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F. Q. Chen · D. Prehn · P. M. Hayes
D. Mulrooney · A. Corey · H. Vivar

Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*)

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Abstract Two genes conferring resistance to the barley stripe rust found in Mexico and South America, previously identified as race 24, were mapped to the M arms of barley chromosomes 7 and 4 in a doubled haploid population using molecular markers and the quantitative trait loci (QTL) mapping approach. The resistance gene on chromosome 7 had a major effect, accounting for 57% of the variation in disease severity. The resistance gene on chromosome 4 had a minor effect, accounting for 10% of the variation in trait expression. Two pairs of restriction fragment length polymorphism markers are being used to introgress the resistance genes to North American spring barley using molecular marker-assisted backcrossing.

Key words Barley stripe rust · RFLP · QTL mapping · Molecular marker-assisted backcrossing

Introduction

A virulent form of barley stripe rust (*Puccinia striiformis* f. sp. *hordei*) is a major threat to barley production in the United States. Barley stripe rust was first observed in Colombia, South America in 1975 and was determined to be the same race 24 that caused an outbreak of disease in Central Europe in the 1960s (Dubin and Stubbs 1986). By 1982, this virulent race of stripe rust had affected all principal barley growing regions in South America and had reduced yields up to 70% (Dubin and Stubbs 1986). A virulent form of barley stripe rust, apparently the same race 24, reached Mexico in 1987; Texas, USA in 1991, and Arizona, USA in 1992. The results of an ongoing screening effort in Bolivia and Ecuador coordinated by the USDA/CSRS are disturbing: most USA spring barley cultivars are highly susceptible (B. Brown, personal communication).

Genetic resistance to barley stripe rust, and to race 24 in particular, is available, but information on it is limited. Bakshi and Luthra (1971) described a dominant resistance gene in Indian germ plasm. Three recessive resistance genes (*Yr*, *Yr2*, and *Yr3*) have been identified in European spring barley, and one dominant resistance gene has been reported in European winter barley (Lehmann et al. 1975). The only stripe rust resistance gene mapped in barley, *Yr4*, is located on chromosome 5 (von Wettstein-Knowles 1992), but this gene does not confer resistance to race 24.

The most economical and environmentally appropriate strategy for disease control is genetic resistance. However, given the dearth of information regarding the genetic basis of resistance and the necessity of introgressing resistance genes from exotic germ plasm, this could represent a protracted endeavor if conventional breeding procedures were used. The recent development of medium density genome maps (Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993) and quantitative trait loci (QTL) mapping analyses in barley (Hayes et al. 1993) offers an alternative approach to locating and subsequently manipulating resistance genes. Molecular markers can be used to expedite exotic germ plasm introgression and minimize linkage drag (Tanksley et al. 1989). Furthermore, molecular marker-based selection procedures are particularly useful when phenotyping is problematic, as is the case with this pathogen. Given the relatively recent arrival of the pathogen in the USA, a sufficient field inoculum for effective discrimination among genotypes does not exist, and artificial inoculation is obviously not appropriate.

Locating one or a few resistance genes need not involve the expense and time required for full genome

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F. Q. Chen (✉) · D. Prehn · P. M. Hayes · D. Mulrooney · A. Corey
Department of Crop and Soil Science, Oregon State University, Corvallis OR 97331-3002, USA

H. Vivar
ICARDA/CIMMYT, El Batan, Mexico

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mapping. Two approaches to the rapid identification of genes of interest, in the absence of near isogenic lines, have been reported. Michelmore et al. (1991) used bulked segregant analysis to identify random amplified polymorphic DNA (RAPD) (Williams et al. 1990) and restriction fragment length polymorphism (RFLP) markers linked to downy mildew resistance genes in lettuce. Lander and Botstein (1989) proposed using selective genotyping of the extreme progeny in a phenotyped population to increase the efficiency of QTL mapping. Both approaches can substantially reduce the cost associated with generating molecular marker data. Given the nature of trait expression in our materials and the catalog of known location molecular markers available in barley, we used the selective genotyping approach with the objective of rapidly locating putative resistance loci before the full population was mapped on regions of putative resistance loci. Our goal is to map barley stripe rust resistance genes and to identify molecular markers that can be used to rapidly introgress resistance genes into North American germ plasm with minimum linkage drag.

