Identification of the Powdery Mildew Resistance in Chinese Wheat Cultivar Heng 4568 and its Evaluation in Marker-Assisted Selection

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Powdery mildew induced by Blumeria graminis f. sp. Tritic (Bgt) has a devastating impact on global wheat yield and quality. Host resistance is the most effective and economical means to control this disease. In this study, Heng 4568, an elite wheat cultivar, shows high resistance to 12 Bgt isolates from different regions in China at the seedling stage. Genetic analysis demonstrates that the powdery mildew resistance in Heng 4568 is conferred by a single dominant locus, temporarily designated PmH4568. Furthermore, PmH4568 is mapped to the reported Pm2 interval on chromosome 5DS with five Pm2 linked markers and flanked by the markers Bwm20 and Bwm21 with a genetic distance of 0.3 and 0.6 cM, respectively. To further investigate the relationship between PmH4568 and Pm2, the diagnostic marker Pm2b-map-3 of Pm2 is used to genotype the F2:3 population derived from the cross Heng 4568 × Daimai 2173. Notably, there is no recombination found, indicating that PmH4568 is also probably a Pm2 allele. In addition, five closely linked markers as well as one diagnostic marker are successfully developed and tested in 16 wheat cultivars from different agro-ecological areas in China, which have potential applications in molecular breeding by marker-assisted selection.

Keywords: wheat powdery mildew, Heng 4568, molecular markers, MAS, Pm2

INTRODUCTION

Wheat powdery mildew incited by the biotrophic fungus Blumeria graminis f. sp. tritici (Bgt) is a foliar disease worldwide (Wu et al., 2019). The rapid spread of powdery mildew will cause severe wheat yield losses in a short time, especially in the winter wheat-growing regions with high inputs of irrigation and fertilizers (Luo et al., 2009; Hao et al., 2014). In China alone, the area of winter wheat affected annually by powdery mildew has exceeded 6 mha during recent decades, causing 300,000 tons of crop loss each year (http://cb.natesc.gov.cn/sites/cb/). With the climate getting warmer, the epidemics of wheat powdery mildew in China are growing more severe, which will always be a serious threat to national food security.

Given the significant yield-limiting effects of powdery mildew, the research and exploration of effective prevention and control technology has become urgent in wheat production. Currently, chemical control, biological control, and cultivation of disease-resistant varieties are common means. Chemical control is mainly by spraying fungicides to kill the Bgt isolates; however, it always pollutes the environment and accelerates variation of the Bgt isolates (Ma et al., 2018, 2019). Biological
control mainly relies on some natural beneficial microorganisms and/or some existing substances in nature, to act as a natural antagonist of pathogen to resist other plant pathogens (Curtis et al., 2012). In comparison, host resistance is relatively the most effective, economical, and environmental way to control wheat powdery mildew, including broadening wheat resistance sources, polymerizing disease-resistant genes, and spreading disease-resistant cultivars (Felsenstein et al., 2010; Ma et al., 2018, Ma et al., 2019).

To date, more than 100 powdery mildew resistance (Pm) genes/alleles have been identified at 63 loci in wheat and its relatives (Li et al., 2019; McIntosh et al., 2019; He et al., 2021a; He et al., 2021b). Although several Pm genes have been widely used in production and provided high protection at both seedling and adult plant stages, more and more Pm genes are no longer effective against powdery mildew due to virulent mutants of the Bgt isolates (Xiao et al., 2013). Therefore, it is necessary to increase the genetic diversity of the resistant genes and characterize more effective alleles in wheat germplasms.

