Characterization of a New Pm2 Allele Conferring Powdery Mildew Resistance in the Wheat Germplasm Line FG-1

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Powdery mildew has a negative impact on wheat production. Novel host resistance increases the diversity of resistance genes and helps to control the disease. In this study, wheat line FG-1 imported from France showed a high level of powdery mildew resistance at both the seedling and adult stages. An F₂ population and F₂:3 families from the cross FG-1 × Mingxian 169 both fit Mendelian ratios for a single dominant resistance gene when tested against multiple avirulent Blumeria graminis f. sp. tritici (Bgt) races. This gene was temporarily designated PmFG. PmFG was mapped on the multi-allelic Pm2 locus of chromosome 5DS using seven SSR, 10 single nucleotide polymorphism (SNP)-derived and two SCAR markers with the flanking markers Xbwm21/Xcfd81/Xscar112 (distal) and Xbwm25 (proximal) at 0.3 and 0.5 cM being the closest. Marker SCAR203 co-segregated with PmFG. Allelism tests between PmFG and documented Pm2 alleles confirmed that PmFG was allelic with Pm2. Line FG-1 produced a significantly different reaction pattern compared to other lines with genes at or near Pm2 when tested against 49 Bgt isolates. The PmFG-linked marker alleles detected by the SNP-derived markers revealed significant variation between FG-1 and other lines with genes at or near Pm2. It was concluded that PmFG is a new allele at the Pm2 locus. Data from seven closely linked markers tested on 31 wheat cultivars indicated opportunities for marker-assisted pyramiding of this gene with other genes for powdery mildew resistance and additional traits.

Keywords: allelic variation, Blumeria graminis, MAS, Triticum aestivum, Pm2 locus

INTRODUCTION

Powdery mildew of wheat, caused by Blumeria graminis f. sp. tritici (Bgt), is a foliar disease that occurs worldwide, especially in wheat-producing regions with maritime or semi-continental climates (Cowger et al., 2012). In China, wheat yields and quality have been affected by this disease since the 1970s, especially high-yielding cultivars grown with high-inputs of irrigation and fertilization (Bennett, 1984; Sun et al., 2015). Over the past decade, the area affected by powdery
mildew in China has ranged from 6 to 8 million hectares each year, resulting in estimated grain losses of 300,000 metric tons.1

Although, fungicides can reduce losses in yield and quality caused by powdery mildew, resistant cultivars are the preferred means for control (Horst, 2013; Wang et al., 2015b). More than 70 formally named and about 20 temporarily designated powdery mildew (Pm) resistance genes/alleles have been identified (McIntosh et al., 2013; Hao et al., 2015). However, due to the race-specific nature of resistance and excessive deployment of single resistance genes, the effectiveness of Pm genes is often short-lived as they are defeated by virulent mutants of the pathogen (Hsam and Zeller, 2002; Xiao et al., 2013). Detailed studies have indicated that most current wheat cultivars grown in China have non-effective or no Pm genes (Li et al., 2011). It is therefore urgent to identify effective sources of resistance among germplasm from around the world to increase the existing genetic diversity.

Some of the documented Pm loci have multiple resistance alleles, such as Pm1 (Hsam et al., 1998; Singrün et al., 2003), Pm2 (Ma et al., 2015a; Xu et al., 2015), Pm3 (Zeller and Hsam, 1998), Pm4 (Schmolke et al., 2012), Pm5 (Hsam et al., 2001; Huang et al., 2003), and Pm24 (Huang et al., 2000; Xue et al., 2012). Although, some alleles at these loci may have lost their effectiveness, further allelic variation may be present in other germplasm. The gene Pm2 was identified several decades ago (Pugsley and Carter, 1953), and was used as an effective resistance source in some countries (Li et al., 2011). Although, the avirulence frequency remains low in some regions of China and other parts of the world after decades of deployment, several new alleles (e.g., Pm2b, Pm2c, PmLX66, and PmW14) were identified, thereby increasing the diversity of available resistance genes (Ma et al., 2015a; Sun et al., 2015; Xu et al., 2015).

