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# Candidate markers for powdery mildew resistance genes from wild barley PI284752

**Jana Řepková · Antonín Dreiseitl**

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**Abstract** PI284752, an accession of wild barley (*Hordeum vulgare* ssp. *spontaneum*) resistant to powdery mildew caused by *Blumeria graminis* f.sp. *hordei*, was studied with the aim of identifying genes involved in powdery mildew resistance. An $F_2$ population (456 plants) was established from a cross between the winter barley variety 'Tiffany' and PI284752. This cross demonstrated a two-locus model of resistance. Linkage analysis using polymorphic DNA markers was carried out on 180 plants. The *RGH1a* gene sequence from the *Mla* locus was used as a source for developing the *RGH1aE2I2* marker. By interval mapping on chromosome 1HS, one resistance gene was found to be tightly linked with *RGH1aE2I2* and it was found to be located 2 cM from *GBMS062*. In $F_2$ plants exhibiting resistance reaction type (RT) 0, specific DNA fragments for the *RGH1aE2I2* marker were amplified. In plants with RT1 to RT2-3, the resistance was conferred exclusively by the second *R* gene that we identified, which is linked with *Bmac0134* and *GBMS247* on chromosome 2HS. The aforementioned markers may be valuable candidates for marker-assisted selection of resistant genotypes conferred by one or both genes.

**Keywords** *Blumeria graminis* f.sp. *hordei* · Genetic mapping · *Hordeum vulgare* ssp. *spontaneum* · Resistance gene homolog · Marker-assisted selection

J. Řepková (✉)
Department of Experimental Biology, Faculty of Science, Masaryk University Brno, Kotlářská 2, 61137 Brno, Czech Republic
e-mail: repkova@sci.muni.cz

A. Dreiseitl
Agricultural Research Institute Kroměříž Ltd., Havlíčkova 2787, 76701 Kroměříž, Czech Republic

## Introduction

Barley powdery mildew, caused by *Blumeria graminis* (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal (=*Bgh*), ranks high among cereal pathogens for its adaptability and ability to cause crop loss (McDonald and Linde 2002; Dreiseitl 2003). The efficacy of powdery mildew resistances in barley cultivars (*Hordeum vulgare* L.) registered in the Czech Republic is generally very low (Dreiseitl 2008). To combat this problem, barley must be bred with effective resistance genes to which there is little, or no, corresponding virulence in the existing pathogen population. The use of two or more fully effective genes in one cultivar should limit the speed of pathogen adaptation and thus extend the effectiveness of cultivar resistance to powdery mildew in winter barley. Current methods, such as marker-assisted selection (MAS), enable breeders to pyramid combinations of these resistance genes.

The rapidly evolving technology of DNA markers helps to open a real possibility for developing

functional markers as reliable genetic markers for use in plant breeding. Following high resolution mapping of a gene conferring an important agronomic trait in a crop, researchers can then identify tightly linked markers that co-segregate with the trait and are therefore candidate markers for plant breeding. In barley, consensus and integrated maps (Hearnden et al. 2007; Stein et al. 2007; Varshney et al. 2007) offer the best saturation of the chromosomal regions and are a valuable resource for identifying functional markers for candidate genes at agronomically important loci. Simple sequence repeats (SSRs) have become the preferred markers for the genetic analysis of cereals (Hearnden et al. 2007); they form the basis of several barley genetic maps (Ramsay et al. 2000; Thiel et al. 2003; Varshney et al. 2006). EST-derived (expressed sequence tag) SSR markers derived from highly conserved, transcribed regions have increased considerably coverage of the barley genome (Beaubien and Smith 2006; Varshney et al. 2006; Hearnden et al. 2007). These EST-derived SSRs are the low number of multilocus markers representing unique positions in genetic maps. Moreover, EST-based markers are syntenic to known collinear linkage groups in cereals (Stein et al. 2007). Another type of markers, single nucleotide polymorphisms (SNPs), is the most abundant form of variation in all genomes, including those of plants. The first integrated SNP-based linkage map of barley was constructed by Rostoks et al. (2005). DNA markers offer great potential to increase our knowledge of the sequences underlying traits of interest. From this information, sequence-tagged sites (STSs) and cleaved amplified polymorphic sequence (CAPS) markers can be determined.

