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COMBINED USE OF LINKED MARKERS FOR GENOTYPING THE *Pm1* LOCUS IN COMMON WHEAT

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Abstract: Genotyping of 98 wheat cultivars/lines was carried out with molecular markers that are linked to the *Pm1* locus: two bi-allelic (dominant) markers: the sequence-tagged site *Xsts638-7A* and the amplified fragment length polymorphism *XE39M58-77-7A*; and the multi-allelic simple sequence repeat marker *Xgwm344-7A*. Employing segregation data recorded in the population Chinese Spring x Virest (*Pm1e*), genetic mapping revealed that *Xgwm344-7A* and *XE39M58-77-7A* were distally linked to *Pm1e* in the repulsion phase with respective linkage distances of 0.9 cM and 4.8 cM, while *Xsts638-7A* was found to co-segregate with *Pm1e* in the coupling phase. The genotyping results of *Xsts638-7A* and *XE39M58-77-7A* confirmed disease scoring, except for the accessions of cultivars Omega, Remus and Weihenstephan Stamm M1N. The SSR marker *Xgwm344* amplified 15 different fragments ranging from 102 bp to 147 bp, with 15 entries being null-allelic at the 7A and 7B homoeoloci. It was found that wheat lines having resistance alleles at the *Pm1* locus mainly show the null allele at the *Xgwm344-7A* locus. Due to their fast-evolving nature, the use of multi-allelic SSRs for genotype determination may be complicated. However, the combined use of multiple linked marker alleles seems to be a promising approach for genotyping a broad range of plant materials.

Key Words: *Triticum aestivum*, Wheat, Powdery Mildew Resistance, *Pm1*, STS, SSR, AFLP

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Abbreviations used: SSR – simple sequence repeat; AFLP – amplified fragment length polymorphism; STS – sequence tagged site.

INTRODUCTION

Powdery mildew disease caused by the fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a major threat to wheat production, especially in areas with a cool or maritime climate. Up to now, 32 major gene loci conferring resistance to wheat powdery mildew have been detected, mapped to specific chromosomes, and given official designations (*Pm1-Pm32*; [1-3]). Among them, *Pm1* was first identified in the Canadian wheat cultivar Axminster [4] and was allocated to the long arm of chromosome 7A [5]. Extensive studies on *Pm1* resulted in the description of four alleles at this locus [6]. Very recently, Singrün *et al.* [7] used molecular markers to identify the dominant resistance gene in Italian cultivar Virest as belonging to the *Pm1* locus; the gene was designated *Pm1e*.

A great number of molecular markers that can be instrumental in the identification of *Pm1* genotypes is now available [7-9]. However, a large-scaled genotyping of diversified germplasm is required to prove the reliability of molecular markers as a valuable tool in the selection process. Allele-specificity was shown for the AFLP marker *XS11M20-134-7A*, which was exclusively amplified in Weihenstephan Stamm MIN, possessing allele *Pm1c*, and wheat line Blaukorn Weihenstephan, exhibiting the identical response pattern to 104 differential *Bgt* isolates, but not in lines carrying different *Pm1* alleles [8].

In this study, a panel of 98 wheats was genotyped using three molecular markers mapped in the vicinity of *Pm1*: STS (sequence-tagged site) *Xsts638-7A*, AFLP (amplified fragment length polymorphism) *XE39M58-77-7A* and SSR (simple sequence repeat) *Xgwm344-7A*. The main goal was to examine if the combined use of tightly linked marker alleles may increase the efficiency of identifying *Pm1* genotypes.

