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ORIGINAL PAPER

Molecular mapping of a new powdery mildew resistance gene *Pm2b* in Chinese breeding line KM2939

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Abstract

Key message **An allele of *Pm2* for wheat powdery mildew resistance was identified in a putative *Agropyron cristatum*-derived line and used in wheat breeding programs.**

Abstract Powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*, *Bgt*) is one of the most devastating wheat diseases worldwide. It is important to exploit varied sources of resistance from common wheat and its relatives in resistance breeding. KM2939, a Chinese breeding line, exhibits high resistance to powdery mildew at both the seedling and adult stages. It carries a single dominant powdery mildew resistance *(Pm)* allele of *Pm2*, designated *Pm2b*, the previous allelic designation *Pm2* will be re-designated as *Pm2a*. *Pm2b* was mapped to chromosome arm 5DS and flanked by sequence characterized amplified region (SCAR) markers *SCAR112* and *SCAR203* with genetic distances of 0.5 and 1.3 cM, respectively. Sequence tagged site (STS) marker *Mag6176* and simple sequence repeat (SSR) marker *Cfd*81 co-segregated with *SCAR203*. *Pm2b* differs in specificity from donors of *Pm2a*, *Pm46* and *PmLX66* on chromosome arm 5DS. Allelism tests indicated that *Pm2b*, *Pm2a* and *PmLX66* are allelic. Therefore, *Pm2b* appears to be a new allele at the *Pm2* locus. The closely linked markers were used to accelerate transfer of *Pm2b* to wheat cultivars in current production.

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Abbreviations

AFLP	Amplified fragment length polymorphism
Bgt	*Blumeria graminis* f. sp. *tritici*
BSA	Bulked segregant analysis
GISH	Genomic in situ hybridization
MAS	Marker-assisted selection
NIL	Near-isogenic line
Pm	Powdery mildew resistance gene
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RGC	Recipient genome composition
SCAR	Sequence characterized amplified region
SSR	Simple sequence repeat
STS	Sequence tagged site

Introduction

Powdery mildew, caused by the fungal ascomycete *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a devastating disease of wheat, affecting grain yield and quality (Morgounov et al. 2012). With popularization of semi-dwarf, high-yielding cultivars, increased utilization of nitrogen fertilizer

and improved irrigation, the disease has increased in importance in wet and warm areas (Bennett 1984; Zhuang and Li 1993; Zhuang 2003). Certain agricultural practices and chemical controls have been followed to reduce the disease, but these approaches have also increased production costs, polluted the environment and speeded to encourage the occurrence of new pathogen variants (Hardwick et al. 1994; Ryabchenko et al. 2003; Parks et al. 2008). The most effective, economical, environmentally sound and consistently used method for control is host resistance (Huang et al. 2000; Huang and Röder 2004).

More than 60 *Pm* genes/alleles located at 45 loci have been identified, including the formally designated genes *Pm1–Pm50*, and a few temporarily designated genes (e.g., McIntosh et al. 2012; Mohler et al. 2013; Xiao et al. 2013). However, when a qualitative resistance gene is over popularized in production, new isolates capable of overcoming the resistance usually appear some years later, and may induce epidemic 'boom-bust' cycles such as occurred for *Pm8* and *Yr9* in China following increases in the respective virulence genes and loss of resistance to powdery mildew and stripe rust, respectively (Graybosch 2001; Ryabchenko et al. 2003; Li and Zeng 2002; Wan et al. 2007). In fact, many *Pm* genes have lost effectiveness due to the race-specific nature of resistance and excessive deployment of single resistance genes (Xiao et al. 2013). Therefore, a constant search and transfer of new and effective sources of powdery mildew resistance are necessary to counter the continuous evolution of virulence in *Bgt*.

In the past, exploitation of resistance genes/alleles mainly focused on cultivated wheat. Over time, the genetic base for disease resistance has been narrowed by replacing highly variable landraces with high yielding, pure-line cultivars in many parts of the world, and the more homogeneous genetic backgrounds of cultivated species have led to unprecedented challenges in resistance breeding (Johnson 1992; Gupta et al. 2010; Muhammad et al. 2011; Karsai et al. 2012). Progenies of distant hybridization between common wheat and its wild species could be important sources of wheat disease resistance. A few *Pm* genes from non-progenitor (that is, other than A, B and D genome diploids) species have been identified, such as *Pm7*, *Pm8*, *Pm17* and *Pm20* from *Secale cereale* (Heun et al. 1990; Friebe et al. 1994, 1996; Hsam and Zeller 1997) and *Pm21* from *Haynaldia villosa* (Chen et al. 1995). These genes were introgressed into the wheat genome in large alien translocation or addition lines, and can be detected by genomic in situ hybridization (GISH). Some other genes were also identified in the progenies from distant hybridizations, but no cytologically detectable signals were observed by genomic in situ hybridization techniques. Such genes include *Pm40* and *Pm43* (He et al. 2009; Luo et al. 2009) and stripe rust resistance genes *Yr50* and *YrL693* (Liu et al. 2013; Huang et al. 2014) putatively derived from *Thinopyrum intermedium*. In all cases, the resistant lines containing these genes were selected several generations after the original inter-specific crosses with selection for disease resistance, but without monitoring for alien chromatin by cytological or marker methods.

