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ORIGINAL PAPER

pmX: a recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi

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Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is one of the most devastating foliar diseases of wheat and imposes a constant challenge on wheat breeders. Xiaohongpi, a Chinese landrace of wheat (*Triticum aestivum* L.), shows resistance to powdery mildew during the entire growth stage in the field and under controlled conditions. The F_1 plants from cross of the powdery mildew susceptible cultivar Yangmai158 with Xiaohongpi were susceptible to isolate Bgt19, the locally most prevalent Bgt isolate. In the derived F_2 population and F_3 progenies, the resistance segregation deviated significantly from the one-gene Mendelian ratio. However, marker analysis indicated that only one recessive gene conferred the resistance, which co-segregated with *Xsts-bcd1231* that showed co-segregation with *Pm4a* in different studies. Allelism test indicated that this recessive resistance gene, designated as *pmX*, is either allelic or tightly linked to *Pm4a*. The *pmX* gene was different from *Pm4* alleles in resistance spectrum. Examination of the genotype frequencies at *pmX* and the linked marker loci in the F_2 population showed that a genetic variation favoring the transmission of Xiaohongpi alleles could be the cause of deviated segregation. Mapping of the *pmX*-linked markers using Chinese Spring deletion lines indicated that it resides in the 0.85–1.00 bin of chromosome 2AL.

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Introduction

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* (Bgt), is one of the most devastating foliar diseases of wheat (*Triticum aestivum* L.), and occurs frequently in regions with maritime or semi-continental climate (Bennett 1984). Deployment of disease-resistant cultivars is the most efficient and environmentally friendly approach to curb this disease. Since most of the reported powdery mildew resistance genes in wheat are race-specific and liable to resistance loss once being widely used in deployed cultivars, wheat breeders are working hard on identification and utilization of new genes from various wheat germplasm resources.

The use of molecular markers has greatly facilitated novel gene identification and utilization. Currently, 72 powdery mildew resistance genes of wheat have been documented (He et al. 2009; Hua et al. 2009; Li et al. 2009; Luo et al. 2009; McIntosh et al. 2008; Ben-David et al. 2010; Ma et al. 2011), which map to 41 loci distributing on all wheat chromosomes, but 3A and 4D. Of these genes, *Pm38* and *Pm39* and *MlRE* confer quantitative resistance (Chantret et al. 2000; Lillemo et al. 2008; Spielmeyer et al. 2008), the remaining confer qualitative resistance. The dominant nature seems to be the norm for powdery mildew resistance genes, but more than a dozen of them confer recessive resistance, such as *Pm5* (*Pm5a-Pm5e*) (Hsam et al. 2001; Huang et al. 2003), *Pm9* (Schneider et al.

1991), *Pm26* (Rong et al. 2000), *mlRD30* (Singrün et al. 2004), *pmY212* (Sun et al. 2006), *pm2026* (Xu et al. 2008), *pm42* (Hua et al. 2009), *mlxbd* (Xue et al. 2009) and *PmLK906* (Niu et al. 2008). This suggests the mechanism diversity of powdery mildew resistance.

Increasing evidence implies that multiple alleles exist for many of the powdery mildew resistance genes. This is not unexpected based on the cluster feature of resistance genes as revealed in the sequenced plant genomes and the cloned resistance gene loci. At *Pm1*, in addition to the five allelic genes from *Pm1a* to *Pm1e*, *MlIM72*, *Mlm2033*, *Mlm80*, *MIAG12*, and *PmG16* are also likely allelic to this locus (Hsam et al. 1998; Yao et al. 2007; Ji et al. 2008; Maxwell et al. 2009; Ben-David et al. 2010). Five alleles have been reported for *Pm5* (Huang et al. 2000; Hsam et al. 2001; Huang et al. 2003) and *mlxbd* could be a new allele of this locus (Xue et al. 2009). Schmolke et al. (2012) just reported the fourth allele of the *Pm4* locus, i.e. *Pm4d*. Zhu et al. (2005) showed that *PmPS5A* is also a member of the *Pm4* complex. *Pm3* is the best characterized wheat powdery mildew resistance gene locus, at which 15 resistance alleles have been identified (Yahiaoui et al. 2006; Bhullar et al. 2009; Yahiaoui et al. 2009).

