

# Mapping multiple disease resistance genes using a barley mapping population evaluated in Peru, Mexico, and the USA

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**Abstract** We used a well-characterized barley mapping population (BCD 47 × Baronesse) to determine if barley stripe rust (BSR) resistance quantitative trait loci (QTL) mapped in Mexico and the USA were effective against a reported new race in Peru. Essentially the same resistance QTL were detected using data from each of the three environments, indicating that these resistance alleles are effective against the spectrum of naturally occurring races at these sites. In addition to the mapping population, we evaluated a germplasm array consisting of lines with different numbers of mapped BSR resistance alleles. A higher BSR

disease severity on CI10587, which has a single qualitative resistance gene, in Peru versus Mexico suggests there are differences in pathogen virulence between the two locations. Confirmation of a new race in Peru will require characterization using a standard set of differentials, an experiment that is underway. The highest levels of resistance in Peru were observed when the qualitative resistance gene was pyramided with quantitative resistance alleles. We also used the mapping population to locate QTL conferring resistance to barley leaf rust and barley powdery mildew. For mildew, we identified resistance QTL under field conditions in Peru that

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are distinct from the *Mla* resistance that we mapped using specific isolates under controlled conditions. These results demonstrate the long-term utility of a reference mapping population and a well-characterized germplasm array for locating and validating genes conferring quantitative and qualitative resistance to multiple pathogens.

**Keywords** *Hordeum vulgare* subsp. *vulgare* · *Puccinia striiformis* f. sp. *hordei* · *Puccinia hordei* · *Blumeria graminis* f. sp. *hordei* · Quantitative resistance · Qualitative resistance

## Introduction

One of the principal challenges faced by plant breeders is achieving durable disease resistance. Qualitative resistance has been widely used because it shows Mendelian inheritance and can be easily managed in a breeding program. Unfortunately, it is usually not durable and its effective use requires constant monitoring of pathogen virulence and the identification and introgression of new host resistance genes (Vanderplank 1968). There are examples of qualitative (single gene) durability, such as the stem rust resistance in barley conferred by *Rpg1* gene (Ji et al. 1994). However, quantitative resistance is generally thought to have a higher probability of durability than qualitative resistance.

We have mapped genes conferring quantitative and qualitative resistance to barley stripe rust (BSR; incited by *Puccinia striiformis* f. sp. *hordei*) in multiple germplasm combinations (Chen et al. 1994; Hayes et al. 1996; Toojinda et al. 2000; Castro et al. 2003a). We have introgressed these genes singly, and in combination, into susceptible backgrounds and demonstrated that they confer acceptable levels of resistance (Toojinda et al. 1998; Castro et al. 2003a, b; Vales et al. 2005). Similar progress has been made in other crop-pathogen systems, e.g. rice (Hittalmani et al. 2000; Narayanan et al. 2004; Yi et al. 2004) and soybean (Walker et al. 2004).

In 2000, there were reports of a new BSR race in the Andean region (H. Vivar, ICARDA/CIMMYT, personal communication). The same year, we received data from the USDA Barley

Stripe Rust Screening Nursery [coordinated by Dr. R. Brown and grown at Huancayo, Peru (HP)] that included some dramatic changes in resistance phenotype for some entries. For example, the BSR severity for CI10587 at HP was 60%, while in repeated tests in the Toluca Valley of Mexico (TVM), CI10587 had shown no disease or very low levels of disease (typically <1%). We have mapped the CI10587 resistance, using phenotype data from TVM, as a single gene on chromosome 7H (Castro et al. 2002).

In this study, we used a well-characterized mapping population to test the effectiveness of BSR resistance quantitative trait locus (QTL) alleles at HP. These QTL were mapped based on disease severity data from TVM and Washington State, USA (WUSA) (Vales et al. 2005). We hypothesized that if we detected the same QTL in Peru that we had mapped using TVM and WUSA data, this would mean that (1) there was no new race, or (2) there is a new race but the same QTL resistance alleles are effective. If the population was uniformly susceptible, this would confirm the presence of a new race and it would demonstrate race-specificity of the QTL. If different resistance QTL were detected in Peru than in Mexico and the USA, this would suggest a shift in virulence, as well as race-specificity of QTL. In addition to the mapping population, we included a set of germplasm of known BSR resistance gene architecture and disease reaction, including CI10587. During the course of the field experiment at HP, the mapping population showed a range of phenotypic responses to natural infection by powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*) and leaf rust (caused by *Puccinia hordei*). We therefore used these data to map QTL conferring resistance to these diseases, and in the case of mildew, we compared the QTL results with those obtained using defined isolates under controlled environment conditions.

