Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Mapping of powdery mildew resistance genes in a newly determined accession of *Hordeum vulgare* ssp. *spontaneum*

K. Teturová[1], J. Řepková[1], P. Lízal[1] & A. Dreiseitl[2]

1 Masaryk University, Faculty of Science, Department of Experimental Biology, Kotlářská 2, CZ-611 37 Brno, Czech Republic
2 Agricultural Research Institute Kroměříž Ltd., Department of Integrated Plant Protection, Havlíčkova 2787/121, CZ-767 01 Kroměříž, Czech Republic

Keywords
Barley; *Blumeria graminis* f. sp. *hordei*; DNA markers; genetic mapping; *Hordeum vulgare* ssp. *spontaneum*; *Mla*; resistance genes

Correspondence
Dr Jana Řepková, Masaryk University, Faculty of Science, Department of Experimental Biology, Kotlářská 2, CZ-611 37 Brno, Czech Republic.
Email: repkova@sci.muni.cz

Received: 14 August 2008; revised version accepted: 30 August 2009

doi:10.1111/j.1744-7348.2009.00375.x

Abstract

The accession PI466197 of wild barley (*Hordeum vulgare* ssp. *spontaneum*) with a newly identified resistance to powdery mildew caused by *Blumeria graminis* f.sp. *hordei* was studied with the aim to localise the genes determining resistance on a barley genetic map using DNA markers. Molecular analysis was performed in the F_2 population of the cross between the winter variety 'Tiffany' and the resistant accession PI466197, consisting of 113 plants. DNA markers, 17 simple sequence repeats (SSRs), four sequence-tagged sites (STSs) and one cleaved amplified polymorphic sequence (CAPS) marker developed from the *Mla* locus sequence were used for genetic mapping and a two-locus model of resistance was shown. One of the resistance genes originating from *H. vulgare* ssp. *spontaneum* PI466197 was localised between the markers RGH1aE1 and Bmac0213 on the short arm of chromosome 1H, which is the position consistent with the *Mla* locus. The other gene was proven to be highly significantly linked with GBMS247, Bmac0134 and MWG878 on the short arm of chromosome 2H. The flanking markers were Bmac0134 and MWG878, assigned 4 and 8 cM from the resistance gene, respectively. Until now, no gene conferring powdery mildew resistance originating from *H. vulgare* has been located on the short arm of barley chromosome 2H.

Introduction

Barley (*Hordeum vulgare* L.) is an important crop worldwide, grown mostly for stock feed and malt production, and to a small extent for human consumption. Powdery mildew caused by the fungal pathogen *Blumeria graminis* (D.C.) Golovin ex. Speer f.sp. *hordei* Em. Marchal (*Bgh*) is one of the major diseases of barley in the temperate regions of Europe that can result in losses of grain yield and quality. In conventional agriculture, disease control is often governed by fungicides. Growing resistant varieties is a cost-effective and environment-friendly means of disease control. Therefore, breeding of barley for resistance to powdery mildew is one of the main objectives of crop improvement for both conventional and organic breeding programmes.

A way to retain the qualities of a good variety, while adding desirable traits such as resistance to diseases from either land races or wild germplasm sources, is the introgression of one or more genes from a donor parent into the background of an elite variety. The domestication of cereals has resulted in much lower genetic variation of presently cultivated crops than in original natural populations. Wild barley (*H. vulgare* ssp. *spontaneum*), the ancestor of cultivated barley, has proven to be a good source of new resistance genes to powdery mildew (Dreiseitl & Bockelman, 2003; Fetch *et al*., 2003).

The identification and characterisation of resistance (*R*) genes is usually based on the gene-for-gene relationship (Flor, 1956), which indicates that for a specific resistance gene in the host, there is a corresponding avirulence gene in the pathogen. Barley powdery mildew resistance genes can be postulated using a set of defined *Bgh* pathotypes that possess known avirulence genes. Over the past decades, a considerable number of powdery mildew resistance genes have been discovered and most

of them have also been mapped in the barley genome. Jørgensen (1994) summarised the following identified loci responsible for resistance to powdery mildew (using *Ml*-based symbols): *Mla*, *Mlat*, *MlGa*, *Mlk*, *Mlnn* and *Mlra* on chromosome 1H, *MlLa* on 2H, *MlBo*, *Mlg* and *mlo* on 4H and *Mlh* on 6H. Later, the gene *Mlhb* from *H. bulbosum* was detected on chromosome 2H (Pickering *et al.*, 1995), the gene *Mlj* on 5H and *Mlf* and *mlt* on 7H (Schönfeld *et al.*, 1996).

The *Mla* locus is genetically one of the most thoroughly characterised race-specific *R* loci, encoding 32 known *Bgh* resistance specificities on chromosome 1H (Wei *et al.*, 1999). Another important and most-studied locus is *mlo*, which is race-nonspecific and confers durable resistance to all isolates of *Bgh* (Lyngkjaer *et al.*, 2000). There were also found additional genes required for powdery mildew resistance. The *Rar1* and *Rar2* genes are essential for the function of different *Mla* alleles and for other unlinked race-specific *R* genes (Freialdenhoven *et al.*, 1994; Jørgensen, 1996), and the *Ror1* and *Ror2* genes are necessary for the function of *mlo* (Freialdenhoven *et al.*, 1996). Quantitative trait loci (QTL) for powdery mildew resistance have also been detected on all barley chromosomes (Backes *et al.*, 2003; von Korff *et al.*, 2005; Yun *et al.*, 2005).

