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Mapping of *Rym14^{Hb}*, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley

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Abstract *Hordeum bulbosum* represents the secondary gene pool of barley and constitutes a potential source of various disease resistances in barley breeding. Interspecific crosses of *H. vulgare* × *H. bulbosum* resulted in recombinant diploid-barley progeny with immunity to BaMMV after mechanical inoculation. Tests on fields contaminated with different viruses demonstrated that resistance was effective against all European viruses of the soil-borne virus complex (BaMMV, BaYMV-1, -2). Genetic analysis revealed that resistance was dominantly inherited. Marker analysis in a F5 mapping family was performed to map the introgression in the barley genome and to estimate its size after several rounds of recombination. RFLP anchor-marker alleles indicative of an *H. bulbosum* introgression were found to cover an interval 2.9 cM in length on chromosome 6HS. The soil-borne virus resistance locus harboured by this introgressed segment was designated *Rym14^{Hb}*. For marker-assisted selection of *Rym14^{Hb}* carriers, a diagnostic codominant STS marker was derived from an AFLP fragment amplified from leaf cDNA of homozygous-resistant genotypes inoculated with BaMMV.

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Introduction

The soil-borne virus complex (BaMMV, BaYMV-1, BaYMV-2) is transmitted by the fungus *Polymyxa graminis* and represents one of the most-important diseases in cultivated barley. Chemical measures to defeat the vector of the disease are neither efficient nor acceptable for ecological and economical reasons. Breeding for disease resistance represents the sole alternative. To-date, 13 virus resistance genes with recessive inheritance were derived from the primary gene pool of barley. Molecular mapping led to the localization of these genes on chromosomes 1H (Graner et al. 1999b), 3H (Graner and Bauer 1993; Ordon and Friedt 1993; Graner et al. 1999a), 4H (Bauer et al. 1997; Werner et al. 2001), 5H and 7H (Saeki et al. 1999). Virus resistance of recent European cultivars is based on the resistance genes *rym4* and *rym5*, with the latter being effective to BaMMV, BaYMV-1 and BaYMV-2. Besides *rym5*, three other virus resistance genes, namely *rym2, rym11* and *rym12*, were described which also confer resistance to the three soil-borne viruses reported for Germany (Konishi et al. 2002).

Continuous deployment of plant genetic resources (PGRs) is a prerequisite for maintaining a broad genetic basis in the breeding of our crops. In addition, the use of PGRs by breeders to provide novel resistance genes to diseases, pests and abiotic stresses constitutes an essential component in concepts towards an environmentally compatible and sustainable agriculture. In barley breeding, the wild species *Hordeum bulbosum* presents a novel resource for valuable trait genes which has scarcely been used to-date. *H. bulbosum* makes up the secondary gene pool for barley breeding (Zeller 1998). Despite the existence of prezygotic and postzygotic cross barriers like interspecific incompatibility, chromosome elimination and sterility of the offspring, hybrids and fertile

offspring may be obtained from crosses between *Hordeum vulgare* and *H. bulbosum* (Pickering 1988, 1992; Szigat and Szigat 1991; Kasha et al. 1996). A number of disease resistances were reported to be conferred by introgressions of *H. bulbosum* into barley. Resistances to powdery mildew (Pickering et al. 1995; Kasha et al. 1996) or leaf rust (Pickering et al. 1998, 2000) were found to be introgressed on chromosomes 2HS and 2HL, respectively. An introgression on chromosome 2HS was reported to confer both resistance to powdery mildew and to leaf rust (Pickering et al. 1998; Ruge et al. 2000). In the present study a locus introgressed from *H. bulbosum* and conferring resistance to soil-borne viruses in barley is described in terms of effectiveness, inheritance and genomic mapping.

Materials and methods

Plant material

The F3, F4 and F5 selfed families used in the present study originated in a single diploid-resistant F2 recombinant, VV^B, which had been obtained from an interspecific tetraploid *H. vulgare* cv 'Borwina' × *H. bulbosum* hybrid (VVBB; Szigat and Szigat 1991). Previously obtained evidence from a segregating F3 selfed family suggested that this hybrid displayed digenic resistance to the soil-borne virus complex (Michel 1996). From the F3 family, monogenically segregating F4 families (*BAZ-3005, BAZ-3006* and *BAZ-3009*) as well as nine F5 families (*BAZ-4006* through *BAZ-4014*) were derived by single-seed descent.