DNA marker technology could facilitate the rapid introgression of new race-specific genes conferring resistance to *Bgh* from their donor sources into cultivated barley with a powdery mildew susceptible background. Contrary to the race-specific resistance to single pathogen strains, resistance mediated by a recessive *mlo* allele of the *Mlo* locus confers a broad spectrum resistance (non-specific) to almost all known isolates of the *Bgh* and the resistance is apparently durable in the field. While qualitative resistance is easily used in breeding because it follows Mendelian inheritance patterns, quantitative resistance is thought to be more durable. It has been shown that the powdery mildew resistance is coded by one or a few major genes and also by quantitative trait loci (QTL) resistances (Backes et al. 2003; von Korff et al. 2005; Yun et al. 2005). Wenzel et al. (2001) hypothesised that both QTL and qualitative loci may form tightly linked clusters that act as functional units. It is therefore necessary to identify novel major powdery mildew resistance loci or quantitative loci that can be combined with known genes.

The present study was undertaken to genetically characterize an accession of wild barley resistant to powdery mildew. PI284752 is the newly identified powdery mildew resistance donor from the USDA National Small Grains Collection originating from the Near East (Dreiseitl and Bockelman 2003; Dreiseitl and Dinoor 2004). The objectives of this investigation were: (1) to find the number of genes/loci in wild barley accession PI284752 conferring powdery mildew resistance; (2) to find the identity of resistance genes by means of their chromosomal locations; and (3) to identify tightly-linked polymorphic DNA markers as candidates for marker-assisted selection.

## Materials and methods

### Plant material and population development

The barley variety ‘Tiffany’, which possesses already overcome powdery mildew resistance genes *Mla7* and *MlaMu2* (Dreiseitl 2007), was crossed as a female parent with the wild barley accession PI284752, which is resistant to powdery mildew (Dreiseitl and Bockelman 2003; Dreiseitl and Dinoor 2004). The dormancy of harvested $F_1$ generation was routinely interrupted by keeping the seeds at 38°C for 48 h, and the seeds were consecutively sown in vegetation pots. During vernalisation, young plants were grown in a cool room at 5 ± 2°C for 42 days and then moved into a greenhouse until harvest. The $F_2$ population was established after self-pollination of the $F_1$ generation.

### Pathogen isolates and resistance tests

Two pathotypes of *Bgh* held in the pathogen gene bank at the Agricultural Research Institute Kroměříž (Czech Republic) were used for the inoculation of the young plants to be tested. Twelve plants from each parent, 30 $F_1$ plants and 456 $F_2$ plants were grown in the greenhouse and evaluated. The resistance tests were done on leaf segments: a virulent (*Va7, VaMu2*)

pathotype 5715 was used to identify the number of resistance genes in the accession and its mode of inheritance, and an avirulent (*Aa7*) pathotype 1002 was used to test the allelism of the unknown and the *Mla* loci as described by Řepková et al. (2006). Reaction types (RTs) of individual $F_2$ plants were scored on the 0–4 scale (Torp et al. 1978) where RTs 2–3 and lower are considered resistant. If the evaluation of phenotypic manifestation of the trait in the $F_2$ population revealed both resistant and susceptible plants after inoculation with the *Va7* pathotype and all plants showed only the resistant phenotype after inoculation with the *Aa7* pathotype, the resistance was considered to be determined by alleles of the *Mla* locus. If resistant and also susceptible $F_2$ plants were identified after inoculation with the *Aa7* pathotype, the resistance genes were considered to be different from the *Mla* locus.

## Inheritance of resistance genes

The plant numbers of the two phenotypic categories (resistant and susceptible) found in the $F_2$ population were compared with theoretical Mendelian segregation ratios by a chi-square test, using the data for the observed values and the expected values, and the number of resistance genes was estimated. The comparison of RTs between parental and $F_1$ generations enabled the determination of the inheritance type of resistance genes.

## DNA analysis and linkage detection

Plants (180) from the $F_2$ population were used for molecular analysis. It was a random selection from the 456 $F_2$ plants. DNA extractions from the leaves of parental and $F_2$ plants were performed using the Gene Elute Plant Genomic DNA Miniprep Kit (Sigma–Aldrich Co., Czech Republic). Altogether 44 SSR markers (Table 1) polymorphic for both parents were used to find markers linked to the gene of interest. Those markers were found using a modified bulk segregant analysis (BSA) in which each resistant (RT0) and susceptible bulk (RTs 3 to 4) consisted of 18 individual plants and their DNA without DNA pooling. A new STS marker was developed from the *RGH1a* gene sequence (Fig. 1a) from chromosome 1H (*R*-gene homolog; accession number AF427791, http://www.ncbi.nlm.nih.-gov/) and included into BSA. A pair of primers was designed using the PRIMER3 program.

