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Molecular mapping of novel resistance genes against Barley Mild Mosaic Virus (BaMMV)

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Abstract In the present study three novel genes from barley accessions 10247 (*ym8*), Bulgarian 347 (*ym9*), and Russia 57 (*ym11*), which confer resistance to Barley Mild Mosaic Virus (BaMMV), were mapped using molecular markers. Bulked segregant analysis of four progenies segregating for resistance to BaMMV was followed by fine-scale mapping of the resistance genes using individual F_2 or BC_1F_2 plants. The resistance genes are inherited recessively and are located on the long arm of barley chromosome 4HL. A series of closely linked molecular markers are available for marker-assisted breeding programs. A marker (MWG2134) linked with resistance gene *ym11* from Russia 57 was identified, which is diagnostic for the resistance gene.

Key words Barley Mild Mosaic Virus (BaMMV) · *Hordeum vulgare* · Molecular markers · Marker-assisted selection · Genetic mapping

Introduction

Barley Yellow Mosaic Virus (strains BaYMV-1 and BaYMV-2) and Barley Mild Mosaic Virus (BaMMV) cause one of the major diseases in European winter barley. Virus particles are transmitted into the root cells of susceptible plants via the fungal vector *Polymyxa graminis* (Toyama and Kusaba 1970; Adams et al. 1988). Due to the soil-borne nature of the pathogen the only way to prevent yield losses involves the cultivation of resistant varieties. Resistance of European winter-barley germplasm rests mainly on the recessive *ym4* gene, which has been mapped on the long arm of chromosome 3H using RFLP and RAPD markers (Graner and Bauer 1993; Weyen et al. 1996). However, this resistance gene is only effective against BaMMV and BaYMV-1 and has been overcome by a new strain, BaYMV-2 (Huth 1989). Evaluation of a large number of exotic accessions revealed various resistant lines displaying different specificity against the individual members of the Barley Yellow Mosaic Virus complex (Götz and Friedt 1993; Ordon and Friedt 1993). Also, a series of resistant accessions originating mainly from Japan and China have been described which carry the resistance genes *ym1* (Konishi et al. 1997), *Ym2* (Takahashi et al. 1973), *ym3* (Ukai 1984), and *ym5* (Graner et al. 1995; Konishi et al. 1997); as yet, however, little information is available on the localization of these genes in molecular linkage maps.

In an attempt to investigate the genetic basis of resistance to these bymoviruses, segregating populations have been scored for resistance to BaMMV and subsequently examined using bulked-segregant analysis. Linkage maps have been constructed around the corresponding virus resistance genes, since the availability of molecular markers flanking the genes of interest provides a very efficient tool for their rapid introgression into adapted germplasm by marker-assisted selection.

Materials and methods

Plant material

Four crosses involving three different BaMMV-resistant varieties and three susceptible cultivars were used for genetic analysis

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Table 1 Progenies studied for genetic analysis of resistance to BaMMV

Cross	Resistant parent	Resistance to	Susceptible parent	No. of progeny plants
1101	10247	BaMMV, BaYMV-1	Trixi	108 F_2
1132	Bulgarian 347	BaMMV	Alraune	63 F_2
1289	Russia 57	BaMMV, BaYMV-1, BaYMV-2	Magie	48 F_2
1292	F_2 plant from Russia 57 × Alraune	BaMMV, BaYMV-1, BaYMV-2	Alraune	101 BC_1F_2

(Table 1). Previous analyses demonstrated that the BaMMV resistance genes of Bulgarian 347, accession 10247 and Russia 57 are not allelic (Ordon and Friedt 1993).

Resistance testing

Mechanical inoculation with BaMMV was performed at the 4–5 leaf stage as described earlier (Ordon and Friedt 1993). Briefly, inoculum was prepared from leaves of infected plants (cv Gerbel) by adding carborundum (mesh 300) to the plant sap which was diluted (1 : 10) with K_2HPO_4 buffer (0.1 M, pH 9.1). After spraying the two youngest leaves with a spraygun, plants were grown for 4 weeks at approximately 12°C. The presence of virus particles was assessed by DAS-ELISA according to Clark and Adams (1977) using a BaMMV-specific antiserum (provided by Dr. W. Huth, Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany). Plants were scored for resistance in the F_2 for the first time and ten progenies of each F_2 plant were re-tested in the F_3 in order to confirm the results based on a single F_2 plant and to differentiate homozygous and heterozygous carriers of the genes.