Molecular markers have numerous applications in plant genetics and breeding, with one of the main uses being the construction of linkage maps for localisation of loci for important traits. Detection of resistance genes using DNA markers is more effective than conventional methods based on phenotyping. One of the advantages of molecular analysis is that individuals with desirable genotypes can be selected in early generations through the use of codominant markers that can distinguish homozygotes and heterozygotes. In the past, a variety of DNA markers were developed for detecting sequence polymorphisms in plants, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), cleaved amplified polymorphic sequence (CAPS) and single nucleotide polymorphism (SNP) (reviewed, e.g. in Semagn *et al.*, 2006). PCR-based SSR and SNP markers became the preferred marker types in the last decade. A number of genetic maps have been generated in barley (e.g. Pillen *et al.*, 2000; Ramsay *et al.*, 2000; Li *et al.*, 2003); the latest consensus map by Varshney *et al.* (2007a) comprised 775 mapped SSR markers. High-density maps present the essential source of information for genetic mapping of various target genes. A major focus has recently been put on the generation of functional markers (Andersen & Lübberstedt, 2003). Expressed sequence tags (ESTs) can be utilised as a source for the development of new markers (RFLP, SSR, SNP, CAPS) at lower cost (Kota *et al.*, 2001; Thiel *et al.*, 2003). Recently, Stein *et al.* (2007) generated a barley transcript map of 1055 loci detected by 1032 EST-based markers. In addition, diversity array technology (DArT) is a novel high-throughput marker system that can be used to prepare a whole genome map without the availability of sequence data (Jaccoud *et al.*, 2001; Wenzl *et al.*, 2004).

The present work follows the previous research concerning the identification of powdery mildew resistance genes from *H. vulgare* ssp. *spontaneum*. In the previous study, the resistance tests were done, two genes with dominant inheritance were found to determine resistance in the accession PI466197, and the preliminary linkage analysis roughly localised these genes on chromosomes 1H and 2H (Řepková *et al.*, 2006). The aim of this work was to assign the position of the identified resistance genes more precisely on the genetic map of barley and find more tightly linked DNA markers for the two *R* loci.

Materials and methods

Plant material and mapping population

The populations used for the analyses were developed at the Agricultural Research Institute Kroměříž, Ltd. The F_1 and F_2 generations were obtained from a cross between the variety 'Tiffany' and the wild barley accession PI466197. 'Tiffany' is a two-rowed winter barley carrying the ineffective powdery mildew resistance genes *Mla7* and *MlaMu2* (Dreiseitl, 2007). The accession PI466197 is one of the newly identified powdery mildew resistance sources from the USDA National Small Grains Collection originating from the Near East (Dreiseitl & Bockelman, 2003; Dreiseitl & Dinoor, 2004). The genetic mapping was carried out with 113 plants of the segregating F_2 generation.

Resistance tests

The virulent (*Va7*) pathotype 0323 and the avirulent (*Aa7*) pathotype 1002 of *Bgh* from the pathogen gene bank at the Agricultural Research Institute Kroměříž were used for the inoculation of tested plants used for mapping as have previously been described by Řepková *et al.* (2006).

DNA analysis and DNA markers

DNA was isolated from leaves using the Gene Elute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich Co., Prague, Czech Republic). Microsatellite markers by Ramsay *et al.* (2000), Karakousis *et al.* (2003), Li *et al.* (2003), Thiel *et al.* (2003), von Korff *et al.* (2004),

Ann Appl Biol (2010) 157–165 © 2009 The Authors
Journal compilation © 2009 Association of Applied Biologists

Table 1 Primer sequences of barley markers from chromosome 1H and 2H newly designed in Primer3 program

Marker	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)
RGH1aE1	TCACGACAACACCGACAGAT	CAATTCCGCAAGCTCTGAGT
ABG8	TTTCCAATTCATCCCAATCC	GACAATGACTGTGTAAGCATGTGA
cMWG682	GCACACGCCAACACAAAGT	TCTCAGCATCCAACAATCCA
MWG2094	CGATTTCGGCCTATTGGTTA	CGGGGGATCCACTAGTTCT

Beaubien & Smith (2006) and Varshney *et al*. (2006) were used. The primer sequences of the markers GBM1007, GBMS002, GBMS062 and GBMS247 were gained from IPK Gatersleben. One STS marker (ABG53) from chromosome 1H and five STSs (ABG8, ABG58, cMWG682, MWG878 and MWG2094) from chromosome 2H were chosen to be converted into CAPSs. The primers for ABG53, ABG58 and MWG878 were taken from the GrainGenes database (http://wheat.pw.usda.gov/GG2) and for ABG8, cMWG682 and MWG2094 generated by the Primer3 program (Rozen & Skaletsky, 2000) using the ABG8-5, cMWG682-5 and MWG2094-3 sequences given in the database with the STS markers. The primers for RGH1aE1 were designed from the sequence of the *RGH1a* gene (exon 1) at the *Mla* locus (accession no. AF427791) on chromosome 1H (http://www.ncbi.nlm.nih.gov). The primer sequences newly developed in Primer3 are listed in Table 1.