With the development of molecular markers, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), sequence tagged site (STS) and amplified fragment length polymorphism (AFLP) markers, the above resistance genes were mapped to wheat chromosomes. The wheat-derived markers tightly linked to these resistance genes can be used for marker-assisted selection (MAS) to improve the efficiency of selection in wheat breeding (Gupta et al. 2010). MAS has been successfully performed in wheat breeding programs to transfer *Th. intermedium*-derived barley yellow dwarf virus resistance gene *Bdv2* into commercial cultivars (Zhang et al. 2004), but in this case there is evidence demonstrating that a small alien segment is present. Alien-derived leaf rust resistance genes *Lr9* (from *Ae. umbellulata*), *Lr24* (from *Th. ponticum*) and *Lr47* (from *Ae. speltoides*) have been transferred to other common wheat cultivars by use of markers (Nocente et al. 2007) that were either within the non-combining alien segments, and therefore behaving as 'perfect' markers, or were linked wheat-derived markers.

Agropyron cristatum ($2n = 4x = 28$, PPPP), a wild relative of wheat, possesses many desirable traits for improving wheat, including resistance to major diseases, such as powdery mildew, rusts and barley yellow dwarf virus, tolerance to drought and salinity, and various high-yielding characteristics, e.g., high tiller number, and larger floret and high kernel numbers (Dong et al. 1992; Li et al. 1997; Martín et al. 1999; Wu et al. 2006, 2010). Transfer of these alien traits to wheat is important for wheat improvement, including powdery mildew resistance (Luan et al. 2010; Chen et al. 2013).

KM2939, a putative *A. cristatum*-derived breeding line, shows high resistance to powdery mildew at both the seedling and adult stages. The objectives of this study were to study the inheritance of powdery mildew resistance in KM2939, determine the chromosomal location of the resistance gene, investigate the utility of closely linked markers of the resistance gene in different genetic backgrounds, and rapidly transfer the resistance gene to susceptible cultivars in current production.

Materials and methods

Plant materials

The materials used in this study included wheat lines KM2939, X3986-2, Huixianhong, Mingxian 169, Shimai

15, Fukuho, Laizhou 953 and CMH83.605, and *A. cristatum* accession Z559. KM2939 was derived from the cross 4201/CMH83.605//FC. Line 4201 was selected from a cross of Laizhou 953 and a glossy wheat-*A. cristatum* line selected from selfed hybrids of *A. cristatum* accession Z559 and Japanese introduction Fukuho. Line FC was derived from selfed hybrids of *A. cristatum* accession Z559 and Fukuho, and CMH83.605 was a wheat line introduced from Mexico. X3986-2 is an indigenous germplasm that has a known *Pm* gene *PmX3986-2* discovered by our laboratory (Ma et al. 2014). KM2939 showed high resistance to powdery mildew, while Shimai 15, Huixianhong and Mingxian 169 were all tested by different *Bgt* isolates and showed highly susceptible with infection type (IT) 4.

To analyze the inheritance of powdery mildew resistance, KM2939 was crossed with Huixianhong, Mingxian 169 and Shimai 15 to construct segregating populations, and F_1, F_2, F_3, BC_1F_1 and BC_2F_1 materials were tested for resistance to the *Bgt* isolate E09 that is avirulent on KM2939 and virulent on Huixianhong, Mingxian 169 and Shimai 15. The BC_2F_1 population from cross with KM2939 and Shimai 15 were used as mapping population.

Disease assessment at the seedling stage

KM2939 was tested for reaction to 27 single-pustule-derived powdery mildew isolates by separate inoculations in a temperature-controlled greenhouse at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing (Supplementary Table S1). Meanwhile, 40 wheat lines carrying known *Pm* genes or gene combinations were used to compare their reaction patterns with KM2939 (Supplementary Table S1) (Ma et al. 2014). These *Bgt* isolates were collected from different wheat ecological regions of China, and each isolate contains a different combination of virulences to resistance genes (Zhou et al. 2002). The method of disease infection and evaluation was described in An et al. (2013).

