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RFLP mapping of the *ym4* virus resistance gene in barley

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Abstract. RFLP (restriction fragment length polymorphism) mapping of a recessive gene (*ym4*) conferring resistance to barley yellow mosaic and barley mild mosaic virus was performed using progeny of 86 F_1 anther-derived doubled haploid lines. Two closely linked RFLP markers that flank the gene at a distance of 1.2 centiMorgans were identified. Using one of these markers (MWG10) we obtained a clear differentiation between resistant and susceptible German cultivars. An analysis of a series of unrelated barley lines with probe MWG10 did not reveal additional RFLP fragments. The use of this probe for both marker-assisted selection and the generation of a high-density map around the resistance locus is discussed.

Key words: Restriction fragment length polymorphism – Linkage map – BaYMV – BaMMV

Introduction

Barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) are major pathogens of winter barley in central Europe and Japan. Due to their soil-borne transmission by *Polymyxa graminis* (Adams et al. 1988) the two viruses represent a serious threat to the cultivation of winter barley since plants can not be protected by chemical means. Therefore, the development of disease-resistant cultivars is an indispensable prerequisite for the cultivation of winter barley in infested areas.

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The resistance of European winter barleys to BaMMV and BaYMV rests entirely on a single recessive gene (*ym4*), which confers complete immunity (Friedt and Foroughi-Wehr 1987). More recently, however, a second strain of BaYMV (BaYMV-2) has been detected in Germany that is virulent on hitherto resistant cultivars (Huth 1989), thus necessitating further attempts to breed resistant varieties.

In contrast to this narrow genetic basis of virus resistance in cultivated material, germ-plasm surveys have resulted in the identification of new sources of resistance to BaMMV and BaYMV. Three types of resistance generally occur: (1) resistance only to BaMMV, (2) resistance to both BaMMV and BaYMV-1, and (3) resistance to BaMMV, BaYMV-1, and BaYMV-2 (Ordon et al. 1992). Several independently inherited genes have been identified, most of them in germ-plasm originating from Japan or China (Ordon and Friedt 1993). Although the respective lines provide an appropriate starting material to combine individual resistance genes, introgression into adapted material requires time-consuming greenhouse or field tests that sometimes prove unreliable at the single plant level. Because of the recessive nature of most of the genes conferring resistance to BaYMV and/or BaMMV, heterozygous carriers can not be identified at the phenotypic level. Hence, efficient breeding strategies require both a refined knowledge of the genetic basis of resistance and improved diagnostic procedures. The availability of saturated restriction fragment length polymorphism (RFLP) maps of the barley genome (Graner et al. 1991; Heun et al. 1991) now provides a means for the detailed genetic location of genes and the identification of closely linked, selectable markers.

In this report we describe RFLP mapping of the *ym4* gene conferring resistance to BaMMV and

BaYMV using progeny of 86 anther-derived doubled haploid barley lines and the characterization of a RFLP marker useful for marker-assisted selection.

Materials and methods

Plant material

Genetic data are based on progeny comprising 86 F_1 anther-derived, doubled haploid (DH) barley lines of a cross between the winter barley cvs 'Igri' (susceptible) and 'Franka' (resistant). In vitro regeneration of anthers was conducted as described by Foroughi-Wehr and Friedt (1984). Resistance tests were performed in two replications. In each replication five plants of a DH-line were subjected to mechanical inoculation with BaMMV according to the procedure described by Friedt (1983). The presence or absence of BaMMV was tested by ELISA using antibodies kindly supplied by W. Huth (Braunschweig, Germany).

RFLP and linkage analysis

DNA extraction, digestion, and hybridization were performed according to Graner et al. (1991). RFLP probes originate from several genomic *Pst*I libraries (prefix "MWG") or a cDNA library generated from leaf mesophyll RNA (prefix "cMWG") as described in Graner et al. (1990, 1991). Linkage analysis was conducted using Mapmaker computer software (Lander et al. 1987). Crossover units were converted into map distances (centi-Morgans, cM) by applying the Kosambi function (Kosambi 1944).

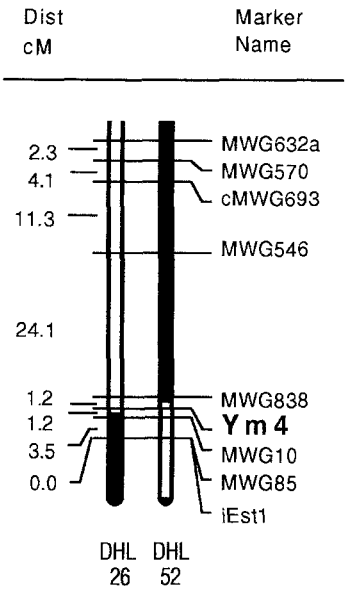

Fig. 1. Distal portion of the RFLP map of the long arm of barley chromosome 3H. Genetic distances are based on an analysis of 86 DH-lines (esterase locus iEst1, 71 DH-lines) and given in centiMorgans (*cM*). Graphical genotypes of DH-lines 26 and 52, each carrying a recombination between Ym4 and a flanking RFLP locus, are indicated. *Black bars* represent chromosomal segments derived from 'Igri' (susceptible), *white bars* those from 'Franka' (resistant). Recombination breakpoints are assumed to be in the *middle* between two loci

