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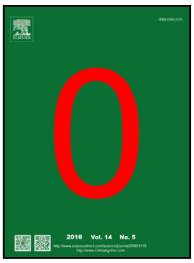

RESEARCH ARTICLE

Fine mapping of powdery mildew resistance gene *PmTm4* in wheat using comparative genomics

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Abstract

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most severe wheat diseases. Mining powdery mildew resistance genes in wheat cultivars and their appliance in breeding program is a promising way to control this disease. Genetic analysis revealed that a single dominant resistance gene named *PmTm4* originated from Chinese wheat line Tangmai 4 confers resistance to prevailing isolates of *B. graminis* f. sp. *tritici* isolate E09. Detailed comparative genomics analyses helped to develop closely linked markers to *PmTm4* and a fine genetic map was constructed using large F_2 population, in which *PmTm4* was located into a 0.66-cM genetic interval. The orthologous subgenome region of *PmTm4* in *Aegilops tauschii* was identified, and two resistance gene analogs (RGA) were characterized from the corresponding sequence scaffolds of *Ae. tauschii* draft assembly. The closely linked markers and identified *Ae. tauschii* orthologs in the mapping interval provide an entry point for chromosome landing and map-based cloning of *PmTm4*.

Keywords: powdery mildew resistance gene, *PmTm4*, genetic mapping, comparative genomic analysis

1. Introduction

Common wheat (*Triticum aestivum* L., 2n=6x=42) is one of the most major cultivated crops of the world, along with rice and maize, and this crop alone provides 20% of total food calories and protein humans need (FAO 2015a). Demand for wheat is estimated to rise more than 50% by 2050 (CRP 2014; FAO 2015b). Many strategies are being applied to meet this demand, including preventing yield losses *via* taking control of wheat diseases. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most common diseases in wheat worldwide, which under favorable conditions can lead to serious yield losses (Cowger *et al*. 2012). Breeding and growing powdery mildew resistant cultivars are the most effective, economic and environmentally safe approach to control this disease. Marker assisted selection (MAS) used in breeding program is able to accelerate development of new resistant variet-

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ies. Identification of powdery mildew resistance genes and development of molecular markers closely linked to them are essential for MAS processes. So far, more than 60 powdery mildew resistance genes or alleles were identified and genetically mapped on 20 chromosomes in wheat (*Pm1* to *Pm54*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) (Alam *et al*. 2011; McIntosh *et al*. 2013), some of which have already been utilized in breeding program. From the catalogued wheat powdery mildew resistance genes, most of them are race-specific and only a few of them, such as *Lr34/Yr18/Pm38*, confer partial resistance or adult plant resistance (APR) in a non race-specific manner. Since race-specific resistance genes tend to be ineffective for the emergence of new virulence *Bgt* isolates, more resistance genes are needed to catch up with the fast pace of pathogen evolution.

Wheat landraces are valuable sources of many agronomically important traits (Cavanagh *et al*. 2013). Breeding for powdery mildew disease resistance in wheat utilizing landraces is an effective way to fight this disease. Rich genetic diversity of powdery mildew resistance was found in Chinese landraces (Huang *et al*. 1997). Presently, several powdery mildew resistance genes or alleles were identified in Chinese landrace, including *Pm5e* in Fuzhuang 30 (Huang *et al*. 2003), *Pm5d* in IGV1–455 (Nematollahi *et al*. 2008), *Pm24* in Chiyacao (Huang and Röder 2011), *Pm24b* in Baihulu (Xue *et al*. 2012), *Pm45* in D57 (Ma *et al*. 2011), *Pm47* in Hongyanglazi (Xiao *et al*. 2013), *pmX* in Xiaohongpi (Fu *et al*. 2013), *MLHLT* in Hulutou (Wang *et al*. 2015), *PmH* in Hongquanmang (Zhou *et al*. 2005), and *Mlxbd* in Xiaobaidong (Xue *et al*. 2009). These genes are beneficial targets of wheat breeding, especially in China. Common wheat line Tangmai 4 is such a breeding line containing a powdery mildew resistance gene *PmTm4* originating from a Chinese wheat landrace (Hu *et al*. 2008). It seems that the *Pm5* locus contains diversified allelic variation in wheat germplasm, especially in Chinese wheat landrace. Developing high-density genetic linkage map of *PmTm4* would be beneficial for cloning the *Pm5* locus and characterizing its allelic variation that would be interested for wheat breeders.

Fine mapping and map-based cloning of important genes is a challenging task in common wheat not only because its genome is hexaploid (AABBDD), highly receptive (90%) and huge (17Gb), but also no reference sequence is available. With the release of wheat expressed sequence tags (EST) database (Mochida *et al*. 2006; Coordinators 2016), reference sequences of wheat closely related species like *Brachypodium* (IBI 2010), rice (IRGSP 2005) and sorghum (Paterson *et al*. 2009), and sequence resource in genome sequencing projects of barley (Ariyadasa *et al*. 2014; IBGSC *et al*. 2012; Poursarebani *et al*. 2013; Zeng *et al*. 2015), *Aegilops tauschii* (Jia *et al*. 2013; Luo *et al*. 2013), *Triticum urartu* (Ling *et al*. 2013), *Triticum aestivum* (Belova *et al*. 2013; Choulet *et al*. 2014; IWGSC 2014), and synthetic wheat w7984 (Chapman *et al*. 2015), new routes to clone genes in wheat are emerging (Borrill *et al*. 2015). One of them is comparative genomics analysis, which use the conserved collinearity between wheat and its related species to facilitate the development of markers linked closer with target genes in wheat (IBI 2010; Catalan *et al*. 2012; Brutnell *et al*. 2015; Mandadi and Scholthof 2015). To date, many powdery mildew resistance genes were genetically mapped making use of comparative genomics analysis, such as *Pm6* (Qin *et al*. 2011), *Pm41* (Wang *et al*. 2014), *MlIW172* (Ouyang *et al*. 2014), and *MlWE4* (Zhang *et al*. 2015). Recently, the physical map of *Ae*. *taus*chii (Luo *et al*. 2013), the D genome donor of common wheat, and its mainly anchored shotgun genome assembly (Jia *et al*. 2013) were constructed, making comparative genomics analysis in grass families and gene mapping in wheat more informative. With this information in D genome, powdery mildew genes *MlIW170* (Liang *et al*. 2015) and *MLHLT* (Wang *et al*. 2015) were successfully mapped into a narrow genetic interval.

In this study, we applied comparative genomics analysis to develop closely linked markers for fine mapping the powdery mildew resistance gene *PmTm4* in Chinese wheat line Tangmai 4.

2. Materials and methods

2.1. Plant materials

A powdery mildew resistant common wheat line Tangmai 4 (Li *et al*. 2004; Hu *et al*. 2008) was crossed with a highly susceptible common wheat line Xuezao (Zhang *et al*. 2010) to produce F_1 hybrids, F_2 segregating populations and their $F_{2:3}$ families for fine mapping of powdery mildew resistance gene *PmTm4* in Tangmai 4. Fuzhuang 30, Xiaobaidongmai, Hongquanmang, Mazhamai, and Laozaomai were used as donors of powdery mildew resistance genes *Pm5e*, *xbd*, *PmH*, *Pmmz*, and *PmTm4*. Partial of the Chinese mini-core collections (MCC) were kindly provided by Dr. Zhang Xueyong of Chinese Academy of Agricultural Sciences.

