High-Resolution Mapping of the Hor1/Mla/Hor2 Region on Chromosome 5S in Barley

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Received 27 July 1993. Accepted 25 May 1994.

A high-resolution mapping population consisting of 270 individual lines, each representing an independent recombination event between the Hor1 and Hor2 loci, was used to construct a high-density restriction fragment length polymorphism (RFLP) map of the Hor1/Mla/Hor2 region. To identify informative markers for screening the recombinant population, the Franger (containing Mla6 and Mla14) and Rupee (containing Mla13) parental lines were screened for RFLPs using 12 restriction endonucleases and 35 cDNA and genomic clones, that had been shown to map to barley chromosome 5 or wheat group 1. As a result, 61 probe/enzyme combinations were chosen to screen the recombinant population. Analysis of the RFLP mapping data indicated nine of the 21 polymorphic probes mapped within the Hor1/Hor2 interval. Map distance between Hor1 and Hor2 was calculated to be approximately 8.1 cM. Nine of the remaining probes mapped outside of the Hor1/Hor2 interval and proximal to Hor1. The remaining three probes mapped to separate linkage groups. Sixteen of the recombinant lines had regions of DNA that were heterozygous at one or more RFLP loci within the Hor1/Hor2 interval. Data from the high-resolution RFLP map of the Hor1/Hor2 region was integrated with previous recombination data which positioned the Mla6, Mla13, and Mla14, resistance alleles, in relation to Hor1 and Hor2. Five of the probes hybridized to multiple sites. BCD249, a cDNA-derived barley clone, hybridized to two sequences 0.3 cM apart, indicating a duplication event within the Hor1/Hor2 interval. One of these sites, Xbed249.2, was positioned within 1.6 cM from the Mla alleles.

Fundamental knowledge of the genetics of host-pathogen interaction is crucial to effectively utilize genetic resistance to control plant disease. Powdery mildew of barley is an ideal model to study these interactions. Because the genetics of the system have been extensively characterized, it is an excellent system for investigating specific recognition in gene-for-gene interactions among small grains and obligate fungal pathogens (Flor 1955; Keen 1990; Thompson and Burdon 1992). In addition, there are many genes for resistance in the host, each giving a unique reaction to one or more isolates of the pathogen. This phenomenon results in a large supply of naturally occurring variation.

A number of M1 genes have been identified in barley, Hordeum vulgare L., which confer resistance to the powdery mildew fungus, Erysiphe graminis DC. Merat f. sp. hordei em. Marchal (Jørgensen and Moseman 1972; Jørgensen and Jensen 1976; Hinze et al. 1991; Görg et al. 1993). The Mla (powdery mildew resistance) locus, located near the telomeric end of chromosome 5S, homoeologous group 1 of the family Gramineae, is of particular interest because of its highly variable, multicomponent nature. There is a large cluster of alleles or tightly linked genes at the Mla locus, and at least twenty-three have been differentiated by their specific reaction to unique isolates of E. graminis, making this locus suitable to detailed genetic analysis (Jørgensen and Moseman 1972; Moseman and Jørgensen 1973; Giese 1981; Giese et al. 1981; Wise and Ellingboe 1983, 1985; Jahoor and Fischbeck 1987; Jahoor et al. 1993).

