Theor Appl Genet (2006) 113:867–873
DOI 10.1007/s00122-006-0345-8

ORIGINAL PAPER

Mapping of *Rym16^{Hb}*, the second soil-borne virus-resistance gene introgressed from *Hordeum bulbosum*

B. Ruge-Wehling · A. Linz · A. Habekuß · P. Wehling

Received: 5 October 2005 / Accepted: 12 June 2006 / Published online: 13 July 2006

Abstract *Rym16^{Hb}*, a gene conferring resistance to soil-borne viruses, was introgressed from *Hordeum bulbosum* to barley chromosome 2HL. Mechanical inoculation with BaMMV and field tests on a plot contaminated with different viruses demonstrated that *Rym16^{Hb}* is effective against all European viruses of the soil-borne virus complex (BaMMV, BaYMV-1, -2). Genetic analysis revealed a dominant inheritance of the resistance controlled by *Rym16^{Hb}*. Using 2HL anchor markers, the size of the introgression was estimated to be about 30 M. In its proximal part, the introgression was characterized by a rearrangement of markers *Xbcd266*, *ABC153* and *ABC252*, accompanied with pronounced linkage drag by factor 4 in segregating mapping populations. The introgression was found to be associated with a recessive lethality factor, *l^{Hb}*, which was closely linked to the markers mentioned above. Recombination occurring within the introgressed *H. bulbosum* segment allowed us to separate *l^{Hb}* from *Rym16^{Hb}* and to reduce the size of the introgression to 23 cM or less.

Communicated by J. Reif.

B. Ruge-Wehling (✉) · A. Linz · P. Wehling
Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute of Agricultural Crops,
Rudolf-Schick-Platz 3a, 18190 Groß Lüsewitz, Germany
e-mail: bafz-lk@bafz.de

A. Habekuß
Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute of Epidemiology and Resistance Resources,
Theodor-Roemer-Weg 4, 06449 Aschersleben, Germany

Introduction

The complex of *Barley Mild Mosaic Virus* (BaMMV) and *Barley Yellow Mosaic Virus* (BaYMV-1 and -2) (Huth 1989) represents the most severe threat to the growing of winter barley in Europe. The disease is transmitted to the roots of barley plants upon cold stress during the winter via the soil-borne fungus *Polymyxa graminis* and is easily spread across fields and over larger distances via tilling and wind drifting of soil. In Germany, BaYMV-1 and BaMMV mainly occur conjointly in most of the barley-growing areas. Meanwhile, most of these areas have also been contaminated with BaYMV-2. Highly infested fields are likely to remain infectious for 20–50 years, depending on the type of soil (Huth 2000). Yield losses caused by BaMMV and BaYMV can be significant and yield advantages of resistant over susceptible varieties have been reported to be in the range of 38–50% in European barley-growing areas (Adams et al. 1992).

The only way to reduce the impact of BaMMV/BaYMV on yield performance is to grow resistant varieties. To-date, at least 14 virus-resistance genes with recessive inheritance have been introduced from the primary genepool into barley. A resistance gene which had tentatively been named *Ym2* was introduced from cv. 'Mihori Hadaka 3' and initially, seemed to be inherited by a partially dominant gene (Takahashi et al. 1973). More recently, though, the resistance from 'Mihori Hadaka 3' was reported to exhibit a recessive inheritance (Ordon and Friedt 1993). Molecular mapping led to the localization of *rym* genes on barley chromosomes 1H, 3H, 4H, 5H, 6H and 7H, respectively (Konishi et al. 2002; Le Gois et al. 2004; Werner et al. 2003). Besides these recessive resistance genes

stemming from the primary genepool, a dominantly expressed resistance gene, *Rym14*^{Hb}, has been identified and mapped onto barley chromosome 6HS. This resistance gene was introduced from the secondary genepool of barley, i.e., *Hordeum bulbosum*, and confers complete resistance against the BaMMV/BaYMV-1/BaYMV-2 virus complex (Ruge et al. 2003). In the present study, we report on a second virus-resistance gene introgressed from *H. bulbosum* and present data on its inheritance, molecular mapping, and effectiveness.

