Identification of a New Powdery Mildew Resistance Gene *pmDHT* at or Closely Linked to the *Pm5* Locus in the Chinese Wheat Landrace Dahongtuo

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Abstract

Chinese wheat landrace Dahongtuo was resistant to 35 of 38 tested Chinese isolates of *Blumeria graminis* f. sp. *tritici* at the seedling stage. Genetic analysis of the F₂ populations and their derived F_{2:3} families of crosses of Dahongtuo with the susceptible varieties Mingxian 169 and Huixianhong indicated that the resistance of Dahongtuo to *B. graminis* f. sp. *tritici* isolate E09 was conferred by a single recessive gene, tentatively designated as *pmDHT*. The gene was mapped to chromosome arm 7BL and flanked by markers *Xwmc526/XBE443877* and *Xgwm611/Xwmc511* at genetic distances of 0.8 and 0.3 cM, respectively. The chromosomal position of *pmDHT* was similar to the multi-allelic *Pm5* locus on 7BL. Allelism tests with crosses of Dahongtuo with Fuzhuang 30 (*Pm5e*) and Xiaobaidong

(*mlxbd*) indicated that *pmDHT* was allelic to both *Pm5e* and *mlxbd*. However, *pmDHT* showed a different pattern of resistance to the 38 *B. graminis* f. sp. *tritici* isolates compared with wheat lines with *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, and *PmHYM* and also differed from *PmSGA*. Thus, *pmDHT* was identified most likely as a new allele or at least a closely linked gene of the *Pm5* locus. This gene can be transferred into susceptible wheat cultivars/lines and pyramided with other resistance genes through marker-assisted selection to improve powdery mildew resistance.

Keywords: powdery mildew, *pmDHT*, mapping, Chinese wheat landrace, *Pm5* locus

Powdery mildew, caused by the *Blumeria graminis* (DC) Speer f. sp. *tritici* emend É J Marchal, is an important foliar disease, and it can cause significant grain yield reductions in many wheat-growing regions of the world (Bennett 1984; Johnson et al. 1979; Morgounov et al. 2012). So far, applying fungicides and growing resistant cultivars are most effective methods for managing powdery mildew (Deising et al. 2002). Although fungicides are effective for reducing yield losses, use of fungicides adds significant costs for wheat production and may have adverse effects on the environment (Bennett 1984; Bliffeld et al. 1999). Developing and growing resistant wheat cultivars are considered to be the most effective, economical, easy to use, and environmentally friendly approaches for controlling wheat powdery mildew. The most widely used and researched resistance sources are the major resistance genes conferring race-specific resistance (Xing et al. 2018; Zou et al. 2018). However, such resistance genes can be defeated by new virulent races or races that have been present at very low frequencies in the pathogen population but can become widespread under new selection pressure (McDonald and Linde 2002). Therefore, it is essential to explore, identify, and utilize

new sources of broad-spectrum resistance on a continuing basis to obtain more durable resistance to powdery mildew (He et al. 2018).

More than 70 formally designated powdery mildew (*Pm*) resistance genes *Pm1* to *Pm63* at 59 loci (*Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, and *Pm31* = *Pm21*) and >20 temporarily named *Pm* genes have been reported (Hao et al. 2015; Li et al. 2018; McIntosh et al. 2017; Sun et al. 2018; Tan et al. 2018; Zou et al. 2018). These genes are distributed on different wheat chromosomes, and most of them are race specific following Flor's gene-for-gene concept (Flor 1971; McIntosh et al. 2013, 2017). Some of these gene loci, such as *Pm1* (Hsam et al. 1998), *Pm2* (Ma et al. 2015; Xu et al. 2015), *Pm3* (Briggle 1966a, 1969; Sourdille et al. 1999; Zeller et al. 1993), *Pm4* (Briggle 1966b; Hao et al. 2008; Schmolke et al. 2010; The et al. 1979), *Pm5* (Hsam et al. 2001; Huang et al. 2003; Lebsack and Briggle 1974), *Pm8/Pm17* (Hsam and Zeller 1997; Lukaszewski 2000), and *Pm24* (Huang et al. 2000; Xue et al. 2012), have more than one resistance allele.