Results

Localization of the ym4 gene

Single locus segregation of the *ym4* gene fitted the expected 1:1 ratio ($\chi^2 = 0.19$, $P = 0.25$–0.5). Five RFLP probes located on the long arm of chromosome 3H displayed linkage to the *ym4* gene with genetic distances between 1.2 cM and 25.5 cM (Table 1).

Using multipoint analysis we included the Ym4 locus into our RFLP map where it is located near the distal end of chromosome 3HL (Fig. 1), flanked at a genetic distance of 1.2 cM by two RFLP markers. Each probe detected only one recombination event with the resistance locus among the 86 meioses analyzed in this study. Both recombinants occurred, however, in different DH-lines. The correct single locus segregation and the nearly additive genetic distances (cf. Table 1, Fig. 1) provide evidence for a correct evaluation of the phenotypic (resistance) data.

Characterization of a RFLP probe closely linked to the ym4 gene

Because of its clear RFLP pattern and its tight linkage to the *ym4* gene, probe MWG10 was selected to examine a number of barley cultivars that are susceptible or carry the *ym4* gene. As is shown in Fig. 2, the discrimi-

Table 1. Two-point recombination values (given in cM, above diagonal) and chi-square contingency values (below diagonal) of RFLP loci linked to the Ym4 resistance locus

	MWG546	MWG838	Ym4	MWG10	MWG85
MWG546		22.9 ± 5.5	25.5 ± 5.9	27.1 ± 6.2	27.5 ± 6.3
MWG838	27.4		1.2 ± 1.2	2.4 ± 1.6	5.9 ± 2.6
Ym4	23.8	81.1		1.2 ± 1.2	4.7 ± 2.3
MWG10	21.7	77.2	82.1		3.5 ± 2.0
MWG85	21.0	66.2	69.8	73.5	

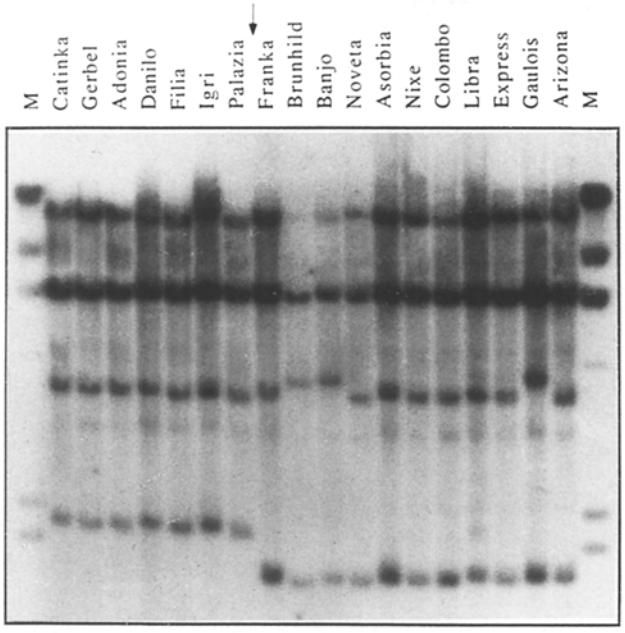

Fig. 2. Southern analysis using probe MWG10 of *Bam*HI-digested DNA from susceptible (*left of arrow*) and resistant (*right of arrow*) barley cultivars *M*, DNA size marker λ *Hin*dIII (23.1, 9.4, 6.7, 4.4, 2.3, 2.0 kbp)

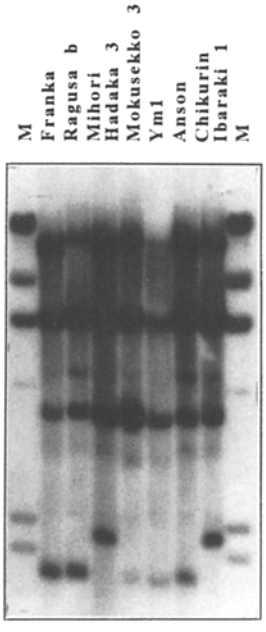

Fig. 3. Southern analysis using probe MWG10 of *Bam*HI-restricted DNA of BaMMV/BaYMV resistant barley cultivars representing a cross section of unrelated germ-plasm. 'Chikurin Ibaraki 1', 'Mihori Hadaka 3', 'Resistant Ym No. 1' (Ym1) (Japan); 'Mokusekko 3' (China); 'Franka' (Germany); 'Ragusa b' (Yugoslavia); 'Anson' (USA). *M*, DNA size marker λ *Hin*dIII