Materials and methods

Plant materials

An F_1-derived doubled haploid (DH) population of 110 lines was developed for mapping using the in vitro floret culture-assisted *Hordeum bulbosum* method (Chen and Hayes 1989). The resistant parent was an elite six-row spring feed barley (LBlran/UNA8271//'Gloria'/'Come') developed by ICARDA/CIMMYT. The first three parents in the pedigree are resistant to the virulence spectrum of *Puccinia striiformis* f. sp. *hordei* found in Mexico and South America. The genetic basis of the resistance is not known. The susceptible parent was a backcross derivative of 'Bowman', a two-row spring feed barley released by North Dakota State University. The DH population was evaluated for response to barley stripe rust at CIMMYT, Mexico in 1991 and 1992, with three planting dates in the second year. Each DH line was planted in a 3-m, one-row plot in 1991, and a 5-m, two-row plot in 1992. Data were taken at flowering and grain filling. Disease was rated by the modified Cobb Scale (Melchers and Parker 1922) on a whole-plot basis.

RFLP mapping

DNA samples were prepared from 4 g of fresh leaf tissue of greenhouse-grown plants using the proteinase K procedure (Draper et al. 1988). Six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII, and *Xba*I) were used for parental screening and subsequent mapping. Digested DNA samples (10 µg per lane) were subjected to overnight electrophoresis in 0.9% agarose gels in TBE buffer. The gels were treated using standard procedures (Maniatis et al. 1982) before DNA was transferred with vacuum to MagnaGraph nylon transfer membranes (MSI) using 10 × SSPE.

Previously mapped cDNA and genomic DNA clones from barley, wheat, and oats were acquired from Cornell University, Kansas State University, the North American Barley Genome Mapping Project (USA), and the Institut für Resistenzgenetik (FRG). Clone selection was based on our objective of providing a good coverage of the barley genome. DNA inserts in cloning vectors were isolated from low-melting-point (LMP) agarose gels after being either digested with appropriate enzymes or amplified by the polymerase chain reaction (PCR) using primers of T3/T7 or Sp6/T7 promoter sequences. The inserts were then labelled with [^{32}P] using the Oligolabelling Kit (Pharmacia LKB). DNA hybridization was conducted at 65 °C in a hybridization incubator in a buffer consisting of 7% SDS, 0.25 *M* NaH_2PO_4 (pH 7.4), and 1% BSA. The membranes were washed at 65 °C with three changes of buffer (2 × SSC + 0.1% SDS, 1 × SSC + 0.1% SDS, and 0.5 × SSC + 0.1% SDS), each for 30 min. Films were exposed at − 80 °C for 24–72 h with double intensifying screens.

PCR mapping

Primer PR168 (5'-CAAGGACGCGCTCGAGCT-3') was derived from a partial sequence of clone ABC168 and used in the mapping. PCR amplifications were performed in a 50 µl reaction volume consisting of 25 ng genomic DNA, 15 pmol primer, 200 µ*M* of each dNTP, 1.5 units *Taq* polymerase (Promega), and 1 × reaction buffer supplied with the enzyme. The amplification profile was 1 cycle of 4 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C. Amplification products were analyzed by electrophoresis in 1% agarose gels.

Linkage and QTL analysis

Informative markers identified from parental screening were first mapped by selective genotyping using a sample of the 25 most resistant and the 25 most susceptible DH lines. Mapping data were then combined with phenotypic data for preliminary QTL analysis using the interval analysis procedures of QTL-STAT (B. Liu and S. J. Knapp, unpublished). We did not, however, use the missing-data method as described by Lander and Botstein (1989) since the Mapmaker/QTL program was not available to our project at the time of preliminary analysis. Markers linked to putative resistance loci were then mapped on the entire population. Mapmaker/Exp 3.0 and Mapmaker/QTL 1.1 (Lander et al. 1987; Lincoln et al. 1992) were used in the final QTL analysis of the population.

