Genetic mapping of three alleles at the *Pm3* locus conferring powdery mildew resistance in common wheat (*Triticum aestivum* L.)

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Abstract: A set of differential isolates of *Blumeria graminis* f.sp. *tritici* was used to identify 10 alleles at the *Pm3* locus on the short arm of chromosome 1A. Three F_3 populations were used to map *Pm3h* in Abessi, *Pm3i* in line N324, and *Pm3j* alleles in GUS 122 relative to microsatellite markers. In total, 13 marker loci were mapped on chromosome 1AS and 1 marker on 1AL. The order of marker loci in the 3 mapping populations is consistent with previously published maps. All 3 alleles were mapped in the distal region of chromosome 1AS. The present study indicated that microsatellite markers are an ideal marker system for comparative mapping of alleles at the same gene locus in different mapping populations. The linkage distances of the closest microsatellite marker, *Xgwm905–1A,* to *Pm3h*, *Pm3i*, and *Pm3j* were 3.7 cM, 7.2 cM, and 1.2 cM, respectively. The microsatellite marker *Xgwm905–1A* cannot be used to distinguish between *Pm3* alleles. The development of specific markers for individual *Pm3* alleles is discussed on the basis of the recently cloned *Pm3b* allele.

Key words: genetic mapping, marker-assisted selection, microsatellite markers, *Pm3* locus, powdery mildew resistance, *Triticum aestivum*.

Résumé : Une collection d'isolats différentiels du *Blumeria graminis* f.sp. *tritici* a été employée afin d'identifier dix allèles au locus *Pm3* sur le bras court du chromosome 1A. Trois populations F_3 ont été utilisées afin de réaliser la cartographie des allèles suivants de ce locus à l'aide de marqueurs microsatellites : *Pm3h* chez Abessi, *Pm3i* chez N324 et *Pm3j* chez GUS 122. Au total, 13 marqueurs liés étaient situés sur le chromosome 1AS et un marqueur sur le chromosome 1AL. L'ordre des marqueurs chez les trois populations de cartographie était conforme à celui rapporté dans les travaux antérieurs. Les trois allèles ont été situés sur la région distale du chromosome 1AS. Le présent travail montre que les microsatellites constituent un type idéal de marqueur pour la cartographie comparée d'allèles du même locus à l'aide de différentes populations de cartographie. Les distances entre le marqueur le plus rapproché, *Xgwm905-1A*, et les allèles *Pm3h*, *Pm3i* et *Pm3j* étaient respectivement de 3,7 cM, 7,2 cM et 1,2 cM. Le marqueur *Xgwm905-1A* ne peut être employé pour distinguer les allèles de *Pm3*. Le développement de marqueurs spécifiques pour les divers allèles de *Pm3* est discuté sur la base des connaissances acquises avec le clonage récent de l'allèle *Pm3b.*

Mots clés : cartographie génétique, sélection assistée de marqueurs, marqueurs microsatellites, locus *Pm3*, résistance à l'oïdium, *Triticum aestivum*.

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Introduction

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f.sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f.sp. *tritici*), is one of the most devastating diseases of common wheat world-wide and occurs in many areas with a cool or maritime climate. Growing resistant cultivars is the most economical and environmentally safe approach to eliminate the use of fungicides and to reduce production losses due to this disease. To date, 29 gene loci (*Pm1–Pm32*) for resistance to powdery mildew have been identified and located on chromosomes (Hsam et al. 2003; Zeller et al. 2002; Hsam

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Table 1. Segregation analysis of powdery mildew resistance alleles *Pm3h, Pm3i*, and *Pm3j* in F_3 populations from 3 wheat crosses.

Cross	Pathogen isolate no.	Population type and size	F_2 genotypes	$\chi^2_{(1:2:1)}$	*P*
Abessi (*Pm3h*)// Asosan/8*Cc (*Pm3a*)	12	74 F_3 families	24RR:39Rr:11rr	4.78	0.05–0.10
CS/N324 (*Pm3i*)	10	77 F_3 families	21RR:43Rr:13rr	2.71	0.10–0.20
GUS 122 (*Pm3j*)/Ralle (*Pm3d*)	2	82 F_3 families	33RR:40Rr:9rr	14.10	<0.001

and Zeller 2002; Huang and Röder 2004), the dominant resistance gene *Pm3* being located on the short arm of chromosome 1A (McIntosh and Baker 1968).

