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ORIGINAL ARTICLE

Pm61: a recessive gene for resistance to powdery mildew in wheat landrace Xuxusanyuehuang identified by comparative genomics analysis

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Abstract

Key message **A single recessive powdery mildew resistance gene *Pm61* from wheat landrace Xuxusanyuehuang was mapped within a 0.46-cM genetic interval spanning a 1.3-Mb interval of the genomic region of chromosome arm 4AL.**

Abstract Epidemics of powdery mildew incited by the biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*) have caused significant yield reductions in many wheat (*Triticum aestivum*)-producing regions. Identification of powdery mildew resistance genes is required for sustainable improvement of wheat for disease resistance. Chinese wheat landrace Xuxusanyuehuang was resistant to several *Bgt* isolates at the seedling stage. Genetic analysis based on the inoculation of *Bgt* isolate E09 on the F_1, F_2, and $F_{2:3}$ populations produced by crossing Xuxusanyuehuang to susceptible cultivar Mingxian 169 revealed that the resistance of Xuxusanyuehuang was controlled by a single recessive gene. Bulked segregant analysis and simple sequence repeat (SSR) mapping placed the gene on chromosome bin 4AL-4-0.80-1.00. Comparative genomics analysis was performed to detect the collinear genomic regions of *Brachypodium distachyon*, rice, sorghum, *Aegilops tauschii*, *T. urartu*, and *T. turgidum* ssp. *dicoccoides*. Based on the use of 454 contig sequences and the International Wheat Genome Sequence Consortium survey sequence of Chinese Spring wheat, four EST-SSR and seven SSR markers were linked to the gene. An F_5 recombinant inbred line population derived from Xuxusanyuehuang × Mingxian 169 cross was used to develop the genetic linkage map. The gene was localized in a 0.46-cM genetic interval between *Xgwm160* and *Xicsx79* corresponding to 1.3-Mb interval of the genomic region in wheat genome. This is a new locus for powdery mildew resistance on chromosome arm 4AL and is designated *Pm61*.

Introduction

Wheat (*Triticum aestivum* L.) is widely grown as a staple crop in many temperate regions of the world. Wheat production, in terms of yield and stability, is constantly challenged by many diseases, and wheat powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici* (DC.) Speer, *Bgt*, is an epidemic foliar disease in many maritime or semi-continental climates (Morgounov et al. 2012). Reported yield reductions caused by powdery mildew range from 5 to 40% and can be as high as 62% in severely infected fields (Singh et al. 2016). Decreases in quality due to powdery mildew infection have also been reported (Samobor et al. 2006). In China, powdery mildew has affected an area of around 6–8 million hectares in recent years (http://cb.natesc.gov.cn/sites/cb/). Fungicides such as triadimefon are often used by farmers to prevent powdery mildew in fields, but fungicide resistance has been detected in the pathogen population (Shi

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et al. 2015). This, together with the environmental pollution concern, discourages the continuous application of fungicides in protection of wheat from the disease. The improvement of powdery mildew resistance is the preferred method for limiting disease epidemics and minimizing the economic losses caused by the disease.

Breeding for powdery mildew-resistant cultivars relies on the availability of resistant resources. Currently, designated powdery mildew (*Pm*) resistance genes or alleles from *Pm1* to *Pm60* have been mapped on specific chromosomes (https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp). Among them, some were identified in *T. aestivum* and the rest originated from close or distant relatives of wheat (Guo et al. 2017). Additionally, many temporarily designated *Pm* or *Ml* resistance genes have been located on different chromosomes.

Some resistance genes can be effective against powdery mildew only for a period in agriculture because of virulence shift in the pathogen populations (Hsam and Zeller 2002). Others are not useful in cultivar development due to the linkage drag caused by association between powdery mildew resistance and certain deleterious traits (Summers and Brown 2013). Identification of new resistance genes is a continuous objective in breeding programs. Since the 1990s, various classes of molecular markers have been used to saturate genetic maps and identify powdery mildew resistance genes (Huang and Röder 2004; McIntosh et al. 2013). The wheat consensus SSR map has integrated 3700 loci (http://wheat.pw.usda.gov). Many designated powdery mildew resistance genes were initially identified with the aid of SSR markers. However, the wheat SSR markers are scattered on chromosomes and due to the huge size of the wheat genome (~ 17 Gb), they are not numerous enough for the fine mapping of target genes. Many wheat bin-mapped ESTs have been used to develop STS and SSR markers for locating the chromosomal bins of the resistance genes (Qi et al. 2004). The EST sequences are suitable for comparative genomics analysis due to the consensus that exists among the EST sequences of grass species.

Conserved synteny exists between wheat and its close relatives; and, the wheat EST database (Mochida et al. 2006; Coordinators 2016), genomic sequences of *Brachypodium distachyon* L. (International Brachypodium Initiative 2010), rice (*Oryza sativa* L.) (International Rice Genome Sequencing Project 2005), and sorghum [*Sorghum bicolor* (L.) Moench] (Paterson et al. 2009) are available. So, comparative genomics analysis has become an effective method to develop more molecular markers for the genetic mapping or fine mapping of *Pm* genes. For example, saturated linkage maps have been developed for *Pm6* (Qin et al. 2011), *MlIW172* (Ouyang et al. 2014), *Pm41* (Wang et al. 2014), *MlHLT* (Wang et al. 2015), *MlWE4* (Zhang et al. 2015), and *PmTm4* (Xie et al. 2017) using this strategy. The release of genomic sequences of *T. aestivum* cv. Chinese Spring (AABBDD genome) (Belova et al. 2013; Choulet et al. 2014; International Wheat Genome Sequencing Consortium 2014; Zimin et al. 2017), *Aegilops tauschii* (DD genome) (Jia et al. 2013; Zhao et al. 2017; Luo et al. 2013, 2017), *T. urartu* (AA genome) (Ling et al. 2013, 2018), and wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) (AABB genome) (Avni et al. 2017) makes comparative genomics analysis and map-based cloning in wheat more informative.

