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ORIGINAL PAPER

Pm23: a new allele of *Pm4* located on chromosome 2AL in wheat

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Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the major diseases of common wheat (*Triticum aestivum*) worldwide. The powdery mildew resistance gene *Pm23*, identified in the common wheat Line 81-7241 and originally assigned to wheat chromosome 5A, was relocated on chromosome 2AL with the aid of molecular markers. Mapping of microsatellite markers in two wheat crosses segregating for *Pm23* and *Pm4b*, respectively, in combination with the reported mapping of *Pm4a*, indicated that the three genes were all linked to the marker *Xgwm356* with a distance of 3–5 cM. Allelism between *Pm4b* and *Pm23* was then confirmed, when the progenies of a cross between VPM1 (*Pm4b*) and Line 81-7241, were shown to be all resistant to a *B. graminis* isolate avirulent to the both parents. *Pm23* is therefore a new allele of the *Pm4* locus, and was redesignated as *Pm4c*.

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Introduction

Powdery mildew of commom wheat (*Triticum aestivum* L.), caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the major diseases of wheat worldwide, especially in highly productive areas with a maritime or semi-continental climate (Bennett 1984). Although fungicides controlling powdery mildew are available, utilization of resistant cultivars is the most efficient and environmentally friendly approach to control this disease, as long as sources of resistance are available (Hulbert et al. 2001).

Currently, more and more powdery mildew resistance genes have been identified from the wild relatives or distantly related species of wheat, and some of them have been transferred into bread wheat. Until now, 52 formally designated *Pm* resistance genes mapped at 36 loci (*Pm1*-*Pm39*), including three recessive genes (*Pm5*, *Pm9*, *Pm26*), have been reported and located on various chromosomes (McIntosh et al. 2003, 2007; Perugini et al. 2008; Spielmeyer et al. 2008; Lillemo et al. 2008). Apart from the *Pm* named genes, several tentatively designated genes, for example *mlRD30*, *PmU*, *Mlm2033* and *Mlm80*, *MlIW72*, were also reported recently (Singrün et al. 2004; Qiu et al. 2005; Yao et al. 2007; Ji et al. 2008).

Molecular markers have been utilized successfully in mapping powdery mildew resistance genes in wheat (Michelmore 1995). Microsatellites, also termed simple sequence repeats (SSRs), have the advantages of being easy to handle, inexpensive and reliable. Moreover microsatellites reveal much higher polymorphisms in wheat compared with other marker system, and are now being preferentially used for genetic mapping (Landjeva et al. 2007). So far, the resistance genes *Pm1e* (Singrün et al. 2003), *Pm2* (Qiu et al. 2006), *Pm3g* (Bougot et al.

2002), *Pm3h*, *Pm3i*, *Pm3j* (Huang et al. 2004), *Pm4a* (Ma et al. 2004), *Pm5d* (Nematollahi et al. 2008), *Pm5e* (Huang et al. 2003), *Pm12* (Song et al. 2007), *Pm16* (Chen et al. 2005b), *Pm24* (Huang et al. 2000), *Pm27* (Järve et al. 2000), *Pm30* (Liu et al. 2002), *Pm31* (Xie et al. 2003), *Pm33* (Zhu et al. 2005), *Pm34* (Miranda et al. 2006), *Pm35* (Miranda et al. 2007), *Pm36* (Blanco et al. 2008), *Pm37* (Perugini et al. 2008), *Pm38* (Spielmeyer et al. 2008), *Pm39* (Lillemo et al. 2008) all have been tagged successfully using microsatellite markers.

The powdery mildew resistance gene *Pm23*, derived from the common wheat Line 81-7241, was originally assigned to chromosome 5A using monosomic of Chinese Spring analysis (Yang and Ren, personal communication; McIntosh et al. 1998), and has shown a very broad spectrum of resistance to the isolates of *B. graminis* f.sp. *tritici* in China (Zhou et al. 2005). In this study, we report the genetic characterization, microsatellite linkage mapping and allelism test performed for *Pm23* and *Pm4b*, describe the relocation of *Pm23* to chromosome 2AL and its relationship to the complex *Pm4* locus.