Resistance tests

Testing for viruses resistance was done by growing plants in fields contaminated with BaMMV and BaYMV (see below). Alternatively, plants were mechanically inoculated with BaMMV in the greenhouse. For segregation analyses, all plants were assessed by DAS-ELISA (Proeseler 1993). Testing for BaYMV-1 and BaYMV-2 resistance was performed on four test fields contaminated with different combinations of viruses. Fields A (Aschersleben, Saxony-Anhalt) and D (Martinsheim, Bavaria) were infested with BaMMV/BaYMV-1, whereas fields B (Aschersleben, Saxony-Anhalt) and C (Bornum, Lower Saxony) contained the combination BaMMV/BaYMV-1/BaYMV-2. Plant material to be field-tested was sown in autumn 1999 and visually assessed for virus symptoms in April 2000, together with cv 'Borwina' as the susceptible standard. For segregation analyses on test fields A and B, plants with a resistant or susceptible phenotype were individually assessed by DAS-ELISA for the absence or presence of virus particles, respectively, according to Proeseler (1993). Test fields C and D were used to define the range of effectiveness of the introgressed resistance on a larger scale. Plots each containing 100 plants of one out of eight F5 families were assessed. All F5 families had been derived from *BAZ-3005* by single-seed descent. Scoring was performed visually for all plots and random samples were tested by DAS-ELISA. Each plot was given an overall score on each of two scoring dates. Plots containing plants without mosaic symptoms were scored as resistant (score 1), whereas plots showing typical chlorotic streaks were assessed to be susceptible (score 9).

In situ hybridization (FISH)

Chromosome preparations were made from actively dividing root-tip cells as described by Anamthawat-Jónsson et al. (1993) and Pickering et al. (1997). Air-dried slide preparations were rinsed twice (5 min) in 2× SSC (1× SSC: 0.15 M NaCl, 0.015 M trisodium citrate, pH 7) and then incubated in 50 μg/ml of DNase-free RNase in 2× SSC for 40 min at 37 °C followed by three washes (5 min each) in 2× SSC. The slides were fixed in freshly de-polymerised paraformaldehyde (3 g/75 ml 2 × phosphate buffered saline) for 10 min at RT and washed three times in 2× SSC before dehydrating in an ethanol series and air drying. The DNA probe, pSc119.2, was a gift from Professor K.J. Kasha (Guelph University, Canada) and is a subclone from the rye repetitive sequence pSc119 (Bedbrook et al. 1980; McIntyre et al. 1990). pSc119.2 hybridizes preferentially to distal sites on some chromosome arms of *H. bulbosum* but only weakly to sites on *H. vulgare* chromosomes (Xu et al. 1990; Taketa et al. 2000). The insert of pSc119.2 was removed from plasmid pUC18 after restriction enzyme digestion and gel electrophoresis, using procedures similar to those of Xu et al. (1990). The insert was labeled with Rhodamine-5-dUTP by nick translation following the manufacturer's (Roche Diagnostics) instructions. The probe was mixed to a final concentration of 1.75 or 2.5 ng/μl in a solution of 50% formamide, 2× SSC, 10% dextran sulphate, 0.1% SDS and 325 μg/ml of sonicated salmon-sperm DNA. The probe mixture was denatured for 7 min at 95 °C, chilled on ice for 3 min, and 20 μl applied to each slide preparation and covered with a plastic coverslip. The chromosomes and probe mixture were denatured for 6.5 min at 80 °C and hybridized overnight at 37 °C. After hybridization, slides were washed in 2× SSC (2 × 5 min at 42 °C) followed by a stringency wash (20% formamide in 0.1× SSC) at 42 °C for 10 min, and then washed in 2× SSC twice for 5 min at 42 °C. Subsequent detection (without amplification), epi-fluorescence microscopy and image preparation followed the method of Pickering et al. (2000), except that counterstaining with 4′, 6-diamidino-2-phenylindole (DAPI) (1 μg/ml of Vectashield antifade) was carried out.

