Theor Appl Genet (2000) 101:580–589

T. Toojinda · L.H. Broers · X.M. Chen · P.M. Hayes
A. Kleinhofs · J. Korte · D. Kudrna · H. Leung
R.F Line · W. Powell · L. Ramsay · H. Vivar · R. Waugh

Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare)*

Received: 2 February 1999 / Accepted: 30 December 1999

Abstract Stripe rust, leaf rust, and Barley Yellow Dwarf Virus (BYDV) are important diseases of barley (*Hordeum vulgare* L). Using 94 doubled-haploid lines (DH) from the cross of Shyri x Galena, multiple disease phenotype datasets, and a 99-marker linkage map, we determined the number, genome location, and effects of genes conferring resistance to these diseases. We also mapped Resistance Gene Analog Polymorphism (RGAP) loci, based on degenerate motifs of cloned disease resistance genes, in the same population. Leaf rust resistance was determined by a single gene on chromosome 1 (7H). QTLs on chromosomes 2 (2H), 3 (3H), 5 (1H), and 6 (6H) were the principal determinants of resistance to stripe rust. Two-locus QTL interactions were significant determinants of resistance to this disease. Resistance to the MAV and PAV serotypes of BYDV was determined by coincident QTLs on chromosomes 1 (7H), 4 (4H), and 5 (1H). QTL interactions were not significant for BYDV resistance. The associations of molecular markers with qualitative and quantitative disease resistance loci will be a useful information for marker-assisted selection.

Key words Barley · Genome mapping · Stripe rust · Leaf rust · BYDV · Resistance Gene Analog Polymorphism · QTL

Communicated by M.A. Saghai Maroof

T. Toojinda
DNA Fingerprinting Unit,
National Center for Genetic Engineering and Biotechnology,
Kasetsart University, Kampangsaen Campus,
Nakorn Pathom, Thailand

L.H. Broers
Nunhems Zaden BV, P.O. Box 4005,
6080 AA Haelen, the Netherlands

X.M. Chen · R.F. Line
US Department of Agriculture, Agricultural Research Service,
Washington State University, Pullman, WA 99164–6430, USA

P.M. Hayes (✉)
Department of Crop and Soil Sciences,
Oregon State University, Corvallis, OR 97331, USA
e-mail: patrick.m.hayes@orst.edu, Fax:+1-541-737-1589

A. Kleinhofs · D. Kudrna
Department of Crop and Soil Sciences,
Washington State University, Pullman, WA 99164–6420, USA

J. Korte
AgriBioTech, 530 Liberty lane, West Kingston, RI 02892, USA

H. Leung
IRRI, P.O. Box933, Manila, 1099, Philippines

L. Ramsay · W. Powell · R. Waugh
Scottish Crop Research Institute Invergowrie,
Dundee DD2 5DA, Scotland

H. Vivar
Barley program, ICARDA/CIMMYT,
Apdo. 370, Mexico 6, D.F. Mexico

Introduction

Genetic resistance is the most cost-effective and environmentally appropriate approach to disease management in crop plants. While genetic variation for disease resistance to many diseases is still available within the cultivated germplasm pool of many crop species, in many cases restricted genetic variance has led to searches for new resistance genes in crop ancestors and relatives (Conner et al. 1989; Ordon and Friedt 1993). The introgression of exotic genes is an expensive and difficult process (Hoffbeck et al. 1995; Tanksley and Nelson 1996). Resistance genes are, accordingly, precious commodities. In this context, the durability of resistance is of great importance. While durability can only be demonstrated in hindsight, theory and some historical evidence support the contention that quantitative resistance is often more durable than qualitative resistance (Browning et al. 1977; Line 1993).

Qualitative disease resistance genes have been extensively studied in terms of genome location (Giese et al. 1993; Graner and Tekauz 1996) and specificity (Thomas et al. 1995). In other plant models, resistance genes have been characterized in terms of structure and function (Lamb 1994; Martin 1996). Until the recent development

of quantitative trait locus (QTL) analysis tools, the study of quantitative resistance genes focused on biometrics and epidemiology. QTL tools allow for the systematic dissection of quantitative resistance into estimates of locus number, location, effect, and interaction (Michelmore 1995; Young 1996). Disease resistance QTLs have been described for a number of host pathogen systems (Williamson et al. 1994; El-Kharbotly et al. 1994; Maisonneuve et al. 1994), including barley (Graner and Bauer 1993; Hayes et al. 1996). However, the structure and function of quantitative resistance genes is still a matter of conjecture. They could represent the effects of alternative alleles at qualitative resistance loci (Dingerdissen et al. 1996) or they could represent an entirely different class of genes (Pryor and Ellis 1993).

Genes conferring resistance to different specificities of the same pathogen, and to different pathogens, are known to cluster in a range of plants (Michelmore 1995; Ellis et al. 1998). These clusters are particularly dynamic regions of the genome, although the mechanisms leading to variation are still a matter of debate (Pryor 1987; Pryor and Ellis 1993; Leister et al. 1998). The discovery of common motifs in cloned resistance genes – leucine-rich repeats (LRR), nucleotide-binding sites (NBS), and serine/threonine kinase domains – has served as a basis for a generalized approach to resistance genes analysis. Degenerate primers based on these motifs can be used to amplify specific genomic DNA sequences known as resistance gene analogs (RGAs). The RGA approach has been used to map plant disease resistance genes (Kanazin et al. 1996; Yu et al. 1996; Leister et al. 1996). Degenerate primers can also be used to generate Resistance Gene Analog Polymorphisms (RGAPs) for linkage map construction and QTL analysis (Chen et al. 1998).