2.2. Evaluation of powdery mildew resistance

Blumeria graminis f. sp. *tritici* (*Bgt*) isolate E09 is a prevailing pathotype on common wheat at the region of Beijing, and is virulent to resistance genes *Pm1*, *Pm3a*, *Pm3c*, *Pm5a*, *Pm7*, *Pm8*, *Pm17*, *Pm19* and Xuezao (Zhou *et al*. 2005; Zhang *et al*. 2010, 2015). It was used in this study to evaluate the resistance of Tangmai 4, Xuezao, their F_1 hybrids, F_2 plants, and $F_{2:3}$ families, as well as the MCC lines to powdery mildew disease. The source of *Bgt* isolate E09 was kindly provided

by Dr. Zhou Yilin at the Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing, China. Xuezao was used as host for reproduction and preservation of *Bgt* isolate E09.

Powdery mildew reaction test was conducted under controlled greenhouse condition with temperature of 22°C/15°C for the day/night cycle. Two replicates, 15 seeds for each, were planted and tested for the parental lines and their progenies. The seedlings were inoculated with *Bgt* isolate E09 through brushing conidia from neighbor highly susceptible seedlings of Xuezao onto the tested leaf surface when the first leaf of tested plants was fully opened. Infection type (IT) was scored 8–15 days after inoculation when the control (Xuezao) displayed severe symptoms. ITs were recorded as a scales of 0, 0;, 1, 2, 3, and 4, representing no visible symptoms, necrotic flecks, highly resistant, moderately resistant, moderately susceptible, and highly susceptible, respectively (Liu *et al*. 1999). Phenotypes were classified into two groups, resistant (R, IT 0–2) and susceptible (S, IT 3–4). Only families with consistent phenotype in two replicates were used for construction of genetic map of *PmTm4*.

2.3. DNA extraction, pool construction and polymerase chain reaction (PCR)

Genomic DNA was extracted from the uninfected seedling tender leaves using cetyltrimethylammonium bromide (CTAB) method (Allen *et al*. 2006). For screening polymorphic and linked markers in bulked segregant analysis (Michelmore *et al*. 1991), resistant and susceptible DNA bulks were constructed through pooling equal amount of DNA from 10 homozygous resistant and 10 homozygous susceptible F_3 families, respectively. PCR experiments were taken place in a 10-μL reaction system, including 10 mmol L^{-1} Tris-HCl, 50 mmol L^{-1} KCl, 1.5 mmol L^{-1} MgCl, 0.2 mmol L^{-1} dNTP, 20 ng of each primer, 50 ng genomic DNA, and 0.75 U *Taq* DNA polymerase. PCR amplification program were designed as follows: 94°C for 5 min, followed by 35 cycles at 94°C for 35 s, 55–62°C (depending on annealing temperature of the primer pair) for 35 s, and 75°C for 60 s, with a final extension at 72°C for 10 min. PCR amplification assays were conducted on Applied Biosystems GeneAmp PCR System 9700. Three μL of each PCR product was mixed with 2.5 μL loading buffer, and then was separated on 8% non-denaturing polyacrylamide gels with a 39:1 acrylamide:bisacrylamid and 1× TBE (Tris-borate-EDTA) buffer. PCR products were visualized after the gels were silver-stained and photographed.

2.4. Comparative genomic analysis and marker development

The sequences and annotations of *T. aestivum*, *Ae. tauschii*, *Brachypodium*, rice, and sorghum were fetched from ensembl plant database release 25 (Kersey *et al*. 2014) and data published by Belova *et al*. (2013). Wheat expressed sequence tags (ESTs) were downloaded from dbEST database (Mochida *et al*. 2006; Coordinators 2016). Physical map data of *Ae. tauschii* was obtained from Wheat D Markers Database (http://probes.pw.usda.gov/WheatDMarker/)(Luo *et al*. 2013). Mutual best hits in BLAST (Camacho *et al*. 2009) output were considered as orthologous sequence contigs or scaffold pairs by aligning their sequences to each other using BLASTn module (e-value<1e–10, query coverage>15%, and identity>85). Orthologous gene pairs were identified using software OrthoMCL (Fischer *et al*. 2011) with default parameters. Genomic regions with high level of synteny (high proportion of orthologous gene pairs maintain same gene order and orientation) were regarded as orthologous genomic regions. The gene pairs with high level of synteny among *Ae. tauschii*, *Brachypodium*, rice, and sorghum were priorly used to find the orthologous sequences in improved *T. aestivum* 7BL assembly (Belova *et al*. 2013) to develop new polymorphic markers. If no hits were found in common wheat 7BL, the wheat EST sequences were used for primer design instead. The primers were designed using software BatchPrimer3 (You *et al*. 2008) and GSP (http://probes.pw.usda.gov/GSP/index.php) with default settings.

2.5. Independency test, linkage analysis and genetic map construction

Pearson's Chi-squared (χ^2) tests were performed to determine whether there is a significant difference between the expected frequencies and the observed frequencies. Function chisq.test in R language was used to do χ^2 tests (R Development Core Team 2011). Linkage analysis of molecular markers and the *PmTm4* locus were conducted using software MAPMAKER 3.0 with a LOD score threshold 3.0 (Lander *et al*. 1987). The genetic map was constructed by software Mapdraw v2.1 with default parameters (Liu and Meng 2003).

3. Results

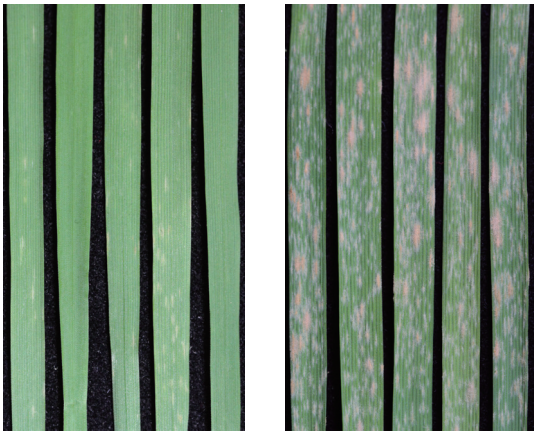
3.1. Genetic analysis of *PmTm4* in a F_2 population

The parental lines Tangmai 4 and Xuezao, their F_1 hybrids, 112 F_2 plants and F_2-derived ($F_{2:3}$) families were evaluated for powdery mildew resistance to *Bgt* isolate E09. Tangmai 4 was highly resistant (IT 0), while Xuezao was highly susceptible (IT 4; Fig. 1). All the F_1 seedlings were resistant (IT 0), suggesting dominant feature of the resistance gene in Tangmai 4. The F_2 population segregated as 80 resistant

and 32 susceptible, which fits 3:1 ratio ($\chi^2_{3:1}$=0.76, P>0.05). The $F_{2:3}$ progenies segregated as 27 homozygous resistant:53 segregating:32 homozygous susceptible (Table 1). This fits the expected 1:2:1 ratio of single Mendelian locus ($\chi^2_{1:2:1}$=0.77, P>0.05), which is consistent with previous result, a single powdery mildew resistance gene *PmTm4* in Tangmai 4 (Hu *et al*. 2008).