PCR amplification and marker analysis

Genomic DNA was extracted from the leaf tissues of young seedlings following a phenol/chloroform method (Sharp et al. 1988). Equal amounts of DNA from 10 resistant and 10 susceptible BC_2F_1 plants of cross KM2939/Shimai 15 were bulked to produce resistant and susceptible pools for bulked segregant analysis (BSA) (Michelmore et al. 1991). Simple sequence repeat (SSR) primers randomly distributed on all 21 chromosomes were used to amplify DNA from the two parents, and resistant and susceptible bulks in a polymorphic marker survey.

Twenty-five expressed sequence tags (ESTs) mapping to the *Pm2*-containing bin of chromosome arm 5DS were converted to STS markers and tested against both the parents and bulks; these included BE498794, BG604817, BE352603, BE404490, BE498665, BE636795, BE499257, BF291319, BG313707, BE591974, BE500291, BE498878, BE446058, BE591275, BQ167501, BE496976, BE443538, BE435260, BF291319, BE498665, BE499257, BE636795, BE443751, BE352603 and BG604817 (Ma et al. 2011; Gao et al. 2012; http://wheat.pw.usda.gov/cgi-bin/gbrowse/WheatPhysicalESTMaps/#search).

The resulting polymorphic markers were genotyped on the BC_2F_1 plants of KM2939/3*Shimai 15. PCR amplification and separation and visualization of the PCR products followed Xu et al. (2012). The PCR profile was as follows: 1 cycle at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 50–60 °C (depending on specific primers) for 40 s, 72 °C for 40 s, and a final extension at 72 °C for 5 min. Appropriate amounts of PCR products mixed with loading buffer (98 % formamide, 0.25 % bromophenol, 0.25 % xylene cyanol and 10 mM EDTA) were separated on 8 % non-denaturing polyacrylamide gels (Acrylamide: Bisacrylamide = 25:1 or 39:1) with 1× TBE buffer (90 mM tris–borate, 2 mM EDTA, pH 8.3), and visualized by silver straining (Xu et al. 2012).

Linkage mapping

Chi squared (χ^2) tests for goodness-of-fit were made to evaluate deviations of observed phenotypic data from theoretically expected segregation ratios. Linkage maps with genetic distances between markers and the resistance gene were constructed by the software MAPMAKER 3.0 with an LOD threshold of 3.0 (Lincoln et al. 1992). Map distances were derived from recombination values using the Kosambi function (Kosambi 1944).

Application of the resistance gene in wheat breeding programs

KM2939 was crossed with susceptible recurrent parents Shimai 15, Shixin 828, Kenong 199 and Gaocheng 8901, followed by two backcrosses to the recurrent parents. The closely linked markers were evaluated for applicability in diagnosing the resistance gene in each of the recipients. Progeny plants with the marker alleles from the resistant donor were retained for MAS. Finally, the resistances of selected progeny plants were verified using *Bgt* isolate E09.

A near isogenic line (NIL) was developed using susceptible cultivar Shimai 15 as the recurrent parent. To assess the genetic similarity of selected lines to the backcross parent, they were surveyed with 284 SSR markers distributed across all 21 chromosomes. Markers with polymorphism between KM2939 and Shimai 15 were selected from the 284 SSR markers, and used to screen the segregating

Table 1 Genetic analysis of resistance to *Bgt* isolate E09 in F_1, F_2, F_3, BC_2F_1 progeny from crosses of KM2939 and susceptible parents Shimai 15, Mingxian 169 and Huixianhong

Parent and cross	Generation	Observed ratio			Expected ratio	χ^{2a}	*P*
		HR	Seg	HS			
KM2939	P_R	10					
Shimai 15	P_S			10			
Mingxian 169	P_S			10			
Huixianhong	P_S			10			
KM2939/Shimai 15	F_1	10					
KM2939/Mingxian 169	F_1	10					
KM2939/Huixianhong	F_1	10					
KM2939/Shimai 15	F_2	3,275		1,079	3:1	0.11	0.74
KM2939/Mingxian 169	F_2	190		60	3:1	0.80	0.37
KM2939/Huixianhong	F_2	255		76	3:1	0.73	0.39
KM2939/Mingxian 169	F_3	54	115	45	1:2:1	1.95	0.37
KM2939/Huixianhong	F_3	67	147	79	1:2:1	0.99	0.61
KM2939/2*Shimai 15	BC_1F_1		36	29	1:1	0.75	0.39
KM2939/2*Mingxian 169	BC_1F_1		236	209	1:1	1.52	0.22
KM2939/3*Shimai 15	BC_2F_1		236	255	1:1	0.66	0.42

P_R resistant parent, P_s susceptible parent, *HR* homozygous resistant, *Seg* segregating (heterozygous resistant), *HS* homozygous susceptible

[a] Values for significance at $P = 0.05$ are 3.84 ($df = 1$) and 5.99 ($df = 2$)

materials. Estimation of the recipient genomic composition (RGC) followed the method of Xue et al. (2010), and was calculated by the formula: RGC = (2BB + AB) × 100 %/2 (BB + AB), where BB represents homozygous marker loci of the recurrent parent Shimai 15, and AB represents heterozygous loci.