## Materials and methods

### Plant material

This research used two types of germplasm resources: a mapping population and a germplasm

array. The ORO doubled haploid (DH) mapping population (Vales et al. 2005) was derived from the F<sub>1</sub> of the cross of BCD47 and Baronesse. This population consists of 409 lines; 94 were used in the current study. BCD47 is a two-rowed, spring growth habit DH line, developed via marker-assisted selection (MAS) for BSR resistance alleles at QTL on chromosomes 4H and 5H (Castro et al. 2003a). Baronesse is a two-rowed, spring growth habit developed by Nordsaat Saatzucht GmbH and introduced by Westbred, LLC to the Pacific Northwest of the USA, where the variety is widely grown. The germplasm array (Table 1) consisted of 23 varieties and genetic stocks of known BSR resistance gene architecture Castro et al. (2003a, b).

#### Disease assessments under field conditions

Ninety-four ORO maplines, the two parents, and the germplasm array were evaluated for disease resistance phenotypes at the Universidad Nacional Agraria La Molina research farm at HP in 2005 and 2006 using separate two-replicate Randomized

Complete Block Designs. Each plot consisted of two 1-m rows. The HP facility is located at an elevation of 3,320 m, with latitude 11°49' South and longitude of 75°23' West. The following diseases occurred in response to natural infection without supplemental inoculation: stripe rust, leaf rust, and powdery mildew. Stripe rust is an endemic disease in this area and susceptible check lines produce the inoculum necessary for infection. All three diseases were scored for disease severity on a plot basis using visual assessment of the percentage of crop canopy infected. Ratings were made when the majority of the test genotypes were at growth stage 55 on the Zadocks scale. For the purposes of comparing resistance QTL number, location, and effect, we used the BSR severity data reported by Vales et al. (2005) for the same 94 lines from the TVM, and Pullman and Mt. Vernon, WUSA.

#### Disease assessments under controlled conditions

*Blumeria graminis* f. sp. *hordei* (*Bgh*) isolates 5874 (Torp et al. 1978; Wei et al. 1999; *AvrMla1*, *AvrMla6*, *AvrMla12*) and A27 (Giese et al. 1981;

**Table 1** Barley stripe rust disease severity (%) for 23 barley accessions evaluated at Toluca Valley, Mexico in 2000, and at HP in 2000 and 2004

Accession	Stripe rust resistance alleles	Mexico 2000	Peru 2000	Peru 2004
Harrington	None	77	86	40
Galena	None	73	95	50
Baronesse	2H, 5H, 7H	76	84	70
Calicuchima-sib	<b>4H</b> , <b>5H</b> , 6H, 7H	20	60	20
Shyri	<b>1H</b> , 2H, 3H, 6H	0	0	0
CI10587	<b>7H</b>	0	60	20
D1-72	<b>1H</b>	15	20	8
D3-6	<b>7H</b>	Trace	NA	8
Orca	<b>4H</b> , <b>5H</b>	17	72	19
BCD47	3H, <b>4H</b> , <b>5H</b> , 6H	19	43	20
BCD12	<b>1H</b>	30	60	25
D3-6/B-23	<b>7H</b>	8	30	15
D3-6/B-61	<b>7H</b>	4	70	15
OPS 19	<b>1H</b> , <b>7H</b>	Trace	0	5
OPS 66	<b>1H</b> , <b>7H</b>	Trace	0.1	10
OPS 78	<b>1H</b> , <b>7H</b>	Trace	0	10
AJO 44	<b>4H</b> , <b>5H</b> , <b>7H</b>	8	20	25
AJO 59	<b>4H</b> , <b>5H</b> , <b>7H</b>	2	0	8
BU 16	<b>4H</b> , <b>5H</b> , <b>7H</b>	3	10	13
BU 27	<b>4H</b> , <b>5H</b> , <b>7H</b>	Trace	0	8
BU 37	<b>4H</b> , <b>5H</b> , <b>7H</b>	Trace	0.1	0
BU 38	<b>4H</b> , <b>5H</b> , <b>7H</b>	0	0	3
BU 45	<b>4H</b> , <b>5H</b> , <b>7H</b>	0	0.1	5

Stripe rust resistance QTL alleles are numbered according to their chromosomal locations (1–5)

