Genetic Analysis of Thirteen Accessions of *Hordeum vulgare* ssp. *spontaneum* Resistant to Powdery Mildew

A. Dreiseitl, J. Řepková* and P. Lízał

1Agricultural Research Institute Kroměříž Ltd., Kroměříž, Czech Republic
2Department of Genetics and Molecular Biology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

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Thirteen accessions of wild barley (*Hordeum vulgare* ssp. *spontaneum*) resistant to powdery mildew caused by the fungus *Blumeria graminis* f. sp. *hordei* were studied with the aim of determining the number of resistance genes and their allelic relationships to the *Mla* locus on the short arm of chromosome 1H. In five accessions (PI391130, PI466193, PI466200, PI466495 and PI466510), the resistance was caused by one gene, in seven accessions (PI354949, PI391081, PI466158, PI466197, PI466211, PI466297 and PI466461) by two independent genes and in PI301004 by three independent genes. The type of inheritance of all analysed genes except two was dominant or semi-dominant; only one of two genes in PI391081 and PI466297 was recessive. Allelism tests confirmed that in 10 accessions one gene was allelic with the *Mla* locus, and in three accessions (PI391081, PI466193 and PI466297) the resistance genes were different from the *Mla* locus.

**Keywords:** allelism, barley, *Blumeria graminis* f. sp. *hordei*, *Mla* locus, resistance, wild barley

**Introduction**

Barley (*Hordeum vulgare* L.) is one of the most wide-spread crops in the world and its production is adversely affected by many diseases. In the Czech Republic, powdery mildew caused by *Blumeria graminis* f. sp. *hordei* DC. f. sp. *hordei* Ém. Marchal (*Bgh*) is the most common and economically important disease of barley (Dreiseitl 2003a). Powdery mildew epidemics lead to the reduction of grain yield,
feeding and malting quality, and profitability for growers. The high frequency of virulences to resistance genes carried by current winter barley varieties (Dreiseitl 2004) contributes to a frequent and heavy infection of both spring and winter barley. Winter barley serves as an important source of inoculum where new and widely virulent pathotypes of the powdery mildew pathogen can arise and reproduce, subsequently attacking both barley types. This results in a faster adaptation of the pathogen to the resistances present in cultivated barley varieties (Dreiseitl 2003b).

Jørgensen (1994) summarised known genes for powdery mildew resistance in barley, including their location on four out of seven barley chromosomes. Later, Schönfeld et al. (1996) mapped three genes on other two chromosomes, 5H and 7H, and Pickering et al. (1995) mapped one gene on chromosome 2H. The complex Mla locus on the short arm of chromosome 1H is the most important among the known powdery mildew resistance genes (Panstruga and Schulze-Lefert 2002).

Resistance to powdery mildew plays a significant role in the breeding of barley. To counter the pathogen, varieties should be bred with resistance genes for which no corresponding virulence factor in the pathogen population has been found within a given epidemiological unit. Wild barley *H. vulgare* ssp. *spontaneum* and *H. bulbosum* represent promising sources of resistance to important barley diseases (Williams 2003). Screening of wild barley accessions from the USDA National Small Grains Collection revealed that a high proportion of these accessions exhibited useful resistance to powdery mildew (Dreiseitl and Bockelman 2003).

We studied a set of wild barley accessions resistant to powdery mildew aiming: (1) to find the number of genes/loci conferring the resistance, (2) to identify the modes of inheritance of these genes, and (3) to define their relationships to the Mla locus.

**Materials and Methods**

*Plant material and population development*

Preparation of biological material and all experiments were carried out at the Agricultural Research Institute Kroměříž Ltd. Thirteen wild barley accessions (PI354949, PI391004, PI391081, PI3191130, PI466158, PI466193, PI466197, PI466200, PI466211, PI466297, PI466461, PI466495 and PI466510) from the USDA National Small Grains Collection, with resistances to powdery mildew.
(Dreiseitl and Bockelman 2003, Dreiseitl and Dinoor 2004), and the two-row winter barley variety ‘Tiffany’ carrying powdery mildew resistance genes *Mla7, MlaMu2* were used. ‘Tiffany’, as a female parent, was crossed with the thirteen resistant accessions. The dormancy of harvested seed was routinely interrupted at 38 °C for 48 h, and F1 generations 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 seeds of F2 generations were obtained after selfing of F1 plants.