MATERIALS AND METHODS

Plant material

Ninety-eight cultivars/lines were genotyped with molecular markers that are linked to the *Pm1* locus. Czech cultivars and the varieties Canon, Timmo, Remus, Sicco and Thew were provided by the Research Institute of Crop Production, Prague-Ruzyně, Czech Republic. Seeds of Mexican wheat varieties and Japanese variety Gogatsu Komugi were supplied by CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo). Polish cultivars and the varieties Kris, Slade, Aron, Glockner and Zentos were from the collection of the Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland. The remaining wheat accessions are maintained at the Chair of Agronomy and Plant Breeding, the Technical University of Munich, Germany. Information on pedigrees, resistance genes and alleles was obtained at http://genbank.vurv.cz/wheat/pedigree/gene1_2.asp and http://biodiv.iao.florence.it/proceedings/pen_gib/martynov.PDF. Since *Pm1* was reported to be completely linked to the leaf rust resistance gene *Lr20* and the stem rust

resistance gene *Sr15* [1], data from surveys for leaf and stem rusts was included, in the case of genotypes with no *Pm* gene postulations.

Molecular marker and mapping techniques

DNA was extracted from 7-day-old seedling leaves, essentially following the procedure of Stępień *et al.* [10]. Fluorescent *EcoRI*+*ANN*/*MseI*+*CNN* AFLPs were generated according to Schwarz *et al.* [11]. AFLP marker designation was based on the primer combination used and the fragment size estimated with reference to the internal lane size standard GeneScan-500 ROX (Applied Biosystems). Codes for AFLP primers can be viewed in the standard list for AFLP primer nomenclature (<http://wheat.pw.usda.gov/ggpages/keygene/AFLPs.html>). Detected loci were marked with an 'X', the basic symbol for molecular marker loci of unknown function in wheat. The PCR protocol for wheat SSR marker *Xgwm344* (Tab. 1) was as described in Röder *et al.* [12]. Both molecular marker types were detected on an ABI PRISM 377 sequencer (Applied Biosystems). Fragment size-calling was carried out with GENESCAN analysis software version 3.0, and allele-calling with GENOTYPER DNA fragment analysis software version 2.0 (Applied Biosystems). Primer sequences for marker *Xsts638* were reported in Neu *et al.* [9]. Linkage mapping was performed with the computer programme JOINMAP 3.0 [13] using the Haldane mapping function.

RESULTS

Two bi-allelic markers, *Xsts638-7A* and *XE39M58-77-7A*, and the multi-allelic SSR marker *Xgwm344-7A*, which had been shown to be linked to the *Pm1* locus [7, 9, 14], were chosen to be evaluated in a diverse set of wheat lines. To determine the linkage relationship between the chosen markers and *Pm1*, segregation data recorded in the population Chinese Spring x Virest (*Pm1e*) were used for genetic map construction. Using this data subset, the repulsion phase markers *Xgwm344-7A* and *XE39M58-77-7A* were linked distally to *Pm1e* with respective linkage distances of 0.9 cM and 4.8 cM, while the coupling phase marker *Xsts638-7A* was found to co-segregate with *Pm1e*.

Of 98 cultivars/lines that were tested with markers *Xsts638-7A*, *XE39M58-77-7A* and *Xgwm344-7A*, 22 were supposed to carry the *Pm1* locus. Generally, results of *Xsts638-7A* (amplifying a 542-bp fragment) and *XE39M58-77-7A* (showing a null allele), linked in opposite phases to resistance alleles at the *Pm1* locus, confirmed the disease scoring, except for the accessions of the cultivars Omega, Remus and line Weihenstephan Stamm M1N. The first two genotypes were scored as lacking the *Pm1* locus using both marker systems, whereas the STS marker locus *Xsts638-7A* was not amplified with the DNA of Weihenstephan Stamm M1N, the original source of the *Pm1c* allele (Tab. 1).

SSR marker *Xgwm344* amplified 15 different fragments ranging from 102 bp to 147 bp in the wheat samples tested, with 15 entries being null-allelic at 7A and

Tab. 1. Genotyping of 98 wheat lines using three different markers linked to the *Pm1* locus.