Molecular markers

For RFLP genotyping, total plant DNA was isolated as described by Wilkie (1989). Genomic DNA was digested with *Dra*I, *Xba*I or *Hin*dIII, electrophoresed on 0.8% agarose gels and transferred onto nylon membranes (Roche) by capillary blotting (Sambrook et al. 1989). Labeling of the probes and non-radioactive Southern hybridizations were carried out according to the protocols of the manufacturer (Roche). Marker analysis with RFLP probes located on the barley consensus map of chromosome 6H (Qi et al. 1996) comprised MWG and ABG clones from barley (Graner et al. 1991; Kasha and Kleinhofs 1994) as well as PSR clones from wheat (Gale et al. 1995).

For AFLP analysis, genomic DNA was digested with *Mse*I and *Eco*RI. After adaptor ligation, non-selective amplification of DNA fragments was performed using primers with one-base-pair extension. Selective amplification was carried out using various primer combinations of M and E (three-base-pair extension) of AFLP Analysis System I (Life Technologies). Amplification products were separated on denaturing polyacrylamide gels (6%) for 3.5 h. After electrophoresis, gels were silver-stained (Budowle et al. 1991).

For cDNA-AFLP analysis, 100 F5 plants were separated into two groups of genotypes homozygous for either the *H. vulgare* or *H. bulbosum* allele of marker *Xiac501*(*Got*) (see below). All plants were inoculated mechanically with BaMMV. The mRNADirect Kit (Dynal) was applied for the isolation of intact $polyA^+$ RNA using Dynabeads Oligo $(dT)_{25}$ according to the protocols of the manufacturer. $PolyA^+$ RNA was isolated from bulks of ten individuals per genotypic bulk by using equal amounts of leaf tissue from homozygous *Xiac501* genotypes 15 days after inoculation with BaMMV. Analysis of cDNA-AFLPs was performed using the displayProfile Kit (Appligene). cDNA synthesis and fragmentation (*Taq*I), adaptor ligation and cDNA amplification with different ^{33}P-labeled primers (64 combinations) were performed according to the manufacturer's recommendations. PCR products were separated on denaturing 6% polyacrylamide gels for 3.5 h, dried on chromatography paper and exposed to an X-ray film (Roche). Differentially expressed cDNA-AFLP fragments were TA-cloned into the plasmid vector pCRII-TOPO according to the manufacturer's intro-

ductions (Invitrogen) and used as probes for RFLP analysis in the mapping population *BAZ-4006*.

For the establishment of *Xiac500*, cDNA-AFLP fragments were sequenced and PCR primers were designed. Using Oligo 5.0 (Rychlik 1994) additional PCR primers were designed based on GenBank acc. AF017431 to convert a glutamate oxaloacetate transaminase (GOT) isozyme marker located on chromosome 6H (Islam and Shepherd 1981) into the STS marker *Xiac501*(*Got*). The forward and reverse primers for *Xiac501*(*Got*) were 5′-GGATCGC-CGGCAACCTCAACA-3′ and 5′-CCGGCAGGAAACGCTTCTG-GA-3′, respectively, and the annealing temperature was set at 60 °C. For each PCR assay, 100 ng of genomic DNA was used in a solution containing 1 × reaction buffer (Promega), 200 μM of each dNTP, 5 pmol of primers and 0.5 U of *Taq* DNA Polymerase (Promega). PCR products were separated on 10% non-denaturing polyarcylamide gels followed by silver staining (Budowle et al. 1991).

Mapping

Linkage analysis was performed using 168 individuals of the F5 family *BAZ-4006*. Of these, 124 plants with a resistant phenotype and ten randomly selected plants with a susceptible phenotype had been genotypically assessed by progeny tests using selfed offspring of each plant. The remaining 34 susceptible plants of the mapping population were not included in progeny testing. Mapmaker 3.0 (Lander et al. 1987) was applied for mapping. Recombination values were calculated based on the model of codominance and converted into genetic-map distances (cM) by use of the Kosambi function.