**Pathogen isolates**

Two selected pathotypes of *Bgh* held in the gene bank of the pathogen at the Agricultural Research Institute Kroměříž Ltd. were used for the inoculation of the tested plants. A virulent (*Va7, VaMu2*) pathotype 0323 was used for determination gene number conferring resistance in each accession and their inheritance. An avirulent (*Aa7*) pathotype 1002 was employed for the tests of allelism for the *Mla* locus. Each pathotype had previously been purified, verified for the correct virulence/avirulence phenotype on the differential hosts and increased on the varieties ‘Tiffany’ (0323) or ‘Algerian’ (1002).

**Resistance tests**

Four seeds per genotype (parental, F1 and F2 generations) were sown in pots (80 mm upper diameter) in the greenhouse, and the plants were grown at a continuous temperature of 17±2 °C and under natural daylight. Four segments of about 25 mm in length were cut from the central part of each fully expanded primary leaf of eighteen-day-old plants and placed in four dishes with 0.6% agar and 35 ppm of benzimidazole; inoculation was carried out with each pathotype separately with two replications (Dreiseitl and Dinoor 2004) with inoculum density of ca. 8 conidia mm⁻². Eight days after inoculation, reaction types (RTs) of leaf segments were scored on the 0–4 scale (Torp et al. 1978). Reaction types 2–3 and lower were considered resistant. Twenty to forty plants of each parent, 22 to 74 F1 plants and 190 to 542 F2 plants of individual crosses were evaluated.

**Inheritance of resistance genes**

The numbers of plants in the two phenotypic categories (resistant and susceptible) found in F2 populations were compared with theoretical Mendelian segregation ratios by a chi-square test, and the number of resistance genes in each accession
was estimated. The comparison of RTs between parental and F1 generations enabled the determination of the modes of inheritance of resistance genes (dominant, semi-dominant or recessive).

Allelism tests

The results of resistance tests of F2 populations with Va7 and Aa7 pathotypes were compared and conclusions on allelism for the Mla locus were drawn. If both resistant and susceptible plants in the F2 population were found after inoculation with the Va7 pathotype and all F2 plants showed only the resistant phenotype after inoculation with the Aa7 pathotype, the resistance was considered to be determined by an allele of the Mla locus. If resistant and also susceptible F2 plants were identified after inoculation with the Aa7 pathotype, the resistance genes were considered to be different from the Mla locus.

Results

Table 1 summarises the numbers of resistant and susceptible plants in the F2 populations of 13 powdery mildew resistant accessions and significance of considered segregation ratios. The numbers of F2 plants sorted according to evaluated RTs are given in Figure 1. The evaluation of plants of F2 populations after inoculation with the Va7 pathotype revealed the whole range of RTs, including the susceptible ones. Only resistant plants were found after inoculation with the Aa7 pathotype with the exception of PI391081, PI466193 and PI466297. This indicated the presence of an allele of the Mla locus in all accessions excluding the three exceptions. The following conclusions were drawn for individual, tested, resistant accessions after analysis of the parental, F1 and F2 plants.

