	4	46 b	62 ab
'Emir'	—	4	34 ab	67 a	4	45 bc	57 b
102C2/14	'Emir'	4	21 c	27 c	4	35 cd	46 bcd
119Y4	'Emir'	4	34 ab	54 ab	4	56 ab	77 a
171J1	'Emir'	4	26 bc	32 bc	4	63 a	81 a
177L20	'Emir'	0	0 d	0 d	0 (4)	3 e	5 e
181P158	'Emir'	4	26 bc	46 abc	4	36 cd	48 bcd
200A3	'Emir'	4	22 bc	40 bc	4	24 d	38 cd
120G4	'Emir'	4	31 abc	55 ab	4	65 a	80 a
129F2	'Emir'	4	25 bc	39 bc	4	27 d	43 bcd
169P15	'Emir'	4	24 bc	44 abc	4	23 d	35 d
170R1	'Emir'	4	26 bc	56 ab	4	65 a	77 a
36L36	'Emir'	4	40 a	69 a	4	32 cd	49 bcd
38P18	'Emir'	4	24 bc	50 abc	4	42 bc	52 bc
203S1	'Emir'	4	25 bc	28 c	4	42 bc	57 b
216U3	'Emir'	0	0 d	0 d	0	0 e	0 e
219W4	'Emir'	0	0 d	0 d	0	0 e	0 e

[1]RLs are separated into groups according to their genetic background.
[2]Infection type (IT) on a scale of 0–4 (Moseman 1965).
[3]Disease severity (DS) estimated as the percentage of leaf area covered by powdery mildew colonies, infection frequency (IF) calculated as number of powdery mildew colonies per cm^2.
[4]Data with the same letter per column per group, according to their genetic background, do not differ significantly (Duncan, P = 0.05).

Table 6: Infection type[1] on eight barley recombinant lines with DNA segments introgressed from *Hordeum bulbosum* after inoculation with 24 isolates of *Blumeria graminis* f.sp. *hordei*

		Isolate																							
Barley line	Gene(s)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
'Vada'	*MlLa*	3	3	4	3	2	3	3	3	3	3	4	4	4	3	3	3	4	4	3	4	2	4	4	3
81882		1	1	2	1	0	0	1	2	2	2	2	1	1	1	2	2	2	2	1	0	0	2	1	1
'Vada'	*MlLa*	3	3	4	3	2	3	3	3	3	3	4	4	4	3	3	3	4	4	3	4	2	4	4	3
81882		1	1	2	1	0	0	1	2	2	2	2	1	1	1	2	2	2	2	1	0	0	2	1	1
'Golden Promise'	None	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4
53A8		4	4	4	4	3	3	4	3	4	4	4	3	4	4	4	4	4	4	4	4	4	3	4	4
182Q20		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4
212Y1		4	2	4	3	2	3	2	3	3	3	3	3	3	4	3	4	3	4	3	2	2	2	3	3
'Emir'	*Mla12*	1	3	3	2	1	3	2	3	2	4	2	2	4	2	2	2	4	4	4	3	3	4	4	4
177L20		0	2	0	0	0	2	0	1	0	2	0	0	1	2	0	0	4	2	3	2	2	0	2	2
200A3		0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	3	2	0	0	2	2	2	2
216U3		0	1	0	4	0	2	0	2	0	2	0	4	1	0	1	1	3	2	3	3	0	0	2	0
219W4		0	0	0	1	0	1	0	1	0	0	0	0	2	1	0	0	3	0	3	0	1	0	0	0

[1]Infection type (IT) on a scale of 0–4, where 0–2 = resistant and 3–4 = susceptible (Moseman 1965).

reaction from 38P18 and 102C2/14, indicating that this DNA fragment is not exactly the same size in all three RLs, or that the *H. bulbosum* parent contains different alleles or, finally, that resistance alleles transferred from *H. bulbosum* to *H. vulgare* do not behave the same in different genetic backgrounds as they do in the *H. bulbosum* background (Xu and Kasha 1992).

Resistance to powdery mildew

There was little resistance among the 'Golden Promise' RLs to powdery mildew in the seedling tests, indicating that there are no effective minor or major gene(s) for resistance against powdery mildew in the introgressed *H. bulbosum* DNA fragments. However, 212Y1 was significantly more resistant

Table 7: Microscopic components of resistance to *Blumeria graminis* f.sp. *hordei* in barley recombinant lines (RLs) with introgressed segments from *Hordeum bulbosum*

Barley line	Genetic Background	Isolates CC1 EA+[1]	EA−[1]	EST−[1]	CO-02 EA+	EA−	EST−
'Vada'	—	2.4 a[2]	72.9 b	24.7 a	0.0 b	76.3 b	23.7 a
81882	'Vada'	3.3 a	96.7 a	0.0 b	6.0 a	84.1 a	9.9 b
'Emir'	—	2.0 c	71.7 c	26.3 a	0.0 a	89.6 b	10.4 a
177L20	'Emir'	7.1 b	91.1 a	1.8 b	0.0 a	95.2 a	4.8 b
216U3	'Emir'	8.4 b	90.8 a	0.8 b	0.0 a	97.7 a	2.3 c
219W4	'Emir'	18.2 a	78.9 b	2.9 b	1.8 a	98.2 a	0.0 c

[1]Expressed as percentages of early aborting colonies associated with host cell necrosis (EA+), percentage of early aborting colonies without host cell necrosis (EA−) and established colonies without host cell necrosis (EST−).
[2]Data with the same letter per column per group, according to their genetic background, do not differ significantly (Duncan, P = 0.05).

than 'Golden Promise' in the field. Furthermore, the non-significant trend towards partial resistance in 53A8 has been borne out in field trials in New Zealand, Denmark and the UK.

Several 'Emir' RLs were, however, resistant (low IT) to one or many isolates of powdery mildew. Their resistance against powdery mildew was due to a high percentage of early aborting colonies without host cell necrosis. 219W4 'Emir' RL, with a distal introgression on chromosome 7HL, gave a hypersensitive reaction to one isolate of leaf rust (TU-03); it was also more resistant to ten isolates of powdery mildew indicating that the *H. bulbosum* DNA fragment in 219W4 has resistance genes against at least two barley foliar diseases.

The 'Vada' RL 81882 and the 'Emir' RL 200A3 showed effective hypersensitive resistance in seedlings to all isolates tested as well as partial resistance. Their reaction patterns in the seedling test differed from the patterns of all Pallas differential lines. They must, therefore, carry new and effective resistance genes that could be used for developing mildew-resistant cultivars.

From these results one can conclude that *H. bulbosum* is an important source of resistance against powdery mildew and leaf rust. Effective major gene(s) for resistance against leaf rust can be transferred from *H. bulbosum* to *H. vulgare* as many RLs showed resistance to the most virulent isolate TU-03. For future research, it will be important to study allelism among genes located on chromosome 2HL, which confer hypersensitive resistance to leaf rust in some of the 'Emir' RLs and non-hypersensitive resistance in 182Q20 'Golden Promise' RL. Preliminary inheritance and allelism studies indicate that the alleles conferring resistance to powdery mildew in 177L20, 216U3 and 219W4 are simply inherited and allelic, although 216U3 was susceptible to five isolates compared with susceptibility to only two isolates for 177L20 and 219W4. These differences may be due to the heterozygous nature of the common *H. bulbosum* parent. Inheritance studies of these resistance gene(s) will continue to determine their relationship to other mapped resistance genes and to establish how many new major resistance gene(s) to powdery mildew and leaf rust are available for breeders.

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