Qualitative and quantitative resistance to rust fungi (*Puccinia* sp.) has been an area of extensive study in the Triticeae. In barley (*Hordeum vulgare* L.), there is an especially rich literature on resistance to leaf rust (*Puccinia hordei*) (Feuerstein et al. 1990; Jin et al. 1996; Qi 1998). The impetus for this research was the fact that race-specific qualitative resistance genes lacked durability to this pathogen of worldwide importance. There is less information on the basis of genetic resistance to stripe rust (*Puccinia striiformis*, fsp. *hordei*). Due to the relatively recent arrival and importance of this pathogen in the Americas, we have been systematically mapping resistance in a range of germplasm (Chen et al. 1994; Hayes et al. 1996). Barley Yellow Dwarf Virus (BYDV) is an aphid-vectored luteovirus of worldwide importance (D'Arcy 1995; Collins et al. 1996). These three diseases tend to be episodic in response to environmental conditions, but in any given environment at least one disease is usually a principal production constraint. In other situations, the use of cultivars resistant to multiple diseases is a necessity. The development of varieties with resistance to multiple diseases can be expedited by information on the number, location, and effect of the determinant genes.

Our objective was to map genes contributing to resistance to leaf rust (caused by the fungus *Puccinia hordei*), stripe rust (caused by the fungus *Puccinia striiformis* fsp. *hordei*), and two serotypes (MAV and PAV) of Barley Yellow Dwarf Virus (BYDV) in barley. We used restriction fragment length polymorphisms (RFLPs) and simple-sequence repeats (SSRs) as framework markers, and amplified fragment length polymorphisms (AFLPs) and RGAPs as high-throughput filler markers, to construct the linkage maps that would allow us to localize the determinants of resistance to these diseases in the barley genome.

Materials and methods

Plant material

One hundred doubled haploid (DH) lines were derived from the F_1 of the cross Shyri/Galena, using the *Hordeum bulbosum* technique as described by Chen and Hayes (1989). Shyri is a two-rowed feed barley developed by ICARDA/CIMMYT (Mexico) and released by INIAP (Ecuador). Shyri is a source of resistance to a range of diseases, including stripe rust, leaf rust, scald, and net blotch. Galena is a proprietary two-rowed malting barley belonging to the Coors Brewing Company, Inc.

Genotyping and map construction

Ninety-four of the DH lines were genotyped with a total of 810 markers. These included 41 RFLPs, 51 SSRs, 562 AFLPs, 155 RGAPs, and one morphological marker. The RFLP marker nomenclature follows that employed by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993; Hayes et al. 1996). RFLPs were assayed as described by Kleinhofs et al. (1993). Fifty-one SSR markers (designated as *BMAC*, *EBMAC*, *HVM*, *HVC*, *HVP*, *HTT* plus an arbitrary number) were assayed as described by Liu et al. (1996) and Russell et al. (1997). The majority of the SSRs were developed by the Scottish Crop Research Institute (SCRI) (http://www.scri.sari.ac.uk/SSR/). The AFLP assays were performed using 16 *Pst*I/*Mse*I and 16 *Eco*RI/*Mse*I primer combinations as described by Zabeau and Vos (1993) and were designated as *E_M_* for *Eco*RI/*Mse*I, *T_M_* for *Pst*I/*Mse*I. The RGAP markers were generated using degenerate primers derived from resistance gene homologs as described by Chen et al. (1998) and, following the nomenclature in that report, are designated as NLFR, LM637, PK1, PK2, RLRfr, NLRIN, and S2AS3IN. Polymerase chain reaction (PCR) amplification was performed in a Perkin Elmer 9600 thermal cycler. The reaction mixture and the polyacrylamide gel electrophoresis procedures were as described by Chen et al. (1998). The morphological marker, rachilla hair length (*mSrh*), was scored under a dissecting microscope.

The base map (Fig. 1) was constructed using a subset of the 810 markers to achieve a target interval distance of 10–15 cM. Markers in common with published maps were retained, when possible, in order to facilitate map integration. Markers showing significant segregation distortion, markers with missing observations and markers causing map expansion were discarded. Linkage analysis was performed on the remaining subset of 138 markers using GMENDEL 3.0 (Holloway and Knapp 1994). Linkage groups were first calculated using a maximum recombination percentage of 25% and a LOD score of 7. Cosegregating and tightly linked markers were then dropped, and markers with the most complete data were retained. Linkage groups were then recalculated using LOD 3.8 and a recombination percentage of 35%. Marker orders were checked by Monte Carlo and Bootstrap simulations, using annealing temperatures of 300 "inner" and 200 "outer". The assignment of linkage groups to chromosomes was based on mark-

ers in common with previously published maps (Kleinhofs et al. 1993; Qi et al. 1996). The final map, consisting of 99 markers (Fig. 1) was used together with the phenotype datasets for QTL analysis.

Disease resistance phenotyping

Stripe rust

The 94 DH lines and parents were assessed for adult plant resistance in four tests at Toluca, Mexico and one test at Celaya, Mexico. At Celaya, the DH lines and parents were grown in unireplicate hill plots in the winter of 1995. A field epidemic was initiated by inoculating spreader rows (formed from a mixture of 15 extremely susceptible genotypes) 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 at DGS59 (Feekes stage 10.5) as percentage severity on a plot basis. Reaction type was scored using a 0–9 scale (0 = immune, 9 = susceptible). At Toluca, the DH lines and the parents were planted in one-row, 3-m plots in 1994, 1995 and at two planting dates in 1996. 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. Stripe rust was rated as percentage severity on a plot basis.

Leaf rust

The 94 DH lines and parents were assessed for adult plant leaf rust resistance in field tests at Ciudad Obregon, Mexico in 1994, 1995 and 1996 using two-row, 3-m plots. Epidemics were initiated by inoculating spreader rows, a mixture of several very susceptible lines, with fresh spores of a mixture of leaf rust races 8, 19, and 30 collected from a susceptible variety. The inoculum was applied by two methods: (1) a water and surfactant suspension was injected by syringe into the stems of boot stage plants in spreader rows and (2) a talc carrier was blown into the nursery with a backpack duster multiple times during the growing cycle. Disease was rated by infection type – resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) – and percentage severity on a plot basis. For mapping purposes, a rust index was calculated by equating the infection type rating with a numerical score (R =1, MR = 2, MS = 3, and S = 4) and multiplying this value by the percentage severity.

