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A high-resolution genetic map and a diagnostic RFLP marker for the *Mlg* resistance locus to powdery mildew in barley

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Abstract The semi-dominantly acting *Mlg* resistance locus in barley confers race-specific resistance to the obligate biotrophic fungus *Erysiphe graminis* f.sp. *hordei*. A high-resolution genetic map was constructed at *Mlg* based on a cross between the near-isogenic barley lines Pallas BC_5 *Mlg* and Pallas *mlg*. A total of 2000 F_2 progeny were inspected by cleaved amplified polymorphic sequence (CAPS) analysis, defining a 4.47 cM interval encompassing the resistance locus. Pathogen challenge of the segregants with multiple powdery mildew isolates uncovered a novel resistance specificity in Pallas BC_5 *Mlg*. Probes from within 4.0 cM of *Mlg* were mapped in rice, revealing orthologues on five different rice chromosomes and suggesting multiple breaks of chromosomal collinearity in this region between the two grass species. The most tightly *Mlg*-linked RFLP marker, MWG032, was shown to reliably detect the presence of the resistance allele in a collection of 30 European barley cultivars.

Keywords High-resolution map · Powdery mildew fungus · CAPS · Resistance gene · Diagnostic marker

Introduction

Erysiphe graminis f. sp. *hordei* is the causal agent of powdery mildew in barley. This obligate biotrophic fungus causes significant yield losses in the field and represents one of the common foliar diseases in temperate climates. Because the air-borne pathogen has rapidly acquired virulence to every fungicide developed, a better understanding of innate resistance mechanisms to this fungus promises alternative routes of pathogen control.

At least two genetically separable pathways control resistance to powdery mildew in barley (Jørgensen 1994; Peterhänsel et al. 1997). In the first pathway, resistance is mediated by recessive alleles at the *Mlo* locus (*mlo* resistance alleles). This resistance is effective against all tested powdery mildew isolates and requires for its function at least two further host genes, designated *Ror1* and *Ror2* (Freialdenhoven et al. 1996; Büschges et al. 1997). The resistance reaction is tightly linked with a rapid cell-wall remodelling in host epidermal cells in response to attempted fungal penetration (Freialdenhoven et al. 1996; von Röpenack et al. 1998). The second resistance pathway can be triggered by a number of race-specific resistance genes (*R* genes; e.g. *Mla*, *Mlg*, *Mlk*) (Jørgensen 1994), and is almost invariably associated with the activation of rapid host cell death at attempted infection sites (Freialdenhoven et al. 1994). Signalling of *R* gene-triggered resistance in barley is dependent on at least two additional genes, *Rar1* and *Rar2* (Jørgensen 1996). Mutants in these two genes abolish the *R* gene-triggered cell death response (Freialdenhoven et al. 1994; Shirasu et al. 1999).

The predominant class of plant *R* genes encode proteins sharing a nucleotide-binding (NB) domain and various leucine-rich repeats (LRRs) (Ellis and Jones 1998). The barley *Mla* resistance locus on chromosome 1H was shown to contain, within an interval of 240 kb, at least 11 NB-LRR protein-encoding genes that form three distinct families (Wei et al. 1999). The first molecularly isolated *Mla* resistance specificity, *Mla-1*, is a gene located at the edge of the complex *Mla* locus, and is predicted to encode a 115-kDa NB-LRR protein (Zhou and Schulze-Lefert, unpublished).

The semi-dominantly acting *Mlg* resistance locus on barley chromosome 4H is of interest for several reasons. Comparative analysis of *Mlg*- and *Mla-12*-dependent resistance responses revealed that the two genes act at different stages during the fungal infection process (Görg et

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al. 1993). Interestingly, the resistance response in *Mlg* plants is associated with both rapid cell-wall remodelling and activation of host cell death (Görg et al. 1993). Time-course analyses, gene-dosage experiments, and pharmacological studies have shown that host cell death is dispensable for *Mlg* but not for *Mla*-triggered resistance (Görg et al. 1993; Schiffer et al. 1997).

Further insights into the molecular mechanisms of *Mlg* resistance are expected from the isolation of the gene. A previous study located *Mlg* on barley chromosome 4H but failed to define a DNA marker interval suitable for later fine mapping and map-based gene isolation (Görg et al. 1993). Here we describe the construction of a high-resolution linkage map at *Mlg* and show that the most-tightly linked RFLP marker can be used as a reliable indicator for the presence of the *Mlg* resistance allele among European barley cultivars.