3.2. Comparative genomics analysis of the *PmTm4* genomic region

PmTm4 was previously mapped in a 33.6 cM genetic interval on 7BL between EST-SSR marker *Xcau12* and EST marker *XEST92* (Hu *et al*. 2008). The corresponding EST sequences (Mochida *et al*. 2006) of these two markers were used to find orthologous genes in *Brachypodium*, rice and sorghum. *CJ584170* (*Xcau12*) is orthologous to *Bradi1g30850*, *Os06g43990* and *Sb10g025570* in *Brachypodium*, rice and sorghum, respectively, while *CJ729392* (*XEST92*) is orthologous to *Bradi1g29800* in *Brachypodium* and *Os06g51150* in rice. No ortholog of *CJ729392* (*XEST92*) was detected in sorghum. In order to develop a fine genetic map of *PmTm4*, the 631 kb genomic region ranging from *Bradi1g30850* to *Bradi1g29800* in *Brachypodium* was selected for detailed comparative genomics analyses with the corresponding orthologous genomic regions in rice and sorghum. Middle level of collinearity was observed between genomic regions of *Brachypodium Bradi1g30610–Bradi1g29800*, rice *Os06g43120–Os06g51150* (966 kb), and sorghum *Sb10g025010–Sb10g030820* (983 kb). Within these regions, 48 of 82 predicted *Brachypodium* genes are found orthologous to 45 of 159 predicted rice genes and 32 of 97 predicted sorghum genes (Table 2). Meanwhile, only 29 orthologous gene pairs between *Brachypodium*, rice and sorghum were found, suggesting a relatively low micro-collinearity level in these genomic regions. Additionally, the collinear regions can be separated into two blocks. In the first block, genomic region of *Bradi1g30610–Bradi1g30260* in *Brachypodium* (36 genes, 291 kb) is collinear to rice genomic region of *Os06g43760–Os06g43120* (65 genes, 426 kb), and sorghum genomic region of *Sb10g025330–Sb10g025010* (33 genes, 418 kb). An insertion may arise in rice genomic region *Os06g43560–Os06g43280*. In the second block, genomic region of *Bradi1g30250–Bradi1g29800* in *Brachypodium* (46 genes, 316 kb) is collinear to rice genomic region *Os06g50180–Os06g51150* (94 genes, 539 kb), and sorghum genomic region *Sb10g031180–Sb10g030820* (64 genes, 565 kb). These two blocks showed similar proportion of orthologous gene pairs. However, compared to the conserved collinearity between rice and sorghum, two obvious small-scale inversions occurred in *Brachypodium* (*Bradi1g30250–Bradi1g30110* and *Bradi1g30090–Bradi1g29910*) in the second block, in which a deletion may have happened between *Bradi1g29920* and *Bradi1g29910*, and *Bradi1g30220* may have relocated. No inversion was observed between rice and sorghum in these regions. Furthermore, when comparing to improved chromosome-sorted assemblies of *T. aestivum cv*. Chinese Spring 7AL, 7BL and 7DL (Belova *et al*. 2013; International Wheat Genome Sequencing Consortium 2014), 15 more *Brachypodium* genes were found specifically orthologous to wheat genes. Besides of these, 15 genetically anchored genes of *Ae. tauschii* were orthologous and highly collinear to *Brachypodium* genes in above analyzed genomic regions (Table 2). These results suggested that the collinear regions of *PmTm4* in *Brachypodium* experienced lots of rearrangements after divergence from rice, and high gene conservation and closer relation-



Tangmai 4 Xuezao

Fig. 1 Phenotype of resistant parent Tangmai 4 and susceptible parent Xuezao at 15 days post inoculation (DPI) with *Bgt* isolate E09.

Table 1 Genetic analysis of the powdery mildew resistance gene *PmTm4*[1)]

Mapping population	Resistance	Susceptible	Total	χ^2	$\chi^2_{0.05}$
Tangmai 4	20				
Xuezao		20			
Tangmai 4/Xuezao F_1	20				
Tangmai 4/Xuezao F_2	80	32	112	0.76	3.84
Tangmai 4/Xuezao F_3	27(A)+53(H)	32(B)	112	0.77	5.99

A, H and B represent the homozygous resistant, heterozygous resistant and homozygous susceptible individuals, respectively.

Table 2 Orthologous gene pairs among collinear genomic regions of *Aegilops tauschii*, *Brachypodium*, rice and sorghum

Ae. tauschii SNP marker	*Brachypodium*	Rice	Sorghum	*Ae. tauschii* SNP marker	*Brachypodium*	Rice	Sorghum
AT7D7122_5	Bradi1g30610	Os06g43760			Bradi1g30170	Os06g50350	Sb10g030290
	Bradi1g30600	Os06g43710			Bradi1g30150	Os06g50330	Sb10g030260
AT7D7122_3	Bradi1g30590	Os06g43700	Sb10g025330		Bradi1g30140	Os06g50310	Sb10g030250
	Bradi1g30580	Os06g43690	Sb10g025320		Bradi1g30110	Os06g50220	Sb10g030190
AT7D7123_2	Bradi1g30560				Bradi1g30090	Os06g51010	Sb10g030610
AT7D7123_3	Bradi1g30550				Bradi1g30070	Os06g50980	Sb10g030580
	Bradi1g30540	Os06g43650	Sb10g025260		Bradi1g30030	Os06g50940	Sb10g030540
AT7D7124_2	Bradi1g30530	Os06g43640			Bradi1g30020	Os06g50930	Sb10g030520
	Bradi1g30510	Os06g43620	Sb10g025230	AT7D7151_1	Bradi1g30010	Os06g50910	
AT7D7127_1	Bradi1g30490	Os06g43610	Sb10g025220		Bradi1g30000	Os06g50900	Sb10g030470
	Bradi1g30440	Os06g43570			Bradi1g29990	Os06g50890	Sb10g030460
	Bradi1g30430	Os06g43590			Bradi1g29980	Os06g50880	Sb10g030450
	Bradi1g30410	Os06g43560	Sb10g025180		Bradi1g29970	Os06g50870	Sb10g030440
	Bradi1g30390		Sb10g025150		Bradi1g29960	Os06g50860	
AT7D7129_1	Bradi1g30370	Os06g43280	Sb10g025080		Bradi1g29950	Os06g50850	Sb10g030420
AT7D7129_2	Bradi1g30360	Os06g43270	Sb10g025070		Bradi1g29930	Os06g50840	Sb10g030410
	Bradi1g30350	Os06g43250	Sb10g025060		Bradi1g29920	Os06g50830	
AT7D7135_2	Bradi1g30330	Os06g43210	Sb10g025050		Bradi1g29910	Os06g50420	
AT7D7134_1	Bradi1g30310		Sb10g025040		Bradi1g29900	Os06g51029	
AT7D7133_1	Bradi1g30280		Sb10g025010		Bradi1g29890	Os06g51060	
AT7D7136_1	Bradi1g30260	Os06g43120			Bradi1g29850	Os06g51084	
	Bradi1g30230	Os06g50390	Sb10g030330		Bradi1g29840	Os06g51100	
	Bradi1g30220	Os06g50180	Sb10g030180	AT7D7155_1	Bradi1g29830	Os06g51110	
	Bradi1g30190	Os06g50370	Sb10g030310		Bradi1g29820	Os06g51140	Sb10g030820
AT7D7142_2	Bradi1g30180	Os06g50360	Sb10g030300		Bradi1g29800	Os06g51150	

ships remained for *Brachypodium*, *Ae*. *tauschii* and wheat.