Briggle (1969) developed near-isogenic lines (NILs) in the genetic background of the susceptible wheat cultivar Chancellor (Cc) carrying the *Pm3a* allele from the Japanese 'Asosan', *Pm3b* from the Russian 'Chul', and *Pm3c* from the Mexican 'Sonora'. Seven more alleles at the *Pm3* locus were reported by Zeller et al. (1993) and Zeller and Hsam (1998). Through monosomic analysis and allelism tests, Zeller et al. (1993) described 3 *Pm3* alleles: *Pm3d* in the German spring wheat 'Kolibri', *Pm3e* in the Australian wheat strain W150, and *Pm3f* in the NIL Michigan Amber/8*Cc. Four alleles, *Pm3g*, *Pm3h*, *Pm3i,* and *Pm3j*, were identified, respectively, in the French wheat 'Aristide'; lines Abessi, whose resistance was derived from an Ethiopian durum wheat; N324 from Nepal; and GUS 122 from Russia (Zeller and Hsam 1998; Sourdille et al. 1999). The traditional method of gene identification using race-specific pathogen isolates becomes more laborious and prohibitive with the increased number of alleles at a gene locus. It is anticipated that molecular markers will enhance the differentiation and use of these alleles in resistance breeding.

Molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA, amplified fragment length polymorphism, and simple sequence repeat (SSR), have been widely used for mapping powdery mildew resistance genes in wheat (Huang and Röder 2004). SSRs, also termed microsatellites, as a type of genetic marker reveal a much higher polymorphism in wheat than any other marker system (Plaschke et al. 1995; Huang et al. 2002). Over 1000 microsatellites from wheat are currently available (Gupta et al. 2002; Guyomarc'h et al. 2002; Huang et al. 2003*a*, 2004; Pestsova et al. 2000; Röder et al. 1998, 2004; Song et al. 2002; Stephenson et al. 1998). Because microsatellites are codominant, PCR based, and amenable to automation, they are most suitable for marker-assisted selection in wheat breeding program (Huang et al. 2000*a*, 2003*b*).

Among the DNA marker systems, only RFLP markers are suitable for comparative genetics of cereal species, because RFLP probes from cereals can often be mapped to all 3 homoeologous wheat chromosomes (e.g., 1A, 1B, or 1D) as well as to syntenic chromosomes of other cereal species (Gale and Devos 1998; Van Deynze et al. 1995). Microsatellites are multiallelic at a given locus, and their polymorphisms are based on size differences between alleles, usually the result of variable numbers of repeat units (Di Rienzo et al. 1994; Huang et al. 2002). Hence, microsatellite markers are suitable for comparative mapping of different alleles at the same gene locus. Here, we report on identification of microsatellite markers linked to 3 alleles, *Pm3h*, *Pm3i*, and *Pm3j*, comparative mapping of these 3 alleles at the *Pm3* locus, and testing the ability of a single microsatellite to distinguish different *Pm3* alleles.

Materials and methods

Plant materials and genomic DNA extraction

The near-isogenic line Asosan/8*Cc (*Pm3a*) was provided by R.A. McIntosh, University of Sydney, Australia. Wheat cultivar Ralle (*Pm3d*) was obtained from K. Brunckhorst, von Lochow-Petkus GmbH, Wetze, Germany. The wheat lines N324 (*Pm3i*) from Nepal, GUS 122 (*Pm3j*) from Russia, and a powdery-mildew-resistant durum line BGRC 2026 from Ethiopia were provided by Genebank Gatersleben (Germany). Abessi (*Pm3h*) is a hexaploid derivative from a hybrid of the durum wheat mentioned earlier and the German spring wheat 'Amor'. F_3 populations were used for linkage analysis between microsatellite markers and the 3 *Pm3* alleles. F_2 plants were selfed to produce F_3 families. Twenty plants of each F_3 family were tested to identify the genotype of corresponding F_2 plants. Table 1 lists crosses and sizes of 3 mapping populations used in this study.

Three nulli-tetrasomics (N1AT1B, N1BT1A, and N1DT1A) and 2 ditelosomics (DT1AS and DT1AL) of Chinese Spring (CS), originally obtained from the late Dr. E.R. Sears, University of Missouri, USA, were used for chromosome arm assignments of SSR markers.

Total genomic DNA was extracted from young leaf tissue and frozen in liquid nitrogen, as previously described by Huang et al. (2000*b*). The DNA was diluted to a concentration of 5–10 ng/μL before microsatellite analysis.

Powdery mildew resistance tests

B. graminis f.sp. *tritici* (*Bgt*) isolates avirulent to the parents carrying *Pm3h*, *Pm3i,* and *Pm3j* and virulent to their crossing partners were used to test 3 mapping populations (Table 1). Powdery mildew resistance tests on F_3 families of the mapping populations were conducted on primary leaf segments cultured on 6 g agar/L and 35 mg benzimidazole/L in plastic boxes in a growth chamber. The methods of inoculation, conditions of incubation, and disease assessment were carried out according to Huang et al. (1997). Based on the reaction of F_3 families to powdery mildew, the genotypes of the corresponding F_2 individual plants were determined, i.e., homozygous resistant (*RR*), heterozygous resistant (*Rr*), and homozygous susceptible (*rr*). Chi-squared tests for goodness of fit were used to test for the deviation of observed data from theoretically expected segregation ratios.