Materials and methods

Plant material

The F4, F5 and F6 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 carried two different resistance genes effective to the soil-borne virus complex (Michel 1996), with one of the genes designated *Rym14*^{Hb} later on (Ruge et al. 2003). From the F3 family, the F4 family *BAZ-3010* was generated. This family had been selected among other F4 selfed families because it (1) segregated monogenically for virus resistance and (2) was homozygous for the *H. vulgare* allele at *Xiac501*, a marker which is closely linked to *Rym14*^{Hb} (Ruge et al. 2003). Thus, *BAZ-3010* was assumed to segregate with the second, hitherto unknown virus-resistance gene. A F5 family, *BAZ-4034*, was derived from *BAZ-3010* by selfing a heterozygous-resistant plant (*BAZ-3010/46*). F6 families (*BAZ-5044* through *-5078*) were derived by single-seed descent.

Resistance tests

Mapping population *BAZ-4034* was mechanically inoculated with BaMMV in the growth chamber. For segregation analyses, all plants were assessed by DAS-ELISA (Proeseler 1993). For progeny testing, plants were selfed and 15–20 offspring individuals per selfed parent were inoculated with BaMMV. Testing for BaYMV-1 and BaYMV-2 resistance was performed by growing plants in a field plot in Aschersleben (Germany, Saxony-Anhalt) contaminated with BaMMV, BaYMV-1 and -2. Plant material to be field-tested was sown in autumn 2003 and individually assessed by DAS-ELISA in May 2004 using polyclonal BaMMV and BaYMV specific antibodies of the Serum Bank of the Institute of Resistance Research and Pathogen Diagnostics, Aschersleben. Virus titers were estimated via extinction at 405 m using a DYNATECH MR 5000 microtiter-plate reader.

Molecular markers

RFLP genotyping was performed as described by Ruge et al. (2003). RFLP probes located on the Barley-Consensus2map of chromosome 2H (Qi et al. 1996) comprised MWG and ABC clones from barley (Graner et al. 1991; Kasha and Kleinhofs 1995). The EST-derived SSR anchor marker *GBM1019* (Thiel et al. 2003) was kindly provided by A. Graner (IPK, Gatersleben). The STS marker *Xbcd266*, representing the barley-anchor RFLP marker *BCD266*, corresponded to *TC108778* (Hackauf and Wehling 2005). PCR primers (F-ACTTCATGCCCAATGTTG; R-TGATTCG AGATCGGAACTTTA) for STS marker *Xiac502* were derived from the sequence of RFLP probe *MWG866* (GenBank acc. no. AJ234601) and marker *Xmwg2076* was obtained using the primers published for *MWG2076* (Sayed-Tabatabaei et al. 1998). *Xiac507* primers (F-TCTACATACAAGGGGAAAG; R-TTT TTACTACTTCGTCACTGT) were derived from a barley EST (GenBank acc. no. AV945298), which was identified by querying the sequence of *cMWG720* against all ESTs in GenBank (NCBI BLAST). The markers *TC116908, TC101821* and *TC89057* were based on tentative consensus (TC) sequences from the TIGR Barley Gene Index (HvGI) (http://www.tigr.org/tdb/tgi/plant.shtml) and had been in-silico mapped together with the barley anchor marker *Xbcd266* on rice chromosome 4 as well as mapped on rye chromosome 2RL (Hackauf and Wehling 2005).

Primers were designed using the software package Primer3 (Rozen and Skaletsky 2000), which can be accessed via the webpage of the Whitehead Institute for Biomedical Research (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). For PCR of STS and SSR markers, 50–100 g of genomic DNA was used in a solution containing 1× reaction buffer (Qiagen), 200 µM dNTPs, 5 pmol primers and 0.5 U of *Taq* DNA polymerase (Qiagen). PCR products were separated either on 1% agarose gels or 10% polyacrylamide gels followed by ethidium bromide and silver staining (Budowle et al. 1991), respectively. A 5-µl aliqout of the PCR was digested with 1 U of *Rsa*I and *Hae*III, respectively, to exhibit the CAPS markers *TC101821* and *TC89057*.