Molecular markers are powerful tools for tagging resistance genes. Almost all the designated Pm genes have been mapped to specific chromosomal loci (McIntosh et al., 2013). Microsatellites or simple sequence repeats (SSRs) provide a simple and effective marker system for molecular mapping in wheat (Somers et al., 2004; Sourdille et al., 2004; Xue et al., 2008). To develop high-density marker assays, high-throughput single nucleotide polymorphism (SNP) genotyping platforms based on wheat 9K, 90K, and even 660K SNP chips are now available (Bérad et al., 2009; Lai et al., 2012; Avni et al., 2014; Wang et al., 2014), and these will greatly increase the numbers of markers closely linked to targeted resistance genes.

Molecular markers closely linked to targeted genes controlling valuable traits can be used to rapidly transfer them to other cultivars. Hence, marker-assisted selection (MAS) has been practiced in many parts of the world (USA, Australia, Canada, India, and Europe) to complement conventional breeding programs (Gupta et al., 2010). A number of markers associated with documented QTL/genes for some major economic traits, such as disease resistance, grain protein content and pre-harvest sprouting tolerance, have also been used for MAS in wheat breeding programs (e.g., de Bustos et al., 2001; Davies et al., 2006; Nocente et al., 2007; Badea et al., 2008; Zhang et al., 2009).

In this study, the wheat germplasm line FG-1 imported from France showed a high level of powdery mildew resistance in China. To make better use of this resistance resource, the following research was carried out to: (1) determine the inheritance of powdery mildew resistance in FG-1 using an array of Bgt isolates, (2) determine the chromosomal location of the resistance gene using different kinds of molecular markers and allelism tests, (3) compare response spectra of FG-1 and lines carrying documented Pm genes using Bgt isolates, (4) to compare allelic variation between FG-1 and genotypes with documented Pm genes using SNP-derived markers, and (5) investigate the applicability of closely linked markers for MAS.

**MATERIALS AND METHODS**

**Plant Materials**

FG-1 is a common wheat line that was imported from France and maintained in the germplasm bank of Shijiazhuang Academy of Agricultural and Forestry Sciences (Shijiazhuang, Hebei Province, China) of unknown pedigree. It has been grown in Northern China since it was imported from France. It has resistance to powdery mildew at both the seedling and adult growth stages based on observations over many years. The susceptible Chinese cultivar (cv.) Mingxian 169 was crossed with FG-1 to study the inheritance of powdery mildew resistance. Mingxian 169 and Huixianhong were used as susceptible controls for test of powdery mildew resistance. Several stocks with documented Pm genes on chromosome arm 5DS, such as Ulka/8*Cc with Pm2a, KM2939 with Pm2b, Niaomai with Pm2c, Tabasco with Pm48, Liangxing 66 with PmLX66, Wenrong 14 with PmW14, YingBo700 with PmYB, Zhongmai 155 with PmZ155, X3986-2 with PmX3986-2, Wanfengjian 34 with PmWFl, PB3558 with PmPB3558, Brock with MBrock and D57-5D with PmD57-5D were used in multi-race response comparisons with FG-1. Thirty-one wheat cultivars representing Chinese elite germplasm were tested using molecular markers closely linked to the Pm gene in FG-1 to validate their applicability for MAS.

**Phenotyping Reactions to Powdery Mildew**

Forty-nine Bgt isolates with different avirulence/virulence arrays (races) collected from different regions of China (Supplementary Table S1) were used to inoculate FG-1 and various host lines to determine the breadth of effectiveness of the resistance in FG-1 and compare the response spectrum of FG-1 to wheat stocks with documented Pm genes. Five seedlings per plot were planted in rectangular trays with 128 cells (3 cm × 3 cm) in a growth chamber. The susceptible check Mingxian 169 and Huixianhong were randomly planted in the trays. Three replications were included in each test. When the seedlings reached the one-to two-leaf stage, fresh conidiospores from Mingxian 169 seedlings were dusted on the trays. The trays were then placed in a greenhouse with a daily cycle of 14 h light at 22 ± 2°C and 10 h of darkness at 18 ± 2°C. The inoculation operations were performed once a day for three consecutive days. When the disease was fully developed.

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1http://cb.natesc.gov.cn
on Mingxian 169, the infection types (ITs) on each plant were assessed on a 0–4 scale based on the IT scale described by Si et al. (1992), with ITs 0, 0, 1, and 2 being regarded as resistant, and ITs 3 and 4 as susceptible. All the phenotypic experiments were repeated three times to confirm their reactions to each Bgt isolate.