Materials and methods

Plant materials

Common wheat Line 81-7241 with the pedigree Fan7/Aurora//77S-3521, carrying resistance genes *Pm23* and *Pm8*, was identified by Yang and Ren (personal communication). *Pm8* has been overcome by nearly all the races in China and elsewhere (Duan et al. 2002), but the present evidence indicated that it was suppressed in Line 81-7241 (Huang et al. 1997; McIntosh et al. 1998). In this study, Line 81-7241 was crossed with powdery mildew susceptible cultivar Chancellor in greenhouse, and the F_1 was backcrossed to Chancellor to obtain the BC_1 in field. A total of 143 BC_1 plants were grown in greenhouse and used for genetic analysis and linkage mapping. In addition, 148 F_2 plants of Chancellor/Line 81-7241 were tested against *Bgt* isolate to validate the inheritance of this resistance gene. Likewise, an F_2 population and the derived $F_{2:3}$ families from Chancellor/VPM1 (*Pm4b*) (Bariana and McIntosh 1993) were created to further map powdery mildew resistance gene *Pm4b* using microsatellite markers.

In addition, F_1 seeds were obtained from cross between VPM1 and Line 81-7241 in 2002, and subsequently, the F_2 population and the derived $F_{2:3}$ families were developed.

Evaluation of powdery mildew resistance

Fourteen isolates of *B. graminis* (DC.) E.O. Speer f. sp. *tritici* (*Bgt*) were selected to differentiate the resistance genes *Pm4a*, *Pm4b*, *Pm8* and *Pm23*. The *Bgt* isolate E09, avirulent to VPM1 (*Pm4b*) and Line 81-7241 (*Pm23*), but virulent to Chancellor, was used for disease response tests. One hundred and forty-three BC_1 and 148 F_2 plants from Chancellor*2/Line 81-7241, and 109 $F_{2:3}$ lines derived from Chancellor/VPM1 were evaluated at the seedling stage in the inoculated greenhouse for response to powdery mildew. Inoculations were performed by dusting or brushing conidia from neighboring sporulating susceptible seedlings of 'Mingxian 169,' the highly susceptible control, onto the test seedlings. Reactions were scored 15 days after inoculation, when 'Mingxian 169' and the susceptible parent were heavily infected. Infection types (ITs) were scored on a 0, 0; and 1 to 4 scale, with 0 representing no visible symptoms, 0; representing necrotic flecks, and 1, 2, 3, 4 for highly resistant (necrosis with low sporulation), resistant (necrosis with medium sporulation), susceptible (no necrosis with medium to high sporulation), and highly susceptible (no necrosis with full sporulation) reactions, respectively (Liu et al. 2002). Reactions were classified into two groups, with resistant (R) (IT = 0–2) and susceptible (S) (IT = 3 and 4) (Chen et al. 2005b).

In addition, the F_2 population and some of the derived $F_{2:3}$ families were evaluated using *Bgt* isolates E09 and E15 (avirulent to Line 81-7241/virulent to VPM1) for the cross of VPM1/Line 81-7241 to test the allelism of mildew resistance genes *Pm4b* and *Pm23*.

Microsatellite markers analysis

One hundred and forty-three individuals of BC_1 population from Chancellor*2/Line 81-7241 and 109 F_2 plants derived from Chancellor/VPM1 were used for SSR analysis. Wheat seedling leaf tissues were collected for DNA extraction prior to inoculation with powdery mildew. Genomic DNA was extracted following the procedure described by Stein et al. (2001) with minor modifications, using 70% ethanol as washing solution.

Wheat microsatellite markers, mapped to chromosome 5A (92 SSRs) and 2AL (76 SSRs) were screened for polymorphism between the two parental lines Chancellor and Line 81-7241. They were divided into two groups, one was genomic-SSRs, including GWM, GDM, WMC, BARC, CFA, CFD and PSP and the other was EST-SSRs, including CFE (Zhang et al. 2005), CWEM (Peng and Lapitan 2005), DuPw (Eujayl et al. 2002), KSUM, CNL (Yu et al. 2004) and SWES (Chen et al. 2005a). Relevant information for these markers was published on the GrainGenes website (http://wheat.pw.usda.gov) or in reference articles. Each PCR reaction was conducted in a total volume of 25 μl in a TakaRa PCR thermal cycler or in a Bio-Rad 9600 thermal cycler, following the proportion described by Röder et al. (1998a), amplifications were

performed using a touchdown PCR protocol with the following conditions: 94°C for 3 min, 15 cycles of 94°C for 45 s, 65°C for 50 s (−1°C per cycle) and 72°C for 55 s, followed by 30 cycles of 94°C for 40 s, 50°C for 40 s and 72°C for 40 s and a final extension step at 72°C for 5 min. The PCR products were separated in 6% non-denaturing polyacrylamide gels. Gels were then silver stained and photographed.