In PI354949, dominant alleles of two independent genes were present (P = 0.37), and the allelism test indicated that one gene was located at the Mla locus (Table 1). All parental plants showed RT0; F1 plants showed RTs 0 and 1 as a consequence of one gene resembling a dominant and the other a semi-dominant type of inheritance (Figure 1A). In PI391004, three independent resistance genes (P = 0.70) were detected, and the allelism test confirmed at least one resistance gene at the Mla locus. The parental plants proved to be of RTs 0 and 1 and the same RTs were found in plants of the F1 generation. This clearly indicated dominant inheritance (Figure 1B). In PI391081, two independent resistance genes (P = 0.16) were confirmed, one dominant and the other recessive. The allelism test also revealed susceptible plants, which indicated that none of the genes was at the Mla locus. The parental plants with the RT1-2 and the F1 generation with RTs ranging be-
between 1-2 and 2-3 reflected a semi-dominant mode of inheritance (Figure 1C). In PI391130, only one dominant resistance allele was estimated (P = 0.46) and the allelism test confirmed its location at the Mla locus. RT1-2 was found in the parental generation and also in the F1 plants (Figure 1D). In PI466158, two independent resistance genes (P = 0.76) were found; one of them was allelic with the Mla locus. The two genes exhibited semi-dominant inheritance (Figure 1E). In PI466193, one gene conferring resistance (P = 0.48) was detected. Screening with the avirulent pathotype revealed both resistant and susceptible plants, which indicated that the gene was not tightly linked with the Mla locus. RT1-2 of the parental plants and RTs 2 and 2-3 of the F1 plants were in agreement with a semi-dominant mode of inheritance (Figure 1F). In PI466197, two resistance genes (P = 0.68) were estimated, one allelic with the Mla locus. Their inheritance was semi-dominant because the RTs were 0 and 1 in the resistant accession and 1-2 in the F1 plants (Figure 1G). In PI466200, one resistance gene allelic with the Mla locus might be expected (P = 0.16). The paternal and the F1 plants exhibited the same RT0, which was indicative of dominant inheritance (Figure 1H). In PI466211, two independent resistance genes (P = 0.08), one allelic with the Mla locus, were confirmed. Semi-dominance was concluded from the fact that parent-

### Table 1. The number of resistant and susceptible plants in the F2 populations of 13 powdery mildew resistant accessions of *Hordeum vulgare* ssp. *spontaneum* after inoculation with virulent (Va7) and avirulent (Aa7) pathotypes of *Blumeria graminis* f. sp. *hordei* and the significance of considered segregation ratios

<table>
<thead>
<tr>
<th>Resistant accession</th>
<th>Va7 pathotype</th>
<th>Segregation Ratio</th>
<th>Aa7 pathotype</th>
<th>( \chi^2 )</th>
<th>Segregation Ratio</th>
<th>No. plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI354949</td>
<td>422</td>
<td>33</td>
<td>15:1</td>
<td>0.78*</td>
<td>455</td>
<td>0</td>
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<tr>
<td>PI391004</td>
<td>206</td>
<td>4</td>
<td>63:1</td>
<td>0.16*</td>
<td>210</td>
<td>0</td>
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<tr>
<td>PI391081</td>
<td>162</td>
<td>28</td>
<td>13:3</td>
<td>2.01*</td>
<td>183</td>
<td>7</td>
</tr>
<tr>
<td>PI391130</td>
<td>370</td>
<td>114</td>
<td>3:1</td>
<td>0.54*</td>
<td>484</td>
<td>0</td>
</tr>
<tr>
<td>PI466158</td>
<td>352</td>
<td>22</td>
<td>15:1</td>
<td>0.09*</td>
<td>374</td>
<td>0</td>
</tr>
<tr>
<td>PI466193</td>
<td>241</td>
<td>73</td>
<td>3:1</td>
<td>0.51*</td>
<td>300</td>
<td>14</td>
</tr>
<tr>
<td>PI466197</td>
<td>431</td>
<td>31</td>
<td>15:1</td>
<td>0.17*</td>
<td>462</td>
<td>0</td>
</tr>
<tr>
<td>PI466200</td>
<td>175</td>
<td>71</td>
<td>3:1</td>
<td>1.96*</td>
<td>246</td>
<td>0</td>
</tr>
<tr>
<td>PI466211</td>
<td>372</td>
<td>16</td>
<td>15:1</td>
<td>2.99*</td>
<td>389</td>
<td>0</td>
</tr>
<tr>
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<td>425</td>
<td>117</td>
<td>13:3</td>
<td>2.86*</td>
<td>532</td>
<td>10</td>
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<td>468</td>
<td>30</td>
<td>15:1</td>
<td>0.04*</td>
<td>498</td>
<td>0</td>
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<tr>
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<td>318</td>
<td>94</td>
<td>3:1</td>
<td>1.05*</td>
<td>412</td>
<td>0</td>
</tr>
<tr>
<td>PI466510</td>
<td>145</td>
<td>53</td>
<td>3:1</td>
<td>0.33*</td>
<td>198</td>
<td>0</td>
</tr>
</tbody>
</table>