Molecular mapping

For RFLP analysis, DNA extraction, digestion and hybridization were carried out essentially as described previously (Graner et al. 1991), except for substituting the restriction endonuclease *Sac*I by *Dra*I. Phenotypic pools (bulks) for bulked segregant analysis (Michelmore et al. 1991) comprised equal amounts of DNA from ten susceptible and ten resistant F_2 plants, respectively for crosses 1101, 1132 and 1289. Pools for cross 1292 consisted of DNA from 15 plants each. RAPD primer screening and RAPD analyses followed the protocol described by Ordon et al. (1995). Amplification using the SSR (simple sequence repeat) primer pair HVM3 (Saghai Maroof et al. 1994) was carried out in a 25-µl reaction volume containing 50 ng of DNA, 1 × reaction buffer (Pharmacia, 1.5 mM $MgCl_2$), 100 µM of each dNTP, 200 nM of each primer, and 1 U of *Taq* DNA polymerase (Pharmacia). PCR reactions were performed in a Hybaid Omn-E cycler using the following conditions: the start denaturation (94°C for 3 min) was followed by 35 cycles of amplification (94°C for 30 s, 55°C for 30s and 72°C for 1 min) and by a final extension step (72°C for 5 min). Amplification products were separated on 1.8% agarose gels (MetaPhor™, Biozym) and stained with ethidium bromide. Linkage analysis was carried out using MAPMAKER (Lander et al. 1987) and MAPMANAGER (Manly 1993) computer software.

Results

Resistance tests and genetic mapping

In a first step the resistance reaction of F_2 plants against BaMMV was monitored. In all crosses the segregation displayed a 1 : 3 ratio (resistant : susceptible) as expected from previous experiments which indicated the presence of single recessive genes for all of the resistant parents (Ordon and Friedt 1993). Since infection rates varied between 90 and 95%, the progeny of resistant F_2 plants was re-tested in the F_3 generation in order to clarify whether resistant plants had escaped infection. Segregation ratios for resistance to BaMMV are summarized in Table 2.