Polymorphic markers were rechecked among a preferred small group (PSG) made up of ten typical resistant individuals and ten typical susceptible ones instead of bulked segregant analysis. The resulting polymorphic SSRs, which displayed the general consistency between genotype and phenotype, were further used to assay the mapping populations (the criteria was that the estimated recombination frequency was lower than 40%).

Data analysis

Chi-square (χ^2) tests were used to determine the goodness-of-fit of the observed data with the expected segregation ratios. Linkage analysis was performed using MAPMAKER/Exp version 3.0b (Lincoln et al. 1993). Map distance were determined using the Kosambi mapping function (Kosambi 1944) and loci were ordered using the 'sequence' and 'compare' commands, with an LOD threshold score $\geq$3.0 and a maximum distance allowed between markers set to 37.5.

Results

Inheritance of the powdery mildew resistance gene in common wheat Line 81-7241

Reaction to *Bgt* isolate E09 was determined in BC_1 and F_2 populations derived from the cross of Chancellor and Line 81-7241. A total of 76 resistant and 67 susceptible plants were found among the 143 BC_1 population (Table 1), and among 148 plants of the F_2 population, 108 were resistant, 40 were susceptible. Thus, the resistance segregation in both populations fits either 1:1 or 3:1 ratios ($\chi^2 = 0.57$ and 0.32, $df = 1$, $P = 0.45$ and 0.57), indicating that the powdery mildew resistance in common wheat Line 81-7241 is conditioned by a single dominant gene, which should be *Pm23* (Table 1).

Microsatellite markers linked to *Pm23*

Initially, 92 wheat SSR markers on chromosome 5A of bread wheat were screened for polymorphism between the parental lines Chancellor and Line 81-7241. Forty-five genomic-SSRs markers showed polymorphism, and no EST-SSRs marker was identified. But only 26 of 45 were selected to check the PSG of BC_1 on the basis that they were easily scored. Only one marker, *Xbarc122*, exhibited a general consistency between genotype and phenotype (Fig. 1), whereas the others deviated from the set criteria.

Table 1 Segregation for resistance and microsatellite markers linked to the genes *Pm23* and *Pm4b*

Population	Gene/markers	No. of BC_1/F_2	Observation no.[a]			Expected ratio	χ^2	*P* value
			A	H	B			
Chancellor*2/Line 81-7241	*Pm23*	143	–	76	67	1:1	0.57	0.45
	Xbarc076	143	–	79	64	1:1	1.57	0.21
	Xbarc122	143	–	78	65	1:1	1.18	0.28
	Xgwm311	143	–	76	67	1:1	0.57	0.45
	Xgwm356	143	–	77	66	1:1	0.85	0.36
	Xgwm382	143	–	76	67	1:1	0.57	0.45
	Xgdm93	143	–	75	68	1:1	0.34	0.56
	Xcfd267	143	–	76	67	1:1	0.57	0.45
	Xwmc170	143	–	69	74	1:1	0.17	0.68
Chancellor/VPM1 F_2	*Pm4b*	109	25	56	28	1:2:1	0.25	0.88
	Xbarc76	109	26	58	25	1:2:1	0.47	0.79
	Xbarc122	109	24	55	30	1:2:1	0.67	0.72
	Xgwm356	109	79[b]	–	30	3:1	0.37	0.54
	Xgdm93	109	84[b]	–	25	3:1	0.25	0.62

Values for significance at $P = 0.05$, $\chi^2 = 3.84$, $df = 1$; $\chi^2 = 5.99$, $df = 2$

[a] Phenotype or genotype: *A* homozygous resistant, *H* heterozygous, *B* homozygous susceptible

[b] Summation of 'A' and 'H,' replaced with the default code 'D' in MAPMAKER 3.0b

