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ORIGINAL ARTICLE

# Molecular tagging of a new broad-spectrum powdery mildew resistance allele *Pm2c* in Chinese wheat landrace Niaomai

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**Abstract**

***Key message*** **A new broad-spectrum powdery mildew resistance allele *Pm2c* was identified and mapped in Chinese wheat landrace Niaomai.**

*Abstract* Chinese wheat landrace Niaomai showed resistance to 27 of 28 Chinese *Blumeria graminis* f. sp *tritici* (*Bgt*) races. Genetic analysis of an $F_2$ population and its derived $F_{2:3}$ families from the cross Niaomai × Mingxian 169 and backcross population, Niaomai/2*Mingxian 169, indicated that the resistance of Niaomai to *Bgt* races was conferred by a single dominant resistance gene, temporarily designated *PmNM*. Molecular tagging showed that *PmNM* was located on chromosome 5DS and flanked by SSR markers *Xcfd81* and *Xcfd78* with the genetic distances of 0.1/0.4 cM and 4.9/7.5 cM, respectively. Niaomai showed a different array of responses compared to lines with *Pm2a*, *Pm2b*, *PmD57-5D*, *PmLX66*, *PmX3986-2* and *Pm48* genes, sharing the same *Xcfd81* allele but differing from *Xcfd78* allele for *Pm2a* and *Pm2b* lines. Allelism tests based on crosses of Niaomai with Ulka/8*Cc and KM2939 showed that *PmNM* is allelic to *Pm2a* and *Pm2b*. We concluded that *PmNM* is a new allele of *Pm2*, re-designated *Pm2c*. *Pm2c* could be transferred into wheat cultivars by marker-assisted selection to improve the powdery mildew resistance of breeding cultivars/lines.

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## Introduction

Powdery mildew, caused by the *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a devastating foliar disease occurring throughout the wheat growing regions of the world. Resistant cultivars and resistance genes are the most effective measure to curb this disease. The most widely used resistance genes are major genes conferring race-specific resistance. However, such resistance genes can be defeated by new virulent races or by races that were previously present at very low frequencies in the pathogen population (McDonald and Linde 2002). Therefore, it is essential to explore and identify new sources of broad-spectrum resistance on a continuing basis.

At present, about 69 formally designated powdery mildew (*Pm*) resistance genes *Pm1–Pm54* at 49 loci (*Pm8* is allelic to *Pm17*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, and *Pm31* = *Pm21*) and more than 20 temporarily named *Pm* genes have been reported. These genes are distributed on all chromosomes except 3D (McIntosh et al. 2013; Mohler et al. 2013; Petersen et al. 2015; Hao et al. 2015). Seven of these loci, namely *Pm1* (Hsam et al. 1998; Peusha et al. 1995), *Pm2* (Briggle 1969; Ma et al. 2015), *Pm3* (Tommasini et al. 2006; Bhullar et al. 2009), *Pm4* (Singrün et al. 2003; Hao et al. 2008; Schmolke et al. 2012), *Pm5* (Hsam et al. 2001; Huang et al. 2003), *Pm8*/*Pm17* (Hsam and Zeller 1997) and *Pm24* (Huang et al. 2000b; Xue et al. 2012) have more than one resistance allele. Most of these major resistance genes are dominant and easily used in breeding for resistance. However, relatively few *Pm* genes have been

successfully applied in breeding or in development of resistant cultivars in China. The genes that have been used include *Pm2a*, *Pm4a*, *Pm6*, *Pm8* and *Pm21* (Zhang et al. 2010; Huang et al. 2012).

