Theor Appl Genet (1993) 85:897–900

THEORETICAL AND APPLIED GENETICS

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Localization of the Laevigatum powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers

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Received April 12, 1992; Accepted June 26, 1992
Communicated by P. M. A. Tigerstedt

Summary. The powdery mildew disease resistance gene *Ml*(*La*) was found to belong to a locus on barely chromosome 2. We suggest that this locus be designated *MlLa*. Linkage analysis was carried out on 72 chromosome-doubled, spring-type progeny lines from a cross between the winter var 'Vogelsanger Gold' and the spring var 'Alf'. A map of chromosome 2 spanning 119 cM and flanked by two peroxidase gene loci was constructed. In addition to the Laevigatum resistance locus the map includes nine RFLP markers, the two peroxidase gene loci and the six-row locus in barley.

Key words: Barley leaf stripe – *Erysiphe graminis* f.sp *hordei* – *Heterodera avena* – Linkage map – *Ml*(*La*)

Introduction

The gene derived from the barley line *Hordeum laevigatum* conditioning resistance to the fungus *Erysiphe graminis* DS. f.sp. *hordei* Em Marshal causing powdery mildew in barley has attracted considerable interest among barley breeders. Two varieties, 'Minerva' and 'Vada', developed from a cross between *Hordeum laevigatum* and var. 'Gold' (Dros 1957), have caused the introduction of this resistance gene into modern barley varieties.

It has been found to be linked to a gene conditioning resistance to barley leaf stripe (*Drechslera graminea*) that is derived from 'Vada' (Haahr et al. 1989) and is often found in combination with other powdery mildew resistance genes in modern barley varieties (Jensen et al. 1992).

Unlike most of the other specific powdery mildew resistance genes in barley, this gene conditions is resistance to *E. graminis* f.sp. *hordei* Em Marshal which is characterized by an intermediate type of reaction. The infection type is modified by genetic background and possibly also by environment, rendering the disease reaction difficult to register (Torp et al. 1978; Röbbelen et al. 1983). This gene has been effective against mildew for a number of years, and it has been speculated that this durability is caused in part by the intermediate reaction type (Jørgenesen 1983). The resistance is reported by Torp et al. (1978) to be due to a single gene designated *Ml*(*La*). No linkage to previously localized powdery mildew resistance genes or other markers has been reported, but linkage analysis of doubled haploid progeny lines using 12 marker loci have shown statistically significant associations to loci on chromosome 2 and 6 (Jensen et al. 1991).

The study presented here reports on the genetical assignment of the Laevigatum gene to barley chromosome 2 and on the development of an RFLP-based linkage map of this chromosome.

Materials and methods

Plant material

Genetical analysis was carried out on 72 chromosome doubled haploid progeny spring lines produced by means of the Bulbosum technique on the F_1 generation of the cross between spring var 'Alf' and winter var 'Vogelsanger Gold' (Doll et al. 1989). Two plants of var 'Alf' and three of var 'Vogelsanger Gold' were used in the crosses. Wheat-barley addition lines (Islam et al. 1981) were employed to determine chromosomal location of the markers, and barley var 'Pallas' was used in the

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construction of a genomic library. Leaves from about 50 greenhouse-grown seedlings of each line were harvested in liquid nitrogen and stored at $-20\,^{\circ}$C until use.

Disease resistance and morphological marker

Two isolates of the barley powdery mildew fungus, HL-3 and JEH28 (see Giese et al. 1990), both avirulent to the Laevigatum resistance, were used. Thirty plants of each of the individual offsping lines were germinated in the greenhouse and tested for mildew resistance at the primary leaf stage. Two independent tests were carried out, the first with isolate HL-3; and the second with the JEH28 isolate. All of the offspring lines were scored for the two- six-rowed character.

DNA preparations and cloning of genomic barley DNA

High-molecular-weight DNA was extracted as described by Sharp et al. (1988). For cloning 'Pallas' DNA was cut to completion with restriction enzyme *Pst*I and ligated into the *Pst*I site of the PUC12 vector using T4 ligase (B.R.L.) (Maniatis et al. 1982; Messing 1983). Prior to transformation the ligated barley PUC12 DNA was given a protease K (Boehringer-Mannheim) treatment. The ligation mix was then used to transform *Escherichia coli* JM83. The transformants were selected by Amp r, Lac-phenotypes and further analysed by alkaline lysis mini-preparations (Maniatis et al. 1982).