Microsatellite analysis

A total of 20 microsatellite markers for the short arm of chromosome 1A were used for checking polymorphism between the 2 parents of each mapping population. One gdm and 14 gwm microsatellite markers were mapped on wheat

Table 2. Differential reactions of 10 wheat cultivars and (or) lines possessing powdery mildew resistance alleles at the *Pm3* locus after inoculation with 13 isolates of *Blumeria graminis* f.sp. *tritici*.

	Blumeria graminis f.sp. *tritici* isolate													
Cultivar and (or) line	2	5	6	9	10	12	13	14	15	16	17	42	90	*Pm*
'Asosan'/8*Cc[a]	r	s	r	r	r	s	r	r	s	s	r	i	r	*3a*
'Chul'/8*Cc	r	s	s	r	r	r	r	r	s	r	i,s	s	r	*3b*
'Sonora'/8*Cc	r	s	s	i	r	s	r	i,s	s	s	s	s	s	*3c*
Ralle	s	s	s	r	s	r	s	r	r	s	r	s	r	*3d*
W150	s	i,s	i,s	i	r	i,s	r	r,s	s	s	s	s	i	*3e*
Mich. Amb./8*Cc	r	s	s	s	r	s	r	i,s	s	s	s	i,s	s	*3f*
'Aristide'	r	i	s	r	s	i	s	i	r	s	s	s	i	*3g*
Abessi	r	r	r,i	r	r	r	i	r	r	i	r	i	i	*3h*
N324	r	r,i	i	r	r	r	r	r	r	i,s	i	r	r	*3i*
GUS 122	r	r	r	r	r	r	r	r	r	r	i	s	r	*3j*

Note: r, resistant; s, susceptible; i, intermediate.
[a]Seven times backcrossed to 'Chancellor'.

Table 3. Annealing temperatures (T_m) and fragment sizes (bp) of polymorphic microsatellite markers from chromosome 1AS in 3 mapping populations.

Locus	T_m (°C)	Abessi//Asosan/8*Cc	CS/N324	GUS 122/Ralle
Xbarc148	55	198/202	204/198	194/198
Xbarc263	55	204	236/204	204
Xcfd59b	60	null/276	276	276/282
Xgwm164	55	122/116	116/118	118/122
Xgwm357	55	119/115	117/119	119
Xgwm691	60	153/null	151	147/151
Xgwm752	55	127/117	129/127	115/127
Xgwm791	60	null/173	179/171	167/null
Xgwm905	55	252/264	248/258	244/252
Xgwm1097	60	147/151	149/159	147
Xgwm1104	50	194	168/194	166/194
Xgwm1111	55	144/146	149/141	146/141
Xgwm1148	60	140/null	129/null	null/141
Xwmc24	60	140	158/140	140

chromosome 1A in the International Triticeae Mapping Initiative population (Pestsova et al. 2000; Röder et al. 1998, 2004); 2 microsatellite primer pairs gwm1097 and gwm1148 were provided by Dr. M. Ganal, TraitGenetics GmbH, Am Schwabeplan 1b, 06466 Gatersleben, Germany. Two microsatellite primer pairs cfd21 and cfd59 (Guyomarc'h et al. 2002) were provided by Dr. P. Sourdille, INRA-UBP, Clermont-Ferrand, France. Two microsatellite primer pairs barc148 and barc263 (Song et al. 2002) were available from the website http://www.scabusa.org/research_bio.html. One microsatellite primer pair wmc24 was available from Gupta et al. (2002).

Forward primers of all primer pairs were labelled using Cy5. The PCR was performed according to Röder et al. (1998) and Huang et al. (2003*b*). Microsatellite fragments were detected on an automated laser fluorescence express sequencer and analysed using the computer program Fragment Analyser Version 1.02 (Amersham Biosciences, Freiburg, Germany) by comparison with internal size standards (Huang et al. 2002, 2003*b*).