The specific interaction between different host alleles and their corresponding pathogen isolates has proven advantageous for the genetic analyses of many resistance gene clusters. These include the Rpl cluster in maize for resistance to the maize rust pathogen, Puccinia sorghi Schw. (Saxena and Hooker 1968; Pryor 1987; Hulbert and Bennetzen 1991; Sudupak et al., 1993), the L and M gene clusters in flax (Linum usitatissimum L.) for resistance to the flax rust pathogen, Melampsora lini (Ehrenb.) Lev. (Shepherd and Mayo 1972; Mayo and Shepherd 1980; Islam and Shepherd 1991), a Pc gene cluster in diploid Avena for resistance to the oat crown rust pathogen, Puccinia coronata Corda f. sp. avenae Eriks (Rayapati et al. 1994), and various Dm loci in lettuce (Lactuca sativa L.) for resistance to the downy mildew pathogen, Bremia lactucae Regel (Hulbert and Michelmore 1985; Farrara et al. 1987; Paran et al. 1991). Research on the genetic mechanisms responsible for linkage between genes in resistance clusters is ongoing, but no specific process has been confirmed (Sudupak et al. 1993; Robbins et al. 1991). However, the clustering of many resistance gene families, often associated with seed storage protein gene families, suggests a conserved mechanism in which tandem duplication, followed by multiple recombination or mutation events, could be involved (Giese et al. 1981; Wise and Ellingboe 1985; Hulbert and Bennetzen 1991; Singh et al. 1990; Islam and Shepherd 1991; Paran et al. 1992; Sudupak et al. 1993). Similarly, a mechanism of unequal exchange may account for the observed instability of many race-specific resistance loci.

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MPMI Vol. 7, No. 5, 1994, pp. 657-666
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located within these clusters (Wise and Ellingboe 1985; Pryor 1987; Sudupak et al. 1993). These mechanisms, occurring singly or in combination may also account for the generation of new resistance specificities (Islam and Shepherd 1991).

Specific disease-resistant alleles have been mapped to general chromosomal regions in many crops. Construction of high-resolution restriction fragment length polymorphism (RFLP) maps has allowed the precise location of some of these disease resistance genes, including the Pto locus in tomato (Martin et al. 1993), the Rpl locus in maize (Hulbert and Bennetzen 1991), the Xa21 locus in rice (Ronald et al. 1993), and the Dm3 locus in lettuce (Paran et al. 1991). Four RFLP maps have been reported for barley which assign locations to a number of molecular and morphological markers (Shin et al. 1990; Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993). Of the twenty-three Mla variants, the Mla6 allele has been mapped near the Xmcg036 RFLP locus (Graner et al. 1991; Schüller et al. 1992), and the Mla12

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**Fig. 1.** Derivation of mapping population recombinant between Horl and Hor2. The majority of the selected recombinants will be homozygous in the region spanning the Horl/Hor2 interval, indicated as derived via 1A. However, selection of homozygous recombinants based on the flanking Horl and Hor2 markers can result in the selection of apparent homozygous recombinants containing internal heterozygous regions. These are indicated as derived via 1B. The F₃ segregating progeny from selfed apparent homozygous recombinants were screened to select for true homozygous recombinants derived via 1C. The symbol Θ indicates self-pollination. The top and bottom of the vertical lines represent Horl and Hor2, respectively.
allele was shown to be linked to the Xbcd249 RFLP locus (Heun et al. 1991). However, a limited number of detailed, high-resolution RFLP maps have been developed for small grains.

Previous research in our laboratory was directed towards developing a high-resolution mapping population for the region between the Hor1 and Hor2 endosperm storage-protein genes on the short arm of barley chromosome 5. The Hor1 and Hor2 genes are approximately 8–10 centimorgans (cM) apart (Jensen et al. 1980), and flank the Mla powdery mildew-resistance locus. This high-resolution mapping population consists of 270 individual lines, each representing an independent recombination event between the Hor1 and Hor2 loci (Fig. 1). This population was used to map precisely the Mla6, Mla13, and Mla14 alleles, and to investigate the genetic organization of the locus (Mahadevappa, DeScenzo, and Wise 1994). These experiments indicated that the Mla6 and Mla13 alleles are functionally distinct. In this report we describe the use of these recombinant lines to construct a high-resolution RFLP map of the Hor1/Hor2 region. In addition, we define the recombination sites in each of the 270 lines at a more detailed level. Characterized in this manner, this high-resolution mapping population is an essential component towards our long term goal of map-based cloning at the Mla locus.

RESULTS

Identification of polymorphic markers in the (Hor1/Hor2) region.