3.3. Fine genetic mapping of powdery mildew resistance gene *PmTm4*

Based on the above detailed comparative genomics analyses, the coding sequences (CDS) of orthologous genes showing good collinearity between *Brachypodium*, rice and sorghum were preferentially used to search for orthologous Chinese Spring sequence contigs (Belova *et al*. 2013; IWGSC 2014) to develop molecular markers linked to *PmTm4*. The designed wheat markers were screened for polymorphisms between Tangmai 4 and Xuezao as well as the resistant and susceptible F_2 DNA bulks. Altogether, 8 polymorphic markers, *XWGGC7532*, *XWGGC403*, *XWGGC2127*, *XWGGC5974*, *XWGGC5262*, *XWGGC6892*, *XWGGC5746*, and *XWGGC891*, were developed (Table 3). Using the 112 plants F_2 segregating population, *PmTm4* was mapped into a 1.5-cM genetic interval between markers *XWGGC5262* and *XWGGC5746*. In order to construct a fine genetic linkage map of *PmTm4*, a larger F_2 mapping population comprising 1 499 F_2 individuals was developed and genotyped using markers *XWGGC7532* and *XWGGC891*, resulting in 139 F_2 recombinants between them. These recombinants' F_3 families were tested for powdery mildew resistance and genotyped using the newly developed polymorphic markers. The *PmTm4* was thus mapped in a 0.66-cM genetic interval flanked by *XWGGC6892* and *XWGGC5746* with genetic distance of 0.63 and 0.03 cM, respectively (Fig. 2).

XWGGC6892 and *XWGGC5746* were tested on powdery mildew resistance wheat lines Fuzhuang 30 (*Pm5e*), Xiaobaidongmai (*mlxbd*), Hongquanmang (*PmH*), Mazhamai (*Pmmz*), Laozaomai (*PmTm4*), and 141 cultivars from Chinese wheat mini-core collections (MCC). *XWGGC6892* detected *PmTm4* pattern of on most of the landraces (either resistant or susceptible to powdery mildew) and Xuezao pattern on all the susceptible breeding cultivars. Same amplification patterns were detected for *XWGGC5746* on *PmTm4*, *Pm5e*, *mlxbd*, and *PmH*, *Pmmz*, Laozaomai and Qiangchangmai (a powdery mildew resistant landrace also originated from Shaanxi, China), but not on other 139 Chinese wheat mini-core collections (Fuzhuang 30 is one of the MCC), indicating possible single haplotype type for this powdery mildew resistance locus.

3.4. Identifying orthologous sub-genomic regions of *PmTm4* in *Ae. tauschii*

The 4.2 cM genetic interval of *PmTm4* between markers

Table 3 Markers linked to the powdery mildew resistance gene *PmTm4*

Markers	Template	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size (bp)[1)]	Dominance
WGGC7532	*Bradi1g30610*	CAACATGCATAGATGTCTCAA	GAAGTACCCCAGTACCCTTTA	197	Co-dominant
WGGC403	*Bradi1g30590*	CGGAAAGCGTCAAGTAAA	GAGCTTGGGACATTTCAGT	356	Co-dominant
WGGC2127	*Bradi1g30550*	CCAACTCCACCTTCCTCTT	CCAAACCCTAACCCTGATT	562	Co-dominant
WGGC5974	*Bradi1g30530*	TCCGGTACGAGAGCCTCC	ACTACTATATGCCTCCGTGTCG	351	Co-dominant
WGGC5262	*Bradi1g30360*	GGAGCGTCATCAAAGGCAG	AGTAGGTAGCAGATGGACTTCA	426	Co-dominant
WGGC6892	*BM137749*	TGCTGTCAAGATGGCAAACT	TGCAGGCCACTATGATTTGTG	680	Co-dominant
WGGC5746	*Bradi1g30310*	TTCAGCCGTCCATCTCCTTT	GGAGAGATCAACTGCAACGAC	304	Co-dominant
WGGC891	*Bradi1g30330*	CTCTGGGTGGACCTGATGAT	GCGGAGATTCAGAATTTTCAT	232	Co-dominant

[1)] The fragments sizes for some polymorphic markers cannot be accurately estimated because of distinct DNA conformations.

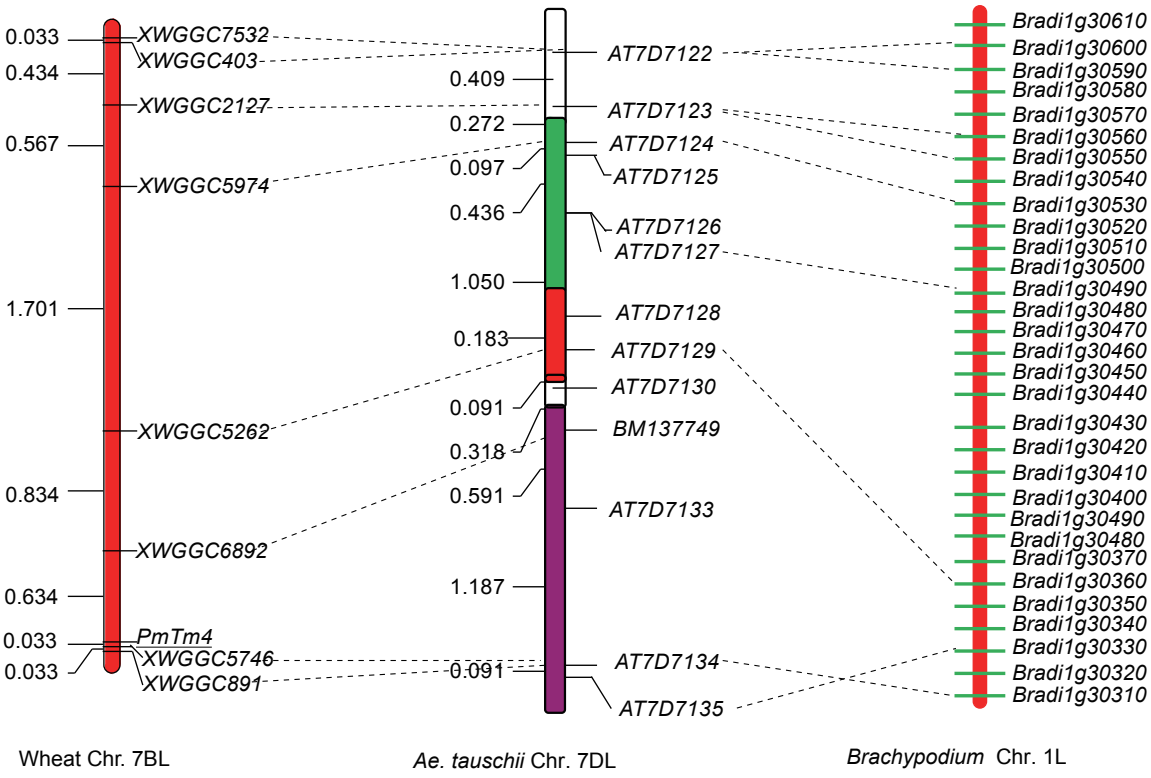


Fig. 2 Comparative genomics mapping of the powdery mildew resistance gene *PmTm4*. Genetic maps of wheat chromosome arm 7BL and *Aegilops tauschii* chromosome arm 7DL in *PmTm4* region were represented with genetic distance in cM shown on the left and marker names displayed on the right. The *PmTm4* locus was underlined. Different colors in genetic map of *Ae. tauschii* Chr. 7DL were used to distinguish different BAC contigs in physical map. The collinear region of *PmTm4* in *Brachypodium* chromosome 1L (238 kb) was presented with gene name on the right. The orthologous gene/marker pairs were linked by dashed line.