Mapping

Linkage analysis was performed using the F5 family *BAZ-4034* ($n = 55$) and the F6 family *BAZ-5061* ($n = 80$) as mapping populations. Mapping and construction of an integrated map were accomplished with JoinMap (Van Ooijen and Voorrips 2001).

Lethality factor

Genetic analysis of the lethality factor was performed using F6 families segregating with *H. bulbosum* introgressions of various sizes. Seeds of segregating families were grown in the greenhouse at 20/16°C. Lethality of plants was assessed after the emergence of the cotyledon. Genotypes expressing the lethality factor displayed necroses proceeding from the tips to the bases of the primary and the second leaves and died off soon.

Results

Virus resistance is dominantly inherited and effective to BaMMV/BaYMV-1/BaYMV-2

Upon mechanical inoculation with BaMMV, F6 families *BAZ-5044* and *-5061* revealed segregations consistent with a 3:1 ratio of resistant versus susceptible individuals (Table 1). Subsets of these two families were also tested in a field plot contaminated with BaYMV-1 and -2, as well as BaMMV. Again, about three fourths of the plants proved to be resistant according to DAS-ELISA (Table 1). In addition to families segregating with respect to virus resistance, families *BAZ-5059* and *−5077* were field-tested. The former F6 family had been derived from a progeny-tested, homozygous-resistant individual of F5 family *BAZ-4034* (see below). In contrast, *BAZ-5077* originated in a progeny-tested, susceptible plant of the same F5 family. Of the 23 plants tested from *BAZ-5059*, all turned out to be resistant in the field plot according to DAS-ELISA. In contrast, 35 of 39 plants (90%) of *BAZ-5077* became infected with the BaMMV and BaYMV virus according to DAS-ELISA, indicating that the infection pressure in the field plot was high. The segregation data corroborated the conclusion that the *H. bulbosum* introgression present in F4 family *BAZ-3010* and its F5 and F6 offspring contributed a dominant resistance which was effective against the entire virus complex of BaMMV/BaYMV-1/BaYMV-2. The dominant resistance locus was designated *Rym16*^{Hb}.

Rym16^{Hb} is introgressed distal on barley chromosome 2HL

A total of 38 RFLP markers anchored in the Barley-Consensus2 map for barley chromosome 2H were tested for the mapping of *Rym16*^{Hb}. Five RFLP anchor markers located on chromosome 2HL were polymorphic and segregated with alleles present in *H. vulgare* cv. 'Borwina' (*Hv* marker alleles) and in the *H. bulbosum* parent (*Hb* marker alleles), respectively, of the original hybrid (not shown). The order of the three most distal RFLP markers in the integrated map of BAZ-4034/-5061 deviated from that in Barley-Consensus2-2H but was consistent with the order in the Hordeum-Graner2-2H map. As a consequence, *MWG949* was the most distal marker in our integrated map. *MWG949* mapped proximal of the resistance locus in 3.6 cM distance (Fig. 1a). Since this anchor marker is located on the long arm of chromosome 2H at position 147.0 (Qi et al. 1996) we can

Table 1 Segregation analysis of resistance to different viruses of the soil-borne virus complex (BaMMV, BaYMV)

Family	Generation	Viruses tested	*N*	Segregation res:susc	$\chi^2_{3:1}$
BAZ-5044	F6	BaMMV[a]	81	66:15	1.8
		BaMMV, BaYMV-1, −2[b]	44	28:16	3.0
BAZ-5061	F6	BaMMV[a]	80	61:19	0.1
		BaMMV, BaYMV-1, −2[b]	45	31:14	0.9
BAZ-4034	F5	BaMMV[a]	55	42: 13	0.05
4034PT	F6	BaMMV[a]	40	1:33:6[c]	–
BAZ-5049	F6	BaMMV[a]	108	66:42	11.1
BAZ-5059	F6	BaMMV, BaYMV-1, −2[b]	23	23:0	–
BAZ-5077	F6	BaMMV, BaYMV-1, −2[b]	39	4: 35	–

PT progeny-tested

[a] Mechanically inoculated (growth chamber)

