

Genetic mapping of a leaf rust resistance gene in the former Yugoslavian barley landrace MBR1012

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Abstract Leaf rust, caused by *Puccinia hordei* Otth, is an important disease of barley (*Hordeum vulgare* L.) in many areas of the world. The appearance of new virulent races necessitates the identification of new resistance genes in barley. Screening of spring barley landraces from former Yugoslavia led to the identification of an accession (MBR1012) carrying resistance to the most widespread virulent leaf rust pathotypes in

Europe. Ninety-one doubled haploid lines derived from a cross between landrace MBR1012 and the susceptible German cultivar Scarlett were evaluated for resistance to *P. hordei* isolate I-80 and segregated 48 resistant : 43 susceptible ($\chi^2_{1:1} = 0.29$, $p = 0.6$), indicating a monogenic inheritance of resistance. Using simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers, the resistance gene in MBR1012 was mapped to the telomeric region of chromosome 1HS. This gene is assigned the temporary locus designation of Rph_{MBR1012} until it can be unequivocally determined to be different from all previously reported resistance genes. The closest flanking markers for Rph_{MBR1012} are located 0.8 cM distal (SNP marker GBS546 and SSR marker GBMS187) and 6.0 cM proximal (SSR marker GMS21). The diagnostic value of the closest linked markers was assessed in a genetically diverse collection of 51 susceptible and resistant barley lines and cultivars. The SSR GBMS187 predicted the presence of Rph_{MBR1012} with 100% accuracy. However, this marker could not be used singly for the rapid incorporation of resistance into high yielding barley cultivars, since it detects a null allele in MBR1012. Therefore, simultaneous use of the markers closely linked to Rph_{MBR1012} is needed for transferring Rph_{MBR1012} into adapted cultivars.

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Introduction

Leaf rust, caused by the biotrophic pathogen *Puccinia hordei* Otth, is an economically important foliar disease of barley in many temperate regions worldwide (Mathre 1997). Under experimental conditions, yield losses as high as 60% can occur in highly susceptible barley cultivars, but losses of about half that level are common in practice (Clifford 1985; Cotterill et al. 1992; Das et al. 2007). The deployment of single race-specific resistance genes alone or in combination by pyramiding (Werner et al. 2005) can be an effective and environmentally-friendly method of disease control. In cultivated barley (*Hordeum vulgare* ssp. *vulgare*) and wild barley (*H. vulgare* ssp. *spontaneum*), 19 major genes (*Rph1* to *Rph19*) effective in the seedling stage have been described (Golegaonkar et al. 2009). These major genes are effective against different pathotypes of *P. hordei*, but when deployed singly have often been overcome by new pathotypes. The leaf rust resistance genes *Rph7*, *Rph15*, and *Rph16* are still effective in Europe (Niks et al. 2000; Perovic et al. 2004), but are potentially vulnerable because pathotypes with virulence for *Rph7* are known in Israel (Golan et al. 1978), Morocco (Parlevliet 1981) and USA (Steffenson et al. 1993). Therefore, it may be just a matter of time until these resistance genes are overcome in Europe in the same way as *Rph3* and *Rph10* (Dreiseitl 1990; Fetch et al. 1998). It appears that the frequency of mutation in asexually reproducing populations of *P. hordei* is sufficient to overcome many resistance genes in barley (Cromey and Viljanen Rollinson 1995). Thus, the economic impact of leaf rust on barley production and the ability of the fungus to generate new virulent pathotypes in a short period of time highlights the importance of searching for new resistance genes and, in parallel, for the development of tools for their efficient deployment in barley breeding programs.

Within the primary gene-pool of barley, which includes cultivars, landraces and wild barley accessions (*H. vulgare* ssp. *spontaneum*), the latter two are of particular importance regarding breeding for disease resistance. Incorporation of new leaf rust resistance genes from landraces was reported only for the genes *Rph3* and *Rph7* (Walther and Lehmann 1980; Jin et al. 1995), while a much greater number (*Rph2*, *Rph10*, *Rph11*, *Rph12*, *Rph13*, *Rph15* and *Rph16*) was identified in the wild progenitor *H. vulgare* ssp.

spontaneum (Franckowiak et al. 1996; Ivandic et al. 1998).

To identify new sources of leaf rust resistance, barley germplasm from eco-geographically diverse collection sites in former Yugoslavia was evaluated (Perovic et al. 2001), as this is a region of high disease pressure (Reinhold and Sharp 1980; Boskovic and Boskovic 2009). One of the landraces from this collection (MBR1012) exhibited a strong resistant (hypersensitive) reaction to *P. hordei* isolate I-80 (Perovic et al. 2001), which is virulent to all known major resistance genes in the European barley gene pool, except *Rph7*, *Rph15* and *Rph16* (Weerasena et al. 2004). Therefore, the aims of this study were to investigate the genetics of leaf rust resistance in MBR1012 and develop molecular markers for this resistance gene, facilitating efficient marker-based selection procedures.

