Theor Appl Genet (2006) 113:485–495
DOI 10.1007/s00122-006-0314-2

ORIGINAL PAPER

Pyramiding and dissecting disease resistance QTL to barley stripe rust

K. L. Richardson · M. I. Vales · J. G. Kling · C. C. Mundt · P. M. Hayes

Received: 28 October 2005 / Accepted: 6 May 2006 / Published online: 31 May 2006

Abstract Quantitative resistance (QR) to disease is usually more durable than qualitative resistance, but its genetic basis is not well understood. We used the barley/barley stripe rust pathosystem as a model for the characterization of the QR phenotype and associated genomic regions. As an intermediate step in the preparation of near-isogenic lines representing individual QTL alleles and combinations of QTL alleles in a homogeneous genetic background, we developed a set of QTL introgression lines in a susceptible background. These intermediate barley near-isogenic (i-BISON) lines represent disease resistance QTL combined in one-, two-, and three-way combinations in a susceptible background. We measured four components of disease resistance on the i-BISON lines: latent period, infection efficiency, lesion size, and pustule density. The greatest differences between the target QTL introgressions and the susceptible controls were for the latter three traits. On average, however, the QTL introgressions also had longer latent periods than the susceptible parent (Baronesse). There were significant differences in the magnitudes of effects of different QTL alleles. The 4H QTL allele had the largest effect, followed by the alleles on 1H and 5H. Pyramiding multiple QTL alleles led to higher levels of resistance in terms of all components of QR except latent period.

Communicated by F. Ordon

K. L. Richardson · M. I. Vales · J. G. Kling · P. M. Hayes (✉)
Department of Crop and Soil Science,
Oregon State University, Corvallis,
OR 97331-3002, USA
e-mail: patrick.m.hayes@oregonstate.edu

C. C. Mundt
Department of Botany and Plant Pathology,
Oregon State University, Corvallis,
OR 97331-2902, USA

Introduction

Host plant genetic resistance is typically the most cost-effective and sustainable approach to the control of plant diseases. Plant resistance to biotic stresses can be classified as qualitative or quantitative. Generally speaking, these terms refer to the resistance phenotype and its inheritance. Qualitative resistance shows simple, "major" gene inheritance, i.e. progeny of resistant × susceptible crosses fall into discrete resistant and susceptible classes. Quantitative resistance shows more complex, usually polygenic, inheritance. Progeny of resistant × susceptible crosses show a range, often continuous, of phenotypes. Parlevliet (1979) more explicitly defined the terms in the context of resistance to cereal rusts (incited by *Puccinia* species). Strictly speaking, qualitative resistance is race-specific and involves gene-for-gene interactions between the host and pathogen. It is usually complete, or nearly complete, in that there is little or no spore production. Quantitative resistance is race-nonspecific with no gene-for-gene interaction between the host and pathogen. Quantitative resistance is incomplete: host plants are infected but spore production is reduced. The qualitative–quantitative resistance debate is of importance because of the probable durability of disease resistance. Durability is an attribute that can only be defined in an historical context: durable resistance remains effective while a cultivar possessing it is widely cultivated (Johnson 1981). In

theory, quantitative resistance has a higher probability of being stable and durable (Niks and Rubiales 2002), although there are examples of durable qualitative resistance genes, such as *Rpg1* of barley that confers resistance to stem rust (Brueggeman et al. 2002).

Though quantitative disease resistance is highly valued for its higher probability of durability, the genetic basis and underlying mechanisms are not as well understood as in the case of qualitative resistance, where a number of genes have been cloned and characterized (Pflieger et al. 2001). Quantitative resistance may be due to uncharacterized classes of resistance genes or to the presence of alternative alleles at loci where other alleles correspond to known classes of resistance genes (for example, NBS-LRR) (Lefebvre and Chèvre 1995; Qi et al. 1998; Li et al. 1999; Gebhardt and Valkonen 2001). According to Wisser et al. (2005), about half of the genetically defined rice genome is involved in quantitative disease resistance. The coincidence of disease resistance quantitative trait loci (QTL) with qualitative resistance gene clusters may indicate functional and evolutionary relationships or simply association due to linkage disequilibrium.

The availability of whole genome sequences from plant model systems and tools for positional cloning in large-genome species will ultimately allow us to determine the genetics of quantitative and qualitative resistance. In the interim, quantitative resistance to stripe rust (incited by *Puccinia striiformis* Westend. f.sp. *hordei*) in barley (*Hordeum vulgare* subsp. *vulgare*) provides a model system for characterizing the quantitative resistance phenotype and association with genomic regions (Toojinda et al. 2000; Castro et al. 2002, 2003a, b, c; Vales et al. 2005). The quantitative resistance phenotype used in the preceding citations was disease severity— the area of plant tissue affected by disease, expressed as a percentage of the total area assessed (Parlevliet 1979). With polycyclic diseases such as stripe rust, in the absence of qualitative resistance genes, disease severity is the cumulative result of several component mechanisms conferring partial resistance (Osman-Ghani and Manners 1985). For example, Broers (1997) showed that quantitative resistance to wheat stripe rust (caused by *Puccinia striiformis* Westend. f.sp. *tritici*) could be dissected into mechanistic components: latent period, infection efficiency, lesion size, and pustule density. Accordingly, a logical next step in our quantitative resistance research is the assignment of components of disease severity to QTL. Unfortunately, the time required for phenotyping each of the components precludes conducting the experiment at the population level: Vales et al. (2005) showed that a large ($n \geq 300$) mapping population is needed to detect the most barley stripe rust (BSR) resistance QTL with the least bias. Our alternative was to develop a set of lines, of known disease severity QTL allele architecture, and to measure the components on this smaller set of germplasm.

