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Identification of a Recessive Gene *PmQ* Conferring Resistance to Powdery Mildew in Wheat Landrace Qingxinmai Using BSR-Seq Analysis

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Abstract

Wheat powdery mildew is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), a biotrophic fungal species. It is very important to mine new powdery mildew (*Pm*) resistance genes for developing resistant wheat cultivars to reduce the deleterious effects of the disease. This study was carried out to characterize the *Pm* gene in Qingxinmai, a winter wheat landrace from Xinjiang, China. Qingxinmai is resistant to many *Bgt* isolates collected from different wheat fields in China. F_1, F_2, and $F_{2:3}$ generations of the cross between Qingxinmai and powdery mildew susceptible line 041133 were developed. It was confirmed that a single recessive gene, *PmQ*, conferred the seedling resistance to a *Bgt* isolate in Qingxinmai. Bulked segregant analysis-RNA-Seq (BSR-Seq) was performed on the bulked homozygous resistant and susceptible $F_{2:3}$ families, which detected 57 single nucleotide polymorphism (SNP) variants that were enriched in a 40 Mb genomic interval on chromosome arm 2BL. Based on the flanking sequences of the candidate SNPs extracted from the Chinese Spring reference genome, 485 simple sequence repeat (SSR) markers were designed. Six polymorphic SSR markers, together with nine markers that were anchored on chromosome arm 2BL, were used to construct a genetic linkage map for *PmQ*. This gene was placed in a 1.4 cM genetic interval between markers *Xicsq405* and *WGGBH913* corresponding to 4.9 Mb physical region in the Chinese Spring reference genome. *PmQ* differed from most of the other *Pm* genes identified on chromosome arm 2BL based on its position and/or origin. However, this gene and *Pm63* from an Iranian common wheat landrace were located in a similar genomic region, so they may be allelic.

Keywords: *Triticum aestivum* L., *Blumeria graminis* f. sp. *tritici*, landrace, BSR-Seq

Powdery mildew of wheat (*Triticum aestivum* L.) is globally an important disease incited by the fungus *Blumeria graminis* f. sp. *tritici* (DC.) Speer (*Bgt*) (Dubin and Duveiller 2011). Epidemics of powdery mildew and economic losses of wheat are enhanced by high plant density, heavy use of nitrogen fertilizers, and irrigation conditions (Cowger et al. 2012). Infection of powdery mildew at the early growth stages may result in seedling or tiller death, and thus a severe yield reduction (Cunfer 2002). Yield loss estimates in wheat due to powdery mildew range from 5 to 40% and can be even higher when symptoms occur early in the growing season (Mehta 2014; Singh et al. 2016). Other deleterious effects of powdery mildew on wheat include reduced seed quality and processing properties of flour (Samobor et al. 2006).

In China, wheat powdery mildew has been an economically important disease since the 1970s and it was reported earlier in isolated wheat fields. At present, this disease occurs on an acreage ranging from 6 to 8 million ha where both winter wheat and spring wheat are grown (available online: https://www.natesc.org.cn/sites/cb/). Symptoms of powdery mildew can be seen on all the above-ground parts, including leaf, leaf sheath, spike, and awn, but the leaves are the main tissues on which symptoms occur. So, fungicides, such as triadimefon, are often top-dressed on leaves to control powdery mildew when the host resistance cannot provide sufficient protection from the disease. However, powdery mildew-resistant cultivars are preferred by wheat growers for the sake of reducing production costs and better environmental protection (Johnson et al. 1979).

Powdery mildew (*Pm*) resistance genes have been detected on all wheat chromosomes except 3D and 4D. The permanently designated *Pm* genes include *Pm1* through *Pm65* (https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp). Also, there are some tentatively named *Pm* genes, but the *Pm* genes are not evenly distributed on the wheat chromosomes. Some of them appear to reside in clusters on certain chromosomes. For example, *Pm6* (Jørgensen and Jensen 1973), *Pm33* (Zhu et al. 2005), *Pm51* (Zhan et al. 2014), *Pm52* (Wu et al. 2019), *Pm64* (Zhang et al. 2019), *MlZec1* (Mohler et al. 2005), and *MlAB10* (Maxwell et al. 2010) have been identified on chromosome arm 2BL.