To identify informative markers for screening the recombinant population, the Franger (containing Mla6 and Mla14) and Rupee (containing Mla13) parental lines were screened for RFLPs by digesting their respective DNA with 12 restriction endonucleases (BamHI, EcoRI, EcoRV, HindIII, Kpnl, DraI, XbaI, XhoI, Apal, StyI, BclI, and BglII). Southern filters carrying the digested DNA samples were hybridized with 35 cDNA and genomic clones, that had been shown to detect polymorphisms on barley chromosome 5 or wheat group 1 (Granner et al. 1991; Heun et al. 1991; Kleinhofs 1993). Numerous polymorphisms were detected between the two parental lines. As a result of the parental screening, 61 probe/enzyme combinations were chosen to screen the recombinant population; selected based on homozygosity at Hor1 and Hor2 (Table 1). The restriction endonucleases HindIII, DraI, EcoRI, and EcoRV were selected for screening the mapping population because they, in conjunction with the available probes, yielded the greatest number of easily scored polymorphisms between the parental lines. Fifteen of the probes screened detected polymorphisms between the parents with at least one of these enzymes (Table 1, group A). Six additional probes detected polymorphisms with the restriction endonucleases BclI, BamHI, XbaI, or StyI (Table 1, group B). The remaining probes did not detect any easily scored polymorphisms.

Linkage analysis.

RFLP analysis within the Hor1/Hor2 interval was completed on the mapping population of 270 F2 recombinant lines (Fig. 1). The 270 individual recombinant lines were representative of an F2 population size of 1,800 (3,600 gametes). Therefore, calculation of map distance within the Hor1/Hor2 interval was based on an effective population size of 1,800. This population size was appropriate for calculating linkage distances within the Hor1/Hor2 interval, because the 270 recombinant lines were obtained by screening 1,800 F2 segregants and selecting the lines that contained a recombination event between these two markers (Mahadevappa et al. 1994). We could presume the remaining F2 segregants had no recombination between Hor1 and Hor2 with the exception of

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<th>Probes used to generate a high resolution map of the Hor1/Mla/Hor2 region on barley chromosome 5S</th>
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<td>ABC152</td>
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<td>BCD371</td>
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* Restriction enzymes: R1, EcoRI; RV, EcoRV; H3, HindIII; D1, DraI; S1, StyI; BHI, BamHI; BclI; BclI; X1, XbaI.
rare double-crossover events. This permitted us to estimate the map distance between Hor1 and Hor2 to be 8.1 cM. Additional RFLP analysis for probes which mapped outside of the Hor1/Hor2 interval was conducted on two discrete subsets of the mapping population and a linear order was determined (Fig. 2). DNA from the primary subset (n = 115) was digested with the restriction endonucleases HindIII, DraI, EcoRI, and EcoRV, whereas DNA from the secondary subset (n = 115) was digested with the restriction endonucleases BclI, BamHI, XbaI, or StyI.

The F3 intercross function of Mapmaker/Exp version 3.0b (Lander et al. 1987; Lincoln et al. 1992) was used to establish genetic linkage. This function was used because the recombinant population had been through an additional meiosis in the F2 prior to selecting the individuals homozygous at both flanking markers. Nine of 21 markers were placed within the Hor1/Hor2 interval (Fig. 2). Nine of the remaining markers were mapped outside of the Hor1/Hor2 interval and proximal to Hor1. The remaining three probes did not map to barley chromosome 5 in our mapping population. Within the Hor1/Hor2 interval the framework assigned to chromosome 5 was Hor1, Xmwg068, Xbcd249.1, Xbcd249.2, Xmouse36, Xmouse060, Xmouse645, and Hor2. Markers outside the interval were positioned using a combination of the “place” command along with ordering and overlapping additional subsets. Linkage analysis indicated four loci, Xmouse645.2, Xmouse075.2, XChs1.1, and XChs1.2 were unlinked to the Hor1/Hor2 interval.

The Mla6, Mla13, and Mla14 disease resistance alleles were previously ordered in the recombinant population (Mahadaveppa et al. 1994). Data from these experiments were integrated with the results from the present work in order to position the Mla locus relative to the RFLP markers.

Sequence duplication.