Although many *Pm* genes/alleles have been identified and named, not all identified resistance genes can be readily used in breeding programs. Many known powdery mildew resistance genes originated from rye (*Secale cereale*) and wheat's wild relatives, such as *Aegilops tauschii*, *Triticum dicoccoides*, *Triticum timopheevii*, and *Haynaldia villosa*. Breeders need to eliminate some adverse factors owing to linkage drag to use alien species-derived resistance and other desirable traits. It often takes many years for wheat breeders to eliminate the linkage drag of undesirable genes on alien chromosome segments (Klindworth et al. 2013; Summers and Brown 2013). So far, relatively few *Pm* resistance genes have been successfully utilized in developing powdery mildew-resistant wheat cultivars in China; these include *Pm2a*, *Pm4a*, *Pm6*, *Pm8*, and *Pm21* (Gao et al. 2005; Huang et al. 2012; Li et al. 2011; Liu et al. 2000).

Molecular markers have been widely used for mapping *Pm* resistance genes in wheat (Alam et al. 2011; Fu et al. 2017; McIntosh et al. 2013; Petersen et al. 2015; Xiao et al. 2013; Xie et al. 2017; Xu et al. 2015, 2018). Diagnostic molecular markers that are tightly linked to *Pm* genes have contributed to the development of near-isogenic lines for powdery mildew resistance (Zhou et al. 2005) and marker-assisted selection (MAS) for gene transfer and the pyramiding of

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resistance genes into released cultivars (Ma et al. 2018; Yildirim et al. 2004; Zhang et al. 2002). Furthermore, closely linked flanking markers have been useful for the map-based cloning of the resistance genes (Yahiaoui et al. 2004; Zou et al. 2018).

Chinese wheat landraces have been shown to be valuable genetic resources for crop improvement (Hazen et al. 2002; Ma et al. 2017). There are valuable resistance genes in the landraces, such as *Pm2c* in Niaomai (Xu et al. 2015), *Pm5e* in Fuzhuang 30 (Huang et al. 2003), *Pm24a* in Chiyacao (Huang et al. 2000), *Pm24b* in Baihulu (Xue et al. 2012), *Pm47* in Hongyanglazi (Xiao et al. 2013), *mlxbd* in Xiaobaidong (Xue et al. 2009), *PmH* in Hongquanmang (Zhou et al. 2005), *PmX* in Xiaohongpi (Fu et al. 2013), *MIHLT* in Hulutou (Wang et al. 2015), *PmT4* in Tangmai 4 (Xie et al. 2017), *PmHYM* in Hongyoumai (Fu et al. 2017), and *PmSGD* in Shangeda (Xu et al. 2018). More unidentified resistance genes likely are present in Chinese wheat landraces, which should be explored.

Chinese wheat landrace Dahongtuo is highly resistant to powdery mildew in various wheat-growing regions (Zhao et al. 2012). The major objectives of this study were to identify and map the powdery mildew resistance gene(s) in Dahongtuo, determine the relationship with previously reported alleles at the *Pm5* locus on chromosome arm 7BL, and test tightly linked markers for their usefulness in MAS.

Materials and Methods

Plant materials. Chinese wheat landrace Dahongtuo (S761) was provided by the National Crop Genebank of China (Beijing, China). F₁, F₂, and F_{2:3} seeds were obtained from the crosses of Dahongtuo with the susceptible varieties Huixianhong (HXH) and Mingxian 169 (MX169). When the resistance was tested at seedling stage in the greenhouse, F₁ and F₂ plants were inoculated using *B. graminis* f. sp. *tritici* isolate E09, representing the prevalent population in northern China (Li et al. 2011), and after recording the responses and removing sporulating leaves, the plants were transplanted into a field for the production of seeds of the next generation. The two segregating populations consisting of 208 F₂ plants from the cross of HXH × Dahongtuo and 267 F₂ plants from Dahongtuo × MX169 and their derived F_{2:3} lines were used in the genetic analysis of powdery mildew resistance. Ten randomly selected homozygous-resistant F_{2:3} lines from the cross of Dahongtuo × MX169 were tested with 34 *B. graminis* f. sp. *tritici* isolates that were avirulent to Dahongtuo, not including the E09 isolate.