Materials and methods

Plant materials

An F1-derived doubled haploid (DH) population, produced via anther culture, comprising 91 lines of a cross between the resistant landrace MBR1012 and the susceptible German cultivar Scarlett was used for mapping. In addition, a collection of 51 barley accessions was evaluated for resistance to several leaf rust isolates to determine the diagnostic value of the closest linked molecular markers to the gene identified in MBR1012. This barley collection consisted of the original sources of described *Rph* genes, i.e. near-isogenic lines of the cultivar Bowman carrying the introgressed *Rph* genes, two lines with partial leaf rust resistance, and 10 susceptible genotypes. A set of 7 barley genotypes [MBR1012, Scarlett, Gold (*Rph4*); Cebada Capa (*Rph7*), NIL Bowman*Rph15* (*Rph15*), Hsp680 (*Rph16*) and L94] was used to determine the leaf rust infection patterns (see below).

Phenotyping

Five to ten plants of each DH line and the parents were inoculated with the leaf rust isolate I-80 in the greenhouse. Plants were inoculated at the seedling stage, according to Ivandic et al. (1998). The inoculated plants were placed in a growth chamber (20–22°C) under a plastic cover for 24 h to provide

a moist environment for successful infection. Infection types were recorded between 10 and 12 days after inoculation according to the 0–4 scale of Levine and Cherewick (1952). The DH lines were classified based on their infection types. Plants exhibiting infection types from 0, 0_{nc} (hypersensitive reactions with necrotic/chlorotic ‘flecks’), 1, 2-, or 0-2- were considered resistant, while those exhibiting infection types 2+, 3, 3-4 and 4 were considered susceptible. The Chi-squared test was used to assess segregation ratios for goodness of fit to expected ratios.

Additionally, a collection of 13 European leaf rust isolates (Walther 1987) was tested on the parental lines and accessions of Gold (carrying leaf rust resistance gene *Rph4*); Cebada Capa (*Rph7*), NIL Bowman-*Rph15* (*Rph15*) Hsp680 (*Rph16*), and L94 (susceptible control) in order to determine whether the resistance of MBR1012 is different from previously described resistance genes. Inoculation and scoring was performed as described for isolate I-80. The leaf rust isolates were collected from different parts of Europe and selected from single spore progenies (Walther 1979, 1987). The isolates are maintained at the Julius Kuehn-Institute, Federal Research Institute for Cultivated Plants (JKI), Institute for Resistance Research and Stress Tolerance, in Quedlinburg, Germany.

Marker analyses

Genomic DNA was extracted from leaves of 14-day-old plants according to Stein et al. (2001). The concentration and quality of DNA was determined using the NanoDrop ND-100 spectrophotometer (PeQLab, Erlangen, Germany) and gel electrophoresis. All samples were adjusted to a final concentration of 20 ng/ μ l. For bulk segregant analysis (BSA) (Michelmore et al. 1991), equal aliquots (10 μ l) of DNA from nine resistant and nine susceptible DH lines were pooled.

A total of 175 SSRs and 73 SNPs were screened for polymorphism between the parents and between the bulks. The sequences of the SSR primer pairs and amplification protocols were obtained from Struss and Plieske (1998), Ramsay et al. (2000), Maccaulay et al. (2001), Thiel et al. (2003), and Varshney et al. (2007), while the sequences of the SNP primer pairs, amplification protocol and restriction sites were obtained from Kota et al. (2008) and Perovic et al. (2007). All

markers which generated polymorphic PCR products between the parental lines and between the bulks were assayed in all DH lines (Table 1).