BYDV

The population and parents were assessed for resistance to the BYDV-MAV-"Mex" and BYDV-PAV-"Mex" serotypes. In all tests, one replication of two-row, 1-m plots was used. The infected treatment plots were infested twice with five to ten aphids harboring the MAV and PAV serotypes, respectively, at 3-weeks intervals. Aphids were removed from plants by applying an insecticide 1 week after the second inoculation. Control plots were chemically protected against aphid attack during the entire growing cycle. MAV resistance was described with a dwarfness score (0–9) and a tillering score (0–9) in four tests at Toluca, Mexico in 1995 and 1996 (two planting dates per year). In 1996, plant height reduction (control – infected) was also measured. PAV resistance was measured in one test at Toluca in 1996. The same dwarfing and tillering scores used for MAV were used to measure PAV resistance.

QTL analysis

QTLs were mapped using the interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of MQTL (Tinker and Mather 1995) and regression procedures. For MQTL, each dataset was analyzed with 1,000 permutations, a 5-cM walk speed, and a Type-I error rate of 5%. For sCIM, 18 background markers with approximately even spacing were specified, with a maximum of 3 background markers per linkage group. Approximate estimates of heritability were computed by substituting environments for replications as: $H^2 = \sigma^2g/(\sigma^2g + \sigma^2e/r)$ where σ^2g is the variance among DH lines, σ^2e is the error variance among DH lines, and r is the number of environments.

Results and discussion

Linkage map construction

The base map of 1316.5 cM is shown in Fig. 1. Single linkage groups were assigned to each chromosome, except for chromosomes 3 (3H) and 7 (5H). There are unmapped regions on the long arm of chromosome 3 (3H); the long and short arms of 7 (5H); and the long arms of chromosomes 5 (1H) and 6 (6H). Locus ordering and distance are in agreement with published maps (Kleinhofs et al. 1993; Qi et al. 1996). Based on comparisons of markers in common with the Steptoe/Morex map (Kleinhofs et al. 1993) and the merged barley map (Qi et al. 1996), the unmapped region on the short arm of chromosome 7 (5H) is approximately 40 cM long, and the unmapped region on chromosome 3 (3H) is approximately 39 cM long. Telomeric RFLP loci on chromosomes 5 and 6 (*ABG387* and *MWG798*, respectively) were not associated with the corresponding linkage groups. Two-locus recombination distances between these telomeric markers and nearest markers (*HvHVA1* and *PK2/4–1*) were 36 cM and 35 cM, respectively. RFLPs and SSRs served as anchor markers, and the higher throughput AFLPs and RGAPs were used to fill gaps. However, only 16 AFLPs and 12 RGAPs are included in the final base map, due primarily to segregation distortion and clustering, phenomena reported in the literature (Becker et al. 1995). Markers used for basemap construction are shown on the left-hand side of each linkage group (Fig. 1).

QTL detection

The phenotypic distribution of stripe rust severity, averaged over the five environments, did not show discrete classes allowing for Mendelian analysis (Fig. 2a). Similar distributions were observed for each of the individual environments (data not shown). Only 5 of the DH lines were as resistant as Shyri (1.5 $\pm$ 0.9%), while there were 33 susceptible transgressive segregants with severities higher than Galena (62.5±7.4). These data suggest that unique configurations of multiple alleles may be required for high levels of resistance and that the susceptible parent has some resistance alleles. The consistency of the disease severity ratings (h^2 = 94%) confirms that resistance was stable in the face of the stripe rust virulence present during the years these tests were conducted. While we do not have extensive data on pathogen

Fig. 1 Linkage maps based on 94 DH progeny from the cross of Shyri × Galena. Marker loci on the *left side* of each linkage group were used for linkage map construction. Distances are in Kosambi centiMorgan (cM) units. QTLs for stripe rust (*SR*), the leaf rust resistance gene (*LR*), and Barley Yellow Dwarf Virus (BYDV) are shown in *bold italics*. *Shaded regions* are gaps