nation obtained by the hybridization pattern agreed perfectly with the phenotype (resistant/susceptible) of the cultivars tested. All resistant plants showed an allele approximately 1.8 kb in size, while all susceptible plants shared an allele of a higher molecular weight (ca. 2.2 kb). To examine the variability of the restriction fragments detected at the MWG10 locus, unrelated, BaMMV-resistant barley cultivars from a wide range of origins were tested (Fig. 3). Again, only two alleles were detected at the RFLP locus. The cv 'Ragusa b', which is considered to be a candidate donor of the BaMMV resistance gene in European material, displayed the typical *ym4*-related resistance allele. Correspondingly, cv 'Chikurin Ibaraki 1', which carries a recessive-resistance gene that is unlinked to the Ym4 locus (Götz 1991), was characterized by the absence of the 1.8-kb band. No correlation between this band and the genotype at the Ym4 locus was observed for cvs 'Anson' and 'Mihori Hadaka 3'. The first variety displayed the 1.8-kb despite containing a resistance gene non-allelic to *ym4* and vice versa this band was absent in 'Mihori Hadaka 3', which in turn contains several recessive resistance genes, including *ym4* (Götz 1991). 'Resistant Ym No. 1' and 'Mokusekko 3' displayed only weak bands of 1.8 kb, which are presumably the result of an inhomogeneity in the respective cultivar (see below).

Discussion

RFLP mapping in this study confirms previous results about the assignment of the Ym4 locus to the long arm of chromosome 3H by means of trisomic and telotrisomic analysis (Kaiser and Friedt 1989, 1992). Similar to the Ym1 resistance locus that is present in the cvs 'Mokusekko 3' and 'Resistant Ym No. 1', the Ym4 locus is closely linked to the Est1 locus. The map distance between Est 1 and Ym4 corresponds to that determined for the Est1-Ym4 interval by Konishi et al. (1989) and Kawada (1991). This is in accordance with the assumption that *ym1* and *ym4* are either allelic or tightly linked genes (Friedt and Foroughi-Wehr 1987). In fact, probe MWG10 detected the *ym4*-related RFLP fragment in both Ym1-carrying cultivars analyzed in this study ('Mokusekko 3', 'Resistant Ym No. 1') It should be noted, however, that RFLP analysis of four different accessions of 'Mokusekko 3' and three different accessions of 'Resistant Ym No. 1' with this probe revealed lines carrying a null allele, which correlated with a resistant phenotype (results not shown). This inhomogeneity might contribute to the difficulties encountered in determining the number of resistance genes present in 'Mokusekko' and 'Resistant Ym No. 1' (Götz 1991).

RFLP analysis using probe MWG10 showed a perfect discrimination between resistant and susceptible European cultivars, which is not unexpected because of its tight linkage to the Ym4 locus. It has previously been postulated from the analysis of pedigree data and resistance testings that the Dalmatian landrace 'Ragusa' represents the source of the ym4 resistance in German varieties (Huth 1985). The occurrence of the resistance-specific RFLP allele in 'Ragusa b', a breeding line from this landrace, lends further strength to this hypothesis. With respect to the cvs 'Anson' (USA) and 'Mihori Hadaka 3' (Japan) the presence of the 1.8-kb RFLP band is not indicative of the presence of the *ym4* gene. At this time, this observation can only be explained as being the result of a (rare) crossover event in the chromosomal interval between MWG10 and Ym4. Nevertheless, marker MWG10 seems to be appropriate for marker-assisted selection in practical breeding programs because (1) it is closely linked to the Ym4 locus and (2) the probe, although not derived from a single-copy sequence, displays a fairly stable hybridization pattern, even if it is used in un-related germplasm.

The use of this probe in combination with genomic fingerprinting and haploid techniques will facilitate a strong selection in backcross programs, which is required in order to disrupt the apparent linkage between *ym1/ym4* resistance and susceptibility to leaf spots (Foroughi-Wehr and Wenzel 1990). RFLP mapping of further resistance genes will facilitate the combination of individual resistance genes by marker-assisted selection without time-consuming progeny tests.

The inclusion of the Ym4 locus into the RFLP map of chromosome 3H provides the basis for a more detailed molecular characterization of the resistance gene. This approach requires a high-resolution map based on a large number of progeny, far exceeding the 86 DH-lines that were used in this study. The use of the two flanking markers allows the rapid selection of plants carrying crossovers in close proximity to the locus, thus leading to a drastic reduction in the number of plants needed to be analyzed at the phenotypic level in order to construct a high-resolution map (e.g., in the present case more than 97% of all the progeny plants are not expected to be informative for the chromosomal segment between MWG10 and MWG838). To further saturate the map of the chromosomal segment comprising the *ym4* gene, work is in progress to select RFLP clones derived from sub-genomic libraries generated via the microdissection of barley chromosomes (Schondelmaier et al. 1993).

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