Our long-term goal is to develop a set of near-isogenic lines (NILs) representing resistance alleles at individual QTL and combinations of QTL. NILs not only provide a better estimate for the effect of single QTL alleles, but also provide a better insight into QTL × pathogen and QTL × environment interactions (van Berloo et al. 2001). Furthermore, QTL–NILs provide a starting point for positional cloning of quantitative resistance gene candidates. As an intermediate step between the QTL allele introgression lines described by Castro et al. (2003a), which represent resistant alleles at individual QTL and QTL combinations in variable genetic backgrounds, and a set of QTL–NILs, previously described, we developed a set of QTL resistance allele introgression lines in a more homogenous genetic background. These lines contain resistant alleles at disease severity QTL that were mapped in different backgrounds and then combined in one-, two-, and three-way combinations in an elite agronomic background. In this report, we describe the results of an experiment in which we measured the components of disease severity on these lines (the intermediate barley NILs; i-BISON). Our goals were to (1) determine if the disease components are QTL-specific, and (2) if pyramiding resistance alleles at multiple QTL leads to higher levels of resistance.

Materials and methods

Germplasm development

A set of intermediate QTL resistance allele introgression lines, i-BISON, was developed by molecular marker assisted introgression of BSR resistance QTL alleles into a BSR susceptible background, the variety "Baronesse." Baronesse is a two-rowed, spring growth habit, feed barley developed by Nordsaat in Germany from the cross Mentor/Minerva//mutant of Vada//// Carlsberg/Union///Opavsky/Salle//Richard/////Oriol/ 6153 P40. The variety was introduced into the United States by Western Plant Breeders, Inc. in 1991 and is grown extensively in the Pacific Northwest of the USA. Based on repeated tests in Mexico, Baronesse is susceptible to BSR, although under less disease pressure in the Pacific Northwest it is not as susceptible as other varieties (Vales et al. 2005). The donors of the resistance alleles were BCD47 and BCD12. These are two-

rowed, spring growth habit doubled-haploid (DH) experimental lines developed via marker-assisted selection (MAS) for BSR QTL resistance alleles. BCD47 contains resistance alleles at the QTL on chromosomes 4H and 5H, and BCD12 on 1H (Castro et al. 2003a). The i-BISON lines (Table 1) were derived from the cross of BCD47/Baronesse, F1//BCD12/Baronesse, F1 (Fig. 1). Resistance alleles at QTL on three chromosomes (designated as targets 1H, 4H, and 5H) were tagged for introgression. In addition, we developed two controls: (1) a "0–QTL" line selected for the susceptible (Baronesse) alleles at the 1H, 4H, and 5H targets and (2) a qualitative resistance gene i-BISON containing a major gene on chromosome 7H, derived from the experimental line D3-6/B23 (Castro et al. 2003a). These controls were developed as checks to test the effectiveness of MAS and to compare the effects of resistance alleles at QTL in a susceptible background.

Other approaches to the development of near-isogenic QTL would have been more efficient if the breeding program did not have other objectives. Other objectives of our breeding program at that time were to accumulate all possible BSR QTL resistance alleles into single, agronomically favorable genotypes and to combine the high yield of Baronesse with the Barley Yellow Dwarf resistance of BCD12 and the malt quality profile of BCD47. Further, the initial crosses from which the i-BISON lines were eventually derived were made prior to the availability of detailed information on BSR resistance QTL location and effect.

Table 1 The i-BISON lines and their respective introgression assignments

Line	Introgression	Graphical Genotype 1H	4H	5H
69	1H QTL			
128	1H QTL			
191	1H QTL			
104	4H QTL			
129	4H QTL			
18	4H QTL			
87	5H QTL			
111	5H QTL			
136	5H QTL			
157	5H QTL			
217	5H QTL			
2-20	7H major gene (control)			
2-22	7H major gene (control)			
3-50	7H major gene (control)			
216-4	1H and 4H QTL			
243-4	1H and 4H QTL			
136-2	1H and 4H QTL			
218-1	1H and 5H QTL			
174	1H and 5H QTL			
110-3	1H and 5H QTL			
217-2	4H and 5H QTL			
108	4H and 5H QTL			
22-4	4H and 5H QTL			
95-2	1H, 4H, and 5H QTL			
130	No QTL (control)			

Each graphical genotype represents the QTL present in the line (1H, 4H, 5H, or their combinations) based on genome screening. Black indicates the presence of the resistance allele, white the absence of the allele, and gray the possibility of the presence of the resistance allele (based on the inability to differentiate between two alleles)

DNA extraction and genotyping

DNA was extracted from 30–50 mg of young leaf tissue harvested from greenhouse-grown plants using a Qiagen/Retsch MM300 mixer Mill and the Qiagen DNeasy 96 Plant Kit (Qiagen Inc, Valencia, CA). Simple sequence repeat (SSR) markers (Liu et al. 1996; Ramsay et al. 2000) were amplified by polymerase chain reaction (PCR) using a fluorescently tagged reverse primer and a non-labeled forward primer. Twelve SSRs linked to the target regions were used for foreground screening and MAS (Fig. 2). For the F1 i-BISON generation, one to three PCR products, with non-overlapping sizes, were analyzed simultaneously with an internal size standard using ABI PRISM DNA sequencers equipped with Genescan® and Genotyper® software (PE Biosystems, Foster City, CA). PCR products for generations F2–F5 were analyzed on 6% polyacrylamide gels (Wang et al. 2003).