Cultivars/lines	R genes	<i>Xsts638</i>	<i>XE39M58-77</i>	<i>Xgwm344</i>	
				7A	7B
Axminster/8*Cc	<i>Pm1a</i>	+	-	null	null
MocZlatka	<i>Pm1b</i>	+	-	null	null
<i>T. spelta</i> var. <i>duhamelianum</i> TRI 2258	<i>Pm1d</i>	+	-	null	null
Virest	<i>Pm1e</i>	+	-	null	null
Attis	<i>Pm1+2+4b+9</i>	+	-	null	null
Hanno	<i>Pm1+4b+9</i>	+	-	null	null
Helia	<i>Pm1+2+4b+9</i>	+	-	null	null
Hezja	<i>Pm1+3d+4b</i>	+	-	null	null
Normandie	<i>Pm1+2+9</i>	+	-	null	null
Quattro	<i>Pm1+3d+4b</i>	+	-	null	null
Sappo	<i>Pm1+2+4b+9</i>	+	-	null	null
Timmo	<i>Pm1</i>	+	-	null	null
Triso	<i>Pm1+4b+5</i>	+	-	null	null
Troll	<i>Pm1+2+4b</i>	+	-	null	null
Zhengzhou 871124	<i>Pm1</i>	+	-	null	null
Weihenstephan Stamm MIN	<i>Pm1c</i>	-	-	117	102
Canon	<i>Pm1</i>	+	-	null	106
Velos	<i>Pm1+3d</i>	+	-	null	106
Thew	<i>Pm1</i>	+	-	null	106/110
Planet	<i>Pm1+2+4b+9</i>	+	-	127	null
Remus	<i>Pm1+4b+9</i>	-	+	129	null
Omega	<i>Pm1+3d+4b</i>	-	+	127/131	null
Marquis	-	-	+	127	null
Sicco	<i>Pm5</i>	-	+	127	null
Aron	<i>Pm4b</i>	-	+	127	null
Zyta	n.a.	-	+	127	null
Rayon 89	<i>Lr13+34</i>	-	+	127	null
Tui	<i>Lr13+26</i>	-	+	127	null
IGV 455	<i>Pm5d</i>	-	+	127	null
Kolibri	<i>Pm3d</i>	-	+	127	null
Ralle	<i>Pm3d</i>	-	+	127	null
Amor	-	-	+	127	null
Viza	<i>mlviz</i>	-	+	127	null
Sofia	<i>Pm2+4b+8</i>	-	+	129	null
Slade	<i>Pm4b</i>	-	+	129	null
Sparta	<i>Pm2+4b+8</i>	-	+	129	null
Contra	<i>Pm2+4b+5+6</i>	-	+	129	null

Cultivars/lines	R genes	<i>Xsts638</i>	<i>XE39M58-77</i>	<i>Xgwm344</i>	
				7A	7B
Glockner	<i>Pm4b</i>	-	+	129	null
Zentos	-	-	+	129	null
Wilga	<i>Pm4b+8</i>	-	+	129	null
Jasna	<i>Pm3d</i>	-	+	129	null
Polna	<i>Pm2+3d+4b</i>	-	+	129	null
Kris	<i>Pm2+6</i>	-	+	129	null
Mewa	-	-	+	129	null
Ritmo	<i>Pm2+6</i>	-	+	129	null
Moldau	<i>Pm5+mlTa2</i>	-	+	129	null
Olymp	<i>Pm8</i>	-	+	129	null
Tower	<i>Pm2</i>	-	+	129	null
Kontrast	<i>Pm5a</i>	-	+	129	null
Kronjuwel	<i>Pm8</i>	-	+	129	null
Flair	-	-	+	129	null
Toronto	<i>Pm4b+8</i>	-	+	129	null
Siete Cerros 66	<i>Sr6+10+11+7</i>	-	+	129	null
Bacanora 88	<i>Lr26+34</i>	-	+	129	null
Kauz	<i>Lr26</i>	-	+	129	null
Pavon F76	<i>Lr1+10+13+14a+47</i>	-	+	129	null
Gogatsu Komugi	n.a.	-	+	129	null
Hanseat	<i>mlHa2</i>	-	+	129	null
Amigo	<i>Pm17</i>	-	+	129	null
Hussar	<i>Pm2+4b+8</i>	-	+	129	null
Longos	<i>Pm5+6</i>	-	+	129	null
Borneo	<i>Pm4b+5+6</i>	-	+	129	null
W150	<i>Pm3e</i>	-	+	129	null
Khapli/8*Chancellor	<i>Pm4b</i>	-	+	131	null
Moro	<i>Yr10, YrMoro</i>	-	+	131	null
Michigan	<i>Pm3f</i>	-	+	131	null
Amber/8*Chancellor					
Sonora/8*Chancellor	<i>Pm3c</i>	-	+	131	null
RD30	<i>mlRD30</i>	-	+	131	null
Disponent	<i>Pm8</i>	-	+	133	null
Chiyacao	<i>Pm24</i>	-	+	133	null
Chinese Spring	-	-	+	133	null
Chinese Spring (Marquis2B)	-	-	+	133	null
Xiaobaidong	<i>mlxbd (Pm5 allele)</i>	-	+	135	null
Khapli	<i>Pm4b</i>	-	+	137	null
Tercja	<i>Pm4b+6</i>	-	+	145	null
Mona	<i>Pm3d+4b</i>	-	+	146	null
Simona	-	-	+	147	null