Wheat landraces from China have provided several powdery mildew resistance genes. The first gene, *Pm5e*, was identified on chromosome arm 7BL in Fuzhuang 30, which was selected from a cross between the two landraces Liquan Heshangtou and Huaxian Qisifeng (Huang et al. 2003). Another allele in this locus, *Pm5d*, was identified in IGV1-556, which was derived from the accession CI 10904 that was introduced from Jinling University, Nanjing (Hsam et al. 2001). The provisionally designated genes *PmH* (Hongquanmang, Zhou et al. 2005), *PmTm4* (Tangmai 4, Hu et al. 2008), *Mlmz* (Mazhamai, Zhai et al. 2008), *Mlxbd* (Xiaobaidong, Xue et al. 2009), *pmHYM* (Hongyoumai, Fu et al. 2017), *PmBYYT* (Baiyouyantiao, Xu et al. 2018a), and *PmSGD* (Shangeda, Xu et al. 2018b) were also localized in the chromosomal region around the *Pm5* locus. *PmTm4* was believed to have originated from the landrace Laozaomai (Hu et al. 2008). There are two alleles on locus *Pm24* on chromosome arm 1DS, *Pm24a* in Chiyacao (Huang et al. 2000) and *Pm24b* in Baihulu (Xue et al. 2012). *Pm47* was located on chromosome arm 7BS in Hongyanglazi (Xiao et al. 2013). *PmX* in Xiaohongpi (Fu et al. 2013), *MlHLT* in Hulutou (Wang et al. 2015), *Pm2c* in Niaomai (Xu et al. 2015), and *Pm45* in D57 (Wuzhaomai) (Ma et al. 2011) were mapped on chromosome arms 2AL, 1DS, 5DS, and 6DS, respectively.

A landrace, Xuxusanyuehuang (XXSYH), collected from Fengdu County, Sichuan province, appeared to be highly resistant against different *Bgt* isolates. The aims of this study were to examine (1) the effectiveness of the XXSYH gene(s) to *Bgt* isolates from wheat-producing regions of China; and (2) the inheritance and molecular mapping of the *Pm* gene(s) in XXSYH by means of comparative genomics analysis.

Materials and methods

Plant materials

The F_1, F_2, and $F_{2:3}$ populations, and F_5 recombinant inbred lines (RILs) were developed by crossing XXSYH to the susceptible cultivar Mingxian 169 for the genetic analysis and molecular detection of the *Pm* gene in XXSYH. Chromosome arm assignment of the target resistance gene-linked markers was performed using the Chinese Spring (CS)

nullisomic–tetrasomic, ditelosomic, and deletion lines. Twenty-five wheat accessions that carry known *Pm* genes or gene combinations were used to differentiate the *Bgt* isolates. Zhongzuo 9504 was included in this study for maintaining and increasing *Bgt* isolates, and it was used as the susceptible control in all assessments of the powdery mildew reactions.

Powdery mildew evaluations

Fifteen single-colony cultures of *Bgt* isolates, collected from different wheat fields in China, were used to evaluate the resistance of XXSYH to powdery mildew (Table 1). Isolate E09 was used to phenotype the mapping populations and the two parents for genetic analysis of the target resistance gene. Evaluations of powdery mildew reactions to the *Bgt* isolates at the seedling stage were conducted in a greenhouse set at 22 °C day/18 °C night with 60% relative humidity and a 12-h light/12-h dark photoperiod. Xuxusanyuehuang, Mingxian 169, the F_1, 286 F_2 plants, 159 $F_{2:3}$ families, and 200 F_5 RILs were tested. At least 15 plants from each $F_{2:3}$ family and F_5 line were examined. Two independent tests were conducted for the RIL population. Seedlings at the one leaf stage were artificially inoculated with *Bgt* isolates by dusting conidiospores that were multiplied on the susceptible plants of Zhongzuo 9504. Infection types (ITs) of all plants were rated on a 0–4 scale 15 days after inoculation (Liu et al. 1999). The inoculated plants were divided into either a resistant group (IT 0–2) or a susceptible group (IT 3–4).

Molecular marker analysis

Genomic DNA was extracted from the young leaves using the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). Resistant and susceptible DNA bulks were composed of equal amounts of DNA from the representative plants of 10 homozygous resistant and 10 homozygous susceptible $F_{2:3}$ families for bulked segregant analysis (BSA) (Michelmore et al. 1991). Polymorphisms of wheat genomic SSRs (i.e., *Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*,

Table 1 Reactions of Xuxusanyuehuang, Mingxian 169, wheat entries possessing known powdery mildew resistance genes or gene combinations, and the susceptible control Zhongzuo 9504 after inoculation with 15 isolates of *Blumeria graminis* f. sp. *tritici* (*Bgt*)