PCR was performed in a volume of 10 μ l, containing 1 μ l of $10 \times$ buffer, 1 μ l of 25 mM $MgCl_2$, 0.2 μ l of each 10 mM dNTPs, forward primer (1.0 pmol/ μ l) and reverse primer (10.0 pmol/ μ l), 0.08 μ l 5U Hot FIREPol[®] DNA polymerase (Solis BioDyne, Tartu, Estonia), 6.12 μ l HPLC gradient grade water (Carl Roth, Karlsruhe, Germany) and 1 μ l template DNA. For SSR amplification, M13 tailed forward primers were used, so that 0.1 μ l of M13 primer (10.0 pmol/ μ l) (5'-CACGACGTTGTAAACGAC-3') labelled with 5' fluorescent dyes was added to the reaction mix (Boutin-Ganache et al. 2001). DNA amplification was performed in a Gene Amp[®] PCR System 9700 (Applied Biosystems, Darmstadt, Germany). The following PCR conditions were used for all primers: 94°C for 5 min followed by a touchdown PCR (–0.5°C/cycle) with 12 cycles of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C; and then 35 cycles with 30 s at 94°C, 30 s at 56°C, 30 s at 72°C, and a final extension at 72°C for 10 min. For marker GBMS187, the second amplification phase was extended to 50 cycles, while the other parameters remained the same. Detection of allele sizes for the SSR marker was conducted using a capillary electrophoresis ABI PRISM[®] 3100 genetic analyzer (Applied Biosystems) or a CEQ[™] 8000 Genetic Analysis System (Beckman). SNP markers (Kota et al. 2008) were amplified in a volume of 20 μ l, containing 2.0 μ l of $10 \times$ PCR buffer with $MgCl_2$ (25 mM), 0.4 μ l of each 10 mM dNTPs, 0.5 μ l forward primer (1.0 pmol/ μ l) and reverse primer (10.0 pmol/ μ l), 0.16 μ l 5U Taq DNA-polymerase (Qiagen, Hilden, Germany), 6.12 μ l HPLC gradient grade water and 2.0 μ l template DNA using the same PCR conditions as described for the SSRs. The applied SNP markers were converted to cleaved amplified polymorphic sequences (CAPS) markers by digesting PCR products with corresponding restriction endonucleases. A restriction site for the GBS546 SNP marker was previously described by Kota et al. (2008). The other two markers were firstly sequenced to detect SNPs and then converted into CAPS markers by the use of the SNP2CAPS tool (<http://pgrc.ipk-gatersleben.de/snp2caps/>; Thiel et al. 2004). The PCR products were digested with a set of three restriction endonucleases to generate CAPS. Three enzymes, *HpyCH4IV*, *HhaI* and *SsiII*, were used according to the

Table 1 Size of alleles detected in the resistant (MBR1012) and susceptible (Scarlett) parents for 17 polymorphic markers located on chromosome 1H of barley

Marker	Endonuclease	Allele size (bp)		References
		MBR1012	Scarlett	
QBS2	GBR218 derived	361	328	Stein et al. (2007); this publication
GBS546	<i>CAPS-HhaI</i>	424	308/116	Kota et al. (2008)
GBMS187		Null	161	Li et al. (2003)
GMS21		183	Null	Struss and Plieske (1998)
GBM1007		218	216	Thiel et al. (2003)
GBM1042		312	308	Thiel et al. (2003)
Bmag872		134	118	Ramsay et al. (2000)
GBM1032		115	117	Thiel et al. (2003)
Bmag211		204	200	Ramsay et al. (2000)
GBM1070		180	182	Thiel et al. (2003)
Bmag347		129	125	Ramsay et al. (2000)
GBM1336		147	143	Varshney et al. (2007)
Bmac63		150	146	Ramsay et al. (2000)
GBS528	<i>CAPS-HpyCH4IV</i>	390	282/108	Kota et al. (2008)
Bmac32		243	231	Ramsay et al. (2000)
GBS582	<i>CAPS-SsiII</i>	446	219/196	Kota et al. (2008)
HvHVA1		153	157	Maccaulay et al. (2001)

manufacturer's instructions (New England Biolabs) and performed in a total volume of 20 μ l with 10 μ l PCR-product, 2 μ l of the relevant NE Buffer, 7.9 μ l HPLC gradient grade water and 0.1 μ l enzyme. The cleaved DNA fragments were separated on 1.8% agarose gels as described previously. Conversion of restriction fragment length polymorphism (RFLP) marker GBR218 into PCR-based marker QBS2 (QBS2f 5'-AGCTGAATCCAACCCAACAC-3' and QBSr 5'-AGTCGCAGAGCCACAAGTTC-3') was performed according to Perovic et al. (2007).

Map construction

The genetic map was constructed using JoinMap 4.0 (van Ooijen 2006) applying the Kosambi function (Kosambi 1944). Only markers with a LOD score of 3 were integrated into the map.

Results

Phenotypic analysis

The infection type of the susceptible parent Scarlett was 2-3, whereas that of the resistant parent MBR1012 was

0-2-. The 91 DH lines derived from the cross of the resistant landrace MBR1012 and the susceptible German cultivar Scarlett segregated 48 resistant: 43 susceptible ($\chi^2 = 0.29$, $p = 0.6$). This segregation pattern fits well to the expected 1 resistant : 1 susceptible ratio, indicating that leaf rust resistance against isolate I-80 in MBR1012 is inherited in a monogenic manner.