Materials and methods

Plant material

The *Mlg* backcross (BC) line Ingrid BC_7*Mlg*, carrying the *Mlg* resistance gene in the genetic background of the susceptible cultivar Ingrid, was kindly provided by J. McKey, University of Upsala, Sweden. This line was generated through seven backcrosses with *Hordeum vulgare* cv Ingrid followed by at least seven self-pollinations. The *Mlg* BC line 'P21' carries the *Mlg* resistance gene in the genetic background of cultivar Pallas and was a gift from L. Munk, Royal Veterinary and Agricultural University, Copenhagen, Denmark. The generation of this line has been previously described (Kølster et al. 1986).

We used three *Oryza* mapping populations to locate orthologous rice loci of *Mlg*-linked barley RFLP probes. A population consisting of 123 individuals derived from the cross IR20 × 63–83 was employed to map probe b4–104/1 (Quarrie et al. 1997). The remaining orthologous loci were mapped on the basis of wide crosses between *Oryza sativa* × *Oryza longistaminata* (Causse et al. 1994) and *Oryza sativa japonica* (cultivar Nipponbare) × *Oryza sativa indica* (cultivar Kasalath) (Kurata et al. 1994).

Resistance tests

Tests for resistance on barley plants were carried out as described previously (Görg et al. 1993). Infection phenotypes of DNA marker-selected recombinant F_2 plants were determined after self-pollination in F_3-families. At least 20 seedlings of each family were challenged with powdery mildew spores. Fungal isolates R146 and A6 were provided by H. Giese, Agricultural Research Department, Risø, Denmark. Isolates GF1, CC1, CC2, CC47 and CC23, all avirulent on *Mlg*-containing lines, were provided by J. K. M. Brown, John Innes Centre, Norwich, UK.

CAPS analysis

Plant genomic DNA for cleaved amplified polymorphic sequence (CAPS) analysis was isolated according to Steward and Via (1993). CAPS marker 2036 was developed from RFLP probe MWG2036. The recombinant MWG2036 plasmid contains two *Pst*I fragments that were cloned separately, sequenced and mapped. Only one of the two *PstI* fragments mapped to the same locus as the original MWG2036 probe. The plasmid containing this fragment, MWG2036/2, was then used to develop a CAPS for locus MWG2036 (Table 1). CAPS analysis was performed in a volume of 20 µl [100 pmol of each primer, 200 µM dNTPs, 10 mM Tris-HCl pH 8.3, 2 mM $MgCl_2$, 50 mM KCl, 0.5 U of *Taq* DNA Polymerase (Boehringer)] using 50 ng of barley genomic DNA. The digested PCR products were size-fractionated on 1% (w/v) agarose gels.

Southern analysis

The RFLP probes bAL57, CDO541, MWG032, MWG2036, and b4–104/1 utilized in this study have been described before (Görg et al. 1993) (Table 1). RFLP probes MWG058 and Osc147 were provided by A. Graner, Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany. Mapping filters

Table 1 Oligonucleotide sequences and PCR conditions used for CAPS analysis and amplicon generation of RFLP loci linked to *Mlg*

RFLP/CAPS locus	Oligonucleotide primer sequence	PCR conditions[a]	Size of amplicon (bp)	Restriction enzymes used for CAPS
MWG2036	5′CGAGAGTTGTCTGGACACGGC 5′GCACGACCGGACGCCAATCA	94°C, 20 s 57°C, 60 s 72°C, 90 s 35 cycles	490	*Bsr*I
MWG032	5′CATGGCTACATACAACCTAGTG 5′TGTCAACTGGTAGGCATGGTTA	94°C, 20 s 60°C, 60 s 72°C, 90 s 35 cycles	450	–
b4-104/1	5′GTCGATGGATTGTCCTGGGAA 5′CGCCGATGCCCCCACCAACGT	94°C, 20 s 57°C, 60 s 72°C, 90 s 35 cycles	280	–
MWG058	5′GCCGCCGCGCCGAATTGCTTGG 5′TAGACCTCACACTGATTCATGC	94°C, 20 s 57°C, 60 s 72°C, 90 s 35 cycles	800	–
bAL57	5′GGGTCCAGCGAAAGGGAAGCAAGA 5′GTGTTGTTGCGCTCCTTTGGGTCC	94°C, 20 s 58°C, 60 s 72°C, 90 s 35 cycles	1600	*Hae*III

[a] PCR reaction for MWG2036 included 10% (w/v) DMSO

were prepared as described in Gebhardt et al. (1989). DNA restriction enzymes used for RFLP analysis were *Hae*III (for marker MWG032) and *Rsa*I (for marker b4–104/1). Probe labelling and hybridizations were performed as described in Görg et al. (1993).