Wheat landraces, mostly the products of natural and farmer's selections, have abundant genetic variations. Many genes useful for improvement of environmental adaptation, for instance tolerance to abiotic stress and resistance to biotic threats, exist in these germplasms. Up to now, five powdery mildew resistance genes, including *Pm5d*, *Pm5e*, *mlxbd*, *Pm24* and *Pm45,* have been identified in wheat landraces (Hua et al. 2009; Xue et al. 2009; Huang et al. 2000; Ma et al. 2011). Thus, exploitation of this type of wheat germplasm is valuable for enrichment of the gene pool toward wheat improvement. In this study, a recessive powdery mildew resistance gene in the Chinese wheat landrace Xiaohongpi was identified and mapped, and its allelic relationship to the *Pm4* locus was investigated.

Materials and methods

Plant materials

Bread wheat cultivar Xiaohongpi is a Chinese landrace and shows resistance to powdery mildew. Seeds of Xiaohongpi were deposited in the China Wheat Seed Bank (Institute of Crop Science, Chinese Academy of Agricultural Sciences, China). Yangmai158 is an elite wheat cultivar developed by Yangzhou Lixiahe regional Institute of Agricultural Sciences, China, and is susceptible to powdery mildew. Resistance segregation population was created by crossing Yangmai158 with Xiaohongpi. CI14123 (Khapli/8*CC) and CI14124 (Yuma/8*CC) are *Pm4a* near-isogenic lines developed using 'Chancellor' as the recurrent parent (Briggle 1969). Cultivar Armada possesses *Pm4b* and 81-7241 is a line possessing *Pm4c* (Hao et al. 2008). All these lines or cultivars were used in resistance spectrum investigation. Allelism test was performed using the materials derived from the cross of CI14123 with Xiaohongpi.

Bread wheat cultivar 'Chinese Spring' (CS) and its deletion lines del2AS-5 and del2AL-1, which lack the terminal 22 % length of the short arm and 15 % length of the long arm of chromosome 2A, respectively (Endo and Gill 1996), were used for bin assignment of the linked molecular markers.

The mapping population and data for *Pm4a* were the same as reported in Ma et al. (2004).

Powdery mildew resistance evaluation

Field resistance of Xiaohongpi, Sumai no. 3 and Yangmai158 was investigated in a field of Jiangpu agricultural experiment station of Nanjing Agricultural University that has annual occurrence of powdery mildew in the Spring. The segregating populations were evaluated following method described in Yao et al. (2007). The seedlings were grown in rectangular trays placed in a growth chamber and inoculated at the one-leaf stage with pathogen isolate Bgt19, the locally most prevalent Bgt isolate. Bread wheat cultivar Sumai No. 3 was used as the susceptible control. In progeny test, 15–20 plants were inoculated for each family. Resistance level was scored at the seventh day after the inoculation, using a 0–5 scale (Yao et al. 2007), which represents no visible symptom, necrosis without sporulation, sparse sporulation, moderate sporulation, abundant sporulation, and abundant sporulation with more than 80 % of the leaf area covered with mycelia, respectively.

Resistance spectrum was investigated using the detached leaf assay following Limpert et al. (1988) with some modification as described in Ma et al. (2011). Fifteen Bgt isolates derived from single-spore isolation from the composite collected from Nanjing, China were tested.

Marker analysis

DNA was extracted from young seedling tissues following the procedures described in Ma et al. (1994). For bulked segregant analysis, DNA bulks were made for individual $F_{2:3}$ families through combining equal quantities of DNA from six resistant and six susceptible plants.

Polymorphism survey was initially conducted with markers linked to known powdery mildew resistance genes (Huang and Röder 2004; Ma et al. 2004; McIntosh et al. 2011). Other markers using in the survey included SSR markers mapping to the region surrounding the *Pm4* locus

on chromosome 2AL (Röder et al. 1998; Pestsova et al. 2000, http://www.wheat.pw.usda.gov). PCR was performed in a PE9600 thermal cycler (Perkin Elmer, Norwalk, CT, USA) in a volume of 10 µl containing 10–20 ng of template, 2 pmol of each of the primers, 2 nmol of each of the dNTPs, 15 nmol of $MgCl_2$, 0.1 U *Taq* DNA polymerase and 1× PCR buffer. The PCR profile included one cycle of 94 °C 3 min, 35 cycles of 94 °C 30 s, 50–60 °C (depending on the specific primers) 30 s and 72 °C 50 s, and a final extension at 72 °C for 5 min. PCR products were separated in 8 % non-denaturing polyacrylamide gels with a 19:1, 25:1 or 39:1 acrylamide/bisacrylamide ratio, and then silver-stained as described by Santos et al. (1993). PCR products from amplification using the BCD1231-derived STS marker that co-segregated with *Pm4a* were digested with *Msp*I (Ma et al. 2004) and separated in 1.2 % agarose gels run in 0.5× NEB buffer (50 mM Tris, 0.5 mM EDTA, 6.3 mM NaAc, pH 8.1). PCR amplification and the product separation of the *XResPm4* locus that co-segregated with *Pm4d* followed the procedures described in Schmolke et al. (2012).