The common wheat cultivars/lines CI14125 (*Pm5a*), Aquila (*Pm5b*), Xiaobaidong (*mlxbd*), Hongyoumai (*PmHYM*), Fuzhuang 30 (*Pm5e*), HXH, and MX169 plus 178 wheat cultivars/lines (Supplementary Table S1) were used in this study. The isolates of *B. graminis* f. sp. *tritici* were preserved separately in culture tubes and multiplied on seedlings of MX169 before being used in the seedling tests for powdery mildew resistance.

Powdery mildew phenotyping. Dahongtuo, HXH, MX169, and their F₁, F₂, and F_{2:3} progenies were evaluated for their powdery mildew reactions using *B. graminis* f. sp. *tritici* isolate E09. Thirty-eight single spore-derived *B. graminis* f. sp. *tritici* isolates, including isolate E09, were used to compare the reaction patterns of Dahongtuo and five wheat cultivars/lines with documented *Pm* genes on chromosome arm 7BL. Reactions of the plant materials to these *B. graminis* f. sp. *tritici* isolates were determined twice in the greenhouse. For each of the cultivars/lines, 20 seeds were sown in a 128-well (each well was 3 cm in length × 3 cm in width × 4.2 cm in height) rectangular tray (each tray was 54 cm in length × 28 cm in width × 4.2 cm in height) in a growth chamber; four seeds in four wells of the susceptible checks MX169 and HXH were also sown randomly in each tray. Seed of Dahongtuo and five wheat cultivar/lines with documented *Pm* genes on chromosome 7BL were planted in 38 rectangular trays, which were tested separately for their reaction patterns against the 38 *B. graminis* f. sp. *tritici* isolates. At the one-leaf stage (10 to 15 days old), all seedlings in a tray were inoculated with a single *B. graminis* f. sp. *tritici* isolate and then, maintained in a separate growth chamber in the greenhouse. All seedlings were inoculated with fresh spores that were multiplied on MX169 and incubated in a growth chamber at 18°C with a relative humidity >80% for 24 h; then, they were placed in the greenhouse with a diurnal cycle of 14 h of light at 22°C and 10 h of darkness

at 18°C and a relative humidity >80%. Seedling reactions were recorded 8 to 10 days after inoculation when MX169 displayed heavy sporulation. The infection type (IT) on each plant was recorded on a 0 to 4 scale: IT 0, 0; 1, and 2 were considered resistant, and IT 3 and 4 were susceptible (Sheng 1988; Wang et al. 2005).

Molecular marker linkage mapping. Total genomic DNA was extracted from wheat leaf tissues as previously described (Ma et al. 1994). DNA of resistant and susceptible bulks was prepared for bulk segregant analysis (Michelmore et al. 1991) by combining equal amounts of DNA from 10 resistant (IT = 0) or 10 susceptible (IT = 4) F₂ plants from the cross of HXH × Dahongtuo.

Five hundred simple sequence repeat (SSR) markers that were evenly distributed across all of the wheat chromosomes (Somers et al. 2004) were selected for an initial screening for polymorphic markers. The SSR markers showing polymorphisms between the two bulks were used to genotype the F₂ population. Based on their genomic location on the consensus linkage map, additional expressed sequence tag (EST)-sequence tagged site (STS) markers in the target region of chromosome 7BL were designed and synthesized in the author's laboratory, and also, they were used to genotype the F₂ population.

PCR was performed in a Veriti thermal cycler (Applied Biosystems) in a 10-μl reaction mixture containing 10 to 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 (50 mM/liter KCl, 10 mM/liter Tris-HCl, and 1.5 mM/liter MgCl₂). The PCR profile included one cycle of 94°C for 3 min followed by 36 cycles of 94°C for 30 s, 50 to 65°C (depending on the specific primers) for 40 s, and 72°C for 40 s and a final extension at 72°C for 5 min. PCR products were separated in 8% nondenaturing polyacrylamide gels with 19:1, 25:1, or 39:1 ratios of acrylamide and bis-acrylamide; then, they were silver stained and visualized as described by Santos et al. (1993).