Linkage analysis

The parents and the two bulks were analysed with a set of 248 co-dominant molecular markers, which were evenly distributed along the seven chromosomes of barley. Of these, 89 SSR (51%) and 21 SNP markers (29%) were polymorphic. Polymorphisms between the resistant and susceptible bulk were detected on chromosome 1H. Overall, out of the 32 SSR and 11 SNP markers localized on chromosome 1H, 14 and 3 were polymorphic, respectively. Based on these markers, a final genetic map of 119 cM of chromosome 1H was constructed (Fig. 1). The leaf rust resistance gene in MBR1012 was mapped in the telomeric region of the short arm of chromosome 1H. The closest linked markers are GBMS187 and GBS546, which map 0.8 cM distal to the resistance gene. On the proximal side, the closest marker

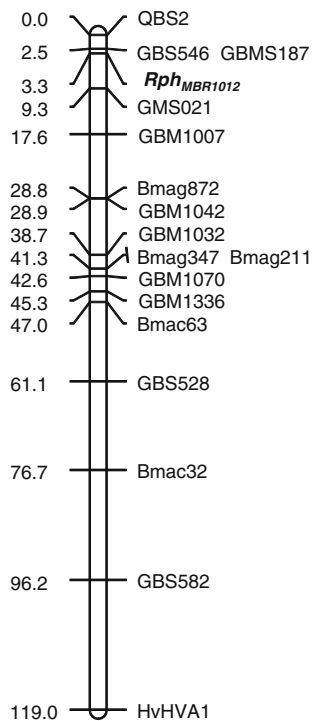


Fig. 1 Genetic map of chromosome 1H including the resistance locus *Rph*_{MBR1012}

identified is GMS21, which maps 6.0 cM from the resistance locus. The allele sizes and the restriction patterns of all mapped markers from chromosome 1H are given in Table 1.

Leaf rust infection patterns

The seven barley accessions displayed very different reaction patterns in response to the collection of 13 European leaf rust isolates (Table 2). Susceptible L94 and Gold carrying *Rph4* were highly susceptible to the entire collection of leaf rust isolates, while susceptible parental cultivar Scarlet exhibited resistance to 12 isolates and was only susceptible to isolate I-80. Although Cebada Capa (*Rph7*), H.sp.680 (*Rph16*) and MBR1012 turned out to be resistant to all isolates, their reaction patterns varied from 0_c (chlorosis), 0_n (necrosis) to 0-2-, respectively.

Diagnostic value of closest markers

To assess the diagnostic value of the four closest linked markers to the leaf rust resistance gene in MBR1012, a set of 51 barley accessions was assayed. This barley collection consisted of the original sources of the described *Rph* genes, near-isogenic lines of cultivar Bowman with introgressed *Rph* genes, two lines with partial leaf rust resistance, and 10 susceptible lines with no known *Rph* genes (Table 3). The number of detected alleles per marker in this analysed collection varied from three alleles for GBS546 and six alleles for GMS21 to nine and eleven alleles for QBS2 and GBMS187, respectively. Restriction pattern B of SNP marker GBS546 (Fig. 2), characteristic

Table 2 Differential reactions of seven barley cultivars/lines possessing known leaf rust resistance genes after inoculation with 13 *Puccinia hordei* isolates

Cultivar Isolate/gene	Gold <i>Rph4</i>	Cebada capa <i>Rph7</i>	Bowmann <i>Rph15</i>	Scarlett <i>Rph3/Rph9/Rph12</i>	H.sp. 680 <i>Rph16</i>	MBR1012 <i>Rph</i> _{MBR1012}	L 94 Susceptible
R 8-1	3-4	0 _c	0	0	0 _n	0	3-4
R 8-2	3-4	0 _c	0	0	0 _n	0	3-4
R 14-1	3-4	0 _c	0	0	0 _n	0	3-4
R 14-2	3-4	0 _c	0	0	0 _n	0	3-4
R 16-1	3-4	0 _c	0	0	0 _n	0	3-4
R 34-3	3-4	0 _c	0	0	0 _n	0	3-4
R 54-3	3-4	0 _c	0	0	0 _n	0	3-4
I 80	3-4	0 _c	0-2-	3	0 _n	0-2-	3-4
30-1*	3-4	0 _c	0-2-	0	0 _n	0, 0-1	3-4
30-1/2*	3-4	0 _c	0	0, 0-2-	0 _n	0, 0-2-	3-4
23- 3	3-4	0 _c	0	0	0 _n	0	3-4
23-1/2/3*	3-4	0 _c	0	0	0 _n	2-	3-4
23-1+3*	3-4	0 _c	0	0	0 _n	0, 0-2-	3-4

* Additional virulence to the Trumpf (*Rph12*)