Results

Disease reaction

Intense barley stripe rust epidemics were observed in the DH population both in 1991 and 1992. The average disease severities were 59% in 1991 and 42% in 1992. Although there was a considerable between-year variation, the performance of individual DH lines was rather consistent in the 2 years, as indicated by correlation analysis ($r = 0.85$). A small variation was observed between the planting dates in 1992 ($r = 0.91$). The resistant parent was rated from 9% to 18%, while the susceptible parent was rated from 68% to 90%. Variation among the DH lines was continuous in all four environments, ranging from 3% to 90% (Fig. 1). Given the type of reaction observed for the parents under the same environments and the homozygous nature of doubled haploids, the frequency distribution of disease severity in the DH population indicates that the resistance can not be explained by a monogenic model.

Despite the large variation between 1991 and 1992, no QTL × environment interaction was observed in the combined analysis over environments using QTL-

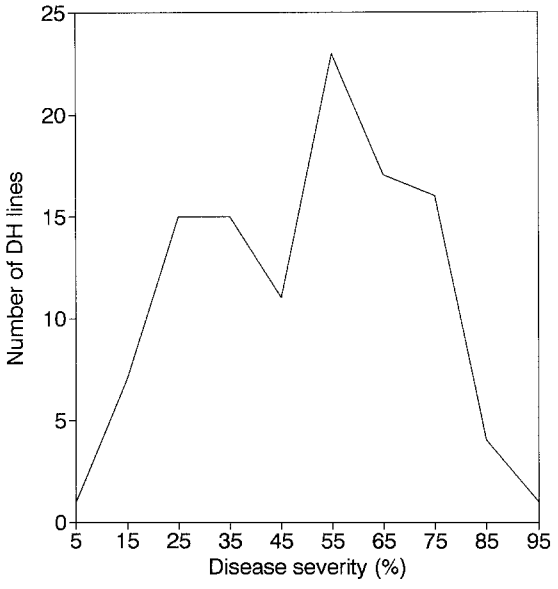

Fig. 1 Frequency distribution of stripe rust severity (%) in a barley doubled haploid (DH) population. Data are the average of four environments. Average disease severities for the resistant and susceptible parents were 13% and 75%, respectively

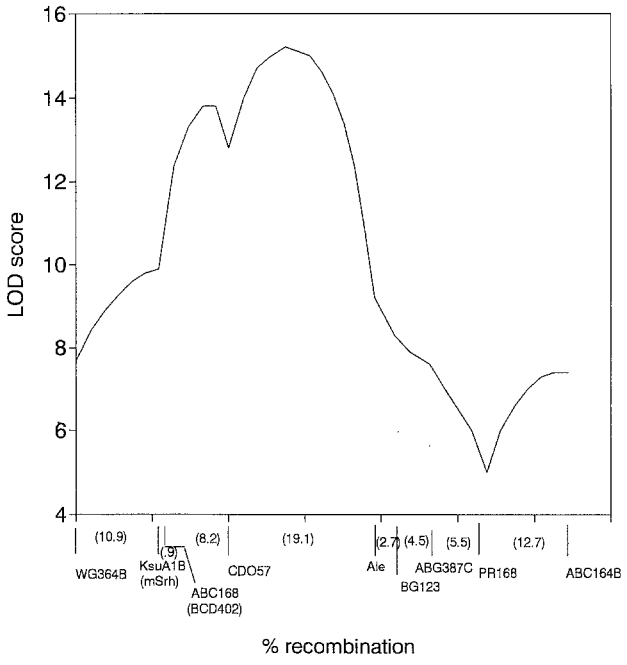

Fig. 2 LOD scores from the QTL analysis of stripe rust severity in a barley doubled haploid population and markers on the M arm of barley chromosome 7. Complete cosegregation was observed for *KsuA1 B/mSrh* and *ABC168/BCD402*

STAT. This is consistent with the results of correlation analysis. Thus, for individual DH lines, mean values of disease severity over the four environments were used in the final QTL analysis.

Mapping resistance loci

Of 99 cDNA and genomic DNA clones screened, 78 were informative in the two parents. The informative markers were first mapped on the 50 DH lines sampled. These markers spanned approximately 900 cM among the seven barley chromosomes (data not shown). In general, the linkage profile of these markers in the sample paralleled the published maps (Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993). Using the mapping data, we detected four putative resistance loci in the preliminary QTL analysis: two of the putative loci were detected on the "minus" (M) arms of chromosomes 7 and 4; the other two, which had small effects and marginally significant log-likelihood (LOD) scores, were detected on the "plus" (P) arms of chromosomes 6 and 5. The minus and plus chromosome arm designations were based on the proposal of Linde-Laursen and Jensen (1992). Full population analysis, however, did not confirm the putative resistance loci on chromosomes 6 and 5. Their LOD scores (≤ 0.8) were far less than a threshold of 2.0.