XWGGC7532 and *XWGGC891* at 7BL of *T. aestivum* is corresponding to a similar genetic interval of 4.7 cM between *AT7D7122* and *AT7D7135* at 7DL of *Ae. tauschii* (Luo *et al*. 2013). Same order of orthologous genetic markers in the two genetic linkage maps was observed as well (Fig. 2). This may imply that the physical map of *Ae. tauschii* chromosome arm 7DL can be used as the framework for fine mapping of *PmTm4* on 7BL in *T. aestivum*. *PmTm4* is mapped between *XWGGC6892* and *XWGGC5746* in *T. aestivum*, which is orthologous to the anchored SNP markers *BM137749* and *AT7D7134* of BAC contig 3554 in *Ae. tauschii* chromosome 7D physical map (Luo *et al*. 2013). This BAC contig was estimated as 1 246 kb containing 10 overlapping minimum tiling path (MTP) BACs and 4 anchored SNP markers, *BM137749*, *AT7D7133*, *AT7D7134*, and *AT7D7135* (Fig. 3). Three overlapping BACs existed between *BM137749* and *AT7D7134*.

The 4 anchored SNP marker extended sequences contigs were aligned to the draft genome assembly of *Ae. tauschii* (Jia *et al*. 2013). *BM137749* and *AT7D7133* were

mapped on scaffold 61742 (109 kb) and scaffold 6693 (102 kb), respectively, and both *AT7D7134* and *AT7D7135* were aligned on scaffold 9246 (122 kb). Scaffold 61742 and 6693, coupled with scaffold 11110 (177 kb) and 2506 (330 kb), were located on the same recombination bin 273 (129.201 cM) in another high density genetic map of *Ae*. *tauschii* (Jia *et al*. 2013). Annotations of all these five scaffolds showed that two disease resistance gene analogs, *F775_24889* (LRR receptor-like serine/threonine-protein kinase) and *F775_15095* (putative disease resistance RPP13-like protein 3), on 7DL of *Ae*. *tauschii* may serve as homologous starting point for chromosome landing towards map-based cloning of the powdery mildew resistance gene *PmTm4* on 7BL (Table 4).

4. Discussion

4.1. A fine genetic linkage map of powdery mildew resistance gene *PmTm4*

So far, no well-assembled reference genome sequence is available for common wheat, and the released draft assemblies are too fragmented, limiting its application in gene mapping and cloning process. Usually, comparative genomics approach using reference sequences of grass species was considered as a fast way to develop closely linked markers for a gene, such as fine mapping powdery mildew resistance genes *MlIW172* (Ouyang *et al*. 2014), *Pm41* (Wang *et al*. 2014), *MlIW170* (Liang *et al*. 2015), and

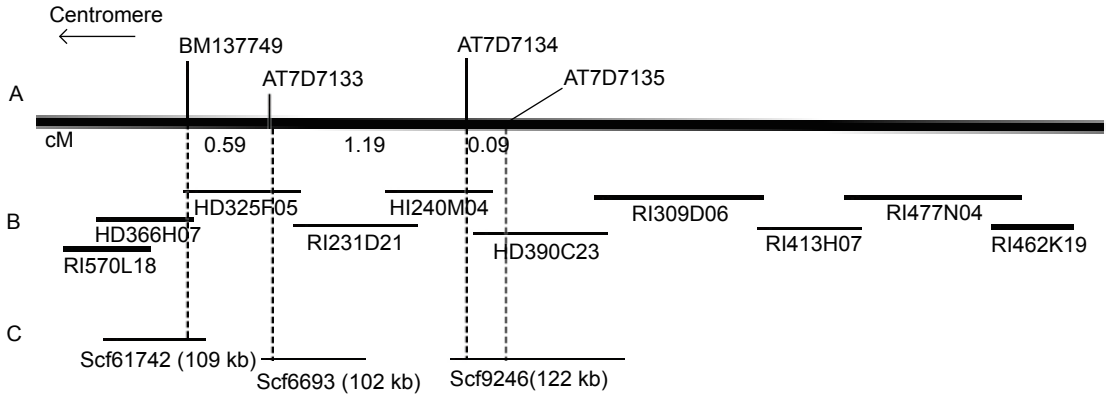


Fig. 3 Genetic map, bacteria artificial chromosome (BAC) layout and homologous scaffolds on BAC contig 3554 of *Ae. tauschii* physical map in *PmTm4* region. A, genetic map of anchored markers on BAC contig 3554 with genetic distance in cM shown below. B, the layout of BACs on minimum tilling path (MTP) of BAC contig 3554. C, scaffolds of draft assembly aligned on marker extend contigs.

Table 4 Gene annotations of *Ae. tauschii* scaffolds corresponding to bacteria artificial chromosome (BAC) contig 3554 on 7DL

Gene name	Scaffold ID[1)]	Start (bp)	End (bp)	Strand	Genetic position (cM)	Annotation
F775_30199	Scaffold11110	46 519	48 903	+	169.201	E3 ubiquitin-protein ligase KEG
F775_08233	Scaffold11110	49 635	52 567	–	169.201	Elongator complex protein 4
F775_03262	Scaffold11110	79 603	81 149	+	169.201	Cytochrome P450 71D7
F775_17491	Scaffold11110	113 052	116 425	–	169.201	Cytochrome P450 71D6
F775_08234	Scaffold11110	125 918	126 310	+	169.201	Uncharacterized protein
F775_17492	Scaffold11110	133 352	134 443	+	169.201	Cytochrome P450 71D6
F775_17493	Scaffold11110	140 536	141 036	+	169.201	Cytochrome P450 71D10
F775_24889	Scaffold6693	99 806	101 666	+	169.201	LRR receptor-like serine/threonine-protein kinase
F775_08978	Scaffold2506	47 912	50 194	+	169.201	Uncharacterized protein
F775_24902	Scaffold2506	56 234	59 250	+	169.201	Uncharacterized protein
F775_15093	Scaffold9246	63 250	69 272	+	170.283	Uncharacterized protein
F775_07189	Scaffold9246	73 348	79 438	+	170.283	RING/U-box superfamily protein
F775_15094	Scaffold9246	82 410	83 393	–	170.283	Germin-like protein 5-1
F775_02399	Scaffold9246	92 837	97 061	+	170.283	Calcium-dependent lipid-binding (CaLB domain) family protein
F775_15095	Scaffold9246	99 010	102 911	–	170.283	Putative disease resistance RPP13-like protein 3
F775_28784	Scaffold9246	103 570	105 525	+	170.283	Uncharacterized protein
F775_15096	Scaffold9246	108 674	109 679	–	170.283	Uncharacterized protein
F775_07190	Scaffold9246	118 814	121 606	+	170.283	Jasmonate O-methyltransferase

[1)] Scaffold61742 was not listed as no gene was annotated on it.

MLHLT (Wang *et al*. 2015), wax inhibitor gene *Iw1* (Wu *et al*. 2013), spot biotch resistance gene *Sb3* (Lu *et al*. 2016) and cloning leaf rust resistance gene *Lr67* (Moore *et al*. 2015). In the case of *Lr67*, it was first mapped into a small interval with the closest marker *Xgwm165* being located at 0.4 cM (Herrera-Foessel *et al*. 2011), which is routinely supposed to be a large region including tens of genes in wheat genome. After isolating the positive BAC with marker *Xgwm165*, comparative genomic analysis between corresponding regions in *Brachypodium* and rice was carried out, and the resulted conserved orthologs were identified and served as starting point to develop closer linked markers. With the help of this collinearity information, the newly developed markers on syntenic genes quickly facilitated narrowing down the number of candidate genes, so that the time consuming process of BAC walking was avoided (Moore *et al*. 2015).