[b] Field test

[c] Homogeneous-resistant:segregating:homogeneous-susceptible test progeny

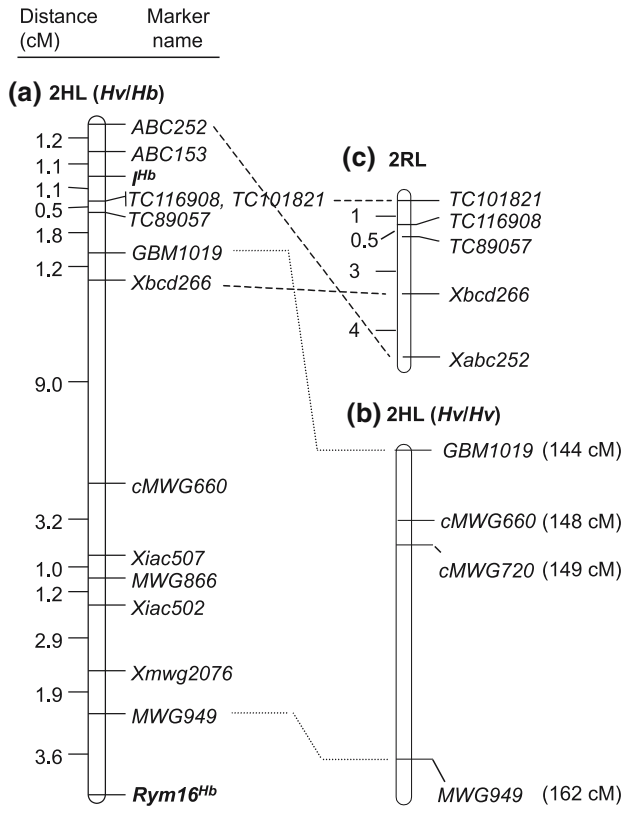

Fig. 1 Mapping of resistance gene *Rym16*^{Hb} and lethality factor *l*^{Hb} on barley chromosome 2HL. **a** integrated map based on introgression families *BAZ-4034/-5061*; **b** genetic-map positions of barley anchor markers in a pure barley-genetic background (*Hv/Hv*) as reported by Thiel et al. 2003; **c** genetic distances of markers on rye chromosome 2RL as reported by Hackauf and Wehling (2005)

infer *Rym16*^{Hb} to be localized in the distal part of a *H. bulbosum* segment which was introgressed on barley chromosome 2HL. *MWG949* was flanked in proximal direction in 1.9 cM distance by another 2HL-anchored marker, *Xmwg2076*. To assess the size of the introgression, additional markers were checked for the presence of *Hb* alleles. The most proximal marker which was found to carry *Hb* alleles was the 2HL anchor marker *ABC252*, which mapped 29.7 cM away from the resistance (Fig. 1a). This genetic distance gives, thus, the minimum size of the introgression in families *BAZ-4034/-5061*. Altogether, 13 molecular markers were mapped on the introgressed segment (Fig. 1a). Development of further markers by exploiting barley/rice orthology of subgenomic regions was beyond the scope of the present study.

For comparison purposes the mapping data by Thiel et al. (2003) was used, which describes the marker interval *MWG949/GBM1019* in a pure-barley genetic background. In the integrated map of *BAZ-4034/-5061* this interval spans a genetic distance of 20.4 cM, which corresponds well with the data published by Thiel et al. (2003) for this genomic interval (18 cM; Fig. 1b). Thus, there was no evidence for pronounced suppression of recombination between the introgressed *H. bulbosum* segment and its homoeologous *H. vulgare* counterpart in the distal part of the 2HL introgression.

The situation was different in the proximal marker interval *ABC153-Xbcd266*. In the Barley-Consensus2-2H map by Qi et al. (1996) this interval spans approximately 28 cM. This distance compares with 7.3 cM in the integrated map of mapping populations *BAZ-4034/-5061* (Fig. 1a), suggesting that recombination in this interval was suppressed by a factor of 4. This linkage drag was accompanied with a reversed order of markers. In our integrated map, *Xbcd266* mapped distal of *ABC153* and *ABC252* (Fig. 1a) while in the Barley-Consensus2-2H map by Qi et al. (1996) the latter two markers were located distal of *BCD266* in 15.1 and 28.2 M distance, respectively (not shown). In rye, *Xabc252* was also reported to map distal of *Xbcd266* (Fig. 1c; Hackauf and Wehling 2005).