In this study, χ^2 tests for goodness of fit were used to evaluate deviations of observed phenotypic data from the expected segregation ratios of the F₂ and F_{2:3} generations. Software MAPMAKER/Exp (version 3.0b) was used to determine linkage using an LOD (logarithm of the odds) 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 1943).

Physical mapping. DNA samples of the Chinese Spring nullite-trasomic (NT) lines (lacking one chromosome pair but compensated by the presence of an additional pair of homologous chromosomes) and ditelosomic lines (Dt; having 21 pairs of chromosomes but with one chromosome pair comprising only either the short or long arms) (Devos et al. 1999) were amplified with molecular markers to determine the chromosome arm locations of the target gene. Then, DNA samples of Chinese Spring deletion lines, in which one pair of chromosomes has different lengths of terminal deletions, were amplified with molecular markers to physically assign genes to small chromosomal regions (Qi et al. 2003).

As the powdery mildew resistance gene in Dahongtuo, tentatively named *pmDHT*, was mapped to the long arm of chromosome 7B (7BL), molecular markers flanking the gene as well as other powdery mildew resistance genes previously identified on 7BL were used to genotype Chinese Spring and its five NT lines (N7AT7B, N7BT7A, N7BT7D, N7DT7A, and N7DT7B), two Dt lines (Dt7BS and Dt7BL), and seven 7BL deletion lines (7BS-1, 7BL-14, 7BL-2, 7BL-9, 7BL-7, 7BL-10, and 7BL-3) to determine the chromosomal location of the markers and the resistance genes as described by Werner et al. (1992).

Allelism testing for determining the relationships of *pmDHT* with *Pm5e* and *mlxbd*. Crosses were made between Dahongtuo (*pmDHT*) and Fuzhuang 30 (*Pm5e*) and between Dahongtuo and Xiaobaidong (*mlxbd*), which carried the powdery mildew resistance genes on 7BL. F₁ and F₂ plants of the crosses were tested with *B. graminis* f. sp. *tritici* isolate E09, which was avirulent to resistance genes *pmDHT*, *Pm5e*, and *mlxbd*.

Results

Inheritance of the powdery mildew resistance in Dahongtuo. When the parents and F₁, F₂, and F_{2:3} progeny populations from

HXH × Dahongtong and Dahongtong × MX169 were tested with *B. graminis* f. sp. *tritici* isolate E09 at the seedling stage, Dahongtong was nearly immune with an IT 0, and both HXH and MX169 were susceptible with IT 4 (Table 1). The F₁ plants were all susceptible with an IT 4. Among 267 F₂ plants of the Dahongtong × MX169 cross, 64 were resistant with IT 0 to 2, and 203 were susceptible with IT 3 to 4, fitting a 1:3 ratio ($\chi^2 = 0.10$, $P = 0.75$) (Table 1). Among the 267 F_{2,3} lines of the Dahongtong × MX169 cross, 61 were homozygous resistant, 128 were segregating, and 78 lines were homozygous susceptible, fitting a 1:2:1 segregation ratio ($\chi^2 = 2.62$, $P = 0.27$). Among 208 F₂ plants of the HXH × Dahongtong cross, 61 were resistant with IT 0 to 2, and 147 were susceptible with IT 3 to 4, fitting a 1:3 ratio ($\chi^2 = 1.85$, $P = 0.17$). Among the 202 F_{2,3} lines of HXH ×

Dahongtong, 50 were homozygous resistant, 109 were segregating, and 43 were homozygous susceptible, fitting a 1:2:1 segregation ratio ($\chi^2 = 1.75$, $P = 0.42$). These results showed that the resistance of Dahongtong was conferred by a single recessive gene that was tentatively designated as *pmDHT*. When tested by the other 37 *B. graminis* f. sp. *tritici* isolates, Dahongtong was susceptible to only 3 isolates (B45, B46, and B47), and therefore, Dahongtong with *pmDHT* was resistant to 35 of 38 isolates, including E09 (Table 2). Ten randomly selected homozygous resistant F_{2,3} lines from Dahongtong × MX169 were tested with the 34 isolates that were avirulent to Dahongtong; not including E09 isolate, all were homozygous resistant to all 34 isolates, showing that *pmDHT* conferred the resistance to all of the avirulent isolates.

