A search for 4 specific markers linked to Pm3 alleles for resistance to powdery mildew (Blumeria graminis) in rye (Secale cereale)

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Abstract: To investigate powdery mildew resistance in rye (Secale cereale), 397 inbred lines of winter rye were tested for susceptibility to infection with Blumeria graminis f. sp. secalis. The 50 most tolerant lines and 50 most infected lines were chosen for comparison. They were next tested for the presence of 4 markers linked to 4 alleles for resistance to powdery mildew, identified earlier in common wheat (Triticum aestivum). We found Pm3a only in 3 susceptible genotypes of winter rye, although this marker is linked to the powdery mildew resistance gene in wheat. The other 3 markers linked to Blumeria graminis f. sp. secalis resistance genes (Pm3b, Pm3c, Pm3d) were found in neither resistant nor susceptible rye genotypes.

Keywords: Blumeria graminis f. sp. secalis, molecular markers, powdery mildew, resistance genes

INTRODUCTION

Winter rye (Secale cereale) is still one of major crops grown in Poland. The most important fungal pathogen affecting its leaves is Blumeria graminis f. sp. secalis, which causes a disease called powdery mildew. This pathogen may contribute to losses in rye yields as high as 20% (SAWICKA 2011), and is most serious in cool and wet climates. There are appropriate fungicides, but fungicide-resistant strains of powdery mildew have emerged (JORGENSEN 1988). The most commonly used environmentally safe method is to cultivate rye varieties resistant to this pathogen.

Ten genes determining resistance to powdery mildew infection have been recognized in rye: Pm1a, Pm1b, Pm7 (1RS), Pm2 and Pm8 (2RL), Pm3 (3RS), Pm6 (4R), Pm4 (5RL), Pm5 and Pm(?) (6RL) (MELZ et al. 1992; SCHLEGEL et al. 1998;
In contrast, more than 60 various alleles of powdery mildew resistance genes (Pm) have been identified in wheat (Triticum aestivum), for Blumeria graminis f. sp. tritici. For most of them, molecular markers closely linked to those alleles were developed.

The wheat ResPm4 marker is present in resistant rye genotypes (Jurkowski et al. 2014a). This has encouraged us to continue the search for markers linked to powdery mildew resistance genes in rye, by examining the resistance markers developed for wheat. This strategy is far less expensive than the testing of thousands of new markers. The aim of this study was to examine the utility of 4 wheat resistance markers in identifying powdery mildew resistance genes in rye.

MATERIALS AND METHODS

Plant material included 397 inbred lines of rye provided by breeding companies (Danko Plant Breeding Ltd. and Poznan Plant Breeding Ltd.) or coming from the working collection of the Department of Genetics, Plant Breeding, and Seed Production, Wroclaw University of Environmental and Life Sciences.

The evaluation of the degree of rye genotype susceptibility to infection with powdery mildew was made according to Bujak & Jurkowski (2013) and Jurkowski et al. (2014b).

For winter rye, there is no information about the linkage of molecular markers of individual powdery mildew resistance genes, so we decided to check the markers developed for wheat (Tommasini et al. 2006). The primers used in PCR reactions for individual molecular markers linked to powdery mildew resistance genes in wheat are summarized in Table 1. PCR reaction conditions were described in Tommasini et al. (2006). The amplification products were analysed electrophoretically. Fragment size was estimated with MassRuler DNA Ladder (Thermo Fisher Scientific), and a refer-

Table 1. Characteristics of primers used for PCR reaction (Tommasini et al. 2006)

