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Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development

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Abstract Genome-analysis tools are useful for dissecting complex phenotypes and manipulating determinants of these phenotypes in breeding programs. Quantitative trait locus (QTL)-analysis tools were used to map QTLs conferring adult plant resistance to stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) in barley. The resistance QTLs were introgressed into a genetic background unrelated to the mapping population with one cycle of marker-assisted backcrossing. Doubled-haploid lines were derived from selected backcross lines, phenotyped for stripe-rust resistance, and genotyped with an array of molecular markers. The resistance QTLs that were introgressed were significant determinants of resistance in the new genetic background. Additional resistance QTLs were also detected. The susceptible parent contributed resistance alleles at two of these new QTLs. We hypothesize that favorable alleles were fixed at these new QTLs in the original mapping population. Genetic background may, therefore, have an important role in QTL-transfer experiments. A breeding system is described that integrates single-copy and multiplex markers with confirmation of the target phenotype in doubled-haploid lines phenotyped in field tests. This approach may be useful for simultaneously producing agronomically useful germplasm and contributing to an understanding of quantitatively inherited traits.

Key words QTL · AFLP · Marker-assisted selection · Barley · *Puccinia striiformis* f.sp. *hordei*

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Introduction

Genome analysis based on DNA polymorphisms can reveal the genetic determinants of complex phenotypes and provide tools for manipulating these determinants to maximize selection response. We are using molecular markers in barley (*Hordeum vulgare* L.) in an attempt to rapidly develop cultivars adapted to the Pacific Northwest of the United States that are resistant to barley stripe rust. Barley stripe rust is caused by *Puccinia striiformis* f.sp. *hordei*. The disease was first reported in the Americas in 1975 (Dubin and Stubbs 1986) and in the United States in 1991 (Marshall and Sutton 1995). By 1995, the disease was reported throughout the western United States, where localized epidemics have caused severe losses in yield and quality. While the disease can be controlled by fungicides, economic and environmental considerations favor genetic resistance.

There is limited information on the genetics of resistance to stripe rust in barley. Bakshi and Luthra (1971) described a dominant resistance gene in Indian germplasm but did not map it. Three recessive resistance genes (*Yr*, *Yr2*, and *Yr3*) are reported in European spring barley germplasm, and one dominant resistance gene is reported in European winter barley. These genes have not been mapped (Lehmann et al. 1975).

The only stripe rust resistance gene showing Mendelian inheritance that has been mapped in barley is *Yr4*. This locus is located on chromosome 5 (1H). It does not confer resistance to race 24 (Von Wettstein-Knowles 1992). The virulence of stripe rust in the Americas was first described in terms of race 24 (Dubin and Stubbs 1986). Chen et al. (1994) and Hayes et al. (1996 c) reported adult plant stripe-rust resistance QTLs on chromosomes 4 (4H) and 7 (5H). The race composition of the field inoculum was not known. Thomas et al. (1995) reported QTLs for adult plant stripe-rust resistance to an uncharacterized field inoculum on chromosomes 1 (7H), 5 (1H), and 7 (5H). The chromosome-5 (1H) QTL was hypothesized to be due to the *Yr4* locus and the chromosome-7 (5H) QTL to be the same QTL reported by Chen et al. (1994) and Hayes et al. (1996 c). In contrast, the genetics of stripe rust in wheat is an area of extensive study (reviewed by Line et al. 1993). Based on the homoeology of the two crop species, there are likely to be parallels in the two host/pathogen interaction systems. For example, quantitative, adult-plant resistance will probably be more durable than race-specific resistance (Line 1993).

We have, therefore, focused our attention on introgressing resistance genes from genotypes that, under field conditions, allow limited disease development on adult plants. This type of disease reaction may indicate durable, adult-plant resistance (Parlevliet and Van Ommeren 1975). When such genotypes, developed by the International Center for Agricultural Research in the Dry Areas (ICARDA) program based at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, are crossed with susceptible genotypes, the progeny show a range of disease-reaction phenotypes that do not fall into discrete classes. This quantitative inheritance can be studied through the techniques of quantitative trait locus (QTL) analysis (Hayes et al. 1996 c). Based on restriction fragment length (RFLP) linkage data and adult-plant disease-reaction phenotype data, we reported stripe rust resistance QTLs on chromosomes 4 (4H) and 7 (5H) of Calicuchima-sib (Chen et al. 1994; Hayes et al. 1996 c).

Backcrossing is an approach to introgressing target loci, such as stripe rust resistance QTLs, into adapted backgrounds. The contribution of the donor parent is reduced by half with each generation of backcrossing, assuming no linkage. Molecular markers can increase the efficiency of the process in several ways. Flanking markers can be used to identify the backcross lines that are heterozygous for target genome regions. Advancing only these selected lines will also have the effect of reducing linkage drag (Young and Tanksley 1989; Tanksley and Nelson 1996). Single-copy, or low-copy, markers with defined map locations, such as RFLPs and simple-sequence repeats (SSRs), are ideal for this step. Molecular markers could also increase the efficiency of backcrossing by allowing for the selection of genotypes with the maximum percentage of the recurrent parent genome. Markers with higher information content per reaction, such as amplified fragment length polymorphisms (AFLPs), are ideal for this step (Waugh et al. 1997).