In addition, STS markers converted from RFLP markers BCD410 and BCD292 (http://www.wheat.pw.usda.gov) and seven expressed sequence tags (EST) (BE423792, BE490763, BE591763, BE499251, BF291539, BE442849 and BE446530) mapping to the chromosome bin 2AL1-0.85–1.00 (Qi et al. 2004) were also surveyed for polymorphism. The primer sequences of STS marker MAG8342 converted from BCD410 were 5′ CGATATACCAGAA ACAACTACC 3′ (F) and 5′ CCGGCTGGACACGTTC CGGCT 3′ (R). PCR with these markers was performed in the procedure similar to the SSR marker survey described above and the PCR products were separated on 8 % non-denaturing polyacrylamide gels with a 25:1 acrylamide/bisacrylamide ratio and silver-stained.

Linkage analysis

MAPMAKER Macintosh V2.0 (Lander et al. 1987) was used to construct the linkage map with map distance calculated using the Kosambi function. A LOD score of 3.0 was used as the threshold for declaration of linkage.

Results

Powdery mildew resistance of Xiaohongpi and its inheritance

The field resistance of Xiaohongpi to powdery mildew was investigated for two seasons in a field of Jiangpu agricultural experiment station. At heading stage, when the lower three to four leaves of the susceptible cultivars, such as Sumai No. 3 and Yangmai 158 had more than 60 % of the leaf area covered with mycelia, Xiaohongpi displayed a nearly immune phenotype. In the growth chamber, the seedling leaves of Xiaohongpi showed no visible symptom or a few necroses without sporulation in some plants when challenged with 15 different Bgt isolates. These results indicated that Xiaohongpi possesses resistance gene(s) conferring powdery mildew resistance.

To investigate the inheritance of powdery mildew resistance in Xiaohongpi, F_1 and F_2 plants from Yangmai158 × Xiaohongpi were challenged with isolate Bgt19. Like Yangmai158, the F_1 plants were susceptible and had a score of 3–4. At first, 118 F_2 plants were evaluated, of which 46 had a resistance phenotype with a score of 0–1 and 72 had a susceptible phenotype with a score of 3–5. Obviously, the resistance segregation did not fit the Mendelian ratio of one recessive resistance gene ($\chi^2_{1:3} = 11.6$, $P < 0.01$). To verify this result, another 156 F_2 plants were evaluated with Bgt19. The result was similar. There were 66 resistant plants with a score of 0–1 and 90 susceptible plants with a score of 3–5. In the progeny test, the $F_{2:3}$ progenies of 112 resistant F_2 plants did not segregate and were resistant; among the 162 families from the susceptible F_2 plants, only 18 did not segregate and were susceptible. Thus, proportions of the three types of resistance genotypes in the F_2 population fit the Mendelian segregation ratio of two independent recessive genes ($\chi^2_{7:8:1} = 0.92$, $P = 0.63$).

When two independent recessive genes segregated in a F_2 population, about half of the $F_{2:3}$ families with phenotype segregation would have had only one quarter of plants with a recessive phenotype. However, it was noted that within each of the 144 $F_{2:3}$ families with phenotype segregation, there were nearly half of the plants with a recessive phenotype. Because only 15–20 plants per family were evaluated in the progeny test, to obtain convincing segregation ratio estimate, the seeds of seven $F_{2:3}$ families that segregated in resistance and had more than 30 seeds available were planted and grown to maturity to harvest $F_{3:4}$ seeds. Progeny test was then conducted for the seven sibling populations. The results showed that the genotype segregation proportions in all the seven populations still only fit the 7:8:1 ratio (Table 1). Statistically, there was 99 % probability that at least one of the seven families segregated in a 1:2:1 ratio if two independent recessive genes stood. It was therefore speculated that abnormal segregation might exist in the population and the conclusion of two independent recessive genes might not be correct.