* a Reaction types 0 to 2-3
  b Reaction types 3 to 4
  c The reaction type 3 is considered resistant
  * Significance of the tested segregation ratio was confirmed at P > 0.05
tal plants were of two RTs, 0 and 0-1, and the F1 plants were of RTs 1-2 and 2 (Figure 1I). In PI466297, two independent genes (P = 0.09), one probably semi-dominant and the other recessive, were considered. Allelism with the \( Mla \) locus was not supported, owing to the identification of susceptible plants in the F2 generation after avirulent pathotype testing. The parental plants exhibited up to three different RTs (Figure 1J). In PI466461, two independent resistance genes (P = 0.84) were confirmed; one was allelic with the \( Mla \) locus. Two RTs, 0 and 1, were detected in the parental plants. In the F1 generation, only RT1 was observed, which indicated semi-dominant inheritance for one gene and dominant inheritance for the other (Figure 1K). In PI466495, one resistance gene (P = 0.30) allelic with the \( Mla \) locus was estimated. Semi-dominance of the gene could be assumed on the basis of the RTs’ shifting from 1 in the resistant parent to 1-2 and 2 in the F1 plants (Figure 1L). Also in PI466510, one resistance gene (P = 0.58) allelic with the \( Mla \) locus was confirmed. Partly overlapping RTs of the resistant accession (1 and 1-2) and

\[ X \text{ – scored reaction types (RTs) of leaf segments, } Y \text{ – the number of plants for individual RTs } \]

- Resistance accession
- Variety ‘Tiffany’
- \( F_1 \) generation
- \( F_2 \) population

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the $F_1$ plants (1-2 and 2) indicated the semi-dominant inheritance of the resistance allele (Figure 1M).

**Discussion**

*Blumeria graminis* f. sp. *hordei* ranks high among cereal pathogens for its adaptability and potential to cause crop losses (McDonald and Linde 2002). It is desirable to combine more effective resistance genes in one variety. Therefore, localisation of such genes in the barley genome is important.

We used 13 genetic resources selected from a large group of wild barley accessions fully resistant to powdery mildew (Dreiseitl and Bockelman 2003, Dreiseitl and Dinoor 2004). Our genetic analyses showed that 10 out of these 13 accessions contained an allele of the *Mla* locus. It confirmed the unique significance of the *Mla* locus among other barley loci conditioning resistance to powdery mildew.

The size of the evaluated $F_2$ populations was sufficient for individual ratios testing, including differentiation between 13:3 and 3:1. Nevertheless, owing to large differences in RTs of resistant plants in the $F_2$ generation (particularly those shown in Figures 1A, 1E, 1G, 1I, 1J and 1L), each locus, either the Mla or another, could include additional linked resistance gene(s). This means that the trait might not be determined in a simple manner similarly 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).

The number of resistance genes was determined and confirmed. In most cases, a correlation was observed between the gene number in the $F_2$ generation and the number of RTs determined for parental plants of a corresponding resistant accession (two genes in PI466158, PI466197, PI466211, PI466297 and PI466461; one gene in PI391130, PI466193, PI466200 and PI466495). For two crosses (PI354949 and PI391081), the number of genes determined in the $F_2$ generation was higher than the number of RTs determined for the parental plants.

The identities of resistance genes in the 13 accessions are not known yet and at present, we cannot determine which of the genes are already known or new. Obtaining genetically characterised $F_2$ populations with a known number of genes determining resistance of the accessions to powdery mildew and knowledge about the alleles’ modes of inheritance will enable us to answer the question of gene identity. Recombinant analyses utilising DNA markers will help us to localise these genes on the barley genetic map and to compare their positions with those of known resistance genes.
Current methods of molecular biology enable, among others, development of various types of DNA markers and genetic map construction, thus opening new possibilities for effective and fast breeding of new varieties. The identification of DNA markers tightly linked with individual resistance genes will facilitate the purposeful selection of offspring (marker-assisted selection) and the combination of fully effective resistance genes in one genotype.

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References