Results

Inheritance of virus resistance

Resistance tests in families *BAZ-3005, -3006, -3009, -4006* and *-4007* mechanically inoculated with BaMMV revealed segregations of the resistance trait that were consistent with a 3:1 ratio of resistant to susceptible individuals, respectively (Table 1). Thus, resistance to BaMMV in these families proved to be dominantly inherited. Of the 236 plants of *BAZ-4006* that had been challenged with BaMMV, 168 plants were subjected to progeny tests to identify resistant genotypes. The segregation obtained among the progeny tested (PT) fraction of *BAZ-4006* was in accordance with a 1:2:1 ratio (Table 1). Hence, a single resistance locus was identified that was associated with BaMMV resistance. When grown on test fields A and B, related F5 families, *BAZ-4008* and *BAZ-4009*, also segregated 3:1 for resistance vs susceptibility (Table 1). Since field B was contaminated with all three viruses it may be concluded that the virus resistance present in these introgression families is effective against the entire soil-borne complex of BaMMV, BaYMV-1 and BaYMV-2. The dominant factor responsible for this resistance introduced from *H. bulbosum* was designated *Rym14^{Hb}*.

Effectiveness of *Rym14^{Hb}* was confirmed by larger-scale tests on contamination fields C and D, which are used for selection by commercial plant breeders. Infection of the plants grown in these uniformly contaminated fields was highly efficient, and resistant plants were easily distinguishable from susceptible ones. Plants of the original cv ‘Borwina’ invariably developed chlorotic streaks typical of virus infection (score 9). In contrast, plots of F5 introgression families generally remained symptomless, although four plots on the field located in Bornum contained a number of plants developing bright leaf areas (Table 2). The latter observation was not

Table 1 Segregation analysis of resistance to different viruses of the soil-borne virus complex (BaMMV, BaYMV). *Progeny test; $^{\#}\chi^2_{1:2:1}$

Family	Generation	Viruses tested	N	Segregation	$\chi^2_{3:1}$
BAZ-3005	F4	BaMMV[a]	40	30:10	0.00
BAZ-3006	F4	BaMMV[a]	32	27:5	1.41
BAZ-3009	F4	BaMMV[a]	66	51:15	0.19
BAZ-4006	F5	BaMMV[a]	236	181:55	0.36
*4006 PT**	F6	BaMMV[a]	168	32:92:44	3.09[#]
BAZ-4007	F5	BaMMV[a]	30	26:4	2.18
BAZ-4008	F5	BaMMV, BaYMV-1[b]	39	28:11	0.21
BAZ-4009	F5	BaMMV, BaYMV-1, -2[c]	44	35:9	0.48

[a] Mechanically inoculated (greenhouse)
[b] Test field A
[c] Test field B

Table 2 Effectiveness of *Rym14^{Hb}* resistance on test fields C and D infested with different viruses. *Tested with two repetitions; bl, leaves displaying bright areas; score 1, leaves without symptoms; score 9, leaves with chlorotic streaks

Families	Field C BaMMV, BaYMV-1		Field D BaMMV, BaYMV-1, -2	
	First score 31.01.00	Second score 28.02.00	First score 24.01.00	Second score 07.03.00
BAZ-4007	1	1	1*	1 (bl)*
BAZ-4008	1	1	1	1
BAZ-4009	1	1	1	1
BAZ-4010	1	1	1 (bl)*	1*
BAZ-4011	1	1	1 (bl)*	1*
BAZ-4012	1	1	1 (bl)*	1*
BAZ-4013	1	1	1	1
BAZ-4014	1	1	1	1
cv ‘Borwina’	9	9	9	9