The *Pm* genes differ in their positions on chromosomes, origins, and reactions to different *Bgt* isolates. Molecular markers are effective for localizing *Pm* genes. Several classes of molecular markers, particularly simple sequence repeats (SSR), frequently have been used in molecular mapping of *Pm* genes (Shah et al. 2018). Because of the limited number and sporadic distribution of SSR markers in the wheat genome, it is difficult to establish a high-resolution genetic linkage map for a target gene based only on the existing molecular markers. The expressed sequence sites (EST) that are anchored on the wheat chromosomes provide an additional source of markers for saturating the genetic linkage map of target genes (Lazo et al. 2004). Single nucleotide polymorphisms (SNP) are a newly developed class of markers that offer abundant numbers and high-throughput detection for tagging genes conferring disease resistance and other traits of agronomic importance (Wang et al. 2014).

Bulked segregant analysis (BSA) is a rapid and efficient method to identify molecular markers that are linked to the target genes (Michelmore et al. 1991). Next-generation sequencing offers a

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high-throughput method and sources of genomic sequences for large scale development of molecular markers. The combination of BSA and RNA-seq technology, which is referred to as bulked segregant analysis-RNA-Seq (BSR-Seq), can effectively develop molecular markers for localizing target genes within a segregating population (Trick et al. 2012). This approach has been used to quickly detect genes that confer resistance to wheat powdery mildew and stripe rust (caused by *Puccinia striiformis* f. sp. *tritici* Eriks.) (Hu et al. 2019; Wang et al. 2018; Wu et al. 2018a, b; Zhang et al. 2019).

Wheat landraces were widely grown before the initiation of modern hybridization breeding. They are rich in disease resistance genes that can be used in current breeding programs, although they have been replaced by improved cultivars with higher productivity. More than 20 *Pm* genes or alleles have been detected in some Chinese wheat landraces (Hu et al. 2019). New loci conferring resistance to powdery mildew were also identified in wheat landraces from other countries, e.g., *Pm63* in PI 628024 from Iran (Tan et al. 2019), *Pm223899* in PI 223899 (Li et al. 2018), and *Pm59* in PI 181356 from Afghanistan (Tan et al. 2018). Those findings have demonstrated the potential for identifying more *Pm* genes from wheat landraces.

A large number of wheat landraces have been collected from all over the country and conserved in the China National Gene Bank in Beijing (Liu et al. 2000). A project was initiated to deploy *Pm* genes from Chinese wheat landraces, which has led to the identification of *Pm47* in Hongyanglazi from Shanxi Province (Xiao et al. 2013) and *Pm61* in Xuxusanyuehuang from Sichuan Province (Sun et al. 2018). Here, the molecular characterization of a *Pm* gene in Qingxinmai, a winter wheat landrace originating from Manasi, Xinjiang, China, is reported. The objectives of the present study were to: (1) determine the mode of inheritance of powdery mildew resistance; and (2) develop SNP and SSR markers using BSR-Seq analysis for localizing the *Pm* gene in Qingxinmai.

Materials and Methods

Plant materials and *Bgt* isolates. Qingxinmai was crossed to the powdery mildew susceptible spring wheat line 041133 to develop F_1, F_2, and $F_{2:3}$ populations, which were used in the genetic and molecular analyses of its *Pm* gene. The powdery mildew-susceptible winter wheat cultivar Zhongzuo 9504 was included as the control in the powdery mildew assessments. *Bgt* isolates were collected from wheat fields in Shandong, Hebei, Henan, Sichuan, Beijing, and Shanxi provinces of China. They were subjected to three rounds of single-pustule culture on Zhongzuo 9504 seedlings prior to inoculating wheat seedlings in the powdery mildew tests. DNA from wheat-*Thinopyrum ponticum* (Podp.) Barkworth and D. R. Dewey line CH7086 (Zhan et al. 2014) and the Iranian wheat landrace PI 628024 (Tan et al. 2018), which carry *Pm51* and *Pm63*, respectively, were included in the molecular analysis of the markers developed in the present study.