Molecular markers, especially simple sequence repeat (SSR) markers, were widely used for locating and tagging *Pm* genes in wheat (McIntosh et al. 2013; Li et al. 2014; Peng et al. 2014; Zhao et al. 2014; Hao et al. 2015; Petersen et al. 2015). High-density SSR maps of wheat (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005) greatly facilitate the location and mapping of *Pm* resistance genes. More than 30 formally designated and most of the temporarily named *Pm* genes have been mapped with SSR markers (Huang and Röder 2004; Alam et al. 2011; McIntosh et al. 2013). Diagnostic SSR markers tightly linked to *Pm* genes contribute to the development of near-isogenic lines (Zhou et al. 2005), and transfer and pyramiding of resistance genes into released cultivars (Liu et al. 2000; Xu et al. 2008; Ma et al. 2015) through marker-assisted selection (MAS). Furthermore, closely linked flanking markers will also aid in fine-mapping and map-based cloning of the resistance genes (Yahiaoui et al. 2004; Qin et al. 2011; Ouyang et al. 2014; Wang et al. 2014).

Wheat landraces are valuable resistance genetic resources. Six documented *Pm* resistance genes, *Pm5e* (Huang et al. 2003), *Pm24a* (Huang et al. 2000b), *Pm24b* (Xue et al. 2012), *Pm47* (Xiao et al. 2013), *mlxbd* (Huang et al. 2000a) and *pmX* (Fu et al. 2013) are derived from wheat landraces Fuzhuang 30, Chiyacao, Baihulu, Hongyanglazi, Xiaobaidong and Xiaohongpi, respectively. Niaomai, a Chinese wheat landrace, was highly resistant in the field to a composite of *Bgt* races in north China at the adult stage. The major objective of this study was to identify and map the resistance gene(s) in Niaomai and to compare the relationship with documented alleles at the locus of the resistance gene(s).

## Materials and methods

### Plant materials and pathogen isolates

The Chinese wheat landrace Niaomai was highly resistant to powdery mildew in wheat and Mingxian 169 was highly susceptible. The populations derived from the cross of Niaomai and Mingxian 169 were used to map the powdery mildew resistance gene(s) in Niaomai. Niaomai and common wheat cultivars/lines KM2939 (*Pm2b*, Ma et al. 2015), Liangxing 66 (*PmLX66*, Huang et al. 2012), X3986-2 (*PmX3986-2*, Ma et al. 2014), Zhongmai 155 (*PmZ155*, Sun et al. 2015), Wennong 14 (*PmW14*, Song et al. 2014), Liangxing 99 (*Pm52*, Zhao et al. 2014), Jimai 22 (*PmJM22*, Yin et al. 2009), Shixin 828, Kenong 199, Shimai 15, Yangmai 158, Zhengmai 366, Zhou 8425B, Lumai 21 and Mingxian 169 are maintained in our laboratory. Seed of Niaomai have been provided to R.A. McIntosh, the author of catalogue of gene symbols for wheat and are publicly available. Seed of Ulka/8*Cc (*Pm2a*), Brock (*MlBrock*, Li et al. 2009), Maris Huntsman (*Pm2a+Pm6*) and Maris Dove (*Pm2a+PmMLD*) were kindly provided by Professor Hongyan Liu, Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhenzhou, D57-5D (*PmD57-5D*, Ma et al. 2011) was provided by Professor Zhengqiang Ma, Nangjing Agricultural University, Nanjing, and the German cultivar Tabasco (*Pm48*, Gao et al. 2012) was provided by Professor Shibin Cai, Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Nanjing. Twenty-eight single spore-derived *Bgt* cultures were used to compare the reaction patterns of Niaomai and the lines with documented alleles at the locus of the resistance gene(s) in Niaomai. *Bgt* race E09, a predominant powdery mildew race in North China, was used to evaluate the mapping populations and allelic tests populations.

### Resistance phenotyping

Reactions of plant materials to *Bgt* races were determined in a greenhouse. Seedlings were grown in rectangular trays in a growth chamber; each tray had 128 cells (3 × 3 cm) and the susceptible check Mingxian 169 was planted randomly in the trays. All seedlings were inoculated with fresh conidiospores increased on Mingxian 169 seedlings and incubated in a chamber at 18 °C for 24 h and then placed at a greenhouse with a daily cycle of 14 h of light at 22 °C and 10 h of darkness at 18 °C. Response data were scored 8–10 days after inoculation. Infection types (ITs) on each plant were assessed on a 0–4 scale, of which 0 = no visible symptoms and signs, 0 = necrotic flecks without sporulation, 1 = sparse aerial hypha and little sporulation, diameter of colonies less than 1 mm, 2 = moderate aerial hypha and sporulation, diameter of colonies less than 1 mm, 3 = thick aerial hypha and abundant sporulation, diameter of colonies more than 1 mm, and 4 = abundant sporulation with more than 80 % of the leaf area covered with aerial hypha, with IT 0, 0;, 1 and 2 being regarded as resistant, and IT 3 and 4 as susceptible (Fig. 1; Si et al. 1992). All tests were repeated to assure reliability of the data.