Results

Inheritance of powdery mildew resistance in KM2939

When inoculated with *Bgt* isolate E09, KM2939 showed a hypersensitive reaction with IT 0, whereas Shimai 15, Huixianhong and Mingxian 169 were highly susceptible to IT 4. F_1 seedlings of KM2939/Shimai 15, KM2939/Huixianhong, and KM2939/Mingxian 169 were all resistant to IT 0, and the F_2 populations segregated 3,275 resistant to 1,079 susceptible plants ($\chi^2_{3:1} = 0.11$, $df = 1$, $P = 0.74$), 255 to 76 ($\chi^2_{3:1} = 0.73$, $df = 1$, $P = 0.39$) and 190 to 60 ($\chi^2_{3:1} = 0.80$, $df = 1$, $P = 0.37$), respectively, all fitting single dominant gene segregation ratios (Table 1). When F_2 populations of KM2939/Huixianhong and KM2939/Mingxian 169 were transplanted to the field, 293 and 214 plants survived to produce F_3 seeds, respectively. Twenty-four plants of each $F_{2:3}$ family were evaluated for powdery mildew response. The ratios of homozygous resistant (RR): segregating (Rr):homozygous susceptible (rr) families from KM2939/Huixianhong and KM2939/Mingxian 169 were consistent with the expected 1:2:1 (Table 1). BC_1F_1 populations of KM2939/2*Shimai 15 and KM2939/2*Mingxian 169, and a BC_2F_1 population of KM2939/3*Shimai 15 all segregated with expected ratios of 1:1 (Table 1). We concluded that powdery mildew resistance in KM2939 was conferred by a single dominant gene, which was designated *Pm2b*.

Molecular mapping of *Pm2b*

Initially, 284 SSR markers randomly located on all 21 wheat chromosomes were tested for polymorphism between the parents and bulks, and only the marker *Cfd40* located on both chromosome arms 5DS and 5AS showed a consistent polymorphism between both the parents and resistant and susceptible bulks (Supplementary Fig. S1). To determine and saturate the genomic region near *Pm2b* based on the location of *Cfd40*, 42 and 36 SSR markers on 5DS and 5AS, respectively, were tested on the parents and bulks. Only SSR markers *Cfd81*, *Cfd78* and *Gpw302* on 5DS showed polymorphisms between the parents and bulks (Supplementary Fig. S1), whereas none of the markers located on 5AS displayed such polymorphisms. Primer pairs CFD40, CFD81, CFD78 and GPW302 were further used to genotype the BC_2F_1 population of KM2939/3*Shimai 15 to determine the genetic distance between the four marker loci and *Pm2b* (Fig. 1). Marker loci *Xcfd40*, *Xcfd81*, *Xcfd78* and *Xgpw302* were closely linked to *Pm2b* with genetic distances 9.1, 1.3, 4.5 and 10.2 cM, respectively (Fig. 2a). AFLP-derived SCAR markers *SCAR112* and *SCAR203* linked to the known *Pm* gene *MlBrock* (Li et al. 2009) also mapped close to *Pm2b* with genetic distances of 0.4 and 1.3 cM, respectively (Fig. 2a). Twenty-five ESTs, previously

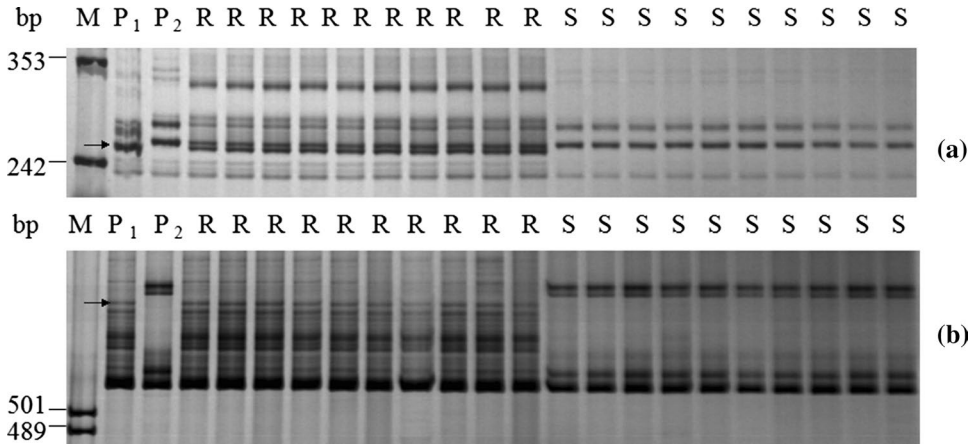

Fig. 1 Examples of amplification patterns of *Pm2b*-linked polymorphic markers *Cfd81* (**a**), and *Mag6176* (**b**) from selected KM2939/3*Shimai 15 F_1 plants in 8 % silver-stained non-denaturing polyacrylamide gels. *Lane M*, pUC18/*Msp*I; P_1, P_2 KM2939 and Shimai 15; *Lanes*: *R* heterozygous resistant plants, *S* homozygous susceptible plants. *Arrows* indicate polymorphic bands

