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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;

Wakuliński et al. 2007). 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)

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

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*

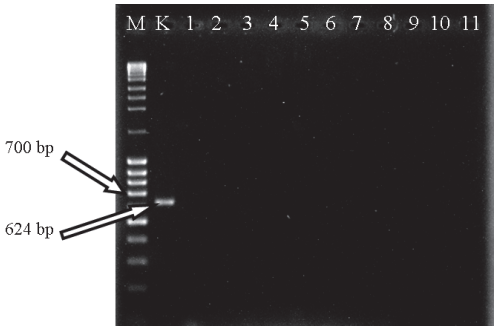

Fig. 1. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3a* in inbred lines of winter rye with the lowest susceptibility to powdery mildew infection. M = marker; K = control (Asosan/8*CC); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366; 6 = UP382; 7 = UP2; 8 = UP76; 9 = UP90; 10 = UP260; 11 = UP287

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

Using primers appropriate for the marker specific for *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 *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.

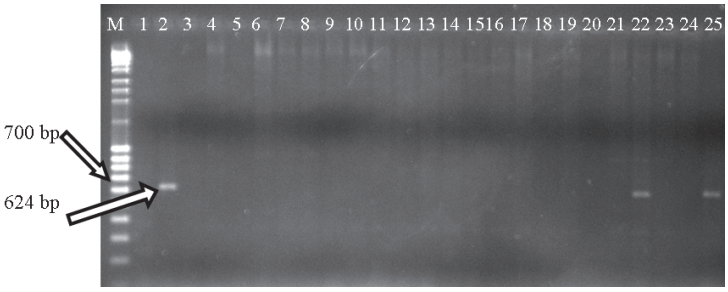

Fig. 2. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3a* in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; 1 = UP332; 2 = UP333; 3 = UP337; 4 = UP338; 5 = UP340; 6 = UP342; 7 = UP346; 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

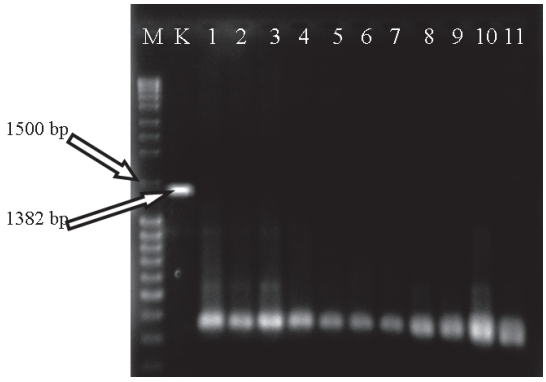

Fig. 3. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3b* in inbred lines of winter rye with the lowest susceptibility to powdery mildew infection. M = marker; K = control (Chul/8*CC); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366;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

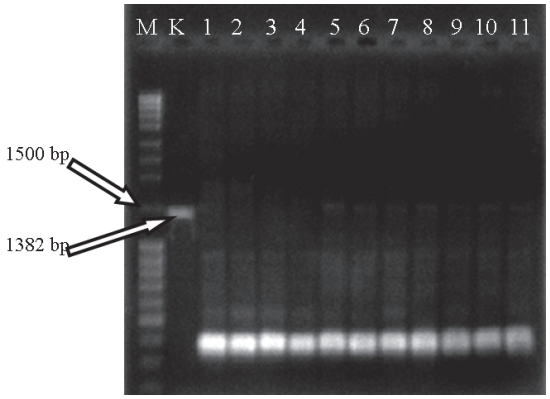

Fig. 4. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3b* in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; K = control (Chul/8*CC); 1 = UP365; 2 = UP367; 3 = UP368; 4 = UP369; 5 = UP371; 6 = UP374; 7 = UP377; 8 = UP378; 9 = UP379; 10 = UP384; 11 = UP385

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-

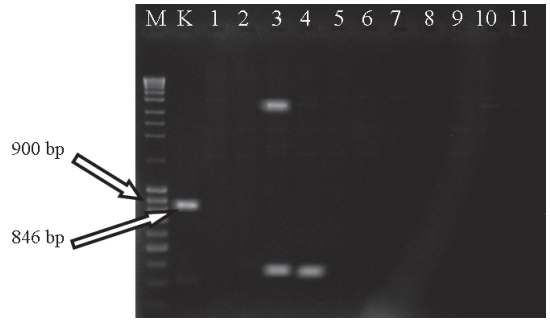

Fig. 5. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3c* in inbred lines of winter rye with the lowest susceptibility to powdery mildew infection. M = marker; K = control (Sanora/8*CC); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366; 6 = UP382; 7 = UP2; 8 = UP76; 9 = UP90; 10 = UP260; 11 = UP287