The SSR markers were amplified according to their source protocols, except for AWBMS80 (Karakousis *et al*., 2003), for which the PCR cycle was designed as follows: 94°C for 3 min, 59°C for 1 min, 72°C for 1 min, 35 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, and an additional extension step for 5 min at 72°C. In addition to the markers previously used for linkage detection (Bmac0213, MGB402 and Bmac0134) (Řepková *et al*., 2006), the polymorphisms between the parents ('Tiffany' and PI466197) were screened for 13 SSRs on chromosome 1H and 23 SSRs on chromosome 2H. The amplification products were separated on 3% agarose gels using ethidium bromide staining, except for GBMS062, which was visualised on 10% polyacrylamide gels. For the STS markers ABG53, ABG8, cMWG682 and MWG878, the PCR cycle corresponded to the cycle E by Ramsay *et al*. (2000). The amplification cycle for RGH1aE1 was designed as follows: 94°C for 3 min, 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and the final extension at 72°C for 5 min, and for ABG58 as follows: 94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min 25 s, and 72°C for 5 min. For MWG2094, the cycle F by Ramsay *et al*. (2000) was used and two other variants were proposed, but none of them yielded detectable products.

Table 2 Restriction enzymes used for digestion of CAPS markers derived from barley STSs on chromosome 1H and 2H

Marker	Restriction Enzymes
ABG53	*Alu*I, *Bsa*AI, *Dde*I, *Dpn*I, *Hin*fI, *Mbo*I, *Mse*I, *Nla*III, *Rsa*I, *Taq*I
RGH1aE1	*Dde*I, *Dpn*I, *Mbo*I, *Nla*III, *Scr*FI, *Taq*I
ABG8	*Afl*II, *Dde*I, *Dpn*I, *Hin*fI, *Mbo*I, *Rsa*I, *Xba*I
ABG58	*Acc*I, *Alu*I, *Bst*NI, *Btg*I, *Cla*I, *Dde*I, *Dpn*I, *Dra*I, *Mbo*I, *Rsa*I, *Scr*FI, *Sna*BI, *Taq*I
cMWG682	*Alu*I, *Ava*II, *Ban*II, *Bst*UI, *Btg*I, *Dde*I, *Rsa*I, *Scr*FI, *Taq*I

CAPS, cleaved amplified polymorphic sequence; STSs, sequence-tagged sites.

The expected size of the PCR products was 505 bp for RGH1aE1, 463 bp for ABG53, 314 bp for ABG8, 1024 bp for ABG58, 504 bp for cMWG682, 272 bp for MWG878 and 320 bp for MWG2094. The polymorphism between the parents was tested using restriction enzyme digestion of the PCR products obtained for individual markers. The restriction enzymes were selected according to *in silico* restriction analysis of the particular marker sequence. The restriction enzymes used for the digestion are summarised in Table 2. The cleaved DNA fragments were separated on 3% agarose gels, the undigested PCR products of MWG878 were separated on 10% polyacrylamide gels using ethidium bromide staining.

Linkage analysis and mapping

The data of polymorphic markers obtained for 113 F_2 plants of all reaction types (RTs) scored were analysed in the Map Manager QTX (version b20) program (Manly *et al*., 2001) based on interval mapping. Partial linkage groups were constructed and the orders and map distances for each group of markers were established, one for chromosome 1H and the other for chromosome 2H. Markers were grouped together using LOD > 3. The estimate of map distance (*D*, cM) was determined using the Kosambi map function (Kosambi, 1944). The statistical significance of linkage between a resistance gene and a particular marker was proved using the likelihood ratio statistic (LRS), which was calculated by means of marker regression. The criterion $P < 0.001$ was used as the probability required for a highly significant linkage.