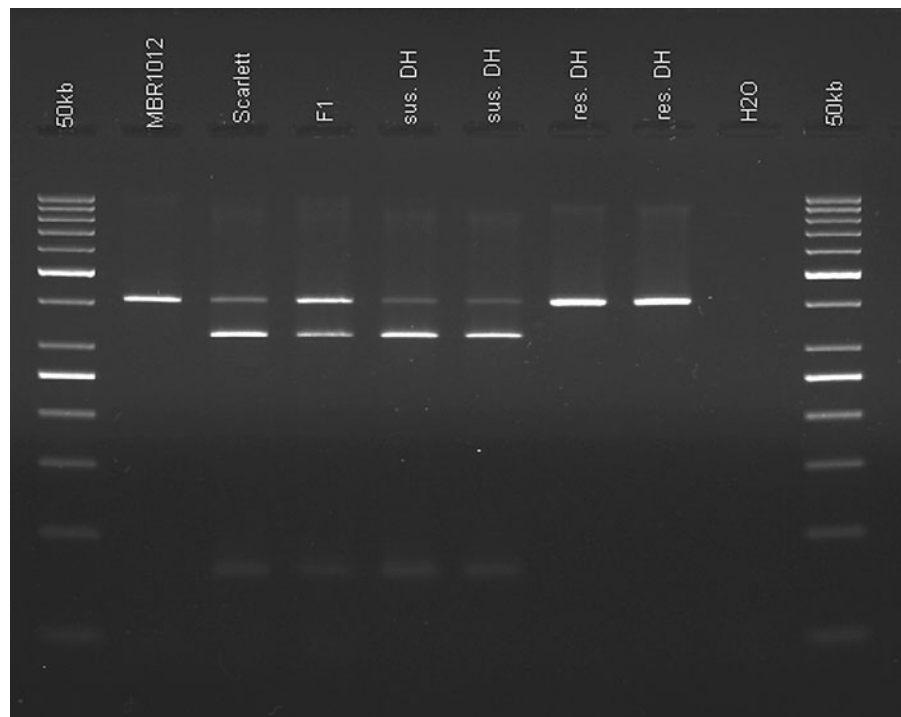
Table 3 Size of alleles and restriction patterns detected on 52 barley lines screened with the four markers most closely linked to the resistance locus on chromosome 1H

Cultivar/accession	Gene	Gene-locus	QBS2	GBMS187	GBS546	GMS21	Origin	Genbank no.	Locus
1 MBR1012	Resistant/Rph _{MBR1012}	–	361	Null	B	183	YU	Landrace	–
2 Scarlett	Rph3/Rph9/Rph12	7HL/5HS/5HL	328	161	A	Null	GER	Cultivar	Jin et al. (1993)/Borovkova et al. (1998)
3 Bowman	Susceptible	–	–	176	A	175	USA	Cultivar	–
4 Bowman Rph15	Rph15	2HS	–	176	A	175	USA	NIL Bowman	Weerasena et al. (2004)
5 195-264-1	Rph1a	2H	328/361	178	A	175	USA	NIL Bowman	Roane and Starling (1967)
6 195-266-1	Rph2.b	5HS	361	178	A	175	USA	NIL Bowman	Borovkova et al. (1997)
7 195-343-1	Rph2.y	5HS	328	148/178	A	170	USA	NIL Bowman	Borovkova et al. (1997)
8 195-267-2	Rph3.c	7HL	328/361	178	A	175	USA	NIL Bowman	Jin et al. (1993)
9 195-268-4	Rph4.d	1HS	328	178	B	170	USA	NIL Bowman	McDaniel and Hathcock (1969)
10 195-269-1	Rph5.e	3HS	361	178	B	175	USA	NIL Bowman	Mammadov et al. (2003)
11 195-270-2	Rph6.f	3HS	361	178	A	175	USA	NIL Bowman	Brunner et al. (2000)
12 196-424-2	Rph7.ac	3HS	321	178	A	175	USA	NIL Bowman	Brunner et al. (2000)
13 193-21	Rph7.g	3HS	361	178	A	178	USA	NIL Bowman	Brunner et al. (2000)
14 195-349-4	Rph8.h	7HS	328	178	A	175	USA	NIL Bowman	Borovkova et al. (1997)
15 194-224	Rph9.i	5HS	361	178	A	183	USA	NIL Bowman	Borovkova et al. (1998)
16 195-274-1	Rph9.z	5HS	361	178	A	175	USA	NIL Bowman	Borovkova et al. (1998)
17 195-273-2	Rph10.o	3HL	361	178	A	175	USA	NIL Bowman	Feuerstein et al. (1990)
18 195-272-1	Rph11.p	6HS	323	178	A	183	USA	NIL Bowman	Feuerstein et al. (1990)
19 195-288-2	Rph13.x	7HS	328/361	178	A	175	USA	NIL Bowman	Sun and Neate (2007)
20 195-290-2	Rph14.ab	7HS	361	148/178	A	183	USA	NIL Bowman	Golegaonkar et al. (2009)
21 195-282-2	Rph15.ad	2HS	361	178	A	175	USA	NIL Bowman	Weerasena et al. (2004)
22 Sudan	Rph1	2H	328	178	A	175	USA	AHOR 307	Roane and Starling (1967)
23 Peruvian	Rph2	5HS	374	178	B	183	USA	AHOR 255	Borovkova et al. (1997)
24 Rika x FI	Rph3	7HL	361	178/161	A	178	GER	AHOR 1308	Jin et al. (1993)
25 Gold	Rph4	1HS	327	178	B	178	DNK	AHOR 118	McDaniel and Hathcock (1969)
26 Cebada Capa	Rph7	3HS	327	178	A	175	TUR	AHOR 4270	Brunner et al. (2000)
27 Quinn	Rph2+Rph5	5HS+3HS	321/327/363	178	A	175	USA	AHOR 2397	Borovkova et al. (1997)/Mammadov et al. (2003)
28 Bolivia	Rph2+Rph6	5HS+3HS	321/341	178	B	183	USA	AHOR 4506	Borovkova et al. (1997)/Brunner et al. (2000)

