Genetical and RFLP studies at the *Mla* locus conferring powdery mildew resistance in barley

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Summary. The complex structure of the multigene family at the *Mla* locus conferring powdery mildew resistance in barley was studied by making diallel crosses between several near-isogenic lines carrying different *Mla* alleles. The mode of inheritance of the *Mla* alleles investigated was determined to be dominant for *Mla1*, *Mla6*, *Mla7* and *Mla13* and semidominant for *Mla3*, *Mla12* and *Mla20*. *F*1 plants were backcrossed to the susceptible recurrent parent in order to identify susceptible and double-resistant recombinants in the BC1*F*1 generation. Out of 17 605 progenies tested in the BC1*F*1 generation, two susceptible recombinants, one between *Mla1* and *Mla12* and one between *Mla13* and *Mla20* were confirmed. The former was also verified by RFLP analysis.

Key words: Barley – Multigene family – *Mla* locus – Recombination – RFLP marker

Introduction

The genes conferring resistance to plant diseases are not distributed randomly over the genome, rather they occur frequently in groups on particular chromosomes. In the case of barley powdery mildew, multiple allelism has been found on three loci, namely *Mla*, *Mlp* and *mlo* (Moseman and Jørgensen 1971; Giese et al. 1981; Jahoor et al. 1989; Hentrich 1979). The *Mla* locus of barley has been the focus of much interest because of its complex polymorphism; up to now 23 alleles or several closely linked loci have been confirmed for this locus (Jahoor et al. 1991a, b). Jørgensen and Moseman (1972) reported one susceptible recombinant plant originating from a cross involving the *Mla1* and *Mla3* alleles. Wise and Ellingboe (1985) studied the complex structure of the multigene family in the *Mla* region, a region which should allow intra-allelic recombination.

RFLP markers are phenotypically neutral and independent of allelic and nonallelic interaction. Due to this fact, they enable the investigator to detect the exact genetic constitution of an individual plant in a segregating population. RFLP-based genetic maps for barley are at present being developed at several laboratories (Heun et al. 1991; Jahoor et al. 1991a; Graner et al. 1991; Blake et al. 1991). Schüller et al. (1992) have been able to separate the *Mla* alleles into 11 different groups by polymorphism obtained with only one RFLP marker. This marker is very closely linked to the *Mla* locus (0.7 cM). In addition, two more markers have been identified that tightly flank the *Mla* locus on both sides.

The purpose of the study presented here was to detect the mode of inheritance and intralocus recombination at the *Mla* locus conferring powdery mildew resistance in barley.

Materials and methods

In order to minimise effects which might arise from the genetic background of the parental lines carrying different alleles of the *Mla* locus, near-isogenic lines (NILs) in the ‘Pallas’ background (Kölster et al. 1986) were used. For our study seven NILs carrying *Mla* alleles which originated from different geographic areas were chosen, together with one line derived from a *Hordeum spontaneum* collection from Israel that carried the *Mla20* allele (Table 1).

Single-spore isolates of powdery mildew which are able to distinguish individual resistance genes were selected from a
collection of mildew cultures maintained at the Institute of Agronomy and Plant Breeding, Weiheenstein; 9 out of the 13 isolates originated from European mildew populations and 4 originated from the wild population of *H. spontaneum* in Israel. For each of the two parents of the crosses that were made one virulent and one avirulent isolate was chosen to determine double-resistant as well as double-susceptible recombinant plants. Diallele crosses among lines carrying *Mla1, Mla3, Mla6, Mla7, Mla12, Mla13* and *Mla20* were made, and *F1* progenies were used to determine the mode of inheritance.

To study the fine structure of the *Mla* locus, *F1* plants of the diallele crosses were backcrossed to the susceptible recurrent parent of the near-isogenic lines, cv 'Pallas' (which carries the ineffective allele *Mla8*), in order to allow the identification of susceptible and double-resistant plants in the BC1F1 generation.