Powdery mildew resistance assessments. Qingxinmai and line 041133 were tested against different *Bgt* isolates. The F_1 (15 plants), F_2 (486 plants), and F_3 (376 families) populations were phenotyped against isolate *Bgt1*, collected from Yuncheng, Shandong Province, which was avirulent on Qingxinmai, but virulent on 041133. Fifteen plants from each $F_{2:3}$ family were tested, and this test was conducted twice. Wheat seeds were sown in plastic pots (6.5 × 6.5 × 6.5 cm in size). Seedlings at the two-leaf stage were dusted with freshly increased condiospores of the *Bgt* isolates separately and incubated in a dew plastic bag for 24 h. Inoculated plants were grown in a greenhouse set at 15 to 18°C with 60% relative humidity and 12 h light/12 h dark for symptom development. Two weeks after inoculation, infection type (IT) on the primary leaves was scored on a 0 to 4 scale (Liu et al. 1999). Plants that were categorized into the resistant group had IT 0 (free of any symptom), 0; (hypersensitive reaction), 1 (sparse aerial hypha and limited sporulation, with colony diameters <1 mm), or 2 (moderate hypha and sporulation, with colony diameters <1 mm), and those into the susceptible group had IT 3 (thick aerial hypha and abundant sporulation, with colony diameters >1 mm) or 4 (abundant sporulation, with more than 80% of the leaf area covered with aerial hypha).

BSR-Seq analysis. The phenotypically contrasting $F_{2:3}$ families against isolate *Bgt1* were used to construct the resistant and susceptible RNA pools for RNA-seq analysis. Fifty homozygous resistant and homozygous susceptible families each, represented by equal sized segments of the primary leaves from single plants of each family, were pooled for conducting bulked segregant analysis. RNA was extracted with the Illumina TruSeq RNA sample preparation kit (Illumina, Inc., San Diego, CA) and subjected to RNA-sequencing on the Illumina HiSeq 4000 platform at the Beijing Southern Genome Research Technology (Beijing, China). Quality control was applied for the generated raw data to filter out the adapter sequences and low-quality sequences using software Trimmomatic v0.36 (Bolger et al. 2014). Software STARv2.5.1b (Qureshi et al. 2018) was used to align the trimmed high-quality reads against the Chinese Spring reference genome sequence v1.0 (International Wheat Genome Sequencing Consortium 2018). The PCR optical duplicates and the mapped reads that spanned introns were removed. The unique and confident alignments were used to identify SNP variants using "HaplotypeCaller" module in the GATK v3.6 software (McKenna et al. 2010). The SNP variants with *P*-values of Fisher's Exact Test (FET) <1e–8 and allele frequency difference (AFD) >0.6 were regarded to be associated with the disease resistance and used as templates for developing SNP markers.

Development of SNP and SSR markers. The 3 kb flanking sequences of the candidate SNPs were used to blast against the Chinese Spring genome sequence assembly (https://urgi.versailles.inra.fr/blast). Sequences of the resulting homologous scaffolds were used as templates to develop SNP markers on the GSP website (https://probes.pw.usda.gov/GSP/) (Bolger et al. 2014). At least one variant site at the 3′ end was included in the primers and the expected sizes of the amplicons ranged from 300 to 800 bp. All the SSR loci in the region of the target gene generated by the BSR-Seq analysis were obtained from JBrowse in the Triticeae Multiomics Center (http://202.194.139.32), which were used to develop SSR markers with the Primerserver tools.