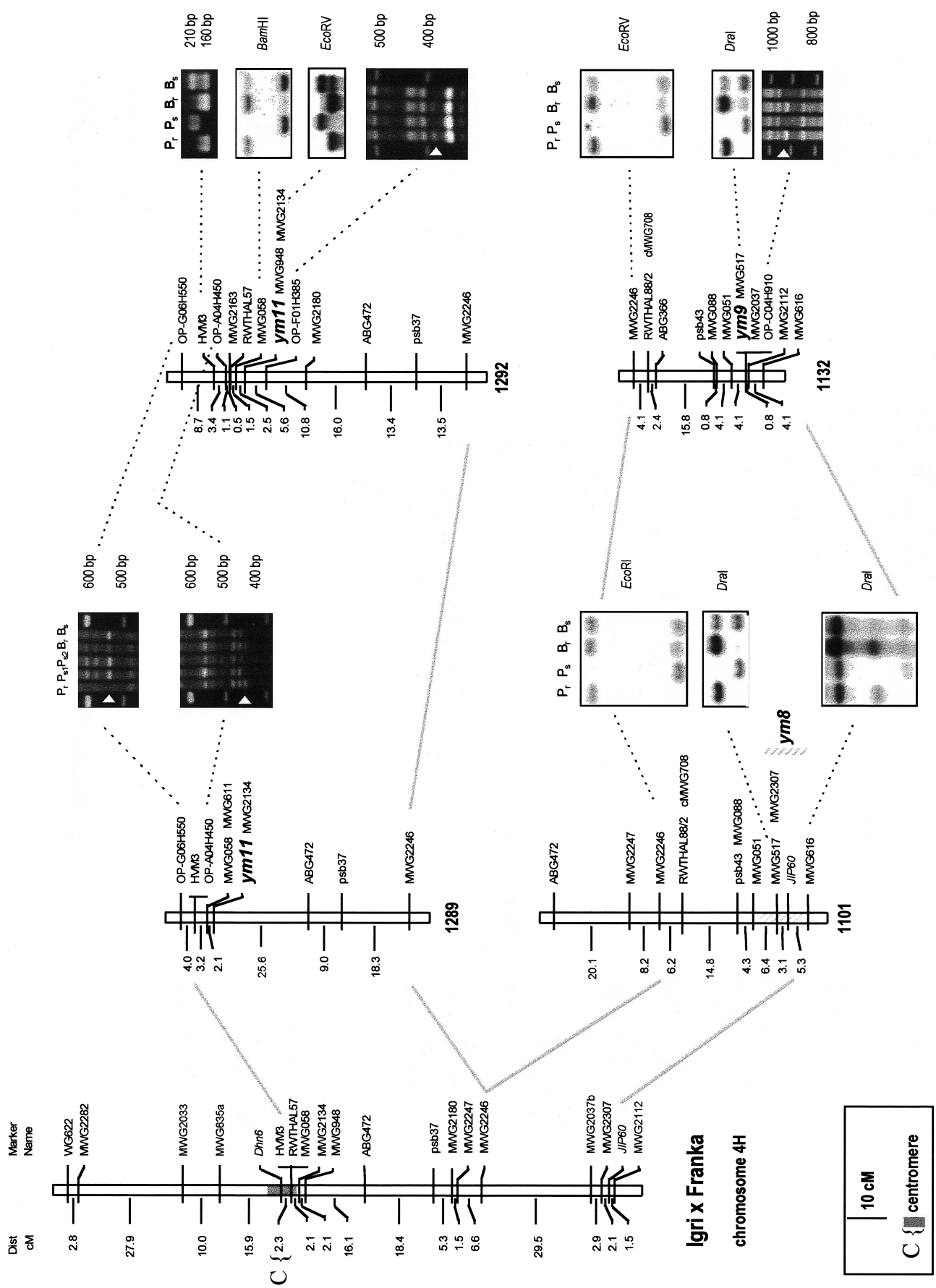
Table 2 Segregation ratios for BaMMV resistance

Cross	Segregation F_2/F_3 r : s	χ^2 1r : 3s	Infection rate (%)
1101	29 : 78[a]	0.25	95
1132	16 : 47	0.005	94
1289	12 : 36	0.00	90
1292	18 : 83	2.77	95

[a] Results only from F_2 generation

According to the resistance data from the F_2-generation, phenotypic pools were assembled as described above. In a first step, previously mapped molecular markers were used for a RFLP scan over the whole genome until a region was identified in each cross where the markers showed a clear differentiation between resistant and susceptible pools (for bulked-segregant results for selected markers see Fig. 1). As expected for recessive genes, closely linked markers detect only the allele of the resistant parent in the

Fig. 1 Molecular linkage maps of the chromosomal regions harbouring the resistance genes *ym11* (crosses 1289 and 1292), *ym8* (cross 1101), and *ym9* (cross 1132). The approximate position of *ym8* is indicated by *hatched bars* in the map of cross 1101. As a reference, a skeletal map of Igri × Franka chromosome 4H (cf. Graner et al. 1994) is shown on the left side, connecting common markers with *solid grey lines* between the different crosses. The interval which covers the centromere is *shaded grey* in the Igri × Franka map. Results from bulked segregant analysis are presented for selected markers, indicated by *black dotted lines*. P_r, P_s: resistant and susceptible parents of the corresponding cross (for markers OP-G06H550 and OP-A04H450: P_r = Russia 57, P_{s1} = Alraune, P_{s2} = Magie); B_r, B_s: resistant and susceptible bulks. In the case of RAPD markers the *first and last lane* (not labelled) of each gel is part of a 100-bp ladder with the corresponding fragment sizes indicated on the right side. A *white arrowhead* shows the differentiating RAPD fragment. The sizes of the two alleles of SSR marker HVM3 are presented in cross 1292. Restriction enzymes are listed near the autoradiographs of RFLP markers