Manipulation of QTLs can be problematic due to loss of target loci though recombination, incorrect information regarding the location of the QTLs, and/or negatively altered expression of the QTLs in new genetic backgrounds (Hayes et al. 1996 b). Therefore, a marker-assisted QTL backcrossing scheme for a self-pollinated crop, such as barley, might: (1) use flanking markers to select progeny with a probability of carrying the target QTL allele(s), (2) confirm the target phenotype in the selected progeny, and (3) use multiplex markers to identify those selections with the maximum percentage of the recurrent-parent genome. In crops where a rapid approach to homozygosity (such as the doubled-haploid technique) is possible, the efficiency of the second step can be increased by phenotyping on a plot, rather than an individual-line, basis. See Powell et al. (1996) for additional details on the idea of integrating single- and multi-locus markers in barley breeding.

Our long-term practical objective is to develop six-row, spring-habit germplasm adapted to the Pacific Northwest of the United States that has durable resistance to stripe rust. In doing so, we sought to: (1) validate the effects of mapped stripe-rust resistance QTLs, (2) determine if there were different resistance QTLs in an unrelated genotype, and (3) pilot a marker-assisted backcrossing scheme incorporating RFLP, AFLP, and random amplified polymorphic DNA (RAPD) markers, doubled-haploids, and field phenotyping.

Materials and methods

Germplasm

The germplasm derivation process is shown in Fig. 1. BSR41, a 6-row mapline from the Calicuchima-sib × Bowman mapping population (Chen et al. 1994; Hayes et al. 1996 c) was used as the donor parent in a single backcross to the variety Steptoe. Steptoe is the most widely grown six-row feed barley in the Pacific Northwest United States. It has been the subject of intensive genome mapping efforts by the North American Barley Genome Mapping Project (see Hayes et al. 1996 b for a review). Steptoe is susceptible to stripe rust.

Four RFLP markers bracketing the stripe-rust resistance QTLs on chromosomes 4 (4H) and 7 (5H), described by Chen et al. (1994) and Hayes et al. (1996 c), were screened in a population of 66 backcross-one (BC1)-generation plants. Only a subset of the RFLP markers available at the time this experiment was conducted showed polymorphism between Steptoe and BSR41. The target regions on chromosome 4 (4H) and chromosome 7 (5H) were poorly populated with markers when this work was carried out, and they remain sparsely populated on the current barley consensus map of Qi et al. (1996). *ABG366* and *Bmy1* flank the resistance QTLs on chromosome

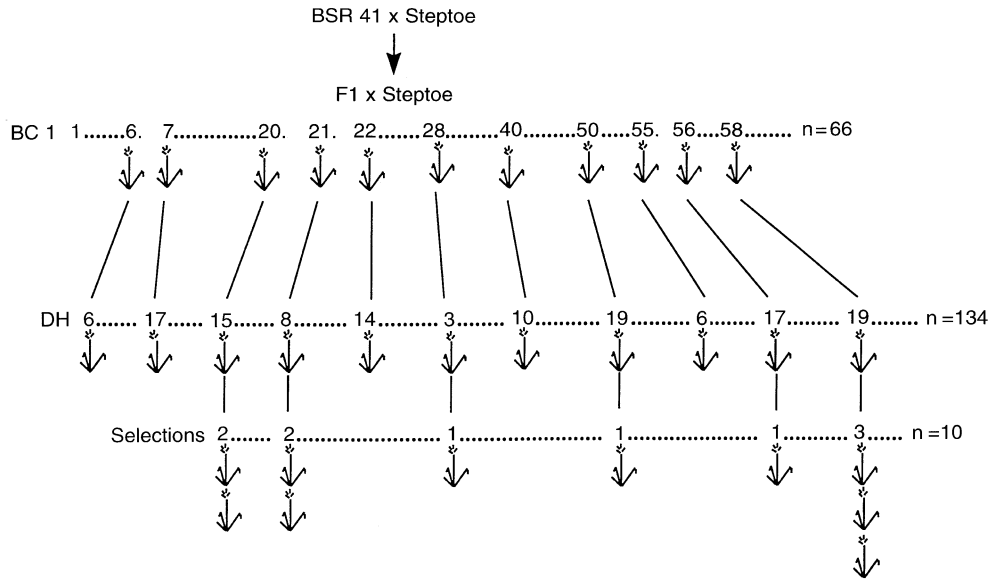

Fig. 1 Derivation of doubled-haploid (DH) germplasm from a marker-assisted selection program for adult plant stripe rust resistance. BC1 refers to the first backcross generation and the *numbers* identify the BC1 plants that were selected based on their genotypes at marker loci flanking stripe rust resistance QTLs on chromosomes 4 (4H) and 7 (5H). DH refers to doubled-haploid and the *numbers* indicate the number of DH lines produced from each BC1 plant. Selections refer to the ten most resistant DH lines advanced to extensive phenotyping. See text for additional details on line derivation and genotyping procedures

4 (4H). *ABG366* was not mapped in the reference mapping population, Calicuchima-sib × Bowman, where the resistance QTL peak was detected between *Bmy1* and *ABG397*, a distance of 28.1 cM. *ABG366* and *Bmy1* are 32.7 cM apart on the Steptoe × Morex map (Mather 1995). In the Steptoe × Morex cross, *ABG366* is 3.7 cM proximal to *ABG397* (Mather 1995). *WG530* and *CDO57* flank the resistance QTL on chromosome 7 (5H). *WG530* was not mapped in Calicuchima-sib × Bowman, where the resistance QTL peak was detected in the *Ale-CDO57* interval, a distance of 20.3 cM. *WG530* and *CDO57* are 31.4-cM apart on the Steptoe × Morex map (Mather 1995).