Polymorphism analysis of the SNP and SSR markers. Leaves of the $F_{2:3}$ families after phenotyping were sampled by extracting DNA using a CTAB method. The phenotypically contrasting DNA bulks included separate pools of equal quantities of DNA (50 $ng \cdot ml^{-1}$) from eight resistant or eight susceptible $F_{2:3}$ families and were used to identify the polymorphic markers. DNA amplification was performed in a Biometra T3000 Thermocycler (ABI, New York, NY). Each reaction volume (10 μl) comprised 5 μl PCR mixture (including *Taq* polymerase, dNTPs, and 10× PCR buffer with Mg^{2+}), 2 μl ddH_2O, 1 μl DNA, and 1 μl 10 μM each of the forward and reverse primers. The PCR profile included an initial denaturation for 3 min at 98°C; for 10 s at 98°C, annealing for 10 s at 54 to 62°C (depending on the specific primers), and 72°C for 25 s, 35 cycles; and extension for 10 min at 72°C. Polymorphisms of the SNP markers were determined by analyzing the sequences of amplicons from the two parents and the two DNA bulks. Polymorphisms of SSR markers were determined by comparing the amplification patterns on 2% agarose gel or 8% nondenaturing polyacrylamide gel (Acr: Bis = 19:1 or 39:1).

Linkage analysis, genetic linkage map construction, and statistical analysis. The polymorphic SNP and SSR primers identified, together with previously published molecular markers that were closely linked to the *Pm* resistance genes on chromosome arm 2BL, were used to genotype the $F_{2:3}$ mapping population to construct the genetic linkage map using software Mapdraw V2.1 (Liu and Meng 2003). The genetic distance between the polymorphic markers and the target gene was estimated using Mapmaker 3.0 software (Lincoln et al. 1993), using a logarithm of the odd ratio (LOD) of 3.0 and the maximum genetic distance allowed of 50 cM. The goodness of fit for the observed separation data from the expected separation ratio of the F_2 and $F_{2:3}$ populations was examined with the χ^2 test in the SAS statistical software package (version 8.01; SAS Institute Inc., Cary, NC).

Results

Reaction of Qingxinmai and line 041133 to *Bgt* isolates. Twenty-three *Bgt* isolates collected from Shandong, Hebei, Henan,

Sichuan, Beijing, and Shanxi provinces were separately inoculated on 2-leaf-stage wheat seedlings. Qinxinmai produced a resistant phenotype against 12 isolates (IT 0 to 2) and a susceptible phenotype to 11 isolates (IT 3 or 4). Line 041133 was as susceptible as the susceptible control Zhongzuo 9504 to all isolates (Table 1).

Inheritance of the powdery mildew resistance in Qingxinmai. When tested at the seedling stage, isolate *Bgt1* from Shandong Province clearly differentiated the reactions to powdery mildew between Qingxinmai (IT 1) and line 041133 (IT 4) (Fig. 1). Therefore, it was used to test the F_1, F_2, and $F_{2:3}$ populations to perform the genetic analysis of powdery mildew resistance in Qingxinmai. The infection type of F_1 plants did not differ from that of the susceptible parent 041133 (Fig. 1). The F_2 plants exhibited a segregation ratio of 1:3 for resistant and susceptible plants ($\chi^2_{1:3} = 0.0764$, $P = 0.7822$). The $F_{2:3}$ population consisting of 273 families showed a segregation of 76 (homozygous resistant):127 (heterozygous):70 (homozygous susceptible) ($\chi^2_{1:2:1} = 1.5861$, $P = 0.4525$) (Table 2). Results of the genetic analysis support the conclusion that a single recessive gene, tentatively designated *PmQ*, confers the resistance to isolate *Bgt1* in Qingxinmai.

BSR-Seq analysis of the RNA bulks with contrasting responses to isolate *Bgt1*. The RNA samples isolated from the powdery mildew resistant and susceptible $F_{2:3}$ families, designated Bulk-R and Bulk-S, respectively, were subjected to RNA-Seq analysis on an Illumina HiSeq 4000 platform, which generated 24,005,361 and 24,344,440 raw read pairs, respectively. After quality control, the Bulk-R was changed to 24,004,147 high-quality read pairs and 21,668,815 (90.27%) of them were mapped to the wheat reference genome assembly IWGSC RefSeq v1.0. There were 24,343,474 high-quality reads and 21,871,257 (89.84%) uniquely mapped reads for the Bulk-S sample. The distribution of SNPs on different wheat chromosomes and chromosome 2B is shown in Fig. 2A and Fig. 2B, respectively. Sixty-seven SNP variants ($P <$ 1e-10 and AFD > 0.6) were identified from those reads by the GATK software. Fifty-three of them were enriched in a 40 Mb genomic interval (710,063,176–750,260,469) on chromosome arm 2BL in the Chinese Spring reference genome, which were regarded as the candidate SNPs linked to *PmQ*.