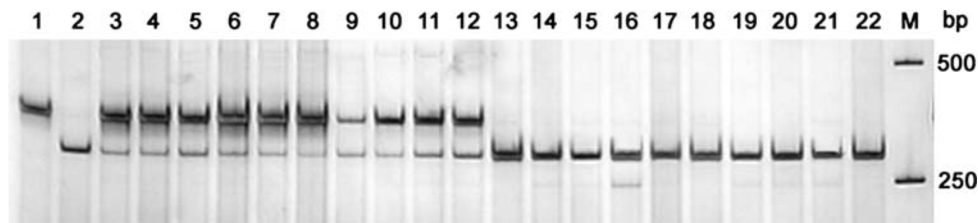

Fig. 1 Amplification products by wheat microsatellite primer *Xbarc122* in resistant parent Line 81-7241 (*Lane 1*), susceptible parent Chancellor (*Lane 2*), 10 typical resistant individuals of BC_1 (*Lane 3–12*) and 10 typical susceptible individuals of BC_1 (*Lane 13–22*). M, DL 2000 marker

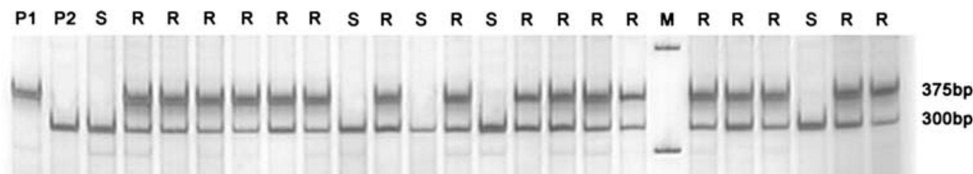

Fig. 2 Genotyping the BC_1 population using the *Xbarc122* marker, *R* indicates heterozygous resistant plants, *S* indicates homozygous susceptible plants. P1, Line 81-7241. P2, Chancellor. M, DL 2000 marker

The polymorphic fragments *Xbarc122*/375 bp and *Xbarc122*/300 bp were observed in Line 81-7241 and Chancellor, respectively. The fragment *Xbarc122*/375 bp was only present in typical resistant individuals, and the fragment *Xbarc122*/300 bp was amplified in all the plants of the PSG, thus, the genotype was completely consistent with the phenotype, *Xbarc122* was a candidate marker tightly linked to the *Pm23* resistance gene in common wheat Line 81-7241.

We found two loci for the marker *Xbarc122*, one located on 5AS (map data: Wheat, Composite, 2004, together with the mapping population Synthetic × Opata) and the other assigned to 2AL (map data: Wheat, Grandin × BR34) (http://wheat.pw.usda.gov). Further, another 76 markers located on 2AL were tested for polymorphism between the parental lines Chancellor and Line 81-7241, and the resulting polymorphic markers were then used to check the PSG. Seven polymorphic markers (*Xgwm311*, *Xgwm356*, *Xgwm382*, *Xgdm93*, *Xwmc170*, *Xbarc76*, and *Xcfd267*) were got, and the estimated recombination frequencies were all lower than 40% when checked using the PSG. They were all genomic-SSRs markers, and concerning 17 EST-SSRs markers, they all showed no polymorphism between the parental lines. The polymorphic fragments amplified by the seven microsatellite primer pairs were *Xgwm311*/110, *Xgwm356*/170, *Xgwm382*/90, *Xgdm93*/220, *Xwmc170*/300, *Xbarc76*/230 and *Xcfd267*/530, respectively, which mostly segregated with the resistance gene *Pm23*, and the polymorphic fragments *Xgwm311*/175, *Xgwm356*/300, *Xgwm382*/130, *Xgdm93*/200, *Xwmc170*/235, *Xbarc76*/220 and *Xcfd267*/500 were associated with the Chancellor allele.

These seven polymorphic microsatellite markers were further used to test all 143 BC_1 individuals from Chancellor*2/Line 81-7241 together with the marker *Xbarc122*. A typical amplification pattern generated by *Xbarc122* is shown in Fig. 2. All markers showed 1:1 segregation ratios (Table 1). A microsatellite genetic map involving the *Pm23* along with the eight microsatellite markers was constructed with a total map length of 54.1 cM using MAPMAKER 3.0b, *Pm23* was flanked by *Xbarc122* and *Xgwm356* with the genetic distances of 1.4 cM and 3.5 cM, respectively (Fig. 3d).