Cultivars/lines	R genes	<i>Xsts638</i>	<i>XE39M58-77</i>	<i>Xgwm344</i>	
				7A	7B
Rysa	n.a.	-	+	147	null
Greif	<i>Pm5+6</i>	-	+	147	null
Herzog	<i>Pm4b+8</i>	-	+	147	null
Caribo	<i>Pm8</i> (suppressed)	-	+	147	null
Kanzler	-	-	+	147	null
Lynx	-	-	+	147	null
Mercia	<i>Pm5</i>	-	+	147	null
Trakos	-	-	+	147	null
Irena	n.a.	-	+	127	106
Seri M82	<i>Lr23+26</i>	-	+	127	106
Attila	<i>Lr26</i>	-	+	127	106
Opata	-	-	+	129	106
Baviacora	n.a.	-	+	129	106
Embrapa 16	<i>Lr13</i>	-	+	129	106
Ciano 67	n.a.	-	+	129	106
Roque 73	<i>Lr1+3a+13</i>	-	+	131	106
Sonora 64	<i>Lr1+2a+3</i>	-	+	131	106
Don Ernesto	<i>Lr26</i>	-	+	145	106
Turbo	<i>Pm3d+4b</i>	-	+	127	112
Nourin 61	n.a.	-	+	131	114
Ibis	<i>Pm4b+5</i>	-	+	147	114

n.a. = not available; + = present; - = absent; SSR alleles with sizes of 106 bp, 127 bp, 129 bp, and 133 bp correspond to the ones of 104 bp, 128 bp, 130 bp and 134 bp, respectively, reported in [15].

7B homoeoloci. The majority of lines amplified fragments from a single locus (*Xgwm344-7A*: 66 lines; *Xgwm344-7B*: 3 lines), whereas 14 varieties amplified alleles from both SSR loci. Marker alleles with sizes of 117 bp, 127 bp, 129 bp, 131 bp and 133 bp were recently assigned to chromosome 7A and one of 106 bp to chromosome 7B [7, 14, 15]. Since SSR alleles with sizes of 102 bp, 112 bp and 114 bp were co-amplified with those mapped on chromosome 7A, we assume their location to be on chromosome 7B and, similarly, those with sizes of 145 bp and 147 bp to come from chromosome 7A. After assigning the allele ladders to the respective SSR loci, it is assumed that the allele of 110 bp (cv. Thew) originates from *Xgwm344-7B* and those with sizes of 135 bp (cv. Xiaobaidong) and 137 bp (cv. Khapli) from *Xgwm344-7A*. Affiliation to the *Xgwm344-7A* locus also applies to the 146-bp allele from cv. Mona, provided that a single nucleotide insertion/deletion occurred in the SSR bracketing regions. Based on these observations, we classified the cultivars Omega and Thew to be heterozygous at the 7A and the 7B locus, respectively. Except for cultivar Planet, which displays an allele of 127 bp at the *Xgwm344-7A* locus, all

the *Pm1* cultivars/lines that were properly genotyped with *XE39M58-77-7A* and *Xsts638-7A* exhibited a null allele at *Xgwm344-7A*.