Development of SNP markers linked to *PmQ*. Flanking sequences of the 53 candidate SNPs were blasted against the Chinese Spring reference genome, and 33 homologous scaffolds were identified. Twenty-eight SNP primer pairs were developed based on the 3 kb sequences of the homologous scaffolds that consisted of the candidate SNPs. Analysis of the amplicon sequences amplified by these SNP markers resulted in the detection of four polymorphic markers between the two parents, Qingxinmai and line 041133, and also between Bulk-R and Bulk-S. Linkage analysis indicated that *Xicsn32* was potentially mapped on the proximal side, and *Xicsn93*, *Xicsn136*, and *Xicsn171* on the distal side of the target gene (Table 3). *PmQ* was localized in a 20 Mb physical region (710,523,186–730,866,838) between SNP markers *Xicsn32* and *Xicsn93* on chromosome arm 2BL.

Development of SSR markers linked to *PmQ*. All the SSR loci in the 710–750 Mb sequences of chromosome arm 2BL were retrieved from the Triticeae Multi-Omics Center (http://202.194.139.32). From the physical positions of 710, 720, 730, 740, and 750 Mb in the reference genome sequence, 50, 111, 151, 85, and 88 pairs of SSR primer pairs, respectively, were developed, and 6, 13, 18, 9, and 13 of them were polymorphic between Qingxinmai and 041133. Six markers *Xicsq405* (Fig. 3A), *Xicsq347*, *Xicsq253*, *Xicsq453*, *Xicsq129*, and *Xicsq10* (Table 4) were further shown to be polymorphic between the two contrasting DNA bulks and proved to be linked to *PmQ* based on the analysis of the Qingxinmai × 041133 $F_{2:3}$ mapping population.