Largest-effect QTL are shown in bold font. 7H denotes a major gene

*AvrMla1*, *AvrMla7*, *AvrMla10*, *AvrMla13*) were propagated at Iowa State University on *H. vulgare* cv. Manchuria (C.I. 2330) in separate growth chambers at 18°C (16 h light/8 h darkness). The same 94 DH lines that were characterized in Peru were grown in three, 36-cell flats. Groups of three seedlings per DH line were sown per cell in each flat. The Baronesse and BCD47 parents, C.I. 16137 (*Mla1*), C.I. 16151 (*Mla6*), C.I. 16149 (*Mla10*), Sultan5 (*Mla12*), C.I. 16155 (*Mla13*), in addition to the fully susceptible Manchuria (C.I. 2330), were used as checks (Moseman 1972). Seedlings were grown to the second leaf stage with the first leaf unfolded, and inoculation was performed at 16:00 h. US Central Standard Time by tipping the flats at 45° angle and dusting the plants with a high density of fresh conidiospores ( $84 \pm 19$  spores/mm<sup>2</sup>). This conidial density per unit leaf area routinely results in greater than 50% of epidermal cells that are successfully infected (Bushnell 2002; Collinge et al. 2002). Groups of flats were placed at 18°C (16 h light, 8 h darkness) in separate controlled growth chambers corresponding to the two *Bgh* isolates (5874 and A27). Infection types were scored at 7 and 9 days post-inoculation as described in Wei et al. (1999). The infection types 0, 1, or 2 are considered resistant reactions while the infection types 3 or 4 are considered susceptible.

#### Genotyping, map construction, and QTL analysis

Markers reported by Vales et al. (2005) that co-segregated or that had  $\geq 10$  missing data points were eliminated. Twenty additional markers were added to the ORO data set. The new markers are shown in bold in Fig. 1. Markers k04435, k03512, k08433, k08302, k06257, k04261, k03878, k00688, k04489, k07339, k00088, k02892, k03352, and k07229 are expressed sequence tag (EST)-based marker loci of known location on the barley transcript map (Sato et al. 2004). Markers MWG2180 and ABG54 were developed by the North American Barley Genome Project and were originally scored as Restriction Fragment Length Polymorphisms (RFLPs) (Kleinhofs et al. 1993). They were converted to the Sequence Tagged Site (STS) markers based on the sequences available at the GrainGenes website (<http://wheat.pw.usda.gov/>

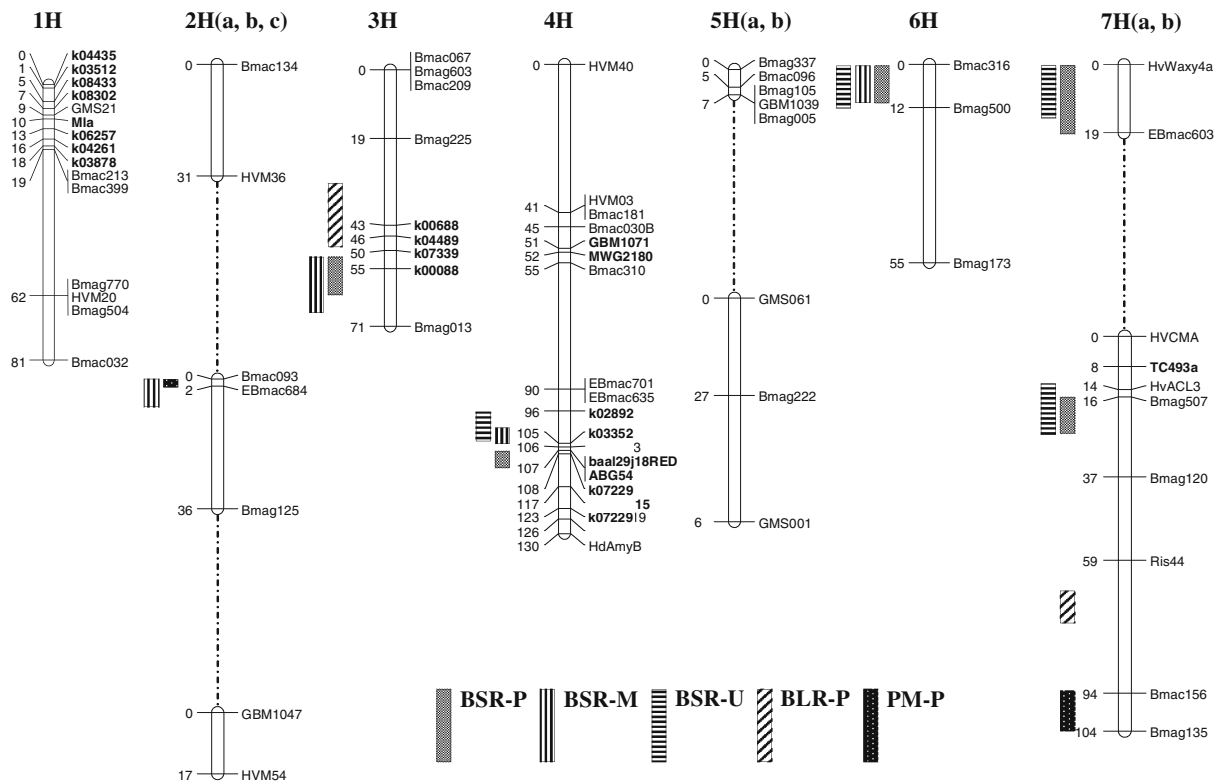
GG2/index.shtml). Primers for the kbaal29j18 marker were designed based on unpublished RIB EST sequence of clone rbaal29j18. The EST-derived SSR-markers GBM1071 and GBM1015 were developed by Thiel et al. (2003). The HvSnf2 locus was mapped using the primers and procedures reported by Yan et al. (2002). TC493a locus sequence was obtained from the tentative contig TC112493 sequence in The Institute for Genome Research (TIGR) web page (<http://www.tigr.org>). The *Mla* locus was mapped using primers based on the sequence for *Mla12* (GenBank Accession AY196347). Primer sequences for unpublished markers are shown in Table 2.