DISCUSSION

Of the 98 wheats tested, 22 were postulated to carry the *Pm1* locus. Twenty of them were lacking the AFLP marker *XE39M58-77-7A* supporting the linkage of the null allele with the *Pm1* locus. In addition, except for line Weihenstephan Stamm M1N, all of them amplified the 542-bp fragment of STS marker *Xsts638-7A*, indicating the lines for which the presence of the *Pm1* locus has been postulated. Both, the AFLP and STS marker genotypes of cultivars Remus and Omega did not confirm the presence of *Pm1* as has been suggested by phenotypic resistance gene analyses. Since the results of both markers support one another, two interpretations are proposed: (a) the accessions of the cultivars Remus and Omega were misclassified with respect to the *Pm1* phenotype; or (b) the seeds used for analyses were mixed up. After examining the pedigrees of the cultivars, we have found the first true for cultivar Omega, whereas the latter may be the case for cultivar Remus. The pedigree of cultivar Omega [Kadett (*Pm3d*, *Pm4b*)/Jara (*Pm3d*)] indicates a lack of a *Pm1* locus, for which reason the marker scores were considered to be correct. Concerning cultivar Remus, the seeds must have been mixed up, since Cultivar Sappo, the putative *Pm1* donor for cultivar Remus [Sappo/Mex//Famos], was properly genotyped (Tab. 1). The line Weihenstephan Stamm M1N, which is known to carry *Pm1c*, displayed a unique haplotype of closely linked markers *Xsts638-7A* and *Xgwm344-7A*, suggesting a diversified evolution of the *Pm1* region compared to lines with other *Pm1* alleles.

SSRs or microsatellites are tandem repeats of short nucleotide sequence motifs (1-6 bp) and ubiquitous in eukaryotic genomes [16]. SSR loci are extremely variable in the number of repeat units among individuals of a given species and can be easily typed via STS PCR [17]. The opportunity to tag highly variable DNA sequence motifs which are evenly spaced throughout the genome as simple PCR markers represented the ideal resource for the development of a marker class to be applied in practical breeding. The wheat SSR marker *Xgwm344* is able to detect homoeoloci on the long arms of chromosomes 7A and 7B, for which the presence of null alleles also have been reported. The prevailing SSR allele at *Xgwm344-7A* had a size of 129 bp and was found in 35 cultivars, followed by the null and the 127-bp alleles that were present in 18 and 17 cultivars, respectively. We also detected alleles with sizes of 131 bp, 133 bp, 145 bp and 147 bp that were present in 9, 4, 2 and 10 cultivars, respectively. The 146-bp allele of cv. Mona belongs to the allele group of either 145 bp or 147 bp. Looking at *Xgwm344-7B*, 81 cultivars exhibited the null allele and 13 entries had the mapped 106-bp allele. SSR marker alleles with sizes of 102 bp, 110 bp, 112 bp and 114 bp were assumed to represent rare alleles due to their very low

frequencies. Referring to the data set, this may also be true for SSR alleles having sizes of 117 bp, 135 bp and 137 bp from *Xgwm344-7A*.

It was found that wheat lines having resistance alleles at the *Pm1* locus mainly show the null allele at *Xgwm344-7A*, which means that the microsatellite locus is not physically present in these lines or was not amplified due to mutation(s) at the primer annealing site(s). Therefore, the occurrence of a *Pm1* carrier with a detectable SSR allele (cv. Planet) suggests that this line may have acquired the SSR locus by a recombination event rather than the locus having been deleted in wheats possessing the *Pm1* region. Due to their fast-evolving nature, the use of multi-allelic SSRs for genotype determination may be complicated. However, in our case, the *Pm1* locus was linked to the null allele of SSR locus *Xgwm344-7A*, making this SSR similar to bi-allelic markers and therefore, reducing the problems associated with allele diversification.