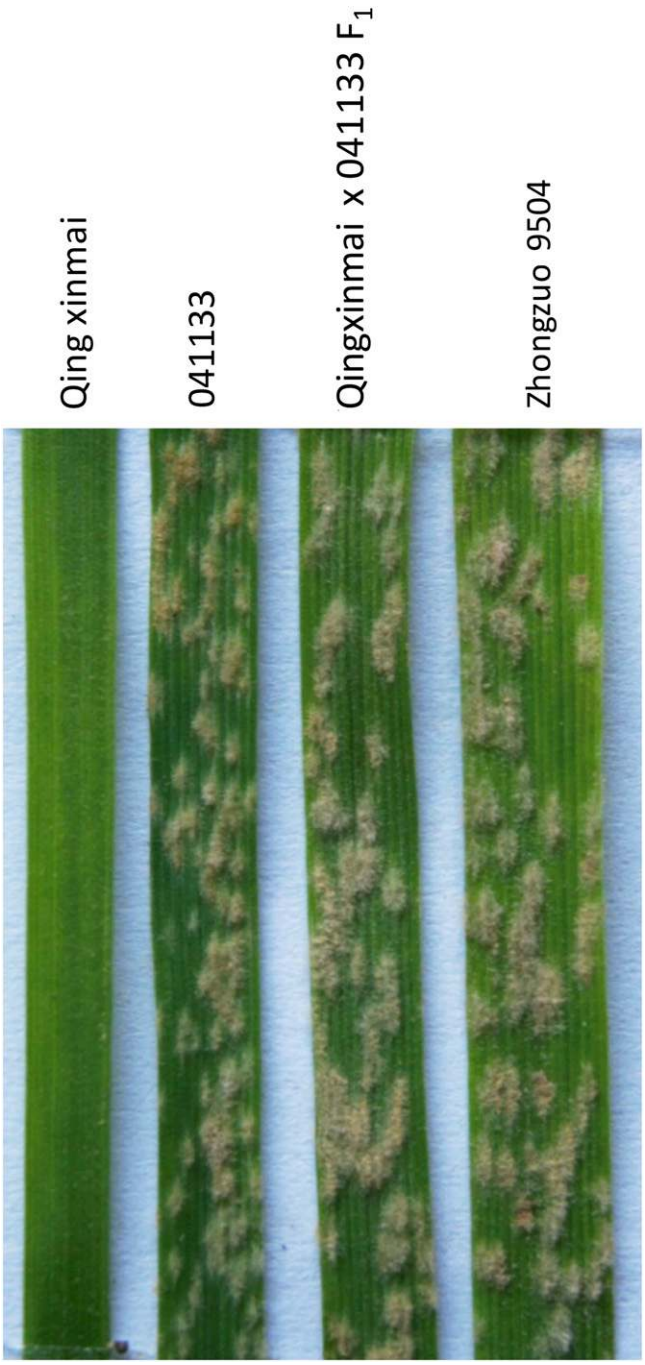

Fig. 1. The phenotypic reactions of resistant parent Qingxinmai, susceptible parent 041133, and their F_1 progeny to *Bgt* isolate *Bgt1*.

Table 1. Infection types of Qingxinmai, 041133, and the susceptible control Zhongzuo 9504 to 23 *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates from different provinces in China

	***Bgt* isolates**																						
Wheat entry	**1[y]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Qingxinmai	R[z]	S	R	S	S	R	R	S	S	S	R	S	S	R	S	R	S	R	R	R	S	R	R
041133	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Zhongzuo 9504	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[y] *Bgt* 1–2 from Shandong, *Bgt* 3–10 from Hebei, *Bgt* 11–12 from Henan, *Bgt* 13–14 from Sichuan, *Bgt* 15–19 from Beijing, and *Bgt* 20–23 from Shanxi.
[z] R: resistant, plants with IT 0–2; S: susceptible, plants with IT 3–4.

Polymorphic analysis of the molecular markers linked to the *Pm* genes previously assigned to chromosome arm 2BL. Polymorphisms of the 48 molecular markers closely linked to known genes on chromosome arm 2BL were determined using the parents and the DNA bulks from the $F_{2:3}$ mapping population of Qingxinmai × 041133 cross (Table 5). The three *Pm6*-linked markers showed no polymorphisms between the parents or the DNA bulks. Three of the six *Pm52*-linked markers were polymorphic between Qingxinmai and line 041133, but only *Xwmc441* and *Xgwm120* were polymorphic between the DNA bulks. Among the 14 *Pm64*-linked markers, seven were polymorphic between the parents. Only markers *WGGBGB47-2*, *WGGBH1099*, *WGGBH913* (Fig. 3B), and *WGGBH686* were also polymorphic between the DNA bulks. Analysis of the 15 markers linked to *Pm51* resulted in six polymorphic markers between the parents and two of them, *BQ246670* and *Xwmc332*, also were polymorphic between the DNA bulks. Five *Pm63* linkage markers were examined. Two markers *Xstars382* and *Xstars419* were polymorphic between the parents, but only *Xstars419* was polymorphic between the DNA bulks. Although three of the five molecular markers linked to *MlZec1* and *Pm33* were polymorphic between the two parents, they did not display any polymorphisms between the DNA bulks. Altogether, 11 polymorphic molecular markers between the DNA bulks were incorporated into the *PmQ* linkage map after genotyping the whole set of Qingxinmai × 041133 $F_{2:3}$ population. Finally, *PmQ* was placed in a 1.4 cM genetic interval between markers *Xicsq405* and *WGGBH913* (Fig. 4A), which corresponded to a 4.9 Mb physical region (710.1–715.0 Mb) of the Chinese Spring reference genome (Fig. 4B).