Cultivar/line	Pm gene	*Bgt* isolate														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	E09
Festival	*Pm1a*	0	0	0	0	0	0	3	0	0	3	0	0	0	3	3
Pm1c	*Pm1c*	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
Ulka/*8Cc	*Pm2*	0	0	1	0	0	0	0	1	0	3	0	0	0	0	0
Asosan	*Pm3a*	3	3	0	0	3	3	0	3	3	3	3	3	3	3	3
CI 14121	*Pm3b*	4	3	3	3	0	3	1	0	3	0	3	0	0	3	0
Sonora/8*Cc	*Pm3c*	4	3	3	3	3	3	0	3	3	3	3	0	3	3	3
7P580	*Pm3e*	4	3	3	3	3	3	1	3	3	3	3	3	4	3	3
Courtot	*Pm3g*	3	0	3	3	3	3	0	3	3	0	3	3	3	3	3
Khaphi/8*Cc	*Pm4a*	0	0	0	0	4	3	1	3	3	0	3	0	3	3	0
VPM1	*Pm4b*	0	0	0	0	3	3	0	3	3	0	3	0	3	3	0
Pm4c	*Pm4c*	0	0	0	0	3	3	1	3	3	0	3	0	3	0	0
CI 14125	*Pm5a*	3	3	4	3	4	3	1	3	3	3	3	3	3	3	4
Fuzhuang 30	*Pm5e*	0	0	0	3	0	0	1	0	0	0	0	1	0	0	0
Hongquanmang	*PmH*	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0
Coker 747	*Pm6*	1	3	3	3	4	3	0	3	4	0	3	1	3	0	3
CI 141879	*Pm7*	3	3	3	3	2	3	1	3	3	3	3	3	3	3	3
PI 361879	*Pm8*	3	3	3	3	3	1	3	3	3	3	3	3	3	0	4
96-282	*Pm13*	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
96-283	*Pm16*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96-286	*Pm19*	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3
Chiyacao	*Pm24*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
L2632-32R	*Pm33*	0	0	0	0	3	3	3	3	3	0	3	0	3	0	0
Liangxing 99	*Pm52*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CI 12632	*Pm2+6*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coker	*Pm5+6*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Xuxusanyuehuang	*Pm61*	3	0	3	0	2	3	0	0	0	0	0	0	0	3	0
Mingxian 169	Susceptible parent	3	3	4	3	3	3	3	4	3	3	3	3	3	3	4
Zhongzuo 9504	Susceptible control	3	3	4	3	3	3	3	4	3	3	3	3	3	3	4

and *Xcfd* series) and EST markers (http://wheat.pw.usda.gov) were examined. The reaction mixture (10 µl) for DNA amplification was prepared by mixing 50 ng DNA, 0.2 mM dNTPs, 0.2 µM of each primer, 1 U of *Taq* polymerase, and 1× assay buffer. The following conditions were used for DNA amplification: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 53–60 °C (depending on primers used) for 30 s, 72 °C for 30 s; and 72 °C for 10 min. The amplification products were visualized on 8% non-denaturing polyacrylamide gels (Acr/Bis = 39:1) after silver staining.

Chromosome arm assignment of the target resistance gene, comparative genomics analysis, and marker development

The resistance gene-linked markers were localized by comparing the banding patterns amplified from the Chinese Spring nullisomic–tetrasomic, ditelosomic, and deletion lines. Assignment of polymorphic markers to chromosome bins was conducted by determining the smallest deletion bin that possesses them.

The EST sequences flanking the target gene on bin 4AL-4-0.80-1.00 were used to search for the orthologous genes in the CDS sequences of *Ae. tauschii*, *B. distachyon* (http://mips.helmholtz-muenchen.de/plant/Brachypodium/), rice (http://rice.plantbiology.msu.edu/), and sorghum (http://mips.helmholtzmuenchen.de/plant/sorghum/) genomic sequences. Then, the wheat EST sequences homologous to CDS sequences of *Brachypodium* within the homologous genomic regions were used to develop EST-SSR markers polymorphic between the two parents and the contrasting DNA bulks. The *Brachypodium* orthologous gene sequences flanking the polymorphic EST-SSR markers were used as query to search the Chinese Spring genomic sequences released by the International Wheat Genome Sequencing Consortium (IWGSC) (http://www.wheatgenome.org/) to determine the homologous contigs or scaffolds on chromosome arm 4AL. The acquired contig sequences were used to develop SSR markers using the software Batchprimer3 (https://probes.pw.usda.gov/batchprimer3/). Polymorphic EST-SSR and SSR markers were mapped on the F_5 RILs to develop the linkage map of the target gene.

Linkage analysis and genetic linkage map construction

The deviations between the observed phenotypic data and the expected segregation ratios in the genetic analysis of the resistance gene using F_2, $F_{2:3}$, or F_5 populations were analyzed with the Chi-squared (χ^2) test. Linkage relationships and distances between the polymorphic markers and the *Pm* gene in XXSYH with the F_5 population were determined using the software Mapmaker version 3.0 with the Kosambi map function and an LOD threshold of 3.0 (Lincoln et al. 1993). A genetic linkage map for the target gene in XXSYH was developed using the software Mapdraw V2.1 (Liu and Meng 2003).

Results

Reactions of XXSYH to *Bgt* isolates

In the seedling tests, all the 15 *Bgt* isolates examined were avirulent on lines carrying genes or gene combinations involving *PmH*, *Pm16*, *Pm24*, *Pm52*, *Pm2* + *6*, and *Pm5* + *6* (Table 1). Lines with genes *Pm1c*, *Pm2*, *Pm5e*, and *Pm13* were resistant against 14 isolates. The virulence frequencies on lines with *Pm1a*, *Pm3b*, *Pm4a-4c*, and *Pm33* ranged from 26.7 to 53.3%. The same reaction patterns were observed between lines carrying *Pm4a* and *Pm4b*. Most *Bgt* isolates were virulent on lines with *Pm3a*, *Pm3c*, *Pm3e*, *Pm3 g*, *Pm5a*, *Pm6*, *Pm7*, and *Pm8*. Line carrying *Pm19* was susceptible to all *Bgt* isolates tested.

Xuxusanyuehuang was resistant to 11 of the 15 isolates examined, but was susceptible to isolates 1, 3, 6, and 14. Compared to the differential wheat entries, XXSYH differed from the lines carrying the known genes *Pm1c* in its reaction to 3 isolates, *PmH*, *Pm16*, *Pm24*, *Pm52*, *Pm5* + *6*, and *Pm2* + *6* to 4 isolates, *Pm2*, *Pm5e*, and *Pm13* to 5 isolates, *Pm4a*, *Pm4b*, and *Pm1a* to 7 isolates, and *Pm4b* and *Pm4c* to 8 isolates (Table 1). Mingxian 169 was as susceptible to all the *Bgt* isolates as the control cultivar Zhongzuo 9504.