Marker assisted selection

Germplasm in the F1 generation was screened with the SSRs GMS021, Bmac0399, Bmac0213, and Bmac0032 spanning the chromosome 1H QTL; EBmac0701, EBmac0635, EBmac0788, and HvMLO3 spanning the chromosome 4H QTL; Bmac0096, Bmag0323, and Bmag0337 spanning the chromosome 5H QTL; and Bmag0120, Ris44, and Bmac0156 flanking the major gene on chromosome 7H to identify and select heterozygotes for the resistance alleles at one, two, or three of the target QTL resistance allele introgressions and heterozygotes for the resistance allele at the major gene. For example, at the F1 generation a line selected for the 1H QTL was heterozygous for the 1H QTL and either heterozygous or homozygous for the susceptible allele at the 4H and 5H QTL. All SSRs were described by Ramsay et al. (2000) except for GMS021, which was first described by Struss and Plieske (1998). Ris44 is a size polymorphism based STS (http://www.wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=probe;name=RIS44). The same SSRs were used to screen lines in the F2 generation to identify and select

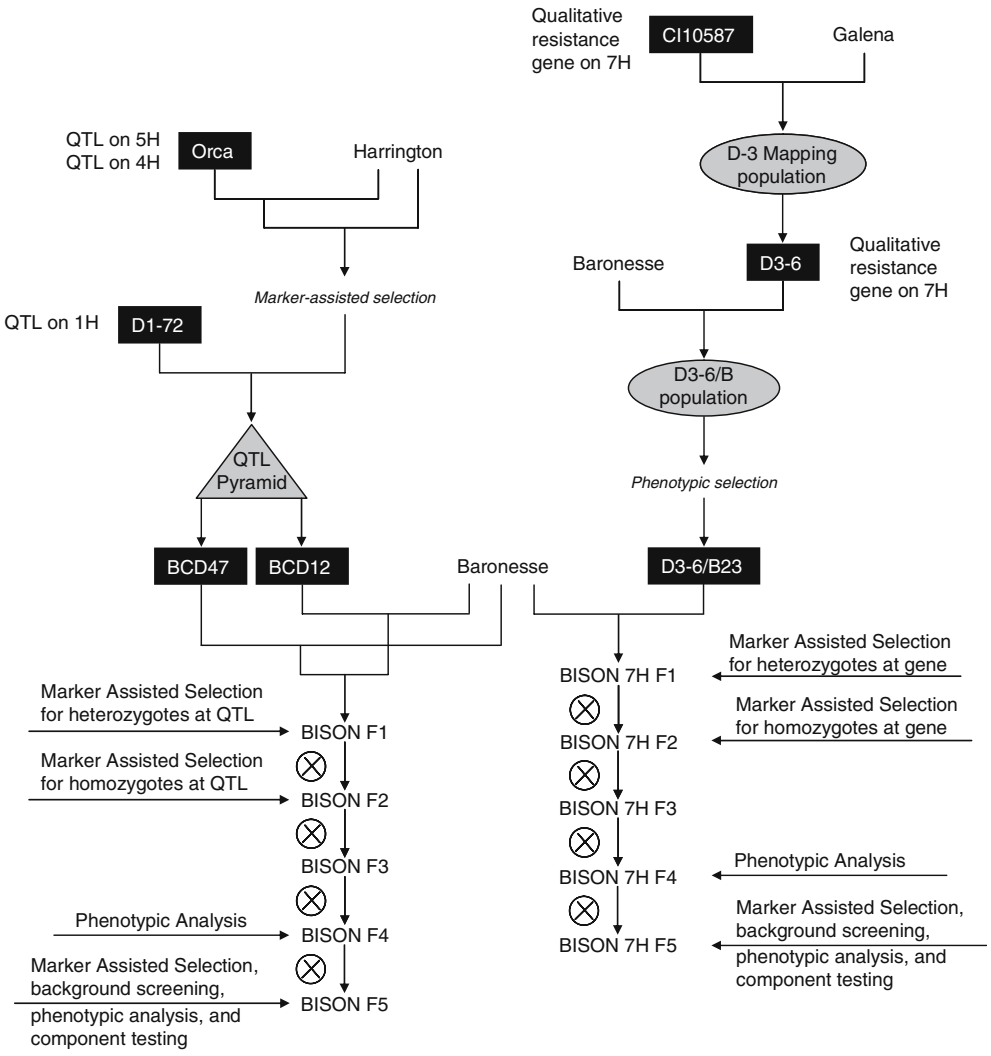

Fig. 1 The pedigree and derivation of the i-BISON lines indicating generations when phenotypic and genotypic screenings were performed. *Black squares* indicate resistance allele donors

homozygotes for the resistance alleles at one, two, or three of the target QTL resistance allele introgressions and homozygotes for the resistance allele at the major gene. The lines identified at the F2 were only selected if, besides being homozygous for the resistance allele at the target QTL, they were also homozygous for the susceptible allele at the other target QTL. The i-BISON lines were again screened with molecular markers at the F5 generation. During the time between the F2 and F5 generations, a new and more concise map (Vales et al. 2005) was generated. Based on this map we selected markers that more closely bracketed each QTL. The F5 generation of the i-BISON lines were screened using GMS021, k06267 (an EST from the Research Institute for Bioresources, Okayama University, Japan), Bmac0213, and Bmac0399 spanning the chromosome 1H QTL; EBmac0679, EBmac0788, and HvMLO3 spanning the chromosome 4H QTL; Bmag0337 and GBM1039 spanning the chromosome 5H QTL; and Bmag0120, Ris44, and Bmac0156 flanking the major gene on chromosome 7H. In addition to these 12 markers in the QTL target regions, 34 additional markers were used to screen the background genome of the F5 generation i-BISON lines (Fig. 2). These markers are described in detail by Vales et al. (2005).