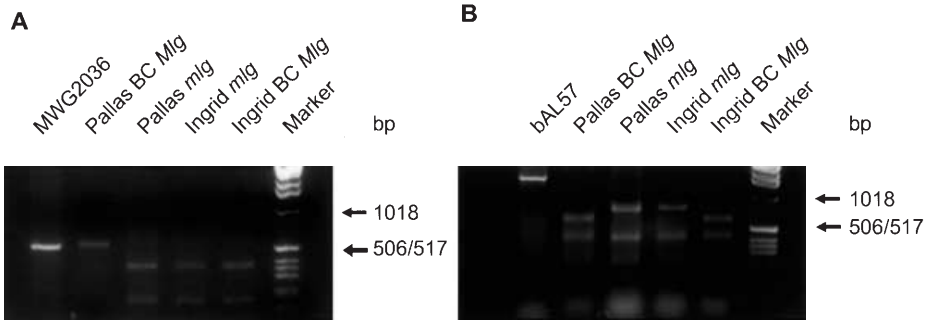
Results

CAPS marker development

The *Mlg* locus was previously mapped close to the centromere on the long arm of barley chromosome 4H using morphological markers and the RFLP markers MWG032, bAL57, and b4–104/1 (Görg et al. 1993). The MWG032, bAL57 and b4–104/1 probes were sequenced, in addition to the probes MWG2036, MWG058 and Osc147 which also detect RFLP loci in the region (Graner et al. 1994, and personal communication). Oligonucleotide primers (listed in Table 1) were designed for PCR-amplification of the corresponding sequences from the genomic DNA of the nearly isogenic barley cultivars used as parents in the mapping crosses Ingrid *mlg* × Ingrid BC_7 *Mlg* and Pallas *mlg* × Pallas BC_5 *Mlg*.

Four primer pairs were used in an attempt to PCR-amplify barley sequences orthologous to the rice probe Osc147, but no product was obtained. Products corresponding to the other five probes were successfully amplified from all parent lines and sequenced directly. The MWG2036 sequence was identical in Pallas, Ingrid and Ingrid BC *Mlg*, whereas it differed by a 7 bp insertion/deletion and a single nucleotide substitution between Pallas BC *Mlg* and the other lines. The 7 bp insertion in Ingrid, Ingrid BC *Mlg* and Pallas creates a *Bsr*I restriction-enzyme recognition site that was used as the basis for a CAPS marker (Fig. 1 A). The bAL57 sequence was polymorphic within a *Hae*III site for both pairs of parents, enabling this marker to be scored by CAPS analysis (Fig. 1B). None of the remaining three marker sequences showed polymorphisms suitable for CAPS marker development.

Fig. 1 A, B PCR-based markers close to *Mlg*. The displayed CAPS data correspond to RFLP loci MWG2036 (**A**) and bAL57 (**B**). Ethidium bromide-stained 1% agarose gels displaying restriction enzyme-digested amplification products using Pallas BC_5*Mlg*, Pallas *mlg*, Ingrid *mlg*, and Ingrid BC_7*Mlg* as template DNA. Undigested PCR products, obtained with a plasmid DNA template containing RFLP probes MWG2036 (**A**) and bAL57 (**B**), are also shown



Mapping the *Mlg* region

Because both of the CAPS markers MWG2036 and bAL57 were polymorphic between Pallas BC *Mlg* and Pallas *mlg*, we used an F_2 family derived from the Pallas BC *Mlg* × Pallas *mlg* cross for mapping *Mlg*. Out of 2000 F_2 individuals analyzed with the MWG2036 and bAL57 CAPS markers, 179 recombinants for the marker interval were identified, corresponding to a genetic interval of 4.47 cM (Fig. 2).