As shown in Fig. 1, 11 of the 66 BC1 plants (plant numbers 6, 7, 20, 21, 22, 28, 40, 50, 55, 56 and 58) were selected as heterozygotes for the target flanking markers. Doubled-haploid (DH) lines were derived from these BC1 plants, using the *Hordeum bulbosum* technique, as described by Chen and Hayes (1989). A total of 134 DH lines were produced, with varying numbers of DHs per BC1 plant, as shown in Fig. 1. For example, six DHs were produced from BC1 plant no. 6, 17 DHs were produced from BC1 plant no. 7, etc.

Genotyping

The 134 DH lines were genotyped for: the four RFLP markers used for resistance QTL introgression and an additional RFLP on chromosome 4 (4H) (*ABG54*); two morphological markers on chromosome 7 (5H), *mSrh* (rachilla hair length) and *mR* (awn texture); 106 AFLPs; and eight RAPDs. RFLPs were assayed as described by Chen et al. (1994). The morphological markers were scored under a dissecting microscope. RAPDs were assayed as described by Barua et al. (1993). The AFLP methodology was essentially as described by Zabeau and Vos (1993) with the following modifications. Template DNA was prepared the using restriction-enzyme combination *Eco*RI/*Mse*I (Boehringer Mannheim/New England Biolabs); 2.5 µg of genomic DNA was digested as outlined by Zabeau and Vos (1993) and two specific double-stranded adapters were ligated to the fragment ends. Neither of the adapters was biotinylated and the selection step using streptavidin-coated magnetic beads was omitted. Adapter sequences were:

*Eco*RI 5′ CTCGTAGACTGCGTACC
5′ AATTGGTACGCAGTC,

*Mse*I 5′ GACGATGAGTCCTGAG
5′ TACTCAAGGACTCAT.

The digested and ligated DNA was pre-amplified using an *Eco*RI-directed primer and an *Mse*I-directed primer. The primers did not have additional selective nucleotides at the 3′ end. The sequences of the primers were

E00 5′ GACTGCGTACCAATTC,
M00 5′ GATGAGTCCTGAGTAA.

Pre-amplification was performed in a total volume of 25 µl containing 75 ng each of primers E00 and M00, 0.2 mM of all four dNTPs (Pharmacia), 1 × PCR buffer (Perkin Elmer Cetus), 1 U of Amplitaq DNA Polymerase LD (Perkin Elmer Cetus) and 30 ng of the digested and ligated DNA. The cycle profile used was as follows; denaturation for 30 s at 94°C, annealing for 30 s at 60°C, extension for 60 s at 72°C , for 30 cycles. After pre-amplification, the product was diluted by the addition of 55 µl of buffer (10 mM Tris pH 8, 0.1 mM EDTA). This mixture was used as a template for selective amplification. Selective amplification was carried out using adapter-directed primers. Nine different primer combinations were used, each combination consisted of one '*Eco*' primer and one '*Mse*' primer. All of the primers had three selective nucleotides at the 3′ end. Primer combinations and base extensions were:

primer combination	*Eco*RI	*Mse*I
e32m34	AAC	AAT
e35m42	ACA	AGT
e36m33	ACC	AAG
e36m36	ACC	ACC
e36m50	ACC	CAT
e38m31	ACT	AAA
e38m50	ACT	CAT
e39m61	AGA	CTG
e41m33	AGG	AAG.

In each case the '*Eco*' primer was radiolabelled using ^{33}P-ATP as described by Vos et al. (1995).

The amplification reactions were carried out in a total volume of 20 µl, comprising 6.7 ng of labeled *Eco*RI primer, 25 ng of unlabelled *Eco*RI primer, 30 ng of *Mse*I primer, 0.2 mM of all four dNTPs, 1 × PCR buffer (Perkin Elmer Cetus), 0.5 U of Amplitaq DNA Polymerase (Perkin Elmer Cetus) and 2 µl of template DNA.

Reactions were carried out using the cycle profile described by Vos et al. (1995). All PCR reactions were performed using a Perkin Elmer 9600 thermocycler. Reactions were stopped by the addition of an equal volume of formamide loading buffer (98% formamide, 10 mM EDTA pH 8, Bromophenol Blue, xylene cyanol). The samples were denatured at 90°C for 5 min. Then, 3.5 µl of each sample was loaded onto a 40-cm, 6% denaturing polyacrylamide gel (Easigel, Scotlab) which had been pre-heated by running at 80 W for 30 min. The samples were then electrophoresed at a constant power of 80 W for 1 h 45 min. Gels were transferred to Whatmann 3 MM paper and dried for 2 h at 80°C on a gel drier (Biorad). They were then exposed to autoradiographic film (X-OMAT S, Kodak) to visualize the results, which were scored manually. The AFLP loci were named based on enzyme, primer sequence, and band size. For example, *e38m31l* refers to band "l" revealed in this germplasm, using primer sequence 38 with *Eco*RI and primer sequence 31 with *Mse*I.