Five of the probes used in this study hybridized to multiple sites (Fig. 2). BCD249, a cDNA derived barley clone, hybridized to two sequences 0.3 cM apart (Xbcd249.1 and Xbcd249.2), indicating a sequence duplication event within the Hor1/Hor2 interval (Fig. 3). Duplicated sequences of ABC160 (Xabc160.1 and Xabc160.2) and CD0580 (Xcd580.1 and Xcd580.2) were detected at two sites, each duplicate pair closely linked, outside the Hor1/Hor2 interval.

Similarly, two other probes, p.CHS11 and MNG645 detected one site, XChs1.3 and Xmouse645.2, respectively, within the Hor1/Hor2 interval and one or more sites that were unlinked. The probe MNG075 hybridized to one site on chromosome 5 (Xmouse075.1) and one unlinked site.

Heterozygous internal regions with homozygous flanking markers.

Sixteen of the recombinant lines had regions of DNA that were heterozygous at one or more RFLP loci within the Hor1/Hor2 interval. These were most likely the result of an additional recombination event in the F2 meiosis (Fig. 1B). Analysis of RFLP data with the output from Map Manager v2.5 indicate these regions were variable in length, but were clustered around the Xbcd249.1/Xbcd249.2 loci within the Hor1/Hor2 interval. In addition, heterozygous regions were found associated with Xmouse060, and Xmouse068. Two classes of heterozygous regions were observed (Fig. 4). The type 1 class contained heterozygous regions that were flanked by one parental sequence on one side and the other parental sequence on the opposite side (Fig. 4; columns 2, 7, and 12). The type 2 class contained heterozygous regions that were inserted into and flanked by a single parental sequence (Fig. 4; column 28). Eleven of the type 1 and five of the type 2 heterozygous regions were observed in the mapping population.

Analysis of F3 lines derived from selfed F2 lines containing type 1 heterozygous regions.

The F3 lines containing type 1 heterozygous regions were analyzed to determine their haplotypes. Homozygous F3 lines that were analyzed are shown in Fig. 3. Southern analysis of probe BCD249 hybridizing to two loci (Xbcd249.1 and Xbcd249.2) within the Hor1/Hor2 interval. The two parental types, observed in lane 1, Franger (Mla6), and lane 2, Rupee (Mla13), were the predominant pattern observed. Two allelic sites were detected at each locus, and designated bcd249.1.Ru/bcd249.1.Ru, and bcd249.2.Fr/bcd249.2.Fr. Eleven lines, out of the 270 analyzed, had recombinant RFLP patterns indicating duplication of the sequence hybridizing with BCD249 (lanes 5 and 6).
were selected by screening segregating progeny from selfed F₁ plants containing type 1 heterozygous regions (Fig. 1). Both RFLP analysis and resistance phenotypes were used to confirm the identity of homozygous lines. At least 16 F₁ progeny derived from each selfed F₁ line were screened for disease resistance. Eight of these progeny were selected for RFLP analysis based on their disease resistance phenotype. Two classes of homozygous recombinants were recovered from each of the selfed F₁ lines. Each class of homozygous recombinants contained one of the two recombination sites which flank the heterozygous region in the F₁ line. Data obtained from analysis of F₂ progeny from each type 1 heterozygous lines (column 7; line H92S6447 and column 12; line H92S6453) (Mahadevappa et al. 1994) are shown in Figure 4. At the present time, F₄ progeny from selfed F₁ lines containing type 2 heterozygous regions have not been analyzed.

**DISCUSSION**

The objectives of this study were to develop a high-resolution RFLP map of the *Hor1/Hor2* region on barley chromosome 5S, and to integrate this RFLP map with previous recombination data which positioned the three *Mia* resistance alleles, *Mia6*, *Mia13*, and *Mia14*, in relation to *Hor1* and *Hor2*. In this report, markers from three previously reported maps (Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993) have been integrated with three alleles of the *Mia* locus into one map using our high-resolution recombinant population. In addition, we positioned three RFLP markers (*Xcd658, Xcd580.1, and Xcd580.2*) proximal to the *Hor1/Hor2* interval, which have not been previously reported as mapped to chromosome 5S.