Genetic analysis of the gene for powdery mildew resistance in XXSYH

A genetic analysis was carried out to characterize the inheritance mode of the powdery mildew resistance gene in XXSYH against *Bgt* isolate E09 using the F_1, $F_{2:3}$, and F_5 populations developed from the XXSYH × Mingxian 169 cross (Table 2). The parental cultivars XXSYH (IT 1) and Mingxian 169 (IT 3) showed distinct phenotypic responses to isolate E09. The IT of F_1 plants was the same as the susceptible parent Mingxian 169 (Fig. 1). The segregation of resistant and susceptible F_2 plants provided a good fit to a 1:3 ratio. The 159 $F_{2:3}$ families and 200 F_5 RILs segregated in ratios of 1:2:1 (homozygous resistant: heterozygous: homozygous susceptible) and 1:1 (resistant: susceptible), respectively (Table 2). These results clearly demonstrate that XXSYH carries a single recessive gene for resistance to *Bgt* isolate E09.

Table 2 Genetic analysis of resistance to isolate E09 of *Blumeria graminis* f. sp. *tritici* in F_1, F_2, $F_{2:3}$, and F_5 progenies derived from Xuxusanyuehuang × Mingxian 169 cross

Parents and cross	Generation	Total number of plants/families	Observed ratio			Expected ratio	χ^2	*P* value
			R	Seg	S			
Xuxusanyuehuang	P_R	15	15		0			
Mingxian 169	P_S	15	0		15			
$P_R \times P_S$	F_1	15	0		15			
	F_2	286	81		205	0.044	1.680	0.195
	$F_{2:3}$	159	39	78	42	0.043	0.016	0.900
	F_5	200	110		90	0.042	2.000	0.160

P_R, resistant parent Xuxusanyuehuang; P_S, susceptible parent Mingxian 169; R, resistant; Seg, heterozygous resistant; S, susceptible

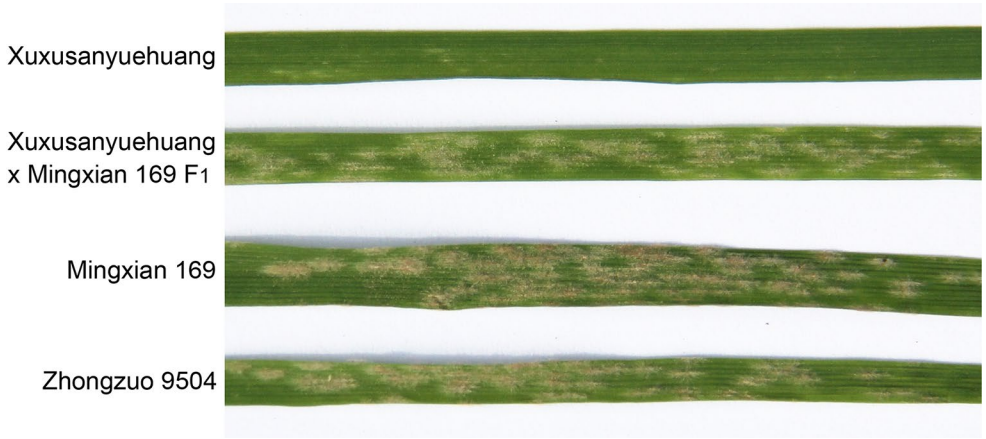

Fig. 1 The phenotypic reactions of resistant parent Xuxusanyuehuang, susceptible parent Mingxian 169, and the susceptible control Zhongzuo 9504 to *Bgt* isolate E09

Localization of the gene for powdery mildew resistance with SSR markers

By analysis of the polymorphisms of 120 SSR primer pairs randomly distributed on different wheat chromosomes, we identified only one marker, *Xgwm160*, located on chromosome arm 4AL that was polymorphic between the parental cultivars, as well as the contrasting DNA bulks. Screening of 104 additional pairs of SSR primers mapped on 4AL produced two more polymorphic markers, *Xbarc52* and *Xbarc327*. Genotype analysis of the F_5 mapping population revealed that the powdery mildew resistance gene was localized between the co-dominant markers *Xgwm160* and *Xbarc327* within a 4.64-cM genetic interval (Fig. 2). Because of its unique position on chromosome arm 4AL, this gene was designated *Pm61*.

Molecular marker development for *Pm61* through comparative genomics analysis

The SSR markers *Xgwm160* and *Xbarc52* were mapped to deletion bin 4AL-4-0.80-1.00, so 20 EST-STS markers on this chromosome region (https://wheat.pw.usda.gov/cgi-bin/westsql/bin_candidates.cgi?bin=4AL4-0.80-1.00) were initially screened for their polymorphisms between the parental cultivars and the contrasting bulked segregants. Unfortunately, no polymorphic EST-STS markers between the parental cultivars or the contrasting DNA bulks were identified. An additional 105 wheat EST sequences that were anchored on the bin 4AL-4-0.80-1.00 were compared to the genomic sequence databases of *Brachypodium*, rice, and sorghum using the batch Blast program hosted at GrainGenes (http://www.graingene/lblgov/cgi-bin/nphblast_interface.cgi). Orthologous genes were found on *Brachypodium* chromosome 1, rice chromosome 6, and sorghum chromosome 10 (Table S1). The wheat EST sequences with high synteny to the orthologous genes were used to design 125 SSR primer pairs. Three co-dominant EST-SSR markers, namely *Xicsx29* (BE490293), *Xicsx65* (BE591440), and *Xicsx79* (BG604834) (Fig. 3), and one dominant EST-SSR marker, *Xicsx73* (BF200736) (Table 3), were polymorphic between the parental cultivars and the contrasting DNA bulks. *Pm61* was re-localized in the genetic interval (4.18 cM) between markers *Xgwm160*