The all PCR-amplified fragments were separated by electrophoresis either in a 3% agarose gel or in a 10% polyacrylamide gel. The DNA fragments were visualized by staining using ethidium bromide. Recombination ratios for individual markers and a particular resistance gene were inferred from marker

**Table 1** The survey of all polymorphic microsatellite markers used for *R* genes identification on chromosomes 1H to 7H for powdery mildew resistance accession PI284752 of species *Hordeum vulgare* ssp. *spontaneum*

| Chromosome | Microsatellite | Chromosome | Microsatellite | Chromosome | Microsatellite |
|---|---|---|---|---|---|
| 1H | *Bmac0213*[a] | 2H | *Bmag0692*[a] | 6H | *Bmag0500*[a] |
| | *Bmac0063*[a] | | *Bmag0140*[a] | | *EBmac674*[a] |
| | *Bmac0093*[a] | | *EBmag0793*[a] | | *EBmac0602*[a] |
| | *Bmac0154*[a] | | *EBmag0415*[a] | 7H | *Bmag0021*[a] |
| | *Bmag0382*[a] | | *MGB391*[b] | | *EBmac0603*[a] |
| | *Bmag0872*[a] | 3H | *Bmag0013*[a] | | *Bmag0516*[a] |
| | *UMB502*[c] | | *EBmac0705*[a] | | *Bmac0187*[a] |
| | *UMB503*[c] | | *Bmag0603*[a] | | *Bmag0011*[a] |
| | *MGB402*[b] | | *Bmag0225*[a] | | *Bmag0507*[a] |
| | *GBM1007*[d] | | *Bmag0841*[a] | | *EBmac0764*[a] |
| | *GBM1042*[d] | | *EBmag0705*[a] | | *EBmac0785*[a] |
| | *GBMS247*[d] | 4H | *EBmac0788*[a] | | *Bmag0120*[a] |
| | *GBMS062*[d] | | *GBM1221*[d] | | *Bmac0156*[a] |
| 2H | *Bmac0134*[a] | 5H | *Bmag0223*[a] | | *GBM1326*[d] |
| | *Bmag0125*[a] | 6H | *Bmac0316*[a] | | |

[a] Ramsay et al. 2000

[b] von Korff et al. 2004

[c] Beaubien and Smith 2006

[d] IPK Gatersleben

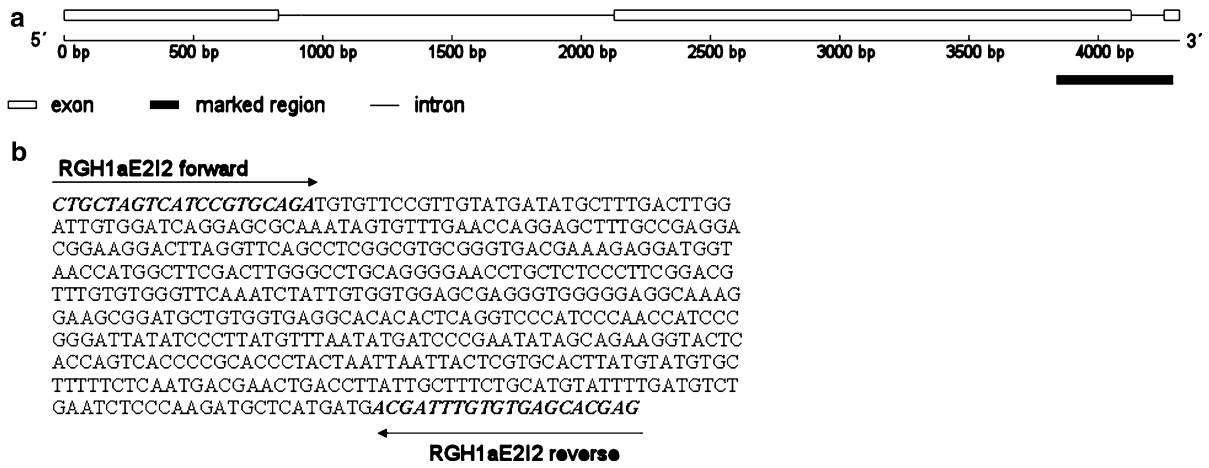


**Fig. 1** Region (*black box*) of the second exon, the second intron and the third exon of the *RGH1a* gene used for *RGH1aE2I2* marker development (**a**) and nucleotide sequence of the *RGH1a* gene used for primer design and amplification (**b**)

genotypes obtained by DNA fragments amplified for a particular marker in the 18 recessive segregants *rr*.