Due to the near-isogenic nature of the two parental lines, we anticipated the majority of the probes detecting polymorphisms would be located in the introgressed region centered around the *Hor1/Hor2* interval. Theoretically, each of the recombinant lines contain approximately 88% of the recurrent parent germplasm, and 12% from the respective donor parents, based on the selection method utilized to generate the lines used as parents for the mapping population (Hanson 1959). However, none of the probes mapped outside the *Hor1/Hor2* interval proximal to *Hor1*. These RFLP data indicate the introgressed region extends to marker *Xcd658* and probably extends to *Xabc160.1* and *Xabc160.2* (Fig. 2). Alternatively, the polymorphisms observed at *Xabc160.1* and *Xabc160.2* could be associated with a second introgressed region.

Fifteen of 155 recombinant lines in the primary mapping subset contained additional recombination events in the *Hor1/Xcd658* segment adjacent to the *Hor1/Hor2* interval. We would expect that interference from a crossover within the *Hor1/Hor2* interval would affect subsequent crossovers adjacent to the *Hor1/Hor2* interval during a single meiosis. However, crossovers occurring in the *F₂* meiosis would not interfere with crossovers in the F₁ meiosis. We are not able to determine if the additional recombination events close to *Hor1* occurred during the *F₂* meiosis, and were subject to interference, or if they occurred in the *F₂* meiosis. However, analysis of these 15 recombinant lines suggest the possibility of interference occurring in this region. For example, lines in which the within-interval recombination occurred close to

![Fig. 4. Map Manager v2.5 output illustrating 40 of the 270 recombinant lines used in this study. An “A” denotes the Franger (*Mia6*) allele, “B” denotes the Rupee (*Mia13*) allele, and “H” denotes a heterozygote for the RFLP or resistance locus indicated on the left side of the table. Individual accessions are in numerical order across the top of the figure. Columns 2, 7, and 12 represent examples of lines containing type 1 heterozygous regions which occur at the junction between the two genotypes. Column 28 represents a line containing a type 2 heterozygous region in which the heterozygous region is flanked by a single parental genotype. Data from RFLP and disease resistance analysis for selfed segregating progeny from the lines represented in columns 7 (line H92S6447) and 12 (line H92S6453) (Mahadevappa et al. 1994) were used to generate haplotypes which are shown in columns 41-48 and 49-56, respectively.](image-url)
Hor2, exhibited the additional recombination close to Hor1, within the Hor1/Xcd0658 segment. Likewise, lines in which the within-interval recombination occurred near Hor1, exhibited the additional recombination in the Hor1/Xcd0658 segment closer to Xcd0658.

The calculated map distance between Hor1 and Hor2 of 8.1 cM is within the range of previous reports: 8.5 cM (Siedlieder and Graner 1991); 13.91 map units (Shin et al. 1990); approximately 10.0 cM (Graner et al. 1991); 12.5 cM (Kleinholfs et al. 1993); and 16.4 cM (Schondelmaier et al. 1993). The majority of the markers we have mapped within the Hor1/Hor2 interval are consistent with orders reported in the previously published maps. However, marker Xabg373.2, which we mapped to a site proximal to Hor1, had been previously placed 153.8 cM proximal to Hor1 (Kleinholfs et al. 1993). Similarly, Xabg452, which mapped identically to Hor1, was previously placed approximately 53.7 cM proximal to Hor1 (Kleinholfs et al. 1993). In addition, the Xbcd249 locus had not previously been positioned on a map with RFLP markers located in the Hor1/Hor2 interval. The remaining markers placed on the same linkage group were mapped outside the Hor1/Hor2 interval, proximal to Hor1 (Fig. 2), and correspond to the map order previously reported.

Of particular interest, towards our long-term goals, are the markers Xbcd249.2 and Xmwc036 that flank Mla6 and Mla13, by 1.6 and 1.5 cM, respectively, on either side. Identifying flank- ing RFLP markers in this proximity to the Mla resistance locus has enabled us to investigate the correlation between distinct RFLP alleles and resistance phenotype. The flanking markers are invaluable in the investigation of lines with altered resistance phenotypes.