### Marker analysis

Total genomic DNA was extracted from leaf tissues following a procedure described by Ma et al. (1994). DNA bulks were prepared for bulked segregant analysis (BSA) by combining equal amounts of DNA from 10 resistant (IT = 0) or 10 susceptible (IT = 4) $F_2$ plants from the cross Niaomai × Mingxian 169.

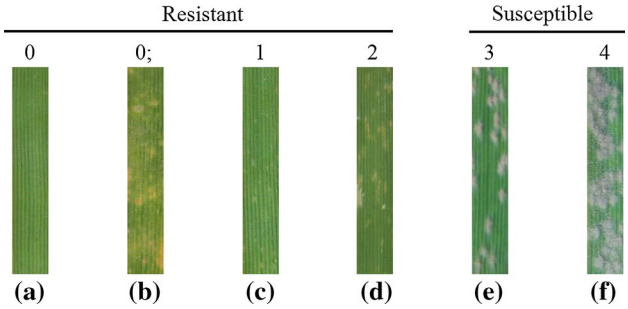


**Fig. 1** Infection types (ITs) of wheat cultivars/lines to *Blumeria graminis* f. sp. *tritici* races at the seedling stage. IT 0, 0;, 1 and 2 were regarded as resistant (**a**–**d**), and IT 3 and 4 as susceptible (**e**, **f**)

SSR markers evenly distributed across all the chromosomes (Röder et al. 1998; Paillard et al. 2003; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005; Xue et al. 2008) were selected for an initial survey of polymorphism. SCAR markers SCAR203 and SCAR112 were developed by Li et al. (2009). PCR was performed in a Veriti® thermal cycler (Applied Biosystems, USA) in 10 µl reaction mixtures containing 10–20 ng of template DNA, 2 pmol of each primer, 2 nmol of each dNTP, 0.1 U of Taq DNA polymerase and 1× PCR buffer (with $MgCl_2$). The PCR profile included: one cycle of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50–65 °C (depending on the specific primers) for 40 s, 72 °C for 40 s, and a final extension at 72 °C for 5 min. PCR products were separated in 8 % non-denaturing polyacrylamide gels with 19:1, 25:1 or 39:1 ratios of acrylamide and bis-acrylamide, and silver-stained prior to visualizing the banding patterns as described by Santos et al. (1993).

### Statistical analysis

Chi-squared ($\chi^2$) tests for goodness-of-fit were used to evaluate deviations of observed data from expected segregation ratios. The software MAPMAKER/Exp (version 3.0b) was used to determine linkage with a LOD score of 3.0 as the threshold for declaration of linkage (Lander et al. 1987). Genetic distances were estimated from recombination values using the Kosambi mapping function (Kosambi 1944).