### Identification of resistance genes on barley chromosomes and their co-segregation with DNA markers

Once a linkage was identified by BSA, it was verified using 180 $F_2$ plants of all genotypes. The linkage between resistance genes and microsatellite markers found by BSA and surrounding markers was detected with marker regression and interval mapping using the Map Manager QTXb17 software package for a $F_2$ population (Meer et al. 2002). The significance of the linkage was measured by likelihood ratio statistic (LRS) analysis and the probability of false linkage, $P = 0.001$, was used as the criterion for evidence of linkage. Two threshold values were calculated by the permutation test supplied in the Map Manager QTX software as evidence of linkage: significant, $P < 0.01$; and highly significant, $P < 0.001$. Maps for resistance locus positions on particular chromosomes were constructed using Map Chart software (Voorrips 2002).

Individual phenotypic values for RTs of individual $F_2$ plants scored on a 0–4 scale were compared with a particular amplified fragment for the tightly linked SSR and STS markers. Genotyping of individual $F_2$ plants was performed and the results were compared with the results of genetic analysis based on phenotypes. Conclusions were then drawn concerning gene number and their identity in individual $F_2$ plants, as well as the reliability of co-segregation.

## Results

### Inheritance of resistance genes and allelism test

A summary of the gene number estimates and their allelic relationship to the *Mla* locus is presented in Table 2. Evaluation of $F_2$ plants following inoculation with the virulent pathotype revealed the whole scale of RTs, including the susceptible types. A ratio of 15:1 ($\chi^2 = 1.58$, $P = 0.21$) in the $F_2$ population resulted from the segregation of 434 resistant and 22 susceptible plants, and this result is consistent with a model of two independent genes with dominant alleles of resistance. An allelism test revealed one of the resistant genes to be at the *Mla* locus, because no susceptible plants were detected after inoculation with an avirulent pathotype of *Bgh* (Table 2). Only the RT0 phenotype was found in the resistant parent and in the $F_1$ generation, which is consistent with the notion that both genes show dominant inheritance (Table 2).

### STS marker development

A pair of primers for the second exon, second intron and third exon of the *RGH1a* gene sequence (Fig. 1a) was designed: 5′-CTGCTAGTCATCCGTGCAGA-3′

**Table 2** The number of resistant and susceptible plants in the $F_2$ population derived from the cross of 'Tiffany' and powdery mildew resistance accession PI284752 of species *Hordeum vulgare* ssp. *spontaneum* following inoculation with virulent (*Va7*) and avirulent (*Aa7*) pathotypes of *Blumeria graminis* f.sp. *hordei*

| Generation | Plants | RT[a] | *Va7* pathotype no. plants | Σ | *Aa7* pathotype no. plants | Σ |
|---|---|---|---|---|---|---|
| Parental | 'Tiffany' | 4 | 12 | 12 | | |
| | PI284752 | 0 | 12 | 12 | | |
| $F_1$ | | 0 | 30 | | | |
| $F_2$ | Resistant | 0 | 331 | 434 | 396 | 456 |
| | | 0–1 | 14 | | 45 | |
| | | 1 | 6 | | 14 | |
| | | 1–2 | 23 | | 1 | |
| | | 2 | 46 | | 0 | |
| | | 2–3 | 14 | | 0 | |
| | Susceptible | 3 | 7 | 22 | 0 | 0 |
| | | 3–4 | 2 | | 0 | |
| | | 4 | 13 | | 0 | |

[a] Reaction type, determined as phenotypic manifestation of powdery mildew resistance after inoculation of tested plants with virulent pathotype 5715

and 5′-ACGATTTGTGTGAG-CACGAG-3′ (Fig. 1b). PCR analysis was performed in a volume of 10 µl with 1× reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.5 units of *Taq* polymerase, and 100 ng of barley genomic template DNA. The following program was designed for the amplification of the 506-bp long DNA fragment: one cycle for 3 min at 94°C; 35 cycles for 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, and a final extension of 5 min at 72°C for amplification. The resulting fragments were detected on a 3% agarose gel.

The marker named *RGH1aE2I2* (*E2*—exon 2, *I2*—intron 2) was amplified with a 395-bp fragment in 'Tiffany' and a 440-bp fragment in PI284752. Due to the distinct length polymorphism between parents, no further analysis was performed.

## Linkage detection

The linkage between resistance genes and various markers was revealed by means of BSA on recessive segregants *rr* (susceptible plants of the susceptible bulk), as resistance was dominant over susceptibility. Forty-four microsatellite primer pairs were screened in 18 susceptible plants; in other words, for diploid barley 36 chromosomes and alleles were tested. All markers tested were co-dominant; therefore, recombinants were evaluated out of the 36 chromosomes. Nine markers on chromosome 1H and four markers on chromosome 2H displayed recombination ratios lower than 0.5, indicating linked loci (Table 3). Tight linkage was determined with the markers *RGH1aE2I2*, *GBMS062*, and *GBMS247*; no recombination was detected using these markers.