Phenotyping

The DH lines were evaluated in four field tests. In the first test, lines and parents were grown in uni-replicate hill plots at Celaya, Mexico, in the winter of 1995. To initiate a field epidemic, spreader rows (formed from a mixture of 15 extremely susceptible genotypes) were inoculated with a stripe rust isolate whose virulence pattern corresponds to the race 24 Varunda-Mazurka type described by Dubin and Stubbs (1986). Stripe rust severity was rated for DGS 59 (Feekes stage 10.5) as the percent severity on a plot basis. The percent severity was estimated visually as the percentage of the total plant canopy in each plot that was covered with stripe rust pustules. DH lines and parents were then assessed at three planting dates at Toluca, Mexico, in the summer of 1995. A single replicate was grown at each planting date, using 3-m, one-row plots. Spreader rows, planted at 5.25-m intervals and consisting of a mixture of 15 susceptible genotypes, were inoculated twice with infected plants placed in the foliage and with applications of spores suspended in oil. Infected plants and spores were collected locally. The race composition of this inoculum was not determined. Genotypes inoculated in this fashion will never escape rust infection (H. Vivar, personal communication). Stripe rust was rated in terms of the percent severity on a plot basis. At the time of rating, genotypes at the three planting dates were at growth stages DGS 75, DGS 59, and DGS 49, respectively. Multiple planting dates were used in an attempt to determine the effect of maturity on the expression of stripe-rust resistance. In the summer of 1996 the ten most resistant and ten most susceptible lines (as measured by average performance in the previous four tests), and the two parents, were grown at Toluca, Mexico. The phenotyping and rating procedures were the same as those employed in 1995, except that at each of the three planting dates, each genotype was grown in a two-row plot.

Data analysis

Of the 134 DH lines that were produced, there was sufficient seed to include 96 in all four 1995 phenotyping experiments. Subsequent analyses were based on these 96 genotypes. Each of the four experiments grown in 1995 were considered replicates for the purpose of obtaining an estimate of the heritability of stripe rust severity. This heritability estimate was calculated as:

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e/r},$$

where $\sigma^2 g$ is the variance among DH lines, $\sigma^2 e$ is the error variance, and $r = 4$, the number of environments sampled.

Alleles at the 120 marker loci were scored as 0 (Steptoe) or 1 (BSR41) and were considered independent variables. Stripe rust severity data were considered as dependent variables and were used for estimating genotype/phenotype associations via simple and multiple regression. These associations were determined for each of the 1995 environments, and from the mean of the four 1995 environments. Individual markers that were significant ($P < 0.05$) determinants of trait expression were included in multiple-regression models. Determining the joint effects of multiple loci could be biased if linked loci are included in a multi-locus model. Therefore, only the most significant single locus from each group of linked loci was included in the multi-locus model. Procedures for grouping linked loci are described in the next paragraph. Multiple-regression models were evaluated using Sawa's Bayesian Information Criterion (BIC), as available in the Statistical Analysis System (SAS 1988). The sign of the slope was used to identify the value of the stripe rust-reaction alleles contributed by each parent. Negative and positive effects indicate that BSR41 contributed resistant and susceptible QTL alleles, respectively. The R^2 value was used as a measure of the total phenotypic variance accounted for by each marker and by the joint analysis of multiple markers. A genotypic coefficient of variation was calculated as

$$R^2 p/H^2,$$

where $R^2 p$ is the proportion of phenotypic variance accounted for by a marker or set of markers, and H^2 is the heritability.

Putative map positions of the AFLP and RAPD loci were established as follows. Homologous AFLP products, identified by fragment sizes, have been shown to map to the same regions in the barley genome in a study of a number of barley crosses (Waugh et al. 1997). Some of the primer combinations used in the current study had also been used in extending the Dicktoo × Morex map (Hayes et al. 1996 a), and inclusion of Dicktoo and Morex on the AFLP gels for the current study enabled the identification of 12 markers also segregating in the Dicktoo × Morex population. The chromosomal locations of these markers were therefore established by reference to the Dicktoo × Morex map. In order to establish tentative linkage-group assignments for AFLP and RAPD markers that could not be directly related to mapped loci in other mapping populations, we employed multivariate analysis of all of the marker data. Similarities between markers in a backcross population are equivalent to $1 - r$, where r = the recombination value (Ramsay and Thomas 1992). For an unselected backcross of 66 individuals, markers showing similarities >0.74 and >0.82 are significantly linked at the 0.05 and 0.01 probability levels, respectively. Groups showing similarities of >0.82 were therefore formed and given a tentative chromosomal assignment if they contained markers mapped in other populations. Because the population assayed with molecular markers is based on 11 selected BC1 genotypes, we cannot be completely sure of the chromosome location of these groups. However, the putative locations provided an objective basis for identifying a single locus from each group of loci for the multilocus-regression models, as described in the previous paragraph.