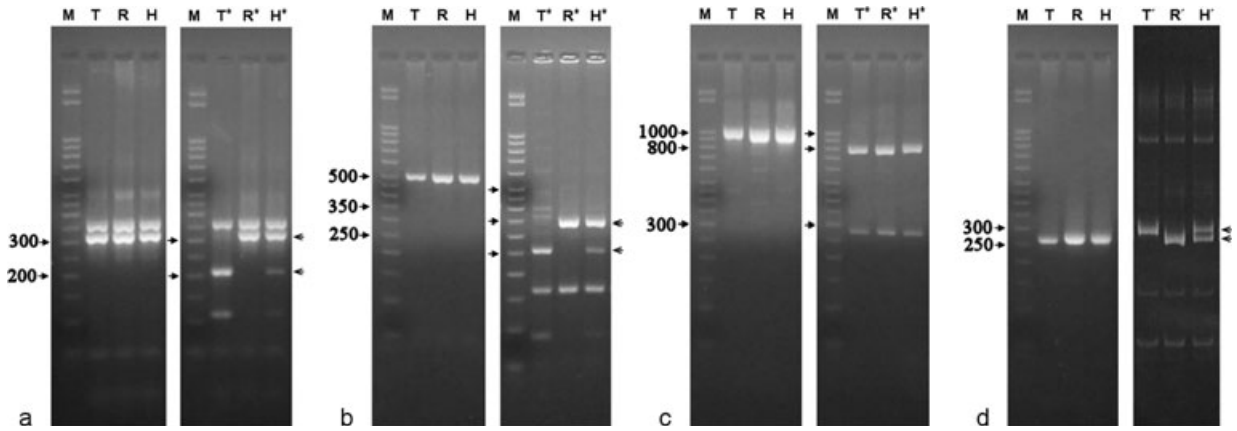

Figure 1 Polymorphism testing for markers ABG8 (a), cMWG682 (b), ABG58 (c) and MWG878 (d) on chromosome 2H. DNA fragments of 'Tiffany' (T), PI466197 (R) and heterozygote (H) obtained after polymerase chain reaction and after digestion with restriction enzymes (T*, R*, H*); in MWG878, PCR products on agarose and polyacrylamide gel (T', R', H'). Size marker 50 bp DNA ladder (M). The arrows on the right (in a, b and d) indicate the polymorphic bands.

Highly significant threshold values were calculated by permutation tests. The data from interval mapping were used in the MapChart software (Voorrips, 2002) to construct the graphical representation of partial genetic maps of barley chromosomes 1H and 2H. The gene positions were established, where LRS score reached local maxima.

Results

DNA markers

The recommended PCR protocols had to be optimised for five SSR markers (Bmac0213, EBmac0501, GBMS247, UMB206 and UMB503). The PCR optimisation for the STS marker MWG2094 was not successful; the marker was excluded from further testing. Out of 36 microsatellites evaluated for polymorphisms, 22 were monomorphic, and 14 markers were polymorphic and were used for mapping. As for the STS markers, four out of five markers were polymorphic. The marker MWG878 was polymorphic without digestion, ABG53 after cleavage with the restriction enzyme *Mbo*I, ABG8 with *Hin*fI and *Xba*I, and cMWG682 with the enzyme *Bst*UI. Only *Hin*fI was used for mapping with ABG8. ABG58 was not polymorphic even after digestion with all enzymes mentioned above (Fig. 1). The marker RGH1aE1 was polymorphic after cleavage with *Nla*III. RGH1aE1 (505 bp) was cleaved into fragments of 279, 91, 72, 35 and 28 bp in 'Tiffany' and 164, 115, 91, 72, 35 and 28 bp in PI466197. Only the longest fragments (279 and 164 bp) detected after digestion of the PCR products were used for subsequent linkage analysis. For ABG53, the PCR products of about 294 bp yielded DNA fragments of approximately 262 bp in 'Tiffany' and 222 bp in PI466197 after digestion, no other fragments were detected. For ABG8, the 314-bp-long PCR products from 'Tiffany' were cleaved into two fragments of 204 and 110 bp, the PCR products from PI466197 remained undigested and therefore polymorphic (Fig. 1a). cMWG682 (504 bp) was cleaved into fragments of 256, 162 and 86 bp in 'Tiffany' and 342 and 162 bp in PI466197 (Fig. 1b). ABG58 (1024 bp) yielded fragments of 750 and 274 bp in both parents (Fig. 1c).

Resistance genes localisation

Fig. 2 demonstrates the order and map distances of nine SSR and two newly developed CAPS markers mapped into a linkage group on chromosome 1HS and eight SSR, one STS and two CAPS markers mapped into a linkage group on chromosome 2H in the cross 'Tiffany' × PI466197. The data from the 113 plants analysed in the Map Manager confirmed a highly significant linkage with the markers UMB503, MGB402, GBMS062, RGH1aE1, Bmac0213 and GBM1007 on chromosome 1H and with GBMS247, Bmac0134 and MWG878 on chromosome 2H. Significant linkage was revealed with ABG8, cMWG682 and GBMS002 on chromosome 2H and suggestive linkage with ABG53 and Bmag0872 on chromosome 1H and UMB206 on 2H. The genotypes of GBM1121 were the same as in UMB206 for all plants, so this marker was excluded from the MapManager analysis. The LRS values are given in Table 3. The LRS thresholds counted for 10,000 permutations were 21.1, 12.2 and 5.4 for a highly significant, significant and suggestive linkage, respectively.

Ann Appl Biol (2010) 157–165

Table 3 Statistical analysis of markers associated with powdery mildew resistance genes in 'Tiffany' × PI466197

Marker	Chromosome[a]	LRS[b]	Significance[c]	P[d]	CI[e]
UMB503	1HS	25.1	***	0.00000	24
MGB402	1HS	26.6	***	0.00000	22
GBMS062	1HS	52.3	***	0.00000	13
RGH1aE1	1HS	56.7	***	0.00000	12
Bmac0213	1HS	30.6	***	0.00000	20
GBM1007	1HS	36.6	***	0.00000	17
ABG53	1HS	11.7	**	0.00294	48
Bmag0872	1HS	8.4	*	0.01519	66
cMWG682	2HS	20.9	***	0.00003	28
GBMS247	2HS	27.3	***	0.00000	22
Bmac0134	2HS	35.2	***	0.00000	18
MWG878	2HS	21.8	***	0.00002	27
ABG8	2HS	19.7	***	0.00005	29
UMB206	2HS	8.6	*	0.01374	64
GBMS002	2HS	12.7	**	0.00170	44

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ for highly significant, significant and suggestive linkage.
[a]Chromosome (S, short arm).
[b]Likelihood ratio statistic.
[c]Significance thresholds.
[d]Probability of linkage.
[e]Confidence interval (95%).