The adult plant reactions to powdery mildew of FG-1 and the susceptible controls Mingxian 169 and Huixianhong were evaluated under field conditions using a mixture of the Bgt isolates prevalent in northern China. The tests with the mixture of the isolates were conducted using the procedures described by An et al. (2013) at Luancheng Agro-Ecological Experimental Station, Chinese Academy of Sciences, Shijiazhuang, China. Disease reactions were assessed on a 0–9 scale, where 0–4 was considered resistant and 5–9 susceptible (An et al., 2013). The adult plant reactions test was repeated over 4 years' growing season using the same procedure.

Bgt race B03 was chosen to inoculate seedlings of the segregating materials and parents for genetic analysis. Twenty-four plants of each F$_2$$_3$ family were inoculated with this race. To confirm that the same resistance gene conferred the resistance to all avirulent Bgt isolates, random sets of 10 homozygous resistant and 10 segregating F$_2$$_3$ families to the isolate B03 were tested with all of the 42 Bgt isolates that were avirulent on FG-1. Twenty-four plants of each F$_2$$_3$ family were inoculated with each tested race. The number of resistant and susceptible plants was counted to confirm the phenotype of each F$_2$$_3$ family. Tests on F$_2$$_3$ families with intermediate ITs 2 and 3 were repeated to confirm the results of the previous tests for each Bgt isolate.

**Molecular Genotyping of the Mapping Population**

Genomic DNA of FG-1, Mingxian 169 and the F$_2$ plants were isolated using the phenol/chloroform method (Sharp et al., 1988) following evaluation of their powdery mildew reactions. Resistant and susceptible DNA bulks were produced using equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F$_2$ plants, respectively, following progeny testing. To screen for molecular markers potentially linked to the Pm gene in FG-1, the parents FG-1 and Mingxian 169 and resistant and susceptible bulks were assessed for polymorphisms, initially using 50 SSR markers linked to 35 documented Pm genes. To saturate the linkage map, different types of molecular markers were screened further. They included about 100 SSR markers on chromosomes arms 4DL, 5DS, and 7BS from Somers et al. (2004), 25 SNP-derived markers located on chromosome 5DS developed from the wheat 90K SNP array and its sequence alignment with the Aegilops tauschii draft genome sequence (Lu et al., 2015), 25 EST-derived markers located on chromosome arm 5DS and two sequence characterized amplified region (SCAR) markers linked to documented Pm2 alleles (Li et al., 2009; Huang et al., 2012). Polymorphic markers were then genotyped on the F$_2$ population of FG-1 × Mingxian 169. After confirming the response genotypes through progeny testing of F$_2$$_3$ families, a linkage map for the resistance gene in FG-1 was then produced using the software MAPMAKER 3.0 with a LOD threshold of 3.0 and a maximum map distance 37.5 cM (Lincoln et al., 1993). Kosambi function was carried out to calculate the map distances between linked markers and the targeted gene using a total of 214 F$_2$$_3$ families from the cross FG-1/Mingxian 169 (Kosambi, 1943). Chi-squared tests were performed to determine the goodness of fit of observed and expected segregation ratios of the F$_2$ and F$_2$$_3$ populations for markers and powdery mildew responses.

**Allelism Test**

After the Pm gene(s) in FG-1 was mapped on the short arm of chromosome 5D, FG-1 was crossed with the resistant stocks with

![FIGURE 1](https://www.frontiersin.org) | Examples of leaf segment reactions of FG-1 and various wheat genotypes to 6 of 49 Bgt isolates; cv. Mingxian 169 was used as the susceptible control.
documented $Pm$ genes on the same chromosome arm to obtain F$_2$ populations. The $Bgt$ isolate B03, which was avirulent to FG-1 and all the documented resistant stocks, was selected to inoculate the F$_2$ populations. The susceptible check Mingxian 169 and Huixianhong were randomly planted in the trays. The number of resistant and susceptible plants in each plot was counted after evaluating their phenotyping reactions. Then, the allelic relationships between the $Pm$ gene(s) in FG-1 and documented $Pm$ genes on the same chromosome arm were confirmed based on the ratio of resistant and susceptible F$_2$ plants.