Chromosome localization of the powdery mildew resistance gene

To localize the powdery mildew resistance gene in Xiaohongpi, markers linked to the known powdery mildew

Table 1 Progeny test results of seven $F_{2:3}$ families with phenotype segregation and their Chi square test

$F_{2:3}$ family	Phenotype and number of the tested $F_{3:4}$ families			$\chi^2_{(1:2:1)}$	$\chi^2_{(7:8:1)}$
	All resistant	Segregating	All susceptible		
F_2-24	25	24	4	17.11**	0.52
F_2-39	15	22	2	9.31**	0.65
F_2-44	26	35	3	17.09**	0.67
F_2-57	22	25	5	11.19**	1.01
F_2-66	25	37	5	12.67**	1.16
F_2-107	15	17	2	9.94**	0.01
F_2-149	23	18	2	21.65**	1.68

** Significantly different from 1:2:1 at $P = 0.01$

resistance genes were first surveyed for polymorphism between the crossing parents and resistant and susceptible bulks made for each of the seven $F_{2:3}$ families with progeny test results (Table 1). Among these surveyed markers, the BCD1231-derived STS marker, which co-segregated with *Pm4a* on chromosome 2AL (Ma et al. 2004), distinguished the resistant parent and bulks from the susceptible parent and bulks. Genotyping the seven sibling populations using this marker showed that *Xsts-bcd1231* co-segregated with the resistance genotypes without exception. Illustration of the segregating banding patterns was shown in Fig. 1. When the marker was applied to the initially surveyed 118 F_2 plants, the same result was achieved. Thus, it was concluded that a single recessive gene on chromosome 2A contributed to the powdery mildew resistance in Xiaohongpi. This gene was thereafter named *pmX*.

Allelic relationship of *pmX* to *Pm4a* and the resistance spectrum comparison

To determine the allelic relationship of *pmX* to *Pm4a*, the F_1 and F_2 plants derived from the cross of CI14123 (*Pm4a* carrier) with Xiaohongpi were evaluated with the inoculation of Bgt19. The F_1 plants were immune to this isolate and no susceptible plants were observed among 155 inoculated F_2 plants. This result suggested that *pmX* is either allelic or tightly linked to *Pm4a*.

To compare the resistance spectra of *pmX*, *Pm4a*, *Pm4b* and *Pm4c*, their respective carriers, Xiaohongpi, CI14123 and CI14124 (*Pm4a*), Armada (*Pm4b*) and 81-7241 (*Pm4c*) were tested with 15 Bgt isolates. As shown in Table 2, CI14123 and CI14124 were susceptible to 4 of the 15 isolates, Armada displayed a necrosis phenotype without sporulation, 81-7241 was immune to ten of the isolates but sparse sporulation was observed when challenged with isolates Bgt3 and Bgt4. Xiaohongpi had a resistance spectrum different from all of them. It was either immune to the inoculations or only showed some necrosis on the leaves. Moreover, Xiaohongpi was the only genotype immune to isolates Bgt4 and Bgt22 (Table 2; Fig. 2).

Markers linked to *pmX*

To identify more markers linked to *pmX*, markers mapping to the regions surrounding the *Pm4* locus on the published maps were surveyed between the parents for polymorphism. Moreover, two STS markers converted from RFLP markers BCD410 and BCD292 and seven STS markers converted from ESTs mapping to the chromosome bin 2AL1-0.85-1.00 were also surveyed. Nine markers, including GWM356,