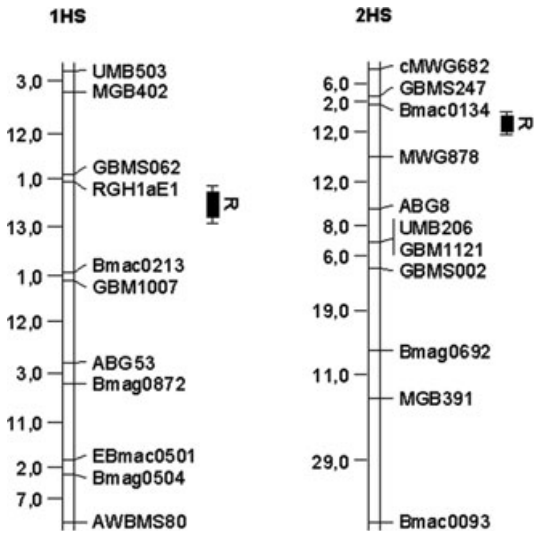

Figure 2 Partial genetic linkage map of chromosomes 1HS and 2HS based on the analysis of F_2 plants of the cross 'Tiffany' × PI466197 showing the positions of the resistance genes (*R*). Map intervals are given in centimorgans (cM) to the left of chromosomes, marker loci are assigned to the right, the bars to the right of chromosomes indicate the *R* gene positions (with confidence intervals).

The resistance gene on the short arm of chromosome 1H was localised between the markers RGH1aE1 and Bmac0213, 4 cM from RGH1aE1 and 10 cM from Bmac0213. The position of the resistance gene on the short arm of chromosome 2H was established between the markers Bmac0134 and MWG878, in the distance of 4 and 8 cM, respectively. The partial genetic map of chromosomes 1H and 2H with the putative positions of the two resistance genes identified in the 'Tiffany' × PI466197 cross is drawn in Fig. 2.

Discussion

Powdery mildew is a disease that often has considerable effects on barley production. Single genes in resistant varieties can soon be overcome by new virulences of the pathogen (Dreiseitl, 2003). A possible way to delay the pathogen from overcoming the host's resistance is to combine two or more fully effective resistance genes in one variety (gene pyramiding), yet this is hardly possible by breeding methods based on phenotypic selection of progeny (Werner *et al*., 2005). Information on barley resistance genes and linked DNA markers therefore play a crucial role in improving the efficiency of barley breeding programmes.

Considering the life cycle of the pathogen (*Bgh*) and the possibility of its transmission from winter barley to spring barley (Dreiseitl, 2007), growing resistant varieties is thus important not only for winter barley itself, but also for the spring crop. *H. vulgare* ssp. *spontaneum* is a rich source of new powdery mildew resistance genes and PI466197 is one of many resistant accessions (Dreiseitl & Bockelman, 2003), which can be used for incorporation of resistance genes into adapted winter barley varieties, of which 'Tiffany' is a possible candidate because it is

the only winter malting variety registered in the Czech Republic up to now, however, without efficient genes for powdery mildew resistance.

The trait of powdery mildew resistance evaluated in this study was genetically characterised using the resistance test on detached barley leaves. The genes participating in resistance were characterised as qualitative with a distinct ratio of 15:1 ($\chi^2 = 0.17$) for two independent resistance genes with semidominant inheritance (Řepková *et al.*, 2006). There were two reasons for the use of the QTL mapping program (Map Manager QTX) to assign the genes to barley chromosomes. Firstly, two genes conferring the trait made it impossible to use a program for the calculation of genetic linkage maps in experimental populations and localisation of individual genes/loci analysed. Such programs, for example the often used JoinMap (Van Ooijen & Voorrips, 2001), are intended for the 3:1 or 1:2:1 ratios. Secondly, due to a large range of RTs in the F_2 generation plants, both loci, either the *Mla* or the other, could include additional linked resistance gene(s). Variation established in the powdery mildew resistance trait was represented by an almost continuous range of RTs (Dreiseitl *et al.*, 2007). This means that the trait might not be determined in a simple manner similar to the *Mla* locus which is not defined by one multiallelic gene but by three distinct, closely linked resistance-gene homologue families (Wei *et al.*, 1999). This should be solved by a QTL computer program.