Igri x Franka
chromosome 4H
Dist cM
Marker Name
10 cM
C centromere
1289
1292
1101
1132
ym11
ym8
ym9
EcoRI
DraI
BamHI
EcoRV
600 bp
500 bp
400 bp
210 bp
160 bp
1000 bp
800 bp

resistant pool (since those plants must be homozygous resistant), whereas in the susceptible pools which consist of homozygous and heterozygous susceptible plants both parental alleles are present. The appearance of faint bands from the susceptible parent in the resistant pools is due to the fact that up to 20% "escapes" were included in the resistant pools, as revealed upon testing of F_3 families (e.g. MWG517 in Fig. 1, cross 1132). The level of polymorphism in all four crosses was comparably high for intraspecific crosses (cf. Graner et al. 1990; Melchinger et al. 1994) and ranged from 63% in cross 1289 to 79% in cross 1101; however, only pre-selected probes have been used. As soon as the genomic region harbouring the resistance gene was detected, genetic mapping was performed by analysing individual F_2 plants.

Resistance gene *ym9* (Bulgarian 347)

The accession Bulgarian 347 is resistant to BaMMV only. A single recessive gene designated *ym9* was mapped in a F_2 population comprising 63 plants (cross 1132). It is located in the distal region of chromosome 4HL where it co-segregates with two RFLP markers (MWG517 and MWG2037a) and a dominant RAPD marker (OP-C04H910; Fig. 1, cross 1132) which is linked in coupling phase.

Resistance gene *ym11* (Russia 57)

Russia 57 confers resistance to all European strains of BaMMV and BaYMV. For genetic mapping of the recessive BaMMV resistance gene of Russia 57 two separate populations (crosses 1289 and 1292) were analysed. The level of polymorphism in cross 1289 was lower than in cross 1292, therefore a smaller number of RFLP probes could be mapped. The resistance gene has been localized on the long arm of chromosome 4H in a proximal position co-segregating with two RFLP markers (MWG948 and MWG2134, Fig. 1, crosses 1292 and 1289). A total of four PCR-based markers have been identified in the *ym11* region. The co-dominant SSR marker HVM3 has been mapped in both populations at a distance of 5.3 cM (cross 1289) and 9 cM (cross 1292), respectively. Two dominant RAPD markers segregated in both progenies, OP-G06H550 being linked to *ym11* in repulsion and OP-A04H450 being linked in coupling phase. Linkage in coupling phase was also observed for RAPD marker OP-F01H385 which was only polymorphic in cross 1292.

Resistance gene *ym8* (accession 10247)

The accession 10247 originates from the territory of former Yugoslavia and confers resistance to BaMMV and BaYMV-1 in infested fields. During bulked-segregant analysis the recessive *ym8* gene was localized in the distal region of chromosome 4HL in the map interval between MWG051 and MWG616 (Fig. 1, cross 1101). However, it was difficult to determine the exact map position of the gene during further genetic mapping due to five double-crossover events involving the resistance locus. Analysis of graphical genotypes indicated that two F_2 plants may have escaped the mechanical infection procedure. On the other hand, three F_2 plants could represent "false positives". This assumption is based on the observation that in resistance tests almost all F_3 families derived from resistant F_2 plants (24 out of 29 F_3 families which have been examined) either segregated or else were completely susceptible. In a control experiment the infection progress was monitored weekly over a 8-week period in 10247 (*ym8*), cv Franka (*ym4*, resistant to BaMMV and BaYMV-1), and the two susceptible control varieties Corona and Igri. Interestingly, virus multiplication in 10247 was only slightly delayed in comparison to the susceptible control plants, indicating that in cultivars carrying *ym4* and *ym8* there may be different resistance mechanisms with regard to BaMMV resistance.

Diagnostic molecular markers for marker-assisted breeding strategies

Several DNA markers have been identified which are useful for marker-assisted selection in breeding programs for the introgression of resistance genes from donor lines into adapted germplasm. RAPD markers OP-A04H450 and OP-F01H385 are linked to *ym11* in Russia 57 in the coupling phase and therefore are suitable for selecting homozygous-resistant as well as heterozygous-susceptible plants, avoiding the necessity of two backcrossing steps which would be indispensable to identify carriers of recessive genes in classical breeding programs. Similarly, RAPD marker OP-C04H910 is useful for the identification of lines carrying the *ym9* gene (Bulgarian 347) in the homozygous or heterozygous state. On the other hand, OP-G06H550 is linked to *ym11* in repulsion and hence allows selection of homozygous-resistant lines which are characterized by the lack of the RAPD fragment. In this case, however, samples that failed to amplify can not be differentiated from resistant plants. Therefore, stringent controls have to be included into the experimental design.