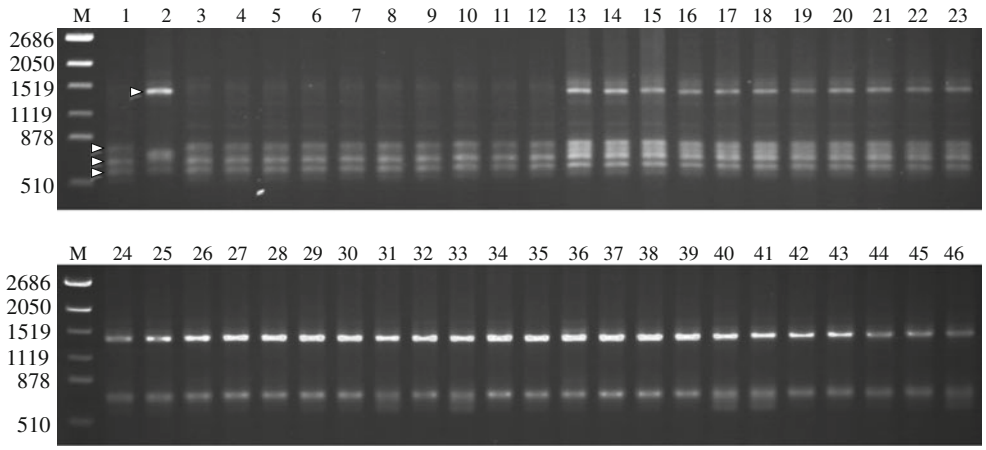

Fig. 1 Polymorphic patterns detected with STS-BCD1231, in 1.2 % agarose gels. *M* molecular size standard, the number to the left is band size (bp). *1* Xiaohongpi, *2* Yangmai158, *3–12* and *13–23* randomly chosen homozygous resistant and segregating $F_{3:4}$ lines shown in Table 1; *24–46*, all the 23 susceptible lines in this table. *Arrows* indicate the mapped polymorphic bands

Table 2 Resistance spectra of CI14123, CI14124, Armada, 81-7241, and Xiaohongpi that carry *Pm4* alleles

Lines	Bgt isolates														
	1	2	3	4	6	7	7-1	8	8-1	17	18	19	20	21	22
CI14123 (*Pm4a*)	0	0	5	5	0	0	1	0	2	2	0	0	1	5	5
CI14124 (*Pm4a*)	0	0	5	5	0	0	1	0	2	2	0	0	1	5	5
Armada (*Pm4b*)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
81-7241 (*Pm4c*)	1	0	2	2	0	0	0	0	0	0	0	0	1	0	1
Xiaohongpi (*pmX*)	1	1	1	0	0	1	1	1	0	0	1	1	1	1	0

This experiment was repeated three times and produced the same results

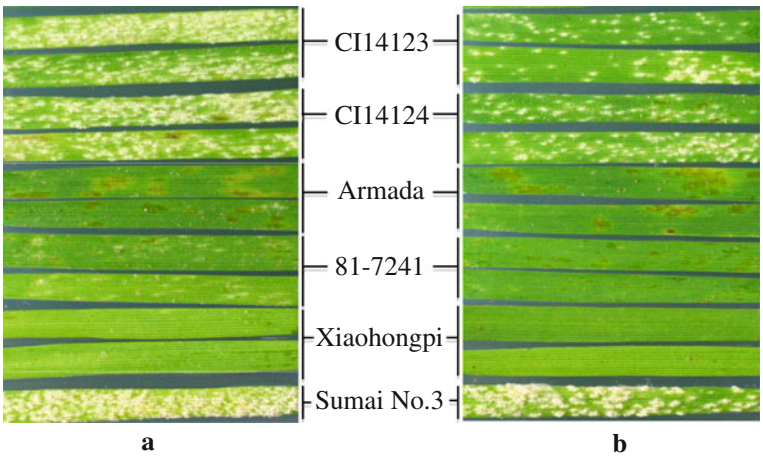

Fig. 2 Resistance of Xiaohongpi, CI14123, CI14124, Armada and 81-7241 to Bgt4 (**a**) and Bgt22 (**b**) with Sumai no.3 as the susceptible control

HBG327, GWM382, GWM311, PSP3039, GPW4474, GPW4456, ResPm4, and the STS marker MAG8342 converted from BCD410, detected polymorphism. Using the data from genotyping the F_2 plants with these markers, a linkage map covering *pmX* was constructed (Fig. 3a). In this map, markers *Xhbg327* and *Xgpw4456*/*XResPm4* flank *pmX* in a distance of 0.6 and 8.9 cM, respectively.

Deletion bin assignment of *pmX*

To determine the physical position of *pmX*, all marker loci, but *Xgwm356* that was assigned to the bin (C-2AL1-0.85) close to the centromere on chromosome 2AL and *Xgwm382* and *Xgwm311* that were assigned to the distal bin (2AL1-0.85-1.00) by Sourdille et al. (2004), were mapped using CS and deletion lines del2AS-5 and del2AL-1. Two marker loci, *Xsts-bcd1231* and *XResPm4* were missing in CS and thus could not be mapped. The remaining five marker loci were all missing in del2AL-1 but present in CS, as illustrated in Fig. 4. Thus, all linked markers, as well as *pmX*, reside in the distal deletion bin (Fig. 3b).