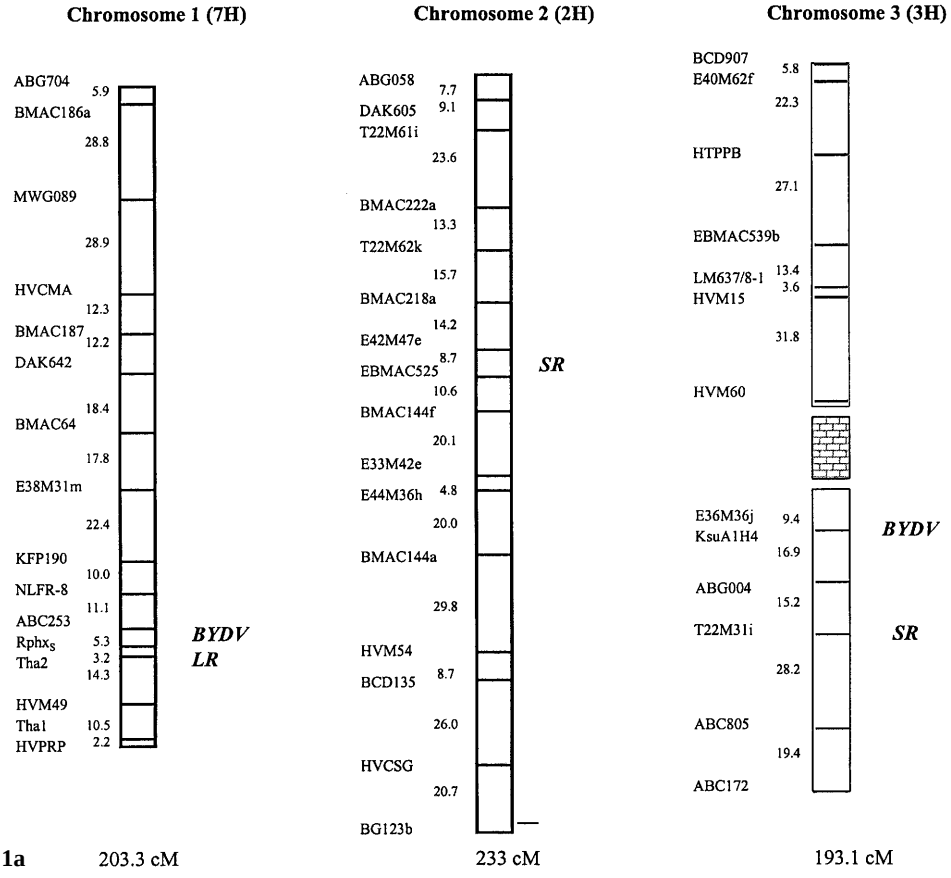


Fig. 1a

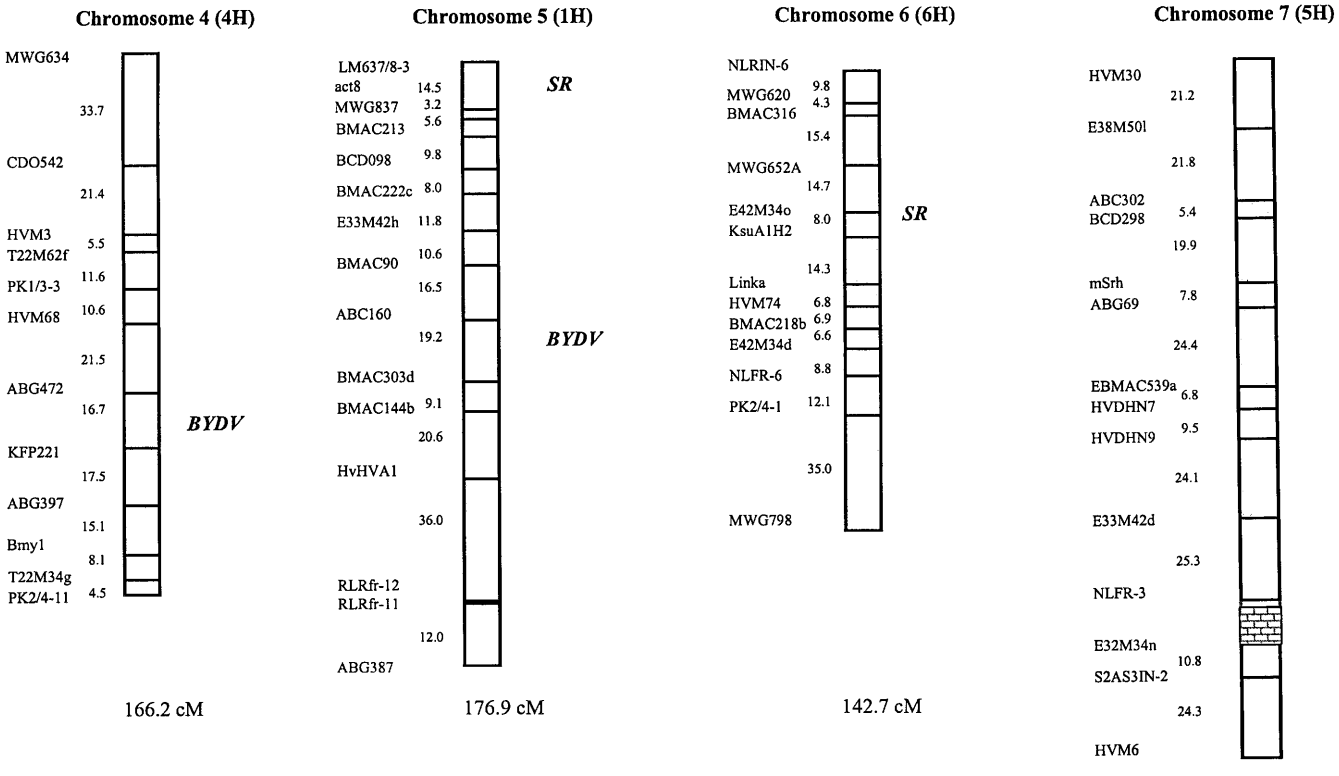


Fig. 1b

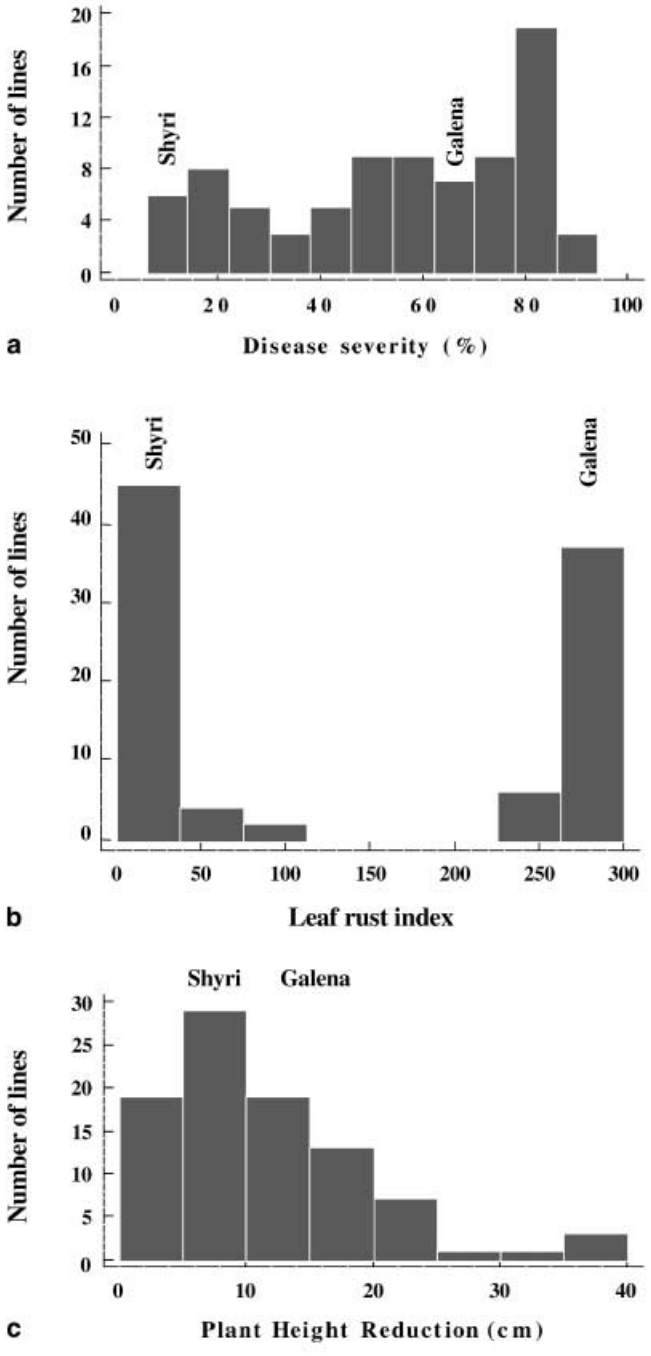
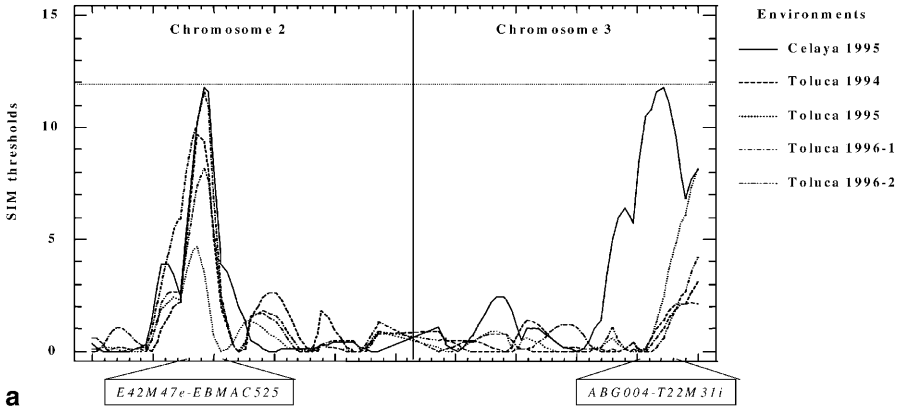