Table 3 continued

	Cultivar/accession	Gene	Gene-locus	QBS2	GBMS187	GBS546	GMS21	Origin	Genbank no.	Locus
29	Egypt4	Rph8	7HS	328/373	178	A	183	USA	AHOR 3875	Borovkova et al. (1997)
30	HOR 500-1	Rph1d+Rph1r	2H	328	178	B	175	TUR	AHOR 500	Roane and Startling (1967)
31	HOR 1132	Rph2r	5HS	328/361	178	C	187	France	AHOR 1132	Borovkova et al. (1997)
32	HOR 2596	Rph9	5HS	–	178	A	183	ETH	Abyssinian	Borovkova et al. (1998)
33	HOR 679-3	Rph3	7HL	361	178	A	183	GRC	AHOR 679	Jin et al. (1993)
34	Estate	Rph3+r	7HL	328	178	C	175	USA	AHOR 2476	Jin et al. (1993)
35	Oderbrucker	Rph1	2H	328	181	A	175	GER	AHOR 1898	Roane and Startling (1967)
36	Rekal	Rph3+?	7HL	328	181	B	183	USA	AHOR 4845	Jin et al. (1993)
37	HOR 4280	Rph1d+Rph1r	2H	328	181	A	183	ITA	AHOR 4280	Roane and Startling (1967)
38	Trumpf	Rph12	5HL	328/361	181	B	170	GER	AHOR 4967	Borovkova et al. (1998)
39	H.spon.680	Rph16	2HS	328	181	A	183	GER	–	Ivandić et al. (1998)
40	Hanka	Rph7	3HS	–	181	A	175	Israel	–	Brunner et al. (2000)
41	Krona	Rph12	5HL	361	181	A	170	GER	–	Borovkova et al. (1998)
42	HOR 1063	Partial res.	–	361	148	C	170	ETA	AHOR 3036	–
43	Vada	Partial res.	–	–	178	B	170	GER	AHOR 10987	–
44	Karat	Susceptible	–	328/361	178	A	175	NLD	–	–
45	L94	Susceptible	–	328	178	A	170	GER	–	–
46	Grossklappige	Susceptible	–	328	148/178	A	175	CSK	AHOR 9796	–
47	Salome	Susceptible	–	328	178	A	175	GER	AHOR 9735	–
48	Lada	Susceptible	–	328/361	181	B	175	GER	HHOR 2276	–
49	Alexis	Susceptible	–	328/348	178	A	170	GER	–	–
50	Igri	Susceptible	–	321	161/178	A	175	TUR	AHOR 1063	–
51	MBR532	Susceptible	–	328	178	A	170	YU	–	–
52	Emir	Susceptible	–	328/361	178	C	170	NLD	AHOR 3548	–

Fig. 2 Pattern of the CAPS marker GBS546 after digestion with *Hha*I closely linked to resistance locus $Rph_{MBR1012}$



of the resistant parent MBR1012, was observed for ten other genotypes. Similarly, for the closest proximal marker SSR GMS21, ten genotypes showed the same allele pattern as the resistant parent MBR1012. Accuracies in prediction of $Rph_{MBR1012}$ were 74.5% for QBS2, 78.5% for GMS21, 80.5% for GBS546 and 100% for GBMS187. Therefore, we conclude that QBS2, GBS546 and GMS21 have no diagnostic value for tagging the resistance gene described in MBR1012, but could be used for the transfer of the resistance gene into elite cultivars. The closely linked marker GBMS187 (Fig. 3) detected a null allele in the resistant parent MBR1012, whereas in the other genotypes examined eight different alleles were identified. Although the null allele of the GBMS187 in the parent MBR1012 is unique in the germplasm investigated, the use of this marker in breeding is of limited value due to its dominant mode of inheritance.