**Allelic Variation of Linked Marker Alleles**

To compare the $Pm$ gene(s) in FG-1 and documented allelic and closely linked $Pm$ genes, several markers that were linked with $PmFG$ were selected to amplify FG-1 and resistant stocks with $Pm$ genes at or near $Pm2$ locus. The allelic sizes of those markers were assessed by the SensiAnsys gel imaging analysis system (Shanghai Peiing Science & Technology Ltd., Shanghai City, China).

**Validation of the Closely Linked Markers in Different Genetic Backgrounds**

To evaluate the potential of the $Pm$ gene(s) in FG-1 for MAS, several closely linked markers were assayed in 31 Chinese wheat cultivars. The patterns or sizes of the polymorphic bands amplified from these cultivars were compared with those amplified from FG-1 to assess the usefulness of the markers in MAS. If the polymorphic band(s) of a marker were all same for FG-1 and the wheat cultivars, it could not be used for MAS. However, patterns or sizes of the polymorphic bands amplified from FG-1 that differed from those in the wheat cultivars indicate the marker can be used to detect $PmFG$ when it was transferred into those cultivars by hybridization.

### RESULTS

**Evaluation and Inheritance of Powdery Mildew Resistance in FG-1**

FG-1 showed resistance to a mixture of $Bgt$ races in the field over 4 years with disease reaction types 0-2 while adjacent controls Huixianhong and Mingxian 169 were susceptible with disease reaction types 8-9. At the seedling stage, FG-1 was resistant to 43 of the 49 $Bgt$ isolates collected from different regions, indicating an avirulence frequency of 87.8% (Figure 1; Supplementary Table S1). Compared with several wheat cultivars currently deployed in different regions of China, FG-1 possessed a broader resistance spectrum, and more significantly, it was resistant to several highly virulent $Bgt$ isolates; for example, B29, B38, B50 and B80, which are virulent on several or all of the five cultivars Liangxing 66, Lianxing 99, Wenhong 14, Zhongmai 155, and Jimai 22 widely planted in Shandong, Hebei, and Henan provinces and Beijing area. Therefore, FG-1 could serve as a valuable resistance donor to add to the current diversity of $Pm$ genes in different wheat production regions.

When tested with $Bgt$ isolate B03, FG-1 was resistant with an IT 0, while the cultivar Mingxian 169 was susceptible with an IT 4. The infection types of the F$_1$s, and the segregation patterns of the F$_2$, and F$_2$;$_3$ populations are shown in Table 1. F$_1$ plants from the cross showed infection types similar to the resistant parent, indicating that the resistance was dominant. We observed segregation of 228 resistant: 74 susceptible in the F$_2$ population, which is consistent with an expected segregations for a single dominant locus. The F$_2$ population was then transplanted to the field and 214 plants produced enough seed for progeny testing. When tested with the same race, segregations of 54 homozygous resistant (RR), 115 segregating (Rr) and 45 homozygous susceptible (rr) F$_2$;$_3$ families confirmed single gene segregation. The gene was tentatively designated $PmFG$.

When tested against 42 other $Bgt$ isolates that were avirulent to FG-1, all the 10 homozygous resistant F$_2$;$_3$ families for B03 were also homozygous resistant to all avirulent isolates and all the 10 segregating F$_2$;$_3$ families for B03 again segregated, including isolates B29 and B38 with IT 2 on FG-1. Therefore, $PmFG$ conferred powdery mildew resistance to all the avirulent $Bgt$ isolates.

**TABLE 1 | Segregation ratios for the powdery mildew reactions of F$_1$ plants, and F$_2$ and F$_2$;$_3$ populations from the cross FG-1 × Mingxian 169 when inoculated with Bgt isolate B03 in the greenhouse.**

| Cross          | Plants observed | Expected ratio | $\chi^2$ | $P$   |
|----------------|-----------------|----------------|---------|-------|
| FG-1 × Mingxian 169 F$_1$ | 20 – 0          | – 0            | – –     | – –   |
| FG-1 × Mingxian 169 F$_2$ | 228 – 74        | 3:1            | 0.02    | 0.89  |
| FG-1 × Mingxian 169 F$_2$;$_3$ | 54 – 115 – 45   | 1:2:1          | 1.95    | 0.37  |

Table values of $\chi^2$ for significance at $P = 0.05$ are 3.84 (1 df), 5.99 (2 df).