Results

The phenotypic distribution of stripe rust severity in the DH lines, averaged over the four environments sampled in 1995, did not show discrete classes (Fig. 2). Similar distributions were observed for each of the individual environments (data not shown). The standard errors for disease severity in BSR41 and Steptoe were $\pm$ 1.5 and $\pm$ 4.5%, respectively. The heritability of stripe rust severity, calculated using environments as replicates, was 0.95.

A total of 120 data points were generated for the 96 genotypes. Of these, four were RFLPs, two were

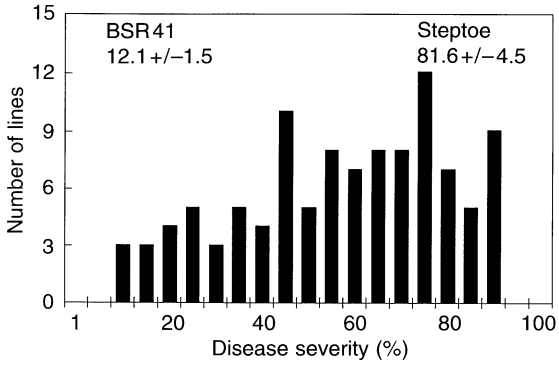

Fig. 2 Average stripe rust disease severity (%) in doubled-haploid lines derived from one cycle of marker-assisted backcrossing using BSR41 and Steptoe as the donor and recurrent parents, respectively

Table 1 Chromosome location, slope, *P*-value, and R^2 for markers significantly associated with stripe rust severity in single-locus regressions. Chromosome locations in italics are putative. Negative and positive slopes indicate that BSR41 contributed resistant and susceptible QTL alleles, respectively

Marker	Chromosome location	Slope	*P*-value	R^2
e36m36a	3	− 17.5	0.000	19.1
e41m33j	3	− 13.1	0.001	10.4
e41m33p	3	− 13.5	0.001	10.8
e36m33j	3	− 12.0	0.009	8.3
e36m36h	3	− 12.6	0.002	9.7
OPD8a	3	− 12.9	0.005	9.6
Bmy1	4	− 11.1	0.006	7.9
e32m24q	4	− 9.4	0.042	5.2
CDO57	7	− 15.6	0.000	15.6
mSrh	7	− 13.9	0.000	12.1
e36m36d	?	13.6	0.011	8.1
e36m50i	?	12.1	0.041	5.4
e38m31l	?	11.9	0.063	4.5

morphological markers, 106 were AFLPs, and eight were RAPDs. The individual markers that were significant determinants of stripe rust severity are shown in Table 1. These include markers used for the introgression of stripe rust resistance QTLs on chromosome 4 (4H) (*Bmy1*) and chromosome 7 (5H) (*CDO57*). The chromosome-4 markers *ABG54* and *ABG366* were not significantly associated with stripe rust severity. *WG530*, which is proximal to *CDO57*, did not have a significant association with stripe rust severity, while *mSrh*, which is distal to *CDO57*, did have a significant association with stripe rust severity. Effects for these flanking loci were negative, indicating that BSR41 contributed the resistant alleles. Nine of the AFLPs and one of the RAPDs also showed significant associations with stripe rust severity. Of these, three were positive effects, indicating that Steptoe contributed the favorable allele.

Table 2 Markers with significant effects in multi-locus regression models of stripe-rust severity in individual environments and in the average of four environments. Negative and positive slopes indicate that BSR41 contributed resistant and susceptible QTL alleles, respectively

Environment	Marker	Slope	*P*-value	R^2
Average	*CDO57*	− 17.7	0.0001	
	e36m36a	− 14.3	0.0001	
	Bmy1	− 12.4	0.0008	
	e38m31l	13.9	0.0032	
	e36m50I	10.4	0.0206	0.54
Celaya	*CDO57*	− 14.6	0.0100	
DGS 59	*e36m33d*	14.1	0.0400	
	e36m50i	16.3	0.0300	0.29
Toluca 1	*CDO57*	− 22.2	0.0001	
DGS 75	*e36m36a*	− 19.4	0.0001	
	e38m31l	15.0	0.0129	0.41
Toluca 2	*CDO57*	− 17.1	0.0005	
DGS 59	*e36m36a*	− 17.4	0.0005	
	Bmy1	− 19.8	0.0001	
	e38m31l	20.9	0.0012	
	e36m50i	14.6	0.0170	0.52
Toluca 3	*CDO57*	− 20.91	0.0001	
DGS 49	*e36m36a*	− 10.26	0.0051	
	Bmy1	− 15.90	0.0001	
	e38m31l	14.36	0.0017	
	e36m50i	8.24	0.0649	0.60

Of the markers that were significant determinants of stripe rust severity in single-locus regressions, five were significant in the multi-locus model for the average of the four 1995 environments (Table 2). These included the *CDO57* and *Bmy1* markers, used for the introgression of resistance QTLs, and three AFLP markers: *e36m36a*, *e38m31l*, and *e36m50i*. Based on the relationship with the Dicktoo × Morex map, the *e36m36a* marker is on chromosome 3 (3H). The chromosome positions of the *e36m50i*, and *e38m31l* markers can only be inferred by the multivariate analysis. The *e38m31l* marker was grouped with the *e39m61k* locus, which is equivalent to the *e39m61s* locus on the Dicktoo × Morex map (Hayes et al. 1996 a). The *e39m61s* locus is 48.8 cM distal to the *mSrh* locus on the Dicktoo × Morex map. Therefore, with reference to the current study, the *e38m31l* locus would be expected to be unlinked with *CDO57*. On the Dicktoo × Morex map, the *e39m61s* locus is 16.5 cM from the *mR* locus. In the current study, there was no consistent pattern of association between alleles at the *mR* and *e38m31l* loci, indicating a lack of linkage. The *e36m50i* locus was grouped with the chromosome-3 (3H) loci in the multivariate analysis.