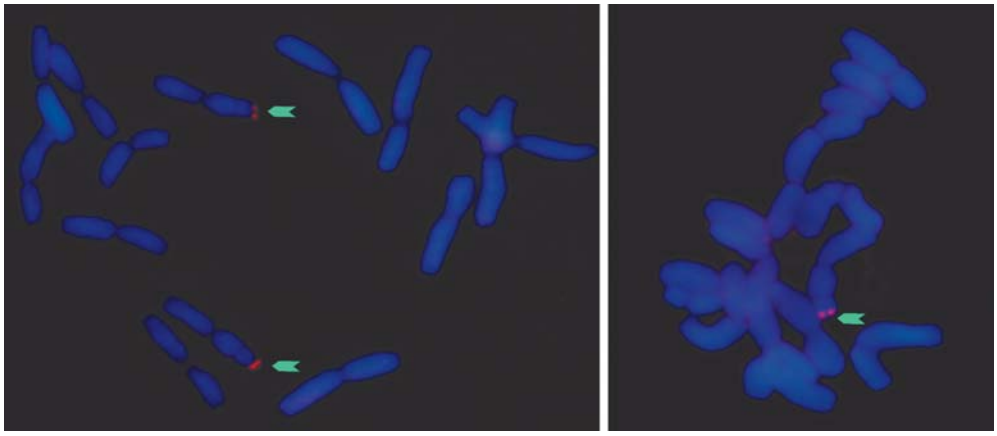

Fig. 1 FISH with rye probe pSC119.2 highlighting the *H. bulbosum* introgression in a homozygous (*left*) and a heterozygous (*right*) virus-resistant plant

unexpected because the F4 parents of the plants growing in the plots had not been selected for homozygosity of *Rym14*^{Hb} resistance. Randomly selected symptomless plants did not contain any virus particles according to the DAS-ELISA test, whereas in plants with typical symptom virus particles were detected.

FISH

FISH revealed a *H. bulbosum* introgression on the short arm of chromosome 6H, which is easily recognised by the presence of a large satellite (Pickering 1992). Heterozygous resistant genotypes of a F4 family (*BAZ-3006*) showed a specific signal on one of the two 6HS homologues, whereas homozygous plants displayed a signal on both (Fig. 1). There were no hybridization signals on the remaining chromosomes suggesting that the introgression on 6HS was the one associated with resistance in the plant material under study, especially as susceptible plants showed no hybridization signals (not illustrated).

Molecular-marker analysis

Among 13 RFLP anchor markers tested on chromosome 6HS, four were polymorphic and segregated with alleles present in barley cv 'Borwina' and in the *H. bulbosum* parent of the original hybrid. Markers *MWG2318* and *ABG466* cosegregated with virus resistance (Fig. 2). *Xpsr167* which was demonstrated to be located in the distal part of chromosome 6HS (Qi et al. 1996) mapped 0.6 cM away from the resistance, whereas *MWG573* mapped 1.2 cM from the locus on the opposite side (Fig. 2). The resistance locus was found to be proximally flanked by the STS marker *Xiac 501 (Got)* at a distance of 0.6 cM. From the results of molecular marker and FISH analysis we conclude that *Rym14*^{Hb} resistance in family *BAZ-4006* was conferred by a *H. bulbosum* introgression on chromosome 6HS.

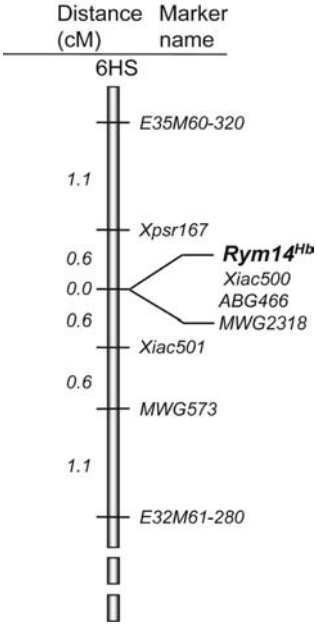

Fig. 2 Mapping of resistance gene *Rym14*^{Hb} on barley chromosome 6HS in mapping population *BAZ-4006*

To develop markers diagnostic for the introgressed *Rym14*^{Hb} resistance, cDNA-AFLP analysis was carried out. Of 64 primer combinations, one led to the amplification of a differentially expressed 250-bp transcript which was exclusively observed for the resistant genotypic bulk (Fig. 3a) after inoculation with BaMMV. When prior inoculation was omitted the 250-bp fragment was not observed (data not shown). Use of the cloned 250-bp amplicon as a hybridization probe in a genomic Southern experiment revealed a single-copy hybridization pattern which displayed a codominant polymorphism between resistant and susceptible genotypes (Fig. 3b). This RFLP marker was designated *Xiac500*. Genetic analysis of *Xiac500* in mapping population *BAZ-4006* revealed cosegregation of *Xiac500* and the resistance locus among 168 individuals. A BLAST search against the Genbank database gave no evidence for sequence similarities to the