**FIGURE 2 | Examples of amplification patterns of PmFG-linked polymorphic SSR marker Cfd81 (A) and SNP-derived markers Bwm20 (B) and Bwm25 (C) in the parents and selected F$_2$;$_3$ families of FG-1 × Mingxian 169 in 8% silver-stained non-denaturing polyacrylamide gels.** Lanes M, pUC18Msp I; lanes 1–2: FG-1 and Mingxian 169; lanes 3–7: homozygous resistant F$_2$;$_3$ families; lanes 8–12, homozygous susceptible F$_2$;$_3$ families; lanes 13–17 heterozygous F$_2$;$_3$ families. White arrows indicate the polymorphic band of FG-1.
Molecular Mapping of PmFG

The Pm2-linked SSR marker Cfd81 was polymorphic for the parents and the bulks. Cfd81 was genotyped on the the F2:3 mapping population (Figure 2). Its linkage with PmFG was estimated at 0.3 cM (Figure 3). Because Cfd81 also can be amplified at loci on chromosome arms 4DL, 5DS and 7BS, 79 SSR markers located on these chromosome arms (Somers et al., 2004) were screened to confirm the location of locus PmFG and to increase the density of the linked markers. SSR markers Cfd40, Cfd78, Gwm159, Wmc608, and Wmc805 on chromosome arms 5DS from the map of Somers et al. (2004) were shown polymorphic and linked to PmFG (Table 2; Figure 3). Markers reportedly located on chromosome arms 4DL and 7BS (Somers et al., 2004) showed no polymorphisms between the parents and the bulks, indicating that PmFG was not located on those chromosome arms. To saturate the linkage map of the PmFG region, 10 SNP-derived markers on chromosome arm 5DS and the SCAR marker SCAR112 which is linked to a documented Pm2 allele were shown polymorphic and linked to PmFG at genetic distances ranging from 0.3 to 16.9 cM (Table 2; Figure 3). SCAR marker SCAR203 co-segregated with PmFG (Figure 3). The reported locations of some of the SNP markers and the SCAR markers were in the deletion bin 5DS1-0-0.63 (Sourdille et al., 2004; Li et al., 2009; Lu et al., 2015), therefore, PmFG should also be located in this chromosome bin.

Allelism between PmFG and Previously Documented Pm2 Alleles

Because PmFG was mapped on the chromosome arm 5DS, FG-1 was crossed with several powdery mildew resistant stocks with documented Pm genes on the same chromosome arm to determine the allelic relationship between PmFG and previously documented Pm genes. These included Ulka/8*Cc with Pm2a, Liangxing 66 with PmLX66 and TD114 with Pm2a+Pm6. Bgt isolate B03 which is avirulent on FG-1 and the other lines was used to inoculate the large F2 populations. The phenotyping reactions of those

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** | Linkage map of PmFG using the F2:3 families of FG-1 x Mingxian 169 (A) and its locus comparison with the documented Pm genes on chromosome arm 5DS using PmFG as point zero with the genetic distances marked off from it based on the anchoring marker locus Xcfd81 (B). Genetic distances in cM are showed to the left. *, closely linked with Pm2; **, allelic with Pm2; ***, allelic relationship with Pm2 was not confirmed.
including all the SNP-derived markers and the SSR marker \( Pm2 \) multi-allelic. To further compare \( PmFG \) with alleles between FG-1 and genotypes of variation in the closely linked marker locus. \( Pm2 \) different from the other and B30 (Supplementary Table S1). Therefore, \( PmFG \) derivative PB3558 that carried a different response pattern from the putative \( Agropyron \) \( Pm2 \) chromosome region for the response to \( Bgt \) isolates from those of other lines with possible \( Pm2 \) alleles and \( PmFG \) alleles including \( PmLX66 \), \( PmW14 \), \( PmZ155 \), \( PmX3986-2 \), \( PmWFJ \), \( MLBrock \), and \( PmD57-5D \) and \( Pm48 \), there were differences in response for \( 6, 5, 8, 5, 6, 7, 8, 12, 5, 4, 6, \) and \( 7 \) \( Bgt \) isolates, respectively (Supplementary Table S1; Figure 1). FG-1 also showed a different response pattern from the putative \( Agropyron cristatum \) derivative PB3558 that carried \( PmPB3558 \) in the \( Pm2 \) chromosome region for the response to \( Bgt \) isolate B07 and B30 (Supplementary Table S1). Therefore, \( PmFG \) was different from the other \( Pm \) genes located at or near the \( Pm2 \) locus.