Although *Mlg* is semi-dominant, it is difficult to distinguish the intermediate infection types of heterozygous *Mlg/mlg* plants from the fully resistant or fully susceptible reactions of *MlgMlg* or *mlgmlg* plants, respectively. Therefore, F_3 progeny of 114 of the recombinants were inoculated with the *Mlg* avirulent powdery mildew isolate GF1 (Caffier et al. 1996) to determine the *Mlg* genotypes of each recombinant. In this way, *Mlg* was located between the two CAPS markers (Fig. 2). For eight of the recombinants, 4–15 inoculated F_3 progeny were also analyzed with the two CAPS markers. Cosegregation of the resistance phenotype with MWG2036 in three of the families, and with bAL57 in the other five families, allowed confirmation of single recombination events in the intervals *Mlg* – bAL57 and MWG2036 – *Mlg*, respectively, and consolidated the placement of *Mlg* between the two markers.

Markers MWG032 and b4–104/1, which could not be mapped as CAPS markers, were instead used to map RFLP loci, by analyzing 61 and 77 of the MWG2036 – bAL57 recombinants, respectively. Both MWG032 and b4–104/1 were located in the MWG2036 – bAL57 interval (Fig. 2). While b4–104/1 mapped between *Mlg* and bAL57, MWG032 was not separated from *Mlg* in any of the 26 recombinants for which the *Mlg* genotype had been reliably determined by the inoculation of significant (>20) numbers of F_3 progeny.

Figure 2 shows the genetic map of the *Mlg* region resulting from the combined CAPS and RFLP analysis, together with the numbers of recombinants observed for each genetic interval. The order MWG032 – b4–104/1 – bAL57 was the same as determined by Görg et al. (1993), and allowed the map to be oriented with respect to the centromere and the telomere of the long arm of chromosome 4H, as shown in Fig. 2.

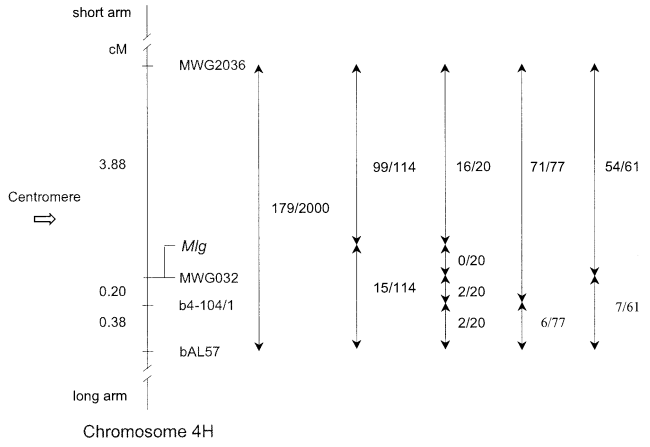


Fig. 2 A high resolution map at *Mlg*. Genetic distances on barley chromosome 4H are given in centiMorgans (cM) and were calculated on the basis of two-point estimates (left). F_2 segregants derived from the cross Pallas *mlg* × PallasBC_5*Mlg* were screened for recombinants between the indicated genetic markers. Vertical arrows (↕) indicate examined two-point marker intervals. Initially, 179 recombinants were identified in the marker interval MWG203 – bAL57 amongst 2000 inspected F_2 segregants (denoted on the right of the corresponding arrow: 179/2000). Randomly chosen groups of these 179 recombinants were then tested with additional markers to pinpoint recombination events as indicated by arrows. Numbers to the right of each arrow indicate, for the respective intervals, the number of identified recombination events and the number of inspected recombinants. Each line of *vertical arrows* denotes groups of recombinants that were simultaneously tested for multiple markers. The presence or absence of *Mlg* alleles was scored in segregating F_3 families of selfed F_2 recombinants. The centromere was previously located between markers MWG2036 and bAL57 (Künzel et al. 2000)