Table 1. Inheritance of resistance to powdery mildew of the segregation populations derived from crosses of Dahongtong with Mingxian 169 (MX169) and Huixianhong (HXH) and phenotypic reactions of the F₂ populations from the crosses of Dahongtong with the documented resistance stocks Fuzhuang 30 (*Pm5e*) and Xiaobaidong (*mlxbd*) to the *Blumeria graminis* f. sp. *tritici* isolate E09. “R” means resistant to the powdery mildew with the infection type (IT) 0, 0+, 1 and 2. “S” means susceptible to the powdery mildew with IT 3 and 4.

Cross	F ₁	F ₂ populations			F _{2,3} lines		
		R:S	$\chi^2_{1:3}$	P value	RR:Rr:rr	$\chi^2_{1:2:1}$	P value
Dahongtong × MX169	S	64:203	0.10	0.75	61:128:78	2.62	0.27
HXH × Dahongtong	S	61:147	1.85	0.17	50:109:43	1.75	0.42
Dahongtong × Fuzhuang 30	R	1,078:0					
Dahongtong × Xiaobaidong	R	1,672:0					

Table 2. Seedling powdery mildew infection types of Dahongtong and genotypes carrying known *Pm* genes at the *Pm5* loci after inoculation with 38 different *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates

<i>Bgt</i> isolates	Cultivars/lines (<i>Pm</i> genes)							
	Huixianhong	Mingxian 169	Dahongtong (<i>pmDHT</i>)	CI14125 (<i>Pm5a</i>)	Aquila (<i>Pm5b</i>)	Fuzhuang 30 (<i>Pm5e</i>)	Xiaobaidong (<i>mlxbd</i>)	Hongyoumai (<i>PmHYM</i>)
E03	4	4	0	4	4	0;	0;	0;
E05	4	4	0;	4	4	0;	0;	0;
E06	4	4	0;	4	4	0;	0;	0;
E09	4	4	0	4	4	0;	0;	0
E11	4	4	0	4	4	0;	0;	0
E13	4	4	0;	4	4	0;	0;	0
E16	4	4	0	4	4	0;	0;	0;
E17	4	4	0;	4	4	0;	0;	0;
E18	4	4	0;	4	4	0;	0;	0;
E21	4	4	0;	4	4	0;	0;	0;
E22	4	4	0;	4	4	0;	0;	0;
E23-1	4	4	0	4	4	0	0;	3
E23-2	4	4	0;	4	4	0;	0;	0;
E26	4	4	0;	4	4	0;	0;	0;
E30-2	4	4	0;	4	2	0	1	0;
E31	4	4	0;	4	4	0;	0;	0;
E32	4	4	0;	4	4	0;	0;	0;
E49	4	4	0;	4	0;	4	4	0;
E50	4	4	0	4	0	4	0;	0
B13	4	4	0	4	4	0;	0;	0
B14	4	4	0;	4	4	0;	0;	0;
B15	4	4	0;	4	4	4	0;	0
B16	4	4	0;	4	2	3	3	0;
B17	4	4	0;	4	4	0;	0;	0;
B41	4	4	0;	4	0;	0;	0;	0
B42	4	4	0;	4	4	0;	0;	0;
B43	4	4	0;	4	4	0;	0;	0;
B44	4	4	0	4	4	0;	0;	0
B45	4	4	3	4	4	0;	0;	0;
B46	4	4	3	4	4	0;	0;	0;
B47	4	4	4	4	4	3	4	4
B48	4	4	0;	4	4	0;	0;	0;
B49	4	4	0	4	4	0;	0;	0
B54	4	4	0	4	4	0;	0;	0
B55	4	4	0	4	4	0;	0;	0
B56	4	4	0	4	4	0	0;	0
B57	4	4	0	4	4	0;	0;	0;
B58	4	4	0	4	4	0;	0;	0

Genetic mapping of *pmDHT*. In the initial screening of SSR markers for polymorphisms between Dahongtong and HXH and the DNA bulks with 500 SSR markers, only 4 markers on chromosome 7B were consistently polymorphic between the parents and between the resistant and susceptible bulks. However, these four polymorphic markers were not closely linked to *pmDHT*. An additional 76 markers previously mapped on chromosome arm 7BL were tested, and 9 of them (*Xwmc311*, *Xbarc32*, *Xwmc526*, *Xgwm611*, *Xcfa2040*, *Xwmc581*, *Xwmc273*, *Xwmc276*, and *Xgwm577*) were polymorphic. All 208 F₂ plants from HXH × Dahongtong were genotyped with the nine markers. A genetic linkage map was constructed with the markers, and *pmDHT* was positioned 0.8 cM proximal to *Xwmc526* and 0.3 cM distal to *Xgwm611/Xwmc581* (Fig. 1A).