A recent report (Ahn and Tankersley 1993) indicates gene duplication frequently occurs throughout the genome in maize as well as wheat, oats, and barley. High-resolution populations enable the detailed genetic investigation of duplicated regions within linkage groups. We have positioned five cDNA probes that hybridize to multiple sites, indicating duplicated regions. The probes hybridized to duplicate sites within and outside the Hor1/Hor2 interval. Identification of duplicate sequences in this interval has been previously reported for sites within the Hor1 and Hor2 loci (Forde et al. 1985), and Schondelmaier et al. (1993) observed a duplicated segment, separated by 20.2 cM, on barley chromosome 5S adjacent to the Hor1/Hor2 interval. We observed several duplicate DNA sequences in the Hor1/Xcd0658 segment of the introgressed region. Some pairs of duplicate sequences (Xcd0580.1 and Xcd0580.2) were tightly linked, while other pairs were not linked. Sudupak et al. (1993) hypothesized that there were duplicate sequences carried in some of the Rp1 alleles in maize, which determine resistance to races of the maize rust fungus, Puccinia sorghi. Further analysis of markers in the Rp1 region revealed an additional duplication of the closely linked NPl285 RFLP locus (Hong et al. 1993).

In addition, Paran et al. (1992) observed duplicate sequences associated with the glycolytic enzyme, triose phosphate isomerase (TPI). The duplicate TPI sequences mapped to separate clusters associated with downy mildew resistance. Martin et al. (1993) observed numerous cross-hybridizing sequences at the tomato Pto locus, indicating sequence duplication occurred at this resistance cluster. Although these observations suggest the areas associated with clusters of resistance genes are prone to gene duplication and rearrangement, there is no direct evidence that these processes function at a higher rate in these areas than in other areas of the genome. However, sequence duplication has been postulated as a component of the mechanism by which new disease resistance alleles are generated (Hulbert and Bennetzen 1991; Sudupak et al. 1993).

The homozygous recombinant population was developed by selecting individual lines in which a recombination event had occurred between the Hor1 and Hor2 loci. Although the mapping population is homozygous at the flanking selected markers, Hor1 and Hor2, heterozygous regions internal to the flanking markers were detected in 16 of the lines during RFLP analysis on the mapping population. The presence of internal heterozygous regions was not detected when the flanking markers were screened to select the recombinant population. In six of the sixteen recombinant lines containing internal heterozygous regions, one or more of the adjacent Mla alleles are heterozygous. In addition, nine other recombinant lines are heterozygous at one or more of the Mla alleles (Mahadevappa et al. 1994). Internal heterozygous regions were observed either at the junction of the two parental sequences (type 1), or they occurred inserted into and flanked on both sides by a single parental sequence (type 2) (Fig. 4). Type 1 heterozygous regions are likely the result of an additional recombination occurring in the F1 meiosis (Fig. 1B). Although the presence of the additional recombination events was not unexpected, it was advantageous to resolve the heterozygous regions in order to obtain a single defined recombination site in these lines. To verify this, F1 progeny were selected from selfed F1 plants containing type 1 heterozygous regions to yield true homozygous recombinant lines (Fig. 1C and Fig. 4).

The mechanisms underlying formation of the type 2 heterozygous region are not presently understood. However, these could be the result of a non-crossover or gene conversion event, as several of the type 2 heterozygous regions only affect one locus. Similar unusual events have been observed to accompany unequal exchange and gene conversion in Saccharomyces cerevisiae (Maloney and Fogel 1987), in Drosophila melanogaster (Peterson and Laughnan 1963; Goldberg et al. 1983; Davis et al. 1987; Hipeau-Jacquette et al. 1989), and in maize (Laughnan 1961; Dooner and Kermicle 1970; Sudupak et al. 1993). The lines containing type 2 heterozygous regions are a valuable source of germplasm to further investigate the formation of these regions.