## Results

### Inheritance of the powdery mildew resistance in Niaomai

Segregating populations from Niaomai × Mingxian 169 and the parents were tested with *Bgt* race E09 at the seedling stage. Niaomai was almost immune with IT 0; and Mingxian169 was susceptible with IT 4. $F_1$ plants were resistant indicating dominance. Among 2983 $F_2$ plants, 2235 were resistant with IT 0–2 (only 24 plants with IT = 2) and 748 was susceptible with IT 3–4, fitting a 3:1 ratio ($\chi^2 = 0.0055$, $P = 0.94$). Two hundred and thirteen $F_{2:3}$ lines with more than 24 seedlings were tested to determine the genotypes of the $F_2$ plants; 53 lines were homozygous resistant, 105 segregated in a dominant manner, and 55 were homozygous susceptible, fitting a 1:2:1 segregation ratio ($\chi^2 = 0.80$, $P = 0.96$). Backcross $F_1$ plants from Niaomai × Mingxian $169^2$ segregated 279 resistant:266 susceptible, fitting a ratio of 1:1 ($\chi^2 = 0.31$, $P = 0.58$). These results clearly indicated that a single dominant resistance gene, temporarily designated *PmNM*, in Niaomai conferred resistance to race E09. When challenged by 27 other *Bgt* races Niaomai was susceptible only to race E32 (Table 1). Ten randomly selected homozygous resistant 10 $F_{2:3}$ lines from Niaomai × Mingxian 169, were challenged by the other 26 races avirulent to Niaomai. All the 10 $F_{2:3}$ lines were homozygous resistant to all 26 races showing that *PmNM* conferred the resistance to all avirulent races.

### Mapping resistance gene *PmNM* in Niaomai

In an initial survey of polymorphism between Niaomai and Mingxian 169 and the DNA bulks with 210 SSR markers distributed across the wheat genome only CFD81 amplified a consistent polymorphism between the parents and bulks. Because marker *Xcfd81* was tightly linked *Pm2* (Qiu et al. 2006) and *Pm48* (Gao et al. 2012) located on chromosome 5DS, we tested a further 30 markers previously mapped on this chromosome arm; 12 markers (*Xscar203*, *Xscar112*, *Xcfd78*, *Xgwm159*, *Xcfd67*, *Xwmc608*, *Xcfd40*, *Xwmc805*, *Xgwm16*, *Xgwm190*, *Xcfd8*, and *Xcfd18*) were polymorphic. Thirty-two $F_{2:3}$ lines from Niaomai × Mingxian 169 were genotyped with the SSR markers CFD81 and CFD78, and close associations of *Xcfd81* and *Xcfd78* with *PmNM* were observed (Fig. 1). All 12 markers were then genotyped on the entire $F_{2:3}$ population. Markers *Xgwm16*, *Xgwm190*, *Xcfd8*, and *Xcfd18* showed no linkage with *PmNM*. A genetic linkage map of *PmNM* constructed with the eight linked marker loci showed that *PmNM* was flanked by *Xcfd81* and *Xcfd78* with genetic distances of 0.4 cM distal and 7.5 cM proximal (Fig. 2a). Seven markers (*Xscar112*, *Xcfd81*, *Xcfd78*, *Xgwm159*, *Xcfd67*, *Xcfd40* and *Xwmc805*) were also genotyped on the $BC_1F_1$ population and in this case the flanking distances of *Xcfd81* and *Xcfd78* were 0.1 and 4.9 cM, respectively (Fig. 2b). Thus *PmNM* was located on chromosome 5DS at a position at or near the *Pm2* locus.

