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RESISTANCE OF TRITICALE HYBRIDS WITH *Pm4b* AND *Pm6* GENES TO POWDERY MILDEW

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Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most important foliar diseases of cereals. Infection by this pathogen on triticale has intensified in Poland in the last few years. In this study we examined resistance to powdery mildew in triticale hybrids possessing resistance genes *Pm4b* and *Pm6* introduced from common wheat. The materials tested were hybrids derived from triticale crosses with common wheat cultivars carrying the desired resistance genes. The presence of the transferred genes was reflected in increased field resistance and shown by the use of molecular markers. The paper discusses the potential introduction of the genes to improve powdery mildew resistance.

Key words: Powdery mildew, *Blumeria graminis*, triticale, resistance genes, *Pm4b*, *Pm6*.

INTRODUCTION

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* Em. Marchal, is one of the most important fungal diseases of wheat, barley and oat. It occurs commonly in many areas of the world with cool, temperate environments. The main reason for the spread of powdery mildew is cultivation of crops with similar or related resistance types over large areas (Srnić et al., 2005). The genetic homogeneity of cultivars, the high adaptability of powdery mildew, transfer by wind over long distances, increased use of pesticides, mutations and recombinations – all these factors have favored the pathogen's evolution into new races with new virulence. Rapid changes in physiological specialization have been noted within the *Blumeria graminis* population. The variability of the powdery mildew population leads to loss of the effectiveness of resistance genes, and the search for new sources of resistance becomes a serious challenge for crop breeding (Zeng et al., 2007). *Blumeria graminis* has high host specialization; each cereal species is infected by very specific forms of powdery mildew. It also creates numerous physiological races with different traits and pathogenicity for only certain host cultivars (Limpert et al., 1987). High genetic diversity and the ability to generate new forms by DNA mutations and recombinations allow the pathogen to overcome resistance and adapt to new environmental conditions (Bayles, 1997). Losses of grain yield caused by powdery mildew can reach up to 30% (Lipps and Madden, 1988). Infection of triticale cultivars by *Blumeria graminis* has intensified in the last few years in Poland. This makes it necessary to identify new resistance sources and to introduce new resistance genes or novel gene combinations effective against the existing pathogen populations.

Using host-pathogen tests, Kowalczyk (data not shown) found that some triticale cultivars can be infected by the wheat pathogen *Blumeria graminis* f.sp. *tritici*. The use of resistant cultivars is the most effective, economical and environmentally safe approach to controlling plant diseases (Bennett, 1984; Ge et al., 1998; Navabi et al., 2004). In triticale breeding, wide crossing is applied to introduce powdery mildew resistance genes, (Strzembicka et al., 2007). Another strategy for improving resistance is to introduce several genes into a single genotype, described as gene pyramidization (Liu et al., 2002).

The *Pm4* locus contains *Pm4a* and *Pm4b* alleles derived from *Triticum dicoccum* and *Triticum carthlicum*, respectively. Both of these genes are on the wheat 2AL chromosome (Rong et al., 2000). Recently the *Pm23* gene from common wheat was

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TABLE 1. Primer sequences for identification of resistance genes *Pm4b* and *Pm6*

Primer	Sequence 5'-3'	Reference
$STS_{-241}F$	CTCATTCTTGTTTTACTTCCTTCAGT	Yi et al., 2008
$STS_{-241}R$	GTCTCGTCTTCAGCATCCTATACA	
$NAU/STS_{BCD135-2}L$	GCTCCCAACCAAGAGAAGAA	Ji et al., 2008
$NAU/STS_{BCD135-2}R$	TCTGTCGGTCCTCTGATGTG	

redesignated *Pm4c* (Hao et al., 2008). The *Pm4b* gene was previously designated *Ml-e* (Heun and Fischbeck, 1987). This gene was one of the most effective, and gave resistance to all populations of *Blumeria graminis* f.sp. *tritici* in Central Europe in the 1989–1992 period (Švec and Miklovicova, 1998).

The *Pm6* gene was transferred from tetraploid wheat *Triticum timopheevii* to common wheat (*Triticum aestivum* L.) through recombination between the B genome of *T. aestivum* and the G genome of *T. timopheevii*, and was mapped on the long arm of chromosome 2B (Rong et al., 2000). *Pm6* gene expression could be recognized in the early development phase, and it is more effective than other genes (Costamilan, 2005).