## Identification of resistance genes on barley chromosomes

All loci associated with either resistance genes are summarized in Table 4. Markers with the highest LRS were included in the table following marker regression analysis; the markers listed show significant and highly significant linkages (thresholds of 11.6 and 23.0, respectively). Regression analysis and interval mapping showed the *RGH1aE2I2* marker to be the most tightly linked with the resistance gene on chromosome 1H, with an LRS value of 228 (Table 4; Fig. 2). From the cross 'Tiffany' × PI284752, the expected result of one resistance locus was established on chromosome 1HS between the microsatellites *MGB402* and *GBMS062* (Fig. 2). The location of the other *R* locus was confirmed between *Bmac0134* and *GBMS247*. All markers used for mapping on chromosomes 1H and 2H segregated in a Mendelian fashion for co-dominance.

**Table 3** Bulk segregant analysis and linkage detection in the $F_2$ population derived from the cross of 'Tiffany' and powdery mildew resistance accession PI284752 of species *Hordeum vulgare* ssp. *spontaneum*

| Markers on 1H | Genotypes[a] | | | %[b] | Markers on 2H | Genotypes[a] | | | %[b] |
|---|---|---|---|---|---|---|---|---|---|
| | $R_1R_1$ | $R_1r_1$ | $r_1r_1$ | | | $R_2R_2$ | $R_2r_2$ | $r_2r_2$ | |
| *UMB502* | 2 | 11 | 5 | 41.7 | *Bmag0140* | 5 | 5 | 8 | 41.7 |
| *Bmac0063* | 1 | 8 | 9 | 27.8 | *Bmag0692* | 3 | 8 | 7 | 38.9 |
| *Bmag0872* | 1 | 7 | 17 | 25.0 | *Bmac0134* | 0 | 2 | 16 | 5.6 |
| *UMB503* | 0 | 4 | 14 | 11.1 | *GBMS247* | 0 | 0 | 18 | 0.0 |
| *MGB402* | 0 | 3 | 15 | 8.3 | | | | | |
| *Bmac0213* | 0 | 3 | 15 | 8.3 | | | | | |
| *GBM1007* | 0 | 3 | 15 | 8.3 | | | | | |
| *GBMS062* | 0 | 0 | 18 | 0.0 | | | | | |
| *RGH1aE2I2* | 0 | 0 | 18 | 0.0 | | | | | |

[a] Based on DNA fragments amplified for a particular marker; inferred from the marker genotype

[b] Rrecombination ratio × 100

**Table 4** The survey of all microsatellite loci associated with resistance loci in the $F_2$ population derived from the cross of 'Tiffany' and powdery mildew resistance accession PI284752 of species *Hordeum vulgare* ssp. *spontaneum*

| Chromosome | Locus[b] | LRS[c] | P[d] |
|---|---|---|---|
| 1HS[a] | *Bmac0063* | 18.6 | 0.00009** |
| | *Bmag0872* | 41.9 | 0.00000*** |
| | *UMB503* | 71.8 | 0.00000*** |
| | *MGB402* | 94.7 | 0.00000*** |
| | *GBM1007* | 95.7 | 0.00000*** |
| | *Bmac0213* | 101.5 | 0.00000*** |
| | *GBMS062* | 205.5 | 0.00000*** |
| | *RGH1aE2I2* | 228.7 | 0.00000*** |
| 2HS[a] | *GBMS247* | 20.1 | 0.00000** |
| | *Bmac0134* | 24.9 | 0.00000*** |

[a] S short arm

[b] Microsatellite marker

[c] Likelihood ratio statistic

[d] The probability of an association between the trait and SSR marker

** $P < 0.01$ and *** $P < 0.001$, the significance thresholds for significant and highly significant evidence of linkage, respectively