**Table 1** Response spectra of Niaomai and lines with documented alleles at or near the *Pm2* locus

| Var./lines | *Pm* gene | E01 | E02 | E03 | E05 | E06 | E07 | E09 | E11 | E13 | E16 | E17 | E18 | E20 | E21 | E22 | E23-1 | E23-2 | E26 | E30-1 | E30-2 | E31 | E32 | E49 | E50 | B13 | B14 | B41 | B45 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mingxian 169 | – | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Niaomai | *PmNM* | 0; | 0; | 0 | 0 | 0 | 0; | 0; | 0; | 0 | 0; | 0; | 0 | 0 | 0; | 0 | 0 | 0; | 0; | 0; | 0; | 0; | 4 | 0; | 0; | 0 | 0 | 0 | 0; |
| Ulka/8*Cc | *Pm2a* | 0; | 0; | 0 | 4 | 0; | 0; | 0; | 0; | 0; | 1 | 0; | 4 | 4 | 0; | 0 | 0; | 0; | 0; | 0; | 4 | 0 | 4 | 0; | 0; | 0 | 4 | 1 | 0; |
| KM2939 | *Pm2b* | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 1 | 2 | 3 | 0 | 0; | 0; | 0; | 0; | 0; | 0; | 3 | 0; | 0; | 0 | 4 | 0; | 0; |
| Maris Huntsman | *Pm2a + 6* | 0; | 0; | 0 | 3 | 0; | 0; | 0; | 0; | 0 | 0 | 0; | 4 | 4 | 0; | 0 | 0; | 0; | 0; | 0; | 3 | 0 | 3 | 0; | 0; | 0 | 4 | 0 | 0; |
| Maris Dove | *Pm2a + MLD* | 0; | 0 | 0 | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 3 | 0; | 0 | 0; | 0 | 0; | 0; | 3 | 0 | 4 | 0 | 0; | 0 | 4 | 0; | 0; |
| D57-5D | *PmD57-5D* | 0; | 0; | 0; | 4 | 0; | 0; | 0 | 0; | 0; | 0; | 0; | 3 | 4 | 0 | 0 | 0; | 0; | 0; | 0; | 4 | 0; | 4 | 0 | 0 | 0 | 4 | 0; | 0; |
| Liangxing 66 | *PmLX66* | 0 | 0; | 0 | 3 | 0; | 0; | 1 | 0; | 0; | 3 | 0; | 4 | 4 | 0; | 0 | 0; | 1 | 0; | 0; | 4 | 0 | 4 | 0 | 0; | 0 | 4 | 0; | 0; |
| X3986-2 | *PmX3986-2* | 0; | 4 | 0 | 4 | 0 | 0; | 4 | 0; | 0; | 0; | 0; | 2 | 3 | 0; | 4 | 0; | 0; | 0; | 0; | 4 | 3 | 4 | 0; | 0; | 4 | 4 | 4 | 0; |
| Tabasco | *Pm48* | 0; | 0; | 0 | 3 | 0; | 0 | 0 | 0 | 0; | 0 | 0; | 3 | 4 | 0 | 0 | 0 | 1 | 0; | 0 | 0; | 0; | 4 | 0; | 0; | 0 | 0 | 0; | 0 |

## Powdery mildew reactions of Niaomai and various resistant lines

Isolates of 28 *Bgt* races (Ma et al. 2014) were used to compare reaction patterns of Niaomai with lines possessing other resistance genes on chromosome 5DS. Niaomai (*PmNM*) was resistant to 27 races and was susceptible only to race E32 (Table 1). Ulka/8*Cc (*Pm2a*) and D57-5D (*PmD57-5D*) were both susceptible to races B14, E05, E18, E20, E30-2 and E32 (Table 1; Fig. 3). In addition to these six races Liangxing 66 (*PmLX66*) was susceptible to race E16 with IT 3 (Table 1). Therefore, Niaomai showed a different reaction to five and six races compared to Ulka/8*Cc (or D57-5D) and Liangxing 66, respectively. Predictably, the reactions of Niaomai also differed from those of Maris Huntsman (*Pm2a+Pm6*) and Maris Dove (*Pm2a+PmMLD*) (Table 1). KM2939 (with *Pm2b*) showed an intermediate reaction to race E20 and was susceptible to E21 and B14, whereas Niaomai was clearly resistant to these races. Eleven races were virulent to X3986-2 (*PmX3986-2*). Tabasco (with *Pm48*) was susceptible to E05, E18 and E20, whereas Niaomai was resistant. Thus, the *PmNM* gene in Niaomai gave a reaction array that was different from other resistance genes at or near the *Pm2* locus (Table 1).

## Allelism of *PmNM* and *Pm2*

$F_2$ populations from reciprocal crosses between Niaomai (*PmNM*) and Ulka/8*Cc (*Pm2a*) were tested with race E09. All 5593 $F_2$ plants from Niaomai//Ulka/8*Cc and 2058 plants from the reciprocal cross were immune, indicating that *PmNM* was allelic to *Pm2a*. Likewise, a population of 1586 $F_2$ plants from Niaomai/KM2939 was also resistant. We concluded that *PmNM* is allelic to *Pm2a* and *Pm2b*.