We mapped one resistance gene originating from PI466197 to the short arm of chromosome 1H in the position consistent with the *Mla* locus, which was proven by the allelism test before (Řepková *et al.*, 2006), and the other gene was localised on the short arm of chromosome 2H. On chromosome 2H, the gene *MlLa* from *Hordeum laevigatum* (Giese *et al.*, 1993) and the genes *Rar1* and *Rar2* were mapped, but on its long arm (Freialdenhoven *et al.*, 1994; Lahaye *et al.*, 1998). On the short arm of chromosome 2H, the gene *Mlhb* transferred from *H. bulbosum* was mapped close to the marker cMWG682 (Pickering *et al.*, 1995). A strong linkage between the powdery mildew resistance gene from *H. bulbosum* and the DNA markers cMWG682 and BCD175 was detected, which may be attributed to the reduced recombination that occurs within the introgressed segment (Pickering *et al.*, 1998). Beyond the major genes, there were some QTLs also localised on chromosome 2H (Backes *et al.*, 2003; von Korff *et al.*, 2005). The position of the gene mapped on chromosome 2H in this study, which is 12 cM proximal from the cMWG682 marker, and the fact that its origin is in *H. vulgare* ssp. *spontaneum* suggest that this locus is a new powdery mildew resistance gene. This conclusion is also supported by different reaction types of the standard line 81882 possessing the gene *Mlhb1* (Pickering *et al.*, 1995) and the plants of the cross 'Tiffany' × PI466197 tested under the given conditions. The RTs of the standard line 81882 were 2 to 3 in our tests and RTs 0 and 1 were recorded in the parental accession PI466197. According to Pickering *et al.* (1998), the RTs of 81882 to powdery mildew were 1, 2 (using the system of Moseman, 1968).

Our mapping effort was targeted on the successive narrowing of the chromosomal regions restricted by DNA markers. The microsatellite markers used for mapping were chosen from five different genetic maps of barley (Ramsay *et al.*, 2000; Karakousis *et al.*, 2003; von Korff *et al.*, 2004; Beaubien & Smith, 2006; Varshney *et al.*, 2007a). In the individual maps, markers were mapped for particular populations and the order and genetic distances between the markers may vary among different crosses. It is therefore better to choose markers from consensus maps because they integrate mapping data from several populations and sometimes even for diverse types of markers (Stein *et al.*, 2007; Varshney *et al.*, 2007a; Wenzl *et al.*, 2006), which, in general, can be utilised more universally.

The polymorphism between 'Tiffany' and PI466197 was detected in 14 markers out of 36 SSRs (38.9%). Only seven markers on chromosome 1H and seven markers on chromosome 2H could be used for further mapping. Therefore, the GrainGenes database was searched for other types of markers, particularly on chromosome 2H, and five STS markers and one marker developed from the *Mla* locus were chosen as to be converted into CAPS markers. For polymorphism detection, up to 13 different restriction enzymes were tested for one marker. The parent sequences were mostly polymorphic after digestion with only one or two restriction enzymes out of 6–13 enzymes tested. On the other hand, the restriction polymorphism between parents for the tested markers was relatively high; only for one marker out of six was no polymorphism detected after cleavage with the set of enzymes. The polymorphism study revealed that the coding sequences are quite conservative within the parental genotypes and there are just a few SNPs.

SNPs represent a unit of genetic variation highly abundant in many plant species (Rafalski, 2002), but their occurrence is greatly variable in barley. In a survey, Kanazin *et al.* (2002) mentioned a mutation available on average every 189 bp in cultivated barley and even more polymorphisms within some *H. vulgare* ssp. *spontaneum* genotypes. Rostoks *et al.* (2005) observed an average frequency of 1 SNP per 200 bp. SNP discovery and the genotyping platforms without automation are still quite expensive, but partial DNA sequencing of particular parental genotypes for SNP detection is still reasonably priced. Moreover, newly identified SNPs can

Ann Appl Biol (2010) 157–165 © 2009 The Authors
Journal compilation © 2009 Association of Applied Biologists

be converted into more economical CAPS markers after the identification of a restriction enzyme capable to distinguish SNP (Varshney *et al.*, 2007b). In our studies, SNP detection within the *Mla* locus revealed just rare SNPs for two particular parental genotypes (Řepková *et al.*, 2009). Any change within a conservative coding sequence could result in a new resistance specificity for powdery mildew.

A high-density linkage map is a prerequisite for the application of marker-assisted selection. To use a molecular marker for the selection of plants with desirable genotypes in breeding programmes, markers tightly linked with a target gene, both genetically and physically, are needed. It should be less than 1 cM in barley, because the average physical/genetic distance ratio is 4.4 Mb per cM, although there is a very heterogeneous distribution of recombination rates among barley chromosomes (Künzel *et al.*, 2000).

One possibility to get a dense and accurate genetic map is to enlarge the size of the mapping population (Ferreira *et al.*, 2006), which, however, proves rather laborious. Efforts are being made to continue and find more tightly linked markers that could be used in breeding programmes for the selection of plants carrying the new resistance gene combined with the gene from the *Mla* locus.

Acknowledgements

The primer sequences of the markers GBM1007, GBMS002, GBMS062 and GBMS247 were kindly provided by IPK Gatersleben, Germany. The work was supported by the Czech Science Foundation (grants No. 522/06/0608 and 204/05/H505).

References

Andersen J.R., Lübberstedt T. (2003) Functional markers in plants. *Trends in Plant Science*, **8**, 554–560.

Backes G., Madsen L.H., Jaiser H., Stougaard J., Herz M., Mohler V., Jahoor A. (2003) Localisation of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. *Theoretical and Applied Genetics*, **106**, 353–362.

