Fine Mapping a Broad-Spectrum Powdery Mildew Resistance Gene in Chinese Landrace Datoumai, *PmDTM*, and Its Relationship with *Pm24*

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Abstract

Powdery mildew, caused by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a globally important wheat disease causing severe yield losses, and deployment of resistant varieties is the preferred choice for managing this disease. Chinese wheat landrace Datoumai was resistant to 22 of 23 *Bgt* isolates at the seedling stage. Genetic analysis based on the inoculation of *Bgt* isolate E09 on the F₁, F₂, and F_{2:3} populations derived from the cross Datoumai × Huixianhong revealed that the powdery mildew resistance of Datoumai is controlled by a single dominant gene, temporarily designated as *PmDTM*. Bulk segregant analysis and simple sequence repeat mapping with 200 F₂ plants showed that *PmDTM* was located in the same genetic region as *Pm24* on chromosome 1DS. To fine map *PmDTM*, 12 critical recombinants were identified from 1,192 F₂ plants and delimited *PmDTM* to a 0.5-cM *Xhnu58800* to *Xhnu59000* interval covering 180.5 Kb (38,728,125 to

38,908,656 bp) on chromosome 1DS, and only one highly confident gene, *TraesCS1D02G058900*, was annotated within this region. *TraesCS1D02G058900* encodes a receptor-like serine/threonine-protein kinase (STK), and a 6-bp deletion in exon 5 may confer the resistance to powdery mildew. Allele frequency analysis indicated that the *STK* allele with 6-bp deletion was only present in three landraces (Datoumai, Chiyacao [*Pm24*], and Hulutou) and was absent in all of the 353 Chinese modern cultivars and 147 foreign cultivars. These results demonstrate that *PmDTM* is mapped to the same locus as *Pm24* and can be widely used to enhance powdery mildew resistance in wheat growing regions worldwide.

Keywords: *Blumeria graminis* f. sp. *tritici*, candidate gene, fine mapping, powdery mildew, *Pm24*, wheat

Bread wheat (*Triticum aestivum* L.) is one of the most important crops and a staple food for about 40% of the world population. Wheat yield is constantly challenged by many diseases, and wheat powdery mildew, caused by the fungus *Blumeria graminis* (DC.) Speer f. sp. *tritici* emend. É. J. Marchal (*Bgt*), is an epidemic foliar disease worldwide, and the pathogen competes for nutrients and reduces the photosynthetic ability of the leaves (Yao et al. 2007). The use of high-yielding dwarf varieties and modern wheat cultivation technologies have further increased the epidemic areas of powdery mildew (Singh et al. 2016). In China, powdery mildew has affected an area of around 6 to 8 million hectares in recent years (Sun et al. 2018). Both yield and quality of wheat suffer seriously from powdery mildew, and the commercial yield losses are 30 to 40% in heavy epidemic years (Conner et al. 2003; Samobor et al. 2006; Singh et al. 2016). Genetically controlled resistance is the basis of breeding for powdery mildew-resistant cultivars, which is critical for combating this disease.