Linkage analysis

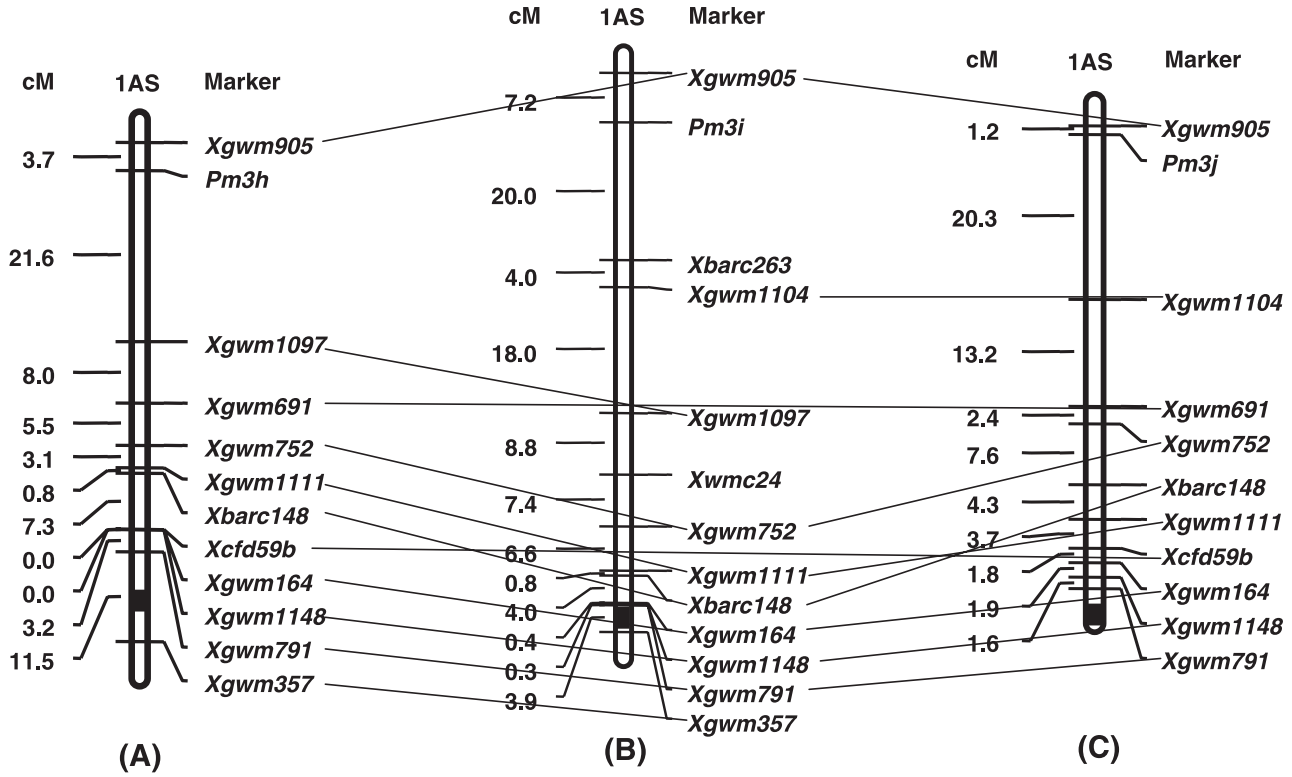
The genetic linkage map was constructed by means of MAPMAKER/Exp version 3.0b (Lander et al. 1987). The commands "group" and "ripple", and LOD scores ≥ 3.0 were used to develop the linkage map. The Kosambi mapping function (Kosambi 1944) was used to convert recombination fractions into centimorgans (cM) as map distances.

Results

Segregation of resistance alleles *Pm3h*, *Pm3i*, and *Pm3j* in 3 mapping populations

A set of 13 *Bgt* isolates was used to characterize the resistant lines Abessi, N324, and GUS 122 in comparison with cultivars or lines carrying different powdery mildew resistance alleles at the *Pm3* locus. All cultivars or lines exhibited patterns of disease reaction distinguishable from one another (Table 2). For the molecular mapping of the resistance alleles *Pm3h* in line Abessi, *Pm3i* in line N324, and *Pm3j* in line GUS 122, the 3 sets of F_3 populations were tested with *Bgt* isolate no. 12 avirulent to Abessi (*Pm3h*) and virulent to Asosan/8*Cc (*Pm3a*), no. 10 avirulent to N324 (*Pm3i*) and virulent to CS (no known *Pm* gene), and no. 2 avirulent to GUS 122 (*Pm3j*) and virulent to Ralle (*Pm3d*), respectively. The observed segregation of homozygous resis-

Fig. 1. Microsatellite linkage maps involving the alleles *Pm3h* (A), *Pm3i* (B), and *Pm3j* (C) of chromosome 1AS. Locus names are indicated on the right side of the map. Kosambi map distances (cM) are shown on the left side. The centromeres are indicated in black. Lines connect common SSR (simple sequence repeat) markers.



tant (*RR*), heterozygous resistant (*Rr*), and homozygous susceptible (*rr*) families from the crosses CS/N324 and Abessi//Asosan/8*Cc (Table 1) fitted a *RR*:*Rr*:*rr* ratio of 1:2:1, whereas the observed segregation in the cross GUS 122/Ralle deviated from the expected ratio of 1:2:1 (χ^2 = 14.10, $P < 0.001$). For genetic mapping, distorted segregation does not affect the recombination values estimated between loci (Singrün et al. 2003).