Microsatellite markers linked to *Pm4b*

The marker *Xgwm356* was reported to be linked to *Pm4a* in CI 14124 at a distance of 4.8 cM by Ma et al. (2004) (Fig. 3a). The complex *Pm4* locus is located on the long arm of chromosome 2A. In this study, we also found *Xgwm356* was closely linked to *Pm23* with a genetic distance of 3.5 cM, thus it was necessary to investigate the allelic relationship of genes *Pm23* and *Pm4*. Since the segregating F_2 population from Chancellor/VPM1 (*Pm4b*) was available, a total of 109 F_2 individuals were inoculated at the seedling stage in the greenhouse with *Bgt* isolate E09. Of these, 81 were resistant and 28 were susceptible, and the χ^2 test indicated a good fit to a 3:1 segregation for resistance ($\chi^2{}_{3:1} = 0.028$, $df = 1$, $P = 0.87$). This segregation was further confirmed by 109 F_2-derived F_3 families. Among 81 F_3 family lines derived from resistant F_2 plants, 25 were homozygous resistant and 56 segregated, whereas the 28 lines derived from susceptible F_2 plants were all homozygous susceptible ($\chi^2_{1:2:1} = 0.25$, $df = 2$, $P = 0.88$) (Table 1). The statistical analysis data indicated that the powdery mildew resistance in VPM1 was controlled by a single dominant gene, which should be *Pm4b* according to its origin.

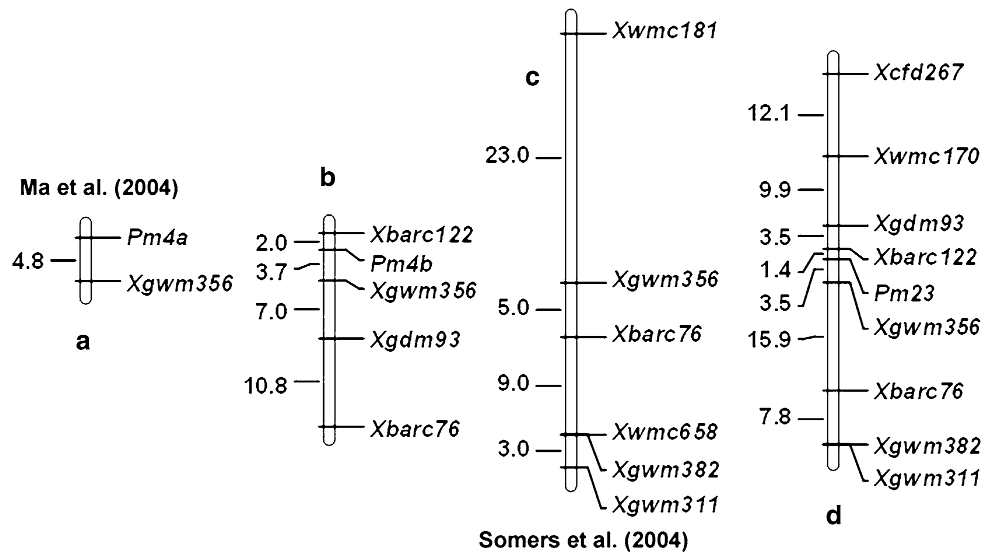

Fig. 3 Comparative view of the *Pm4a* (**a**), *Pm4b* (**b**) and *Pm23* (**d**) linkage maps with respect to the 2AL consensus map (**c**) by Somers et al. (2004)

We used the eight markers linked to *Pm23* to check the polymorphism between the parental lines Chancellor and VPM1. Six of them (except *Xcfd267* and *Xwmc170*) showed polymorphism. When further checked in the PSG of F_2 plants, markers *Xgwm311* and *Xgwm382* showed no linkage with *Pm4b*. And the remaining four markers, *Xgwm356*, *Xgdm93*, *Xbarc76*, *Xbarc122*, exhibited the general consistency between genotype and phenotype in PSG, were the possible markers linked to the *Pm4b* gene, and the sizes of the polymorphic fragments amplified by the four microsatellite primer pairs for VPM1 (*Pm4b*) were similar to the ones amplified for Line 81-7241 (*Pm23*), respectively.