When a Pm gene was identified, its utilization efficiency in wheat production was mainly decided by its effectiveness and the agronomic performance of its donor (Zhao et al., 2013; Ma et al., 2018). There are some reports that several genes cannot be easily used for genetic improvement of powdery mildew resistance because of the linkage drag, competition drag, and adverse pleiotropism (Jia et al., 2020). One example is the gene Pm16, which is able to provide high resistance to different Bgt isolates, but will cause severe reduction of 15% in yield (Summers and Brown, 2013). Usually, commercial wheat cultivars have excellent agronomic performance without significantly bad traits and could be used as donors of valuable genes. In fact, several Pm genes have been identified from the cultivars with broad-spectrum resistance, such as PmJM23 from Jimai 23 (Jia et al., 2020), Pm52 from Liangxing 99 (Zhao et al., 2013), PmW14 from Wennong 14 (Song et al., 2014), and PmTm4 from Tangmai 4 (Xie et al., 2017). Therefore, characterization of powdery mildew resistance in the elite cultivars is important for isolating the underlying genes, which could be rationally used in breeding.

Marker-assisted selection (MAS) has enormous potential to improve the efficiency and precision in wheat breeding. Compared to the conventional breeding, MAS can combine several functional alleles from several individuals into one single genotype more precisely, with less unintentional losses and in fewer selection cycles (Xu and Crouch, 2008; Jiang et al., 2017). To perform MAS, closely linked molecular markers play a key role in tracing the targeted genes in the breeding population. Up to now, many molecular markers closely linked to Pm genes have been developed for MAS and efficiently used in different genetic backgrounds, thereby generating a large number of wheat breeding lines or resistant cultivars (Ma et al., 2018; Shah et al., 2018; Yu et al., 2018; Ye et al., 2019; Jia et al., 2020; Zhang et al., 2021). For instance, using tightly linked markers to Pm2 and Pm21 or co-segregate with Pm4a, three two-gene combinations, namely, Pm2 + Pm4a, Pm2 + Pm21, and Pm4a + Pm21, were successfully transferred into the commercial wheat cultivar “Yangmai 158” and double homozygotes were selected from the F2 population (Liu et al., 2000). In addition, MAS was also applied for other disease resistant in wheat, such as Fusarium head blight and stripe rust (Steiner et al., 2017; Nsabiyera et al., 2018; Su et al., 2018; Randhawa et al., 2019; Maré et al., 2020).

Heng 4568, an elite wheat cultivar, shows high resistance to powdery mildew. A previous study indicated that Heng 4568 most likely carries the known Pm52 inherited from Liangxing 99 (Zou et al., 2017). Notably, Heng 4568 showed a broader resistant spectrum to different Bgt isolates than Liangxing 99 in our evaluation of disease resistance. Therefore, the objectives of this study include (1) analyzing its powdery mildew resistance using different Bgt isolates, (2) clarifying the presence of other Pm genes in Heng 4568 besides Pm52, and (3) developing molecular markers of the new identified Pm gene for MAS.

MATERIALS AND METHODS

Plant Materials

The winter wheat cultivar Heng 4568 crossed by Hengyou 18 and Liangxing 99 was provided by the Institute of Dry Farming Agriculture, Hebei Academy of Agriculture and Forestry Sciences, and used as the donor of resistant gene(s) against powdery mildew. Wheat cultivar Daimai 2173 served as a susceptible parent that was crossed with Heng 4568 to produce F1 hybrids, F2 population, and F2:3 families for genetic analysis and molecular mapping of the Pm gene(s) in Heng 4568. Five wheat cultivars/lines with known Pm genes, namely, Liangxing 99 (Pm52), Wennong 14 (PmW14), Zhongmai 155 (PmZ155), Jimai 22 (Pm52 + PmJ23), and Ulka/8*Cc (Pm2a), were used to compare their reaction patterns to different Bgt isolates with that of Heng 4568. Susceptible wheat cultivar Huixianhong was used as the susceptible control for phenotypic assessment. Sixteen susceptible wheat cultivars from different ecological regions in China (Hebei, Shandong, Henan, Shaanxi, Beijing, Anhui, and Jiangsu provinces in China) were used to evaluate the availability of closely linked markers for MAS.