Genetic rearrangements as the result of base mispairing and unequal crossing-over have been suggested to explain a number of unusual recombination events observed using high-resolution genetic analysis on genomic regions containing disease resistant loci (Hulbert and Bennetzen 1991; Ronald et al. 1992; Sudupak et al. 1993). The results of this research have enabled us to obtain more detailed information on the location of recombination events occurring in the Hor1/Hor2 interval flanking the Mla locus. We have defined the recombination sites in each of the 270 lines in our mapping population using both RFLP and disease resistance analysis. We hope to overcome some of the inherent difficulties associated with chromosome walking in cereal crops by utilizing select lines containing defined recombination sites between specific loci.
In the process of developing a high-resolution mapping population and completing a fine structure map of the Horl/Hor2 interval, we have generated recombinants within the Mla complex and flanked it with probes no more than 1.6 cM on either side. The data from these experiments indicate we need to place more markers in this region prior to initiating physical mapping. It is advantageous to have markers mapped within 0.1 cM of each other to determine the relationship between genetic and physical distance. However, with the current markers we should be able to resolve recombination sites between closely linked loci using pulsed-field-gel-electrophoresis analysis. We have 11 lines containing a recombination event between Xbcd249.1 and Xbcd249.2, which are separated by 0.3 cM. We also have 10 lines containing recombination events between Xmwg068 and Xbcd249.2 which are separated by 0.3 cM. The data obtained from these experiments will be used to determine the resolution of the mapping population for physical mapping of this region.

MATERIALS AND METHODS

RFLP probes.

The RFLP probes and their sources used in these experiments are listed in Table 1.

Plant material.

The F2 recombinant mapping population utilized in this study was developed within our research group (Mahadevappa et al. 1994). The recombinant lines selected were derived from a cross between nearly isogenic barley lines developed for reaction to E. graminis f. sp. hordei (Moseman 1972). Initial crosses were constructed between the Franger (C.I. 16151) and Rupee (C.I. 16155) accessions containing the Mla6 and Mla13 alleles, respectively (Moseman 1972). The mapping population was selected by analyzing endosperm extracts from 1,800 F2 seeds for recombinant C and B hordein polypeptide patterns (Fig. 1). These recombinant patterns indicate genetic recombination between the Horl and Hor2 loci, which flank the Mla region. Embryo halves from the selected F2 heterozygous recombinants were planted in the greenhouse to generate recombinant F3 families. The recombinant F3 families were screened to identify homozygous lines, via analysis of the C and B hordein proteins. These F3 homozygous recombinant lines were planted and leaf tissue was harvested for DNA extraction. F3 seeds were collected and analyzed to confirm that these recombinant lines were homozygous.

DNA preparation.

Barley genomic DNA was isolated from leaves of 3- to 5-wk-old greenhouse grown plants using a modified hexadeyl trimethylammonium bromide (CTAB) method (Saghai-Maroof et al. 1984, Wise and Schnable 1994). DNA suspensions were quantified on a Hoefer mini-fluorometer according to manufacturers instructions.

Southern analysis.

For gel blot analysis, 10 µg of DNA was restricted according to suppliers instructions, using the buffer supplied with the enzyme. Gels (20 × 24 cm) were poured using 0.9% SeaKem LE agarose in 250 ml 1× TPE buffer (36 mM Tris-HCl, 30 mM NaH2PO4, 1 mM EDTA). DNA was transferred for 16–20 hr onto Hybond N+ nylon membrane using 20x SSC (1× SSC is 15mM trisodium citrate, 150 mM NaCl) as the transfer solution in a BIORS blotting unit. DNA was fixed to the membranes by treating for 20 min with 0.4 N NaOH followed by a brief wash in 5× SSPE (1× SSPE, 10 mM NaH2PO4, 180 mM NaCl, 1 mM EDTA, pH 7.4) to neutralize the membranes.

Preparation of probe and hybridization.