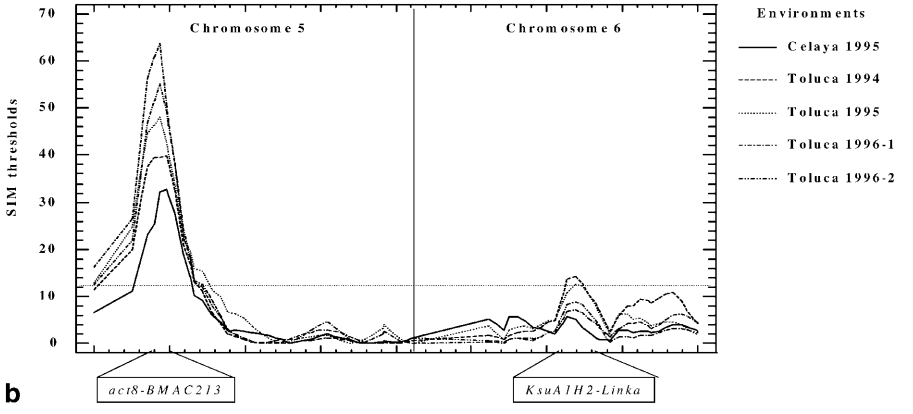
Fig. 2 **a** Average stripe-rust disease severity (%) in the Shyri × Galena population. **b** Average leaf-rust index in the DH progeny of Shyri × Galena. **c** Plant height reduction (cm) after infection with the BYDV-MAV serotype in the Shyri × Galena population

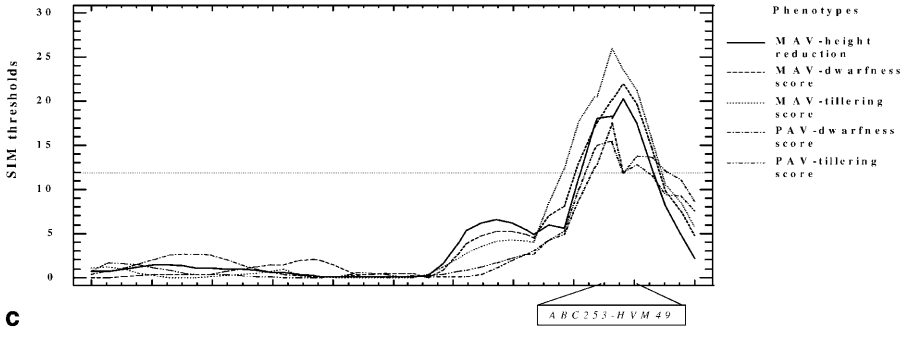
virulence in these tests, studies in the USA have shown considerable variation in stripe rust populations (Chen et al. 1995). We have observed comparable levels of resistance in Shyri in over 10 years of testing at multiple locations throughout the Americas (data not shown). This may be preliminary evidence for durable resistance genes in Shyri.

The QTL data confirm the multi-locus control of stripe rust resistance in Shyri and provide some evidence for a resistance allele in Galena. Shyri contributed resistance alleles at QTLs on chromosomes 3 (3H), 5 (1H), and 6 (6H), while Galena contributed the resistance allele at a QTL on chromosome 2 (2H) that approached, but did not reach, the significance threshold (Fig. 3a, b). The largest effect QTL is on the short arm of chromosome 5 (1H) (*act8-BMAC213*), and it was significant in all five tests. This QTL accounted for 28%–50% of the variation in phenotypic expression (percentage of variance explained = PVE) in the individual environments and 47% PVE on average over the five tests (Table 1). We mapped this QTL to approximately the same position as the *Yr4* locus (von Wettstein-Knowles 1992) and a stripe rust resistance QTL reported by Thomas et al. (1995). The relationship of the Shyri chromosome 5 (1H) QTL to these other loci remains to be determined, although the *Yr4* locus is reported to confer resistance to race 23, while the virulence in the Americas is broadly defined as race 24 (Marshall and Sutton 1995; Chen et al. 1995). The QTLs on chromosomes 3 (3H) and 6 (6H) mapped to the *ABG004-T22M31i* and *KsuA1H2-Linka* intervals, respectively. The QTL main effect on chromosome 3 (3H) was significant only in the Celaya dataset, while the main effect on chromosome 6 (6H) was significant only in the Toluca 1994 and Toluca 1995 datasets. The individual effects of these QTLs were much smaller than the chromosome 5 (1H) QTL. The maximum PVEs for the chromosome 3 (3H) and 6 (6H) QTLs were 12% and 14%, respectively. The relationships of these QTLs to the chromosome 3 (3H) QTLs reported by Toojinda et al. (1998) and the *Yr4* locus on chromosome 6B of wheat (*Triticum aestivum*) reported by Chen et al. (1995) will need to be resolved by additional comparative mapping. Galena contributed the resistance allele at a QTL that approached the significance threshold on chromosome 2 (2H) (*E42M47e-EBMAC525)*. This trend was observed in only 2 of the datasets (Celaya 1995 and Toluca 1996–1). The significant main-effect QTLs, when considered in multi-locus models, accounted for 39–52% and 41–55% of the variation in phenotypic and genotypic expression, respectively. These QTLs should be of considerable value and utility, as they are different from those mapped on chromosomes 4 (4H) and 7 (5H) (Chen et al. 1994; Hayes et al. 1996).