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Two types of powdery mildew resistance, qualitative and quantitative resistance, have been reported in wheat (Huang and Röder 2004). Qualitative resistance, controlled by single genes, is most widely employed in wheat breeding programs. Up to now, 65 permanently designated genes and over 20 temporarily designated *Pm* or *MI* resistance genes or quantitative trait loci have been identified (Li et al. 2019a; McIntosh et al. 2013, 2017). However, due to the complexity of wheat genome (15.4 to 15.8 Gb, IWGSC 2018), only seven genes (*Pm2*, *Pm3*, *Pm8*, *Pm21*, *Pm38*, *Pm46*, and *Pm60*) have been cloned to date (He et al. 2018; Hurni et al. 2013; Krattinger et al. 2009; Moore et al. 2015; Sánchez-Martín et al. 2016; Yahiaoui et al. 2004; Zou et al. 2018). Among them, *Pm3*, *Pm21*, *Pm38*, *Pm46*, and *Pm60* were isolated by standard map-based cloning. These cloned powdery mildew resistance genes can be divided into two categories. Genes of the first category, such as *Pm2*, *Pm3*, *Pm8*, *Pm21*, and *Pm60*, confer race-specific resistance and generally encode a nucleotide-binding and leucine-rich repeat (NB-LRR)-type protein that activates effector-triggered immunity. Genes of the second category such as *Pm38* (Krattinger et al. 2009) and *Pm46* (Moore et al. 2015), confer non-race-specific resistance or adult-plant resistance and encode an ATP-binding cassette transporter or a hexose transporter. The second category genes often provide durable resistance to multiple diseases including powdery mildew, leaf rust, and stripe rust. In addition to these two resistance mechanisms, the plant receptor-like protein kinase superfamily consisting of transmembrane proteins also plays important roles in plant defense (Liu et al. 2019). These receptors recognize microbial-associated or pathogen-associated molecular patterns (PAMPs) and activate PAMP-triggered immunity (Jones and Dangl 2006). For example, wheat gene *Yr15*, comprising two tandem kinase-pseudokinase domains, provides broad-spectrum resistance against stripe rust (Klymiuk et al. 2018). However, the protein kinase genes involved in powdery mildew resistance in wheat have not been cloned and reported to date.

The rapid emergence of virulent *Bgt* isolates has prompted the search for more durable resistance. Broad-spectrum resistance genes are much sought after by breeders, because such genes generally can provide robust protection against diseases. The majority of the mapped genes with broad-spectrum powdery mildew resistance come from wild species or wheat relatives, such as *Pm12* from *Aegilops speltoides* (Jia et al. 1996), *Pm16* from wild emmer (Chen et al. 2005), *Pm20* from rye (Friebe et al. 1994), and *Pm21* and *Pm62* from *Dasypyrum villosum* (He et al. 2018; Zhang et al. 2018). However, no elite cultivars with these genes except *Pm21* have been developed due to the unfavorable linkage drag (Summers and Brown 2013). On the other hand, genes originating from landraces or modern cultivars can be more easily introgressed into new cultivars. There are more than 14,000 wheat landraces in the national seed bank of China, and landraces exhibiting high resistance to *Bgt* isolates have been identified in recent years; some of them confer broad-spectrum powdery mildew resistance, such as Niao-mai (carrying *Pm2c*) (Xu et al. 2015), Chiyacao (carrying *Pm24*) (Huang and Röder 2011), Baihulu (carrying *Pm24b*) (Xue et al. 2012), and Hulutou (carrying *MIHLT*) (Wang et al. 2015). Datou-mai (DTM), a Chinese wheat landrace from the national seed bank, also showed high resistance to more than 20 prevalent *Bgt* isolates in China. In this study, we report: (i) characterization of the DTM powdery mildew resistance gene *PmDTM*; (ii) fine mapping and identification of the candidate genes of *PmDTM*; and (iii) development of breeder-friendly polymerase chain reaction (PCR)-based markers and kompetitive allele-specific PCR (KASP) markers for marker-assisted introgression of *PmDTM* into modern elite lines.

Materials and Methods

Plant materials and pathogen isolates. DTM was crossed with Huixianhong (HXH), which is highly susceptible to powdery mildew. Twenty-three Chinese *Bgt* isolates were used to compare the reactions of DTM, HXH, and 11 wheat accessions with known powdery mildew resistance genes (Table 1). Isolate E09 was used to inoculate parental lines DTM and HXH and the F_1 , F_2 , and $F_{2:3}$ progenies derived from the cross DTM $\times$ HXH. A total of 669 F_2 plants from the cross Chiyacao (*Pm24*) $\times$ DTM were employed to analyze the genetic relationship between *PmDTM* and *Pm24*. Furthermore, 576 core wheat germplasm accessions from the Chinese Academy of Agricultural Sciences were used for frequency analysis of the *PmDTM* candidate gene.