Southern blot analysis

Restriction endonuclease digestion was carried out using enzymes and buffers from B.R.L. or Amersham International. Horizontal gel electrophoresis in TRIS-borate buffer and Southern blotting were performed as described in Maniatis et al. (1982). Amersham Hybond N or Hybond N^{+} membranes were used according to the manufacturers' instructions. Hybridization conditions were as described in Giese et al. (1990). DNA inserts, isolated from agarose gels or in gels from plasmid mini-preparations, were labelled by random priming (Feinberg and Vogelstein 1983) using [α^{32}-P]-dATP (Amersham International). Membranes were washed in $2 \times$ SSC (0.3 M NaCl, 0.03 M Na citrate, pH 7.0) and 0.1% sodium dodecyl sulphate (SDS) for 3×15 min at $42\,^{\circ}$C. The filters were exposed to Hyperfilm-MP (Amersham International) for 16 h to 10 days at $-70\,^{\circ}$C. The filters were stripped as recommended by the manufacturers and reprobed up to 10 times.

RFLP markers

DNA of the parent plants, three 'Vogelsanger Gold' and two 'Alf', were digested to completion with restriction endonucleases *Eco*RV, *Bam*HI, *Hin*dIII and *Eco*RV and separated by agarose gel electrophoresis. Eight duplicate filters were made, and individual clones showing polymorphism were selected. Individual parent plants of the two varieties did not show any polymorphism with regard to the markers employed. RFLP markers located on chromosome 2 were kindly provided by T. Blake (Shin et al. 1990) and A. Graner (Graner et al. 1991). The peroxidase cDNA clones *Prx7* (Rasmussen et al. 1991) and *pBT6-3* (Bryngelson and Collinge 1991) were kindly provided by S. K. Rasmussen and D. B. Collinge, respectively.

Segregation analysis

The double haploid progeny lines were scored for RFLP markers using the appropriate combinations of restriction enzyme and clone as determined by the hybridization to the parent filters. Chi-square tests were carried out to verify the 1:1 single gene segregation ratio and to establish the linkage group. The recombination frequencies between all pairs of markers in the linkage group were calculated and subjected to the procedure described by Jensen (1987) for construction of a linkage map.

Nomenclature

The nomenclature system proposed by Hart and Gale (1988) for wheat was adapted for the designation of RFLP markers developed in this study, i.e. Risø markers will be recognized by *Xris*.

Results

Alleles in all of the 13 loci examined showed a 1:1 segregation ratio. Six RFLP markers, *Xris11*, *Xris16*, *Xris21*, *Xris45a*, *Xris45b*, and a barley peroxidase, *Prx7* (Rasmussen et al. 1991), were found to belong to a linkage group that included the Laevigatum resistance gene (Fig. 1). One marker, *Xris16*, was found to be tightly linked (1.4 cM) to the Laevigatum powdery mildew resistance gene (Fig. 1). Hybridization to restriction digests of DNA from the wheat-barley

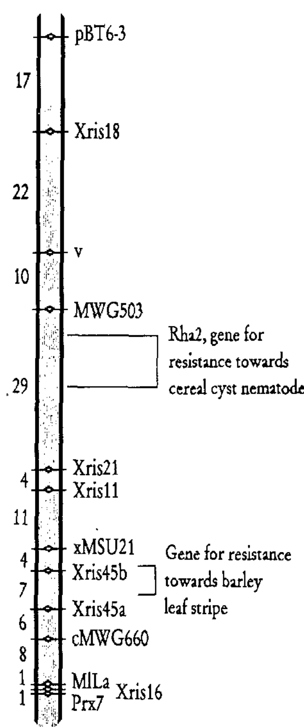

Fig. 1. Linkage map of barley chromosome 2 including RFLP markers, peroxidase gene loci (*pBT6-3* and *Prx7*), the six-row locus (*v*) and the Laevigatum resistance locus (*MlLa*). The likely position of the nematode resistance locus (*Rha2*) and a gene conditioning resistance to barley leaf stripe is indicated

addition lines using the same probes indicated that marker *Xris16* is located on barley chromosome 2.