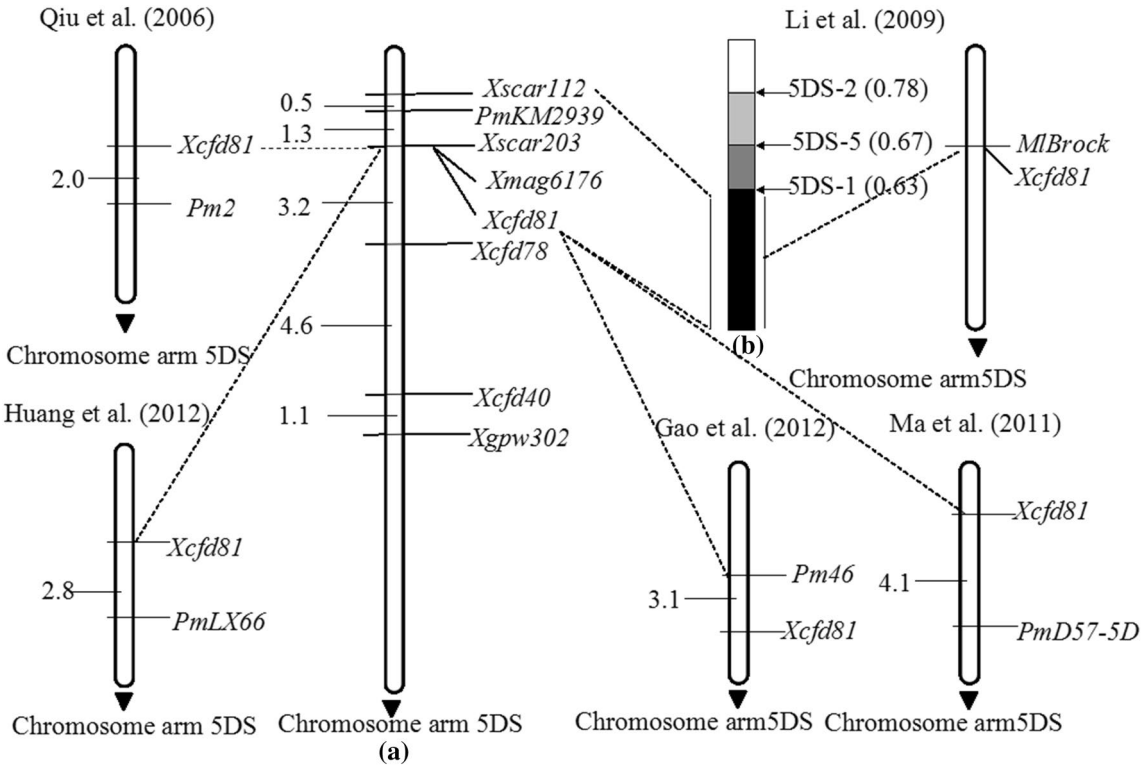

Fig. 2 Linkage map (**a**) and chromosome bin physical map (**b**) of *Pm2b* with its loci comparison with the known *Pm* genes on chromosome arm 5DS. Genetic distances shown on the *left* are in cM. *Black arrow* points to the centromere

mapped to bin 5DS1-0-0.63 containing *Pm2a,* were also tested for polymorphism between the parents and bulks, and polymorphic marker *Mag6176* was derived by conversion from EST BE498794. This is the same as the *Pm2a-linked* marker (*Xmag6176*) described by Ma et al. (2011). It was located 1.3 cM proximal to *Pm2b* (Fig. 2a). The loci order of linked markers was *Xscar112–Pm2b–Xscar203/Xmag6176/Xcfd81–Xcfd78–Xcfd40–Xgpw302*. Based on the previous studies, marker loci *Xcfd81*, *Xscar112* and *Xscar203* were all assigned to the deletion bin 5DS-1-0-0.63 (Ma et al. 1994; Gao et al. 2012; Huang et al. 2012). Therefore, *Pm2b* flanked by these markers should be located in the same chromosome bin (Fig. 2b). As EST BE498794 was previously mapped in bin 5DS-1-0-0.63 (http://wheat.pw.usda.gov/cgi-bin/gbrowse/WheatPhysicalESTMaps/#search), its converted EST-SSR marker locus *Xmag6176* linked with *Pm2b* must be located in the same chromosome position.