### Variation in the Closely Linked Marker Alleles between FG-1 and Genotypes with \( Pm \) Genes Located at or Near the \( Pm2 \) Locus

To further compare \( PmFG \) and documented \( Pm \) alleles in the multi-allelic \( Pm2 \) region, several markers associated with \( PmFG \), including all the SNP-derived markers and the SSR marker Cfd81, were compared for their allelic variations among FG-1, Ulka/8°Cc, KM2939, Niaomai, Tabasco, Liangxing 66, Wennonong 14, Zhongmai 155, YingBo700, X3986-2, Wanfengjian 34, Brock, D57-5D and PB3558 that carried \( Pm \) genes located at or near the \( Pm2 \) locus. The Cfd81, Bwm20 and Bwm25 amplicons were approximately 270, 195, and 208 bp, respectively, in FG-1 and all lines with \( Pm \) genes at or near the \( Pm2 \) locus. However, the presence of eight of 10 SNP-derived markers linked to \( PmFG \) (including Bwm3, Bwm6, Bwm8, Bwm9, Bwm11, Bwm13, Bwm16, and Bwm21) varied in their allelic sizes among FG-1 and various stocks with \( Pm \) genes at or near the \( Pm2 \) locus (Table 4; Figure 4). For example, the two Bwm21 amplicons in FG-1 were different from those in Ulka/8°Cc (\( Pm2a \)), Tobasco (\( Pm48 \)) and Brock (MLBrock); and the 194 bp Bwm13 amplicon was unique to FG-1 (Table 4). Therefore, FG-1 showed a diversity of alleles of the \( PmFG \)-linked markers compared to other resistant stocks with \( Pm \) genes at or near the \( Pm2 \) locus. From our results of allelism tests, allelic variation of the linked markers and response spectrum analysis, we find that \( PmFG \) is a new allele of \( Pm2 \).

### Multi-race Comparisons of FG-1 and Lines with Reported Resistance Alleles at or Near the \( Pm2 \) Locus

FG-1 showed a clearly different response spectrum to the \( Bgt \) isolates from those of other lines with possible \( Pm2 \) alleles and \( Pm48 \) (Supplementary Table S1). Compared to the lines with \( Pm2a \), \( Pm2b \), \( Pm2c \), \( PmLX66 \), \( PmW14 \), \( PmZ155 \), \( PmX3986-2 \), \( PmWFJ \), \( MLBrock \), and \( PmD57-5D \) and \( Pm48 \), there were differences in response for \( 6, 5, 8, 5, 6, 7, 8, 12, 5, 4, 6, \) and \( 7 \) \( Bgt \) isolates, respectively (Supplementary Table S1; Figure 1). FG-1 also showed a different response pattern from the putative \( Agropyron cristatum \) derivative PB3558 that carried \( PmPB3558 \) in the \( Pm2 \) chromosome region for the response to \( Bgt \) isolate B07 and B30 (Supplementary Table S1). Therefore, \( PmFG \) was different from the other \( Pm \) genes located at or near the \( Pm2 \) locus.

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**TABLE 4 |** Sizes of the linked marker alleles of FG-1 and the wheat genotypes with documented Pm genes at or near Pm2 locus on chromosome arm 5DS using SNP-derived markers of PmFG.