## Molecular variability and validation of diagnostic markers flanking *PmNM* in the common wheat cultivars/lines

The markers flanking *PmNM* were used to test 18 common wheat cultivars/lines with or without *Pm2* resistance allele (Fig. 4). Ulka/8*Cc, KM2939, Brock, D57-5D, Liangxing 66, X3986-2, Zhongmai 155 and Wennong 14 were reported as carrying *Pm2* resistance alleles, and Tabasco carried the tightly linked gene *Pm48*. The other nine wheat cultivars/lines, Liangxing 99 (*Pm52*), Jimai 22 (*PmJM22*), Shixin 828, Kenong 199, Shimai 15, Yangmai 158, Zhengmai 366, Zhou 8425B and Lumai 21 did not carry a resistance allele at the *Pm2* locus. The marker CFD81 amplified a band of about 255 bp in Niaomai, Ulka/8*Cc, KM2939, Brock, D57-5D, Liangxing 66, Tabasco, X3986-2, Zhongmai 155, Wennong 14, Liangxing 99 and Jimai 22, whereas

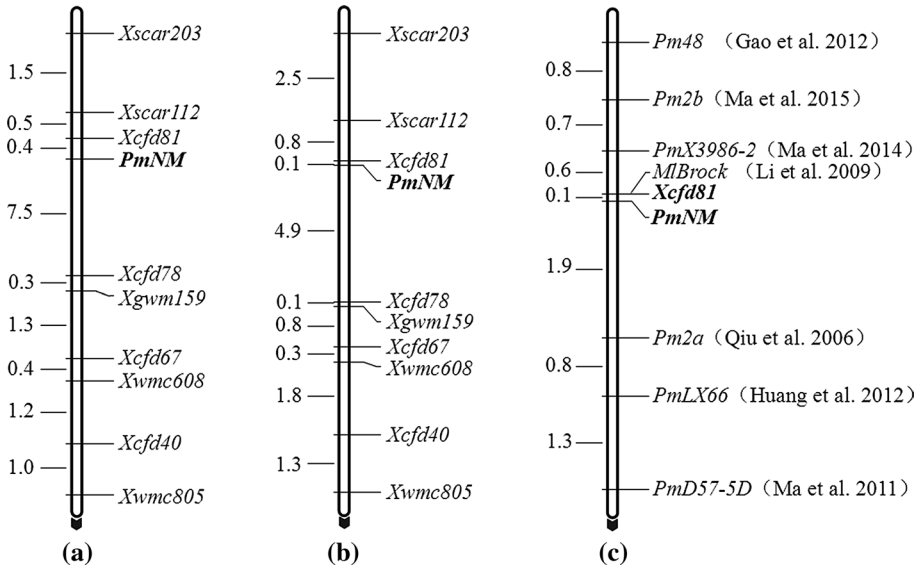


**Fig. 2** Genetic maps for *PmNM* genotyped on $F_{2:3}$ lines of Niaomai/Mingxian 169 (**a**) and backcross population Niaomai//2*Mingxian 169 (**b**), and the positional comparison for documented *Pm* resistance genes on chromosome 5DS (**c**). Genetic distances are shown on the *left*