Table 2 Comparative responses of KM2939 and wheat genotypes with known powdery mildew resistance genes on chromosome arm 5DS to 14 isolates of *Blumeria graminis* f. sp. *tritici* (*Bgt*) virulent differently to these *Pm* genes selected from 27 *Bgt* isolates tested

Cultivar/line	*Pm* gene	*Bgt* isolate[a]													
		E02	E05	E09	E15	E16	E18	E20	E21	E30−1	E30−2	E31	Bg02	Bg03	Bg04
KM2939	*Pm2b*	0[b]	0	0	0	0	1	2	3	0	0	0	0	0	0
Ulka/8*Cc	*Pm2a*	0	4	0	0	1	4	4	4	0	4	3	1	0	4
Tabasco	*Pm46*	0	3	0	2	0	3	4	0	0	0	0	0	0	0
Liangxing 66	*PmLX66*	0	3	1	3	3	4	4	0	0	0	0	0	0	4
X3986-2	*PmX3986-2*	4	4	4	4	0	2	3	0	4	1	3	4	4	4

[a] Each *Bgt* isolate (refer to Supplementary Table S1) represents a different race (Zhou et al. 2002)

[b] Infection types (IT) were scored on a 0–4 scale, where ITs 0, 0;, 1 and 2 were considered resistant and ITs 3 and 4 were considered susceptible (Si et al. 1992)

Comparison of *Pm2b* and known *Pm* genes on chromosome arm 5DS

Twenty-seven *Bgt* isolates were used to compare the seedling stage powdery mildew reactions conferred by *Pm2b*, *Pm2a*, and temporarily designated *Pm* genes *Pm46*, *PmLX66* and *PmX3986-2* (Supplementary Table S1). KM2939 was resistant to 25 of 27 isolates (92.6 %), except for E21 and E32. This was a wider range of effectiveness than conferred by the donor of *Pm2a*, a widely used gene in Chinese wheat breeding programs (19 of 27 isolates (70.4 %) avirulent). *Pm46*, a temporarily designated *Pm* gene in German cultivar Tabasco, conferred resistance to 23 of 27 (85.2 %) isolates, *PmLX66*, a temporarily designated gene in Liangxing 66, one of the most widely grown cultivars in China, conferred resistance to 20 of 27 (74.1 %) isolates, and *PmX3986-2*, a temporarily designated gene in an indigenous germplasm, conferred resistance to 16 of 27 (Ma et al. 2014). All the three genes conferred narrower resistance spectra than *Pm2b* to the isolates tested. Data for 14 differential *Bgt* isolates are shown in Table 2; the total data are provided in Supplementary Table S1. The resistance spectrum of *Pm2b* was clearly different from those for *Pm2a*, *Pm46*, *PmLX66* and *PmX3986-2*. It was concluded that *Pm2b* is different from other *Pm* genes located on chromosome arm 5DS.

To further clarify the allelic relationships of *Pm2b* and other *Pm* genes on chromosome arm 5DS, a total of 6,112 F_2 plants from KM2939//Ulka/8*Cc (*Pm2a*) were inoculated with culture E09 that is avirulent to both the parents. No susceptible F_2 plant was found, demonstrating that *Pm2b* and *Pm2a* are likely at the same locus. Also no susceptible plant occurred among 1,112 and 292 F_2 plants from crosses KM2939/Liangxing 66 and Liangxing 66/KM2939 tested with the same isolate, indicating that *Pm2b* and *PmLX66* were also allelic or closely linked in repulsion.

Applicability of the closely linked markers in MAS and development of an NIL

Of the markers linked to *Pm2b*, *Cfd81*, *SCAR112*, *SCAR203* and *Gpw302* showed polymorphisms between KM2939 and recipient cultivars Shimai 15, Kenong 199, Shixin 828 and Gaocheng 8901, whereas markers *Cfd40*, *Cfd78* and *Mag6176* were polymorphic only between KM2939 and one or two of the recipients.

For MAS, susceptible cultivar Gaocheng 8901 was selected as a high quality wheat recipient. Using the closely linked markers, we selected a high gluten quality, powdery mildew resistant, wheat line after backcrossing and selfing for several generations. This line is now taken part in our own yield comparation test. For transferring *Pm2b* to more susceptible cultivars in current production, BC_2F_1 populations of KM2939/Shimai 15, and BC_1F_1 plants from crosses of KM2939 with Kenong 199 and Shixin 828 are being subjected to MAS using *Pm2b*-linked markers. F_1 hybrids of KM2939 and other cultivars in current production, such as Han 7086, Han 6172, Ji 5265, Luyuan502, Zhou 16, Zhou 8425B and Zhoumai 22, have also been obtained, and will be backcrossed with the cultivars and subjected to MAS in the near future.