Phenotyping components of disease severity

We used a randomized complete block design. Treatments consisted of the QTL target introgressions (1H, 4H, 5H, 1H + 4H, 1H + 5H, 4H + 5H, and 1H + 4H + 5H), two controls (0-QTL and the 7H major gene introgression), and the three parents (Baronesse, BCD12, and BCD47). There were variable numbers of

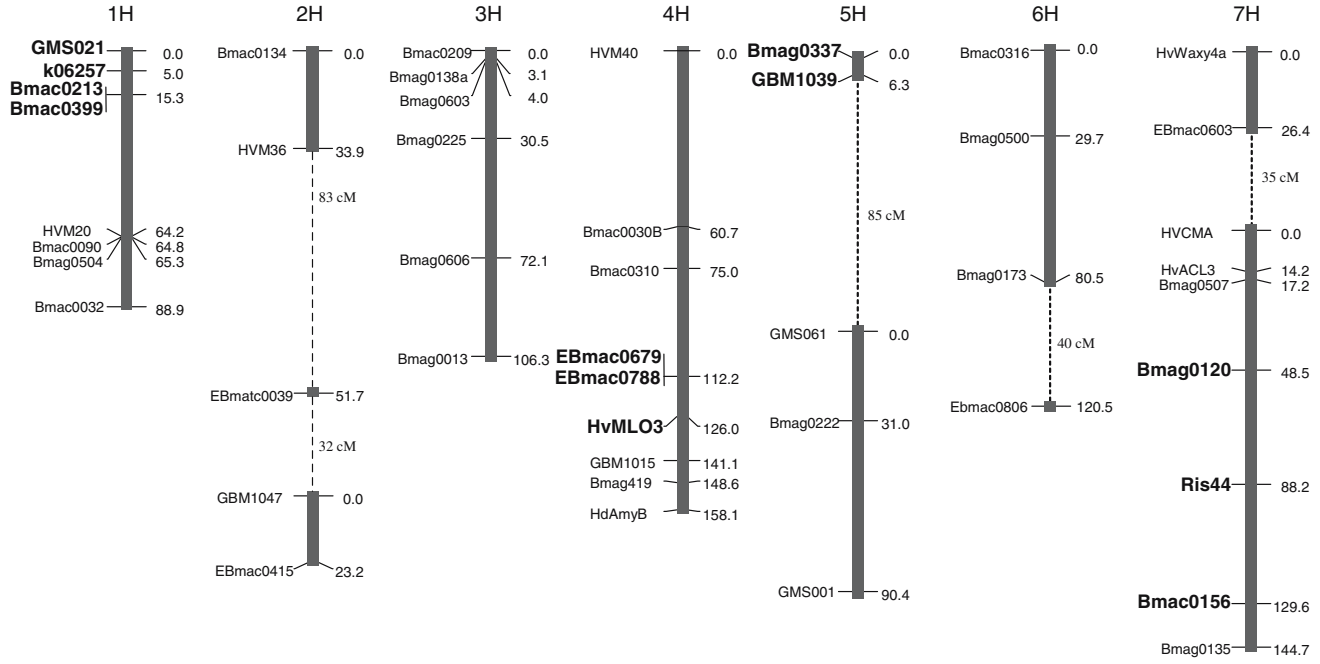

Fig. 2 Linkage map of the BCD47/Baronesse DH population constructed by Vales et al. (2005) showing only markers used for foreground and background screening of the i-BISON lines. The *dotted lines* indicate regions with distances inferred from Ramsay et al. (2000). Markers in *bold* represent those that flank the QTL target introgression regions and were used for foreground screening. All others were used for background screening

lines representing each QTL target introgression, with a total of 28 genotypes (Table 1). Three separate Percival MB-60B growth chambers (Percival Scientific, Inc., Iowa) were used as blocks (replications). Each genotype was grown in a single pot in each growth chamber, with ten individually labeled seedlings per pot. Plants were grown at 15°C with a 16 h light (245 μmol m^{-2} s^{-1})/8 h dark photoperiod. Seedlings were inoculated at the third leaf stage. The 28 pots per replication were divided in two groups for inoculation in order to meet the size limitations of the inoculation chamber. For each replication, 14 pots at a time were placed in an inoculation chamber (45.7 cm long × 45.7 cm wide × 61 cm high) and the 140 seedlings were inoculated with a powdered mixture of 0.36 mg fresh *P. striiformis* f.sp. *hordei* (race PSH-31) spores in 0.58 g talc powder using a DeVilbiss powder blower (Model 119) (Sunrise Medical, Australia) held at the top of the chamber. The powder blower was rotated around the top of the chamber to ensure uniform coverage with the inoculum. Each experimental unit received 26 μg spores per 41 mg talc powder. Inoculated plants were placed in a dew chamber at 13°C and 100% relative humidity for 16 h. The 28 pots per replication were then transferred back to the growth chamber at 15°C with 16 h light (245 μmol m^{-2} s^{-1})/8 h dark per 24 h period.

The second leaf of each plant in each pot was examined daily until the end of the experiment (20 days post-inoculation). The number of sporulating lesions present on the second leaf of each plant was counted on a daily basis until there was no further increase in lesion number. These data were used to calculate *latent period* as the number of days post-inoculation when the median number of sporulating lesions occurred (based on the day the first sporulating lesion appeared until the day when there was no further increase). The highest number of sporulating lesions was used as the estimate of *infection efficiency*. At approximately 1 week after lesion appearance, the length and width of three isolated lesions present on the second leaf of each plant were measured to determine *lesion size* (cm^2). At 18 days post-inoculation, digital images of the second leaf of each plant were taken. Each digital image included a 1 cm^2 guide and based on these images the *pustule density* was determined as the number of pustules per cm^2.