In order to verify this result, RFLP markers previously assigned to chromosome 2 by Shin et al. (1990) and Graner et al. (1991) were employed. Four chromosome-2 markers were kindly supplied by Blake one of which, *xMSU21*, was informative in the present cross. Linkage analysis showed this marker to belong to the same group as the 6 *Xris* markers (Fig. 1). Two out of 4 chromosome 2 markers (*MWG503*, *cMWG660*), kindly provided by Graner, displayed polymorphism. One of these, *cMWG660*, was found to be closely linked to the Laevigatum resistance gene (Fig. 1). The marker *MWG503* showed association to the *Xris21* marker as well as to the 6-row locus, linking up with *Xris18* and another barley peroxidase gene, *pBT6-3* (Bryngelson and Collinge 1991), expanding the map with about 78 cM (Fig. 1). The weakest point of association on the linkage map, that between *MWG503* and *Xris21*, was highly significant ($P < 0.001$).

Thus 9 RFLP markers, 2 peroxidase loci, the 6-row locus and the Laevigatum barley powdery mildew resistance gene were found to be linked. The linkage group spans 119 cM and belongs to barley chromosome 2.

The two point segregation data is available on request.

Discussion

The assignment of the Laevigatum resistance gene to barley chromosome 2 in the present investigation has been based on different types of evidence. RFLP markers localized to chromosome 2 by Graner et al. (1991) and Shin et al. (1990) showed linkage to a group of newly developed RFLP markers that also linked to the Laevigatum resistance gene. The RFLP marker developed by Shin was localized on the basis of linkage to a group of markers containing a peroxidase isozyme locus and the six-row locus (*v*). The two RFLP markers from Graner were assigned to chromosome 2 on the basis of hybridization to the wheat-barley addition line containing chromosome 2. One of our own RFLP markers, *Xris16*, which is very closely linked to the Laevigatum gene, could also be assigned to chromosome 2 in this way. Linkage to the key marker *v* on chromosome 2 as well as cytology (the addition lines) provide evidence for the localization of nine RFLP markers, two peroxidase loci and the Laevigatum resistance locus to barley chromosome 2. The linkage group spans about 119 cM, and linkage estimates are in accordance with the results of Shin et al. (1990) and Graner et al. (1991). In previous investigations no associations were found when 37 translocation lines including marker genes were analyzed, but segregation analysis of double haploid progeny lines using 12 markers did indicate linkage to either chromosome 2 or 6, the first of which is now confirmed. It is also clear that the Laevigatum barley powdery mildew resistance gene is unrelated to any of the previously located mildew resistance genes, as is also indicated in other studies (Jensen et al. 1991). We suggest that the locus be designated *MlLa*.

In the present study two loci, *Xris21* and *Xris11*, showed a statistically significant association to markers on barley chromosome 5. However, as these markers have been shown to reside in the middle of the linkage groups on chromosome 2, we are confident that this is their correct location. Such conflicting results occur relatively frequently when segregation for a large number of markers is analyzed in a single cross. It is a statistical problem, and we only mention it to illustrate that chance deviations are factors which must be taken into account when constructing linkage maps.

The identification of an RFLP marker closely linked to the Laevigatum resistance gene has practical implications. The expression of this resistance gene is difficult to score on a single plant basis, and in the present study 30 plants of each line were used in the resistance test to ensure correct results. It may thus be more efficient to score for an RFLP marker that requires only a few plants and allows for a number of other RFLP tests. Furthermore, in previous genetical studies the Laevigatum resistance gene has been found linked to a barley leaf stripe resistance locus with about 20% recombination (Haahr et al. 1989). It is likely that one of the newly developed RFLP markers, *Xris45a*, or *Xris45b*, are tightly linked to this gene (Fig. 1), thus providing an easier selection procedure for the otherwise difficult barley leaf stripe resistance test. Segregation data for the gene *Rha2*, which conditions resistance towards the nematode *Heterodera avenae* (Andersen and Andersen 1973) was subjected to the linkage estimation procedure by Jensen (1987). This analysis suggests that the *Rha2* locus is linked to the markers *MWG503*, *Xris 21* and *Xris11*. The use of these RFLP markers will probably be a valuable alternative to the very time-consuming nematode test for resistance (Fig. 1). One other agronomic character has been reported to be associated with the Laevigatum powdery mildew resistance, namely milling energy, which in turn is related to malting quality (Swanston 1987). This is probably due to a QTL, the effect of which may be resolved by the use of the RFLP markers.

The identification of RFLP markers in this seemingly important region of chromosome 2 will facilitate the understanding and development of disease-resistant varieties. Markers very closely linked to a disease resistance gene may by chromosome walking, eventually lead to the isolation of the gene and a biochemical understanding of the resistance mechanism.

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