For the mildew tests, seedlings were grown under controlled conditions to exclude undesirable mildew infections. All experiments were carried out in vitro to avoid contamination. Detached leaves from the seedlings were placed upon agar containing 30 mg/1 benzimidazol to delay leaf chlorosis. The inoculation technique, subsequent treatments after inoculation and reading of the infection type were similar to those described by Nover (1972). Type 0 denotes immune reactions showing no visible symptoms of infection, whereas reaction type IV indicates complete susceptibility, showing no visible defense mechanisms. In addition, infection severity of the leaf area covered with powdery mildew was classified in a 0.0–1.0 scale relative to the universal susceptible standard SM4142.

For Southern analysis, DNA from the recombinant plants and parents were isolated according to Graner et al. (1990). The DNA was digested with the restriction enzyme *EcoRV*. The restricted DNA (10 µg/lane) was subjected to electrophoresis in 0.75% agarose gels, and the resulting DNA fragments were then transferred to nylon membranes as described by the supplier (Pall Crop, Dreieich). The inserts from the recombinant plasmids were labeled with 32P-dCTP by random priming (Feinberg and Vogelstein 1983) and subsequently used as probes. Hybridisation and further treatments of the membranes were conducted as described by Jahoor et al. (1991a).

**Results**

**Reaction pattern of parental lines**

The reaction patterns of the parental lines are presented in Table 2. The mildew resistance genes included in the present investigation showed either highly resistant or highly susceptible reactions upon infection with European isolates but when exposed to infection by Israeli isolates most of them reacted in a susceptible manner. Only *Mla7* (*P04B*) developed highly resistant reactions against three Israeli isolates, which reaction type II scored from infection by Ar-4 and AI-1 indicated the presence of the *Mlk* gene in the near-isogenic line *P04B*.

**Interaction between Mla alleles**

*F1* plants derived from the diallele crosses between NILs carrying different *Mla* alleles were infected with isolates that were virulent against one parent and avirulent against the other, and vice versa. The results agreed with the information obtained from crosses between the near-isogenic lines and the recurrent parent 'Pallas'. A dominant mode of inheritance was also observed shown for *Mla6*, for which heterozygous plants from a cross with 'Pallas' were not available. Most of the *Mla* alleles involved in this study maintained a stable mode of inheritance against different avirulent isolates, with the exception of *Mla13*.

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**Table 1.** Near-isogenic lines of *Mla* alleles and geographic origin of their donors, including the recurrent parent Pallas

<table>
<thead>
<tr>
<th><em>Mla</em> allele</th>
<th>Near-isogenic line (Nil)</th>
<th>Donor</th>
<th>Geographic origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mla8</em></td>
<td>Pallas</td>
<td>Hanna</td>
<td>Europe</td>
<td>Moseman (1955)</td>
</tr>
<tr>
<td><em>Mla1</em></td>
<td>P01</td>
<td>Algerian</td>
<td>Algeria</td>
<td>Moseman (1955)</td>
</tr>
<tr>
<td><em>Mla3</em></td>
<td>P02</td>
<td>Ricardo</td>
<td>Uruguay</td>
<td>Moseman (1955)</td>
</tr>
<tr>
<td><em>Mla6, Mla4</em></td>
<td>P03</td>
<td>*H. spontaneum H204</td>
<td>Balkan</td>
<td>Honecker (1936b)</td>
</tr>
<tr>
<td><em>Mla7</em></td>
<td>P04B</td>
<td>Lyallpur 3645</td>
<td>Pakistan</td>
<td>Hoffmann and Nover (1959)</td>
</tr>
<tr>
<td><em>Mla12</em></td>
<td>P10</td>
<td>Arabische</td>
<td>Arabia</td>
<td>Wiberg (1974a)</td>
</tr>
<tr>
<td><em>Mla13, Ml(RU3)</em></td>
<td>P11</td>
<td>Rupee</td>
<td>India</td>
<td>Moseman (1955)</td>
</tr>
</tbody>
</table>

* No NIL was available for *Mla20*