Subsequently, the four candidate markers *Xgwm356*, *Xgdm93*, *Xbarc76*, *Xbarc122*, were used to assay the 109 F_2 plants of the Chancellor/VPM1, all the markers showed the expected segregating ratios in the population (Table 1). In fact, all the four markers were co-dominant in parents, but when checked in the population, the polymorphic band amplified with the marker *Xgwm356* in Chancellor was often indistinct and unrepeatable, this phenomenon was also reported by Gupta and Varshney (2000). And for the marker *Xgdm93*, due to the multiple bands amplified and the very near intervals between the bands, it was a little hard to distinguish the heterozygous from the homozygous using the non-denaturing gels. Hence, we used the default code 'D' to replace 'A' and 'H' in constructing the linkage map using MAPMAKER 3.0b. The microsatellite genetic map of *Pm4b* (Fig. 3b) showed comparability with the map of *Pm23* (Fig. 3d), and *Pm4b* was also flanked by the microsatellite loci *Xbarc122* and *Xgwm356*, only the marker distance was a little different, and the marker *Xgdm93* was located to another position. Comparatively viewing the three genetic maps (Fig. 3a, b, d), we consider that gene *Pm23* in common wheat Line 81-7241 probably belongs to the complex *Pm4* locus.

Allelism of mildew resistance genes *Pm4b* and *Pm23*

To further clarify the relationship of *Pm4b* and *Pm23*, 152 F_2 plants from VPM1/Line 81-7241 were inoculated with *Bgt* isolate E09, avirulent to the both parents, in the greenhouse and 485 F_2 plants were tested with the same isolate in the field. No susceptible individual was obtained (Table 2). In crosses between two resistant lines, the lack of appearance of susceptible plants would indicate that the two lines are carrying resistance genes that are allelic or that the genes are very closely linked (Huang and Röder 2004). There were also no susceptible individuals among 60 F_3 families tested with the same isolate, indicating the two genes were allelic. Another 60 F_3 families were tested in the greenhouse with *Bgt* isolate E15, which was avirulent to *Pm23* but virulent to *Pm4b*. These lines segregated 18 homozygous resistant, 25 segregating and 17 homozygous susceptible (Table 3), and the χ^2 test indicated a good

Table 2 Allelic relationship between powdery mildew resistance genes *Pm4b* and *Pm23* tested against *Bgt* isolate E09 in the F_2 of VPM1 (*Pm4b*)/Line 81-7241 (*Pm23*)

Hybrid	Observed segregation		χ2 (15:1)	*P* value
	Resistant	Susceptible		
VPM1/Line 81-7241 (greenhouse)	152	0	10.13**	0.0015
VPM1/Line 81-7241 (field)	485	0	32.33**	<0.00001

Isolate E09 is avirulent for VPM1 (*Pm4b*) and Line 81-7241 (*Pm23*)
** Significant at $P = 0.01$

Table 3 Test of allelism between resistance genes *Pm4b* and *Pm23* among F_3 lines of VPM1 (*Pm4b*)/Line 81-7241 (*Pm23*)

Hybrid	*Bgt* isolate	No. of F_3 families		
		Resistant	Segregating	Susceptible
VPM1/Line 81-7241	E09[a]	60	0	0
	E15[b]	18	25	17

[a] Avirulent for VPM1 (*Pm4b*) and Line 81-7241 (*Pm23*)

[b] Virulent for VPM1 (*Pm4b*) and avirulent for Line 81-7241 (*Pm23*)

Table 4 Differential reactions of wheat cultivars/lines possessing known powdery mildew resistance genes after inoculation with 14 isolates of *Blumeria graminis* f. sp. *tritici*

Cultivar/Line	*B. graminis* f. sp. *tritici* isolate								*Pm* gene
	E02	E09[c]	E11	E15	E17	E18	E20	E21	
Khapli/8*Cc[a]	s[b]	r	r	s	s	s	s	s	*Pm4a*
VPM1	s	r	s	s	r	s	s	s	*Pm4b*
Kavkaz	s	s	s	s	s	s	s	s	*Pm8*
Line 81-7241	r	r	r	r	r	s	s	s	*Pm23* (*Pm4c*)

[a] Seven times backcrossed to 'Chancellor'

[b] *r* resistant, *s* susceptible

[c] Identical results for E03, E05, E06, E07, E13, E23

fit to a 1:2:1 segregation ($\chi^2_{1:2:1} = 1.7$, $df = 2$, $P = 0.43$). When Khapli/8*Cc (*Pm4a*), VPM1 (*Pm4b*) and Line 81-7241 (*Pm23*), together with the cultivar Kavkaz possessing *Pm8*, were inoculated with 14 isolates of *Bgt* (Table 4), Kavkaz was susceptible to all isolates, and the other three lines showed differential patterns of response indicating the three alleles were different.