JoinMap<sup>®</sup> (van Ooijen and Voorrips 2001) was used for linkage map construction, using the Kosambi mapping function (Kosambi 1944). Linkage groups and locus orders were compared with Vales et al. (2005). The two-replicate means for BSR, barley leaf rust, and barley powdery mildew disease severity and BSR infection from HP and the data for *Bgh* isolates 5874 and A27 (Iowa State University) were used for QTL analysis, as were the datasets from TVM and WUSA for the same 94 lines in this study that were used previously by Vales et al. (2005). QTL analyses were performed using the composite interval mapping (CIM) procedure (Zeng 1994) implemented in Windows QTL Cartographer 2.5 (Wang et al. 2005). A forward-selection backward-elimination stepwise regression procedure was used to identify co-factors for CIM for each trait; the LOD threshold values to declare a QTL significant were obtained based on 1,000 permutations, a 10 cM scan window and a type I error of 5%. Tests for epistasis between QTLs were evaluated using the multiple interval mapping (MIM) method of QTL Cartographer. With MIM, the proportion of the phenotypic variance explained for each trait was calculated by fitting a model using all detected QTL and their significant interactions.

## Results

### Mapping population

The ORO population linkage map (Fig. 1) has 71 markers comprising 11 linkage groups at a LOD



**Fig. 1** Linkage map of the ORO (BCD47 × Baronesse) doubled haploid population ( $n = 94$ ). *Dashed lines* indicate monomorphic regions. Distances are in Kosambi cM. *The bars* to the left of each linkage group indicate 1-LOD intervals for QTL abbreviated as follows: BSR-P, BSR-M, BSR-U (barley stripe rust resistance in Peru, Mexico, and

USA, respectively); BLR-P (barley leaf rust resistance in Peru); PM-P (powdery mildew resistance in Peru); and PM-I (powdery mildew infection type in response to inoculation with *Bgh* isolates 5874 and A27 at Iowa State University)

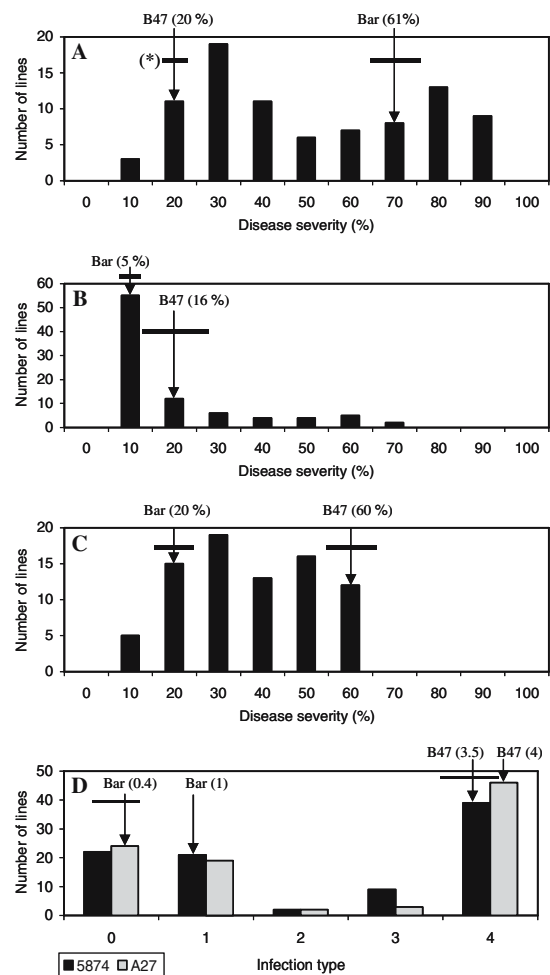
**Table 2** Primer sequences used for mapping new marker loci in the ORO (BCD47/Baronesse) population, listed in linkage map order by chromosome (Chr)