Segregation distortion of *pmX* and its linked markers

It was shown that there was a single recessive gene in Xiaohongpi; however, the segregation ratio of the resistance phenotypes in F_2 and their progenies said otherwise. To clarify this issue, the number of plants corresponding to each genotype at *pmX* and the linked marker loci in the population of 118 F_2 plants was examined. As expected, all loci segregated abnormally. The genotype proportions fit neither the 3:1 ratio (for dominant markers) nor the 1:2:1 ratio (for co-dominant markers) (Table 3).

To find out if this abnormal segregation also existed in the F_2 population of Chancellor × CI14124 that was used in mapping *Pm4a* by Ma et al. (2004), the number of plants corresponding to each genotype at *Pm4a*, *Xsts-bcd1231,* and the flanking marker loci *Xgwm356* and *XResPm4,* which was mapped to the position of 7.9 cM distal from *Pm4a* (Fig. 3c), was also examined. Unlike *pmX* and its linked markers, the genotypic proportions at the individual locus in this population fit either the 3:1 ratio or 1:2:1 ratio perfectly (Table 4).

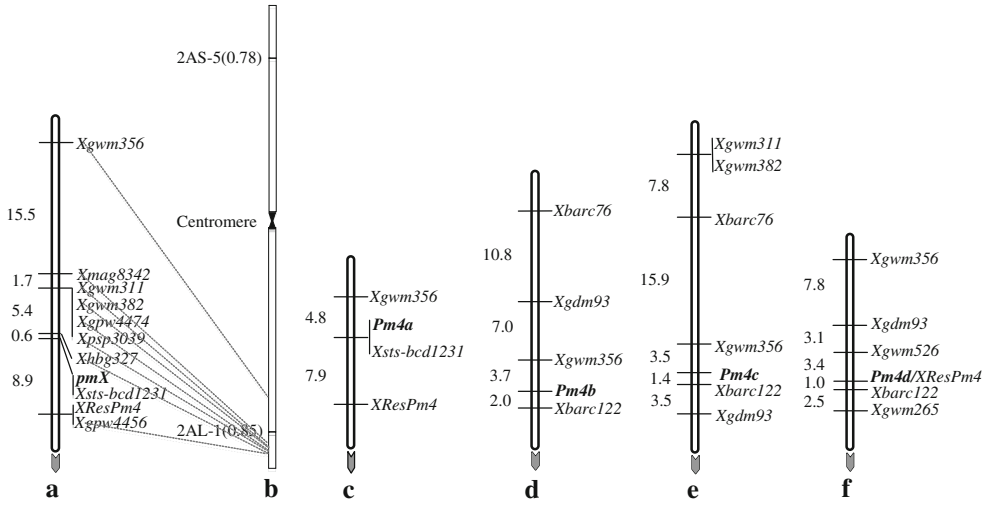

Fig. 3 Genetic and physical maps of the *Pm4*-allelic powdery mildew resistance genes. **a** Linkage map of *pmX* on chromosome 2AL, **b** bin assignment of the *pmX*-linked markers as shown with the *dashed line* connections, breakpoints and fraction-lengths of the bins (within *parentheses*) were shown to the *left*; **c**, **d**, **e** and **f** maps of *Pm4a*, *Pm4b* (Hao et al. 2008), *Pm4c* (Hao et al. 2008) and *Pm4d* (Schmolke et al. 2012), respectively. The *vertical arrows* indicate the direction toward telomere

Interestingly, in the F_2 population of Yangmai158 × Xiaohongpi, marker loci closer to telomere end on chromosome 2AL showed more segregation distortion in comparison to proximal marker loci (Table 3). At *XResPm4* and *Xgpw4456*, of 118 surveyed F_2 plants, there was only one with the Yangmai158 genotype. When compared with the number of plants with the Xiaohongpi genotypes, the heterozygotes for each of these loci was also much less than expected. These results implied that a genetic variation near *XResPm4* and *Xgpw4456* toward the telomere of chromosome 2AL in Xiaohongpi strongly favored the transmission of gametes with it, and thus caused the abnormal segregation of *pmX* and the linked markers.