Genetic mapping of *Pm3h*, *Pm3i*, and *Pm3j*

Twenty microsatellite markers that were mapped on the short arm of chromosome 1A (Röder et al. 1998, unpublished data; Gupta et al. 2002) were used for surveying polymorphism between the parental lines of the 3 mapping populations. Eleven (55%), 12 (60%), and 10 (50%) polymorphic markers were available for mapping *Pm3h*, *Pm3i*, and *Pm3j* on chromosome 1AS in the hybrids of Abessi//Asosan/8*Cc, CS/N324, and GUS 122/Ralle, respectively. The fragment sizes of polymorphic microsatellite markers amplified from the DNA of the parents of the 3 mapping populations are listed in Table 3. Most of the microsatellite markers were inherited in a co-dominant manner. Four microsatellite markers, *Xcfd59b*, *Xgwm691*, *Xgwm791*, and *Xgwm1148,* revealed dominant inheritance in at least 1 population with the single fragment present in 1 parent but absent in the other (Table 3). Segregation of all microsatellite markers in the population Abessi//Asosan/8*Cc conformed to the expected ratios of 1:2:1 for co-dominant markers and 3:1 for dominant markers. However, microsatellite markers *Xgwm357* and *Xgwm1097* did not fit the expected segregation ratio of 1:2:1 ($\chi^2 > 5.99$, df = 2, $P < 0.05$) in the population CS/N324, and the ratios for the 3 microsatellite markers *Xgwm691*, *Xgwm905,* and *Xgwm1104* deviated significantly from the expected segregation ratio of 1:2:1 ($\chi^2 > 5.99$, df = 2, $P < 0.05$) in the population GUS 122/Ralle.

The genetic maps are shown in Fig. 1. Three alleles, *Pm3h*, *Pm3i*, and *Pm3j*, are located between the markers *Xgwm905* and *Xgwm1097*, *Xgwm905* and *Xbarc263*, and *Xgwm905* and *Xgwm1104*, respectively. The linkage distance of the closest marker, *Xgwm905,* to *Pm3h*, *Pm3i*, and *Pm3j* was 3.7 cM, 7.2 cM, and 1.2 cM, respectively. In order to determine the position of the centromere, DNA from CS, 3 nulli-tetrasomics (N1AT1B, N1BT1A, and N1DT1A), and 2 ditelosomics (DT1AS and DT1AL) of CS was amplified with primer pairs of the mapped SSR markers. The centromere was located between the markers *Xgwm791* and *Xgwm357*. Alleles *Pm3h*, *Pm3i*, and *Pm3j* were mapped in the distal region of chromosome 1AS. The genetic distances of *Pm3h*, *Pm3i*, and *Pm3j* to the centromere were approximately 55.0 cM, 70.3 cM, and 56.8 cM, respectively.

Marker validation

In order to identify whether the most closely linked microsatellite marker *Xgwm905* is suitable for marker-assisted selection of *Pm3* alleles, 4 susceptible cultivars and 37 resistant cultivars or lines with known powdery mildew resistance genes or gene combinations were genotyped (Table 4). SSR marker *Xgwm905* amplified 13 fragments ranging from 236 bp to 264 bp in the wheat samples tested, 17

Table 4. Fragment sizes of 41 wheat cultivars and lines with or without known powdery mildew resistance gene(s) after amplification using SSR (simple sequence repeat) primer pair gwm905.

Cultivar and (or) line	Resistance gene/allele	Fragment size (bp)
'Chinese Spring'	—	248
Amor	—	null
Kanzler	—	236
Trakos	—	null
Axminster/8*Cc	*Pm1a*	null
MocZlatka	*Pm1b*	null
M1N	*Pm1c* (*Pm18*)	252
T. spelta var. *duhamelianum*	*Pm1d*	242
Virest	*Pm1e* (*Pm22*)	238
Ulka/8*Cc	*Pm2*	null
'Asosan'/8*Cc	*Pm3a*	264
'Chul'/8*Cc	*Pm3b*	252
'Sonora'/8*Cc	*Pm3c*	null
'Kolibri'	*Pm3d*	252
W150	*Pm3e*	240
Michigan Amber/8*Cc	*Pm3f*	246
'Aristide'	*Pm3g*	244
Abessi	*Pm3h*	252
N324	*Pm3i*	258
GUS 122	*Pm3j*	244
Khapli/8*Cc	*Pm4a*	null
Armada	*Pm4b*	254
Hope	*Pm5a*	256
Selpek	*Pm5a*	null
Kormoran	*Pm5b*	null
IGV 455	*Pm5d*	null
Fuzhuang 30	*Pm5e*	240
Xiaobaidong	*mlxbd* (*Pm5* allele)	240
TP 114	*Pm6*	null
Disponent	*Pm8*	256
Zecoi-4	*Pm16*	250
Amigo	*Pm17*	null
XX186	*Pm19*	null
TAM104-T6BS·6RL	*Pm20*	null
Yangmai 5 line	*Pm21*	256
Chiyacao	*Pm24*	254
Herzog	*Pm4b+Pm8*	null
Piko	*Pm5a+Pm6*	256
Normandie	*Pm1a+Pm2+Pm9*	null
Ritmo	*Pm2+Pm5a+Pm6*	254
Apollo	*Pm2+Pm4b+Pm8*	null