The genetic variance that is not accounted for by the QTLs we have mapped in this population could be due to QTLs in unmapped regions of the genome, QTLs with small effects, and/or to QTL interactions (epistasis). As pointed out by Melchinger et al. (1998), our estimates of QTL effect may also be biased by the use of the same population for both mapping and estimating QTL effects. These authors also point out that larger populations are required for estimating higher order QTL × QTL interactions. Recognizing the limitations of our population of 94 DH lines, we did, however, proceed to test for interactions between significant or nearly significant, main-effects QTLs. Significant interactions between the chro-







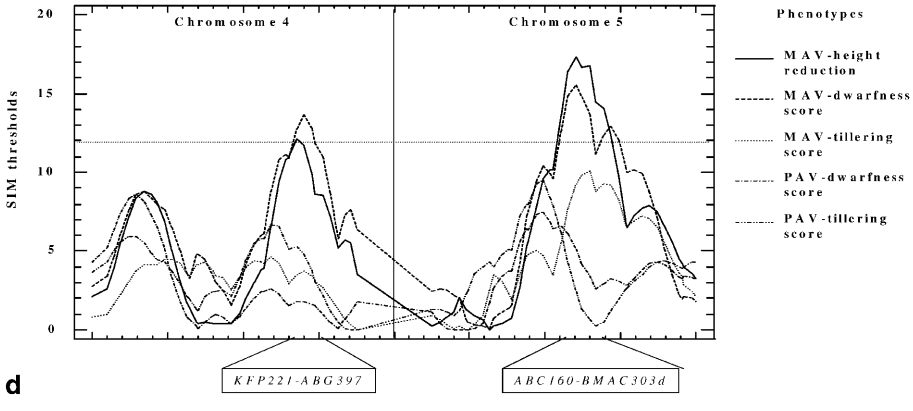


Fig. 3a, b SIM test statistics from the QTL analysis of stripe-rust severity (%) on chromosomes 2 (2H) and 3 (3H) in the Shyri × Galena population. QTL peak intervals are shown on the x-axis. The horizontal bar indicates the maximum significance threshold ($P = 0.05$). **b** SIM test statistics from the QTL analysis of stripe-rust severity (%) on chromosomes 5 (1H) and 6 (6H) in the Shyri × Galena population. QTL peak intervals are shown on the x-axis. The horizontal bar indicates the maximum significance threshold ($P = 0.05$). **c.** SIM test statistics from the QTL analysis of plant height reduction (cm), dwarfness score, and tillering score, attributed to BYDV-MAV and BYDV-PAV infection, on chromosome 1 (7H) in the Shyri × Galena population. QTL peak intervals are shown on the x-axis. The horizontal bar indicates the maximum significance threshold ($P = 0.05$). **d.** SIM test statistics from the QTL analysis of plant height reduction (cm), dwarfness score, and tillering score, attributed to BYDV-MAV and BYDV-PAV infection, on chromosomes 4 (4H) and 5 (1H) in the Shyri × Galena population. QTL peak intervals are shown on the x-axis. The horizontal bar indicates the maximum significance threshold ($P = 0.05$)

Table 1 Chromosome location, allele phase, and effect (expressed as the R^2_p – percentage of phenotypic variance explained) for stripe rust, leaf rust, and BYDV resistance loci in the Shyri × Galena population

Resistance phenotype	Environments or measurments	Type of inheritance	Chromosome	Marker interval	Resistance allele[a]	R^2_p (%)
Stripe rust	Celaya 1995	QTL	2 (2H)	*E42M47e- EBMAC525*	G	12
	Toluca 1996–1					9
Stripe rust	Celaya 1995	QTL	3 (3H)	*ABG004-T22M31i*	S	12
Stripe rust	All tests	QTL	5 (1H)	*act8-BMAC213*	S	28–50
Stripe rust	Toluca 1994	QTL	6 (6H)	*KsuA1H2-Linka*	S	23
	Toluca 1995					15
Leaf rust	All tests	Qualitative	1 (7H)	$Rphx_S$	S	91–96
MAV	Height reduction	QTL	1 (7H')	*ABC253-HVM49*	G	18
MAV, PAV	Dwarfness score	"	"	"	"	20, 11
MAV, PAV	Tillering score	"	"	"	"	22, 11
MAV	Height reduction	QTL	4 $(4H)_a$[b]	*KFP221-ABG397*	G	10
MAV	Height reduction	QTL	4 $(4H)_b$	*MWG634-CDO542*	G	7
MAV, PAV	Dwarfness score	"	"	"	"	7, 6
MAV	Height reduction	QTL	5 (1H)	*ABC160-BMAC303d*	S	15
MAV, PAV	Dwarfness score	"	"	"	"	13, 3
MAV, PAV	Tillering score	"	"	"	"	10, 7

[a] G refers to resistance allele from Galena; S refers to resistance allele from Shyri
[b] 4a and 4b denote the two distinct QTLs on the short and long arms of chromosome 4 (4H), respectively

mosome 2 (2H) and chromosome 5 (1H) QTLs, and between the chromosome 2 (2H) and chromosome 6 (6H) QTLs, were detected in all five environments. The PVEs for the $QTL_{ch2(2H)} \times QTL_{ch5\ (1H)}$ interaction, after accounting for QTL main effects, ranged from 3% to 12% in the analysis of individual environment data. In this interaction, the source of the allele on chromosome 2 (2H) was not important, but Shyri always contributed the resistance allele on chromosome 5 (1H) (Fig. 4a). The PVE for the $QTL_{ch2(2H)} \times QTL_{ch6\ (6H)}$ interaction, after accounting for QTL main effects, was 4%. In the case of this interaction, Galena contributed the resistance allele on chromosome 2 (2H) and Shyri contributed the resistance allele on chromosome 6 (6H) (Fig. 4b). The significant main-effect QTLs and their two locus interactions, when considered in multi-locus models, accounted for 44–59% and 47–63% of the variation in phenotypic and genotypic expression, respectively (Table 2).