The powdery mildew resistance gene of common wheat line Tangmai 4 was mapped on 7BL using a F_2 segregating population derived from a cross between Tangmai 4 and Clement, and was assigned as *PmTm4* (Hu *et al*. 2008). In this study, a larger mapping population was constructed for fine mapping of *PmTm4*. According to the genomic collinearity among grass families (IWGSC 2014; Brutnell *et al*. 2015), detailed comparative genomics analyses were conducted for the *PmTm4* orthologous genomic regions among *Brachypodium* chromosome 1, rice chromosome 6 and sorghum chromosome 10. The conserved collinear orthologous gene pairs were used as guide to design polymorphic markers using the available Chinese Spring 454 contigs (Brenchley *et al*. 2012), International Wheat Genome Sequencing Consortium (IWGSC) chromosome survey sequences (IWGSC 2014), and improved 7B assembly (Belova *et al*. 2013). With the newly developed markers, *PmTm4* was mapped into a 1.5-cM genetic interval between markers *XWGGC5262* and *XWGGC5746*, displaying the power of comparative genomic analysis in wheat gene mapping. However, all orthologous gene pairs among *Brachypodium*, rice and sorghum in this interval cannot help developing further polymorphic markers. Using the SNP extended markers on physical map of *Ae*. *tauschii* (Luo *et al*. 2013), a new closer marker *XWGGC6892* was developed based on sequences of marker *BM137749*, moving forward mapping interval to 0.66 cM, which corresponds to a region with three BACs in BAC contig 3554 in *Ae*. *tauschii* physical map. The anchored shotgun assembly of *Ae*. *tauschii* (Jia *et al*. 2013) further helped to identify three scaffolds (322 kb in all) nearly covering this region. This shows that *Ae*. *tauschii* is more helpful than *Brachypodium* in mapping wheat genes, even for genes on A or B subgenomes of common wheat, just as what was found in the case of *Sb3* (Lu *et al*. 2016). Additionally, the LRR receptor-like serine/threonine-protein kinase (*F775_24889*) in scaffold6693, disease resistance RPP13-like protein (*F775_15095*) in scaffold9246 and their flanking genes in *Ae*. *tauschii* 7DL could be used to search their 7BL orthologs in wheat and serve as a starting point for chromosome landing and map-based cloning of *PmTm4*.

4.2. Comparison of *PmTm4* with other powdery mildew resistance genes in Chinese landraces

High diversity of powdery mildew resistance was observed in Chinese landraces (Huang *et al*. 1997). Currently, more than 10 alleles in 5 powdery mildew resistance loci have been identified in Chinese landraces. Among them, *Pm5d*, *Pm5e*, *PmH*, *Mlxbd*, and *PmTm4* were genetically mapped in the distal region on chromosome arm 7BL (Hsam *et al*. 2000, 2001; Huang *et al*. 2003; Zhou *et al*. 2005; Hu *et al*. 2008; Zhu *et al*. 2008; Xue *et al*. 2009), suggesting a cluster of powdery mildew resistance genes may exist at the *Pm5* locus. In these studies, *PmTm4* was mapped between *Xgwm611* and *Xbar1073* (Hu *et al*. 2008); *Pm5d* was mapped between *Xgwm611* and *Xgwm577* (Nematollahi *et al*. 2008); *Mlxbd* was mapped between *Xgwm1267* and *Xgwm577*/*Xbar1073* (Xue *et al*. 2009); and *Pm5e* was mapped at the distal region of *Xwgm577* and *Xwgm1267* (Huang *et al*. 2003; Zhu *et al*. 2008). These results imply an assumption that *Pm5e* is the closest to telomere, followed by *Mlxbd* and *Pm5d*, and that *PmTm4* may be allelic or closely linked to these three genes. However, all the 277 F_2 individuals of Xiaobaidong (*Mlxbd*)/Fuzhuang 30 (*Pm5e*) are resistance (Hsam *et al*. 2000), and *Xgwm577* is at the distal in *Mlxbd* genetic map (Xue *et al*. 2009), confronting the putative gene order of these genes. This may be caused by the genetic background difference, mis-classification of SSR mapping data and disease reaction scales. The same reaction patterns of *PmTm4* and Xiaobaidongmai (*mlxbd*) to 21 *Bgt* isolates (resistance to 15 out of 21 *Bgt* isolates tested, 2008 data not shown) indicating the *PmTm4* and *mlxbd* may be allelic. The same amplification patterns of the most closely linked marker *XWGGC5746* on *PmTm4*, *Pm5e*, *mlxbd*, *PmH*, *Pmmz*, Laozaomai, and Qiangchangmai, but not on powdery mildew susceptible Chinese wheat mini-core collections cultivars revealed that these alleles might be evolved from same haplotype. The marker *XWGGC5746* closely linked to *PmTm4* developed in this study could be very useful for wheat breeders in developing disease resistance varieties *via* marker assisted selection.

5. Conclusion

Powdery mildew resistance gene *PmTm4* was identified and mapped into a 0.66-cM genetic interval using the fine genetic linkage map developed from a large F_2 population of Tangmai 4×Xuezao. The mapping interval corresponds

to a genomic region of three overlapping BACs on physical map of *Ae*. *tauschii*, in which two disease resistance gene analogs were found.

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References

Alam M A, Xue F, Wang C, Ji W. 2011. Powdery mildew resistance genes in wheat: Identification and genetic analysis. *Journal of Molecular Biology Research*, **1**, 20–39.

Allen G C, Flores-Vergara M A, Krasynanski S, Kumar S, Thompson W F. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols*, **1**, 2320–2325.

Ariyadasa R, Mascher M, Nussbaumer T, Schulte D, Frenkel Z, Poursarebani N, Zhou R, Steuernagel B, Gundlach H, Taudien S, Felder M, Platzer M, Himmelbach A, Schmutzer T, Hedley P E, Muehlbauer G J, Scholz U, Korol A, Mayer K F, Waugh R, *et al*. 2014. A sequence-ready physical map of barley anchored genetically by two million single-nucleotide polymorphisms. *Plant Physiology*, **164**, 412–423.

Belova T, Zhan B, Wright J, Caccamo M, Asp T, Simkova H, Kent M, Bendixen C, Panitz F, Lien S, Dolezel J, Olsen O A, Sandve S R. 2013. Integration of mate pair sequences to improve shotgun assemblies of flow-sorted chromosome arms of hexaploid wheat. *BMC Genomics*, **14**, 222.

Borrill P, Adamski N, Uauy C. 2015. Genomics as the key to unlocking the polyploid potential of wheat. *New Phytologist*, **208**, 1008–1022.

Brenchley R, Spannagl M, Pfeifer M, Barker G L, D'Amore R, Allen A M, McKenzie N, Kramer M, Kerhornou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo M C, Sehgal S, Gill B, Kianian S, *et al*. 2012. Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, **491**, 705–710.

Brutnell T P, Bennetzen J L, Vogel J P. 2015. *Brachypodium distachyon* and *Setaria viridis*: Model genetic systems for the grasses. *Annual Review of Plant Biology*, **66**, 465–485.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden T L. 2009. BLAST+: Architecture and applications. *BMC Bioinformatics*, **10**, 421.