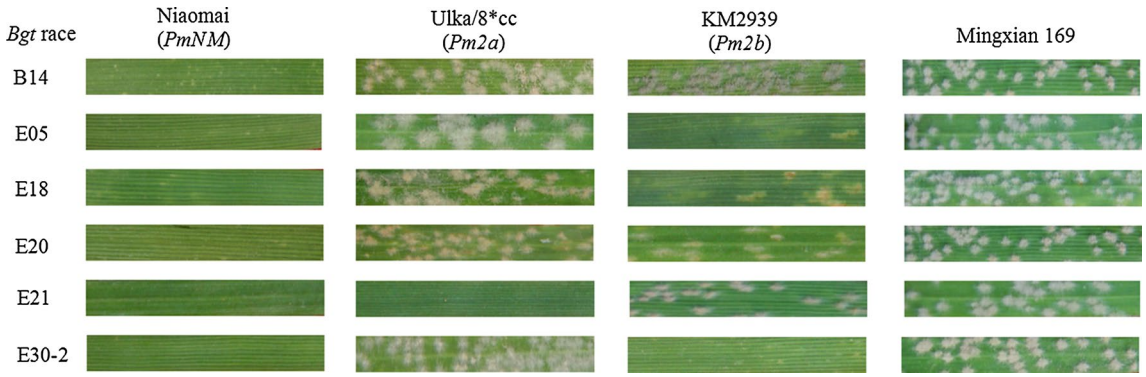


**Fig. 3** Powdery mildew reactions of Niaomai (*PmNM*), Ulka/8*Cc (*Pm2a*), KM2939 (*Pm2b*), Mingxian 169 (susceptible check) to selected *Blumeria graminis* f. sp. *tritici* races

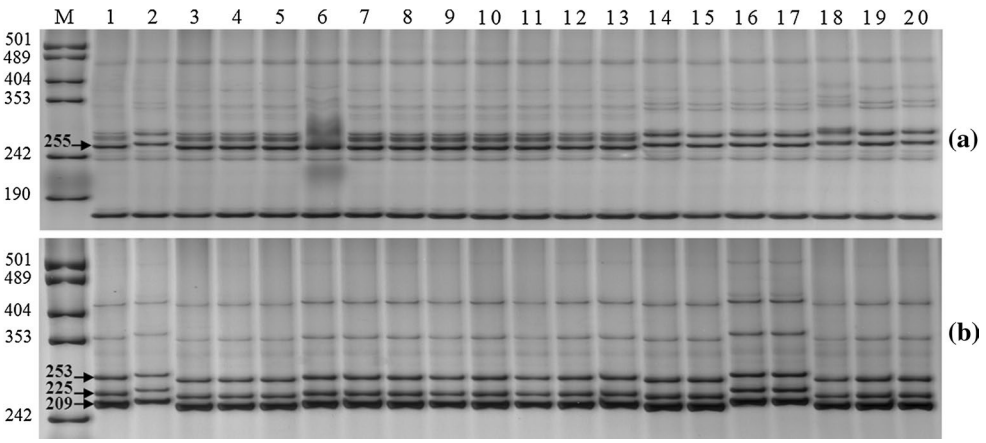


**Fig. 4** Amplification profiles generated by primers CFD81 (**a**) and CFD78 (**b**) on Niaomai, Mingxian 169, Ulka/8*Cc, KM2939, Brock, D57-5D, Liangxing 66, Tabasco, X3986-2, Zhongmai 155, Wennong 14, Liangxing 99, Jimai 22, Shixin 828, Kenong 199, Shimai 15, Yangmai 158, Zhengmai 366, Zhou 8425B, Lumai 21 (*lanes 1–18*). M: pUC18/*Msp*I, *numbers* to the *left* are band sizes (bp), and *black arrows* indicate the polymorphic bands in Niaomai

a band of about 260 bp was present in Mingxian 169, Shixin 828, Kenong 199, Shimai 15, Yangmai 158, Zhengmai 366, Zhou 8425B and Lumai 21 (Fig. 4a). Fifty-four cultivars/lines without a *Pm2* resistance allele were also tested with CFD81 and none amplified the 255 bp band; all amplified the 260 bp band. CFD78 amplified three associated bands of about 253, 225 and 209 bp in Niaomai, D57-5D, Liangxing 66, Tabasco, X3986-2, Zhongmai 155, Wennong 14, Liangxing 99 and Jimai 22, compared to 258, 232 and 231 bp in Mingxian 169, Shimai 15 and Yangmai 158, and 249, 221 and 205 bp in Ulka/8*Cc, KM2939, Brock, Shixin 828, Kenong 199, Zhengmai 366, Zhou 8425B and Lumai 21 (Fig. 4b). Thus all stocks with *Pm2* or a resistance gene at or near the *Pm2* locus shared the same *Xcfd81* allele with Niaomai, but were more variable at the *Xcfd78* locus. We also concluded that CFD81 was a more diagnostic marker than CFD78 for marker-assisted selection of *PmNM* across different genetic backgrounds.