Assessment of Disease Resistance at the Seedling Stage

From 2019 to 2021, the assessment of disease resistance at the seedling stage was carried out in the greenhouse at Hebei University of Science and Technology (Shijiazhuang, China). Twelve Bgt isolates were collected from different wheat production regions in China. They were used to determine the reaction patterns of Heng 4568 and wheat genotypes with known Pm genes. For each Bgt isolate, at least 20 seeds for each genotype were sown in 128-cell rectangular trays in a growth chamber. The susceptible control Huixianhong was randomly planted in the tray. When the first leaves were unfolded, the seedlings were inoculated by Bgt conidiospores that were previously increased on Huixianhong seedlings. Then, the inoculated seedlings were incubated in an airtight dark environment for 24 h and then allowed disease symptom development in a greenhouse with a daily cycle of 14 h of light at 22°C and 10 h of darkness at 18°C (Qu et al., 2020). To perform genetic analysis, Bgt isolate KD07
was selected to inoculate seedling of Heng 4568, the susceptible cultivar Daimai 2173, and their F₁, F₂, and F₂:₃ progenies. For F₁ hybrids, 10 plants were sown; for F₂ population, 177 plants were sown; for F₂:₃ families, 172 families and 20–30 plants per family were sown. Two weeks after inoculation when the spores were fully developed on the susceptible controls, infection types (ITs) on the primary leaves of plants were rated with a scale of 0, 0, 1, 2, 3, and 4. The leaves that displayed ITs 0–2 and 3–4 were regarded as resistant and susceptible, respectively (Liu et al., 1999). Three repeated experiments were carried out using the same procedure.

**Molecular Marker Analysis**

Genomic DNA was extracted from the young leaf tissues following the cetyltrimethylammonium bromide method (Saghai-Maroof et al., 1984). Resistant and susceptible DNA bulks were created by separately mixing equal amount of DNA from 10 homozygously resistant and 10 homozygously susceptible plants, respectively. Forty-eight molecular markers closely linked to 37 known Pm genes were firstly screened for their polymorphisms between Heng 4568, Daimai 2173, and their derived resistant and susceptible bulks (Supplementary Table S1). When the Pm gene in Heng 4568 was preliminarily to Pm2 locus, other Pm2 linked markers Cfd81, Bwm20, Bwm21, Bwm25, and Swgi067 (Lu et al., 2015; Ma et al., 2018) and the diagnostic marker Pm2h-map-3 (Jin et al., 2021) were also used to add the marker density for constructing the linkage map (Supplementary Table S2). PCR was performed in a 10-µl reaction volume containing 1 µl of 40–50 ng/µl template genomic DNA, 4.5 µl of 2×Taq Master Mix (Vazyme, China), and 0.5 µl of 10 µM/µl primer mix. The PCR program used was 95°C for 5 min; 36 cycles of 95°C for 30 s, 50–60°C (depending on specific primers) for 40 s, final extension at 72°C for 5 min; and storage at 4°C. PCR products were separated in 8.0% nondenaturing polyacrylamide gels with 29:1 ratio of acrylamide and bisacrylamide with 1× TBE buffer and then silver-stained and visualized as previously described (Santos et al., 1993).

**Statistical Analysis and Linkage Map Construction**

After confirming genotypes of F₂:₃ families of Heng 4568 and Daimai 2173, the deviations of the observed phenotypic data from theoretically expected segregation ratios for goodness of fit were assessed using χ² test. MAPMAKER 3.0 and the Kosambi function were performed to construct the linkage map of the powdery mildew resistance gene in Heng 4568.

**RESULTS**

**Evaluation of Powdery Mildew Resistance in Heng 4568**

Heng 4568 was highly resistant to 12 Bgt isolates with the ITS 0–2, whereas Daimai 2173 and susceptible control Huixianhong were all highly susceptible to all the tested Bgt isolates (Table 1). Compared with the Pm52 donor Liangxing 99, Heng 4568 was resistant to the Bgt isolates KD03, KD07, KD08, and KD11, while Pm52 was susceptible to these four Bgt isolates, indicating that Heng 4568 contains other Pm gene(s).