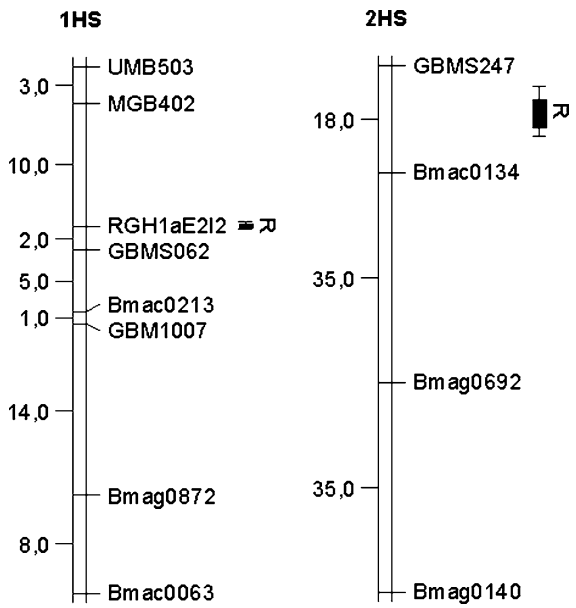


**Fig. 2** A partial genetic map of the barley chromosomes 1HS and 2HS based on the analysis of $F_2$ plants from the cross 'Tiffany' × *Hordeum vulgare* ssp. *spontaneum* PI284752 showing the position of putative loci, *R*, conferring resistance to powdery mildew. Map intervals in centiMorgans are given to the left of chromosomes using Kosambi′s mapping function. DNA marker loci are assigned to the right of the chromosomes. The bars to the right of chromosome map indicate the *R* locus, with the confidence interval positioned by the likelihood maps

## Analysis of co-segregation of DNA markers and powdery mildew resistance

Results summarized in Table 5 and Fig. 3 show that 26 resistant plants with RT1 to RT2-3 have no resistance gene on chromosome 1HS at the *Mla* locus. Linked marker *RGH1aE2I2* amplified only a 395-bp fragment corresponding to the susceptible

**Table 5** Number of genes in individual $F_2$ plants derived from the cross of 'Tiffany' and powdery mildew resistance accession PI284752 of *Hordeum vulgare* ssp. *spontaneum* determined by the marker pairs *Bmac0134/GBMS247* and *RGH1aE2I2/GBMS062*

| RT[a] | No. of plants | No of plants with | | | |
|---|---|---|---|---|---|
| | | 2 genes | 1 gene *Mla* | 1 gene on 2H | No gene |
| 0 and 0-1 | 143 | 116 | 27 | 0 | 0 |
| 1 to 2-3 | 26 | 0 | 0 | 26 | 0 |
| 3 to 4 | 11 | 0 | 1* | 0 | 10 |

[a] Reaction type, determined as phenotypic manifestation of powdery mildew resistance after inoculation of tested plants with virulent pathotype 5715

* Plant with RT3 resembling false genotypic value (resistance) resulted from two recombinations between the *R* gene and the *RGH1aE2I2* marker

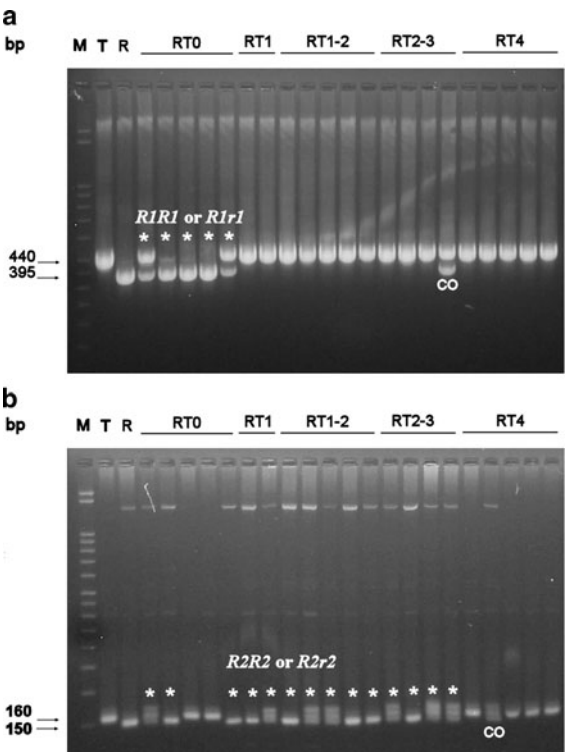


**Fig. 3** Co-segregation analysis for DNA markers **a** *RGH1aE2I2*, **b** *Bmac0134*; Size marker 0.5 kb DNA ladder (M), DNA fragments (bp) obtained after polymerase chain reactions on parents 'Tiffany' (T) and PI284752 (R), and individual $F_2$ plants from the cross 'Tiffany' × *Hordeum vulgare* ssp. *spontaneum* PI284752: Reaction types (RT); RT0, RT1, RT1-2 and RT2-3 resistant plants, RT4 susceptible plants; crossing-over (CO); *asterisks* show plants with the named genotypes

genotype. Resistance in the aforementioned plants is conferred by a gene on chromosome 2H linked to markers *Bmac0134* and *GBMS247*. These results also showed that only resistant plants with RT0 have both *R* genes (116 plants) or only the *Mla* gene (27 plants). Only one exception was found concerning a susceptible plant with RT3 (no resistance gene) but resembling *RR* genotype for the 1HS gene. This resulted from two recombinations between the *R* gene and the *RGH1aE2I2* marker (Table 5).