Discussion

Chromosome location of *Pm23*

Based on the GrainGenes database website (http://wheat.pw.usda.gov) and other integrated and consensus genetic and physical maps (Röder et al. 1998a, 1998b; Somers et al. 2004; Sourdille et al. 2004), marker *Xgwm311* was assigned to chromosomes 2AL, 2B, 2D and 6D, *Xgwm356* to 2AL and 6A, *Xgwm382* to 2AL, 2B and 6D, *Xgdm93* to 2AL, 2B, 4B and 2DL, *Xwmc170* to 2AL, *Xbarc76* to 2AL, 6B and 7D, *Xbarc122* to 2AL and 5A, and *Xcfd267* was located on the chromosome of 2AL, 2B and 2D. Thus, most of the markers detect multiple loci, but 2AL is a common location for all of them. More recently, Singh et al. (2007) has constructed an integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population, five of the eight polymorphic markers linked to *Pm23* were located on the distal end of 2AL, they were *Xcfd267*, *Xwmc170*, *Xgwm382*, *Xgwm311* and *Xbarc122* respectively. The markers order and linkage distance were a little different from the map reported here, maybe it was due to the different mapping population from different species. Considering the above information, we can conclude the resistance gene *Pm23* in Line 81-7241 was located on 2AL.

Pm23: a new allele of *Pm4*

Resistance genes are very abundant in plant genomes, and they are not distributed randomly but rather appear to be clustered on particular chromosomes like, most belong to tightly linked gene families (Hulbert et al. 2001). For example, a high proportion of actively transcribed wheat genes are clustered on chromosome arm 1AS (Gill et al. 1996), where resistance genes for *Pm3* alleles, *Lr10* and *H9* are located. Moreover, multiple genes for resistance to powdery mildew and rusts are clustered on chromosome 7AL, including the *Pm1a* to *Pm1e*, *Pm9*, *Pm37*, *Lr20*, *Sr15*, and other temporarily designated genes (Ji et al. 2008). In addition to the *Pm4* alleles, chromosome 2AL carries a number of genes for resistance to the rusts, including *Yr1*, *Yr32* and *Sr21* (McIntosh et al. 2003). Zhu et al. (2005) reported that powdery mildew resistance gene *PmPS5A*, originating from *T. carthlicum* was also located in a similar chromosomal region to *Pm4*.

The two designated alleles of *Pm4* originated from *T. dicoccum* (*Pm4a*) and *T. carthlicum* (*Pm4b*), which both were independently located on chromosome 2AL (The et al. 1979; Ma et al. 1994). In the present study, *Pm23* in common wheat Line 81-7241 was also placed on 2AL rather than 5A as originally reported. All three genes linked to the marker *Xgwm356* with distances of 3–5 cM, and *Pm23* was allelic to *Pm4b*. Cultivars/lines carrying the three resistance genes exhibited differential patterns of response to an array of *Bgt* isolates indicating that *Pm23* is an allele at the *Pm4* locus (Table 4). It is proposed therefore that *Pm23* be redesignated as *Pm4c*. Moreover, the results offer another evidence that the genes in wheat are present in clusters, the linked markers we have got will facilitate the identification and location of new resistance genes in this region.

To assign the new gene to specific chromosome of wheat, monosomic analysis was most frequently used in conventional genetics (Huang and Röder 2004), but the false location was sometimes unavoidable. Previously, *Pm12*, *Pm16*, *Pm22* (*Pm1e*), *Pm24* and *H9* were firstly located on chromosomes 6A, 4A, 1D, 6D and 5A respectively, using monosomic analysis. However, they were

reassigned to chromosomes T6BS-6SS.6SL (Song et al. 2007), 5BS (Chen et al. 2005b), 7AL (Singrün et al. 2003), 1DS (Huang et al. 2000) and 1AS (Kong et al. 2005), respectively, with chromosome-specific molecular markers. In consideration of these reports, as well as the result obtained in the present study, it is clear that the use of wheat aneuploids in combination with molecular markers is a superior approach for determining the locations of genes in wheat.

The present study has demonstrated the allelism of powdery mildew resistance genes *Pm23* and *Pm4b*, *Pm4a* and *Pm4b* are alleles as we all know, so we can deduce that *Pm23* and *Pm4a* are also alleles, but the allelism test is needed, along with the issue on the using of microsatellite markers linked to the *Pm4* locus in MAS (Marker-assisted selection), that will be discussed in the further study.

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