Powdery mildew evaluations. A protocol described by Xu et al. (2015) was used to evaluate powdery mildew response. In brief, the F_2 populations and 20 to 25 seedlings from each $F_{2:3}$ family were evaluated for responses to *Bgt* isolate E09 in a growth chamber. Each F_2 plant was planted in a single cell (3×3 cm) of a 72-cell tray, and DTM and HXH were used as resistant and susceptible

checks, respectively. All seedlings at the two-leaf stage were inoculated with fresh conidiospores and incubated in a chamber with a daily cycle of 16 h of light at 22°C and 8 h of darkness at 18°C. The infection type (IT) was recorded 7 to 10 days postinoculation when HXH displayed severe symptoms and signed with a 0 to 4 scale; 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 hyphae 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 hyphae. The values of 0 to 2 were classified as resistant, and those of 3 to 4 were classified as susceptible (Xu et al. 2015).

Preliminary and fine mapping of *PmDTM*. Genomic DNA was extracted from young leaves according to the method of Zhao et al. (2019). PCR was performed following the procedure of Zhang et al. (2017). The PCR products were separated in either 8% nonde-naturing polyacrylamide gels (Acrylamide:Bisacrylamide = 19:1 or 29:1) or 1% agarose gels.

DNA bulks were prepared for bulked segregant analysis by pooling equal amounts of DNA from 10 homozygous resistant F_2 plants or 10 homozygous susceptible plants. Together with DNA from the two parents, the resistant and susceptible bulks were genotyped with the Wheat 55K SNP Array (Zhang et al. 2019). Finally, a 42.8-Mb interval on chromosome 1DS was found to be associated with the powdery mildew resistance gene in DTM based on the Chinese Spring (CS) reference sequence IWGSC RefSeq version 1.0 (IWGSC 2018). Linkage analysis with 200 F_2 plants and four known simple sequence repeat (SSR) markers located on chromosome 1DS delimited *PmDTM* to the interval between *Xgwm337* and *Xbarc229*. To fine map *PmDTM*, more recombinants were identified from a large F_2 population by genotyping with these two flanking markers. The CS reference sequence corresponding to the target interval was used to screen for new SSR markers with the Perl script MISA program (<https://webblast.ipk-gatersleben.de/misa/>). Three highly confident genes (*TraesCS1D02G058800*, *TraesCS1D02G058900*, and *TraesCS1D02G059000*) residing within the *PmDTM* region were amplified and sequenced to find single nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (InDel) between DTM and HXH, which were used to develop KASP or PCR-based markers. All primers were designed with MacVector 11.0 (Accelrys, Cambridge, U.K.). Primer information of the markers detecting polymorphisms is shown in Supplementary Table 1.

KASP genotyping assays. KASP assays were performed on a LightCycler 480 II system (Roche, Indianapolis, IN). Reaction mixtures consisted of final volumes of 10 μ l containing 5 μ l of 2 $\times$ KASP Master mix (LGC Genomics), 0.14 μ l of primer mix (12 μ M of each allele-specific primer and 30 μ M of common primer), and 5 μ l

Table 1. Differential reactions of resistant Datoumai (DTM), susceptible Huixianhong (HXH), and 11 accessions with known powdery mildew resistance genes to 23 isolates of *Blumeria graminis* f. sp. *tritici* (*Bgt*) collected from different regions of China