A genotypic ratio of 9:3:3:1 ($\chi^2 = 5.42, P = 0.14$) was calculated (Table 5). The efficiency of MAS was 99%, which was taken to reflect the probability of two recombinations between the marker and the target gene among 360 chromosomes (180 plants) tested. In other words, the probability of a false plant genotyping result is lower than 1%.

## Discussion

Adopting a good resistance model for powdery mildew in barley breeding is an effective method of controlling the spread of *Bgh*. *H. vulgare* ssp. *spontaneum* is a promising source of various resistance genes (Jahoor and Fischbeck 1987; Dreiseitl and Bockelman 2003; Řepková et al. 2006) because it can be crossed with *H. vulgare* and because a high proportion of its accessions express useful resistance to powdery mildew. Contemporary research has adopted direct genetic selection using molecular markers as an alternative to phenotypic selection for breeding resistance due to the speed of the procedure and the lack of a requirement for pathogen inoculum. A fundamental prerequisite for MAS application in conventional breeding is the availability of tightly-linked DNA markers. This can dramatically increase the speed at which resistant varieties are developed and it can thus be an effective tool for plant breeding. MAS was previously reported to be more effective

when alternative selection methods were either less cost-efficient or impossible, or when the trait showed poor heritability (Koebner and Summers 2003).

In our powdery mildew resistant accession PI284752, for which complete genetic and molecular analyses have been performed, two independent race-specific resistance genes were found. Both a phenotypic ratio of 15:1 consisting of plant numbers 434:22 and a genotypic ratio of 9:3:3:1 consisting of plant numbers 116:27:26:10 are in agreement for two *R* genes introgressed from the donor PI284752 and could be a prerequisite to an efficient and long-lasting resistance. A population with two genes also enables evaluation of gene interaction. Our results show that no interaction exists between the two loci determined, since the calculated ratios match the Mendelian ones.

Both resistance genes identified here show a dominant mode of inheritance. A gene corresponding to the *Mla* locus possesses high efficiency of resistance corresponding to RT0. The other resistance gene accounts for the wide expansion of RTs 1 to 2–3 that contribute to powdery mildew resistance and its phenotypic manifestation is overlapped by the *Mla* gene in the $F_1$ generation. Taking into account the wide range of RT values, this locus may even contain additional linked genes. In a segregating population, individual resistant plants can be homozygous or heterozygous for either or both resistance genes. DNA markers enabled the establishment of individual plant genotypes. The marker pairs *Bmac0134*/*GBMS247* and *RGH1aE2I2*/*GBMS062* were used to genotype individual $F_2$ plants; they turned out to be good candidate markers for the found resistance genes. Crossing-over between an *R* gene and a given DNA marker was rare; these recombination events may result in false determination of resistant or susceptible plants. Such an error was calculated to occur at a rate of 0.6%. Our conclusion on highly reliable co-segregation between particular phenotypes represented by RTs and given markers (Table 5) does not correspond to the mapping on chromosome 2HS (Fig. 2). Mapped distances between the *R* gene and the markers *GBMS247* and *Bmac0134* are too large. We deduce that inaccurate mapping results from overlapping of phenotypes RT0 over RT1 to RT2-3 in individual $F_2$ plants possessing both resistance genes.

Pickering et al. (1995) have previously identified a powdery mildew resistance gene *Mlhb* on the short arm of chromosome 2HS. They studied powdery mildew resistance in a recombinant line derived from a *H. vulgare* × *H. bulbosum* hybrid. However, allelic relationship of *Mlhb* to our gene is unknown. As part of our laboratory's continuing effort to screen wild barley for resistance genes using DNA markers, we have found a powdery mildew resistance gene corresponding to a locus on the short arm of chromosome 2HS in five resistant sources of *H. vulgare* ssp. *spontaneum* (PI466197, PI282605, PI284752, PI391126 and PI296935; data not shown) from a set of 1,383 wild barley accessions (Dreiseitl and Bockelman 2003). In addition to this gene, another gene from the *Mla* locus contributes to powdery mildew resistance in these accessions; in PI391126, a third gene also participates in resistance. In addition, different RTs have been detected in individual resistant accessions using the same *Bgh* pathotype (5715), except PI466197, which was inoculated by 323 pathotype. Parental accession PI284752, described here, resembled only RT0 because the phenotypic manifestation of the 2HS resistance gene was over-ridden by an effect of a gene at the *Mla* locus. PI466197 resembled RTs 0 and 1; PI391126, RTs 0 and 0-1; PI282605, RTs 1 and 1-2; and PI296935, RT2. These differences may result from distinct genes conferring resistance or from variations in genetic background. For example, the *Rpt4* gene consistently displayed different levels of seedling resistance in the three backgrounds (Williams et al. 1999). Nevertheless, these resistant accessions appear to be promising sources for resistance gene introgressions in combination with the *Mla* gene, and they are currently being studied in detail in our laboratory.