Statistical analyses

Analysis of variance (ANOVA) was performed using latent period, infection efficiency, lesion size, and pustule density data (Table 2). *F*-tests were performed using the pooled error term as the denominator. The

Table 2 Mean square (MS) and significance results from analysis of variance examining the effects of genotype, parents, introgressions, lines within introgressions, and the contrast of parents versus introgressions using the pooled error as the error term, on latent period (LP), infection efficiency (IE), lesion size (LS), and pustule density (PD)

Source of variation	*df*	MS			
		LP	IE	LS	PD
Reps	2	2.71**	0.87	0.26***	194.02**
Genotypes	24	5.43***	53.84***	0.05**	349.14***
Parents	2	2.69**	199.78***	0.12**	1041.02***
Introgressions	7	10.54***	78.33***	0.09***	468.71***
Parents vs. introgressions contrast	1	2.29*	54.32***	0.08*	37.28
Lines within introgressions	14	3.56	21.11***	0.01	213.25***
Pooled error	48	0.49	1.79	0.02	21.68

*Denotes that F value is significant at $P < 0.05$. **Denotes that F value is significant at $P < 0.01$. ***Denotes that F value is significant at $P < 0.0001$

SAS GLM and Mixed procedures (SAS institute 2001) were employed and produced similar results. Levene's test was performed to confirm the assumptions of ANOVA. We partitioned the Genotype variation [24 degrees of freedom (df)] into four sources of variation: Parents (2 *df*), Introgressions (7 *df*), a Parents versus Introgressions contrast (1 *df*), and Lines within Introgressions (14 *df*). We compared the percentage of Introgressions sums of squares, with respect to the total genotype sums of squares, to the percentage of Lines within Introgressions sum of squares. This allowed us to determine if there was more variation between introgressions than among the lines within introgressions. The additivity of resistance allele pyramiding was tested using SAS GLM procedure with a set of contrasts to determine if the effect of number of QTL on latent period, infection efficiency, lesion size, and pustule density was linear, quadratic, or cubic based on polynomial coefficients. We used the PROC CORR procedure in SAS to determine the correlations between latent period, infection efficiency, lesion size, and pustule density.

Results

The ANOVA revealed significant differences between the introgressions for latent period, infection efficiency, lesion size, and pustule density (Tables 2, 3). BCD12, the donor of the resistance allele on 1H, had a significantly lower infection efficiency, lesion size, and pustule density than the susceptible parent Baronesse, but its latent period was not significantly different. BCD47 was significantly different from Baronesse for all components. The 7H qualitative resistant introgression conferred complete immunity and showed no disease development. Consequently, we did not include the 7H introgressions in the statistical analyses.

When comparing introgressions with the same QTL allele architecture as the resistance donor parents, e.g. 4H + 5H versus BCD47 and 1H versus BCD12, there were no significant differences for latent period, infection efficiency, lesion size, and pustule density. In cases where there are multiple lines per introgression target

Table 3 Comparison of the i-BISON lines to the controls: Baronesse (Bar), 0-QTL (0), BCD12 (B12), and BCD47 (B47) (including the QTL which are present in the controls) for latent period, infection efficiency, lesion size, and pustule density by running all possible pairwise *t* tests on LSmeans when the overall *F*-test for introgressions was significant and focusing only on pre-planned comparisons

			1H	4H	5H	1H + 4H	1H + 5H	4H + 5H	1H + 4H + 5H
Latent period	Bar	(0)		*				*	*
	0	(0)		*				*	*
	B12	(1H)		*				*	*
	B47	(4H + 5H)			*				*
Infection efficiency	Bar	(0)	*	*	*	*	*	*	*
	0	(0)		*		*		*	*
	B12	(1H)			*				
	B47	(4H + 5H)			*				
Lesion size	Bar	(0)	*	*	*	*	*	*	*
	0	(0)	*	*	*	*	*	*	*
	B12	(1H)							
	B47	(4H + 5H)							
Pustule density	Bar	(0)	*	*	*	*	*	*	*
	0	(0)		*		*	*	*	*
	B12	(1H)			*				
	B47	(4H + 5H)	*		*				

*Denotes a significant difference at $P < 0.05$ level

(1H, 4H, 5H, 1H + 4H, 1H + 5H, and 4H + 5H) the variation among lines was always less than the variation between introgression targets.

Individual introgression targets had effects of different magnitudes on the components of resistance (Fig. 3). The 4H introgression had the largest effect on the four components of resistance. Latent period, infection efficiency, and pustule density all exhibited significant differences for pair-wise comparison of alleles (i.e., 1H vs. 4H, 1H vs. 5H, and 4H vs. 5H). The order of magnitude of effects for all three phenotypes was 4H > 1H > 5H. For lesion size, there was no significant difference. The four components of resistance were highly correlated (Table 4). There were significant negative correlations between latent period and infection efficiency, lesion size, and pustule density. There were significant positive correlations between infection efficiency and lesion size and pustule density and between lesion size and pustule density.

Increasing the number of QTL resistance alleles in single genotypes led to more resistant infection efficiency, lesion size, and pustule density, but it did not significantly increase latent period (Fig. 4). However, even the most resistant pyramids did not reach the zero symptom level of the immune 7H qualitative resistance gene introgression.

Forty-six markers were surveyed in the case of the quantitative resistance allele targets (1H, 4H, and 5H). Twelve markers were used for resistance allele introgression and the remaining for background characterization (Fig. 2). There are three possible alleles per locus: Baronesse, BCD12, and BCD47. At five loci for background characterization, identification of the three possible alleles was possible. At the remaining 12 foreground and 29 background loci, alleles from two of the three could not be distinguished. For the eight of the 12 foreground markers it was possible to differentiate the resistance allele from the susceptible alleles. BCD12 and BCD47 were most often identical (29 loci), followed by BCD12 and Baronesse (11) and BCD47 and Baronesse (1). In the case of the 7H introgression, there are only two allele possibilities. Of the 46 markers used for the quantitative resistance introgression, 31 were polymorphic between D3-6/B23, the source of the qualitative resistance gene, and Baronesse (3 flanking and 28 background). Due to the occurrence of identical alleles from two parents in the quantitative resistance allele introgressions, the percentage Baro-

Table 4 Results from the analysis of correlation of latent period, infection efficiency, lesion size, and pustule density