Discussion

The gene pool of cultivated barley is largely depleted of major resistance genes for many fungal and viral pathogens (Graner et al. 2000a). The collection of spring barley landraces from the former Yugoslavia

that was used in this study is a rich resource of important disease resistances (Perovic et al. 2001). Although the diversity present in this barley collection represents a good base for the transfer of many important traits into elite cultivars, it is seldom used in breeding programs. To enhance the use of genetic diversity from this collection, the leaf rust resistance in line MBR1012 was evaluated in detail.

Leaf rust resistance in landrace MBR1012 is conferred by a major gene, which exhibits a hypersensitive reaction to many of the most widespread virulent European *P. hordei* isolates and is located on the distal portion of the short arm of chromosome 1H.

A linkage map was constructed for chromosome 1H. In general, the order of markers in this genetic map was in agreement with previously published maps (Stein et al. 2007; Varshney et al. 2007; Kota et al. 2008). Only slight deviations in the marker order among the different maps were observed and they may be explained by the fact that the maps of Varshney et al. (2007) and Stein et al. (2007) are consensus maps constructed by the integration of four independent genetic maps of barley. In order to convert the RFLP marker GBR218 that is mapped to the most telomeric region of chromosome 1H according to Stein et al. (2007), specific primers were designed for

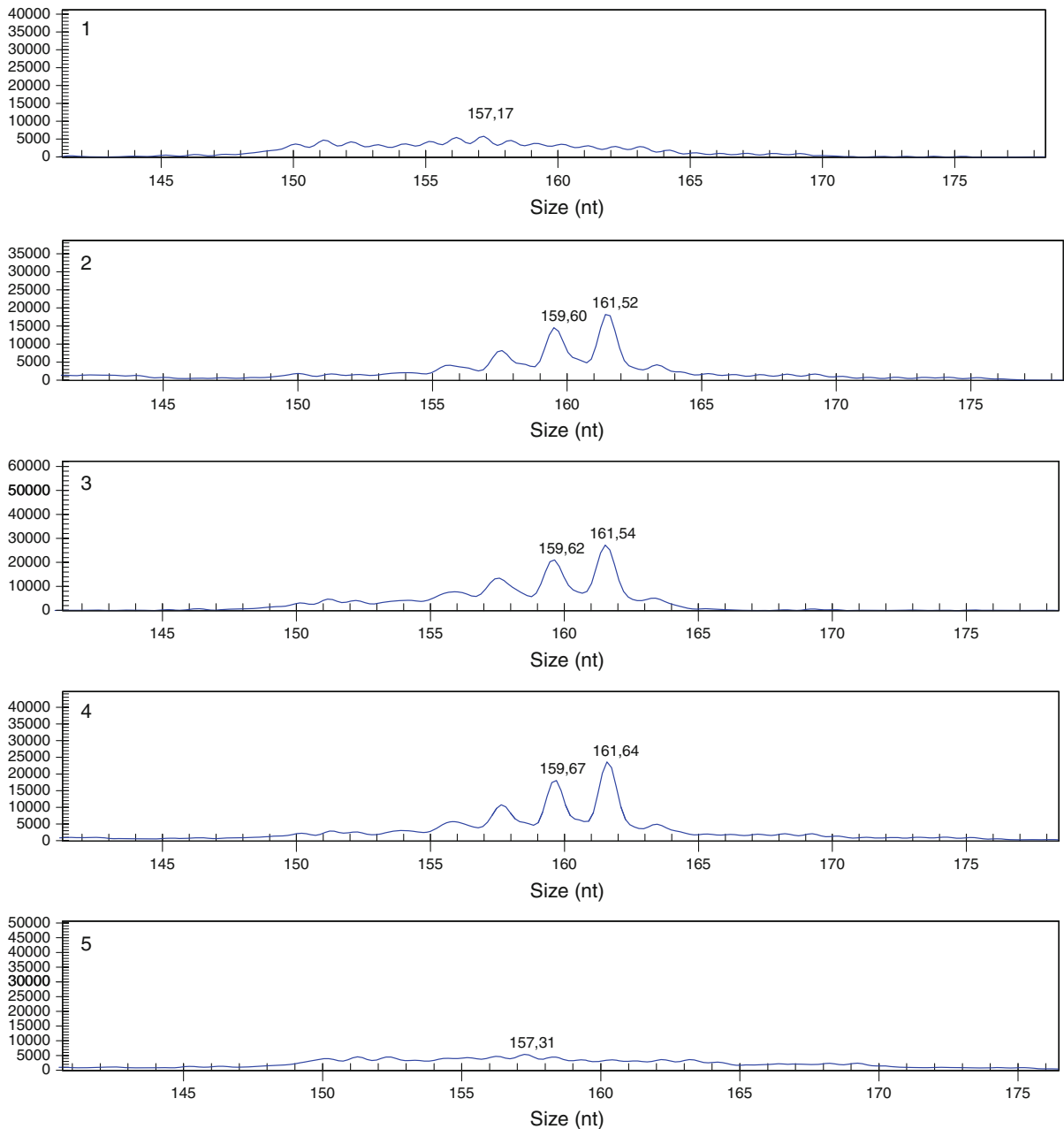


Fig. 3 Chromatograms of the SSR marker GBMS187 closely linked to the resistance gene Rph_{MBR1012} . The order of genotypes are: 1 resistant parent MBR1012, 2 susceptible parent Scarlett, 3 F1-plant, 4 susceptible DH line and 5 resistant DH line

amplification and sequencing. Generally, RFLP markers are very robust markers, but their analysis is expensive and laborious. Here, we converted the GBR marker into an easy-to-use PCR marker. The newly developed PCR marker QBS2 directly detects an insertion/deletion polymorphism of 33 bp between the

parents (Table 1), was locus-specific and showed the same map location as the corresponding RFLP marker.

The leaf rust resistance locus Rph_{MBR1012} was mapped in a 6.8-cM interval between the markers GBMS187/GBS546 and GMS21. GBMS187 and GBS546 co-segregate and are the closest linked

markers (0.8 cM distal) to *Rph*_{MBR1012}. Because of this genetic position, it was assumed that they may be useful as diagnostic molecular markers in breeding programs employing marker-assisted selection (MAS). Although they co-segregated, an assessment of germplasm revealed different accuracies in the prediction of the MBR1012 resistance gene, viz. 80.5% for GBS546 and 100% for GBMS187. It is important to note that GBMS187 (Fig. 3) is a dominant marker in our population; hence, it may not be the best marker for MAS of this leaf rust resistance gene. Since we were able to convert SNP markers into CAPS markers, an easy-to-use PCR MAS scheme can be conducted in combination with flanking markers GBS546/GBMS187 and GMS21.