Genetic mapping of major genes and quantitative trait loci for many major diseases in barley has revealed a heterogeneous distribution of resistance loci on chromosomes, with more than half of the mapped loci occurring in clusters (Williams 2003). The distribution of powdery mildew resistance genes in the barley genome shows a high concentration of genes in a few chromosomal regions. The first *R* gene is located in a region rich in resistance genes in the barley genome. A complex cluster, which contains multiple race-specific genes conferring resistance to a single pathogen, is found on chromosome arm 1HS, at the well-studied *Mla* locus. Comparison of qualitative gene positions and mapped QTLs for powdery mildew resistance revealed strong correspondence for the *Mla* locus (Heun 1992; Backes et al. 2003; Emebiri et al.

2005; von Korff et al. 2005) and also for the chromosome arm 2HS (Backes et al. 2003; von Korff et al. 2005). Such results support the idea that these regions are important for disease resistance in barley.

The use of DNA markers to localize genes controlling disease resistance may allow introgression of these genes into elite materials, even in areas where the disease is uncommon. In barley, marker-assisted backcross introgression of the *Yd2* gene, which confers resistance to barley yellow dwarf virus, was performed by Jefferies et al. (2003). Grewal et al. (2008) developed DNA markers for cover smut resistance and subsequently achieved marker-assisted introgression of loose and covered smut resistance into hulless barley.

We present here both known markers and a newly developed marker capable of differentiating resistant and susceptible alleles of two powdery mildew resistance genes. This procedure enables selection of plants homozygous for alleles of both genes. Our study is also the first to identify a PCR-based DNA marker associated with powdery mildew resistance in barley that is determined by a gene on chromosome 2HS and that may be useful for gene introgression. The new co-dominant marker *RGH1aE2I2*, derived from the known *RGH1a* gene sequence, simplified our search for a tightly-linked marker, and it proved to be diagnostic for a gene that is a part of the *Mla* locus. Amplification of the section of the *RGH1a* gene sequence from which the *RGH1aE2I2* marker was derived—namely the end of the second exon, the second intron, and the third exon—revealed a 45-bp deletion in the variety 'Tiffany' relative to PI284752. In our experience, the *RGH1a* sequence rarely shows only single nucleotide polymorphism based on restriction digestion analysis (Řepková et al. 2009).

The marker *GBMS062* or the tightly-linked marker *RGH1aE2I2*, which were used in this study as diagnostic for a gene at the *Mla* locus, were successful in identifying homozygous or heterozygous resistant plants with RT0. Another marker, *Bmac0134*, was diagnostic for the other resistance gene on chromosome 2HS; this marker enabled identification of heterozygous or homozygous resistant plants with RT1 to RT2-3. Thus, selection of plants with either of the two genes can be achieved using two markers, although it is ideal to use four flanking markers with sufficiently tight linkages. The other two markers, *Bmac0213* and *GBMS247*, are less tightly linked (6 and 8 cM, respectively).

Increasing the availability of DNA markers linked to resistance genes controlling powdery mildew resistance in barley may facilitate MAS in breeding programs aiming to transfer the resistance genes from PI284752 to elite breeding lines. We propose the DNA markers *RGH1aE2I2* and/or *GBMS062* and *GBMS247* and/or *Bmac0134* as the candidate markers for MAS. The value of these markers has been studied in the present study. For effective MAS, the ideal marker linkage distance is smaller than 1 cM. Linkage distance larger than 1 cM is insufficient due to the growing number of false results caused by an increasing rate of recombination. Our results confirm that DNA markers can assist in introgression of powdery mildew resistance genes into elite varieties and gene pyramiding. Pyramiding genes is a difficult and time-consuming task without the use of molecular marker technology (Tuvesson et al. 2007), which is simple, inexpensive, and feasible at the early seedling stage.

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