Probes were radiolabeled by the random hexamer primer method (Feinberg and Vogelstein 1983). Hybridizations and initial washes were done in a Robbins Scientific Hybridization Oven (Robbins Scientific Corporation, Sunnyvale, CA). The blots were prehybridized in 1.0% BSA, 1 mM EDTA, 0.5 M NaHPO4, pH 7.2, 7.0% sodium dodecyl sulfate (Church and Gilbert 1984) for a minimum of 2 hr prior to adding the probe, and then hybridized for 16 hr. Initial washes were done in the hybridization tubes and consisted of two 30-min washes in 1× SSPE, 0.1% SDS, at 65°C followed by transfer to a plastic tray and washed in 1× SSPE, 0.1% SDS for 1 hr at 65°C with shaking. The final wash consisted of 15 min at 65°C in 0.2× SSPE, 0.1% SDS. Membranes were exposed to Kodak X-Omat AR film for 3–7 days at −70°C using two Dupont Cronex Lightening Plus intensifying screens.

Characterization of disease resistance phenotype.

Planting, inoculation with Erysiphe graminis f. sp. hordei, and scoring of disease resistance phenotype for the F2 progeny from selfed F2 lines containing type 1 heterozygous regions were performed as described previously (Mahadevappa et al. 1994).

Data analysis.

RFLP and disease resistance phenotype data were recorded, edited, and transformed into Mapmaker format using the Macintosh compatible program Map Manager (version 2.5), developed by Kenneth Manly at the Roswell Park Cancer Institute (Manly 1993). Map Manager is a program for genetic mapping with selfed recombinant inbred strains or backcrosses. The file output is invaluable for analysis of crossovers and heterozygous regions. The F3 intercross (self) function of Mapmaker/Exp version 3.0b (Lander et al. 1987; Lincoln et al. 1992) was used to analyze the RFLP data to determine linkage. Genetic linkage was calculated by ordering subsets of the linkage group and then overlapping those subsets, mapping any remaining markers relative to those already mapped. The “suggest subset” command was used to produce the initial subset for ordering. Additional subsets were ordered by overlapping existing markers and a framework was produced. The framework was assigned to chromosome 5 and the “place” command was used to position remaining markers within the interval.

The F3 mapping population was selected by screening only for recombinants between Horl and Hor2 and, therefore, was not representative of the entire F3 population. Because the population was not a true F3, but had undergone an additional round of meiosis in the F2, neither the F2 nor F3 analysis functions from Mapmaker 3.0b were considered appropriate for determining the distance between markers in the Horl/Hor2

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interval. To determine distance between markers within the Hor1/Hor2 interval, the distance was calculated between Hor1 and Hor2 in the F2 population. Subsequently, the distribution of recombinants in the F2 was used to define the subintervals within the Hor1/Hor2 interval. Distance between the markers was determined using the following formula:

\[ n \times d / t = s \]

where \( n \) is equal to the number of recombinants in the subinterval between two markers; \( d \) is equal to the distance between Hor1 and Hor2, calculated from the analysis of the F2 population; \( t \) is equal to the total number of homozygous recombinants in the Hor1/Hor2 interval, and \( s \) is equal to the distance between the markers in a particular subinterval.

Markers outside the interval were positioned using a combination of the "place" command along with ordering and overlapping additional subsets. To accurately map markers outside of the Hor1/Hor2 interval, the mapping population was adjusted from 1,800 to 155 or 115, i.e., the size of the primary and secondary subset, respectively. This was done to compensate for the change from a sample of 270 recombinant lines representing a population of 1,800, to a selected sample of 155 or 115 lines used as the mapping population. Analysis on markers outside the interval was done on the primary and secondary data sets consisting of the same 270 individuals used for mapping within the Hor1/Hor2 interval.

ACKNOWLEDGMENTS

We thank Ken Manly for his suggestions on determining subinterval distances, as well as Randy Shoemaker and Tom Blake for their suggestions and reviews of the manuscript. We also thank Carren Dill for her technical assistance with the RFLP analysis. We acknowledge the Iowa State University Computation Center for their support in awarding us a Computing Grant, enabling us to analyze the mapping data. R.A.D. was supported by a USDA-ARS Postdoctoral Research Associateship. Research supported in part by USDA-NRI/CGP grant AMD #800911. Joint Contribution of the Field Crops Research Unit, USDA-Agricultural Research Service, and the Iowa Agriculture and Home Economics Experiment Station. Journal Paper No. J-15473 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project No. 2447.

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