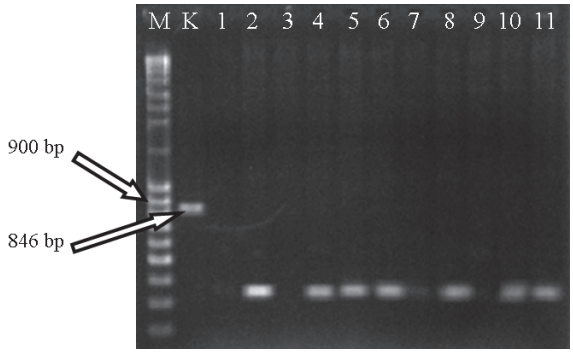

Fig. 6. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3c* in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; K = control (Sanora/8*CC); 1 = UP365; 2 = UP367; 3 = UP368; 4 = UP369; 5 = UP371; 6 = UP374; 7 = UP377; 8 = UP378; 9 = UP379; 10 = UP384; 11 = UP385

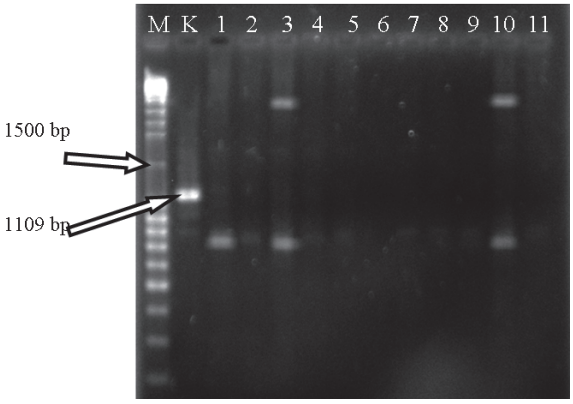

Fig. 7. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3d* in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; K = control (Ralle); 1 = UP77; 2 = UP78; 3 = UP259; 4 = UP269; 5 = UP366; 6 = UP382; 7 = UP2; 8 = UP76; 9 = UP90; 10 = UP260; 11 = UP287

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 *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.

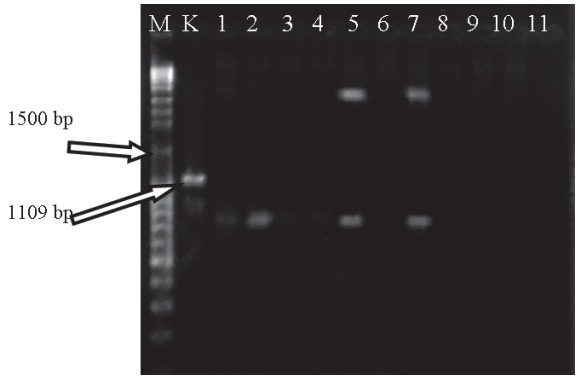

Fig. 8. Electrophoretic separation of amplification products of PCR reaction using primers for a specific marker for *Pm3d* in inbred lines of winter rye with the highest susceptibility to powdery mildew infection. M = marker; K = control (Ralle); 1 = UP365; 2 = UP367; 3 = UP368; 4 = UP369; 5 = UP371; 6 = UP374; 7 = UP377; 8 = UP378; 9 = UP379; 10 = UP384; 11 = UP385

DISCUSSION

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 (Bojnanska 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 (Spetsov 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.

REFERENCES

Ahmadi H., Esmaeili A., Goodarzi D. 2011. Expression of resistance against powdery mildew (*Blumeria graminis* f. sp. *tritici*) in bread wheat (*Triticum aestivum*). Indian J Agri Sci. 81:700–703.

Bojnanska K. 2009. Resistance and genes of resistance against powdery mildew of selected wheat genetic resources. Agriculture (Polnohospodarstvo) 55: 42–48.

Bonnett D. G., Rebetzke G. J., Spielmeyer W. 2005. Strategies for efficient implantation of molecular markers in wheat breeding. Mol Breed. 15: 75-85.

Brunner S., Hurni S., Herren G., Kalinina von Burg O. S., Zeller S. Z., Schmid B., Winzeler M., Keller B. 2011. Transgenic *Pm3b* wheat lines show resistance to powdery mildew in the field. Plant Biotech J. 9: 897–910.