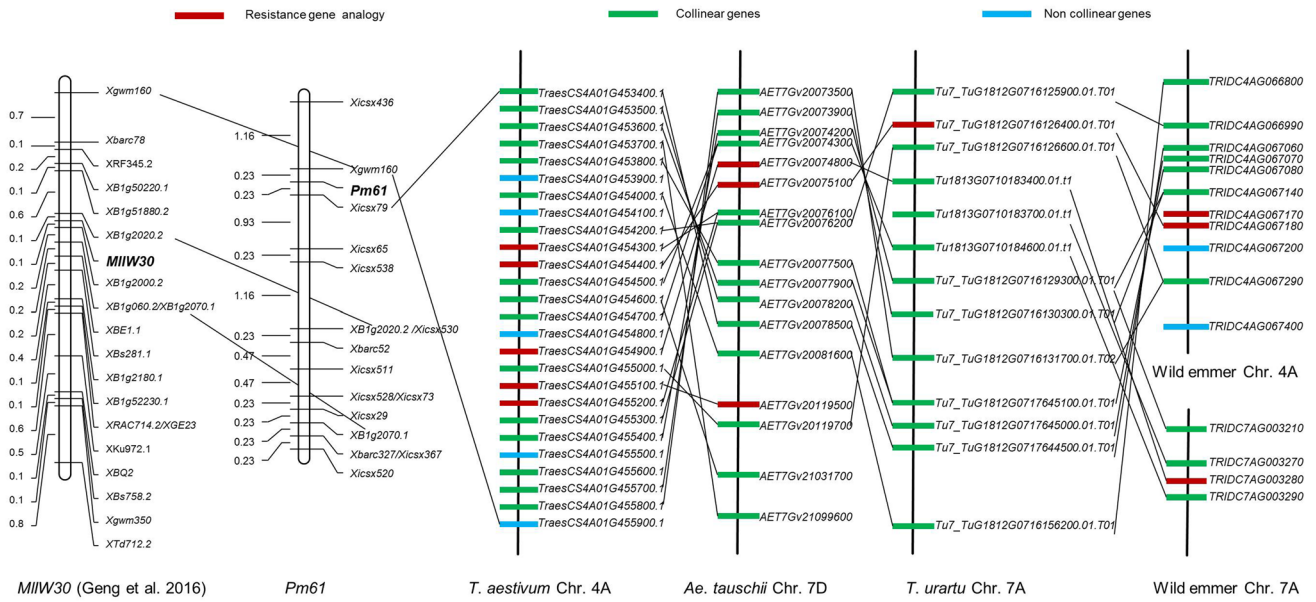

Fig. 2 Comparative genetic linkage and physical maps of powdery mildew resistance gene *Pm61* and the orthologous genomic regions of *Triticum aestivum*, *Aegilops tauschii*, *T. urartu* and wild emmer. The linkage map was constructed using F_5 RIL population derived from Xuxusanyuehuang × Mingxian 169 cross

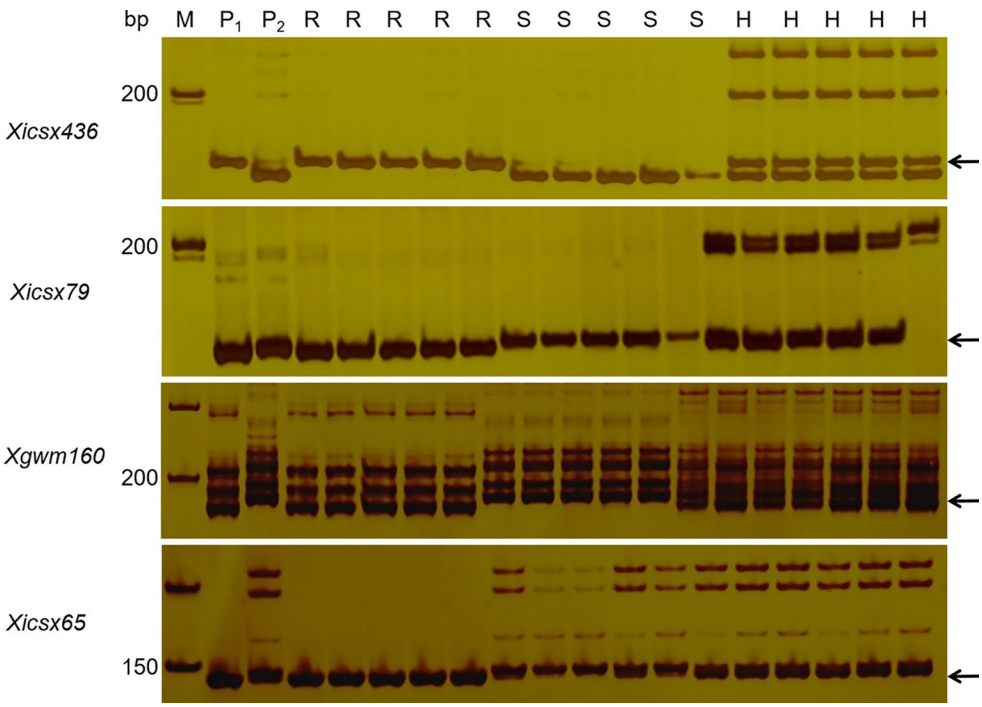

Fig. 3 Amplification patterns of markers *Xicsx436* (SSR), *Xicsx79* (EST-SSR), *Xgwm160* (SSR), and *Xicsx65* (EST-SSR) in Xuxusanyuehuang (P_R), Mingxian 169 (P_S), homozygous resistant (R), heterozygous resistant (H), and homozygous susceptible (S) F_5 recombinant inbred lines derived from Xuxusanyuehuang × Mingxian 169 cross. M: 100 bp DNA ladder

and *Xicsx29* (BE490293) using the F_5 mapping population (Fig. 2).