entries being null-allelic. The 252-bp marker fragment was present in Chul/8*Cc (*Pm3b*), Kolibri (*Pm3d*), Abessi (*Pm3h*), and M1N (*Pm1c*). The 244-bp allele was found in Aristide (*Pm3g*) and GUS 122 (*Pm3j*), whereas the 240-bp allele was shared by W150 (*Pm3e*), Fuzhuang 30 (*Pm5e*), and Xiaobaidong (*mlxbd*, an undesignated *Pm5* allele, Huang et al. 2000*c*). The null allele was observed in Sonora/8*Cc (*Pm3c*) and many wheat cultivars with or without known *Pm* gene(s). Only lines Asosan/8*Cc (*Pm3a*), Michigan Amber/8*Cc (*Pm3f*), and N324 (*Pm3i*) showed unique alleles at SSR locus *Xgwm905*. Thus the microsatellite marker *Xgwm905* could not differentiate between all 10 *Pm3* alleles, nor could it differentiate between *Pm3* alleles and other known *Pm3* genes.

Discussion

Ten alleles (*Pm3a–Pm3j*) have been identified at the *Pm3* locus on the short arm of chromosome 1A. Mapping of *Pm3* alleles in wheat has been accomplished using morphological traits, biochemical markers, and different types of molecular markers. Briggle and Sears (1966) mapped *Pm3c* in cultivar Indian 0.8 crossover units from the *Hg* (hairy glume) locus, whereas *Pm3a* in 'Asosan' was 4.8 ± 0.5 cM from *Hg* (McIntosh and Baker 1968). The biochemical marker *XGli-A5*, coding a gliadin protein, mapped 5.2 cM from *Pm3g* on chromosome 1AS of 'Courtot' (Sourdille et al. 1999). Two RFLP markers, *Xwhs179* and *Xbcd1434*, were linked to the *Pm3* locus. *Xwhs179* was mapped 3.3 ± 1.9 cM from *Pm3b* (Hartl et al. 1993), whereas *Xbcd1434* was mapped 1.3 cM from *Pm3b* (Ma et al. 1994). The cloning of *Mla1*, an allele at the *Mla* locus of powdery mildew resistance on barley chromosome 1HS, allowed mapping of the *Mla* orthologue in wheat at 0.7 cM proximal to *Pm3b* (Zhou et al. 2001). More recently, a high-resolution genetic map involving *Pm3b* in 'Chul'/8*Cc was constructed, and *Pm3b* was mapped to an interval of 0.97 cM (Yahiaoui et al. 2004).

In the present study, the chromosomal locations of the 3 alleles *Pm3h*, *Pm3i*, and *Pm3j* at the *Pm3* locus were confirmed using microsatellite markers. The closest marker was *Xgwm905*, at 3.7 cM, 7.2 cM, and 1.2 cM from *Pm3h*, *Pm3i*, and *Pm3j*, respectively. The integrated genetic maps (Fig. 1) combine microsatellite markers previously mapped in 3 wheat maps (Gupta et al. 2002; Röder et al. 1998, unpublished data; http://www.scabusa.org/research_bio.html). The marker orders of the genetic maps involving the 3 *Pm3* alleles are consistent with those of the earlier maps. Only marker *Xbarc148*, which is located distal to *Xgwm1111* in crosses CS/N324 and Abessi//'Asosan'/8*Cc, was mapped proximal to *Xgwm1111* in population GUS 122/Ralle, suggesting an inversion event involved in GUS 122/Ralle. *Xcfd59* was isolated from *Triticum tauschii* (the D-genome donor of common wheat, Guyomarc'h et al. 2002), but it has loci for the A, B, and D genomes. The locus *Xcfd59a* was mapped on chromosome 1DS (Huang and Röder 2003) and the locus *Xcfd59b* mapped to the homoeologous position of chromosome 1AS (Fig. 1A and 1C). The genetic distances of markers are different in the 3 mapping populations, implying that recombination frequencies are different in the 3 populations mapped. For instance, the genetic distance between *Xgwm791* and *Xgwm357* is 3.9 cM in the population CS/N324 and 11.5 cM in the population Abessi//'Asosan'/8*Cc (Fig. 1). The present study shows that microsatellite markers are ideal for comparative mapping of alleles at the same gene locus.