When considering map positions and distances of *Xbcd266* relative to the EST markers *TC116908, TC10182*, and *TC89057*, a very good correspondence was found between the situation in our barley-introgression map and the partial map reported for rye (Fig. 1c). The genomic regions in rye and barley which are defined by these markers are likely to be orthologous to rice chromosome 4 (Hackauf and Wehling 2005).

A lethality factor associated with the original *H. bulbosum* introgression can be eliminated via recombination

When F5 mapping family *BAZ-4034* initially was tested for resistance an undefined number of plants died off in the early seedling stage. Moreover, in some F6 families segregating for virus resistance there were less resistant individuals than expected, e.g., in family *BAZ-5049* (Table 1). To study this phenomenon in more detail, additional families segregating with the *H. bulbosum* introgression were tested and observed for the occurrence of nonviable plants. Two F6 families, *BAZ-5049* and *BAZ-5078*, were derived from selfed F5 individuals which were heterozygous for the full-sized *H. bulbosum* introgression present in *BAZ-4034*. Among these F6 families, about one fourth of the individuals died off in the early seedling stage (Table 2), indicating the action of a single recessive lethality factor, which was designated *l*^{Hb}. Linkage of *l*^{Hb} with virus resistance was demonstrated by progeny-testing of F5 family *BAZ-4034* (Table 1). Of the 55 individuals of this family, 40 plants could be selfed to F6 (4034PT in Table 1), among them

Table 2 Segregation patterns in respect to vitality in mapping populations carrying different introgression sizes

F6 family	F5 parents		F6 segregation		
	Introgression present (marker alleles)[a]	Resistance genotype[b]	*N*	Nonviable: viable	$\chi^2_{1:3}$
BAZ-5049	Original size (*Hv*/*Hb* at all marker loci)	*Rr*	150	39:111	0.08
BAZ-5078		*Rr*	115	24:91	1.04
BAZ-5060	Proximal part (*Hv*/*Hb* at *GBM1019* and proximal markers; *Hv*/*Hv* at *Xbcd266* and distal markers)	*rr*	94	24:70	0.01
BAZ-5044	Distal part (*Hv*/*Hv* at *Xbcd266* and	*Rr*	81	0:81	–
BAZ-5061	proximal markers; *Hv*/*Hb* at *cMWG660* and distal markers)	*Rr*	80	0:80	–
BAZ-5064	None (*Hv*/*Hv* at all marker loci)	*rr*	10	0:10	–
BAZ-5065		*rr*	10	0:10	–
BAZ-5066		*rr*	10	0:10	–

[a] *Hv*; *Hb*, alleles derived from the *H. vulgare* and *H. bulbosum* parents, respectively

[b] $Rym16^{Hb}$ genotypes, as inferred from progeny tests

34 resistant and 6 susceptible F5 individuals, the latter of which had produced some seed set despite of being virus-diseased. F6 offspring of the 34 resistant selfed parents comprised 1 nonsegregating-resistant family and 33 families segregating for resistance, which is significantly different from the expected frequencies of approximately 11 and 23, respectively. This significant under-representation of homozygous-resistant genotypes among resistant individuals can be expected when $Rym16^{Hb}$ and l^{Hb} are linked. Another example illustrating linkage of resistance and lethality factors was family *BAZ-5049*. This family segregated with 39 nonviable: 111 viable plants (Table 2). Of the 111 viable plants, 108 were tested for resistance and turned out to segregate with 66 resistant and 42 susceptible plants, which means that less than expected resistant individuals were found (Table 1). This result was consistent with the assumption that most of the homozygous-resistant segregants were allocated in the group 39 nonviable (i.e., homozygous for l^{Hb}) plants. Under this assumption, the segregation with resistant and susceptible seedlings would have roughly been 105 (i.e., 39 homozygous + 66 heterozygous) resistant: 42 susceptible, which is consistent with the 3:1 ratio expected for the segregation of a dominant resistance gene before the l^{Hb}-homozygous seedlings died off. We concluded, thus, that the formation of viable homozygous-resistant genotypes was severely compromised by the lethality factor. Only one F6 progeny was obtained which proved to be homozygous-resistant (*BAZ-5059*, which is identical with the homogeneously resistant test progeny of *4034PT*, Table 1). This offspring turned out to be recombinant and carried an introgression of reduced size. In this family, l^{Hb} and $Rym16^{Hb}$ had become genetically separated via recombination. This was inferred from the marker *TC116908* which was heterozygous for the *Hv* allele, indicating that the introgression in *BAZ-5059* was truncated proximal of this marker. A reciprocal outcome of recombination was represented by F6 family *BAZ-5060* which segregated for lethality while being susceptible to the virus (Table 2).