	Infection efficiency	Lesion size	Pustule density
Latent period	−0.78*	−0.83*	−0.68*
Infection efficiency		0.77*	0.87*
Lesion size			0.79*

*Denotes significance at $P < 0.01$ level

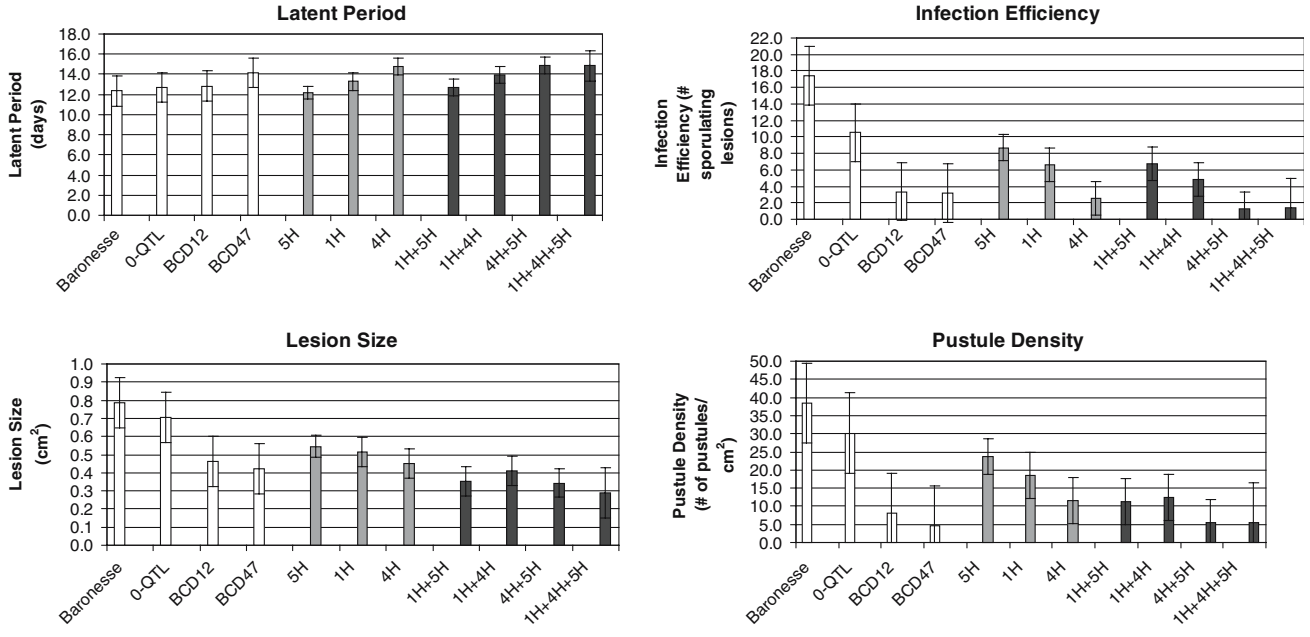

Fig. 3 The least-squares means of treatments for the five components of resistance. *Error bars* indicate 95% confidence intervals. Treatments are separated into three groups: *white* are controls, *gray* are single QTL target introgressions, and *black* are combinations of QTL target introgressions

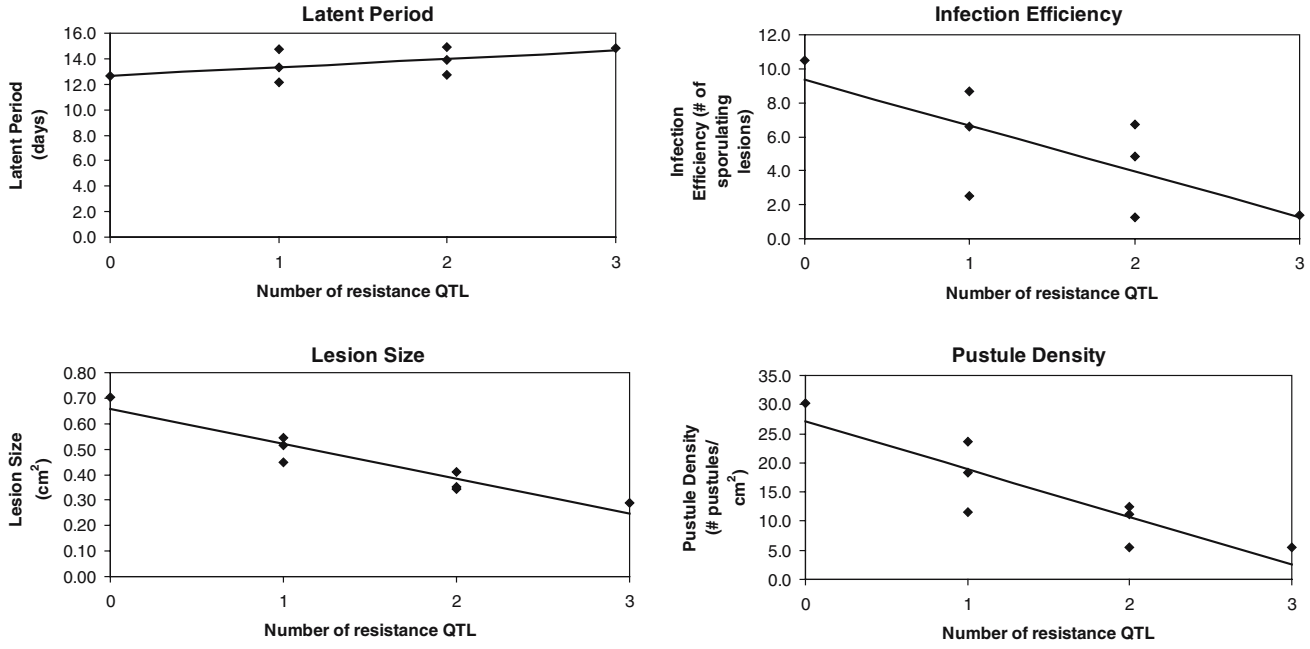

Fig. 4 Regression of each component of resistance on the number of QTL targets per introgression indicating the effectiveness of pyramiding QTL target introgressions for infection efficiency, lesion size, and pustule density