Locus	Ch6	Forward primer	Reverse primer
K04435	1H	TTTCCGGGATAAAGAGTGTGC	CGACTCGGTTGTTTGCTACA
K03512	1H	CATCCCACAGAAAGCAAAT	TACCTACCAGTGACCCCTGC
K08433	1H	TACTGGTCTTGGATGCGACA	GCGCCAAATCTGATAGCACT
K08302	1H	GAACGACAAAGATGATGGCA	CATTGATTGACACCACAGC
K06257	1H	GGCACACATTTACCAAGTGC	CATCCATCAACGTCCTCCTC
K04261	1H	GGGAATTTTCTCCGTTTGGT	AGGTGCTAGACGGTTTCCCT
K03878	1H	AACATGCATGGTGACAAGGA	AAGGGCTTCGTCAATGTTTG
K00688	3H	TAGCCTGGCAGCTTCTGT	CTACTTCCCCCGTTTTCGAC
K04489	3H	GTGGGCATGAAGAACGCTAC	CGAAGTGGTTCCATGGAGTT
K07339	3H	AGGCACAGGCTCTTTTGCTA	GAGCCTTGCTACTGTAATGGG
K00088	3H	ACACGGTCCATGGAAGAAAC	CATAGATGGGCCCTTGAAGA
K02892	4H	AACGTAACAACCGAACGCAT	ATCACGACTGCTCCAATTCC
K03352	4H	CTTTGCATGGCTGATGAAGA	CAATCTGATGGGGAGCACTT
baal29j18	4H	TGTTGATGAATCGCTCTG	CACAACAACCACTACGACGG
ABG54	4H	GTGCTTGGCGGTGACCAAGT	GATGTCCAACGGTGGCTTGA
K07229	4H	AGGCACACAAGCAACACAG	GTTGTAGCCATCGTGAAGA
TC493a	7H	TTTCGGTTTCTGAAATG	AGCTGTGCCAAGGTGAACT

threshold grouping value of 4.0. All linkage groups were assigned to barley chromosomes per Vales et al. (2005), with more than one linkage group for chromosomes 2H, 5H, and 7H. The map covers 611.8 cM, corresponding to an average density of 8.7 cM per marker. Segregation distortion ( $p < 0.05$ ) in favor of Baronesse was observed in chromosome 3H (k00688, k04489, k07339, and k00088) and chromosome 4H (Bmac310 and GBM1015); and in favor of BCD47 in chromosome 1H (Bmag770, HVM20, and Bmag504).

The phenotypic frequency distributions for stripe rust, leaf rust, and powdery mildew at HP in each of two years (Fig. 2a–c) reflect quantitative inheritance and for all three diseases even the most resistant lines showed some level of disease. The frequency distribution for leaf rust may indicate the presence of a qualitative gene (or “major” QTL) and one or more minor QTLs. The frequency distributions for reaction type to mildew after inoculation with the two isolates under controlled conditions were discrete and bimodal (Fig. 2d). Infection types for each mapline were nearly identical for both isolates, except for few cases in which one of the isolates gave variable reaction types in a specific mapline. In these cases, the average of the variable reactions always gave an infection type equal to that observed with the other isolate. As a consequence, the classification of resistant (infection types 0–2) and susceptible infection types (3 and 4) was identical for both isolates. These qualitative data showed excellent fit to a 1:1 ratio ( $\chi^2 = 0.67$ ;  $p = 0.41$ ).

In order to properly compare the BSR resistance QTL results from Peru with QTL detected in Mexico and the USA, we re-analyzed all BSR data sets using the new linkage map. In Peru, the LOD threshold for BSR disease severity was 2.5. BSR resistance QTL with the resistance allele tracing to BCD47 were found on chromosomes 3H, 4H, and 6H, and with the resistance allele tracing to Baronesse on chromosome 7H (linkage groups a and b) (Table 3; Fig. 1). The largest effect QTL was on chromosome 4H, and it accounted for 15% of the phenotypic variance, with an additive effect of 10 (percent disease severity). The five QTL explained 70.8% of the



**Fig. 2** Phenotypic frequency distributions **a** barley stripe rust, **b** barley leaf rust, and **c** powdery mildew disease severity in BCD47, Baronesse and 94 doubled haploid (DH) progeny at Huancayo, Peru in 2004. Panel D shows the phenotypic frequency distribution for mildew infection in response to inoculation with *Bgh* isolates 5874 and A27

phenotypic variance. The reanalysis of the Mexico and USA BSR data with the new linkage map revealed the same total number of QTL as reported by Vales et al. (2005) for a population size of 100. The LOD thresholds were 2.6 and 2.3 for Peru, TVM, and WUSA, respectively.