To develop an NIL with *Pm2b*, Shimai 15, a susceptible cultivar widely grown on the North China Plain, was selected as a recurrent parent. A BC_2F_1 population of KM2939/3*Shimai 15 was obtained, and 100 BC_2F_1 plants possessing resistance to *Bgt* isolate E09 and having the appropriate *Cfd81* allele were selected prior to background selection. To evaluate the genetic similarity of the selected lines to the backcross parent Shimai 15, 97 polymorphic SSR markers randomly distributed on all 21 wheat chromosomes were used for background selection. A BC_2F_1 plant, coded as KS148, with 91 homozygous marker loci identical to Shimai 15 and 6 heterozygous marker loci was selected. RGC of KS148 was thus

96.9 %. Three other BC_2F_1 plants had RGC values of 95.9, 95.5 and 95.4 %, respectively.

Discussion

Exploitation of powdery mildew resistance genes from progenies of distant hybridization

Exploitation of resistance genes from progenies of distant hybridization is an important and effective way of increasing diversity of resistance to powdery mildew in wheat. Unfortunately, many translocation lines have questionable value in wheat improvement, because they often have large and complex chromosome segments that do not fully compensate for lost wheat chromatin or carry additional deleterious features (linkage drag) (Young and Tanksley 1989). If the alien chromosome segment is small, it is more likely to have value in wheat improvement. Occasionally, valuable traits are transferred to recipient genotypes without detectable cytological changes (Kuraparthy et al. 2007a, b). In the Kuraparthy et al. (2007a, b) reports, authenticity of alien origin was supported by the presence of linked markers derived from the alien source and availability of alien addition lines used as the donor sources and experimental controls. Disease resistance genes identified in such lines in wheat also include powdery mildew resistance genes *Pm40* and *Pm43* (He et al. 2009; Luo et al. 2009) putatively derived from *Th. intermedium*, and stripe rust resistance genes *Yr50* and *YrL693* also putatively derived from *Th. intermedium* (Liu et al. 2013; Huang et al. 2014). Irrespective of origin, these genes are important sources of resistance.

Agropyron cristatum is a perennial *Triticeae* species and a valuable source of resistance to many wheat diseases, including powdery mildew (Li et al. 1995). Since it was successfully crossed with common wheat, attempts have been made to introgress useful traits to wheat (Li et al. 1997; Wu et al. 2006; Luan et al. 2010). The Chinese breeding line KM2939 is a derivative of *A. cristatum*. All known common wheat parents in the KM2939 pedigree were tested against *Bgt* isolate E09, and they were all highly susceptible to powdery mildew with the IT 4. However, *Pm2b* is mostly likely a wheat gene rather than an *A. cristatum* derived gene. Firstly, over several years of observations in the field, KM2939 was genetically and agronomically uniform. Secondly, KM2939 has 42 chromosomes and thus far GISH analysis and molecular marker special for the *A. cristatum* genome have failed to detect *A. cristatum* chromatin (data not shown). Thirdly, the powdery mildew resistance gene in KM2939 behaved as a single Mendelian unit and was mapped by wheat-derived markers, with the two closest flanking markers only 0.6 and 1.3 cM from *Pm2b*. Importantly, *Pm2b* appears to be allelic with the known wheat genes. Therefore, *Pm2b* is more likely a wheat gene derived from unknown source.