A very important task in breeding is rapid and accurate identification of genotypes carrying the introduced resistance genes. Powdery mildew resistance genes can be identified by means of host-pathogen tests (Kowalczyk et al., 1998) or by DNA markers (Gupta et al., 1999). The following genes have been mapped using molecular markers: *Pm1e*, *Pm2*, *Pm3g*, *Pm3h*, *Pm3i*, *Pm3j*, *Pm4a*, *Pm4b*, *Pm5d*, *Pm5e*, *Pm 6*, *Pm12*, *Pm13*, *Pm16*, *Pm17*, *Pm22*, *Pm24*, *Pm26*, *Pm27*, *Pm29*, *Pm30*, *Pm31*, *Pm33*, *Pm34*, *Pm35*, *Pm36*, *Pm37*, *Pm38* and *Pm39* (Hao et al., 2008).

Some DNA markers can be used successfully in marker-assisted selection (Tao et al., 2000; Wang et al., 2000; Huang and Röder, 2004; Singrün et al., 2004; Ji et al., 2008). In this study we characterized powdery mildew resistance in triticale (× *Triticosecale* Wittmack) hybrids with introduced resistance genes *Pm4b* and *Pm6* from common wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

Hybrids containing *Pm4b* and *Pm6* genes were obtained by crossing triticale cultivars (Fidelio, Magnat, Lamberto) with wheat cultivars (Meridien, Novalis, Clever, Finezja, Tonacja). Wheat cultivars Meridien and Novalis carry the *Pm4b* gene, and Clever, Finezja and Tonacja contain the gene combination *Pm2+Pm6*. We characterized the powdery mildew resistance of the F_1 and F_2 hybrids phenotypically in field tests as well as by the use of molecular markers.

Field experiments were carried out in the 2007/2008 and 2008/2009 seasons at the Experimental Station in Czesławice. F_1 hybrid kernels were sown in one or two rows per plot, F_2 kernels in four rows per plot. Each line was analyzed in three replicates. During the vegetation period the level of powdery mildew infection was evaluated twice according to COBORU recommendations on a 9-degree scale, where 9 is the most favorable state for agriculture. The obtained data were analyzed statistically with the F test for variance and Tukey's confidence intervals.

The presence of resistance genes in triticale hybrids was confirmed with DNA markers. DNA was extracted by the CTAB method (Aldrich and Cullis, 1993) with modifications. The obtained DNA was resolved in TE buffer (10 mM TrisHCl, pH 8.0; 1 mM EDTA, pH 8.0). Polymerase chain reactions were carried out using a Tprofessional Basic (Biometra) thermal cycler. STS-PCR markers were used to identify resistance genes in the genetic background of the hybrids. The presence of the *Pm4b* gene was analyzed according to the method developed by Yi et al. (2008), and the *Pm6* gene according to Ji et al. (2008). Table 1 gives the sequences of specific primer sets. The reaction products were separated on 1.5% agarose gel with 0.01% ethidium bromide in 1 × TBE buffer. We used GeneRuler 100 bp Plus DNA Ladder (Fermentas) as a marker in electrophoresis.

RESULTS AND DISCUSSION

Field experiments showed that F_1 hybrids were significantly more resistant to powdery mildew than their triticale and wheat parental forms (Tabs. 2, 3). The infection level was highest for the triticale cultivars. Among all the tested hybrids with *Pm4b* introduced, infection was noted only in the Fidelio Novalis cross combination, and it was weak (8.9° on COBORU scale). The other hybrids were totally resistant to *Blumeria graminis* f. sp. *tritici*. Hybrids with the *Pm6* gene showed greater variation. Only the Lamberto × Finezja and the Magnat × Finezja hybrids showed no symptoms of powdery mildew infection. In the other combinations the infection level varied from 8.7° for Fidelio × Finezja to 8.9° for Magnat, Clever and Fidelio × Tonacja.

TABLE 2. Mean values of *Blumeria graminis* f. sp. *tritici* infection for F_1 triticale hybrids with introduced *Pm4b* gene and their parental forms

Hybrid / Cultivar	Powdery mildew infection [9° COBORU scale]
Fidelio × Novalis	8.9[a,b]
Magnat × Novalis	9.0[a,b]
Lamberto × Novalis	9.0[a,b]
Fidelio × Meridien	9.0[a,b]
Magnat × Meridien	9.0[a,b]
Lamberto × Meridien	9.0[a,b]
Fidelio	3.9
Magnat	4.1
Lamberto	3.2
Novalis	7.2
Meridien	7.3

[a] statistically significant difference versus maternal cultivar ($\alpha = 0.05$)
[b] statistically significant difference versus paternal cultivar ($\alpha = 0.05$)

TABLE 3. Mean values of *Blumeria graminis* f. sp. *tritici* infection for F_1 triticale hybrids with introduced *Pm6* gene and their parental forms

Hybrid / Cultivar	Powdery mildew infection [9° COBORU scale]
Fidelio × Clever	8.8[a,b]
Magnat × Clever	8.9[a,b]
Lamberto × Clever	9.0[a,b]
Fidelio × Finezja	8.7[a,b]
Magnat × Finezja	9.0[a,b]
Lamberto × Finezja	9.0[a,b]
Fidelio × Tonacja	8.9[a,b]
Magnat × Tonacja	9.0[a,b]
Lamberto × Tonacja	9.0[a,b]
Fidelio	3.9
Magnat	4.1
Lamberto	3.2
Clever	6.3
Finezja	5.5
Tonacja	6.1