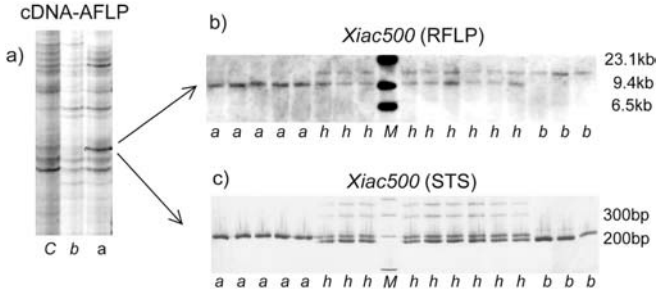

Fig. 3a–c cDNA-AFLP analysis with genotypic bulks of resistant vs susceptible plants challenged with BaMMV. **a** AFLP analysis on a denaturing sequencing gel. One fragment was identified in the bulk of homozygous resistent plants (*a*), that was absent in the bulk of susceptible plants (*b*) as well as in the control 'Borwina' (*C*). **b** Southern hybridization pattern of RFLP marker *Xiac500*. **c** STS derivative of *Xiac500*. Homozygous (*a*) as well as heterozygous resistant plants (*h*) are characterized by the presence of a diagnostic *H. bulbosum* fragment, whereas susceptible plants (*b*) show a *H. vulgare* fragment. *Numbers* on the right indicate the size of DNA markers (*M*)

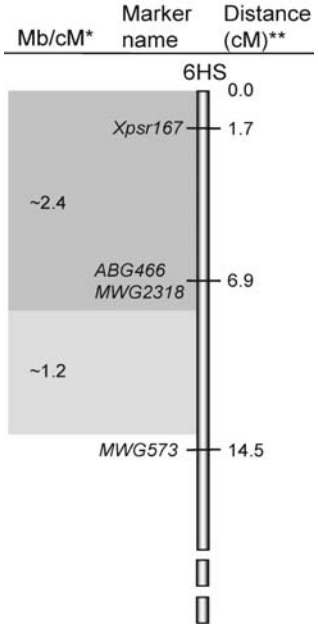

Fig. 4 Genetic distances of anchor markers linked with *Rym14^{Hb}* as given in the barley consensus map of chromosome 6HS and their relation to the physical extensions. *Physical map (Künzel et al. 2000). **Barley consensus map (Qi et al. 1996)

250-bp fragment. The RFLP marker was converted into a 200-bp STS marker, *Xiac500*(*STS*), which also showed codominant inheritance (Fig. 3c) and proved useful for routine PCR-based selection of defined *Rym14^{Hb}* genotypes.

In the barley consensus map (Qi et al. 1996), anchor markers *Xpsr167* and *MWG573* span a genetic distance of 12.8 cM (Fig. 4). In comparison, the distance between these markers was estimated at 1.8 cM in mapping population *BAZ-4006*, suggesting a pronounced linkage drag within the introgressed segment with a recombination frequency reduced by a factor of 7. By relating the distances of common anchor markers in the consensus map to the varying Mb/cM ratios reported for the distal part of chromosome 6HS (Fig. 4; Künzel et al. 2000) allowed us to roughly estimate the physical extension of the introgressed *H. bulbosum* segment in mapping population *BAZ-4006* at 21 Mb. This estimate is based on the assumption that physical distances across the respective genomic intervals are comparable between *H. vulgare* and *H. bulbosum*.

Discussion

Despite pronounced sexual barriers between cultivated barley and *H. bulbosum*, which encompass endosperm degeneration, chromosome instability, low chromosome pairing and crossing-over as well as hybrid infertility (Pickering 1992; Zhang et al. 1999), different attempts to achieve sexual hybridization via triploid or tetraploid interspecific hybrids have been successful (Pohler and Szigat 1982; Pickering 1988; Szigat and Szigat 1991; Xu and Kasha 1992; Pickering et al. 2000a; Szigat 2001). The plant material analyzed in the present study originated from a tetraploid hybrid (Szigat and Szigat 1991) that gave rise to diploid, virus-resistant selfed progeny. Genetic analysis of the latter established the involvement of two dominantly inherited resistance genes (Michel 1996). Based on these results, monogenically segregating populations were developed for a more-detailed characterization of the introgressed resistances (Ruge et al. 2002). The present study demonstrates that a novel resistance factor, *Rym14^{Hb}*, has been introgressed on barley chromosome 6HS.