Microsatellite markers are most suitable for marker-assisted selection, especially for pyramiding 2 or more resistance genes into a single wheat genotype (Huang and Röder 2004). The 204-bp allele of the microsatellite marker *Xgwm337* was found to be specific and diagnostic for the powdery mildew resistance gene *Pm24* on chromosome 1DS (Huang et al. 2000*a*) and is currently used for selection of *Pm24* in breeding programs in Germany. The results in the

present study indicate that it is difficult for linked microsatellite markers to display a specific fragment for each allele at a gene locus with multiple alleles. The closest microsatellite marker, *Xgwm905*, produced 7 fragment alleles (including 1 null-allele) for 10 *Pm3* alleles and could not be used for distinguishing between all *Pm3* alleles or between *Pm3* alleles and other *Pm* genes (Table 4). Similar results were also reported for *Pm5* alleles (Huang et al. 2003*b*). However, it is possible to use *Xgwm905* for combining *Pm3* alleles with other *Pm* genes, for example, *Pm3j* and *Pm24* or *Pm3h* and *Pm5e,* into 1 genotype.

Allele-specific markers must be developed from the resistance allele itself. Small insertion–deletions (indels), even single nucleotide changes in the coding regions of a gene, can lead to functional specificities of different alleles at the same gene locus. Büschges et al. (1997) reported that 8 single nucleotide polymorphisms (SNPs) and 3 small deletions were identified in 11 γ-ray- or EMS (ethyl methane sulfonate) induced *mlo* mutant alleles compared with the *Mlo* wild-type gene. Recently, *Pm3b* was isolated by map-based cloning strategy. A single base deletion in the coding region was detected in an EMS-induced susceptible *Pm3b* mutant, resulting in the loss of function (Yahiaoui et al. 2004). Provided that variability of alleles at the *Pm3* locus is encoded by a single gene, the cloning of *Pm3b* may make it possible to develop diagnostic SNP (single nucleotide polymorphism) markers for marker-assisted selection of *Pm3* alleles in powdery mildew resistance breeding programs. Using gene-specific primers for *Pm3b* and genomic DNA from lines with different *Pm3* alleles as a template for PCR amplification, SNPs and InDels may be detected in the amplified fragments. Understanding the molecular structure may also allow us to elucidate resistance specificities and functions of the different alleles at the *Pm3* locus.

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References

Briggle, L.W. 1969. Near-isogenic lines of wheat with genes for resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci. **9**: 70–72.

Briggle, L.W., and Sears, E.R. 1966. Linkage of resistance to *Erysiphe graminis* f. sp. *tritici* (*Pm3*) and hairy glume (*Hg*) on chromosome 1A of wheat. Crop Sci. **6**: 559–561.

Büschges, R., Hoollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., et al. 1997. The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell, **88**: 695–705.

Di Rienzo, A., Peterson, A.C., Garza, J.C., Valdes, A.M., Slatkin, M., and Freimer, N.B. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. U.S.A. **91**: 3166–3170.

Gale, M.D., and Devos, K.M. 1998. Comparative genetics in the grasses. Proc. Natl. Acad. Sci. U.S.A. **95**: 1971–1974.

Gupta, P.K., Balyan, H.S., Edwards, K.J., Isaac, P., Korzun, V., Röder, M. et al. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor. Appl. Genet. **105**: 413–422.

Guyomarc'h, H., Sourdille, P., Charmet, G., Edwards, K.J., and Bernard, M. 2002. Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor. Appl. Genet. **104**: 1164–1172.

Hartl, L., Weiss, H., Zeller, F.J., and Jahoor, A. 1993. Use of RFLP markers for the identification of alleles at the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **86**: 959–963.

Hsam, S.L.K., and Zeller, F.J. 2002. Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). *In* The powdery mildews, a comprehensive treatise. *Edited by* R.R. Belanger, W.R. Bushnell, A.J. Dik, and T.L.W. Carver. APS Press, St. Paul, Minn. pp. 219–238.

Hsam, S.L.K., Lapochkina, I.F., and Zeller, F.J. 2003. 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**: 367–370.

Huang, X.Q., and Röder, M.S. 2003. High-density genetic and physical mapping of the powdery mildew resistance gene *Pm24* on chromosome 1D of wheat. Proceedings of the 10th International Wheat. *Edited by* H.N. Pogna, M. Romano, E.A. Pogna, and G. Galterio. Paestum, Italy. pp. 961–964.