Bujak H., Jurkowski A. 2013. Estimation of winter rye (*Secale cereale* L.) susceptibility to infection by Powdery mildew (*Blumeria graminis* F. sp. *secalis*). Acta Agrobot. 66: 49–54.

Costamilan L. M. 2005. Variability of the wheat powdery mildew pathogen *Blumeria graminis* f. sp. *tritici* in the 2003 crop season. Fitopat Brasil. 30: 420–422.

Hysing S. C., Meker A., Liljeroth E., Koebner R. M. D., Zeller F. J., Hsam S. L. K. 2007. Powdery mildew resistance in 155 Nordic bread wheat cultivars and landraces. Hereditas 144: 102–119.

Jorgensen J. H. 1988. *Erysiphe graminis,* powdery mildew of cereals and grasses. Adv Plant Pathol. 6: 138–157.

Jurkowski A., Bujak H., Nowosad K. 20014a. Analysis of presence of markers STS638, Xgwm356, ResPm4 in rye (*Secale cereale* L.). Jokull 64: 22–33.

Jurkowski A., Bujak H., Nowosad K. 2014b. Estimation of the level of infection by the powdery mildew *Blumeria graminis* f. sp. *secalis* of winter rye *Secale cereale* L. breeding material. Jokull 64: 332–338.

Kowalczyk K., Hsam S. L. K, Zeller F. J. 1998. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.). XI. Cultivars grown in Poland. J Appl Genet. 39: 225–236.

Liatukas Z., Ruzgas V. 2008. Powdery Mildew Resistance of Winter Wheat Cultivars Registered in Lithuania. Zemdirbyste-Agriculture 95: 327–335.

Liatukas Z., Ruzgas V. 2009. Powdery Mildew Resistance of The Lithuanian Winter Wheat Breeding Material. Proc Latvian Acad Sci, Sect B. 63: 37–44.

Melz G., Schlegel R., Thiele V. 1992. Genetic linkage map of rye *(Secale cereale* L.). Theor Appl Genet. 85: 33-45.

Niewoehner A. S., Leath S. 1998. Virulence of *Blumeria graminis* f. sp. *tritici* on winter wheat in the eastern United States. Plant Dis. 82: 64-68.

Parks R., Carbone I., Murphy J. P., Marshall D., Cowger C. 2008. Virulence structure of the

eastern U.S. wheat powdery mildew population. Plant Dis. 92: 1074–1082.

Sawicka Z. 2011. Wpływ mączniaka prawdziwego i rdzy brunatnej na plonowanie żyta ozimego. Prog. Plant Prot. 51: 1193–1197.

Schlegel R., Melz G., Korzun V. 1998. Genes, marker and linkage data of rye (*Secale cereale* L.): 5th updated inventory. Euphytica 101: 23–67.

Spetsov P., Daskalova N., Plamenow D., Moraliyski T. 2013. Resistance to powdery mildew and leaf rust in wheat lines derived from a *Triticum aestivum*/*Aegilops variabilis* cross. Turk J Field Crops. 18: 128–133.

Svec M., Szunics L., Miklovicova M., Slovakowa T., Tisova V., Hauptvogel P. 2002. Identification of Genes for Resistance to Wheat Powdery Mildew in Hungarian, Polish and Slovak Wheat Cultivars. Plant Protect Sci. 38: 64–72.

Tommasini L., Yahiaoui N., Srichumpa P., Keller B. 2006. Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. Theor Appl Genet. 114: 165–175.

Vechet L. 2006. Reaction of winter wheat cultivars and breeding lines to *Blumeria graminis* f. sp. *tritici*. Plant Prot Sci. 42: 15–20.

Wakulinski W., Zamorski C., Nowicki B. 2007. Podatność odmian i linii hodowlanych pszenżyta na porażenie przez *Blumeria graminis* (DC) Speer. Prog Plant Prot. 47: 361–365.

Zhou R., Zhu Z., Kong X., Huo N., Tian Q., Li P., Jin C., Dong Y., Jia J. 2005. Development of wheat near-isogenic lines for powdery mildew resistance. Theor Appl Genet. 110: 640–648.

Zeller F. J., Lutz J., Reimlein E. I., Limpert E., Koenig J. 1993. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). II. French cultivars. Agronomie 13: 201–207.