All the barley resistance genes to the soil-borne viruses described so far are inherited recessively (Graner and Bauer 1993; Bauer et al. 1997; Konishi et al. 1997; Graner et al. 1999a, b; Iida et al. 1999; Saeki et al. 1999; Werner et al. 2001; Konishi et al. 2002). Compared to these virus-resistance genes, *Rym14^{Hb}* is unique in that: (1) it is the first such resistance gene introduced from the secondary gene pool of barley, (2) it displays dominant inheritance, and (3) is located on barley chromosome 6H. In addition, *Rym14^{Hb}* confers complete resistance to the European viruses of BaMMV, BaYMV-1 and BaYMV-2.

All *H. bulbosum* accessions investigated so far for their reaction to BaMMV and BaYMV were resistant. As discussed for *rym5* by Graner et al. (1999a), separate resistance genes may be involved in the broad-range specificity to distinct viruses like BaMMV and BaYMV. While this possibility also applies to the resistance governed by the *Rym14^{Hb}* locus, addressing this question in genetic studies is difficult because of the considerable linkage drag associated with the *H. bulbosum* introgressions.

Among the *H. bulbosum* introgressions obtained so far, most of them have been assigned to chromosomes 2HS, 2HL, 4HL, 6HS or 7HS, predominantly in their telomeric

regions (Pickering 2000; Pickering et al. 2000a). Only few introgressions were observed for other chromosomes, namely 1H, 3H, 4HS, 5HS and 6HL, which suggests either that homoeologous recombination events between *H. bulbosum* and *H. vulgare* chromatin are not randomly distributed along the barley genome but concentrated at recombination 'hot spots', or that fertilization ability is affected by certain effects (gamete competition) among pollen grains with different genetic constitutions. Despite this apparently non-random production of recombinant lines, several disease resistance genes have already been introgressed from *H. bulbosum* into barley. Besides resistance to the soil-borne viruses, dominant genes from *H. bulbosum* for powdery mildew resistance were mapped on chromosomes 2HL and 2HS (Pickering et al. 1995, 1998; Kasha et al. 1996). Likewise, on chromosome 2HL a dominant resistance gene against leaf rust was described by Pickering et al. (2000b). A recombinant line on 6HS from a hybrid involving cv 'Golden Promise' and *H. bulbosum* genotype Cb2920/4, was also found to be resistant and with dominant inheritance to BaMMV in glasshouse tests (Proeseler and Pickering, unpublished). The markers *Xiac500, Xiac501* and *MWG573* that where polymorphic in this 6HS introgression line displayed genetic distances amongst each other and to the BaMMV resistance, which were similar to those found in mapping population *BAZ-4006* of the present study (Ruge et al., unpublished), suggesting that the same resistance locus has been transferred into cv 'Golden Promise'. While these chromosomal regions of *H. bulbosum* represent a very limited portion of its genome, their potential as a resource for resistance breeding in barley is very important.

In the present study, the size of the *H. bulbosum* introgression on chromosome 6HS and the linkage drag associated with this introgression were estimated by relating molecular-marker data from the mapping population *BAZ-4006* to the barley consensus map. By further relating the genetic distances in specific marker intervals of the consensus map with the physical mapping data by Künzel et al. (2000), an approximation of the physical size of the introgression was obtained. The original size of the introgression was found to be larger than 13 cM in terms of consensus map distances, since the introgressed segment extended distally from anchor marker *MWG573* and beyond anchor marker *Xpsr167*. By identifying further recombination among individuals in family *BAZ-4006* we reduced the size of the introgression. The largest reduction amounted to approximately 1.7 cM in terms of the consensus map or 4 Mb according to the Mb/cM ratios calculated by Künzel et al. (2000). It followed from a recombination between $Rym14^{Hb}$ and *Xpsr167*, resulting in the elimination of the distal part of the introgression including *Xpsr167* (Fig. 4). These recombinants have been introduced into a commercial breeding programme for virus resistance.

In conclusion, unlocking the secondary gene pool to enhance genetic diversity with the aid of molecular markers is beginning to have practical benefits in breeding disease-resistant barley.

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