For some of the RFLP markers which are linked to *ym8*, *ym9* and *ym11*, the alleles in a number of varieties carrying different virus resistance genes were compared. Figure 2 shows the hybridization pattern of MWG2134 (linked to *ym11*). Upon digestion of the DNA with the restriction enzyme *Dra*I, Russia 57 displays a unique restriction fragment (approximately

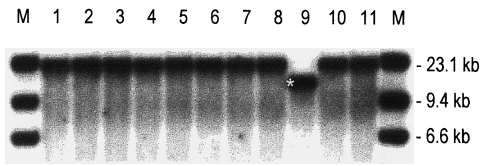


Fig. 2 Hybridization pattern of MWG2134 in different susceptible and resistant cultivars/accessions (*Dra*I digestions). The diagnostic fragment is labelled with an *asterisk*. *Lane 1*: Igri (susceptible), *2*: Franka (*ym4*), *3*: breeding line W122/37.1 (*ym5*), *4*: Iwate Omugi 1 (*ym4*), *5*: H. Hor 3365 (*ym7*), *6*: Bulgarian 347 (*ym9*), *7*: 10247 (*ym8*), *8*: Hiberna (*ym10*), *9*: Russia 57 (*ym11*), *10*: Mihori Hadaka (*Ym2*), *11*: Magie (susceptible), *M*: molecular-weight marker $\lambda \times Hin$dIII

14.5 kb) compared to the other resistant or susceptible varieties.

Discussion

Immediate information on the allele composition at a given locus can be obtained by using flanking, preferrably co-dominant, molecular markers. A large number of mapped RFLP markers are available for barley and other *Triticeae* genomes (cf. GrainGenes Database, http://wheat.pw.usda.gov/graingenes.html) facilitating the mapping of simply inherited traits in segregating populations. In the present study, a series of previously mapped RFLP markers was localized together with four RAPD markers and one microsatellite marker in close linkage to resistance genes effective against Barley Mild Mosaic Virus (BaMMV). Molecular markers can be effectively integrated in backcross breeding programs facilitating selection for the trait(s) of interest directly at the DNA level, thereby avoiding environmental influences. In barley, a large number of resistance genes against fungal and viral diseases have already been tagged by molecular markers (for reviews see Graner 1996; Ordon et al. 1997) including resistance genes against Barley Yellow Dwarf Virus (Collins et al. 1996) and Barley Yellow Mosaic Virus - 1/Barley Mild Mosaic Virus (Graner and Bauer 1993).

With regard to backcross breeding programs, molecular markers are extremely useful when the genes of interest are inherited recessively. In order to select at the phenotypic level, two selfing steps are necessary to obtain the desired genotype, whereas co-dominant molecular markers allow the identification of heterozygous carriers after the first selfing step, which can then be subsequently used for further backcrossing. Besides the introgression of single resistance genes into adapted germplasm, or marker-assisted selection in conventional breeding programs, molecular markers may be of great value for the "pyramiding" of resistance genes with the same or similar specifities against a single pathogen within one genotype. In rice (*Oryza sativa* L.) the accumulation of resistance genes against bacterial blight (*Xanthomonas oryzae* pv *oryzae*) in one genotype has demonstrated that a broader spectrum of resistance than that observed in either parent can be achieved (Yoshimura et al. 1995). In the case of resistance to members of the Barley Yellow Mosaic Virus complex, pyramiding of resistance genes can only be achieved using marker-assisted strategies, preferrably with diagnostic markers, since differentiation between "single" and "multiple" resistant lines is almost impossible at the phenotypic level without extensive progeny testing.