The lethality factor l^{Hb} mapped 27 M proximal of $Rym16^{Hb}$. It proved to be closely linked to the proximal RFLP marker *ABC153* and the distal STS marker *TC116908* (Fig. 1a). *TC116908* was a suitable tool for marker-assisted selection against the lethality gene.

A marker-assisted selection for additional recombinants devoid of the lethality-inducing proximal part of the original introgression was performed and resulted in F6 families *BAZ-5044* and *-5061* which were fixed for the *Hv* alleles at marker locus *Xbcd266* and at all marker loci mapping proximal thereof. These families proved to be completely vital while segregating for virus resistance (Table 2).

Discussion

To-date, *H. bulbosum* has rarely been used for the broadening of the genetic base of breeders' barley germplasm, mainly due to the difficulties in generating hybrids with *H. vulgare* (Pickering 1988, 1992; Zhang et al. 1999). Nevertheless, the secondary genepool of barley contains a number of valuable resistance genes which may be used in the breeding of resistant barley.

The results presented above demonstrate that besides $Rym14^{Hb}$ (Ruge et al. 2003), a second virus-resistance gene has been identified in the secondary genepool and made available for barley breeding. We propose $Rym16^{Hb}$ as a designation for this second gene introduced

from *H. bulbosum*, referring to the recessive gene *rym15* which was reported recently (Le Gouis et al. 2004). Like $Rym14^{Hb}$ (Ruge et al. 2003), $Rym16^{Hb}$ is dominantly inherited and confers resistance to the entire European soil-borne virus complex of BaMMV/BaYMV-1/BaYMV-2. $Rym16^{Hb}$ is the first soil-borne-virus resistance gene which maps to barley chromosome 2H and, thus, represents a novel virus-resistance locus in the barley genome. Altogether, for each of the seven barley chromosomes at least one locus has been reported where resistance gene(s) to soil-borne viruses reside. Combinations of these loci in a common genetic background should be feasible. Besides these genetically independent resistance genes, *rym4* and *rym5* are allelic and located on chromosome 3HL (Graner et al. 1999, 2000). Recently, an "eukaryotic translation factor 4E" (*HveIF4E*) was identified in barley as a candidate for resistance-gene function by physical mapping on a 650 kb AC contig covering the *rym4/5* region (Stein et al. 2005).

The dominant expression of $Rym16^{Hb}$, which is in contrast to the expression of the *rym* genes derived so far from the primary genepool of barley, rises questions as to the resistance mechanism. To check whether $Rym16^{Hb}$ confers resistance to the virus vector, *Polymyxa graminis*, vector-resistance tests are currently performed in a growth chamber. In any case, though, effectiveness of $Rym16^{Hb}$ appears to involve post-transmission steps, since $Rym16^{Hb}$ confers resistance against mechanically inoculated BaMMV (Table 1). Recently, two novel BaMMV viruses isolate were identified in France (Kanyuka et al. 2004) and in Germany (Habekuß et al. 2005), respectively. The BaMMV-Sil virus, which was detected in France overcomes the resistance of *rym3*, *rym5*, and *rym6* (Kanyuka et al. 2004) whereas the virus strain found in Germany overcomes *rym5* only (Habekuß et al. 2005). In contrast, $Rym16^{Hb}$ was found to have kept its effectiveness to this novel virus strain (Habekuß et al. 2005).