| Genotypes | Gene   | Bwm3 | Bwm6 | Bwm8 | Bwm9 | Bwm11 | Bwm13 | Bwm16 | Bwm21 |
|-----------|--------|------|------|------|------|-------|-------|-------|-------|
| FG-1      | PmFG   | 144  | 169  | 210  | 208  | 225   | 194   | 192   | 220, 235 |
| UlKa8*Cc  | Pm2a   | 145  | 160  | 194  | 199  | 225   | 196   | 192   | 210, 220, 225, 235 |
| KM2939    | Pm2b   | 148  | 160  | 190  | 197  | 225   | 197   | 195   | 220, 235 |
| Niaomai   | Pm2c   | 144  | 160  | 210  | 208  | 226   | 196   | 192   | 220, 235 |
| Tabasco   | Pm48   | 144  | 167  | 194  | 197  | 226   | 200   | 187   | 210, 220, 235 |
| Liangying 66 | PmLX66 | 145  | 165  | 196  | 199  | 228   | 201   | 192   | 220, 235 |
| Wennong 14 | PmW14  | 148  | 162  | 190  | 197  | 226   | 200   | 187   | 210, 220, 235 |
| Zhongmai 155 | PmZ155 | 144, 145 | 165  | 196  | 199  | 228   | 200   | 192   | 220, 235 |
| YingBo 700 | PmYB   | 145  | 162  | 196  | 199  | 228   | 200   | 190   | 220, 235 |
| X3986-2 | PmX3986-2 | 144  | 169  | 194  | 197  | 225   | 204   | 192   | 220, 235 |
| Brock    | MlBrock | 144  | 162  | 196  | 199  | 226   | 200   | 187   | 210, 220, 235 |
| D57-SD   | PmD57-SD | 144  | 162  | 196  | 199  | 226   | 200   | 187   | 220, 235 |
| Wanfengjian 34 | PmWFJ  | 148  | 162  | 196  | 199  | 228   | 201   | 192   | 220, 235 |
| PB3558   | PmPB3558 | 146  | 169  | 210  | 208  | 225   | 200   | 190   | 220, 235 |

All the allelic sizes in this table are listed in bp.

Potential of Closely Linked Markers for MAS

To investigate the usefulness of the markers linked to PmFG in MAS, eight closely linked markers flanking PmFG were assayed on 31 Chinese elite cultivars (Table 5; Figure 5). The Bwm20, Bwm21, Bwm25, Cfd81, SCAR112, and SCAR203 alleles in all tested cultivars, except Jimai 22, were different from those in FG-1, demonstrating that the six markers could be used in MAS if PmFG was transferred to these cultivars by conventional hybridization. Other markers such as Bwm13 and Bwm16 also could be used, but only in certain cross combinations.

DISCUSSION

FG-1 was a common wheat line introduced from France and provided by the germplasm bank of Shijiazhuang Academy of Agricultural and Forestry Sciences. It was resistant to many Bgt isolates originating from different wheat-producing regions in...
China (Supplementary Table S1). Compared with the currently deployed cultivars in China, FG-1 is resistant to several highly virulent Bgt isolates that defeated several of the popular wheat cultivars that are currently grown in China. This suggests that FG-1 is a valuable resistant germplasm, which could be used to complement the resistant genes currently deployed in cultivars in China. However, FG-1 was not resistant to all the races present in certain regions of China. Therefore, the gene in FG-1 needs to be combined with other effective resistance genes to increase the durability of resistance.

Genetic analysis demonstrated that a single dominant gene, designated PmFG, conferred resistance to powdery mildew in FG-1 at the seedling stage. Unlike in previous studies (e.g., Gao et al., 2012; Huang et al., 2012; Xue et al., 2012; Xiao et al., 2013; Lu et al., 2015), PmFG was investigated for its resistance to all the avirulent races tested in this study. Based on this information, the single dominant gene in FG-1 was more thoroughly shown to confer powdery mildew resistance to all the avirulent races. Using molecular markers, PmFG was mapped in the Pm2 region on the short arm of chromosome 5D. Its allelic relationship with Pm2 was confirmed by allelism tests.

Compared with previous linkage maps of the Pm2 alleles, more markers were added to the PmFG linkage map particularly the SNP-derived markers, which increased the density of the linked markers at this locus. Many alleles have been identified in the Pm2 chromosome region, such as Pm2a in the wheat landrace Ulka from the former Soviet Union (Pugsley and Carter, 1953; Briggle, 1969; McIntosh and Baker, 1970), Pm2b and PmPB3558 from the putative Agropyron cristatum-derived breeding lines KM2939 and PB3558, respectively (Lu et al., 2015; Ma et al., 2015a), Pm2c from the Chinese landrace Niaomai (Xu et al., 2015), PmX3986-2, PmWFJ, and PmD57-5D from the common wheat lines X3986-2, Wanfengjian 34 and D57-5D in China, respectively (Ma et al., 2011, 2014, 2015b) and PmLX66, PmZ155, PmW14, and PmYB from Chinese wheat cultivars Liangxing 66 (Huang et al., 2012), Zhongmai 155 (Sun et al., 2015), Wennong 14 (Song et al., 2014), and YingBo700 (Ma et al., 2015c),
Another closely linked gene Pm48 is present in the cv. Tabasco (Gao et al., 2012).

