



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

KRZYSZTOF KOWALCZYK*, DANIELA GRUSZECKA, MICHAŁ NOWAK,
AND JUSTYNA LEŚNIEWSKA-NOWAK

*Institute of Plant Genetics, Breeding and Biotechnology,
University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland*

Received August 31, 2010; revision accepted January 5, 2011

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 pyramiding (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

* e-mail: krzysztof.kowalczyk@up.lublin.pl

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	TCTGTCCGGTCTCTGATGTG	

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*, *Pm6*, *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 (\times *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 F₁ and F₂ 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. F₁ hybrid kernels were sown in one or two rows per plot, F₂ 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 \times TBE buffer. We used GeneRuler 100 bp Plus DNA Ladder (Fermentas) as a marker in electrophoresis.

RESULTS AND DISCUSSION

Field experiments showed that F₁ 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 \times Finezja and the Magnat \times Finezja hybrids showed no symptoms of powdery mildew infection. In the other combinations the infection level varied from 8.7° for Fidelio \times Finezja to 8.9° for Magnat, Clever and Fidelio \times Tonacja.

TABLE 2. Mean values of *Blumeria graminis* f. sp. *tritici* infection for F₁ 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 ($\alpha = 0.05$)

In F₂ 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

TABLE 3. Mean values of *Blumeria graminis* f. sp. *tritici* infection for F₁ 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 ($\alpha = 0.05$)

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 F₂ 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 ($\alpha=0.05$)

^b statistically significant difference versus paternal cultivar ($\alpha=0.05$)

TABLE 5. *Blumeria graminis* f. sp. *tritici* infection for F₂ 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 ($\alpha=0.05$)

^b statistically significant difference versus paternal cultivar ($\alpha=0.05$)

ers. PCR amplification with STS₂₄₁F and STS₂₄₁R primers revealed a 241 bp product in a number of F₂ 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 F₂ 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.

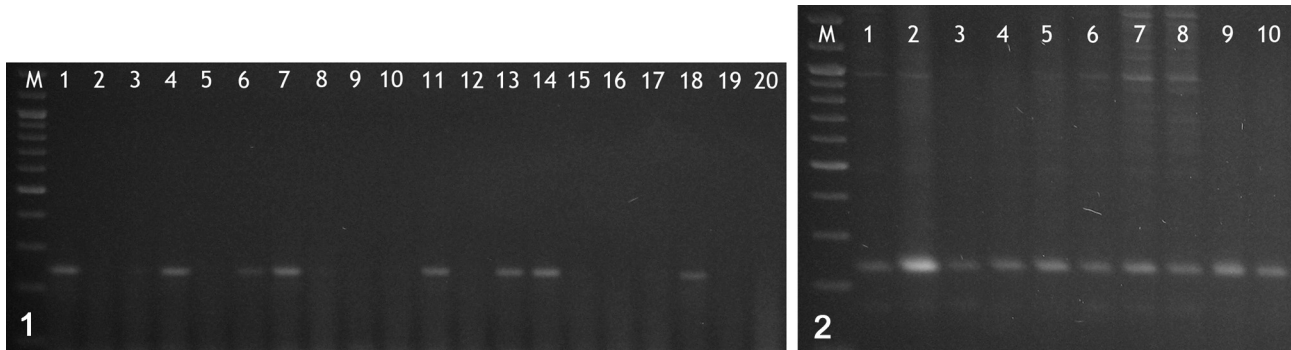


Fig. 1. Electrophoretogram of PCR products obtained with *Pm4F* and *Pm4R* primers for F_2 triticale hybrids. A 241 bp band confirms the presence of *Pm4b* gene. M – GeneRuler™ 100 bp Plus DNA Ladder (Fermentas), lanes 1–20 – triticale F_2 hybrids from Fidelio \times Meridien cross. **Fig. 2.** Electrophoretogram 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™ 100 bp Plus DNA Ladder (Fermentas), lanes 1–10 – triticale F_2 hybrids from Magnat \times Finezja cross.

After PCR with the NAU/STS_{BCD135-2L} and NAU/STS_{BCD135-2R} 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 IG1-463 derived from the cross combination (PI170914/7*Prins) \times 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.

ACKNOWLEDGEMENT

The results reported in this paper were presented at a conference held on September 23–25, 2010, in Szczecin, Poland, organized on the occasion of the 25th anniversary of the University of Szczecin.