nesse in each i-BISON line can only be estimated. To calculate the percentage background Baronesse for each line, a value of 1 was assigned to each non-target locus if the allele could be identified as originating from Baronesse and 0.5 if it could have originated from Baronesse or one of the two resistance donor parents. These scores were summed and divided by the total number of background markers. The number of background markers varied from 37 to 44, depending on the number of target QTL resistance alleles. In the case of the 7H introgression, the percentages of Baronesse background were all based on the number of Baronesse alleles at 43 non-target loci. Estimates of the percentages of background loci at which Baronesse alleles are fixed ranged from 29 to 76% in the quantitative resistance introgressions and from 42 to 61% for the 7H introgression (Fig. 5). Considering all the i-BISON, residual heterozygosity at the F5 was observed at 2% of the loci surveyed.

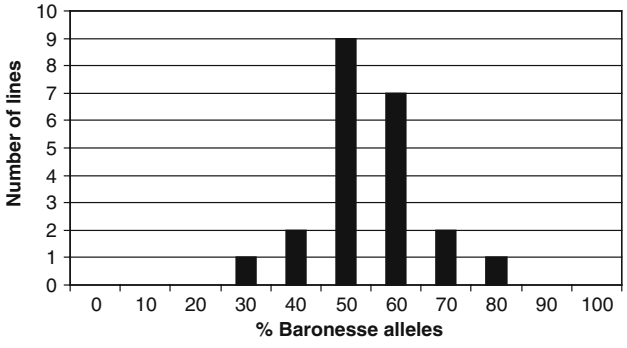

Fig. 5 Distribution of the percentages of background loci at which Baronesse alleles are fixed for the quantitative resistance introgressions

Discussion

We observed a range of resistance phenotypes in the materials tested. The occurrence of some disease symptoms in BCD12 and BCD47 and all QTL allele introgressions confirms that quantitative resistance is present in this germplasm. The 7H major gene control was immune to infection, as expected. Under intense field epidemic conditions in Mexico, we have observed limited symptom development (i.e., trace) at the adult plant stage in CI10587, the line contributing this major gene (Castro et al. 2003a).

We were able to dissect the BSR disease severity QTL reported in previous studies (Toojinda et al. 2000; Castro et al. 2002, 2003a, b, c; Vales et al. 2005) into four components and found that the most notable differences were for infection efficiency, lesion size, and pustule density. On average, the QTL allele introgression lines had longer latent periods than the susceptible parent, Baronesse, but these differences were greater for the other three components. These differences may be due in part to the resistance alleles present in the parental sources; BCD47 had a significantly longer latent period than Baronesse, but BCD12 and Baro-

nesse were not different. These results are similar to those of Parlevliet (1975), who used the barley: *Puccinia hordei* pathosystem and found that there were smaller differences in latent period between resistant and susceptible cultivars than for infection efficiency. The epidemiological importance of latent period was confirmed in studies that showed that changes in latent period produced greater changes in the rate of disease increase than did changes of similar magnitude in other components of resistance (Leonard and Mundt 1984).

We confirmed that marker assisted introgression of resistance alleles is effective. The introgression of the major resistance gene on chromosome 7H gave more clear-cut results (immunity) than the introgression of the QTL with resistance alleles. The introgressed resistance alleles at the target QTL generally led to superior levels of one or more components of resistance, but particularly with some of the single allele introgressions, these effects were modest. There are indications that resistance alleles other than the targets were also introgressed: the 0-QTL control did not always equal Baronesse, the 4H + 5H pyramid did not always equal BCD47, and the 1H did not always equal BCD12. The presence of uncharacterized non-target resistance alleles is one explanation for the variance seen among the lines within an introgression. If there are such QTL, they may trace to BCD12. If they trace to BCD47 or Baronesse, they must have small effects, as they remained undetected in a large mapping population involving the same germplasm (Vales et al. 2005). An alternative explanation is the unintended introgression of favorable alleles due to a lack of markers that could identify all three possible alleles at some of the loci that flanked introgression targets. Although there was some variance among the multiple lines within some of the introgressions, the average effects of the BSR resistance allele introgressions at the QTL were always greater.

The effects of these introgressions with the i-BISON corroborates and extends the findings of Castro et al. (2003a), who targeted the same QTL alleles and introgressed them into more variable genetic backgrounds. The differences in the magnitude of the effect of different QTL alleles also corroborate previous results (Castro et al. 2003b and c). The 4H QTL allele had the largest effects, followed by 1H and 5H. The results of this experiment confirm that multiple phenotypes can be attributed to each QTL allele, as reflected by the high correlations between the four components of resistance. Parlevliet (1979) also reported high correlations between lesion size and infection efficiency. Thus, the data support pleiotropy rather than different and specialized functions attributable to each QTL allele. Nonetheless, the QTL not only vary in effect, but also in their impact on specific components. The 4H QTL allele had the largest effect on all components, as well as a proportionally greater effect on latent period. Therefore, pyramids of multiple resistance QTL alleles where the 4H QTL was present led to lower infection efficiency, lesion size, and pustule density. The i-BISON lines containing three QTL with resistance alleles did not have a significantly longer latent period than those containing only one or two QTL with resistance alleles.