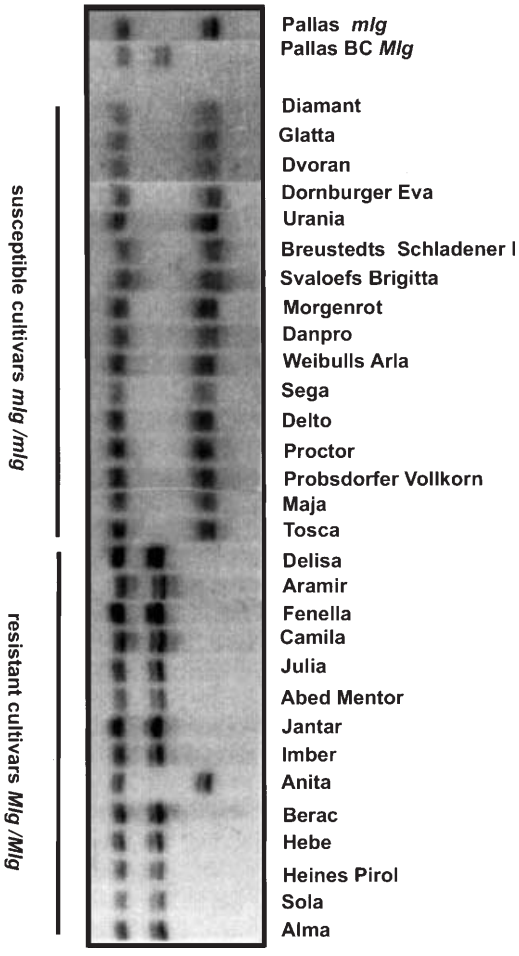


Fig. 3 Marker MWG032 indicates the presence of *Mlg* in European barley cultivars. Southern analysis of a collection of European barley cultivars, recorded as containing *Mlg* or *mlg* alleles, with RFLP probe MWG032. Genomic DNAs were digested with *Hae*III prior to size-fractionation

A diagnostic RFLP marker for the *Mlg* resistance allele

Molecular markers which are tightly linked to a disease resistance locus, and which can nearly always distinguish individuals carrying the resistance allele from those containing a susceptibility allele, can be useful in resistance breeding as they can provide an alternative to often unreliable resistance assays for genotype determination. Probe MWG032 was used in the Southern analysis of European barley cultivars, supplied by the Institut für Pflanzenbau der Bundesanstalt für Landwirtschaft (FAL), Braunschweig, Germany, that were recorded as containing either the *Mlg* resistance allele (14 cultivars), or the *mlg* susceptibility allele (16 cultivars; Fig. 3; a *Hae*III restriction digest was performed prior to size separation). All of the *mlg* cultivars showed the same hybridisation pattern as Pallas *mlg*, and all of the *Mlg* cultivars showed the same hybridisation pattern as Pallas BC_5 *Mlg*, with the exception of Anita (recorded as containing *Mlg*). Anita was tested for its reaction to powdery mildew isolate A6 (Avr*Mlg*) and was found to be susceptible, suggesting that this cultivar may not in fact contain *Mlg*. MWG032, which detected the most-closely linked molecular marker to *Mlg* (Fig. 2), can therefore be regarded as a 'diagnostic probe' for the *Mlg* resistance allele, as it can distinguish *Mlg* lines from *mlg* lines in most, if not all, cases.

Comparative mapping between rice and the *Mlg* region of barley

Southern analyses of rice genomic DNA and hybridisation screens of a library of mapped rice genomic YAC clones (Umehara et al. 1995) were performed to locate sequences in rice that are orthologous to *Mlg*-linked sequences in barley. For this analysis, seven RFLP probes that detect loci in the *Mlg* region were used (CDO541, MWG058, Osc147, MWG032, MWG2036, b4–104/1 and bAL57; Görg et al. 1993; Graner et al. 1994; A. Graner, personal communication, and this study). The results are summarized in Fig. 4. Probes bAL57 and CDO541 were both mapped to chromosome 3 using an *O. sativa* × *O. longistaminata* F_2 population (Causse et al. 1994). The position of bAL57 was also shown by hybridisation to the YAC clone Y5127 from rice chromosome 3. Probe b4–104/1 was used to map an RFLP locus on rice chromosome 11 using an IR20×63–83 F_2 rice mapping population (Quarrie et al. 1997). Probe MWG2036 hybridised to two YAC clones: Y2777 locat-