Cultivar/line	Pm	Bgt isolate																							
		B13	B14	B15	B17	B18	B41	B48	B49	B50	B56	E05	E06	E07	E09	E17	E18	E20	E21	E23	E31	E32	E49	E50	
Axminster/ 8*Cc ^a	Pm1	4	0	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	3	4	4	
Ulka/8*Cc	Pm2	0	4	0	0	4	0	0	0	4	0	0	0	0	0	4	0	4	0	0	0	0	0	0	
Khapli/8*Cc	Pm4a	0	0	0	0	4	1	0	0	4	4	0	1	0	0	4	4	0	4	0	4	0	0	0	
	Pm4b	0	0	0	0	4	0	0	0	4	4	0	0	0	0	4	4	0	4	0	4	0	0	1	
Fuzhuang 30	Pm5e	0	1	4	3	0	4	0	3	0	0	0	4	0	0	1	0	3	0	3	0	0	4	0	
Timgalen	Pm6	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
Yangmai5/ Sub.6V	Pm21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
81-7241	Pm23	0	0	0	0	4	0	0	0	4	4	0	0	0	0	4	4	0	0	0	4	0	0	0	
Chiyacao	Pm24	0	0	0	0	1	0	0	0	0	0	3	1	0	0	0	3	0	3	0	3	0	0	0	
Hulutou	MIHLT	0	0	3	1	0	0	1	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	1	
5P27	Pm30	0	4	0	0	4	0	0	0	4	0	0	0	1	0	0	4	0	3	0	0	0	0	0	
DTM	PmDTM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	1	0	0	0	
HXH	...	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

^a Seven times backcrossed to cultivar Chancellor.

(100 ng) of genomic DNA. The cycling conditions were as follows: 94°C for 15 min, 10 cycles of 94°C for 20 s, touchdown starting at 61°C for 60 s (decreasing 0.6°C per cycle), 32 cycles of 94°C for 20 s, 55°C for 60 s, and a final KASP reading step of 37°C for 60 s. The end-point fluorescence data were visualized and analyzed by LightCycler 480 software version 1.5.1.62.

Isolation and sequencing of the full-length cDNA of *PmDTM* candidate gene. Total RNA was extracted with TRIzol reagent (Invitrogen) according to the method of Su et al. (2019). First-strand cDNA was synthesized with HiScript II (Vazyme, Nanjing, China) using Oligo (dT)₂₃ primer. Nested PCR was carried out first with STK_UTR_F1 and STK_UTR_R1, and then with STK_UTR_F2 and STK_UTR_R2 (Supplementary Table 1). The PCR products were purified, cloned, and sequenced.

Quantitative reverse transcription PCR (qRT-PCR) analysis. For gene expression assays, 7-day-old seedlings of DTM and HXH were treated with *Bgt* isolate E09. Samples from different plants were collected immediately before inoculation (0 h) and at 3, 6, 12, 24, 48, and 72 h postinoculation (hpi). The cDNAs were obtained from 2 µg of total RNA using a reverse transcription kit HiScript Q RT Super-Mix for qPCR (Vazyme). qRT-PCR reactions were performed with primer STK-E5 and AceQ Universal SYBR qPCR Master Mix (Vazyme) following the previously described procedure (Long et al. 2018). The *TaGAPDH* gene was used as an internal control, and the 2^{-ΔΔCt} method was used to calculate the relative gene expression (Livak and Schmittgen 2001). All primers for qRT-PCR assays are listed in Supplementary Table 1.

Frequency analysis of the resistant allele. The PCR-based markers *STK-E5* and *STK-CS* representing the critical variation of the *PmDTM* candidate gene were used to determine the presence or absence of a resistant allele in 576 wheat accessions including 76 Chinese landraces, 353 Chinese modern cultivars, and 147 foreign cultivars. The primers of *STK-E5* and *STK-CS* were designed according to the genomic sequences of DTM and CS, respectively. Their forward primer sequences are highly variable (Supplementary Table 1).

Data analyses. χ^2 tests were performed to test the hypothesis that a dominant powdery mildew resistance allele conferred resistance in the F₂ and F_{2:3} populations. The genetic linkage map (Supplementary Fig. 2) was calculated using the maximum likelihood algorithm and the Kosambi function in JoinMap 4.0 (Van Ooijen 2006). The translated protein sequences of the *PmDTM* candidate gene were used as queries to search the NCBI Conserved Domains Database for domain analysis (https://www.ncbi.nlm.nih.gov/Structure/cdd/docs/cdd_search.html).