Because these markers are located in the physical region 0.86 to 1.00 of 7BL (<https://wheat.pw.usda.gov/GG3/>), *pmDHT* must be located in this chromosomal bin. The sequences of 146 ESTs in this region were used to develop EST-STS markers, and those markers were tested on the two parents, the DNA bulks, and the 208 plants of F₂ population. The primer sequences of the two polymorphic markers *XBE443877* and *XBF473539*, which are listed in Table 3, flanked the resistance locus at genetic distances of 0.8 and 1.2 cM, respectively (Fig. 1A). *pmDHT* was flanked by *Xwmc526/XBE443877* and *Xgwm611/Xwmc581* at genetic distances of 0.8 and 0.3 cM, respectively (Fig. 1A) and located on chromosome 7BL at a position at or near the *Pm5* locus. According to the molecular markers from the linkage map, the genetic distances of *Xgwm611* and *Xgwm577* from *pmDHT* were different from those for *Pm5d*, *Pm5e*, *mlxbd*, and *PmHYM* (Fig. 2).

Physical mapping of *pmDHT*. To determine the physical position of *pmDHT*, the SSR and EST-STS markers linked to *pmDHT* and assigned to the 0.86 to 1.00 terminal bin of 7BL based on Nemolahi et al. (2008) were tested with DNA samples of Chinese Spring and its five NT lines (N7AT7B, N7BT7A, N7BT7D, N7DT7A, and N7DT7B), two Dt lines (DT7BS and DT7BL), and seven deletion lines (7BS-1, 7BL-14, 7BL-2, 7BL-9, 7BL-7, 7BL-10, and 7BL-3). All of the 14 lines tested indicated that marker loci were

missing in the N7BT7A, N7BT7D, DT7BS, 7BS-1, 7BL-14, 7BL-2, 7BL-9, 7BL-7, and 7BL-10, whereas marker loci were detected in the N7AT7B, N7DT7A, N7DT7B, DT7BL, and 7BL-3. Thus, *pmDHT* was mapped to the 7BL-3 chromosomal bin (0.86 to 1.00) (Fig. 1B).

Allelic relationship and resistance spectra. In the cross of Dahongtong × Fuzhuang 30, all 6 F₁ and 1,078 F₂ plants were resistant (Table 1). Similarly, all 8 F₁ and the 1,672 F₂ plants from Dahongtong × Xiaobaidong were also resistant. These results indicated that the *pmDHT*, *Pm5e*, and *mlxbd* were allelic or tightly linked.

Thirty-eight different *B. graminis* f. sp. *tritici* isolates (Table 2) were used to compare the reaction patterns of Dahongtong with wheat lines possessing resistance genes *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, or *PmHYM* on chromosome arm 7BL. Both Dahongtong with *pmDHT* and Xiaobaidong with *mlxbd* were resistant to 35 of the tested 38 isolates but not the same 35 isolates (Fig. 3 and Table 2). Hongyoumai with *PmHYM* was resistant to 36 isolates, Fuzhuang 30 with *Pm5e* was resistant to 33 isolates, and Aquila with *Pm5b* was resistant to five isolates, whereas CI14125 with *Pm5a* was susceptible to all 38 isolates (Table 2). Comparing the reaction pattern of *pmDHT* with *PmSGD* (Xu et al. 2018), among the 11 isolates (E05, E06, E09, E13, E17, E18, E23-1, E23-2, E32, E49, and E50), Dahongtong was resistant to all of them, whereas Shageda with *PmSGD* was resistant to 10 and susceptible to isolate E17 (Xu et al. 2018). Thus, the reaction pattern of *pmDHT* was different from those of *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, *PmHYM*, and *PmSGD*, which are located at or near the *Pm5* locus, and therefore, *pmDHT* should be a new allele of the *Pm5* locus.