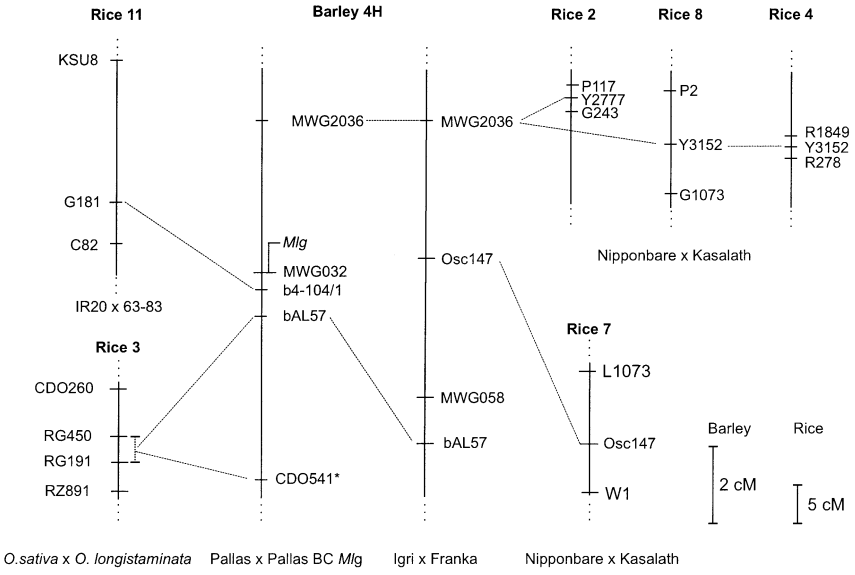


Fig. 4 Comparative mapping of barley probes linked to *Mlg* in rice. Map positions of the indicated barley probes are connected via *dashed lines* with the marker loci of the respective rice probes that map to the same position. Map locations of markers in barley are based on segregant analysis of the cross Pallas *mlg* × PallasBC$_5$ *Mlg* and derived from a general RFLP map (Igri × Franka) (Graner et al. 1994, and personal communication). Orthologous loci of probes CDO541 and bAL57 map on rice chromosome 3 within an interval defined by rice markers RG450 and RG191 (Causse et al. 1994). Probe MWG2036 cross-hybridizes to two anchored rice YAC clones (Y2777 and Y3152) (Umehara et al. 1995). Note different scales (in centiMorgan) of genetic distances in barley and rice chromosomes. * denotes that the map position of barley locus CDO541 is derived from a cross between cultivars Pallas *mlg* and Ingrid BC$_7$ *Mlg* (Gorg et al. 1993)

ed on rice chromosome 2 and a chimeric YAC Y3152 that mapped to rice chromosomes 4 and 8. The rice cDNA clone Osc147 detects a locus mapped on rice chromosome 7 using the Nipponbare × Kasalath F$_2$ population (Kurata et al. 1994). Barley RFLP probes MWG032 and MWG058 showed no cross-hybridisation to rice YAC DNA or rice genomic DNA. Taken together, sequences within 4.0 cM of *Mlg* in barley were shown to have orthologues located on five different rice chromosomes. From this information it is difficult to predict where orthologues of the *Mlg* gene, and sequences immediately surrounding it, may be located in rice.

An additional resistance gene in the line Pallas BC$_5$ *Mlg*

Initially we tried to use the R146 powdery mildew isolate instead of the GF1 isolate to determine the *Mlg* genotypes of segregants from the Pallas BC$_5$ *Mlg* × Pallas *mlg* mapping population. The R146 isolate, reported to be avirulent on *Mlg*-containing lines (Jensen et al. 1992), was used to inoculate F$_3$ progeny of 71 MWG2036-bAL57 recombinants. Of these 71 F$_3$ families tested with R146, 27 contained all resistant individuals, 40 segregated for resistance, and four contained all susceptible individuals. These numbers observed for the three respective phenotypic classes differed significantly from those expected with a 1:2:1 ratio typical of a single locus trait ($\chi^2 = 15.6$; $P < 0.001$), but were consistent with the ratio of 7:8:1 expected for resistance controlled by two unlinked loci ($\chi^2 = 1.14$; $P > 0.5$). The genotypes of these families with respect to the two CAPS markers MWG2036 and bAL57 were also consistent with the R146 isolate containing avirulence genes to both *Mlg* and a second resistance gene unlinked to *Mlg* which was segregating in the Pallas BC$_5$ *Mlg* × Pallas *mlg* mapping population. Because the R146 isolate is virulent on Pallas *mlg*, this additional resistance gene must be present in the Pallas BC$_5$ *Mlg* parent and must have originated from the *Mlg* donor line (cultivar Deba) used to create Pallas BC$_5$ *Mlg*.