In the average of the four environments, the *CDO57*, *e36m36a*, *Bmy1*, *e38m31l* and *e36m50i* loci accounted for 54% of the phenotypic, and 57% of the genotypic, variation in stripe rust severity. As shown in Table 2, *CDO57* was the only locus significant in all four environments. The *e36m33d* locus effect was unique to the

Table 3 Average stripe rust severities in 1995 and 1996 for the ten most-resistant and most-susceptible doubled-haploid lines derived from one cycle of marker-assisted selection. Allelic structure for each line is shown at marker loci significant in multi-locus regressions. Alleles from BSR41 are coded as "1", and this genotype is expected to contribute resistance alleles at QTLs linked to *e36m36a*, *Bmy1*, and *CDO57*. Alleles from Steptoe are coded as "0" and this genotype is expected to contribute resistance alleles at QTLs linked to *e36m50i* and *e38m31l*. Marker-allele genotypes contrary to these expectations are shown in italics

Line number	Mean stripe rust severity 1995	Mean stripe rust severity 1996	Marker				
			e*36m36a*	*Bmy1*	*CDO57*	*e36m50i*	*e38m31l*
Ten most-resistant lines							
SR33	13	8	1	1	1	0	0
SR35	18	18	1	*0*	1	0	0
SR43	23	22	1	*0*	1	0	0
SR47	29	22	1	1	1	0	0
SR66	14	27	1	1	1	*1*	0
SR80	18	22	*0*	1	1	0	0
SR116	18	7	1	1	1	0	0
SR123	14	7	1	1	1	0	0
SR125	18	17	1	1	1	0	0
SR127	20	13	1	*0*	1	0	0
Ten most-susceptible lines							
SR5	77	90	0	0	0	*0*	*0*
SR8	73	91	0	0	0	*0*	*0*
SR34	63	85	*1*	*1*	0	1	*0*
SR46	75	87	0	*1*	0	*0*	*0*
SR50	88	93	0	0	0	1	1
SR72	83	95	0	0	0	1	*0*
SR85	65	70	0	0	*1*	1	*0*
SR93	90	72	0	0	0	*0*	*0*
SR97	65	72	*1*	0	*1*	*0*	*0*
SR120	78	90	0	0	0	*0*	1
Parents							
Steptoe	82	92	0	0	0	0	0
BSR41	12	8	1	1	1	1	1

Celaya environment. The *e36m36a* and *e38m31l* loci were common to all the Toluca environments. The *e36m50i* and *Bmy1* loci were significant in the second and third Toluca environments.

Marker genotypes for loci significant in the average multi-locus regression model of the ten most-resistant and ten most susceptible genotypes, together with their average stripe rust severity ratings in the 1995 and 1996 field trials, illustrate the phenotype/genotype associations (Table 3). The ten most resistant genotypes traced to six different backcross plants (Fig. 1). Three of the four resistant lines selected for accelerated assessment as potential varieties (SR123, SR125, and SR127) traced to BC1 plant no. 58 (Fig. 1). The first three of these selections have favorable marker genotypes at all loci. SR127 has the susceptible marker allele at the chromosome-4 (4H) marker locus. There were five deviations from the predicted favorable-allele genotype among the most-resistant lines and 20 deviations from the predicted unfavorable genotype in the susceptible lines.

Based on 120 data points, the percentage of donor-parent genome in the population ranged from 7 to 56%, with an average of 32.6% (Fig. 3). Considering the ten most resistant genotypes, the percentage of donor genome ranged from 19 to 46%, with an average of 34.8%. The percentage of donor genome in the ten most susceptible lines ranged from 8 to 43%, with an average of 24.6%. The four resistant genotypes selected for accelerated regional assessment as potential varieties were selected before the AFLP data were available. Selection was based on phenotypic resemblance to the recurrent parent (Steptoe). The percentage of donor-parent genome in these genotypes ranged from 32 to 39%.