Results

Inheritance of the powdery mildew resistance in DTM. Segregating populations from DTM × HXH and the parents were tested with *Bgt* isolate E09 at the seedling stage. DTM was immune to

Fig. 1. Powdery mildew reactions of resistant parent Datoumai (DTM), susceptible parent Huixianhong (HXH), and their F₁ hybrids to *Blumeria graminis* f. sp. *tritici* isolate E09.

IT 0, and HXH was highly susceptible with IT 4 (Fig. 1). The F₁ hybrids were highly resistant (IT 0) to E09, indicating the dominant nature of the powdery mildew resistance in DTM. Among 200 F₂ plants, 156 were resistant (IT 0 to 1) and 44 susceptible (IT 3 to 4), fitting a 3:1 ratio ($\chi^2 = 0.96$, $P > 0.05$). The F_{2:3} populations segregated as 50 homozygous resistant, 106 segregating, and 44 homozygous susceptible, fitting a 1:2:1 segregation ratio ($\chi^2 = 1.08$, $P > 0.05$). These results suggested that the powdery mildew resistance in DTM was controlled by a single dominant gene, temporarily designated as *PmDTM*.

Preliminary mapping of *PmDTM* and its relationship with *Pm24*. To localize *PmDTM* in the wheat genome, the parental lines as well as the resistant and susceptible DNA bulks were genotyped with the Wheat 55K SNP Array. Out of 553 polymorphic SNPs identified between the parental lines as well as the DNA bulks, 136 SNPs were distributed on chromosome 1D (Supplementary Fig. 1). Further analysis indicated that 101 SNPs were clustered in a 42.8-Mb genomic region (7.4 to 50.2 Mb) on chromosome 1DS. The known SSR markers located on 1DS were subsequently surveyed for polymorphisms. Linkage mapping with 200 F₂ plants and four polymorphic markers (*Xgwm337*, *Xbarc229*, *Xgpm4503*, and *Xcfd83*) initially localized *PmDTM* into a genetic interval flanked by *Xgwm337* and *Xbarc229* (Fig. 2A). A comparison with published genetic maps suggested that *PmDTM* was located in the same chromosomal region as *Pm24* (Supplementary Fig. 2; Huang and Röder 2011), *Pm24b* (Xue et al. 2012), and *MIHLT* (Wang et al. 2015). The F₂ population consisting of 669 plants derived from Chiyacao (*Pm24*) × DTM was inoculated with isolate E09, and no visible symptom was observed for all F₂ plants. On the basis of these results, we proposed that the resistance gene in DTM was mapped to the same locus as *Pm24*.

Powdery mildew reactions of DTM and accessions with known genes. Differential reactions of DTM, HXH, and 11 wheat accessions possessing known powdery mildew resistance genes to 23 *Bgt* isolates are listed in Table 1. *Pm6* has lost resistance to all Chinese *Bgt* isolates used in this study. DTM was highly resistant to 22 isolates and was susceptible only to isolate E18 (Table 1). Two landraces, Chiyacao and Hulutou, also conferred broad-spectrum

Fig. 2. Map-based cloning of *PmDTM*. **A**, High-density genetic map of the 1DS region containing *PmDTM*. The maps are constructed based on 200 (upper) and 1,192 (lower) F₂ plants. Numbers of crossovers identified between the adjacent marker loci are shown above the rectangle. **B**, Genes annotated within the 180.5-Kb candidate region according to the IWGSC CS RefSeq version 1.1 annotation. **C**, Gene structure of *STK* in resistant Datoumai (DTM) and susceptible Huixianhong (HXH). Orange rectangles represent exons, and black lines represent introns. Four lettered vertical lines represent variations of *STK* between DTM and HXH, including one SSR, one 6-bp InDel, and two SNPs.

resistance to powdery mildew but were susceptible to four and two *Bgt* isolates, respectively (Table 1). The landrace Baihulu was not included in this study due to being unavailable in our laboratory. However, Baihulu has been reported to be resistant to isolate E18 (Xue et al. 2012). Thus, the *PmDTM* gene in DTM gave a unique reaction pattern that was different from other resistance alleles at the *Pm24* locus.