<table>
<thead>
<tr>
<th>Linking to Pm genes</th>
<th>Marker name</th>
<th>Nucleotide sequence of primers (5’→3’)</th>
<th>Length of amplified product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pm3a</td>
<td>Specific for Pm3a</td>
<td>GGA GTC TCT TCG CAT AGA CAG CTT CTA AGA TCA AGG AT</td>
<td>624</td>
</tr>
<tr>
<td>Pm3b</td>
<td>Specific for Pm3b</td>
<td>GGC ACA GAC AAA GCT CTG TCG AGT AGC TCG GGA ATC</td>
<td>1382</td>
</tr>
<tr>
<td>Pm3c</td>
<td>Specific for Pm3c</td>
<td>CTA GTG GAG GTA GTT GAC AGT CGT TCA AGA GAA CGG C</td>
<td>846</td>
</tr>
<tr>
<td>Pm3d</td>
<td>Specific for Pm3d</td>
<td>TGA CTA TTC GTG GGT GCA GAC TGC GGC ACA GTT CAG C</td>
<td>1109</td>
</tr>
</tbody>
</table>

Pm genes = powdery mildew genes; bp = base pairs.
ence line of wheat was used as a control sample of reaction specificity for each series of PCR reaction. Gels were dyed with ethidium bromide (5 μg/ml) for 20 min.

RESULTS

In the greenhouse, 397 genotypes of inbred winter rye lines were evaluated for susceptibility to infection with *Blumeria graminis* f. sp. *secalis*. The lines were marked with symbols UP1 to UP397 (UP = Uniwersytet Przyrodniczy, i.e. the Wrocław University of Environmental and Life Sciences, Table 2). The average degree of infection for the examined rye material varied widely, from 1.0 to 4.0 (data not shown) on a scale from 1 (no symptoms of infection) to 4 (severe infection).

For analysis of the presence of markers linked to powdery mildew resistance genes, we chose 50 genotypes with the lowest degree of infection (UP77, UP78, UP259, UP269, UP366, UP382, UP2, UP76, UP90, UP260, UP287, UP5, UP86, UP89, UP113, UP370, UP8, UP84, UP85, UP234, UP246, UP317, UP352, UP353, UP71, UP72, UP73, UP74, UP75, UP81, UP87, UP91, UP102, UP224, UP225, UP256, UP310, UP350, UP351, UP375, UP380, UP1, UP6, UP7, UP9, UP12, UP16, UP17, UP19, UP20) and 50 genotypes with the highest degree of infection with powdery mildew (UP365, UP367, UP368, UP369, UP371, UP374, UP377, UP378, UP379, UP384, UP385, UP386, UP388, UP389, UP390, UP397, UP321, UP322, UP324, UP325, UP327, UP329, UP332, UP333, UP337, UP338, UP340, UP342, UP346, UP354, UP355, UP356, UP357, UP358, UP359, UP360, UP361, UP362, UP363, UP364, UP28, UP31, UP32, UP34, UP35, UP319, UP320, UP323, UP331, UP3). PCR reactions were conducted on these 100 genotypes to show the presence or absence of the molecular markers.

PCR amplification using primers specific for the *Pm3a* gene revealed the absence of any products in resistant lines (Fig. 1). In contrast, 3 genotypes from the group susceptible to infection showed the presence of the specific marker for *Pm3a*

![Fig. 1. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3a* in inbred lines of winter rye with the lowest susceptibility to powdery mildew infection. M = marker; K = control (Asosan/8*CC); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366; 6 = UP382; 7 = UP2; 8 = UP76; 9 = UP90; 10 = UP260; 11 = UP287](image)
Fig. 2. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for \textit{Pm3a} in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; 1 = UP332; 2 = UP333; 3 = UP337; 4 = UP338; 5 = UP340; 6 = UP342; 7 = UP346; 8 = UP354; 9 = UP355; 10 = UP356; 11 = UP357; 12 = UP358; 13 = UP359; 14 = UP360; 15 = UP361; 16 = UP362; 17 = UP363; 18 = UP364; 19 = UP28; 20 = UP31; 21 = UP32; 22 = UP34; 23 = UP35; 24 = UP319; 25 = UP320

Fig. 3. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for \textit{Pm3b} in inbred lines of winter rye with the lowest susceptibility to powdery mildew infection. M = marker; K = control (Chul/8*CC); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366; 6 = UP382; 7 = UP2; 8 = UP76; 9 = UP90; 10 = UP260; 11 = UP287

(Fig. 2). The reference line of wheat Asosan/8*CC, which carries the \textit{Pm3a} allele, was used as a control.