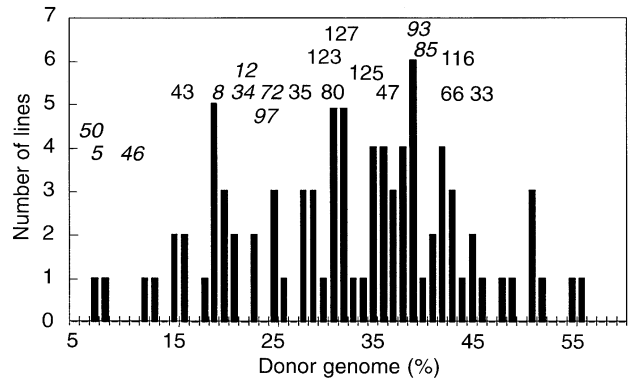

Fig. 3 Percentage of donor-parent genome in doubled-haploid lines derived from one cycle of marker-assisted backcrossing as measured by 120 markers. The *numbers* in standard and italic font are line numbers (see Table 3) of the ten most-resistant and susceptible genotypes, respectively

Discussion

The continuous distribution of disease severity phenotypes (Fig. 2) is probably due to the quantitative expression of the genetic determinants of resistance rather than to experimental error in phenotyping. The heritability of 0.95 is an approximation since environments were used as replicates. However, this high value indicates a high degree of consistency, or repeatability, in the measurement of the stripe rust severity phenotype.

The single-locus regressions underscore the importance of resistance QTLs on chromosomes 7 (5H) and 4 (4H) that were introgressed from BSR41 into the Steptoe background. The significance of *mSrh*, and lack of significance of *WG530*, indicates that the resistance QTL on chromosome 7 (5H) is most likely distal to *CDO57*. Multiple QTL peaks were observed in the original mapping population (Chen et al. 1994). On chromosome 4 (4H), the higher R^2 value for *Bmy1* indicates that the resistance locus is closer to this marker than to the proximal markers *ABG366* and *ABG54*. The significance and sign of the chromosome-4 (4H) and -7 (5H) markers confirms that in these regions of the genome there are loci determining adult-plant reaction to stripe rust.

We recognize that experiment-wise error rates complicate single-locus regression procedures with large marker data sets, and that a selected population is not as appropriate for QTL detection as a defined mapping population. However, the presence of loci significant in both the single-locus and multi-locus models indicates that other regions of the genome were potentially important in determining the reaction to stripe rust in a Steptoe background. There is an important resistance QTL, tentatively mapped to chromosome 3, where BSR41 contributes the favorable allele. Steptoe contributed resistance QTL alleles at markers that could not be assigned genome positions. We hypothesize that favorable (resistant) alleles were fixed at these additional resistance loci in the original mapping population (Calicuchima-sib × Bowman). This underscores the lack of predictability that may be encountered when QTLs detected in a mapping population are transferred to other genetic backgrounds.

Determining the joint effects of multiple resistance loci could be biased if linked marker loci are included in a multi-locus model. In our data, some linkage relationships are documented, as in the case of the marker loci used to introgress resistance QTLs on chromosomes 4 (4H) and 7 (5H). In other cases, we used multivariate analysis to establish tentative linkage groups. In this way, five AFLP loci and one RAPD locus (Table 1) group together and were tentatively assigned to chromosome 3 (3H). One of these loci (*e36m36a*) was significant in the multi-locus model of average stripe rust severity. BSR41 contributed the resistant allele at this locus. In the case of the *e38m31l* and *e36m50i* loci, we could not assign map positions to those AFLPs which showed associations with stripe rust resistance. These associations are particularly intriguing because the susceptible parent (Steptoe) contributed resistance alleles. For the purposes of simultaneous locus discovery and the advance of breeding material through backcrossing, as proposed by Tanksley and Nelson (1996), the ideal marker would have a defined map position. AFLPs are an excellent type of marker for rapidly generating large amounts of polymorphism data (Becker et al. 1995; Waugh et al. 1997). AFLP products of the same size assayed in different genotypes may represent the same locus. However, this needs to be demonstrated on a case-by-case basis.

Five markers accounted for 54% of the variation in the phenotypic expression of stripe-rust severity, averaged over the four environments. Using the heritability estimate of 95%, these markers accounted for 57% of the genetic variation in trait expression. The fact that the marker loci do not account for a higher proportion of phenotypic and genotypic variance is probably attributable to recombination between marker loci and the target QTLs, and to the effects of additional loci that contribute to the expression of resistance. The individual effects of these additional loci cannot be detected at the level of resolution afforded by our experiment. This raises the question of how much variance can be accounted for by QTLs, because the magnitude of the phenotypic, or genotypic, R^2 may be used to determine the likelihood that all loci that are important determinants of trait expression have been detected.

By way of perspective, the *mV* locus determines the fertility of lateral florets in barley. Two-row barley genotypes, with sterile lateral florets, typically have higher kernel weights than six-row barley genotypes. When kernel weight was mapped as a QTL in the doubled-haploid progeny of Calicuchima-sib × Bowman, a two-row × six-row cross, the *mV* locus accounted for 77% of the variation in phenotypic-trait expression (Hayes et al. 1996c). Therefore, having detected 54% and 57% of the phenotypic and genotypic variance, respectively, in the expression of adult plant stripe rust resistance, we believe that we have located the principal genes determining stripe rust resistance in this germplasm. Two are the QTLs that were introgressed, and these are located on chromosomes 4 (4H) and 7 (5H). One is most likely on chromosome 3 (3H), and the map positions of the remaining loci cannot be determined at this time.