Fine mapping of *PmDTM*. For the saturation of the genetic linkage map of the *PmDTM* region, a total of eight polymorphic SSR markers were developed based on the 29.6-Mb CS reference sequence corresponding to the *Xgwm337* to *Xbarc229* interval (Supplementary Figs. 2 and 3). To fine map *PmDTM*, 168 recombinants harboring 173 crossovers were identified between the flanking markers *Xgwm337* and *Xbarc229* from 1,192 DTM × HXH F_2 plants (Fig. 2). These recombinants were genotyped with the newly developed SSR markers and phenotyped with their $F_{2:3}$ progenies. Further linkage analysis indicated that *PmDTM* was flanked by *Xhnu651* and *Xhnu1189*, and cosegregated with *Xhnu1092* and *Xhnu1100* (Fig. 2A; Supplementary Fig. 2). The SSR marker *Xhnu1092* was located in an intergenic region, whereas *Xhnu1100* was located within gene *TraesCS1D02G058900* (Fig. 2B) according to the IWGSC CS RefSeq version 1.1 annotation (https://urgi.versailles.inra.fr/download/iwgs/IWGSC_RefSeq_Annotations/v1.1/). We sequenced and compared the full-length genomic sequences of *TraesCS1D02G058900* and adjacent *TraesCS1D02G058800* and *TraesCS1D02G059000*. A total of six variations were identified between DTM and HXH. There was only one C/T SNP that occurred in *TraesCS1D02G058800* and only one T/C SNP that occurred in *TraesCS1D02G059000*. These two nonsynonymous SNPs were converted to KASP makers (Fig. 3A and C) and were used to genotype the 168 recombinants. We identified 11 crossovers between *Xhnu58800* (*TraesCS1D02G058800*) and *PmDTM*, and only one crossover between *PmDTM* and *Xhnu59000* (*TraesCS1D02G059000*) (Fig. 2A). Except for the SSR marker

Xhnu1100 mentioned above, three additional variations in *TraesCS1D02G058900* were identified between DTM and HXH, including a 6-bp InDel and two SNPs. The 6-bp InDel is located within the predicted exon 5 (Fig. 2C). We developed a KASP marker (*Xhnu58900*, Fig. 3B) and a breeder-friendly PCR marker (*STK-E5*, Fig. 3D) based on the 6-bp variation to genotype all the recombinant plants. As expected, this InDel variation (*Xhnu58900*) was cosegregated with *PmDTM* (Fig. 2A). Because only one recombinant (P5-236) was identified between *Xhnu58900* and *Xhnu59000*, we further checked the genotypes and phenotypes of 43 F_3 plants derived from P5-236 (Supplementary Table 2). All the F_3 plants were resistant to *Bgt* E09, which was in coincidence with the genotypes checked by *Xhnu58900*. Thus, *PmDTM* was finally fine mapped to a 0.5-cM *Xhnu58800* to *Xhnu59000* interval covering 180.5 Kb (38,728,125 to 38,908,656 bp) on chromosome 1DS, and *TraesCS1D02G058900* was the most likely candidate gene for *PmDTM* (Fig. 2; Supplementary Fig. 2).

Candidate gene analysis. We compared a full-length cDNA of *TraesCS1D02G058900* with the corresponding genomic sequence and determined that this gene consists of 11 exons and 10 introns (Fig. 2C). *TraesCS1D02G058900* in DTM spans 10,410 bp from the starting ATG to the termination TAA, with a complete coding sequence of 2,682 bp (Supplementary Fig. 4). *TraesCS1D02G058900* encodes a receptor-like protein with two putative serine/threonine kinase (STK) domains in tandem (Fig. 4) and is designated here as *STK*. Comparison of the complete STK region in DTM revealed a deletion of two amino acids (K [Lys] and G [Gly] at positions 400 and 401) relative to the susceptible parent HXH (Fig. 4). The KG deletion resides in the STK1 domain and may be critical for the resistance to powdery mildew.