Relationships between *Pm2b* and known Pm genes on chromosome 5D

There are three *Pm* genes reported on chromosome arm 5DL; viz. *Pm34* flanked by the SSR markers *Barc177* and *Barc144* (Miranda et al. 2006), *Pm35* linked to *Cfd26* at a distance of 11.9 cM (Miranda et al. 2007) and *Pm-M53* flanked by *Wmc289* and *Gwm292* (Li et al. 2010). These three genes are at genetic distances of about 92, 41 and 55 cM, respectively, from *Pm2b* based on reported maps (Somers et al. 2004). *Pm2b* confers a significantly different response spectrum from *Pm34* and *Pm35* when tested with an array of 27 *Bgt* isolates (Supplementary Table S1). Obviously, *Pm2b* differs from these genes. *Pm2a*, identified in the wheat landrace Ulka and located on chromosome arm 5DS, is linked to SSR marker *Cfd81* with a genetic distance of 2.0 cM (Qiu et al. 2006), and distance of 3.3 cM from *Pm2b* (Fig. 2a). Temporarily designated *Pm* genes reported on chromosome arm 5DS include *MlBrock* which co-segregated with *Cfd81* in cultivar Brock (Li et al. 2009), *PmD57-5D* flanked by *Mag6176* and *Gwm205* in line D57 (Ma et al. 2011), *Pm46* flanked by *Mp510* and *Gwm205* in German cultivar Tabasco (Gao et al. 2012), *PmLX66* flanked by *Cfd81* and *SCAR203* in Chinese wheat cultivar Liangxing 66 (Huang et al. 2012) and *PmX3986-2* flanked by *Cfd81* and *SCAR112* in an indigenous germplasm (Ma et al. 2014). Among these, *PmD57-5D* and *Mlbrock* are likely to be *Pm2a* (Li et al. 2009; Ma et al. 2011). *Pm46*, *PmLX66* and *PmX3986-2* are at genetic distances of 1.8, 4.1 and 0.7 cM from *Pm2b* based on reported maps (Huang et al. 2012; Gao et al. 2012). To clarify the relationships between *Pm2b* and *Pm2a*, *Pm46*, *PmLX66* and *PmX3986-2*, 27 *Bgt* isolates were tested on a panel of wheat genotypes including KM2939, Ulka/8*Cc (with *Pm2a*), Tabasco (with *Pm46*), Liangxing 66 (with *PmLX66*) and X3986-2 (with *PmX3986-2*). The results indicated that *KM2939* confers a different reaction pattern compared to lines with *Pm2a*, *Pm46*, *PmLX66* and *PmX3986-2* (Table 1). Therefore, *Pm2b* is a different specificity from *Pm2a*, *Pm46*, *PmLX66* and *PmX3986-2*. In a test of allelism of *Pm2b* and *Pm2a* using 6,112 F_2 plants from KM2939/Ulka/8*Cc inoculated with isolate E09, no susceptible plant was found. Similar results were obtained for *Pm2b* and *PmLX66* using 1,112 and 292 F_2 plants from reciprocal crosses KM2939/Liangxing 66 and Liangxing 66/KM2939, respectively. Therefore, *Pm2b* is apparently located at the same locus as *Pm2a* and *PmLX66*. *Pm46* was previously shown to be closely linked, but not allelic, to *Pm2a* (Gao et al. 2012). That is, *Pm2b* is also closely linked, but not allelic, to *Pm46*. Thus, based on

response spectrum and allelism tests, *Pm2b* appears to be a novel allele of *Pm2a*, and was therefore designated *Pm2b*; the previous allelic designation *Pm2* will be re-designated as *Pm2a*.

Transferring *Pm2b* into wheat cultivars

Whether a resistance gene has potential in wheat improvement depends, to some extent, on the agronomic traits of the donor. Fortunately, KM2939 possesses some valuable inheritances of high-yield characteristics, e.g., much higher kernel numbers compared with main cultivars in China ($P < 0.01$), and as for other agronomic traits, such as spike length, spike number, spikelet number, thousand kernel weight and sterile spikelet number, KM2939 appears to perform competitively with current leading cultivars (data not shown). Therefore, KM2939 is an attractive high-yielding line that will attract the attention of breeders without the need for intensive pre-breeding prior to incorporation of the resistance gene into breeding populations.

Seven molecular markers were closely linked to *Pm2b* in the BC_2F_1 population KM2939/3*Shimai 15. However, not all the closely linked markers identified in genetic studies diagnose a resistance gene in all genetic backgrounds. To use these markers, it is necessary to trace linked alleles in specific crosses. Likewise, three RAPD markers linked to *Pm25* cannot diagnose *Pm25* in some backgrounds carrying it due to excessive genetic distances between the markers and *Pm25* (Shi et al. 1998). Hartl et al. (1995) reported that it was difficult to identify all cultivars possessing *Pm1* and *Pm2a* using closely linked RFLP markers. A similar result was obtained for *Pm2a* using an STS marker converted from the corresponding RFLP marker linked to *Pm2a* (Mohler and Jahoor 1996). Therefore, it is necessary to confirm the availability of the closely linked markers in different genetic backgrounds for MAS. After verification, four of the seven markers can be used as more effective markers for selection of *Pm2b* in different genetic backgrounds. Work on transferring *Pm2b* into different susceptible cultivars has been carried out for several years by MAS, and we have obtained an advanced line currently in our own yield comparation test, an NIL with RGC of 96.9 %, and several selected populations that show resistance to powdery mildew at the both seedling and adult stages.

Author contribution statement P Ma Experimental implementation, data analysis and manuscript preparation. H. Xu Production of the mapping population and genetic map. Y. Xu Production of the genetic map. Y. Qie MAS analysis. Q. Luo Genomic in situ hybridization and molecular marker detection. X. Zhang, X. Li Germplasm creation and field investigation. Y. Zhou Powdery mildew tests. D. An, L. Li Study concept and design.

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Conflict of interest The authors (Pengtao Ma, Hongxing Xu, Yunfeng Xu, Lihui Li, Yanmin Qie, Qiaoling Luo, Xiaotian Zhang, Xiuquan Li, Yilin Zhou and Diaoguo An) declare that our experiments comply with the current laws of China and we have no conflicts of interest.

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