Molecular variability and validation of closely linked markers flanking *pmDHT*. The markers flanking *pmDHT* were used to test five wheat lines CI14125 (*Pm5a*), Aquila (*Pm5b*), Fuzhuang 30 (*Pm5e*), Xiaobaidong (*mlxbd*), and Hongyoumai (*PmHYM*), which were reported to carry different *Pm5* resistance alleles, and the 178 common wheat cultivars/lines (Fig. 1). The EST-STS marker *XBE443877* and the SSR marker *Xwmc526* amplified bands of 406 and 188 bp, respectively, in Dahongtong, Aquila, Fuzhuang 30, Xiaobaidong, and Hongyoumai but not in CI14125. The same bands appeared in 61 and 63 of 178 cultivars/lines, respectively, but not in the other cultivars/lines. The marker *Xwmc581* amplified a band of 136 bp in Dahongtong, Fuzhuang 30, and 76 of 178 cultivars/lines but not in CI14125, Aquila, Xiaobaidong, Hongyoumai, or the other 102 cultivars/lines. The marker *Xgwm611* amplified bands of 166 bp in Dahongtong but not in the five wheat lines with *Pm5* resistance alleles *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, and *PmHYM* or the 178 cultivars/lines tested.

The markers *XBE443877* and *Xwmc526* could be used to distinguish *pmDHT* from *Pm5a* but could not distinguish *pmDHT* from *Pm5b*, *Pm5e*, *mlxbd*, and *PmHYM*. *Xwmc581* could be used to distinguish *pmDHT* from *Pm5a*, *Pm5b*, *mlxbd*, and *PmHYM* but could not distinguish *pmDHT* from *Pm5e*. *Xgwm611* could be used to distinguish *pmDHT* from *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, and *PmHYM*, and it could also be used as a diagnostic marker for detecting *pmDHT* in the different genomic backgrounds for MAS.

Discussion

Because wheat powdery mildew has become increasingly important and several resistance genes have become ineffective (He et al. 2018; Li et al. 2011; Xing et al. 2018; Zhao et al. 2013; Zhuang and Li 1993), there is an urgent need to identify new effective resistance genes or alleles to develop cultivars with long-lasting resistance through gene pyramiding. In this study, a new recessive gene for resistance to powdery mildew, tentatively named *pmDHT*, was

Fig. 1. Linkage map of *pmDHT*. **A**, Linkage map of *pmDHT* on chromosome arm 7BL. The black arrow points to the centromere, and genetic distances are shown on the left in centimorgans. **B**, Bin assignments of *pmDHT*-linked markers are shown with dashed line connections and breakpoints, and fraction lengths (FL) of the bins are shown to the right.

Table 3. Sequence information of polymorphic expressed sequence tag-sequence tagged site (EST-STS) markers on chromosome arm 7BL used in the Dahongtong resistance gene mapping

EST-STS marker	Chromosome	Forward sequence (5'–3')	Reverse sequence (5'–3')
<i>XBE443877</i>	7BL	GTAGACCAAGCACTATT	AAGCATTTGACATCTCCC
<i>XBF473539</i>	7BL	GGATGGGATGCTGTCTTT	TGTGGTTCAGTCGGTGTAG

identified in the Chinese wheat landrace Dahongtong. Tests in this study have shown that this gene confers a broad spectrum of resistance against *B. graminis* f. sp. *tritici* isolates. Therefore, this gene would be useful to pyramid with other effective *Pm* genes to develop new cultivars with high-level and long-lasting resistance to powdery mildew.