Transcript levels of *STK* relative to *TaGAPDH* were analyzed in DTM and HXH by qRT-PCR (Fig. 5). The basal transcript levels of *STK* in DTM were 1.5-fold higher than in HXH immediately

Fig. 3. KASP and PCR-based markers developed in this study. The KASP markers are developed according to the SNP in *TraesCS1D02G058800* (A), InDel in *TraesCS1D02G058900* (B), and SNP in *TraesCS1D02G059000* (C). The blue (bottom right) and green (upper left) triangles represent resistant Datoumai (DTM) and susceptible Huixianhong (HXH) alleles, respectively. The red (center) triangles represent heterozygous alleles. The gray dots (bottom left) represent the negative control. PCR marker *STK-E5* (D) was developed according to the 6-bp variation in exon 5 of *TraesCS1D02G058900*. R, S, and H indicate resistant, susceptible, and heterozygous plants, respectively, in the F_2 population. M is the DNA size standard.

before the time of inoculation (0 h). The expression of *STK* in DTM was rapidly down-regulated by the presence of the pathogen and reached the lowest level at 12 hpi. The *STK* expression pattern in HXH was similar to that in DTM, although different expression levels were detected between them throughout the time course except 24 hpi (Fig. 5). These results suggested that *STK* was transiently induced by the infection of *Bgt* E09, and the relative abundance of the transcript varied and differed between the resistant and susceptible parents.

Frequency analysis of the *STK* allele with 6-bp deletion. The PCR-based markers *STK-E5* and *STK-CS* developed on the basis of the 6-bp variation were used to analyze the frequency of the resistant *STK* allele in 576 wheat accessions. Single bands were amplified in 429 accessions with *STK-E5*, and the rest were successively amplified with *STK-CS* (data not shown). Surprisingly, the allele with 6-bp deletion was only present in three Chinese landraces: DTM, Chiyacao, and Hulutou. All of the 353 Chinese modern cultivars and 147 foreign cultivars carry the susceptible *stk* allele, implying that there is a great potential to utilize the rare allele with 6-bp deletion for further genetic improvement. To confirm this observation, we cloned the full-length cDNA of *STK* from Chiyacao (*Pm24*), Hulutou (*MIHLT*), and Fielder. As shown in Supplementary Figure 4, Chiyacao and Hulutou have the same coding sequence as DTM, and Fielder (an excellent receptor for wheat genetic transformation) is susceptible to *Bgt* and has the same coding sequence as HXH. These results further demonstrate that *PmDTM*, *Pm24*, and *MIHLT* are mapped to the same locus, and *STK* is the most likely candidate gene of them.

Discussion

Wheat landraces are abundant in China and show wide genetic diversity for powdery mildew resistance. Up to now, more than 10 powdery mildew resistance genes or alleles have been identified from Chinese wheat landraces (Tan et al. 2019). In the present study, a broad-spectrum powdery mildew resistance gene, *PmDTM*, in Chinese landrace DTM was fine mapped using a large population with 1,192 F₂ plants. Based on the genotypes at the gene interval and phenotypes of the identified recombinants, *PmDTM* was placed in the 0.5-cM *Xhnu58800* to *Xhnu59000* interval on 1DS. Chromosome 1DS also harbors three known powdery mildew resistance genes, including *Pm24* (Huang and Röder 2011), *Pm24b* (Xue et al. 2012), and *MIHLT* (Wang et al. 2015). Fine mapping and map-based cloning of these disease resistance genes helps to clarify the exact genetic relationship among them.

Fig. 4. Protein sequence alignment between *STK* in resistant Datoumai (DTM) and *stk* in susceptible Huixianhong (HXH). Domains in the protein sequences were predicted based on the NCBI Conserved Domains Database. Two putative serine/threonine kinase (STK) domains were separately marked with red and blue rectangles.