The homologous region on *Brachypodium* chromosome 1 (Bradi1g50205 to Bragilg52140), corresponding to the genetic interval between markers *Xicsx79* (BG604834) and *Xicsx73* (BF200736) (Fig. 3), was used to blast the *Ae. tauschii* and *T. turgidum* ssp. *dicoccoides* CDS sequence databases and the rice and sorghum genomic sequence databases to determine the region of collinearity. The coding sequences of collinear *Brachypodium* genes were used as queries to search the 454 Chinese Spring contigs and the IWGSC individual chromosome survey sequences

Table 3 Detailed information on EST-SSR and SSR markers linked to the powdery mildew resistance gene *Pm61*

Marker	Source	Type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)	Product size (bp)[a]	References
Xgwm160	–	SSR	TTCAATTCAGTCTTG GCTTGG	CTGCAGGAAAAA AAGTACACCC	55	196	Consensus SSR map[b]
Xbarc52	–	SSR	GCGCCATCCATC AACCGTCATCGT CATA	GCGAGGAAGGCG GCCACCAGAATGA	55	102	Consensus SSR map
Xbarc327	–	SSR	GCGGTATAGGTATAT CGAGGCATAGA	GCGAGAGAGCGG TAGAGATAAATG	52	248	Consensus SSR map
Xicsx29	BE490293	EST-SSR	AAACAACAAACA AACGAACAG	CGGTAATGCATA TTCACCTAT	55	161	Current study
Xicsx65	BE591440	EST-SSR	CGATCTAGGGTT GCTTTTC	GTACTCCTCCTT GGCTTTAAC	55	140	Current study
Xicsx73	BF200736	EST-SSR	TTTTGTACCTTTCTA GCGACA	GAGCTCTGATAC TCGAAACAA	55	152	Current study
Xicsx79	BG604834	EST-SSR	TTTGAAACTAAA GTTGGGTCA	GTTAACTATCCATGT GCCAGA	55	143	Current study
Xicsx367	Bradi1g51960	SSR	TTCTGGTTTATCTTT GCTTTG	CACCGTCTGAAT TCTGATTAC	55	134	Current study
Xicsx436	Bradi1g50280	SSR	AAACACACATGC TAGTTGAGG	TATCTTGTTCCTGCT GTATGG	55	149	Current study
Xicsx511	Bradi1g52040	SSR	TTTCTTCAGTGTTTC TTCCAA	AGTTGACGCTGA CTGAGATAC	55	161	Current study
Xicsx520	Bradi1g52050	SSR	GGGTTGGATCTTCTT CTTCT	ATCTGTGTCCCTGTC TCTGTT	55	143	Current study
Xicsx528	Bradi1g52090	SSR	ATCATATATTTTGGG GAAAGG	AATGCTACAATT AGCGTCTCA	55	149	Current study
Xicsx530	Bradi1g52110	SSR	ATGAGCTAGATTTCA CTCGAC	GTTACTGACGAG CACTCACTT	55	182	Current study
Xicsx538	Bradi1g51750	SSR	TCTAATTAAACA ACGTACCTGCT	CTCTCCGCTTCTCTC TCTAGT	55	148	Current study
XB1g2020.2	Bradi1g52020.1	SSR	TGTTGTGTGTGTGTG TGAGA	GAGAAGAGGAGA GGAGAGGAT	55	150	Geng et al. 2016
XB1g2070.1	Bradi1g52070.1	SSR	TTTCTCCTTCTCCCG CCA	CACTGCCCTCGT CTTTCT	56	204	Geng et al. 2016

[a]Estimated product sizes amplified from Xuxusanyuehuang

[b]Consensus SSR map: http://wheat.pw.usda.gov

(http://www.wheatgenome.org/) for identifying the homologous contigs or scaffolds on chromosome arm 4AL. Based on those sequences, 398 pairs of SSR primers were designed. Seven polymorphic markers developed from different *Brachypodium* orthologous genes, namely *Xicsx367* (Bradi1g51960), *Xicsx436* (Bradi1g50280), *Xicsx511* (Bradi1g52040), *Xicsx520* (Bradi1g52050), *Xicsx528* (Bradi1g52090), *Xicsx530* (Bradi1g52110), and *Xicsx538* (Bradi1g51750), were incorporated into the genetic linkage map (Fig. 3, Table 3). Based on their banding patterns, *Xicsx436* (Fig. 3), *Xicsx511*, *Xicsx520*, *Xicsx528*, *Xicsx530*, and *Xicsx538* were co-dominant, while *Xicsx367* was dominant. *Pm61* was placed to a 0.46-cM interval and flanked by markers *Xgwm160* and *Xicsx79* at genetic distances of 0.23 cM and 0.23 cM at the distal end of chromosome arm 4AL, respectively (Fig. 2).

Comparative genomics analysis of the genetic interval flanking *Pm61* and gene prediction

In the Chinese Spring genomic sequence, the genetic interval between the closest flanking markers *Xgwm160* and *Xicsx79* for *Pm61* (0.46 cM) was mapped on chromosome 4AL within a 1.3-Mb genomic region (717963176–719260469), which contained 26 predicted genes (Table S2). A detailed comparative genomics analysis was conducted to search for the conserved collinear orthologous genes among the CDS sequence databases of *Ae. tauschii*, *T. urartu*, and wild

emmer. The collinear orthologous genomic region corresponding to the genetic interval of *Pm61* spanned an 8.2 kb genomic region consisting of 17 predicted orthologous genes (AET7Gv20073500–AET7Gv21099600) on chromosome 7D of *Ae. tauschii*. This region was collinear with a 1.8-Mb genomic region (Tu7_TuG1812G0716125900.01.T01–Tu7_TuG1812G0716156200.01.T01) consisting of 13 predicted orthologous genes on chromosome 7A of *T. urartu*. Two collinear genomic regions were detected in the wild emmer genome. One was 2.06 Mb (TRIDC4AG066800–TRIDC4AG067400) with 11 predicted orthologous genes, and the other was 484 kb (TRIDC7AG003210–TRIDC7AG003290) with 4 predicted orthologous genes on chromosome 7A (Fig. 2, Table S2). The annotation of the conserved collinear orthologous genes demonstrated that five (TraesCS4A01G454300.1, TraesCS4A01G454400.1, TraesCS4A01G454900.1, TraesCS4A01G455100.1, and TraesCS4A01G455200.1) in Chinese Spring, three (AET7Gv20074800, AET7Gv20075100, and AET7Gv20119500) in *Ae. tauschii*, one (Tu7_TuG1812G0716126400.01.T01) in *T. uraru*, and three (TRIDC4AG067170, TRIDC4AG067180, and TRIDC7AG003280) predicted genes in wild emmer encoded for proteins associated with disease resistance. They included NBS-LRR disease resistance protein, receptor-like kinase family protein, and RPM1-like disease resistance protein (Table S2).