The proximal part of the *H. bulbosum* introgression segregating in mapping families *BAZ-4034/-5061* exhibited an inversed rearrangement of markers *Xbcd266*, *ABC153*, and *ABC252* as compared to the Barley-Consensus2-2H map, which was accompanied with a pronounced linkage drag. In barley, marker *ABC252* defines, together with *BCD453b* and *CDO373*, a chromosomal region of 3.4 cM where collinearity at the macro-level of barley chromosome 2HL with rice chromosome 4 is interrupted (Hackauf and Wehling 2005). This sub-genomic region might delineate a preferential site for recombination between the homoeologous group 2 chromosomes of *H. vulgare* and *H. bulbosum*.

The minimum genetic extension of the original introgression was determined to be 29.7 cM, based on the recombination between $Rym16^{Hb}$ and marker *ABC252*. With regard to the genetic extension of the respective sub-genomic region of pure barley, 29.7 cM appears to be an underestimate, which is due to the linkage drag acting in the mapping families segregating with the non-homologous sub-genomic counterparts. Taking into account that *BCD266* is positioned proximal of *ABC153* and *ABC252* in the Barley-Consensus2-2H map by Qi et al. (1996), the largest part of the introgression can be described by the consensus-map marker interval *MWG949-BCD266*, which in the Barley-Consensus2-2H map spans a distance of 38.3 cM. Considering further that in rye marker *TC101821* maps 4.5 cM proximal of *Xbcd266* and maps also proximal of *ABC252* (Hackauf and Wehling 2005) and assuming that these relative positions of *TC101821* and *ABC252* are also realized in barley, then an estimated extension of ca. 43 cM results for the barley-genomic interval which has been replaced by the full-sized original *H. bulbosum* introgression in families *BAZ-4034/-5061*.

We have demonstrated that a lethality factor, l^{Hb}, leading to early death of seedlings is associated with the presence of the *H. bulbosum* chromatin which was introgressed into the original cv. 'Borwina' barley line, and have mapped l^{Hb} approximately 2 cM distal of *ABC252* (Fig. 1a). Successive selfing of introgression genotypes to F6 allowed us to obtain a *H. bulbosum* introgression which had been recombined proximal of *Xbcd266* (Table 2), leaving a reduced introgression size of no more than 23 cM and devoid of l^{Hb}. Since in this remaining distal region the introgression does not exhibit pronounced linkage drag, its further recombinative tailing appears feasible with the aid of molecular markers. Although not very closely linked to $Rym16^{Hb}$ the marker *Xmwg2076* appears interesting with regard to marker-assisted selection of $Rym16^{Hb}$ genotypes in breeding programmes. Among 17 barley cultivars analyzed so far, this marker exhibited just two *Hv* alleles which are easily distinguishable from the *Hb* allele (not shown), making it a diagnostic selection tool for the distal part of the introgression in a variety of adapted breeding materials. Certainly, additional more closely linked markers for $Rym16^{Hb}$ are desirable. Currently an F2-type mapping population consisting of 1,900 individuals is being prepared for the fine-mapping around $Rym16^{Hb}$.

References

Adams MJ, Grylls J, Hill SA, Jones DR, Morris TI (1992) Soil-borne mosaic viruses in winter barley: effects of variety and management. HGCA Project Report 65, Home-Grown Cereals Authority, London

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of VNTR locus D1S80 by the PCR followed by high resolution PAGE. Am J Hum Genet 48:137–144

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

Graner A, Streng S, Kellermann A, Schiemann A, Bauer E, Waugh R, Pellio B, Ordon F (1999) Molecular mapping and genetic fine-structure of the *rym5* locus encoding resistance to different strains of barley yellow mosaic virus complex. Theor Appl Genet 98:285–290

Graner A, Michalek W, Streng S (2000) Molecular mapping of genes conferring resistance to viral and fungal pathogens. In: Proceedings of the 8th International Barley Genetic Symposium, Adelaine, Australia, vol I, pp 45–53, (inv papers)

Habekuß A, Kühne T, Rabenstein F, Krämer I, Ehrig F, Ruge-Wehling B, Huth, W, Ordon F (2005) Detection of an *rym5* resistance breaking virus strain in Germany. In: Sixth Symp Int Working Group on Plant Viruses with Fungal Vectors (IWGPVFV), 05–07 Sept 2005, Bologna, Italy, p 60