Catalan P, Muller J, Hasterok R, Jenkins G, Mur L A, Langdon T, Betekhtin A, Siwinska D, Pimentel M, Lopez-Alvarez D. 2012. Evolution and taxonomic split of the model grass Brachypodium distachyon. *Annals of Botany*, **109**, 385–405.

Cavanagh C R, Chao S, Wang S, Huang B E, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira G L, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva M L, Bockelman H, Talbert L, *et al*. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 8057–8062.

Chapman J A, Mascher M, Buluc A, Barry K, Georganas E, Session A, Strnadova V, Jenkins J, Sehgal S, Oliker L, Schmutz J, Yelick K A, Scholz U, Waugh R, Poland J A, Muehlbauer G J, Stein N, Rokhsar D S. 2015. A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome. *Genome Biology*, **16**, 26.

Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, Pingault L, Sourdille P, Couloux A, Paux E, Leroy P, Mangenot S, Guilhot N, Le Gouis J, Balfourier F, Alaux M, Jamilloux V, Poulain J, Durand C, Bellec A, *et al*. 2014. Structural and functional partitioning of bread wheat chromosome 3B. *Science*, **345**, 1249721.

Coordinators N R. 2016. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, **44**, D7-D19.

Cowger C, Miranda L, Griffey C, Hall M, Murphy J P, Maxwell J. 2012. In: Sharma I , ed., *Disease Resistance in Wheat*. CAB International, Wallingford. pp. 84–120.

CRP (Consultative Group for International Agricultural Research). 2014. Global wheat science and partnerships for food security and nutrition. CGIAR Research Program on Wheat Annual Report 2014. [2015-09-08]. https://slate.adobe.com/a/J6KpK/

FAO (Food and Agriculture Organization). 2015a. Cereals and us: Time to renew an ancient bond. In: *Save and Grow in Practice: Maize*, *Rice*, *Wheat*, *Chapter 1*. [2015-12-02]. http://www.fao.org/ag/save-and-grow/MRW/en/1/index.html

FAO (Food and Agriculture Organization). 2015b. Online statistical database: Food balance. FAOSTAT. [2015-12-02]. http://faostat3.fao.org/download/FB/*/E

Fischer S, Brunk B P, Chen F, Gao X, Harb O S, Iodice J B, Shanmugam D, Roos D S, Stoeckert Jr C J. 2011. Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups. *Current Protocols in Bioinformatics* (Chapter 6, Unit 6), **12**, 11–19.

Fu B, Chen Y, Li N, Ma H, Kong Z, Zhang L, Jia H, Ma Z. 2013. *pmX*: A recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi. *Theoretical and Applied Genetics*, **126**, 913–921.

Herrera-Foessel S A, Lagudah E S, Huerta-Espino J, Hayden M J, Bariana H S, Singh D, Singh R P. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. *Theoretical and Applied Genetics*, **122**, 239–249.

Hsam S L K, Huang X Q, Zeller F J. 2000. Chromosomal location of powdery mildew resistance genes in Chinese wheat (*Triticum aestivum* L. em. Thell.) landraces Xiaobaidong and Fuzhuang 30. *Journal of Genetics & Breeding*, **54**, 311–317.

Hsam S L K, Huang X Q, Zeller F J. 2001. Chromosomal location of genes for resistance to powdery mildew in common wheat

(*Triticum aestivum* L. em Thell.) 6. Alleles at the *Pm5* locus. *Theoretical and Applied Genetics*, **102**, 127–133.

Hu T Z, Li H J, Xie C J, You M S, Yang Z M, Sun Q X, Liu Z Y. 2008. Molecular mapping and chromosomal location of powdery mildew resistance gene in wheat cultivar Tangmai 4. *Acta Agronomica Sinica*, **34**, 1193–1198.

Huang X Q, Hsam S L K, Zeller F J. 1997. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em Thell.). IX. Cultivars, land races and breeding lines grown in China. *Plant Breeding*, **116**, 233–238.

Huang X Q, Röder M S. 2011. High-density genetic and physical bin mapping of wheat chromosome 1D reveals that the powdery mildew resistance gene *Pm24* is located in a highly recombinogenic region. *Genetica*, **139**, 1179–1187.

Huang X Q, Wang L X, Xu M X, Roder M S. 2003. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, **106**, 858–865.

IBGSC (International Barley Genome Sequencing Consortium), Mayer K F, Waugh R, Brown J W, Schulman A, Langridge P, Platzer M, Fincher G B, Muehlbauer G J, Sato K, Close T J, Wise R P, Stein N. 2012. A physical, genetic and functional sequence assembly of the barley genome. *Nature*, **491**, 711–716.

IBI (International Brachypodium Initiative). 2010. Genome sequencing and analysis of the model grass Brachypodium distachyon. *Nature*, **463**, 763–768.

IRGSP (International Rice Genome Sequencing Project). 2005. The map-based sequence of the rice genome. *Nature*, **436**, 793–800.

IWGSC (International Wheat Genome Sequencing Consortium). 2014. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *Science*, **345**, 1251788.

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X, Jing R, Zhang C, Ma Y, Gao L, Gao C, Spannagl M, Mayer K F, Li D, Pan S, Zheng F, *et al*. 2013. *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature*, **496**, 91–95.

Kersey P J, Allen J E, Christensen M, Davis P, Falin L J, Grabmueller C, Hughes D S T, Humphrey J, Kerhornou A, Khobova J, Langridge N, McDowall M D, Maheswari U, Maslen G, Nuhn M, Ong C K, Paulini M, Pedro H, Toneva I, Tuli M A, *et al*. 2014. Ensembl Genomes 2013: Scaling up access to genome-wide data. *Nucleic Acids Research*, **42**, D546-D552.

Lander E S, Green P, Abrahamson J, Barlow A, Daly M J, Lincoln S E, Newberg L A. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**, 174–181.

Li H J, Conner R L, McCallum B D, Chen X M, Su H, Wen Z Y, Chen Q, Jia X. 2004. Resistance of Tangmai 4 wheat to powdery mildew, stem rust, leaf rust, and stripe rust and its chromosome composition. *Canadian Journal of Plant Science*, **84**, 1015–1023.

Liang Y, Zhang D Y, Ouyang S, Xie J, Wu Q, Wang Z, Cui Y, Lu P, Zhang D, Liu Z J, Zhu J, Chen Y X, Zhang Y, Luo M C, Dvorak J, Huo N, Sun Q, Gu Y Q, Liu Z. 2015. Dynamic evolution of resistance gene analogs in the orthologous genomic regions of powdery mildew resistance gene *MlIW170* in Triticum dicoccoides and *Aegilops tauschii*. *Theoretical and Applied Genetics*, **128**, 1617–1629.

Ling H Q, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y, Gao C, Wu H, Li Y, Cui Y, Guo X, Zheng S, Wang B, Yu K, Liang Q, Yang W, *et al*. 2013. Draft genome of the wheat A-genome progenitor *Triticum urartu*. *Nature*, **496**, 87–90.

Liu R H, Meng J L. 2003. MapDraw: A microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data. *Hereditas* (Beijing), **25**, 317–321.

Liu Z, Sun Q, Ni Z, Yang T, McIntosh R A. 1999. Development of SCAR markers linked to the *Pm21* gene conferring resistance to powdery mildew in common wheat. *Plant Breeding*, **118**, 215–219.