**Genetic Analysis of Pm Genes in Heng 4568**

To explore other Pm gene(s) besides Pm52 in Heng 4568, the isolate KD07 virulent to Liangxing 99 (with Pm52) and avirulent to Heng 4568 was selected to inoculate Heng 4568, Daimai 2173, and their derived F₁, F₂ population, and F₂:₃ families, respectively. All the tested F₁ seedlings were resistant to KD07 similar to their parent Heng 4568. The F₂ population fitted the segregation ratio of a single dominant gene (Table 2). The harvested F₂:₃ families from the F₂ population confirmed the expected ratio of 1:2:1 (Table 2). Therefore, it was concluded that another dominant Pm gene is also involved in Heng 4568, which was temporarily designated PmH4568.

**Molecular Mapping of PmH4568**

To determine the genetic location of PmH4568, 48 molecular markers closely linked to the known Pm genes were firstly used to test their polymorphisms between the parents and the two contrasting bulks. The Pm2-linked marker Cfd81 showed consistent polymorphism between Heng 4568, Daimai 2173, and their derived contrasting bulks. Then, Cfd81 was used to genotype the F₂:₃ families of Heng 4568 and Daimai 2173 and confirmed its linkage relationship with PmH4568 (Figures 1, 2; Supplementary Table S2). This suggested that PmH4568 was most likely located in the Pm2 interval. To confirm this interval,
four additional Pm2-linked markers, Bwm20, Bwm21, Bwm25, and Sg067, were also proved to be closely linked to PmH4568 (Figure 1; Supplementary Table S2). A genetic linkage map was then conducted to locate PmH4568 to the Pm2 interval (Figure 2). To further confirm the relationship between PmH4568 and Pm2, Pm2b-map-3, the diagnostic marker of Pm2, was used to genotype the F2,3 families of Heng4568 and Daimai 2173 (Figure 1; Supplementary Table S2). No recombinants were found, suggesting that PmH4568 was located in the Pm2 locus and most likely a Pm2 allele.

**Evaluation of Closely Linked Markers for Marker-Assisted Selection**

To transfer PmH4568 to susceptible cultivars using MAS, five PmH4568-linked markers, Bwm20, Bwm21, Bwm25, Sg067, and Cfd81, and the diagnostic marker Pm2b-map-3, were used to test Heng 4568 and 16 susceptible cultivars. The results showed that all the tested markers could amplify polymorphic bands between Heng 4568 and these susceptible cultivars, indicating that once PmH4568 is transferred into the susceptible cultivars through conventional hybridization, these markers can be used to detect PmH4568, especially the diagnostic marker Pm2b-map-3 (Figure 3; Table 3).

**DISCUSSION**

Heng 4568 is an elite winter wheat cultivar in Northern China. Due to its superior agronomic performance and powdery mildew resistance, Heng 4568 is considered as an attractive cultivar and serves as a favorable breeding parent for resistance improvement. A previous study indicated that the known Pm52 located on the chromosome 2BL was involved in Heng 4568, which may confer the powdery mildew resistance (Zou et al., 2017). Pm52 is a widely used Pm gene in Chinese cultivars, such as Hanong 2312, Zhongxinmai 99, Shimai 26, and DH51302. Heng 4568 was derived from the cross of Liangxing 99 with Hengyou 18, indicating that Pm52 in Heng 4568 may be derived from its parent Liangxing 99. However, our study demonstrated that Heng 4568 showed significantly broader resistant spectrum than the Pm52 donor Liangxing 99, suggesting that other Pm

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**TABLE 2** Segregation ratios of F2 and F2:3 generations of Heng 4568 × Daimai 2173 following inoculation with Blumeria graminis f. s. tritici (Bgt) isolate KD07 at the seedling stage.