[a] statistically significant difference versus maternal cultivar ($\alpha = 0.05$)
[b] statistically significant difference versus paternal cultivar ($\alpha = 0.05$)

In F_2 progeny, powdery mildew infection of the analyzed forms during the vegetation period was not very high because environmental conditions unfavorable for fungal diseases prevailed: there were warm, dry periods in April and May. The strongest infection of *Blumeria graminis* f. sp. *tritici* was found in the susceptible Lamberto cultivar (4.3° on COBORU scale); the rest of the triticale cultivars showed a slightly lower level of infection (Tab. 4). The analyzed hybrids had significantly lower powdery mildew infection. Most of the plants were not infected, but there were significant differences between hybrids (Tab. 4). Parental wheat cultivars with the *Pm4b* gene had a very low level of infection (Tab. 4). Among all the tested hybrids with the introduced *Pm4b* gene, resistance to powdery mildew was highest for the Fidelio × Novalis cross combination (8.2° on COBORU scale). Infection was strongest (7.84° on COBORU scale) for the Fidelio Meridien combination.

Blumeria graminis f. sp. *tritici* infection was significantly weaker in hybrids with the *Pm6* gene introduced from common wheat than in the triticale cultivars. Most of the plants were not infected, but there were significant differences between hybrids (Tab. 5). Common wheat cultivars with the *Pm2+Pm6* gene combination were characterized by a low level of powdery mildew infection. The one most resistant to this pathogen was Clever (8.52° on COBORU scale).

Crossing triticale genotypes with wheat is the main method of producing lines resistant to powdery mildew. In China, crosses between hexaploid triticale and common wheat produced four triticale lines – Lankao 1, 3, 4 and 5. All of them were shown to be resistant to powdery mildew isolates collected in China and Canada (Li et al., 2007).

Wakuliński et al. (2007) analyzed the powdery mildew susceptibility of 172 triticale genotypes and 169 wheat genotypes derived from Polish plant-breeding companies. The mean level of infection differed significantly between wheat (3.96) and triticale (2.92) on a 6-degree scale where 0 is the absence of infection and 5 is very strong infection. In greenhouse tests they showed that 5.5% of the wheat genotypes and 26% of the triticale genotypes were totally resistant to *Blumeria graminis*. They also field-tested 22 winter triticale cultivars. During that experiment they found that triticale cultivars Disco and Fidelio showed no infection symptoms. The mean level of infection for the rest of the cultivars varied from 0.24 to 1.83.

Strzembicka et al. (2007) described resistance to powdery mildew in triticale hybrids derived from crosses with wild species of the genera *Aegilops* and *Agrotriticum*. The majority of the tested forms were resistant to *Blumeria graminis* races isolated from wheat but were susceptible to races of it isolated from triticale, confirming that new pathogenic races could overcome resistance.

We additionally confirmed the presence of the introduced resistance genes by means of DNA mark-

TABLE 4. *Blumeria graminis* f. sp. *tritici* infection for F_2 triticale hybrids with introduced *Pm4b* gene and their parental forms

Hybrid / Cultivar	Number of plants	Powdery mildew infection range [9° COBORU scale]	Powdery mildew infection mean value [9° COBORU scale]
Fidelio × Novalis	25	3–9	8.20[a]
Magnat × Novalis	33	3–9	8.06[a]
Lamberto × Novalis	44	3–9	7.94[a]
Fidelio × Meridien	25	2–9	7.84[a]
Magnat × Meridien	40	3–9	8.14[a]
Lamberto × Meridien	33	2–9	7.96[a]
Fidelio	30	3–6	4.94
Magnat	30	3–6	4.97
Lamberto	30	2–6	4.30
Novalis	30	7–9	8.20
Meridien	30	6–9	8.00

[a] statistically significant difference versus maternal cultivar (α=0.05)
[b] statistically significant difference versus paternal cultivar (α=005)

TABLE 5. *Blumeria graminis* f. sp. *tritici* infection for F_2 triticale hybrids with introduced *Pm6* gene and their parental forms