REFERENCES

- ALDRICH J, and CULLIS CA. 1993. RAPD analysis in flax: optimization of yield and reproducibility using KlenTaq 1 DNA polymerase, Chelex 100 and gel purification of genomic DNA. *Plant Molecular Biology Reporter* 11(2): 128–141.
- BAYLES R. 1997. Disease development – the interaction of variety resistance and pathogen. *Aspects of Applied Biology* 50: 249–254.
- BENNETT FGA. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. *Plant Pathology* 33: 279–300.
- COSTAMILAN LM. 2005. Variability of the wheat powdery mildew pathogen *Blumeria graminis* f. sp. *tritici* in the 2003 crop season. *Fitopatologia Brasileira* 30: 420–422.
- GE Y, JOHNSON JW, ROBERTS JJ, and RAJARAM S. 1998. Temperature and resistance gene interactions in the expression of resistance to *Blumeria graminis* f. sp. *tritici*. *Euphytica* 99: 103–109.
- GUPTA PK, VARSHNEY RK, SHARMA PC, and RAMESH B. 1999. Molecular markers and their applications in wheat breeding. *Plant Breeding* 118: 369–390.
- HAO Y, LIU A, WANG Y, FENG D, GAO J, LI X, LIU S, and WANG H. 2008. *Pm23*: a new allele of *Pm4* located on chromosome 2AL in wheat. *Theoretical and Applied Genetics* 117: 1205–1212.
- HEUN M, and FISCHBECK G. 1987. Genes for powdery mildew resistance in cultivars of spring wheat. *Plant Breeding* 99: 282–288.
- HUANG XQ, RÖDER MS. 2004. Molecular mapping of powdery mildew resistance genes in wheat: A review. *Euphytica* 137: 203–223.
- Ji J, Qin B, Wang H, Cao A, Wang S, Chen P, Zhuang L, Du Y, Liu D, and Wang X. 2008. STS markers for powdery mildew resistance gene *Pm6* in wheat. *Euphytica* 163: 159–165.
- KOWALCZYK K, HSAM SLK, and ZELLER FJ. 1998. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.) XI. Cultivars grown in Poland. *Journal of Applied Genetics* 39(3): 225–236.
- LI H, CONNER RL, LIU Z, LI Y, CHEN Y, ZHOU Y, SHEN T, CHEN Q, GRAF RJ, and JIA X. 2007. Characterization of wheat-triticale lines resistant to powdery mildew, stem rust, stripe rust, wheat curl mite, and limitation on spread of WSMV. *Plant Disease* 91(4): 368–374.

- LIMPERT E, FELSENSTEIN FG, and ANDRIVON D. 1987. Analysis of virulence in populations of wheat powdery mildew in Europe. *Journal of Phytopathology* 120: 1–8.
- LIPPS PE, and MADDEN LV. 1988. Effect of triadimenol seed treatment and triadimefon foliar treatment on powdery mildew epidemics and grain yield of winter wheat cultivars. *Plant Disease* 72: 887–892.
- LIU Z, SUN O, NI Z, NEVO E, and YANG T. 2002. Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. *Euphytica* 123: 21–29.
- NAVABI A, SINGH RP, TEWARI JP, and BRIGGS KG. 2004. Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. *Crop Science* 44: 1156–1162.
- RONG JK, MILLET E, MANISTERSKI J, and FELDMAN M. 2000. A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP-based mapping. *Euphytica* 115: 121–126.
- SINGRÜN C, HSAM SLK, ZELLER FJ, WENZEL G, and MOHLER V. 2004. Localization of a novel recessive powdery mildew resistance gene from common wheat line RD30 in the terminal region of chromosome 7AL. *Theoretical and Applied Genetics* 109: 210–214.
- SRNIĆ G, MURPHY JP, LYERLY JH, LEATH S, and MARSHALL DS. 2005. Inheritance and chromosomal assignment of powdery mildew resistance genes in two winter wheat germplasm lines. *Crop Science* 45: 1578–1586.
- STRZEMBICKA A, GRUSZECKA D, and GRĄDZIELEWSKA A. 2007. Odporność mieszańców pszenżyta z kozięcami i pszenperzem na rdzę brunatną i żółtą oraz mączniaka prawdziwego. *Zeszyty Problemowe Postępów Nauk Rolniczych* 517: 711–719.
- ŠVEC M, and MIKLOVICOVA M. 1998. Structure of populations of wheat powdery mildew (*Erysiphe graminis* DC f.sp. *tritici* Marchal) in Central Europe in 1993–1996: I. Dynamics of virulence. *European Journal of Plant Pathology* 104: 537–544.
- TAO W, LIU J, LIU D, and CHEN P. 2000. Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. *Theoretical and Applied Genetics* 100: 564–568.
- WAKULIŃKI W, ZAMORSKI C, and NOWICKI B. 2007. Podatność odmian i linii hodowlanych pszenżyta na porażenie przez *Blumeria graminis* (DC) Speer. *Progress in Plant Protection / Postępy w Ochronie Roślin* 47(2): 361–365.
- WANG XY, QI ZJ, MA ZQ, CHEN PD, and LIU DJ. 2000. Identification of RAPD markers tightly linked to wheat powdery mildew resistance gene *Pm6*. *Acta Genetica Sinica* 27(12): 1072–1079.
- YI YJ, LIU HY, HUANG XQ, AN LZ, WANG F, and WANG XL. 2008. Development of molecular markers linked to the wheat powdery mildew resistance gene *Pm4b* and marker validation for molecular breeding. *Plant Breeding* 127: 116–120.
- ZENG Z, FU T, TANG Y, CHEN Y, and REN Z. 2007. Identification and chromosomal locations of novel genes for resistance to powdery mildew and stripe rust in a wheat line 101–103. *Euphytica* 156: 89–94.