Huang, X.Q., and Röder, M.S. 2004. Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica, **137**: 203–223.

Huang, X.Q., Hsam, S.L.K., and Zeller, F.J. 1997. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.) IX. Cultivars, landraces and breeding lines grown in China. Plant Breed. **116**: 233–238.

Huang, X.Q., Hsam, S.L.K., Zeller, F.J., Wenzel, G., and Mohler, V. 2000*a*. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor. Appl. Genet. **101**: 407–414.

Huang, X.Q., Zeller, F.J., Hsam, S.L.K., Wenzel, G., and Mohler, V. 2000*b*. Chromosomal location of AFLP markers in common wheat (*Triticum aestivum* L.) utilizing nulli-tetrasomic stocks. Genome, **43**: 298–305.

Huang, X.Q., Hsam, S.L.K., and Zeller, F.J. 2000*c*. Chromosomal location of two novel genes for resistance to powdery mildew in Chinese landraces (*Triticum aestivum* L. em. Thell.). J. Genet. Breed. **54**: 311–317.

Huang, X.Q., Börner, A., Röder, M.S., and Ganal, M.W. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. **105**: 699–707.

Huang, X.Q., Cöster, H., Ganal, M.W., and Röder, M.S. 2003*a*. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **106**: 1379–1389.

Huang, X.Q., Wang, L.X., Xu, M.X., and Röder, M.S. 2003*b*. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **106**: 858–865.

Huang, X.Q., Kempf, H., Ganal, M.W., and Röder, M.S. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **109**: 933–943.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. **12**: 172–175.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. 1987. MAPMAKER: an interac-

tive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174–181.

Ma, Z.Q., Sorrells, M.E., and Tanksley, S.D. 1994. RELP markers linked to powdery mildew resistance genes *Pm1, Pm2, Pm3*, and *Pm4* in wheat. Genome, **37**: 871–875.

McIntosh, R.A., and Baker, E.P. 1968. Chromosome location and linkage studies involving the *Pm3* locus for powdery mildew resistance in wheat. Proc. Linn. Soc. N.S.W. **93**: 232–238.

Pestsova, E., Ganal, W.M., and Röder, M.S. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome, **43**: 689–697.

Plaschke, J., Ganal, M.W., and Röder, M.S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. **91**: 1001–1007.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., and Ganal, M.W. 1998. A microsatellite map of wheat. Genetics, **149**: 2007–2023.

Röder, M.S., Huang, X.Q., and Ganal, M.W. 2004. Wheat microsatellites in plant breeding — potential and implications. *In* Biotechnology in agriculture and forestry, Vol. 55, Molecular marker systems. *Edited by* H. Lörz and G. Wenzel. Springer Verlag, Heidelberg, Germany. pp. 255–266.

Singrün, C., Hsam, S.L.K., Hartl, L., Zeller, F.J., and Mohler, V. 2003. 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**: 1420–1424.

Song, Q.J., Fickus, E.W., and Cregan, P.B. 2002. Characterization of trinucleotide SSR motifs in wheat. Theor. Appl. Genet. **104**: 286–293.

Sourdille, P., Robe, P., Tixier, M.-H., Doussinault, G., Pavoine, M.-T., and Bernard, M. 1999. Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. Euphytica, **110**: 193–198.

Stephenson, P., Bryan, G., Kirby, J., Collins, A., Devos, K., Busso, C., and Gale, M. 1998. Fifty new microsatellite loci for the wheat genetic map. Theor. Appl. Genet. **97**: 946–949.

Van Deynze, A.E., Dubcovsky, J., Gill, K.S., Nelson, J.C., Sorrells, M.E., Dvorák, J. et al.. 1995. Molecular-genetic maps for group 1 chromosomes of *Triticeae* species and their relation to chromosomes in rice and oat. Genome, **38**: 45–59.

Yahiaoui, N., Srichumpa, P., Dudler, R., and Keller, B. 2004. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J. **37**: 528–538.

Zeller, F.J., and Hsam, S.L.K. 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). Proceedings of the 9th International Wheat Genetics Symposium. *Edited by* A.E. Slinkard. University Extension Press, Saskatoon, Sask. pp. 178–180.

Zeller, F.J., Lutz, J., and Stephan, U. 1993. Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.) 1. *Mlk* and other alleles at the *Pm3* locus. Euphytica, **68**: 223–229.

Zeller, F.J., Kong, L., Hartl, L., Mohler, V., and Hsam, S.L.K. 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 7. Gene *Pm29* in line Pova. Euphytica, **123**: 187–194.

Zhou, F., Kurth, J., Wei, F., Elliot, C., Valè, G., Yahiaoui, N. et al. 2001. Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. Plant Cell, **13**: 337–350.