## Discussion

Wheat landraces are abundant in China and numerous studies have shown that they are highly variable, both molecularly (Hao et al. 2006, 2011) and in regard to agronomic traits. The Chinese wheat landrace Niaomai was not only resistant to powdery mildew but is also highly resistant to stripe rust and leaf rust in China (data not shown). Niaomai was highly resistant to 27 of 28 *Bgt* races that represent the known pathogenic variation for this pathogen in China. Genetic analysis indicated that resistance to *Bgt* races was conferred by a dominant gene *PmNM*, that was mapped on chromosome 5DS and shown to be an allele at the *Pm2* locus.

*PmNM* was closely linked and distal to *Xcfd81* with a genetic distance of 0.1/0.4 cM. The resistance genes *Pm2a* (Qiu et al. 2006), *Pm2b* (Ma et al. 2015), *MlBrock* (Li et al. 2009), *PmD57-5D* (Ma et al. 2011), *PmLX66* (Huang et al. 2012), *PmX3986-2* (Ma et al. 2014), and *Pm48* (Gao et al. 2012) were also localized on chromosome arm 5DS and tightly linked to *Xcfd81*. These genes showed slightly different positions and genetic distances compared to *Xcfd81* (Fig. 2c), but shared the same *Xcfd81* allele with a band size of about 255 bp (Fig. 4a). However, marker locus *Xcfd78* was more variable (Fig. 4b) and no allele was diagnostic of a *Pm2* allele. We concluded that *PmNM* is a member of the complex *Pm2* locus or is very closely linked to that locus.

Of the *Pm* genes on chromosome 5DS, *MlBrock* and *PmD57-5D* are both likely *Pm2a* due to the similar chromosome positions and reaction pattern to different *Bgt* isolates (Fig. 2c; Table 1; Li et al. 2009; Ma et al. 2011). *PmLX66* and *PmX3986-2* both showed different reaction patterns compared to *Pm2a*, so both appear to be new alleles of *Pm2* or tightly linked loci (Huang et al. 2012; Ma et al. 2014). *Pm48* is present in the German cultivar Tabasco and tightly linked to *Pm2* (Gao et al. 2012). In this study, *PmNM* showed a different reaction patterns with respect to 5, 3, 6, 11 and 3 of 28 *Bgt* isolates compared to *Pm2a*, *Pm2b*, *PmLX66*, *PmX3986-2* and *Pm48*, respectively (Table 1; Fig. 3). Allelism tests involving more than 7000 $F_2$ individuals from crosses of Niaomai (*PmNM*) with Ulka/8*Cc (*Pm2a*) and KM2939 (*Pm2b*) showed that *PmNM* was allelic to *Pm2a* and *Pm2b*, respectively. Therefore, allelic tests and resistance spectrum analysis indicated that *PmNM* is a new allele at the *Pm2* locus and is different from *Pm2a*, *Pm2b*, *PmLX66*, *PmX3986-2* and *Pm48*. It was named *Pm2c*.

In this study we identified and mapped *Pm2c*, a new allele of the *Pm2* locus, in Chinese wheat landrace Niaomai. *Pm2c* provided a wider array of resistance to a panel of *Bgt* races than other resistance genes at or close to the *Pm2* locus. *Pm2a* has been used successfully in wheat breeding and Liangxing 66 (with *PmLX66*) is an important commercial variety in China (Huang et al. 2012). Therefore, *Pm2c* could be used instead of *Pm2a* and *PmLX66* and could be transferred into cultivars/lines to improve the powdery mildew resistance. The diagnostic CFD81 marker was absent in 61 of 63 (96.8 %) cultivars/lines without a documented *Pm2* resistance allele so could have application in marker-assisted breeding.

**Author contribution statement** H. Xu and D. An designed the research; Y. Fu and X. Zhang collected the plant materials and constructed the populations; Y. Yi, P. Ma, Y. Qie and Y. Xu performed the experiments; H. Xu and Y. Yi analyzed the data; H. Xu wrote the paper.

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**Conflict of interest** The authors declare that our experiments comply with the current laws of China and we have no conflicts of interest.

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