Using primers appropriate for the marker specific for \textit{Pm3b} for resistant lines showed no product of 1380 bp (base pairs). For all genotypes, a nonspecific product of about 350 bp was present. Fig. 3 shows an example of electrophoresis results from resistant lines. Lines susceptible to infection also failed to amplify the specific marker of \textit{Pm3b}, and the nonspecific product of about 350 bp was seen in 48 genotypes (Fig. 4). As a control of the specificity of PCR, wheat line Chul/8*CC was used.
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Using primers specific to the marker of Pm3c, resistant lines showed no product of 846 bp, whereas 12 genotypes gave a product of about 350 bp and 8 genotypes showed a product of about 1300 bp (Fig. 5). For lines sensitive to infection there were no bands present in the size range expected for the specific marker of Pm3c. A non-specific product of about 350 bp was seen in 34, while a product about 1300 bp was identified in any of the 34 lines (Fig. 6). Wheat line SANOR/8*CC, which contains Pm3c allele, was used as a control.

After electrophoretic separation of PCR amplification products for resistant lines using primers specific for Pm3d (Fig. 7), no products at 1109 bp were found. Non-
specific products of about 750 bp were seen in 27 genotypes, and one of 1450 bp in 8 genotypes. The susceptible lines also did not show the presence of the specific marker for \textit{Pm3d}, but a nonspecific product of 750 bp was demonstrated for 31 genotypes and a product of about 1450 bp was found in 13 lines (Fig. 8). As a control for the specificity of the PCR, wheat variety Ralle was used.
Molecular markers are now widely used for mapping genes and other genetic research. Identification of molecular markers linked to disease resistance genes facilitates breeding of new varieties (Bonnet et al. 2005), development of near-isogenic lines (Zhan et al. 2005), and pyramiding of resistance genes in single genotypes. Genetic progress in breeding programmes is accelerated by the use of molecular markers linked to important agronomic traits, including resistance genes to diseases and pests. A valuable example of comprehensive research towards finding genetic resistance to multiple pathogens, including powdery mildew, is common wheat. This species is an important object of research in many countries. Studies on resistance to powdery mildew in wheat have been conducted in Nordic countries (Hysing et al. 2007), Slovakia (Bojanská 2009), Czech Republic (Svec et al. 2002; Vechet 2006), France (Zeller et al. 1993), Lithuania (Liatukas & Ruzgas 2008, 2009), Brazil (Costamilan 2005), India (Ahmadi et al. 2011), the USA (Niewoehner & Leath 1998; Parks et al. 2008), Poland (Kowalczyk et al. 1998), and Turkey (Spetsos et al. 2013). Those studies mainly located resistance genes on chromosomes and identified molecular markers linked to those genes. Recent reports contain information on obtaining transgenic Pm3 lines with genes that are currently tested in field conditions (Brunner et al. 2011).

The first study of markers linked to resistance genes to powdery mildew in rye has been conducted by our research team (Jurkowski et al. 2014a). The results showed the presence of ResPm4 exclusively in genotypes resistant to powdery mildew. This demonstrated the validity of the search in rye for markers linked to resistance genes.
already described for wheat. However, the present study on 4 other markers linked to resistance genes to powdery mildew, shows that the specific marker for \(Pm3\) appeared only in 3 rye genotypes that were susceptible to infection, whereas the specific markers for \(Pm3b\), \(Pm3c\) and \(Pm3d\) were absent in both resistant and susceptible genotypes.

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Conflict of interest: The authors declare no conflict of interest.

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