Chromosome bin assignment of *Pm61*

Because the synteny genomic regions flanking the *Pm61* locus were found in chromosomes of both homoeologous groups 4 and 7, the Chinese Spring nullisomic–tetrasomic, ditelosomic, and deletion lines for the chromosomes of these homoeologous groups were used to determine the chromosome and the physical bin location of the markers that were linked to *Pm61*. The absence of products from markers *Xicsx65* and *Xicsx79* in the nullisomic–tetrasomic line N4A-T4D, and the deletion lines 4AL-4, 4AL-5, 4AL-12, and 4AL-13 on 4AL-4-0.80-1.00 demonstrated that *Pm61* was located in the distal chromosomal bin 4AL-4-0.8-1.00 (Fig. 4a). *Xicsx65* and *Xicsx79* produced identical products in XXSYH, CS, and the group 7 nullisomic–tetrasomic and ditelosomic lines (Fig. 4b), indicating that *Pm61* was not present on any of the homoeologous group 7 chromosomes.

Comparison of physical positions between *Pm61* and *MlIW30* identified in wild emmer

MlIW30, a single dominant *Pm* gene derived from wild emmer, was mapped on chromosome bin 4AL-4-0.8-1.00 (Geng et al. 2016). The homologous genomic region carrying *MlIW30* in wheat was collinear with the corresponding *Brachypodium* genomic region extending from *Bradi1g50220* to *Bradi1g52230*. Polymorphic markers linked to *MlIW30* were detected between XXSYH and Mingxian 169, as well as the contrasting DNA bulks. Two SSR markers, *XB1g2020.2* and *XB1g2070.1*, developed from genes *Bradi1g52020* and *Bradi1g52070*, were linked to *Pm61*, but they were mapped to the proximal side of *Pm61* at genetic distances of 2.55 cM and 4.18 cM, respectively (Fig. 2). *Pm61* and *MlIW30* were located 0.23 cM and 1.8 cM from the common SSR marker *Xgwm160* on the proximal side, respectively. However, the two nearest flanking markers *XB1g2000.2* and *XB1g2020.2* located *MlIW30* in a 0.1-cM genetic interval corresponding to a 21 kb (732769506–732790522 on chromosome arm 4AL) physical interval in the genome of Chinese Spring, which was

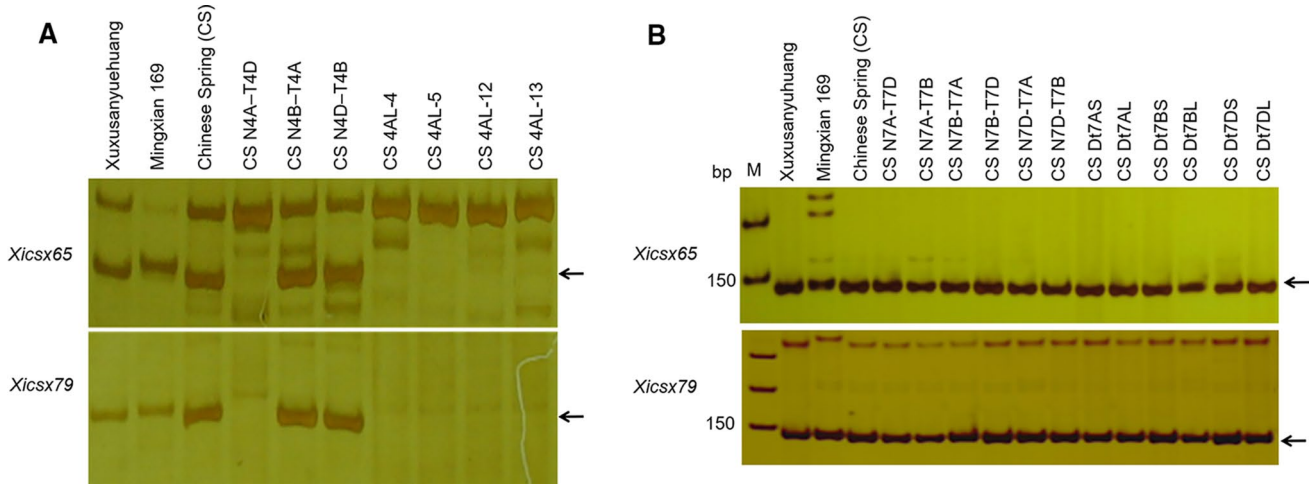

Fig. 4 Amplification patterns of the *Pm61*-linking markers *Xicsx65* and *Xicsx79* in Xuxusanyuehuang, Mingxian 169, Chinese Spring (CS), and CS homoeologous groups 4 (**a**) and 7 (**b**) nullisomic–tetrasomic, ditelosomic, and deletion lines. M: 100 bp DNA ladder

obviously different from the 1.3 Mb physical localization of *Pm61* (717963176–719260469) (Table S3).

Discussion

Chinese landrace XXSYH was resistant to some *Bgt* isolates collected from China in the seedling tests. A recessive gene *Pm61* conferred the resistance to powdery mildew in this cultivar. Molecular marker analysis localized *Pm61* in a 0.46-cM genetic interval on chromosome arm 4AL. Results of physical mapping of the closest flanking markers *Xgwm160* and *Xicsx79* assigned *Pm61* in a 1.3-Mb physical interval in the chromosome 4AL genomic sequence of Chinese Spring.