Positional comparison of *PmQ* with known *Pm* genes identified on chromosome arm 2BL. Based on their physical locations in the Chinese Spring reference genome, genes *Pm6*, *Pm52*, *Pm33*, *PmJM22*, *MlZec1*, and *MlAB10* were far from *PmQ*, indicating that *PmQ* was not located on the same loci as those genes. *Pm64*, derived from wild emmer (*T. turgidum* var. *dicoccoides* Koem. ex. Asch. &. Graebn. Thell., accession G-573-1) and located in a 14.96 Mb physical region at 695.4–710.3 Mb on the Chinese Spring chromosome arm 2BL, was positioned on the proximal side of *PmQ* at an estimated genetic distance of 2.3 cM. *Pm51*, potentially derived from a *Th. ponticum* introgression line and located in a 27.4 Mb corresponding to 706.5–734.3 Mb in the Chinese Spring genome sequence, was located on the distal side of *PmQ* at a genetic distance of 1.5 cM in the integrated map. The physical position of *Pm63*, which originated from the Iranian landrace PI 628024 and was located in a physical interval of 710.3–723.4 Mb on chromosome arm 2BL, overlapped with the *PmQ* (710.7–715.0 Mb) (Fig. 4B). However, only one of the five *Pm63*-linked markers, *Xstars419*, was polymorphic in the mapping population of Qingxinmai × 041133 (Table 5). The marker was 1.7 cM from *PmQ*, while it was 1.1 cM from *Pm63*.

The amplification profile of Qingxinmai (*PmQ*), PI 628024 (*Pm63*), and CH7086 (*Pm51*) was analyzed using the two close markers of *PmQ*. *WGGBH913* produced different banding patterns, but *Xicsq405* amplified similar sized bands from the three accessions (Fig. 5A and 5B). The sequences of the amplicons by *Xicsq405* from Qingxinmai, CH7086, and PI 628024 were identical (data not shown).

Discussion

Winter wheat landrace Qingxinmai was resistant against several *Bgt* isolates. Using the genetic populations developed from Qingxinmai × 041133 cross, a single recessive gene, *PmQ*, was detected in Qingxinmai, which confers seedling resistance to powdery mildew. Two steps were taken to localize this *Pm* gene. BSR-Seq analysis placed the target gene into a ~40 Mb physical interval (710.1–750.3 Mb) on chromosome arm 2BL. Then, the Chinese Spring reference genome sequence of this interval was used as a template to develop SNP and SSR markers for molecular mapping of the target gene. *PmQ* was thus mapped to a 1.4 cM genetic interval corresponding to 4.9 Mb genomic interval (710.1–715.0 Mb) in the terminal part of chromosome 2BL in the Chinese Spring reference genome.

Landraces had been widely grown throughout the wheat producing regions of China before modern wheat cultivars were released in the 1950s (He et al. 2001). Due to the limitations in their productivity, the majority of wheat landraces have been replaced by high-yielding improved cultivars. The landraces were collected and have been conserved in the gene banks as germplasm accessions (Liu

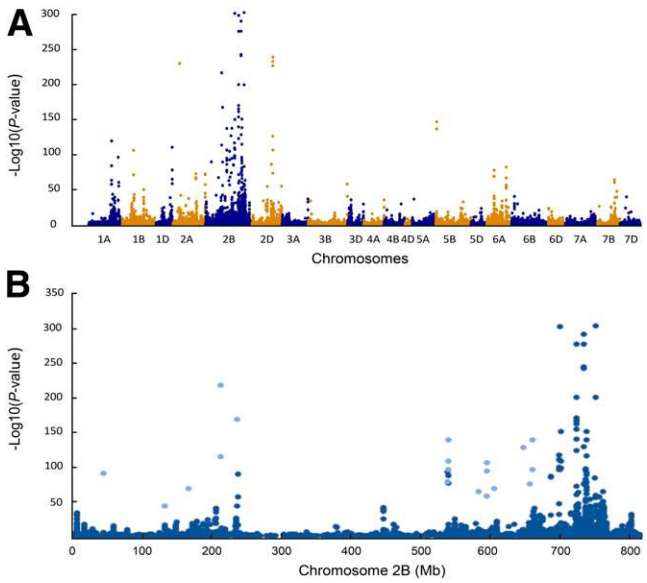

Fig. 2. Number of single nucleotide polymorphisms (SNP) distributed on different wheat chromosomes (**A**) and the SNP variants on chromosome 2B (**B**).