Molecular markers are indispensable tools for the molecular analysis of genes and they can greatly enhance the progress in plant breeding in conventional and backcross breeding programs. However, RFLP analysis still requires relatively large amounts of DNA and either the use of radioisotopes or a relatively sensitive non-radioactive technique. Therefore, the method of choice will be the use of PCR-based markers like RAPDs (preferrably converted into easy reproducible sequence-characterized amplified regions, SCARs, e.g. Nair et al. 1996), sequence-tagged-site markers (STS) derived from RFLP-probes (Tragoonrung et al. 1992), AFLPs (Vos et al. 1995; Becker et al. 1995; Qi and Lindhout 1997), or microsatellites (Liu et al. 1996). Together with increasing sequence information of the barley genome a large number of PCR-based markers will be available in the near future, in some cases allowing multiplex PCR assays for time-saving, economic analyses. The increasing number of molecularly tagged genes of agronomic importance should provide a powerful tool for marker-assisted selection strategies in barley breeding.

There may be differences regarding the mechanisms of resistance between individual resistance genes against the BaYMV complex. This can be deduced from the observation that some resistant lines, such as 10247 or H.Hor 3365 (Graner et al., in preparation), are resistant in the field under natural infection conditions but become infected in the greenhouse upon mechanical inoculation of the leaves. In the first case, the virus particles seem to be unable to infect the plant via the roots; alternatively, inhibition of virus multiplication in root cells or virus movement in the vascular system could be hampered. Obviously this barrier is not present in the latter case, where the pathogen can propagate and move from the leaves to the roots in the phloem. Schenk et al. (1995) concluded from their experiments that cell-to-cell movement is not likely to play a major role for virus transport from roots to leaves, and that movement of BaMMV/BaYMV in the phloem from roots to shoots only takes place under conditions of stress (e.g. low temperatures in the winter). In a study of different sources of resistance against Soilborne Wheat Mosaic Virus (SBWMV), a member of the furoviruses, Rumjaun et al. (1996) identified resistance genes on different *Thinopyrum intermedium* addition

chromosomes which apparently affected different steps during the pathogenesis of wheat plants infected with SBWMV via viruliferous *Polymyxa graminis* zoospores. While in one resistant addition line no virus particles could be detected, either in leaves or in roots, virus particles were present in a second resistant line in roots, but transport to shoots seemed to be inhibited. Since a similar differentiation of resistance mechanisms could be present in barley the combination of diverse resistance genes within one genotype may also prevent the evolution of new resistance-breaking strains of the pathogen.

In order to further characterize the molecular mechanisms of resistance to pathogens high-density molecular linkage maps can be a starting point for cloning of the corresponding genes via a map-based cloning approach. This strategy has been applied by Büschges et al. (1997) for the isolation of the *mlo* gene in barley, and a map-based cloning approach exploiting the synteny between barley and rice has been attempted for the *Rpg1* rust resistance gene by Kilian et al. (1995). A high-resolution mapping population has been established for the *ym4* virus resistance locus on barley chromosome 3HL, aimed at the map-based isolation of the *ym4* gene (Bauer and Graner 1995). The molecular dissection of this virus resistance locus will yield information on the genomic organization of this class of resistance genes as well as providing insights into the principles of plant-virus interactions.

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References

Adams MJ, Swaby AG, Jones P (1988) Confirmation of the transmission of barley yellow mosaic virus (BaYMV) by the fungus *Polymyxa graminis*. Ann Appl Biol 112:133–141

Bauer E, Graner A (1995) Basic and applied aspects of the genetic analysis of the *ym4* virus resistance locus in barley. Agronomie 15:469–473

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van de Lee T, Groenendijk J, Töpsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley *mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Clark MJ, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34:475–483

Collins NC, Paltridge NG, Ford CM, Symons RH (1996) The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. Theor Appl Genet 92:858–864

Götz R, Friedt W (1993) Resistance to the barley yellow mosaic virus complex – differential genotypic reactions and genetics of BaMMV-resistance of barley. Plant Breed 111:125–131

Graner A (1996) Molecular mapping of genes conferring disease resistance: the present state and future aspects. In: Scoles G, Rossnagel B (eds) Proc Vth Int Oat Conf and VIIth Int Barley Genet Symp Inv Papers, Saskatoon, Canada, University Extension Press, Saskatoon, Sakatchewan, pp 157–166