Resistance gene *pmDHT* was mapped in the terminal region of chromosome arm 7BL. To date, several *Pm* genes, including *Pm5* (*a-e*), *mlxbd*, *PmTm4*, *PmHYM*, and *PmSGD*, have been mapped on 7BL. The results of this study indicate that *pmDHT*, *Pm5e*, and *mlxbd* are allelic, and the resistance spectrum of *pmDHT* is different from those of *Pm5a*, *Pm5b*, *Pm5e*, *mlxbd*, *PmHYM*, and *PmSGD*. Genetic analysis indicated that the resistance of Dahongtong to *B. graminis* f. sp. *tritici* isolate E09 was conferred by the recessive gene *pmDHT*, and therefore, *pmDHT* is different from *PmT4*, because *PmT4* was reported as a semidominant gene (Hu et al. 2008; Xie et al. 2017). Kolandi was a *Triticum sphaerococcum*-derived wheat line

(ATRI 2996/74) from India, and it carried *Pm5c*. IG1-455 (CI10904/7*Prins) and IG1-556 (CI10904/7*Starke) were the Kolandi backcross-derived wheat lines, and they also carried *Pm5d* (Hsam et al. 2001; Nematollahi et al. 2008). Based on the genetic backgrounds of these wheat lines, *pmDHT* identified in this study is genetically distant from *Pm5c* and *Pm5d*.

The genetic distances of *Xgwm611* and *Xgwm577* from *pmDHT* were different from those from *Pm5d*, *Pm5e*, *PmHYM*, and *mlxbd* according to the molecular markers on the linkage map (Fig. 2). The sequences of all 17 single-nucleotide polymorphism markers for *PmSGD* (Xu et al. 2018) were BLAST searched against the genomic sequence of Chinese Spring (https://urgi.versailles.inra.fr/blast_iwgc/blast.php). All of them were located in the range of 700.4 to 739.6 Mb and overlapped with *pmDHT* marker sequences of *Xwmc311*, *Xbarc32*, *Xwmc581*, *Xcfa2040*, *Xwmc273*, *Xwmc276*, and *XBE443877*, which were located in the 690.9- to 734.9-Mb regions. The comparison of the molecular markers suggests that

Fig. 2. Comparison of molecular markers linked to genes *Pm5d* (A), *Pm5e* (B), *pmDHT* (C), *mlxbd* (D), and *PmHYM* (E) on chromosome arm 7BL.

Fig. 3. Seedling reactions of Dahongtong, Fuzhuang 30 (*Pm5e*), Xiaobaidong (*mlxbd*), and Huixianhong (susceptible check) after inoculation with seven different isolates of *Blumeria graminis* f. sp. *tritici*.

pmDHT is likely different from *Pm5d*, *Pm5e*, *mlxbd*, and *PmHYM*, whereas *pmDHT* and *PmSGD* overlap in the same genomic region; however, they are different alleles, because these two *Pm* genes possessed different reaction patterns to *B. graminis* f. sp. *tritici* isolates.

Some *Pm* genes from Chinese wheat landraces, such as *Pm5e* in Fuzhuang 30 (Huang et al. 2003), *PmH* in Hongquanmang (Zhou et al. 2005), *mlxbd* in Xiaobaidong (Xue et al. 2009), *Pm47* in Hongyanglazi (Xiao et al. 2013), *PmX* in Xiaohongpi (Fu et al. 2013), *PmHYM* in Hongyoumai (Fu et al. 2017), and *PmSGD* in Shangeda (Xu et al. 2018), were previously reported as recessive genes. Although studies have been conducted to determine the mechanisms controlling the dominant or recessive nature of the genes (Frye et al. 2001; Nishimura et al. 2003), the mechanisms controlling recessive powdery mildew resistance genes in Chinese wheat landraces are still unknown. Additional studies are needed to determine the resistance mechanisms, especially for the alleles at the *Pm5* locus.

In this study, *pmDHT* was identified and mapped as a new allele of the *Pm5* locus or a tightly linked gene with *Pm5* in the Chinese wheat landrace Dahongtuo. *pmDHT* provides a broad spectrum of resistance to different virulent *B. graminis* f. sp. *tritici* isolates from China. This gene can be transferred into susceptible wheat cultivars/lines or combined with other effective resistance genes through MAS, which would be useful for improving powdery mildew resistance. There are several alleles at the *Pm5* locus, especially in the Chinese wheat landraces. Fine mapping to develop a high-density genetic linkage map for *pmDHT* would be beneficial for the further characterization of the allelic variation at *Pm5* locus. Such information and genetic resources should be useful for wheat scientists to study and develop new wheat cultivars with durable resistance to powdery mildew.

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