In contrast to stripe rust resistance, leaf rust resistance in this population is clearly qualitatively inherited. The phenotypic distribution for the leaf rust severity index, averaged over the three environments, is discrete (Fig. 2b). This 1:1 ratio (chi square = 0.68 with a *P*-value <0.01) is clear evidence for monogenic inheritance. The two parents lie at opposite ends of the frequency distribution. The heritability of the rust severity index was 97%. When the quantitative severity index data were mapped using the procedures of MQTL, a single QTL mapped to the long arm of chromosome 1 (7H) in the *ABC253-Tha2* interval. Shyri contributed the resistance allele. The single locus accounted for 84% of the PVE. When the data were treated as bivariate scores (1,0), the single locus ($Rphx_S$), mapped 3.2 cM proximal to *Tha2*. $Rphx_S$ may be allelic with the *Rph3* locus reported by Jin et al. (1993) and the $Rphx_C$ locus reported by Hayes et al. (1996). *Rph* genes have been used extensively in barley breeding programs,

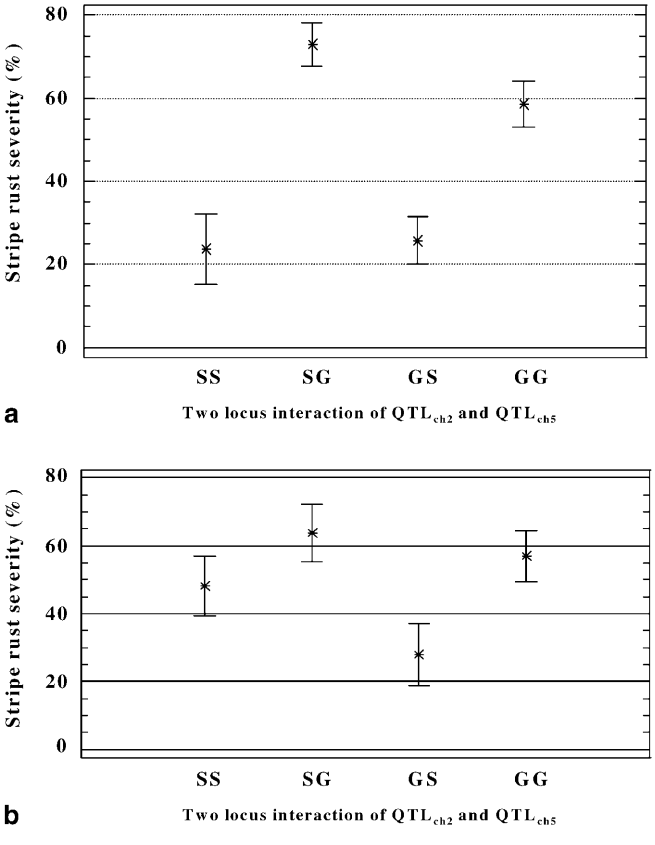


Fig. 4a, b Mean and 95% LSD intervals for the two-locus interactions between the QTLs on chromosomes 2 (2H) and 5 (5H) determining stripe-rust severity (%). SS, SG, GS, and GG refer to the allelic composition of DH lines at QTLs on chromosomes 2 (2H) and 5 (5H), respectively. **b.** Mean and 95% LSD intervals for the two-locus interactions between the QTLs on chromosomes 2 (2H) and 6 (6H) for stripe-rust severity (%). SS, SG, GS, and GG refer to the allelic composition of DH lines at QTLs on chromosomes 2 (2H) and 6 (6H), respectively

Table 2 Percentage of phenotypic variance explained (R^2_p) in multi-locus models involving main-effect QTLs and their interactions (if significant) for stripe rust and BYDV resistance in the Shyri × Galena population.

Disease resistance phenotype	Environment	Main-effect QTLs	Two-locus interaction (P<0.01)	Multi-locus R^2 (%)
Stripe rust	Toluca 1994	$QTL_{ch5\ (5H)}$[a]	$QTL_{ch2\ (2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6\ (6H)}$	55
Stripe rust	Celaya 1995	$QTL_{ch3\ (3H)}$, $QTL_{ch5\ (5H)}$	$QTL_{ch2(2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6(6H)}$	44
Stripe rust	Toluca 1995	$QTL_{ch5\ (5H)}$	$QTL_{ch2(2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6(6H)}$	52
Stripe rust	Toluca 1996–1	$QTL_{ch5\ (5H)}$	$QTL_{ch2(2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6(6H)}$	59
Stripe rust	Toluca 1996–2	$QTL_{ch5\ (5H)}$	$QTL_{ch2(2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6(6H)}$	53
Stripe rust	Average	$QTL_{ch5\ (5H)}$	$QTL_{ch2(2H)}$ x$QTL_{ch5\ (5H)}$ $QTL_{ch2(2H)}$ x$QTL_{ch6(6H)}$	61
BYDV-MAV Height reduction	Toluca 1996	$QTL_{ch1\ (7H)}$, $QTL_{ch4\ (4H)a}$[b], $QTL_{ch4\ (4H)b}$, $QTL_{ch5\ (5H)}$		43
BYDV-MAV Dwarfness score	Toluca 1996	$QTL_{ch1\ (7H)}$, $QTL_{ch4\ (4H)b}$, $QTL_{ch5\ (5H)}$		39
BYDV-MAV Tillering score	Toluca 1996	$QTL_{ch1\ (7H)}$, $QTL_{ch5\ (5H)}$		32
BYDV-PAV Dwarfness score	Toluca 1996	$QTL_{ch1\ (7H)}$, $QTL_{ch4\ (4H)b}$, $QTL_{ch5\ (5H)}$		18
BYDV-PAV Tillering score	Toluca 1996	$QTL_{ch1\ (7H)}$, $QTL_{ch5\ (5H)}$		17