The BSR resistance QTL on chromosomes 4H and 6H were detected in all three environments. Of the QTL that were significant in Peru, three were also significant in Mexico and four were significant in the USA. Considering all three environments and the population size, more BSR

**Table 3** Barley stripe rust disease severity resistance QTL detected in the ORO (BCD47/Baronesse) population at Huancayo, Peru (HP) in 2004; the Toluca Valley, Mexico (TVM) in 2001, 2002, and Pullman and Mt. Vernon WUSA in 2002

Location	Chromos	QTL peak position and 1-LOD interval (cM)	LOD <sup>b</sup>	R <sup>2</sup> (%) <sup>c</sup>	Additive effect <sup>d</sup>
TVM	2H(b) <sup>a</sup>	0.0 (0.0–7.5)	5.4	8.3	7.43
TVM	3H	56.7 (51.6–67.0)	7.7	13.4	–9.70
<b>HP</b>		<b>54.7 (52.0–62.4)</b>	<b>5.9</b>	<b>9.6</b>	<b>–8.03</b>
TVM	4H	103.8 (99.9–104.4)	11.8	24.6	–13.4
<b>HP</b>		<b>108.1 (107.3–111.6)</b>	<b>8.3</b>	<b>15.2</b>	<b>–10.37</b>
WSU		101.8 (95.8–104.2)	4.9	13.0	–3.95
TVM	6H	0.0 (0.0–10.0)	3.0	4.4	–5.78
<b>HP</b>		<b>2.0 (0.0–10.0)</b>	<b>5.0</b>	<b>8.3</b>	<b>–7.30</b>
WSU		0.0 (0.0–6.2)	4.0	9.3	–3.41
<b>HP</b>	7H(a) <sup>a</sup>	<b>18.0 (0.0–19.1)</b>	<b>3.6</b>	<b>5.7</b>	<b>6.07</b>
WSU		0.0 (0.0–11.9)	4.3	9.9	3.29
<b>HP</b>	7H(b) <sup>a</sup>	<b>16.2 (14.8–24.9)</b>	<b>7.1</b>	<b>11.9</b>	<b>8.79</b>
WSU		16.2 (11.6–25.4)	4.9	11.5	3.56
Total (%) <sup>e</sup>	TVM		65.4		
	<b>HP</b>		70.9		
	WSU		52.3		

<sup>a</sup> The letter in parentheses indicates cases where there is more than one linkage group per chromosome (see Fig. 1)

<sup>b</sup> LOD is the log-likelihood at the QTL peak position. The LOD threshold, based on 1,000 permutations and a type I error of 5% was 2.5, 2.6, and 2.3 for Peru, TVM, and WSU, respectively

<sup>c</sup> R<sup>2</sup> is the percentage of phenotypic variation explained by the QTL

<sup>d</sup> Negative and positive values indicate that BCD47 and Baronesse, respectively, contributed the resistance QTL allele

<sup>e</sup> Proportion of the total variance explained by the QTL

resistance QTL were detected in Peru. There were no significant epistatic interactions in any data set.

For barley leaf rust and powdery mildew resistance, the LOD thresholds were 2.6 and 2.5, respectively. Each of the parents contributed a resistance allele at one of two leaf rust resistance QTL (Table 4, Fig. 1). The 7H QTL had the largest effect, accounting for 31% of the phenotypic variance. Considering both QTL and the significant epistatic interaction between them, they accounted for 79% of the phenotypic variance. There were two QTL for powdery mildew resistance (Table 4, Fig. 1); each parent contributed a resistance allele. There was no epistatic interaction between the QTL; the total percentage of phenotypic variance explained was 22%. The results of the segregation based on phenotype data for mildew infection type in response to inoculation with the two isolates and the genotype data based on amplification of the *Mla* sequence were identical and therefore co-segregate. The linkage map position on chromosome 1H (Fig. 1) is indicated as *Mla*.

## Germplasm array

In order to look for changes in disease severity that could be diagnostic of a new race, or race shift, we compared data from TVM-2000 (the most recent season when the full germplasm array was grown at this location) with HP-2000 and HP-2004 data. The correlations between TVM-2000 and HP-2000 and HP-2004 data were 0.79 and 0.90, respectively, indicating that most lines showed consistent levels of disease severity. As shown in Table 1, the susceptible checks (Harrington, Galena, and Baronesse) always had the highest disease severity values. The quantitative resistance donor parents showed a range of disease severities: Calicuchima had 20% disease severity in TNV-2000 and HP-2004 but was rated 60% in 2000. Shyri allowed no disease development. CI10587 showed a 60% increase in disease severity in 2000 but only a 20% increase in HP-2004. Lines derived from CI10587 and not known to carry any other resistance genes besides 7H (e.g. D3-6, D3-6/B-23, and D-3-6/B-61) showed smaller increases in disease severity at HP

**Table 4** Barley leaf rust (A) and powdery mildew (B) disease severity resistance QTL detected in the ORO (BCD47/Baronesse) population of 94 DH lines at HP in 2004