Discussion

Chinese wheat landrace Xiaohongpi shows excellent field resistance to powdery mildew. In this study, *pmX*, a recessive powdery mildew resistance gene either allelic or tightly linked to *Pm4a* located on chromosome 2AL was identified in Xiaohongpi. Among the 72 powdery mildew resistance genes reported so far, 13 are recessive. One of the recessive genes, *pmLK906*, was mapped on chromosome 2AL, but was reported to be 7.6 cM from *Pm4a* (Niu et al. 2008). Obviously, *pmX* is different from this gene. The *pmX* gene is flanked by closely linked PCR-based markers *Xhbg327* and *Xgpw4456*/*XResPm4*, and co-segregated with *Xsts-bcd1231*. These markers could be readily used for marker-assisted selection of *pmX*.

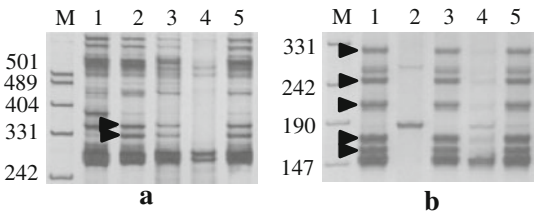

Fig. 4 Amplification of MAG8342 (**a**) and GPW4456 (**b**) in Xiaohongpi (*1*), Yangmai158 (*2*), del2AS-5 (*3*), del2AL-1 (*4*) and Chinese Spring (*5*). *M* pUC19/*Msp*I, the *number to the left* is band size (bp). The *arrow-pointed* polymorphic bands in each figure co-segregated in the segregation populations and were treated as a single locus

Currently, the resistance genes mapping at the *Pm4* locus include *Pm4a* (The et al. 1979), *Pm4b* (The et al. 1979), *Pm4c* (Hao et al. 2008), *Pm4d* (Schmolke et al. 2012), *PmPS5A* (Zhu et al. 2005), and *pmX*. These genes have different origins. *Pm4a* originates from *T. dicoccum* (Briggle 1966); *Pm4b* originates from *T. carthlicum*; *Pm4c* and *Pm4d* were identified in the common wheat line 81-7241 and *T. monococcum* accession Tm27, respectively. In addition, Zhu et al. (2005) mapped the powdery mildew resistance gene *PmPS5A* in the *T. carthlicum* accession PS5 to chromosome 2AL 10.2 cM from *Xgwm356* and proposed that it might be a member of the *Pm4* complex. The identification of multiple resistance genes at the *Pm4* locus is not only valuable for enrichment of genetic diversity, but also for investigation of resistance gene evolution.

When comparing the marker maps of the five resistance genes at the *Pm4* locus (Fig. 3), it was noted that some common markers in the maps have varying distances from the genes. *Xgwm356* is the marker linked to all these genes. It had similar genetic distances from *Pm4a*, *Pm4b* and *Pm4c*, but was much farther from *Pm4d* and *pmX*. Based

Table 3 Plant number for each genotype at *pmX* and the linked marker loci in the population of 118 F_2 plants derived from Yangmai158 × Xiaohongpi and the Chi square test against the expected Mendelian ratio

Loci	Plant number				Expected ratio	χ^2 value
	AA	AB	BB	A_ or B_		
Xgwm356	–	–	17	101	3:1	6.5**
Xmag8342	42	66	10		1:2:1	19.0**
Xgwm311	44	64	10		1:2:1	20.4**
Xgwm382	44	64	10		1:2:1	20.4**
Xgpw4474	44	64	10		1:2:1	20.4**
Xpsp3039	44	64	10		1:2:1	20.4**
Xhbg327	45	–	–	73	1:3	10.2**
Xsts-bcd1231	46	65	7		1:2:1	27.0**
pmX	46	65	7		1:2:1	27.0**
Xgpw4456	–	–	1	117	3:1	26.6**
XResPm4	–	–	1	117	3:1	26.6**

A Xiaohongpi allele, *B* Yangmai158 allele, *A_* The Xiaohongpi allele is dominant and the number includes both Xiaohongpi genotype and heterozygous genotype, *B_* The Yangmai158 allele is dominant and the number includes both Yangmai158 genotype and heterozygous genotype

** Significantly different from the expected ratio at $P = 0.01$

Table 4 Plant number for each genotype at *Pm4a* and the linked marker loci in the population of 85 F_2 plants derived from Chancellor × CI14124 and the Chi square test against the expected Mendelian ratio