Table 2 DNA marker genotypes and infection phenotypes in selected F$_3$ families derived from the cross Pallas BC$_5$*Mlg* × Pallas *mlg*

F$_3$ family	DNA marker		Powdery mildew isolate[a]						
	Alleles MWG2036	Alleles bAL57	GF1 R:S	R146 R:S	CC23 R:S	CC2 R:S	CC47 R:S	A6 R:S	CC1 R:S
1868	Pallas	Heterozygote	8:11	20:0	18:0	n.t.	n.t.	n.t.	n.t.
1153	Pallas	Heterozygote	4:14	21:0	n.t.	18:0	n.t.	n.t.	n.t.
1374	Heterozygote	Pallas	0:17	29:0	n.t.	n.t.	23:0	n.t.	n.t.
1753	Heterozygote	Heterozygote	11:18	45:0	n.t.	n.t.	n.t.	2:8	n.t.
1830	Heterozygote	Pallas	4:15	28:0	n.t.	n.t.	n.t.	n.t.	21:0

[a] n.t. = not tested; R = resistant phenotype; S = susceptible phenotype

The presence of the additional resistance gene from Pallas BC_5 *Mlg* and its corresponding avirulence determinant in the R146 isolate makes R146 unsuitable for mapping the *Mlg* locus in the Pallas BC_5 *Mlg* × Pallas *mlg* mapping population. In contrast, GF1 lacks the additional avirulence gene present in R146, and resistance to this isolate was controlled solely by the *Mlg* locus in this population (Table 2).

To determine whether other powdery mildew isolates may contain the additional avirulence gene in R146, we inoculated >20 members of each of a number of selected F_3 families from the Pallas BC_5 *Mlg* × Pallas *mlg* population with a number of powdery mildew isolates. The results are shown in Table 2. The inheritance of resistance and susceptibility to the isolate A6 was the same as that for GF1, indicating that this isolate contains *AvrMlg* but not the additional avirulence determinant present in R146. All of the families tested with the isolates CC23, CC2, CC47 and CC1 showed the same resistance reaction as R146, indicating that these may contain the additional avirulence gene present in R146.

Discussion

Homing in on *Mlg*

An essential step in the map-based cloning of genes from complex plant genomes is the generation of a high-resolution genetic map that identifies molecular markers flanking the target gene, thereby enabling physical delimitation of a chromosomal segment harboring the gene. It is desirable that these two flanking molecular markers are close enough to be present on a single genomic clone from a library of large genomic DNA fragments constructed using yeast artificial chromosomes or an alternative vector system (''chromosome landing'' Tanksley et al. 1995). This concept was recently validated in barley by the map-based isolation of the genes *Mlo* and *Rar1*, and by the physical delimitation of the *Mla* locus (Büschges et al. 1997; Simons et al. 1997; Lahaye et al. 1998; Shirasu et al. 1999; Wei et al. 1999). Because 1cM genetic distance corresponds to an average length of 3 Mb in barley, our analysis of 2000 progeny segregating for *Mlg* relates to approximately 150 kb between two recombination events, a distance that can be bridged by single clones from available barley genomic DNA libraries (Simons et al. 1997).

However, the relationship between physical and genetic distances can vary considerably along a chromosome. An estimate at the centromeric region near *Mlg* has been inferred from the analysis of chromosome 4H translocation breakpoints (Künzel et al. 2000). The integration of the breakpoints into a RFLP map of chromosome 4H revealed that a segment comprising only 4% of the genetic chromosome length, corresponds to at least 33% of the total physical chromosome length. This study allocated the centromere of chromosome 4H to the same interval (MWG2036 – MWG058). These observations indicate that the ratio of physical to genetic distance in the *Mlg* region is approximately 10-fold higher than the average for the whole genome. This is consistent with other reports indicating suppressed recombination to be a general feature of centromeric regions (Frary et al. 1996). In addition, a 5-fold higher recombination frequency was observed for the interval MWG032 – bAL57 in the *Mlg* region in another mapping population (Graner et al. 1991), suggesting that additional, as yet unknown, factors control recombination rates in this centromeric region.

The next critical step towards the isolation of *Mlg* is aimed at the identification of DNA markers that map at the locus and which are no further apart than 0.05 cM. The 179 recombinants identified in the target interval of 4.47 cM encompassing *Mlg* provide a good resource for future marker enrichment by means of bulked segregant analysis (Giovannoni et al. 1991; Michelmore et al. 1991).