Hackauf B, Wehling P (2005) Approaching the self-incompatibility locus *Z* in rye (*Secale cereale* L.) via comparative genetics. Theor Appl Genet 110:832–845

Huth W (1989) Ein weiterer Stamm des barley yellow mosaic virus aufgefunden. Nachrichtenbl Dtsch Pflanzenschutzdienst 41:6–7

Huth W (2000) Bodenbürtige Viren der Gerste. Mitt Biol Bundesanst Land- Forstwirtsch 373:38–40

Kanyuka K, Mc Grann G, Alhudaib K, Hariri D, Adams MJ (2004) Biological and sequence analysis of a novel European isolate of *Barley mild mosaic virus* that overcomes the barley *rym5* resistance gene. Arch Virol 149:1469–1480

Kasha KJ, Kleinhofs A, North American Barley Genome Mapping Project (1995) Mapping of the barley cross Harrington × TR306. Barley Genet Newsl 23:65–69

Konishi T, Ordon F, Furusho M (2002) Reactions of barley accessions carrying different *rym* genes to BaYMV and BaMMV in Japan and Germany. Barley Genet Newsl 32:46–48

Le Gouis J, Devaux P, Werner K, Hariri D, Bahrmann N, Béghin, Ordon F (2004) *rym15* from the Japanese cultivar Chikurin Ibaraki 1 is a new barley mild mosaic virus (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108:1521–1525

Michel M (1996) Untersuchungen zur Übertragung von Resistenzgenen aus der Wildart *Hordeum bulbosum* L. in die Kulturgerste *Hordeum vulgare* L. PhD Thesis, Lehrstuhl für Pflanzenbau und Pflanzenzüchtung, Technische Universität München, Germany

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

Pickering RA (1988) The production of fertile triploid hybrids between *Hordeum vulgare* L. ($2n = 2x = 14$) and *H. bulbosum* ($2n = 4x = 28$). Barley Genet Newsl 18:25–29

Pickering RA (1992) Monosomic and double monosomic substitutions of *Hordeum bulbosum* L. chromosomes into *H. vulgare* L. Theor Appl Genet 84:466–472

Proeseler G (1993) *Triticum durum* Desf. a further host of barley mild mosaic virus (BaMMV). J Phytopathol 36:262–264

Qi X, Stam P, Lindhout P (1996) Comparison and intergration of four barley genetic maps. Genome 39:379–394

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Ruge B, Linz A, Pickering R, Proeseler G, Greif P, Wehling P (2003) Mapping of $Rym14^{Hb}$, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107:965–971

Sayed-Tabatabaei BE, Komatsuda T, Takaiwa F, Graner A (1998) DNA sequencing and primer designing for RFLP clones evenly distributed in the barley genome. Barley Genet Newsl 28:15–18

Stein N, Perovic D, Kumlehm J, Pellio B, Stracke S, Streng S, Ordon F, Graner A (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive *Bymovirus* resistance in *Hordeum vulgare* (L.) Plant J 42:912–922

Szigat Gi, Szigat Ge (1991) Amphidiploid hybrids between *Hordeum vulgare* and *H. bulbosum*—basis for the development of new initial material for winter barley breeding. Vortr Pflanzenzüchtg 20:34–39

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 Ohara Inst 16:1–17

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR markers in barley (*Hordeum vulgare* L.) Theor Appl Genet 106:411–422

Van Ooijen JW, Voorrips RE (2001) JoinMap® Version 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Werner K, Friedt W, Laubach E, Waugh R, Ordon F (2003) Dissection of resistance to soil-borne yellow-mosaic-inducing viruses of barley (BaMMV, BaYMV, BaYMV-2) in a complex breeders' cross by means of SSRs and simultaneous mapping of BaYMV/BaYMV-2 resistance of var. 'Chikurin Ibaraki 1'. Theor Appl Genet 106: 1425–1432

Zhang L, Pickering R, Murray B (1999) Direct measurement of recombination frequency in interspecific hybrids between *Hordeum vulgare* and *H. bulbosum* using genomic in situ hybridization. Heredity 83:304–309