| Cross                  | Plants observed | Expected ratio | $\chi^2$ | $p$ |
|------------------------|-----------------|----------------|----------|-----|
| Heng4568 × Daimai2173  |                 |                |          |     |
| F2                     | HR 130 Seg 47 HS 3:1 | 0.23 0.63      |          |     |
| F2:3                   | 43 83 46        | 1:2:1          | 0.31     | 0.85|

Note: Values of $\chi^2$ for statistical significance at $p = 0.05$ are 3.84 (1df) and 5.99 (2df); HR, homozygous resistant; Seg, segregating; HS, homozygous susceptible. Discrepancies on the line numbers between F2 and F3 generation are because several F2 plants were died during the growth process.
genes may also be involved in Heng 4568. To clarify the composition of the Pm genes in Heng 4568, a Bgt isolate virulent KD07 was used to identify other Pm gene(s) in Heng 4568. The result showed that another dominant Pm gene PmH4568 also contributed to the powdery mildew resistance in Heng 4568. This result, together with a previous study, provided an explicit genetic constitution for the powdery mildew resistance in Heng 4568, which contributes to scientific parental selection collocation.

Using Pm2-linked markers, PmH4568 was mapped to the known Pm2 interval on chromosome arm 5DS. According to previous studies, a series of Pm2 alleles have been reported in the Pm2 interval, such as Pm2a (Qiu et al., 2006), Pm2b (Ma et al., 2015a), Pm2c (Xu et al., 2015), PmLX66 (Huang et al., 2012), PmX3986-2 (Ma et al., 2014), PmWFJ (Ma et al., 2015b), PmYB (Ma et al., 2015c), PmZ155 (Sun H. et al., 2015), PmW14 (Sun Y. et al., 2015), PmWFJ (Ma et al., 2015a), PmSub (Jin et al., 2018), Pm10V-2 (Ma et al., 2018), PmM23 (Jia et al., 2020), and PmFG (Ma et al., 2016). Using MutChromSeq (Mutant chromosome sequencing) (Sáchez-Martín et al., 2016) and analysis of the fine mapping interval (Chen et al., 2019), Pm2 was cloned and confirmed to encode a CC-NBS-LRR protein. Haplotype analysis of 48 hexaploid common wheat carrying Pm2 alleles showed that all these Pm2 donors have the perfectly consistent as the cloned sequence above. However, different Pm2 alleles derived from hexaploid common wheat have significantly different resistant spectra (Ma et al., 2018; Jia et al., 2020). This may be due to their different genetic backgrounds, and also complex genetic constitution or resistance mechanism may be involved in this interval. Anyway, Pm2 is an elite gene locus that is very valuable for resistance breeding, even though the complex Pm2 locus has not been fully characterized.

In wheat resistance breeding using MAS, the breeding potential of a certain gene depends not only on its resistance but also on the comprehensively agronomic traits, such as yield,
quality, and high combining ability. Thus, although many Pm genes/alleles have been identified, only several have been widely used in breeding programs (Li et al., 2011). The main obstacle that limits the application of these genes is linkage drag in most resistance donors. After transferring these Pm genes into susceptible commercial cultivars, unfavorable traits linked to them will lead to poor agricultural yield or quality performances (Ma et al., 2015b). Therefore, the resistance donors with excellent agricultural performance are very popular for breeders. Fortunately, Heng 4568 is a cultivar with desirable comprehensively agronomic traits. For powdery mildew resistance, Heng 4568 also carries Pm52 besides the Pm2 allele PmH4568. In China, Pm2 and Pm52 are two major Pm genes in many resistance cultivars, such as Liangxing 66, Wennong 14, YingBo 700, Zhongmai 155, Jimai 23 (Jia et al., 2020), and Nongda 399, which all have the Pm2 allele, and Liangxing 99, Hannong 2312, Zhongxinmai 99, DH51302, and Zhimai 26, which all have Pm52 (Qu et al., 2020). Compared with these resistance cultivars, Heng 4568 has two resistance genes, which may show more durable resistance than a single resistance gene; such a situation also involved Jimai 22, a famous cultivar with the largest promotion area in the last 10 years (Jia et al., 2020). For the yield and quality, there was no significant defect in the recent years in our field. Particularly worth mentioning is its high combining ability in breeding; two famous wheat cultivars, Hengmai 28 and Jiamai 361, have been released in production using Heng 4568 as parent (https://www.Chinaseed114.com/seed/16/seed_77094.html; https://www.chinaseed114.com/seed/14/seed_69187.html), and in our lab, Heng 4568 is also a popular breeding parent for both resistance and yield improvement. Therefore, Heng 4568 can be not only directly popularized in region with high incidence of powdery mildew, but also used as a valuable breeding parent to improve powdery mildew resistance.