Loci	Plant number				Expected ratio	χ^2 value
	AA	AB	BB	A_		
Xgwm356	22	39	24		1:2:1	0.67
Xsts-bcd1231	–	–	24	61	3:1	0.32
Pm4a	21	40	24		1:2:1	0.26
XResPm4	–	–	21	64	3:1	0.00

A CI14124 allele, *B* Chancellor allele, *A_* The CI14124 allele is dominant and the number includes both CI14124 genotype and heterozygous genotype

on the marker orders between *Xgwm356* and *Xgwm311*/*Xgwm382* in Fig. 3a and e, it was speculated that an interstitial inversion, relative to the *Pm4c* map, might have occurred in Xiaohongpi, which could cause the distance discrepancies of the markers in the centromere direction from the resistance genes. Schmolke et al. (2012) reported that *XResPm4* was a co-segregating marker of *Pm4d*. However, when applied to *Pm4a* and *pmX* in this study, it was 8.9 and 7.9 cM from the genes in the telomere direction (Fig. 3a, c). Variation of marker/gene genetic distances and orders between different maps is a common phenomenon. It could be affected by several factors, for instance, population size, mapping parents, chromosome structural variation, experimental errors, and so on. Since *Pm4a*, *Pm4d* and *pmX* originate from germplasms at different ploidy levels, variation in recombination rate surrounding them in the hexaploid wheat background is possible. Moreover, as resistance genes in plants often cluster together, the allelic relationships of resistance genes at the *Pm4* locus need to be re-examined using larger populations from crossing among all of them. Ultimately, comparison of the sequence level variations will clarify this issue.

Unlike the other known resistance genes at the *Pm4* locus that are dominant, *pmX* is a recessive gene. This is different from many other reported gene loci having multiple resistance alleles, of which the dominance/recessiveness is consistent. However, the *Pm4* locus is not the sole one having both dominant and recessive resistance genes. In common been (*Phaseolus vulgaris* L.), cultivars Tuscola and Montcalm each carries a gene at the *Co-1* locus conferring resistance to anthracnose; however, a 1R:3S ratio was observed when the F_2 population derived from their cross was inoculated with *Colletotrichum lindemuthianum* race 130 (Muhalet et al. 1981). Kelly and Vallejo (2004) suggested that different degrees of dominance exist for the alleles at this resistance locus. Sidhu and Khush (1978) reported that the dominance of rice *Xa6* conferring

resistance to *Xanthomonas oryzae* isolate PXO61 is dosage dependent. It conditions dominant resistance during flowering, but recessive resistance at the booting stage. Because the expression of resistance could be affected by different factors, including host–pathogen interactions, growth stage, gene–gene interactions, and environmental conditions, it would be interesting to find out the mechanisms governing the dominance/recessiveness of the resistance genes at the *Pm4* locus.

Molecular genetic analysis using F_2 population developed with the cross of Yangmai158 × Xiaohongpi and the derived $F_{2:3}$ progenies demonstrated that Xiaohongpi carries a single recessive gene for powdery mildew resistance. However, no matter in the F_2 population or in the $F_{2:3}$ progenies the phenotype segregation deviated significantly from the 1R:3S ratio. Investigation of the variation pattern for the number of plants with heterozygous genotype or Yangmai158 genotype at the loci along the *pmX* linkage map indicated that a genetic variation linked to *pmX* and favoring the transmission of gametes carrying it could be the cause of the deviated segregation ratio. Segregation distortion is common in plant species. That the homoeologous group 2 chromosomes of *Triticeae* species carry genes favoring the transmission of gametes with them has been well documented. Three gametocidal activity genes, including *Gc1-B1a*, *Gc1-B1b* and *Gc1-Sl1*, all map to chromosomes belonging to this group (Tsujimoto and Tsunewaki 1988). Segregation distortion and recombination suppression has also resulted from alien translocations as reported for *Sr36* and *Sr40* (Tsilo et al. 2008; Wu et al. 2009). However, this was not observed in the *pmX* map since the genetic distance of *XResPm4* from *pmX* was similar to its distance from *Pm4a* (Fig. 3a, c) and *Pm4a* is not known as a translocation. Moreover, no segregation distortion was observed in the *Pm4a* interval (Table 4). The interstitial inversion discussed above was not likely the cause of the segregation distortion, considering the facts that the distortion became less severe as the markers reside closer to the putative inversion interval (Fig. 3a; Table 3) and the most severe distortion occurred in its opposite side. Further studies are still required to determine the *pmX*-linked genetic variation that caused the segregation distortion.

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