Beaubien K.A., Smith K.P. (2006) New SSR markers for barley derived from the EST database. *Barley Genetics Newsletter*, **36**, 30–43.

Dreiseitl A. (2003) Adaptation of *Blumeria graminis* f. sp. *hordei* to barley genetic resistance in the Czech Republic in 1971–2000. *Plant, Soil and Environment*, **49**, 241–248.

Dreiseitl A. (2007) Powdery mildew resistance in winter barley cultivars. *Plant Breeding*, **126**, 268–273.

Dreiseitl A., Bockelman H.E. (2003) Sources of powdery mildew resistance in a wild barley collection. *Genetic Resources and Crop Evolution*, **50**, 345–350.

Dreiseitl A., Dinoor A. (2004) Phenotypic diversity of barley powdery mildew resistance sources. *Genetic Resources and Crop Evolution*, **51**, 251–257.

Dreiseitl A., Řepková J., Lízal P. (2007) Genetic analysis of thirteen accessions of *Hordeum vulgare* ssp. *spontaneum* resistant to powdery mildew. *Cereal Research Communications*, **35**, 1449–1458.

Ferreira A., da Silva M.F., Silva L.D.C.E., Cruz C.D. (2006) Estimating the effects of population size and type on the accuracy of genetic maps. *Genetics and Molecular Biology*, **29**, 187–192.

Fetch T.G., Steffenson B.J., Nevo E. (2003) Diversity and sources of multiple disease resistance in *Hordeum spontaneum*. *Plant Disease*, **87**, 1439–1448.

Flor H.H. (1956) The complementary genic systems in flax and flax rust. *Advances in Genetics*, **8**, 29–54.

Freialdenhoven A., Scherag B., Hollricher K., Collinge D.B., Thordal-Christensen H., Schulze-Lefert P. (1994) *Nar-1* and *Nar-2*, two loci required for *Mla12*-specified race-specific resistance to powdery mildew in barley. *Plant Cell*, **6**, 983–994.

Freialdenhoven A., Peterhänsel C., Kurth J., Kreuzaler F., Schulze-Lefert P. (1996) Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. *Plant Cell*, **8**, 5–14.

Giese H., Holmjensen A.G., Jensen H.P., Jensen J. (1993) Localization of the laevigatum powdery mildew resistance gene to barley chromosome-2 by the use of RFLP markers. *Theoretical and Applied Genetics*, **85**, 897–900.

Jaccoud D., Peng K., Feinstein D., Kilian A. (2001) Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, **29**, e25.

Jørgensen J.H. (1994) Genetics of powdery mildew resistance in barley. *Critical Reviews in Plant Sciences*, **13**, 97–119.

Jørgensen J.H. (1996) Effect of three suppressors on the expression of powdery mildew resistance genes in barley. *Genome*, **39**, 492–498.

Kanazin V., Talbert H., See D., DeCamp P., Nevo E., Blake T. (2002) Discovery and assay of single-nucleotide polymorphisms in barley (*Hordeum vulgare*). *Plant Molecular Biology*, **48**, 529–537.

Karakousis A., Gustafson J.P., Chalmers K.J., Barr A.R., Langridge P. (2003) A consensus map of barley integrating SSR, RFLP, and AFLP markers. *Australian Journal of Agricultural Research*, **54**, 1173–1185.

von Korff M., Plümpe J., Michalek W., Léon J., Pillen K. (2004) Insertion of 18 new SSR markers into the Oregon Wolfe Barley map. *Barley Genetics Newsletter*, **34**, 1–4.

von Korff M., Wang H., Léon J., Pillen K. (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes

against powdery mildew, leaf rust and scald introgressed from wild barley. *Theoretical and Applied Genetics*, **111**, 583–590.

Kosambi D.D. (1944) The estimation of map distance from recombination values. *Annals of Eugenics*, **12**, 172–175.

Kota R., Varshney R.K., Thiel T., Dehmer K.J., Graner A. (2001) Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.). *Hereditas*, **135**, 145–151.

Künzel G., Korzun L., Meister A. (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics*, **154**, 397–412.

Lahaye T., Hartmann S., Töpsch S., Freialdenhoven A., Yano M., Schulze-Lefert P. (1998) High-resolution genetic and physical mapping of the *Rar1* locus in barley. *Theoretical and Applied Genetics*, **97**, 526–534.

Li J.Z., Sjakste T.G., Röder M.S., Ganal M.W. (2003) Development and genetic mapping of 127 new microsatellite markers in barley. *Theoretical and Applied Genetics*, **107**, 1021–1027.

Lyngkjaer M.F., Newton A.C., Atzema J.L., Baker S.J. (2000) The barley mlo-gene: an important powdery mildew resistance source. *Agronomie*, **20**, 745–756.

Manly K.F., Cudmore R.H., Meer J.M. (2001) Map manager QTX, cross-platform software for genetic mapping. *Mammalian Genome*, **12**, 930–932.

Moseman J.G. (1968) Reactions of barley to *Erysiphe graminis* f.sp. *hordei* from North America, England, Ireland and Japan. *Plant Disease Reporter*, **52**, 463–467.