Lu P, Liang Y, Li D, Wang Z, Li W, Wang G, Wang Y, Zhou S, Wu Q, Xie J, Zhang D, Chen Y, Li M, Zhang Y, Sun Q, Han C, Liu Z. 2016. Fine genetic mapping of spot blotch resistance gene *Sb3* in wheat (*Triticum aestivum*). *Theoretical and Applied Genetics*, **129**, 577–589.

Luo M C, Gu Y Q, You F M, Deal K R, Ma Y, Hu Y, Huo N, Wang Y, Wang J, Chen S, Jorgensen C M, Zhang Y, McGuire P E, Pasternak S, Stein J C, Ware D, Kramer M, McCombie W R, Kianian S F, Martis M M, *et al*. 2013. A 4-gigabase physical map unlocks the structure and evolution of the complex genome of Aegilops tauschii, the wheat D-genome progenitor. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 7940–7945.

Ma H, Kong Z, Fu B, Li N, Zhang L, Jia H, Ma Z. 2011. Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. *Theoretical and Applied Genetics*, **123**, 1099–1106.

Mandadi K K, Scholthof K B. 2015. Genome-wide analysis of alternative splicing landscapes modulated during plant-virus interactions in Brachypodium distachyon. *The Plant Cell*, **27**, 71–85.

McIntosh R A, Dubcovsky J, Rogers W J, Morris C. 2013. Catalogue of gene symbols for wheat: 2013–2014. In: *Proceedings of the 12th International Wheat Genetics Symposium*, Yokohama, Japan.

Michelmore R W, Paran I, Kesseli R V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 9828–9832.

Mochida K, Kawaura K, Shimosaka E, Kawakami N, Shin I T, Kohara Y, Yamazaki Y, Ogihara Y. 2006. Tissue expression map of a large number of expressed sequence tags and its

application to in silico screening of stress response genes in common wheat. *Molecular Genetics and Genomics*, **276**, 304–312.

Moore J W, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Lillemo M, Viccars L, Milne R, Periyannan S, Kong X, Spielmeyer W, Talbot M, Bariana H, Patrick J W, Dodds P, Singh R, Lagudah E. 2015. A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nature Genetics*, **47**, 1494–1498.

Nematollahi G, Mohler V, Wenzel G, Zeller F J. 2008. Microsatellite mapping of powdery mildew resistance allele *Pm5d* from common wheat line IGV1–455. *Euphytica*, **159**, 307–313.

Ouyang S, Zhang D, Han J, Zhao X, Cui Y, Song W, Huo N, Liang Y, Xie J, Wang Z, Wu Q, Chen Y X, Lu P, Zhang D Y, Wang L, Sun H, Yang T, Keeble-Gagnere G, Appels R, Dolezel J, *et al*. 2014. Fine physical and genetic mapping of powdery mildew resistance gene *MlIW172* originating from wild emmer (*Triticum dicoccoides*). *PLOS ONE*, **9**, e100160.

Paterson A H, Bowers J E, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti A K, Chapman J, Feltus F A, Gowik U, Grigoriev I V, *et al*. 2009. The sorghum bicolor genome and the diversification of grasses. *Nature*, **457**, 551–556.

Poursarebani N, Ariyadasa R, Zhou R, Schulte D, Steuernagel B, Martis M M, Graner A, Schweizer P, Scholz U, Mayer K, Stein N. 2013. Conserved synteny-based anchoring of the barley genome physical map. *Functional & Integrative Genomics*, **13**, 339–350.

Qin B, Cao A, Wang H, Chen T, You F M, Liu Y, Ji J, Liu D, Chen P, Wang X E. 2011. Collinearity-based marker mining for the fine mapping of *Pm6*, a powdery mildew resistance gene in wheat. *Theoretical and Applied Genetics*, **123**, 207–218.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Wang Z, Cui Y, Chen Y, Zhang D, Liang Y, Zhang D, Wu Q, Xie J, Ouyang S, Li D, Huang Y, Lu P, Wang G, Yu M, Zhou S, Sun Q, Liu Z. 2014. Comparative genetic mapping and genomic region collinearity analysis of the powdery mildew resistance gene *Pm41*. *Theoretical and Applied Genetics*, **127**, 1741–1751.

Wang Z, Li H, Zhang D, Guo L, Chen J, Chen Y, Wu Q, Xie J, Zhang Y, Sun Q, Dvorak J, Luo M C, Liu Z. 2015. Genetic and physical mapping of powdery mildew resistance gene MlHLT in Chinese wheat landrace Hulutou. *Theoretical and Applied Genetics*, **128**, 365–373.

Wu H, Qin J, Han J, Zhao X, Ouyang S, Liang Y, Zhang D, Wang Z, Wu Q, Xie J, Cui Y, Peng H, Sun Q, Liu Z. 2013. Comparative high-resolution mapping of the wax inhibitors *Iw1* and *Iw2* in hexaploid wheat. *PLOS ONE*, **8**, e84691.

Xiao M, Song F, Jiao J, Wang X, Xu H, Li H. 2013. Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theoretical and Applied Genetics*, **126**, 1397–1403.

Xue F, Wang C, Li C, Duan X, Zhou Y, Zhao N, Wang Y, Ji W. 2012. Molecular mapping of a powdery mildew resistance gene in common wheat landrace Baihulu and its allelism with *Pm24*. *Theoretical and Applied Genetics*, **125**, 1425–1432.

Xue F, Zhai W W, Duan X Y, Zhou Y L, Ji W Q. 2009. Microsatellite Mapping of a powdery mildew resistance gene in Wheat Landrace Xiaobaidong. *Acta Agronomica Sinica*, **35**, 1806–1811.

You F M, Huo N, Gu Y Q, Luo M C, Ma Y, Hane D, Lazo G R, Dvorak J, Anderson O D. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics*, **9**, 253.

Zeng X, Long H, Wang Z, Zhao S, Tang Y, Huang Z, Wang Y, Xu Q, Mao L, Deng G, Yao X, Li X, Bai L, Yuan H, Pan Z, Liu R, Chen X, WangMu Q, Chen M, Yu L, *et al*. 2015. The draft genome of Tibetan hulless barley reveals adaptive patterns to the high stressful Tibetan Plateau. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 1095–1100.

Zhang D, Ouyang S H, Wang L L, Cui Y, Wu Q H, Liang Y, Wang Z Z, Xie J Z, Zhang D Y, Wang Y, Chen Y X, Liu Z Y. 2015. Comparative genetic mapping revealed powdery mildew resistance gene *MlWE4* derived from wild emmer is located in same genomic region of *Pm36* and *Ml3D232* on chromosome 5BL. *Journal of Integrative Agriculture*, **14**, 603–609.

Zhang H, Guan H, Li J, Zhu J, Xie C, Zhou Y, Duan X, Yang T, Sun Q, Liu Z. 2010. Genetic and comparative genomics mapping reveals that a powdery mildew resistance gene *Ml3D232* originating from wild emmer co-segregates with an NBS-LRR analog in common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, **121**, 1613–1621.

Zhou R, Zhu Z, Kong X, Huo N, Tian Q, Li P, Jin C, Dong Y, Jia J. 2005. Development of wheat near-isogenic lines for powdery mildew resistance. *Theoretical and Applied Genetics*, **110**, 640–648.

Zhu Y L, Wang L M, Wang H G. 2008. Studies on SSR molecular marker of wheat powdery mildew resistance gene *Pm5e*. *Molecular Plant Breeding*, **6**, 1080–1084.

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