The resistance locus on the M arm of chromosome 7 had a major effect, accounting for 57% of the variation in disease severity in the entire population. As expected, the resistant parent contributed the favorable allele. The maximum LOD score for the locus was 15.2 (Fig. 2), and the predicted location of the gene is 7.4 recombination units from *CD057*. Single marker regression analyses also showed that *CD057* accounted for more variation than any other marker.

The chromosomal location for the resistance locus was defined by a linkage group of one morphological, one PCR, and nine RFLP markers (Fig. 2). The morphological marker is rachilla hair length (*mSrh*). Of the 9 RFLP markers, *BG123*, *Ale*, *CD057*, *KsuA1B*, *ABG168*, and *WG364* had been previously mapped to the same segment in the cross of 'Steptoe' × 'Morex' (Kleinhofs et al. 1993). The order and distance of these RFLP markers in these two crosses were comparable. Four new markers, *PR168*, *ABC164B*, *ABG387C*, and *BCD402*, were mapped to the region. The three RFLP clones also detected additional, multiple RFLP loci elsewhere in the genome.

The resistance locus on the M arm of chromosome 4 had a minor effect, accounting for 10% of the variation in the population. The favorable allele was again contributed by the resistant parent. The LOD score for the locus was 2.2. However, as the LOD scores (those exceeding 2.0) for the locus were very flat across a large interval (Fig. 3), the resolution of the locus is far less than ideal. The chromosomal location of the locus was defined by 7 RFLP markers (Fig. 3). All the markers (*BCD265A*, *ABG472*, *ABG54*, *WG114*, *ABG498*, *ABG397*, and *Bmy1*) had been previously mapped to the same segment in the cross of 'Steptoe' × 'Morex' (Kleinhofs et al. 1993), and linkage data from the two crosses are comparable.

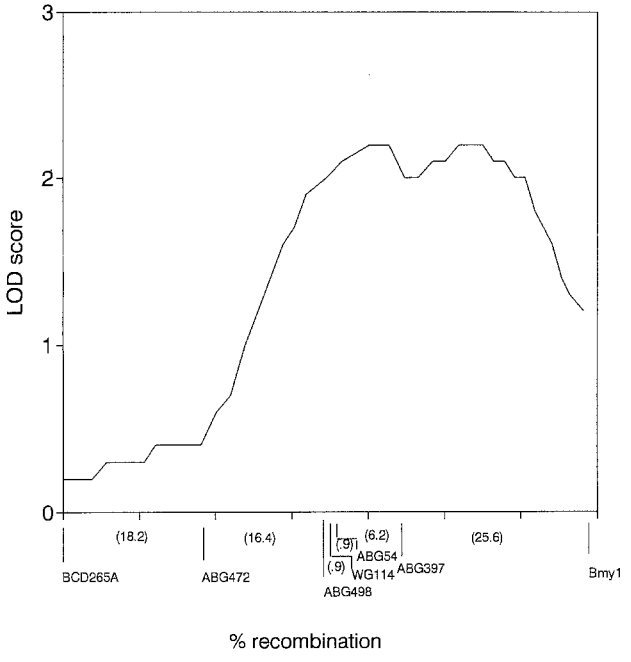

Fig. 3 LOD scores from the QTL analysis of stripe rust severity in a barley doubled haploid population and markers on the M arm of barley chromosome 4

A multilocus model involving both chromosome 7 and 4 gave a small reduction in LOD score (17.0) and r^2 (61%) as compared to the sum of the individual gene effects (LOD = 17.4, r^2 = 67%). The slightly lower LOD score and r^2 may be due to the poor resolution of the chromosome 4 interval, an epistatic interaction between the two loci, or other unknown factors. There is a big gap (approximately 30 cM) between *ABG397* and *Bmy1* in the current chromosome 4 map (Kleinhofs et al. 1993). We are currently searching for additional markers in the region to allow for greater resolution of the resistance locus.