An additional resistance specificity in Pallas BC_5 *Mlg*

Here we have shown that the *Mlg*-containing parental line used for high-resolution mapping must contain an additional resistance gene. The mapping parent Pallas BC_5 *Mlg* was generated by backcrossing the donor line Weihenstephan MRII five times with the recurrent parent Pallas (Kølster et al. 1986). However, the donor line and the resulting backcross line were shown to carry the *Mlg* resistance gene as well as the loosely linked *Ml(CP)* resistance specificity (Wiberg 1974; Kølster et al. 1986), suggesting the presence of a large introgressed fragment in Pallas BC_5 *Mlg* harboring both *R* genes. Since the fungal isolate used here, R146, is known to carry an avirulence function to *Mlg* but virulence to *Ml(CP)* (Jensen et al. 1992), the newly identified resistance specificity in Pallas BC_5 *Mlg* must be different from *Ml(CP)*. This conclusion is further supported by the finding that other fungal isolates different to the ones tested here and virulent on cultivars Goldfoil *(Mlg)* and Julia [*Mlg, Ml(CP)*] were avirulent on Pallas BC_5 *Mlg* (Brown and Jessop 1995; J.K.M. Brown, personal communication).

We have shown that the newly discovered resistance gene must originate from the *Mlg* donor line Weihenstephan MR II and that it is not closely linked to *Mlg*. Southern analysis using RFLP probes from chromosome 4H was performed to determine the length of the introgressed fragment in Pallas BC_5 *Mlg*. This revealed that the *Adh* marker bordered the introgressed fragment on the short chromosome arm and that the introgression extends beyond RFLP bA011 on the long arm, thereby defining an interval of more than 69 cM, half the total genetic length of chromosome 4H (data not shown). Thus, although we failed to detect linkage between *Mlg* and the newly discovered *R* gene, it seems plausible that this *R* gene may reside in the large introgressed fragment of the backcross line Pallas BC_5 *Mlg*. If this is the case, then this *R* gene must be novel since *Mlg* and *Ml(CP)* are the only known race-specific powdery mildew *R* genes on chromosome 4H.

Syntenic relationships between rice and barley at *Mlg*

Collinear orders of genes is a hallmark among grass species and has been extensively investigated both at the genome-wide and local (<1 cM) levels (reviewed in Devos and Gale 1997). Since rice has a small genome size and contains a low amount of repetitive DNA compared to other cereals, we have explored here the potential to utilize rice DNA markers from orthologous segments for mapping at *Mlg*. We found that only two probes from the *Mlg* region (bAL57 and CDO541) detected loci in a similar location in rice (on chromosome 3), while the remainder mapped to separate locations (on chromosomes 11, 7, 2 and 4/8). Probe Osc147 detects at least two barley loci while the remaining probes appear to detect single loci in both barley and rice on the basis of their hybridization patterns. In addition, the YAC library used for locating the markers in rice comprises six genome equivalents (Umehara et al. 1995). Therefore, we are confident that these data represent true breaks in synteny between rice and barley.

The apparently frequent breaks in barley/rice collinearity observed for the *Mlg* region may in part reflect the suppressed recombination in this centromeric region (i.e. the 7.7 cM interval bordered by MWG2036 and MWG058 in this region in fact represents at least 1/3 of the entire barley chromosome 4H). Nevertheless, these findings are reminiscent of experiences at the barley loci *Rpg1* and *Rar1*, in which the barley/rice collinearity at the micro (<1 cM) level was found to be frequently broken (Kilian et al. 1997; Lahaye et al. 1998). These greater than anticipated levels of barley/rice chromosomal rearrangement could hamper attempts to use the small rice genome as a tool in the map-based isolation of the *Mlg* gene. In addition, since mapping of resistance gene homologues of the NB-LRR class has shown extensive rearrangements of *R* gene loci between related grass species (Leister et al. 1998), it may not be possible to identify a *Mlg* candidate *R* gene probe from its mapped location in rice (Han et al. 1999).

A single resistance specificity at *Mlg*?

RFLP MWG032, which is presently the most tightly linked *Mlg* marker, was shown here to reliably detect the presence of the *Mlg* resistance allele in a collection of 30 European barley cultivars. This RFLP marker, and DNA markers indicating the presence of other powdery mildew resistance loci [e.g. *Mla*; (Schüller et al. 1992)], should aid in the molecular fingerprinting of powdery mildew-resistant barley germplasm. The reliability of RFLP MWG032 to predict the presence of *Mlg* in European barley cultivars could be useful to find out whether *Mlg* in European and non-European cultivars such as Goldfoil (Briggs and Stanford 1943) and Palmella Blue (Starling et al. 1963) is derived from a common ancestor. This should be particularly interesting because the latter two lines appear to exhibit different infection phenotypes upon challenge with *AvrMlg*-containing fungal isolates (Wiberg 1974).

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