In this study, PmFG was distinguished from these documented genes by its response spectrum and the allelic variation of the linked marker alleles of the SNP-derived markers. Previous studies indicated that the documented Pm2 alleles shared several similar markers, and the allelic variation of the linked markers could not be detected. However, the advent of the next generation in sequencing technologies significantly reduces sequencing costs, making SNP markers increasingly important due to their abundance in the genome and their very simple genetic mode of inheritance (bi-allelic). Therefore, to further distinguish PmFG from the Pm genes at or near the Pm2 locus, SNP-derived markers of PmFG were screened to distinguish the allelic sizes. For the first time, Pm genes at or near Pm2 locus were studied for their allelic variation using SNP-derived markers. This will contribute to differentiate the variation in the Pm2 locus.

Multiple allelism in disease resistance genes is not uncommon. In the case of powdery mildew resistance in wheat, multiple resistance alleles have been identified at Pm1, Pm2, Pm3, Pm4, Pm5, and Pm24 (McIntosh et al., 2013). So far, 17 functional alleles have been identified at the Pm3 locus, making it one of the largest allelic series of plant resistance genes (Bhullar et al., 2010). For other wheat disease resistance, multi-allelic loci have also been identified. For example, using physical mapping, mutation and complementation, the stem rust resistance gene Sr50 locus revealed extensive diversity, and holds promise for the mining other effective resistance alleles (Mago et al., 2015). These types of genetic diversity may contribute to the genetic improvement of crops and detection of variation in the pathogen and host–pathogen interactions (Prada, 2009; Wicker et al., 2013). In this study, the gene PmFG was identified as a new allele located at the Pm2 allelic cluster. Like the Pm3 allele cluster, more and more Pm2 alleles with different response spectra to Bgt isolates and allelic variation have been identified, increasing the diversity at this locus. However, to further distinguish these alleles, more research should be undertaken in the future, such as fine mapping of all the Pm2 alleles, re-sequencing of the Pm2 region, and even development of functional markers based on the cloning of functional genes of these alleles.

In order to transfer PmFG into the susceptible cultivars or to pyramid multiple R-genes effectively, MAS should be a high-priority in wheat breeding programs. In the previous studies, the SSR marker Cfd81 was shown to be an effective marker for differentiating several Pm2 alleles in MAS (Ma et al., 2015a,b,c). However, Cfd81 serves as only a one-sided marker of Pm2 alleles, and other markers like SCAR112 and SCAR203 also have limited roles because of their dominant characters, which do not allow homozygous and heterozygous genotypes to be distinguished (Ma et al., 2015a,b,c). More closely linked markers need to be screened to increase the density of applicable molecular markers for breeding. SNP markers are based on the variation of a specific nucleotide at a given sequence position between individuals, and therefore their numbers in the wheat genome should be much higher and their detection can be facilitated by cost-efficient based on chips or other array techniques (Colasuonno et al., 2014; Wang et al., 2014, 2015a). In this study, five co-dominant SNP markers were evaluated for their applicability in MAS. The SNP-derived markers Bwm20, Bwm21, and Bwm25 flanked PmFG at genetic distances of only 0.3, 0.3, and 0.5 cM respectively, and they were diagnostic in 96.8% of the tested cultivars in this study. Therefore, these SNP-markers can be used effectively in wheat breeding in the future.

One interesting observation was that the marker alleles of FG-1 by the PmFG-linked markers were all same as those of the cultivar Jimai 22, although PmFG is located on a significantly different genetic locus from that of the powdery mildew resistance gene in Jimai 22, which was mapped on chromosome arm 2BL (Yin et al., 2009). Therefore, PmFG cannot be distinguished in Jimai 22 genomic backgrounds by the PmFG-linked markers. This may ascribe to the insufficient marker density at this locus. Future fining mapping and characterization of the haplotype of this locus may contribute to clarify this issue.

### AUTHOR CONTRIBUTIONS

PM: experimental implementation, data analysis, and manuscript preparation. HX: production of the mapping population and the genetic map. LL: data analysis. HZ: experimental implementation, data analysis, and manuscript preparation. DA: study concept and design.

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### SUPPLEMENTARY MATERIAL

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