Discussion

As differentials were not available at the time these field tests were conducted, the virulence spectrum of barley stripe rust in these environments could not be rigorously defined. However, as phenotypic responses were consistent and previous evaluation of differentials at CIMMYT, Mexico identified the virulence type to be race 24, it is very likely that the disease epidemic in the DH population was due to the same race 24. Other races of barley stripe rust have yet to be identified in the region. However, further investigation using pure isolates of race 24 in a controlled environment is needed before a definite conclusion about the pathogenicity can be drawn.

Molecular markers are now being widely used in mapping resistance genes in many species. In many host-pathogen systems, phenotypic variation can be categorized as resistant versus susceptible and coded like molecular marker data. Mapping this type of resistance can be accomplished by linkage analysis (Timmerman et al. 1993). However, when phenotypic variation is continuous and behaves like quantitative traits, as is the case in the DH population, mapping can not be resolved solely by linkage analysis. The recent development of QTL mapping analyses, particularly those using interval procedures (Lander and Botstein 1989; Knapp et al. 1990), offers an alternative approach for mapping this type of resistance, even though resistance genes may not be true QTLs. Heun (1992) used Mapmaker/QTL to locate genes underlying quantitative resistance to powdery mildew in a barley doubled haploid population. Using the same approach, we have mapped two barley stripe rust resistance loci in the DH population. The evidence for the presence of the resistance gene on chromosome 7 is very strong as supported by the large LOD score (15.2). The evidence for the presence of the resistance gene on chromosome 4 is not as strong (LOD = 2.2). However, considering the large interval between *Bmy1* and *ABG397* and the fact that we did not detect any other region with LOD scores larger than 0.8, we used LOD score 2.0 as a relevant threshold for the locus. Lander and Botstein (1989) pointed out that the selection of an appropriate LOD score threshold must be based on a number of parameters, including the map density, particular genome, and type of cross. In general, a LOD threshold of between 2 and 3 is required to ensure an overall false positive rate not greater than 5%, and a dense map requires a higher LOD threshold than a sparse map (Lander and Botstein 1989). Paterson et al. (1988, 1990) adopted a LOD threshold of 2.4 based on the length and spacing of their tomato RFLP map. Heun (1992) used a LOD threshold of 2.5 in a barley cross. Genes with minor effects may escape detection if a high LOD threshold is used. Further investigation to verify the presence of the resistance locus on chromosome 4 is highly desirable.

The resistance locus on chromosome 7 appears to be a major gene, for it accounted for most of the genetic variation. The resistance locus on chromosome 4 appears to be a minor gene. However, since virulence variation of the pathogen in the region is largly undefined, it is open to question whether the two genes responded to the same pathogenicity. Minor resistance genes have also been reported in wheat stripe rust (Lewellen et al. 1967; Lewellen and Sharp 1968), which was caused by a different formae specialis of the same pathogen species.

Given the pressing need to rapidly introgress resistance genes to North American spring barley germ plasm, we have implemented molecular marker-assisted backcrossing in two genetic backgrounds. We achieved higher resolution of the resistance locus on chromosome 7, and we are using *CD057* and *BG123* as flanking markers for selection. The predicted selection efficiency is greater than 95% due to the rare likelihood of a double crossover in the marker interval. Resolution of

the resistance locus on chromosome 4 is less than ideal. We are currently using *Bmy1* and *ABG397* or *ABG498* as flanking markers. However, the size of this interval could present problems in terms of linkage drag. Hayes et al. (1993) did not detect any agronomic or malting quality trait QTL in the *Ale-CD057* region in the cross of 'Steptoe' × 'Morex', although a significant alpha amylase QTL was detected between *mSrh* and an adjoining upstream molecular marker. On chromosome 4, no critical malting quality QTLs were detected in the *ABG472-Bmy1* interval, although large-effect lodging and grain protein QTLs were detected in this interval. As diverse germ plasm may exhibit distinct agronomic and quality trait QTLs, we are currently phenotyping this population for agronomic and malting quality traits in order to assess the importance of linkage drag associated with the two disease resistance QTLs.

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