Graner A, Bauer E (1993) RFLP mapping of the *ym4* virus resistance gene in barley. Theor Appl Genet 86:689–693

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Graner A, Bauer E, Kellermann A, Kirchner S, Muraya JK, Jahoor A, Wenzel G (1994) Progress of RFLP-map construction in winter barley. Barley Genet Newslett 23:53–59

Graner A, Bauer E, Kellermann A, Proeseler G, Wenzel G, Ordon F (1995) RFLP analysis of resistance to the barley yellow mosaic virus complex. Agronomie 15:475–479

Huth W (1989) Ein neuer Stamm des Barley Yellow Mosaic Virus (BaYMV) gefunden. Nachrichtenbl Dtsch Pflanzenschutzd 41:6–7

Kilian A, Kudrna DA, Kleinhofs A, Yano M, Kurata N, Steffenson B, Sasaki T (1995) Rice-barley synteny and its application to saturation mapping of the barley *Rpg1* region. Nucleic Acids Res 23:2729–2733

Konishi T, Ban T, Iida Y, Yoshimi R (1997) Genetic analysis of disease resistance to all strains of BaYMV in a Chinese barley landrace, Mokusekko3. Theor Appl Genet 94:871–877

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Liu Z-W, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Manly KF (1993) A Macintosh program for storage and analysis of experimental genetic mapping data. Mammalian Genome 4:303–313

Melchinger AE, Graner A, Singh M, Messmer MM (1994) Relationships among European barley germplasm. I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci 34:1191–1199

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using bulked segregant analysis. Proc Natl Acad Sci USA 88:9828–9832

Nair S, Kumar A, Srivastava MN, Mohan M (1996) PCR-based DNA markers linked to a gall midge resistance gene, *Gm4t*, has potential for marker-aided selection in rice. Theor Appl Genet 92:660–665

Ordon F, Friedt W (1993) Mode of inheritance and genetic diversity of BaMMV resistance of exotic barley germplasms carrying genes different from '*ym4*'. Theor Appl Genet 86:229–233

Ordon F, Bauer E, Friedt W, Graner A (1995) Marker-based selection for the *ym4* BaMMV-resistance gene in barley using RAPDs. Agronomie 15:481–485

Ordon, F, Wenzel W, Friedt W (1997) II Recombination: molecular markers for resistance genes in major grain crops. Progr Bot 59 (in press)

Qi X, Lindhout P (1997) Development of AFLP markers in barley. Mol Gen Genet 254:330–336

Rumjaun A, Jahier J, Trottet M, Lapierre H (1996) Identification of a potential source of complete resistance to Soilborne Wheat Mosaic Virus in a *Thinopyrum intermedium* addition line. J Phytopathol 144:135–138

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard W (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. Proc Natl Acad Sci USA 91 : 5466–5470

Schenk PM, Antoniw JF, Batista M deF, Jacobi V, Adams MJ, Steinbiss H-H (1995) Movement of barley mild mosaic and barley yellow mosaic viruses in leaves and roots of barley. Ann Appl Biol 126 : 291–305

Takahashi R, Hayashi J, Inouye T, Moriya I, Hirao C (1973) Studies on resistance to Yellow Mosaic Disease in barley. I. Tests for varietal reactions and genetic analysis of resistance to the disease. Ber Ohar Inst 16 : 1–17

Toyama A, Kusaba T (1970) Transmission of soil-borne barley yellow mosaic virus. 2. *Polymyxa graminis* Led. as vector. Ann Phytopathol Soc Japan 36 : 223–229

Tragoonrung S, Kanazin V, Hayes PM, Blake TK (1992) Sequence-tagged-site-facilitated PCR for barley genome mapping. Theor Appl Genet 84 : 1002–1008

Ukai Y (1984) Genetic analysis of a mutant resistant to barley yellow mosaic virus. Barley Genet Newslett 14 : 31–33

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Frijters A, Pot J, Pelemann J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23 : 4407–4414

Weyen J, Bauer E, Graner A, Friedt W, Ordon F (1996) RAPD-mapping of chromosome 3 of barley including the BaMMV/BaYMV resistance gene *ym4*. Plant Breed 115 : 285–287

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Molecular Breed 1 : 375–387