Other dominant leaf rust resistance genes have been mapped to nearly all barley chromosomes (see overview by Golegaonkar et al. 2009). The genes *Rph7* and *Rph10* were mapped to chromosome 3H (Feuerstein et al. 1990; Graner et al. 2000b; Zhong et al. 2003), *Rph2* to chromosome 5HS (Borovkova et al. 1997), *Rph3* to 7HL (Jin et al. 1993), *Rph11* to 6HL (Feuerstein et al. 1990) and *Rph15* and *Rph16* to 2H (Weerasena et al. 2004). Only the dominant gene *Rph4*, derived from cultivar Gold, was localized on chromosome 1HS through its linkage (~17 cM) with the powdery mildew resistance locus *Mla* (McDaniel and Hathcock 1969). However, this chromosome 1H assignment is probably incorrect, based on comprehensive SNP data from cultivar Bowman and the corresponding near-isogenic line carrying *Rph4* (Arnis Druka, personal communication). The most likely location of *Rph4* according to this information is chromosome 2H. This, coupled with the fact that the leaf rust resistance gene in MBR1012 exhibits a completely different resistance spectrum from *Rph4* to the common European leaf rust isolates (including I-80, Table 2), provides strong evidence that the two genes are different. Before we can unequivocally determine that the leaf rust resistance gene in MBR1012 is different from all other previously described genes in barley, a provisional locus designation of *Rph*_{MBR1012} is being assigned.

*Rph*_{MBR1012} provides effective resistance against leaf rust isolate I-80, which is capable of overcoming all common leaf rust resistance genes in the European barley gene pool, except for *Rph7*, *Rph15* and *Rph16*. While *Rph7* has been incorporated into many European barley cultivars, there are no reports to our

knowledge on the incorporation of *Rph15* and *Rph16*, which are derived from *H. vulgare* ssp. *spontaneum*. In contrast to genes derived from wild barley where linkage drag can be a serious problem, the resistance gene *Rph*_{MBR1012} is derived from a landrace and may be integrated into adapted cultivars much faster, thereby broadening the genetic base of leaf rust resistance. Although further research on this gene is needed, the results of this work open the opportunity to broaden the genetic base of resistance to *P. hordei* in barley and provide breeding programs with genetic markers that will facilitate the faster incorporation of this resistance into elite cultivars.

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