PmDTM was finally mapped to the region between *TraesCS1D02G058800* and *TraesCS1D02G059000*, in a 180,531 bp genomic region based on the CS genome sequence (Fig. 2C). Only one highly confident gene, *TraesCS1D02G058900* (*STK*), was annotated within this region (Fig. 2B). *STK* encodes a receptor-like protein kinase with 893 and 895 amino acids in DTM and HXH, respectively (Fig. 4). The KASP marker *Xhnu58900* developed according to the 6-bp variation in *STK* always cosegregated with the phenotypes. Thus, we concluded that *STK* was the only reasonable candidate gene for *PmDTM*, and further genetic transformation experiments are needed to validate its role in resistance to *Bgt* E09.

To prevent the infection of pathogen, plants have evolved distinct resistance mechanisms including effector-triggered immunity, PAMP-triggered immunity, and quantitative disease resistance (Krattinger and Keller 2016). Most of the powdery mildew resistance genes cloned so far confer race-specific resistance, such as *Pm2*, *Pm3*, *Pm8*, *Pm21*, and *Pm60* (He et al. 2018; Hurni et al. 2013; Sánchez-Martín et al. 2016; Yahiaoui et al. 2004; Zou et al. 2018). They encode NB-LRR-type proteins and activate effector-triggered immunity. By contrast, *STK* encodes a protein containing two putative STK domains (Fig. 4). To date, only two wheat disease resistance genes, *Yr15* (Klymiuk et al. 2018) and *Sr60* (Chen et al. 2020), were found to possess two tandem kinase or pseudokinase domains. The expression pattern of *STK* was similar to *Yr15*; both were down-regulated by the presence of the pathogen (Fig. 5). The deletion of two amino acids (KG) was located within the STK1 domain, and the STK2 domain was identical between DTM and HXH (Fig. 4). For this phenomenon, we speculated that the KG deletion might alter the kinase activity and eventually lead to the different response of the host to *Bgt* isolates.

Cloning and sequencing of *STK* in DTM, Chiyacao, and Hulutou indicated that *PmDTM* was the same as previously designated *Pm24* and *MIHLT*, although they showed some differences in reaction to 23 Chinese *Bgt* isolates (Table 1). The different resistance spectrum may be mainly caused by the variations in genetic background. The same resistance genes but with a different spectrum also occurred at the *Pm2* locus (Chen et al. 2019).

Rare genetic variants are abundant in plants and are expected to contribute to disease resistance and other important agronomical traits. For example, scab resistance gene *Fhb1* was only present in a few Chinese accessions such as Wangshuibai and Sumai 3 (Li et al. 2019b); the *GS2* allele with enlarged grain size and enhanced grain yield came from a rare mutation of the microRNA target site (Hu et al. 2015); the most favorable haplotypes of *crtRB1*, encoding β -carotene hydroxylase 1, were detected only in about 2% of the maize germplasms (Yan et al. 2010). In the present study, the resistant *STK* allele with a 6-bp deletion only existed in three Chinese landraces out of 576 wheat accessions. These results suggested that

Fig. 5. Transcript levels of *STK* in *Blumeria graminis* f. sp. *tritici*-inoculated Huixianhong (HXH) and Datoumai (DTM) plants. Samples were collected immediately before inoculation (0 h) and at 3, 6, 12, 24, 48, and 72 h postinoculation. The expression levels were normalized against the internal control gene *TaGAPDH*. Error bars denote $\pm$ SD ($n = 3$). Asterisk (*) indicates $P < 0.01$ (Student's t test).

the rare allele with a 6-bp deletion has not yet been artificially selected and thus could be widely exploited for wheat resistance improvement. Based on the 6-bp variation in *STK*, we have developed a codominant, breeder-friendly PCR-based marker *STK-E5* for marker-assisted selection and a KASP marker *Xhnu58900* for high-throughput detection of *PmDTM* (Fig. 3B and D). With these two functional markers, we are introgressing *PmDTM* into commercially successful cultivars by implementing both forward selection and background selection. Breeders can use these developed near-isogenic lines for *PmDTM* to produce new powdery mildew resistant cultivars in the near future.

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