Pickering R.A., Hill A.M., Michel M., Timmerman-Vaughan G.M. (1995) The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2(2I). *Theoretical and Applied Genetics*, **91**, 1288–1292.

Pickering R.A., Steffenson B.J., Hill A.M., Borovkova I. (1998) Association of leaf rust and powdery mildew resistance in a recombinant derived from a *Hordeum vulgare* × *H. bulbosum* hybrid. *Plant Breeding*, **117**, 83–84.

Pillen K., Binder A., Kreuzkam B., Ramsay L., Waugh R., Forster J., Leon J. (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. *Theoretical and Applied Genetics*, **101**, 652–660.

Rafalski A. (2002) Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology*, **5**, 94–100.

Ramsay L., Macaulay M., degli Ivanissevich S., MacLean K., Cardle L., Fuller J., Edwards K.J., Tuvesson S., Morgante M., Massari A., Maestri E., Marmiroli N., Sjakste T., Ganal M., Powell W., Waugh R. (2000) A simple sequence repeat-based linkage map of barley. *Genetics*, **156**, 1997–2005.

Řepková J., Dreiseitl A., Lízal P., Kyjovská Z., Teturová K., Psotková R., Jahoor A. (2006) Identification of resistance genes against powdery mildew in four accessions of *Hordeum vulgare* ssp. s*pontaneum*. *Euphytica*, **151**, 23–30.

Řepková J., Lízal P., Dreiseitl A. (2009) New CAPS marker for selection of a barley powdery mildew resistance gene in the *Mla* locus. *Cereal Research Communications*, **37**, 93–99.

Rostoks N., Mudie S., Cardle L., Russell J., Ramsay L., Booth A., Svensson J.T., Wanamaker S.I., Walia H., Rodriguez E.M., Hedley P.E., Liu H., Morris J., Close T.J., Marshall D.F., Waugh R. (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Molecular Genetics and Genomics*, **274**, 515–527.

Rozen S., Skaletsky H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, pp. 365–386. Eds S. Krawetz and S. Misener. Totowa, NJ: Humana Press.

Schönfeld M., Ragni A., Fischbeck G., Jahoor A. (1996) RFLP mapping of three new loci for resistance genes to powdery mildew (*Erysiphe graminis* f.sp. *hordei*) in barley. *Theoretical and Applied Genetics*, **93**, 48–56.

Semagn K., Bjørnstad Å., Ndjiondjop M.N. (2006) An overview of molecular marker methods for plants. *African Journal of Biotechnology*, **5**, 2540–2568.

Stein N., Prasad M., Scholz U., Thiel T., Zhang H.N., Wolf M., Kota R., Varshney R.K., Perovic D., Grosse I., Graner A. (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. *Theoretical and Applied Genetics*, **114**, 823–839.

Thiel T., Michalek W., Varshney R.K., Graner A. (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics*, **106**, 411–422.

Van Ooijen J.W., Voorrips R.E. (2001) *JoinMap® 3.0, Software for the Calculation of Genetic Maps*. Wageningen, Netherlands: Plant Research International.

Varshney R.K., Grosse I., Hähnel U., Siefken R., Prasad M., Stein N., Langridge P., Altschmied L., Graner A. (2006) Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome. *Theoretical and Applied Genetics*, **113**, 239–250.

Varshney R.K., Marcel T.C., Ramsay L., Russell J., Roder M.S., Stein N., Waugh R., Langridge P., Niks R.E., Graner A. (2007a) A high density barley microsatellite consensus map with 775 SSR loci. *Theoretical and Applied Genetics*, **114**, 1091–1103.

Varshney R.K., Beier U., Khlestkina E.K., Kota R., Korzun V., Graner A., Börner A. (2007b) Single

Ann Appl Biol (2010) 157–165

nucleotide polymorphisms in rye (*Secale cereale* L.): discovery, frequency, and applications for genome mapping and diversity studies. *Theoretical and Applied Genetics*, **114**, 1105–1116.

Voorrips R.E. (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, **93**, 77–78.

Wei F., Gobelman-Werner K., Morroll S.M., Kurth J., Mao L., Wing R., Leister D., Schulze-Lefert P., Wise R.P. (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. *Genetics*, **153**, 1929–1948.

Wenzl P., Carling J., Kudrna D., Jaccoud D., Huttner E., Kleinhofs A., Kilian A. (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences of the USA*, **101**, 9915–9920.

Wenzl P., Li H.B., Carling J., Zhou M.X., Raman H., Paul E., Hearnden P., Maier C., Xia L., Caig V., Ovesna J., Cakir M., Poulsen D., Wang J., Raman R., Smith K.P., Muehlbauer G.J., Chalmers K.J., Kleinhofs A., Huttner E., Kilian A. (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics*, **7**, 206.

Werner K., Friedt W., Ordon F. (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). *Molecular Breeding*, **16**, 45–55.

Yun S.J., Gyenis L., Hayes P.M., Matus I., Smith K.P., Steffenson B.J., Muehlbauer G.J. (2005) Quantitative trait loci for multiple disease resistance in wild barley. *Crop Science*, **45**, 2563–2572.