We have verified that MAS is effective for introgressing qualitative and quantitative disease resistance genes into an elite agronomic background. In the case of the single qualitative resistance gene on 7H, similar gains would undoubtedly have been achieved through phenotypic selection and at a reduced cost. In the case of the quantitative resistance allele targets on 1H, 4H, and 5H, it would not have been possible to develop all the single gene and multiple allele combinations without the initial QTL position information and use of markers during the selection process. We have shown that the general barley stripe rust quantitative resistance phenotype "disease severity" can be partitioned into the components of latent period, infection efficiency, lesion size, and pustule density. Our findings concur with previous reports that the four components are highly correlated. Our use of genotype and phenotype information revealed that the QTL with resistance alleles we targeted have pleiotropic effects on all four components, although the QTL alleles varied in the magnitude of their effects. There were linear reductions in infection efficiency, lesion size, and pustule density as more resistance alleles were added to individual genotypes, but resistance pyramiding did not increase latent period. In this study, infection efficiency was the component with the least experimental error, the largest significant differences between resistant and susceptible lines, and was the easiest to measure. Since there is such a high correlation amongst the traits, it would be possible to use only infection efficiency as the selection criterion for quantitative resistance. Latent period is a significant component of resistance and it is important to point out that measurements to determine infection efficiencies will also allow the calculation of latent period.

The introgression of the same resistance alleles at the same QTL into different lines did not always lead to the same resistance: there was more variance among lines within the quantitative resistance allele introgression classes and no variance among lines within the qualitative resistance gene introgression group (all lines were immune). The variance among lines repre-

senting the introgression of the same target QTL alleles may be due to the presence of undiscovered resistance factors. Additional evidence for this possibility is the observation that the 0-QTL line was either equal to or more resistant than the susceptible parent. It is also possible that non-target resistance alleles were inadvertently introgressed due to the lack of completely informative (tri-allelic) markers at all loci. During the course of this research, new markers were identified that provided additional and better resolution than the markers that were available for the first round of resistance allele selection. Therefore, the final characterization of the F5 lines provided the best picture of the allele composition of all lines. This information will be essential in selecting parental lines for the next step in this research—development of the QTL–NILs. From the standpoint of measuring allele effects, however, the variance attributable to inadvertent introgressions of known and unknown resistance alleles was always much less than the variance between introgression classes.

This first set of experiments focused on seedling resistance to a single race. The patterns of QTL allele effects in the i-BISON at the seedling stage in response to multiple races and at the adult plant stage in response to field infection are warranted to explore in more depth in the barley/barley stripe rust pathosystem. This first step in the genetic dissection of quantitative resistance to barley stripe rust into component traits raises interesting questions regarding the nature of quantitative resistance genes. The products of the next stage of this research, the QTL–NILs, should ultimately allow for characterization of these genes in terms of their structure and function.

Acknowledgements This work was supported in part by the USDA-NRI program (Plant–Microbe Interactions) and the United States Barley Genome Project (USBGP). We thank Mary Moore, Tanya Filichkin, Ann Corey, and graduate and undergraduate students in the OSU barley program for their technical support in the lab and greenhouse.

References

van Berloo R, Aalbers H, Werkman A, Niks RE (2001) Resistance QTL confirmed though development of QTL-NILs for barley leaf rust resistance. Mol Breed 8:187–195

Broers LHM (1997) Components of quantitative resistance to yellow rust in ten spring bread wheat cultivars and their relations with field assessments. Euphytica 96:215–223

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Castro AJ, Chen XM, Hayes PM, Knapp SJ, Line RF, Toojinda T, Vivar H (2002) Coincident QTL which determine seedling and adult plant resistance to stripe rust in barley. Crop Sci 42:1701–1708

Castro AJ, Capettini F, Corey AE, Filichkin T, Hayes PM, Kleinhofs A, Kudrna D, Richardson K, Sandoval-Islas S, Rossi C, Vivar H (2003a) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor Appl Genet 107:922–930

Castro AJ, Chen XM, Hayes PM, Johnston M (2003b) Pyramiding quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. Crop Sci 43:651–659

Castro AJ, Chen X, Corey A, Filichkina T, Hayes PM, Mundt C, Richardson K, Sandoval-Islas S, Vivar H (2003c) Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on adult plant resistance. Crop Sci 43:2234–2239

Gebhardt C, Valkonen JP (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

Johnson R (1981) Durable resistance: definition of, genetic control, and attainment in plant breeding. Phytopathology 71:567–568

Lefebvre V, Chèvre A-M (1995) Tools for marking plant disease and resistance genes: a review. Agronomie 15:3–19

Leonard KJ, Mundt CC (1984) Methods for estimating epidemiological effects of quantitative resistance to plant diseases. Theor Appl Genet 67:219–230

Li Z-K, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol Gen Genet 261:58–63

Liu Z-W, Biyashev RM, Maroof MAS (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Niks RE, Rubiales D (2002) Potentially durable resistance mechanisms in plants to specialized fungal pathogens. Euphytica 124:201–216

Osman-Ghani N, Manners JG (1985) Components of partial resistance to *Puccinia striiformis* in barley. Plant Pathol 34:75–82

Parlevliet JE (1975) Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24:21–27

Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17:203–222

Pflieger S, Lefebvre V, Causse M (2001) The candidate gene approach in plant genetics: a review. Mol Breed 7:275–291

Qi X, Niks RE, Stam P, Lindhout P (1998) Identification of QTLs for partial resistance to lead rust (*Puccinia hordei*) in barley. Theor Appl Genet 96:1205–1215

Ramsay L, Macaulay M, Ivanissevich SD, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

SAS Institute (2001) The SAS system for Windows v. 8.02. SAS Institute Inc. Cary, USA

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97:308–315

Toojinda T, Baird E, Broers L, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Vivar

H (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley. Theor Appl Genet 101:580–589

Vales MI, Schön CC, Capettini F, Chen XM, Corey AE, Mather DE, Mundt CC, Richardson KL, Sandoval-Islas JS, Utz HF, Hayes PM (2006) Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. Theor Appl Genet (in press)

Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci 43:1828–1832

Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. Genetics 169:2277–2293