	QTL peak position and 1-LOD interval (cM)	LOD <sup>b</sup>	R <sup>2</sup> (%) <sup>c</sup>	Additive effect <sup>d</sup>
<b>A</b>				
Chromosome				
3H	42.7 (31.6–49.0)	6.2	21.6	9.48
7H(b) <sup>a</sup>	68.6 (67.4–76.3)	9.4	47.1	–13.96
3H × 7H(b)		5.6	10.4	8.42
Total (%) <sup>e</sup>			79.1	
<b>B</b>				
Linkage group				
2H(b) <sup>a</sup>	0.0 (0.0–2.2)	4.2	12.4	5.36
7H(b) <sup>a</sup>	101.6 (93.5–103.6)	3.6	11.6	–5.11
Total (%) <sup>e</sup>			21.8	

Data for leaf rust are based on the multiple interval mapping procedure of QTL Cartographer, due to the presence of significant QTL × QTL interaction

<sup>a</sup> The letter in parentheses indicates cases where there is more than one linkage group per chromosome (see Fig. 3)

<sup>b</sup> LOD is the log-likelihood at the QTL peak position. The LOD thresholds, based on 1,000 permutations and a type I error of 5% was 2

<sup>c</sup> R<sup>2</sup> is the percentage of phenotypic variation explained by the QTL

<sup>d</sup> Negative and positive values indicate that BCD47 and Baronesse, respectively, contributed the resistance QTL allele

<sup>e</sup> Proportion of the total variance explained by the QTL

in 2004 than in 2000. All lines with multiple resistance alleles tracing to Calicuchima-sib, Shyri, and/or CI10587 (OPS, AJO, and BU) had low and consistent disease severities in all three tests.

## Discussion

The coincidence of stripe rust severity QTL detected with the phenotypic data from HP, TVM, and WUSA confirms that the quantitative resistance genes present in the ORO population are effective against the spectrum of virulence encountered in each of the three environments. We had hypothesized that if we detected the same QTL in Peru that we had mapped using TVM and WUSA data, this would mean that (1) there is no new race in Peru, or (2) there is a new race but the same QTL resistance alleles are effective. The changes in disease severity for some genotypes in the germplasm array would lend support to the latter possibility. Additional experiments, including assessment of differentials at HP, are currently underway. Longer term, it would be desirable to develop a more saturated map. More broadly, the effectiveness of these resistance QTL

alleles across environments indicates that their introgression into susceptible, but adapted, germplasm may be justifiable. These genes may have a reasonable expectation of durability: they have proven effective over the past 18 years in repeated tests in Mexico and North America.

Strictly defined, quantitative resistance is non-race specific (Vanderplank 1963, 1968) but it not possible to state definitely that the resistance QTL alleles do not show race specificity. The five resistance QTL that were significant with the HP data were detected with either the TVM or WUSA data, but not all five were significant in all environments. Furthermore, the QTL had different magnitudes of effect in the different environments. In some cases, there were “minor QTL peaks” (e.g., that did not reach the significance threshold); in others there were no trends whatsoever. Interpretation of QTL trends is very subjective, and higher precision may be achieved in the future by re-analysis of the same data sets using more sophisticated analysis tools, by more rigorous phenotyping, and/or by larger population sizes. In the interest of brevity, we present only significant QTL in this report. For finer analyses, the full phenotype and phenotype data sets are

available from the corresponding author. The difference in number and location of significant QTL could be due to a number of causes. Evidence for some race-specificity, and a degree of race specificity has been reported for some leaf rust resistance QTL (Qi et al. 1998, 1999; Lindhout 2002). It is also true that changes in race specificity can show quantitative rather than qualitative effects (Qi et al. 1998, 1999). Alternatively, differences in environmental effects (e.g., temperature, photoperiod, and nutritional status of the crop) could influence the onset of initial infection. Although purely speculative, it is also possible that qualitative resistance against a specific isolate might appear to be quantitative resistance, either by induced resistance or due to a dilution effect. The most prosaic explanation is that the difference in the number of QTL detected, and the differences in estimates of QTL effect, are biases due to small population size.

The BSR QTL are located in resistance gene rich regions of the genome (Toojinda et al. 2000; Hayes et al. 2003). There is ample evidence for the existence of resistance gene clusters in plants (Chelkowski et al. 2003; Williams 2003), and for the occurrence of quantitative and qualitative resistance genes within such clusters (Wisser et al. 2005). Of particular interest is the presence of BSR resistance QTL detected in the same regions as the powdery mildew resistance loci: the *Mla* locus on 1H (Toojinda et al. 2000) and the *mlo* locus on 4H (this study).