More than 13,000 wheat landraces are preserved in the Gene Bank of China in Beijing (Liu et al. 2000). Extensive studies have been conducted to evaluate the resistance to powdery mildew of the Chinese wheat landraces. In the first large scale test, Sheng et al. (1992) identified six immune or highly resistant and 71 moderately resistant landraces in a collection of 3441 accessions from eight provinces in China. Wang et al. (1996) obtained 44 resistant landraces out of 867 accessions indigenous to Henan province. Four cultivars were moderately resistant among 1837 wheat landraces from Jiangsu province (Xiong et al. 1995), and seven landraces were highly resistant in 1152 wheat accessions from Shaanxi province (Hu et al. 2007). Seedling resistance was observed in 46 accessions, and the adult plant resistance was detected in 193 landraces from Gansu province (Cao et al. 2010). Variation in the frequencies of powdery mildew-resistant landraces was observed in different wheat-producing regions (Li et al. 2011). In subsequent studies, more than 20 *Pm* resistance genes/alleles from the Chinese wheat landraces have been identified, and some of them have been mapped on chromosome arms 2AL (Fu et al. 2013), 7BS (Xiao et al. 2013), 7BL (Hsam et al. 2001; Huang et al. 2003; Zhou et al. 2005; Hu et al. 2008; Zhai et al. 2008; Xue et al. 2009; Fu et al. 2017; Xu et al. 2018a, b), 1DS (Huang et al. 2000; Xue et al. 2012; Wang et al. 2015), 5DS (Xu et al. 2015), and 6DS (Ma et al. 2011). Based on its unique position, *Pm61* from XXSYH is a new locus conferring resistance to powdery mildew on chromosome arm 4AL.

Geng et al. (2016) reported a temporarily designated gene *MlIW30* on the distal part of chromosome arm 4AL. Although *Pm61* and *MlIW30* share the same deletion bin 4AL-4-0.8-1.00 on chromosome arm 4AL, they differed obviously in their mode of inheritance, origin, and precise physical localization in the recently released Chinese Spring reference genomic sequence. *Pm61* in the Chinese wheat landrace XXSYH exhibited recessive inheritance when tested with *Bgt* isolate E09, while *MlIW30*, which originated from an Israeli *T. turgidum* ssp. *dicoccoides* accession IW30, showed a dominant mode of inheritance in response to this *Bgt* isolate. Because of their geographic isolation, these genes evolved independently in different ecotypes even though they are located on the same chromosome. Wild emmer is the tetraploid ancestor of common wheat (Nevo et al. 2013). It has been suggested that wild emmer and common wheat have developed an integrated and stable genetic system during their long-term evolution (Shi et al. 2005). The A genomes of these related species are not completely identical, but are homoeologous. The genomic region of *Pm61* that was flanked by the two nearest markers (*Xgwm160* and *Xicsx79*) spans a 1.3 Mb (717963176–719260469) region of chromosome arm 4AL, which is different from the genomic region (732769506–732790522) in which *MlIW30* is located.

Two major QTL for resistance to powdery mildew were identified on wheat chromosome 4A. *QPm.uga-4A* from soft red winter wheat AGS 2000 was located on chromosome arm 4AS (Hao et al. 2015), which is obviously different from *Pm61*. *QPm.tut-4A* was detected on chromosome arm 4AL of the wheat-*T. militinae* introgression line 8.1 (Jakobson et al. 2012). This QTL differed from *Pm61* in its origin from *T. militinae* although they share the common SSR marker *Xgwm160*.

A translocation in the distal region between 4AL and 7BS had occurred during the evolution of *T. aestivum* and *T. turgidum* (Hossain et al. 2004; Miftahudin et al. 2004; Ishikawa et al. 2009; Hernandez et al. 2012). We detected some homoeologous genes around the *Pm61* locus on chromosomes 7AS and 7DS in the common wheat genome. Comparative genomics analysis using the recently released genomic sequences of *Ae. tauschii* (Luo et al. 2017), *T. urartu* (Ling et al. 2018), and wild emmer (Avni et al. 2017) indicated that the orthologous genomic region of the *Pm61* locus was located on chromosome 7D in *Ae. tauschii*, 7A in *T. urartu*, and 4A and 7A in wild emmer (Fig. 3, Table S2). The results of chromosomal and physical bin mapping using Chinese Spring aneuploid and deletion lines for the homoeologous groups 4 and 7 chromosomes confirmed the localization of *Pm61* on 4AL rather than on any of the homoeologous group 7 chromosomes.

A well-assembled genome sequence of common wheat has recently become available (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/). Because of the high levels of macro- and micro-collinearities between wheat genome and *Brachypodium*, rice and sorghum genomes, comparative genomics analysis has often been used as an effective means to develop linked molecular markers for gene mapping in common wheat. In the present study, we mapped *Pm61* in a small genetic interval using the collinear genomic region on *Brachypodium* chromosome 1 generated by comparative genomics analysis. Then, the genetic interval flanking *Pm61* was used to blast

the genomic sequences of *Ae. tauschii*, *T. urartu*, and wild emmer to search for collinear regions, which can serve as a framework for fine mapping and map-based cloning of this gene. Further research is in progress to develop closely linked and/or co-segregating markers for the fine mapping of *Pm61* in the wheat landrace XXSYH.

In summary, Chinese wheat landrace XXSYH carries a new recessive gene for resistance to powdery mildew, which is designated *Pm61*. Molecular mapping analysis located *Pm61* on the distal end of chromosome arm 4AL. Based on the comparative genomics analysis, four EST-SSR and seven SSR polymorphic markers were developed and incorporated in the genetic linkage map, which mapped *Pm61* to a 0.46-cM genetic interval between markers *Xgwm160* and *Xicsx79*, corresponding to a 1.3-Mb interval of the genomic region of 4AL.

Author Contribution Statement HjL and JL conceived and designed the study. HS, JH, WS, DQ, LC, PW, YL, TL, YQ, and WC conducted the experiments. HZ, HwL, LY, YZ, and ZL analyzed data. JL, HjL, and HS wrote the manuscript with the contributions of ZL.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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