The multi-locus analyses of the individual environment data underscore the importance of using multiple measures of phenotype and, potentially, the importance of measuring disease reaction at different growth stages. The heritability estimate of stripe-rust severity was 0.95. This indicates a consistency of response

across the four environments. Therefore, the average of the four environments is one appropriate measure of the stripe rust reaction phenotype. However, individual environment data may also be useful. For example, the Celaya environment was useful in revealing the significance of the resistance QTL linked to *e36m33d*. This locus was not significant in any of the Toluca environments, or in the multi-locus model based on average severity. Markers accounted for the lowest percentage of phenotypic variance at Celaya, and we attribute this to the use of hill plots. Iyamabo and Hayes (1995), in a comparison of hill and row plots for QTL detection, reported that hill plots were best suited to the detection of characters determined by large-effect QTLs.

In terms of growth stage, the chromosome-4 (4H) marker *Bmy1* was significant only at growth stages DGS 49 and DGS 59. If assessments were based only at DGS 75, the effect of the chromosome-4 (4H) resistance QTL would not have been detected. The chromosome-4 (4H) effect may be due to maturity or it may reflect a resistance locus important at earlier stages of plant growth. The *Sh* locus is 2.6 cM from *Bmy1* (Laurie et al. 1995) and a heading date QTL was detected at the same position in the Calicuchima-sib × Bowman population (Hayes et al. 1996 c). On the other hand, in a controlled-environment seedling test of the Calicuchima-sib × Bowman mapping population, a stripe rust resistance QTL was detected proximal to *Bmy1* (Hayes et al. 1996 c).

The patterns of association of genotype and phenotype in the ten most resistant genotypes are additional evidence for the importance of resistance QTLs linked to markers on chromosomes 3 (3H), 4 (4H) and 7 (5H), and to the two unmapped loci (Table 3). The ten most-resistant selections traced to different BC1 plants, but three of the selected genotypes traced to a single backcross plant (Fig. 1). The first three of these selections have the favorable marker genotypes at all loci (Table 3). The fourth selection may represent a crossover event between the resistance locus and the *Bmy1* marker locus on chromosome 4 (4H) , or it may lack the chromosome-4 (4H) QTL. This implies that in marker-assisted backcross projects it may be advisable to advance material derived from multiple backcross plants. This would be even more important when multiple cycles of marker-assisted backcrossing are used without the benefit of phenotypic assessment for the target phenotype.

The higher degree of correspondence between marker genotype and phenotype in the resistant vs the susceptible lines suggests epistasis, as one would expect similar frequencies of crossovers between marker and resistance loci in the two groups. That is, the resistant phenotype resulted only when appropriate resistance alleles were present at multiple loci, while the susceptible phenotype resulted from the presence of only one or a few susceptibility alleles. SR97, for example, was susceptible to stripe rust but had resistant marker alleles at four out of five loci. This suggests that if a complex phenotype, such as adult-plant resistance, is the consequence of a complex multi-locus pathway, it would be relatively easy to disrupt the pathway with susceptible alleles at various points in the pathway. The resistant phenotype would result only with resistant alleles at all, or most, points in the pathway.

AFLP markers were useful for identifying additional resistance loci and they provided information on the genetic structure of the BC1-derived DH population. The population average of 32.6% donor-parent genome is, as would be expected, higher than 25%, as the BC1 plants were selected (Fig. 3). As shown in this figure the percentages of the recurrent parent genome ranged from 7 to 56%. The resistant genotypes had a higher average percentage of donor-parent genome (34.8%) than the susceptible genotypes (24.6%), but the difference was not that great, considering that portions of the genome on at least three chromosomes were introgressed into the resistant lines. Four DH lines were selected, based on their phenotypic resemblance to the recurrent parent, for accelerated assessment as potential varieties before the AFLP data were available. The percentage of donor-parent genome in these lines ranged from 32 to 39%. If resources are available, larger populations of BC1-derived lines could be used. In this case, the percentage of recurrent parent genome would be a more useful selection criterion.

In summary, marker-assisted mapping and the transfer of stripe-rust resistance QTLs allowed us to rapidly develop barley germplasm with a potential for commercial production. Markers that were targets for transfer were significantly associated with resistance in a genetic background different from the reference mapping population. The use of high-throughput markers – primarily AFLPs – allowed us to detect additional resistance QTLs, including QTLs where the susceptible parent contributed resistant alleles. Our findings may be useful in view of the many ongoing efforts in a number of crop species to introgress QTLs.

When QTLs mapped in a reference population are introgressed into new genetic backgrounds, the anticipated selection responses may not be achieved. Precautions can be taken during the introgression process to minimize the loss of QTLs through double crossovers between flanking markers and to guard against the consequences of imprecise positioning of the QTLs in the original mapping population. However, the configuration of alleles in the breeding population at loci where alleles were fixed in the mapping population may not be known. We hypothesize that this was the case with the new resistance QTLs resistance alleles we detected in the Steptoe background.

An intriguing, but unanswered, question is the relationship between disease resistance QTLs and race-specific resistance genes. At this point we know nothing

regarding the kinds of genes that are detected as adult-plant stripe-rust resistance QTLs. Syntenic relationships in the *Triticeae* should allow for useful comparative mapping and an extension of findings from one genus to another. For example, the stripe rust resistance QTL on chromosome 7 (5H) may be homoeologous with one of the durable stripe rust resistance loci in wheat described by Law and Worland (1997).

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