Hybrid / Cultivar	Number of plants	Powdery mildew infection range [9° COBORU scale]	Powdery mildew infection mean value [9° COBORU scale]
Fidelio × Clever	45	3–8	7.16[a]
Magnat × Clever	32	3–9	8.12[a]
Lamberto × Clever	28	3–8	7.54[a]
Fidelio × Finezja	34	4–9	8.12[a]
Magnat × Finezja	42	4–9	8.25[a]
Lamberto × Finezja	35	3–8	7.64[a]
Fidelio × Tonacja	36	3–8	7.28[a]
Magnat × Tonacja	29	3–9	7.94[a]
Lamberto × Tonacja	41	3–9	8.15[a]
Fidelio	30	3–6	4.94
Magnat	30	3–6	4.97
Lamberto	30	2–6	4.30
Clever	30	7–9	8.52
Finezja	30	7–9	8.26
Tonacja	30	7–9	8.15

[a] statistically significant difference versus maternal cultivar (α=0.05
[b] statistically significant difference versus paternal cultivar (α=0.05)

ers. PCR amplification with $STS_{-241}F$ and $STS_{-241}R$ primers revealed a 241 bp product in a number of F_2 hybrids of triticale cultivars Fidelio, Magnat and Lamberto with wheat cultivars Meridien and Novalis, which were resistant in field experiments (Fig. 1). That product indicated the presence of *Pm4b* in their genetic background. Yi et al. (2008) designed a method for *Pm4b* gene detection using DNA markers. They developed PCR primers on the basis of a cloned RAPD fragment sequence, and examined F_2 hybrids obtained by crossing the French resistant cultivar VPM carrying *Pm4b* with susceptible cultivar Yumai 13. In VPM and hybrids resistant to powdery mildew they found a 241 bp DNA fragment. They also analyzed linkage between the gene and the developed STS marker and estimated it at 4.9 cM.

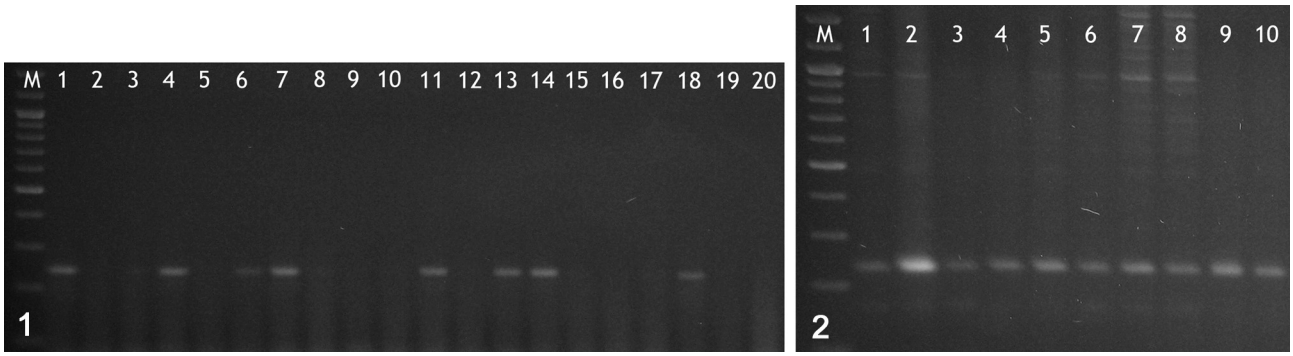

Fig. 1. Electrophoretogram of PCR products obtained with *Pm4*F and *Pm4*R primers for F_2 triticale hybrids. A 241 bp band confirms the presence of *Pm4b* gene. M – GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas), lanes 1–20 – triticale F_2 hybrids from Fidelio × Meridien cross. **Fig. 2.** Electrophoretogram of PCR products obtained with NAU/$STS_{BCD135-2L}$ and NAU/$STS_{BCD135-2R}$ primers for F_2 triticale hybrids. A 230 bp band confirms the presence of *Pm6* gene. M – GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas), lanes 1–10 – triticale F_2 hybrids from Magnat × Finezja cross.

After PCR with the NAU/$STS_{BCD135-2}$L and NAU/$STS_{BCD135-2}$R primer set we found a 230 bp amplification product in a number of F_2 hybrids of triticale cultivars Fidelio, Magnat and Lamberto with wheat cultivars Clever, Finezja and Tonacja (Fig. 2), confirming the presence of the *Pm6* gene in these forms.

Tao et al. (2000) analyzed F_2 hybrids of IGV1-463 derived from the cross combination (PI170914/7*Prins) × Prins. The authors confirmed that the *Pm6* gene is closely linked (1.6 cM) to the *xbcd135* locus, and at 4.8 cM with the *xbcd266* locus. They showed that these markers can be used for selection of genotypes containing *Pm6* in the early steps of wheat development. Ji et al. (2008) converted RFLP marker *Xbcd135* to sequence-tagged sites, and showed that two STS markers, NAU/$STS_{BCD135-1}$ and NAU/$STS_{BCD135-2}$, are closely linked to *Pm6* at 0.8 cM. They also stated that NAU/$STS_{BCD135-2}$ can be used for MAS in cereal breeding programs.

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