This linkage map proximity of BSR resistance QTL and qualitative mildew resistance genes, together with mildew resistance QTL mapped with the Peru phenotype data, prompted us to determine if either *Mla* or *mlo* resistance alleles were present in BCD47 and/or Baronesse. BCD47 had not been characterized for its response to specific isolates of mildew. Baronesse is reported to carry *Mla3* resistance (Hovmøller et al. 2000; Dreiseitl 2003), and analysis of its pedigree (Mentor/Minerva// mutant Vada //// Carlsberg/ Union//Opvasky/Salle//Ricardo//// Oriol/6153P40) reveals that the Mentor, Carlsberg, Oriol, and Ricardo parents are reported to carry *Mla12*, *Mla8*, *Mla7*, and *Mla3* alleles, respectively (www.scri.sari.ac.uk/cprad). The mildew infection type data cosegregate with the

results of the genotyping based on *Mla12*-derived primers. These results clearly confirm the report that Baronesse carries *Mla* resistance, although the exact allele cannot be determined from these data. None of the mildew resistance QTL are coincident with *Mla*. There are no reports of mildew resistance, either qualitative or quantitative, at the position of the 2H(b) QTL. Based on visual alignment of linkage maps, the QTL on chromosome 7H(b) is in the same region as *Mlf* (Schönfeld et al. 1996) and a powdery mildew resistance QTL in *H. vulgare* spp. *spontaneum* (Backes et al. 2003). The two leaf rust resistance QTL alleles, either of which was sufficient to confer resistance, were also found in regions of the barley genome where other resistance genes are reported. The 3H QTL is in the same region as *Rph10* (Feurestein et al. 1990) and the QTL on 7H(b) is located in the same region as the *RphX* gene mapped in Cali-sib and Shyri (Hayes et al. 1996; Toojinda et al. 2000). Also mapping to this region are RphQ9, a QTL with race specificity (Qi et al. 1999; Lindhout 2002), and *Rph3* (Park and Karakousis 2002).

Our rationale for assessing both the mapping population and a germplasm array was that one or more of the genetic stocks could be diagnostic of a new race, or a shift in race frequency. CI10587 showed the most dramatic change in phenotype, with a 60% disease severity rating in HP2000 vs. 0% in TVM2000 (Castro et al. 2003a). However, the disease severity of CI10587 was only 20% in HP2004. This discrepancy merits further study, since the 0–60% difference in disease severity suggests “defeat” of a major gene by a new race whereas a 20% increase in disease is more indicative of a resistance gene behaving as a major gene in response to one race and as a QTL to another race, a phenomenon reported in rice with bacterial blight (Li et al. 1999). Alternatively, CI10587 may possess minor genes for resistance to BSR that have heretofore been undetected.

There was a tendency toward increasing levels of resistance when more resistance QTL alleles were pyramided per line, as reported by Castro et al. (2003a), although the differences were not significant. Of particular interest are the lines with 7H qualitative resistance gene from CI10587. This gene did not confer an acceptable level of



**Fig. 3** The graphical genotype of ORO-019 at the *Mla* locus and at barley stripe rust, barley leaf rust and powdery mildew QTL based on field phenotype data from

resistance in 2000 and more disease was observed in 2004 than expected based on prior ratings in Mexico. However, when deployed in combination with quantitative resistance alleles at 1H or 4H + 5H lines with this gene had some of the lowest levels of disease severity.

Baronesse, of European origin and moderately susceptible to BSR, contributed resistance alleles effective in the Andean environment. Of two parents, it was also the most resistant to leaf rust and mildew. The presence of positive and negative transgressive segregants is reported in many disease QTL studies and thus the contribution of positive alleles from “susceptible” parents is not entirely unexpected (Hayes et al. 2003). The availability of genotype and phenotype information on the ORO population could be useful in introgressing the resistance genes in both Baronesse and BCD47. As shown in Fig. 3, ORO DH line 19 has resistance to all three diseases under field conditions in Peru, and the Baronesse resistance *Mla* allele. This mapline has the expected stripe rust resistance phenotype and allelic configuration at all marker loci bracketing BSR resistance QTL. It is lacking the resistance allele at the small-effect QTL at the 7H(b); this could be remedied by additional crossing. There was a crossover between the markers flanking the 3H QTL, which will assist in future efforts for finer mapping of this locus. This line carries the contrasting target alleles at the two barley leaf rust QTL and the predicted favorable allele at the barley powdery mildew QTL on 7H(b). It is

Huancayo, Peru 2004. The split panel for 3H indicates contrasting alleles at the loci flanking the QTL peak

lacking the mildew resistance allele at the 2H(b) QTL; this could be remedied in a subsequent cycle of crossing.

In conclusion, this research was useful in confirming the value of quantitative stripe rust resistance genes that we have mapped and introgressed, via MAS, into North American germplasm. These resistance genes were discovered through collaborative efforts with the ICARDA/CIMMYT program and National Program scientists in the Andean region. Calicuchima sib and Shyri, were identified as BSR resistant and released in the Andean region. It is thus fitting that these genes are returned to the Andean National Programs, with value added via marker information that will allow for their efficient introgression. Optimistically, this will lead to high levels of durable resistance. However, vigilance and continued gene discovery and introgression are essential, because what appears to be a non-race specific QTL today may, in the face of new virulence, become a “defeated” major gene, or vice versa.

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