To transfer the Pm genes in Heng 4568, MAS is a rapid and effective way (William et al., 2007; Ashra and Foolad, 2013; Collard and Mackill, 2018). Since fine mapping of Pm52 has been carried out, many closely linked markers of Pm52 have been developed for MAS (Wu et al., 2019). In this study, the applicability of five closely linked markers and one diagnostic marker has been investigated in MAS with 16 susceptible wheat cultivars. In particular, the diagnostic marker Pm2b-map-3 is a functional marker designed by SNPs within the Pm2 sequence, suggesting that there is no recombinant in MAS. Therefore, resistance breeding using Heng 4568 as a parent is promising, and more trans-breeding studies using Heng 4568 are under way in our lab.

CONCLUSION

In this study, PmH4568, an effective Pm gene in the elite cultivar Heng 4568, has been identified and proved to be a Pm2 allele. We further clarify the genetic components of the powdery mildew resistance in Heng 4568. The applicability of closely linked markers, including the diagnostic marker, was validated in MAS. Overall, this work will accelerate the utilization of the powdery mildew resistance in Heng 4568.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

PM conceived the study. HG, XX, FL, PA, and PG performed the statistical analysis of the experiment. HG wrote the manuscript. All authors critically read, commented, and approved the final manuscript.

| Cultivars | Region | Bwm20 | Bwm21 | Bwm25 | SWGI067 | Cfd81 | Pm2b-map-3 |
|-----------|--------|-------|-------|-------|---------|-------|------------|
| Heng 4568 | Hebei  | +     | +     | +     | +       | +     | +          |
| Daimai 2173 | Shandong | -     | -     | -     | -       | -     | -          |
| Shannong 1538 | Shandong | -     | -     | -     | -       | -     | -          |
| Hennai 13 | Hebei  | -     | -     | -     | -       | -     | -          |
| Zhounai 27 | Henan  | -     | -     | -     | -       | -     | -          |
| Xinong 979 | Shaanxi | -     | -     | -     | -       | -     | -          |
| Jimai 229 | Shandong | -     | -     | -     | -       | -     | -          |
| Jimai 21 | Shandong | -     | -     | -     | -       | -     | -          |
| Jimai 20 | Shandong | -     | -     | -     | -       | -     | -          |
| Zhongyu 9998 | Henan | -     | -     | -     | -       | -     | -          |
| Wnomai 8 | Anhui  | -     | -     | -     | -       | -     | -          |
| Shimal 15 | Hebei  | -     | -     | -     | -       | -     | -          |
| Xiluo 4 | Henan  | -     | -     | -     | -       | -     | -          |
| Zhengmai 0856 | Henan | -     | -     | -     | -       | -     | -          |
| Wunong 6 | Shaanxi | -     | -     | -     | -       | -     | -          |
| Huaimai 0226 | Jiangsu | -     | -     | -     | -       | -     | -          |
| Luchen 185 | Shandong | -     | -     | -     | -       | -     | -          |
| Zhongyu 1311 | Beijing | -     | -     | -     | -       | -     | -          |

Note: “+” represents that the markers cannot amplify the polymorphic products linked to PmH4568 in the tested genetic backgrounds, and “-” shows the opposite result.
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.819844/supplementary-material