Generally, the combined use of multiple linked markers for genotyping has significant advantages compared to the single marker approach. First, it allows a more meaningful description of the diversification of a genomic region in individual lines and, consequently, the identification of all the existing marker haplotypes. Second, it will be possible to choose markers defining sub-haplotypes that can be used for efficient genotype selection.

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REFERENCES

1. McIntosh, R.A., Hart, G.E., Devos, K.M., Gale, M.D. and Rogers W.J. **Catalogue of gene symbols for wheat.** <http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>, 1998.
2. McIntosh, R.A., Devos, K.M., Dubcovsky, J., Morris, C.F. and Rogers, W.J. (2003) **Catalogue of gene symbols for wheat: 2003 supplement.** <http://wheat.pw.usda.gov/ggpages/wgc/2003upd.html>, 2003.
3. Hsam, S.L.K., Lapochkina, I.F. and Zeller, F.J. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 8. Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. **Euphytica** **133** (2003) 367-370.
4. Pugsley, A.T. and Carter, M.V. The resistance of twelve varieties of *Triticum vulgare* to *Erysiphe graminis tritici*. **Aust. J. Biol. Sci.** **6** (1953) 335-346.
5. Sears, E.R. and Briggles, L.W. Mapping the gene *Pm1* for resistance to *Erysiphe graminis* f. sp. *tritici* on chromosome 7A of wheat. **Crop Sci.** **9** (1969) 96-97.
6. Hsam, S.L.K., Huang, X.Q., Ernst, F., Hartl, L. and Zeller, F.J. Chromosomal location of genes for resistance to powdery mildew in

- common wheat (*Triticum aestivum* L. em Thell.). 5. Alleles at the *Pm1* locus. **Theor. Appl. Genet.** 96 (1998) 1129-1134.
7. Singrün, Ch., Hsam, S.L.K., Hartl, L., Zeller, F.J. and Mohler, V. Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). **Theor. Appl. Genet.** 106 (2003) 1420-1424.
 8. Hartl, L., Mohler, V., Zeller, F.J., Hsam, S.L.K. and Schweizer, G. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). **Genome** 42 (1999) 322-329.
 9. Neu, C., Stein, N. and Keller, B. Genetic mapping of the *Lr20-Pm1* resistance locus reveals suppressed recombination on chromosome arm 7AL in hexaploid wheat. **Genome** 45 (2002) 737-744.
 10. Stępień, Ł., Golka, L. and Chełkowski, J. Leaf rust resistance genes of wheat: identification in cultivars and resistance sources. **J. Appl. Genet.** 44 (2003) 139-149.
 11. Schwarz, G., Herz, M., Huang, X.Q., Michalek, W., Jahoor, A., Wenzel, G. and Mohler, V. Application of fluorescence-based semi-automated AFLP analysis in barley and wheat. **Theor. Appl. Genet.** 100 (2000) 545-551.
 12. Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P. and Ganal, M.W. A microsatellite map of wheat. **Genetics** 149 (1998) 2007-2023.
 13. Van Ooijen, J.W. and Voorrips, R.E. **JOINMAP® 3.0, Software for the calculation of genetic linkage maps**. Plant Research International, Wageningen, The Netherlands, 2001.
 14. Singrün, Ch., Hsam, S.L.K., Zeller, F.J., Wenzel, G. and Mohler, V. Localization of a novel recessive powdery mildew resistance gene from common wheat line RD30 in the terminal region of chromosome 7AL. **Theor. Appl. Genet.** (2004), DOI: 10.1007/s00122-004-1619-7.
 15. Huang, X.Q., Wang, L.X., Xu, M.X. and Röder, M.S. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). **Theor. Appl. Genet.** 106 (2003) 858-865.
 16. Tautz, D. and Renz, M. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. **Nucleic Acids Res.** 12 (1984) 4127-4138.
 17. Weber, J.L. and May, P.E. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. **Am. J. Hum. Genet.** 44 (1989) 388-396.