[a] ch, Chromosome and number; see Table 2 for details
[b] 4 (4H)a, 4 (4H)b refer to the *KFP221-ABG397* and *MWG634-CDO542* intervals, respectively

but these genes often lack durability (Feuerstein et al. 1990; Jin et al. 1996; Qi 1998). When Shyri was released in Ecuador in 1989, it was resistant to leaf rust, with a maximum rating of 20MS. It now has ratings as high 90S (Vivar, personal communication), underscoring the danger of reliance on single qualitative resistance genes. The RGAP marker *S2As3IN-11* is 5.8 cM proximal to the $Rphx_S$ locus. Thus, we found RGAP markers associated with both quantitatively and qualitatively inherited genes conferring resistance to two *Puccinia* species.

We also found markers associated with quantitatively inherited resistance to two serotypes of the aphid-vectored viral pathogen, BYDV. The phenotypic distributions for BYDV-MAV and BYD-PAV did not show discrete classes for any of the three traits used to measure resistance – plant height reduction, dwarfing score or tillering score. The plant height reduction data (Fig. 2c) is representative of these data. These distributions suggest that resistance in this population is not due to the *Ryd2* locus on chromosome 3 (3H), which gives a clear distribution of resistance versus susceptible classes when resistant and susceptible alleles are segregating in a DH mapping population (Hayes et al. 1996). There were large numbers of positive phenotypic transgressive segregants for all measures of resistance, suggesting that both parents contributed resistance alleles. The QTL data support the presence of resistance genes other than *Ryd2*, the contribution of resistance alleles from both parents, a common genetic basis for the three measures of resistance, and a common basis of resistance to the two serotypes.

BYDV resistance QTLs exceeded significance thresholds only in the Toluca 96 date-1 dataset, although trends were apparent in the other datasets. Accordingly, the following discussion is based on the one environment of MAV and PAV data. Four QTLs were detected for BYDV-MAV resistance and three for PAV resistance. None mapped to the centromeric region of chromosome 3 (3H), the site of the *Ryd2* locus (Collins et al. 1996). Coincident large-effect QTLs for all three measures of MAV and the two measures of PAV resistance mapped to chromosome 1 (7H) in the *ABC253-HVM49* interval (Fig. 3c). In all cases Galena contributed the resistance allele. The PVE values for MAV resistance ranged from 18% to 20%. The PVEs for the two measures of PAV resistance were 11%. Two regions on chromosome 3 (4H) were associated with MAV resistance and one with PAV resistance. In all cases, Galena contributed the resistance allele. A MAV dwarfing score QTL mapped to the *KFP221-ABG397* interval (PVE = 10%). Plant height reduction and dwarfing score QTLs for MAV and a PAV dwarfing score QTL coincided in the *MWG634-CDO542* interval. The MAV resistance QTLs had PVEs of 7%, while the PAV QTL had a PVE of 6%. Shyri contributed resistance alleles for all measures of resistance to the two serotypes at coincident QTLs on chromosome 5 (1H) in the *ABC160-BMAC303d* interval (Fig. 3d). The MAV re-

sistance QTLs accounted for PVEs ranging from 10–15%, while the PAV resistance QTL accounted for 3–7% of the PVE. The multi-locus PVE values for MAV dwarfing score, plant height reduction, and tillering score were 43%, 39%, and 32%, respectively. The multi-locus PVE values for PAV dwarfing score and tillering score were 17% and 18%, respectively. No two-locus QTL interactions were significant for resistance to either serotype, indicating that the phenotypic variance that remains unaccounted for may be due to genes in unmapped regions of the genome or to higher order interactions.

The chromosome 1 (7H) BYDV-MAV/PAV resistance QTL may be homoeologous with *Bdv1*, a BYDV resistance gene in wheat. This gene, mapped to chromosome 7D, was reported to be tightly linked to the *Lr34* and *Yr18* genes for resistance to leaf rust and stripe rust, respectively (Singh 1993; Sharma et al. 1995). It is not clear from these reports if these loci are on the long or short arm of chromosome 7D. Barley chromosome 1 (7H) and wheat chromosome 7 (5H) are homoeologous. In this population, the BYDV resistance QTL and the $Rphx_S$ loci are linked, although in repulsion phase. Additional evidence for potential homoeology of these resistance clusters is the presence of a qualitative stripe rust resistance gene in the same region of the genome in CI10587 (Hayes et al. 1999). We found one RGAP to be associated with quantitative resistance to BYDV on chromosome 1 (7H).

In summary, we mapped qualitatively inherited resistance to leaf rust and determinants of quantitative resistance to stripe rust and BYDV. The leaf rust resistance gene may be allelic with a gene mapped in other germplasm (Jin et al. 1993; Hayes et al. 1996), and its linkage relationship with a BYDV resistance QTL may reflect a homoeologous relationship in wheat (Singh, 1993; Sharma et al. 1995). The largest effect stripe rust resistance QTL coincides with a QTL reported in other germplasm (Thomas et al. 1995) and a qualitative resistance gene (von Wettstein-Knowles 1992). RGAPs may be a useful tool for mapping quantitative and qualitative resistance genes. However, their utility must be confirmed by more extensive mapping. Furthermore, the RGAP amplification products need to be sequenced before any relationship to disease resistance genes is inferred. The availability of abundant molecular markers should facilitate the isolation and characterization of both qualitative and quantitative resistance genes and provide tools for answering fundamental questions regarding the genetic basis of the two classes of resistance.

Acknowledgements This work was supported by the North American Barley Genome Mapping Project (NABGMP) and ICARDA/CIMMYT. We would like to thank the Scottish Crop Research Institute (SCRI) for their generosity in providing SSRs. We are indebted to Bernardo Ramirez y Vicente Morales of ICARDA/CIMMYT for their excellent job in disease phenotyping.

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