Table 2. Genetic analysis of resistance to isolate *Bgt1* of *Blumeria graminis* f. sp. *tritici* in F_1, F_2, and $F_{2:3}$ derived from a Qingxinmai × 041133 cross

Parents and cross[w]	Generation[w]	No. of plants/families	Observed ratio			Expected ratio	χ^2	*P*-value
			R[x]	Seg[y]	S[z]			
Qingxinmai	P_R	15	15		0			
041133	P_S	15	0		15			
$P_R \times P_S$	F_1	15	0		15			
	F_2	436	112		324	1:3	0.0764	0.7822
	$F_{2:3}$	273	76	127	70	1:2:1	1.5861	0.4525

[w] P_R: resistant parent Qingxinmai; P_S: susceptible parent 041133.
[x] R: homozygous resistant.
[y] Seg: segregating within an $F_{2:3}$ family.
[z] S: homozygous susceptible.

et al. 2000). Chinese wheat landraces have provided a number of genes against powdery mildew. Several alleles were identified in the *Pm5* locus on chromosome arm 7BL of Chinese landraces, such as *Pm5d* (Nematollahi et al. 2008), *Pm5e* (Huang et al. 2003), *PmH* (Zhou et al. 2005), *PmTm4* (Hu et al. 2008), *Mlmz* (Zhai et al. 2008), *Mlxbd* (Xue et al. 2009), *PmHY* (Fu et al. 2017), *PmBYYT* (Xu et al. 2018a), and *PmSGD* (Xu et al. 2018b). *Pm24a*, *Pm24b*, and *MlHLT* were allelic on chromosome arm 1DS (Huang et al. 2000; Wang et al. 2015; Xue et al. 2012). Genes *Pm47* and *Pm61* were localized on 7BS and 4AL, respectively (Sun et al. 2018; Xiao et al. 2013). *Pm45* (Ma et al. 2011), *PmX* (Fu et al. 2013), and *Pm2c* (Xu et al. 2015) were detected on chromosome arms 2AL, 5DS, and 6DS, respectively. However, *PmQ* on chromosome arm 2BL is different from the *Pm* genes that have been identified in other Chinese wheat landraces.

Besides *PmQ*, nine *Pm* genes have been assigned on chromosome arm 2BL. *Pm6* resides on a *T. timophevii* Zhuk. chromosome segment, which was the first *Pm* gene that was assigned to 2BL (Jørgensen and Jensen 1973; Qin et al. 2011). *Pm52* was detected in a commercial wheat cultivar Liangxing 99 (Wu et al. 2019; Zhao et al. 2013). *PmJM22* was originally located on the distal part of chromosome arm 2BL (Yin et al. 2009). It was confirmed to be identical to *Pm52* in a later study (Qu et al. 2019). *Pm64* was identified from a wheat-wild emmer introgression line WE35 (Zhang et al. 2019). Three genes, *Pm33*, *MlZec1*, and *MlAB10*, were located on the distal side of *PmQ*. *Pm33* originated from *T. persicum* Vav. (Zhu et al. 2005). Both *MlZec1* and *MlAB10* were derived from *T. dicoccoides* (Maxwell et al. 2010; Mohler et al. 2005). All these genes are easily distinguished from *PmQ* based on their chromosome locations and/or origins.

Table 3. Newly developed single nucleotide polymorphism (SNP) markers linked to *PmQ*[z]

$F_{2:3}$ family	*Sicsn32*	Phenotype	*Sicsn93*	*Sicsn136*	*Sicsn171*
282	A	A	A	A	A
127	**H**	B	B	B	B
216	**H**	B	B	B	B
146	**H**	B	B	B	B
235	**H**	B	B	B	B
201	**H**	B	B	B	B
142	H	H	**A**	**A**	**A**
50	H	H	H	**A**	**A**
207	H	H	H	**A**	**A**
11	B	B	B	**H**	**H**
195	B	B	B	B	**H**
246	H	H	H	H	H

[z] A: homozygous resistant; H: heterozygous; B: homozygous susceptible. The recombinant $F_{2:3}$ families are shown in bold.

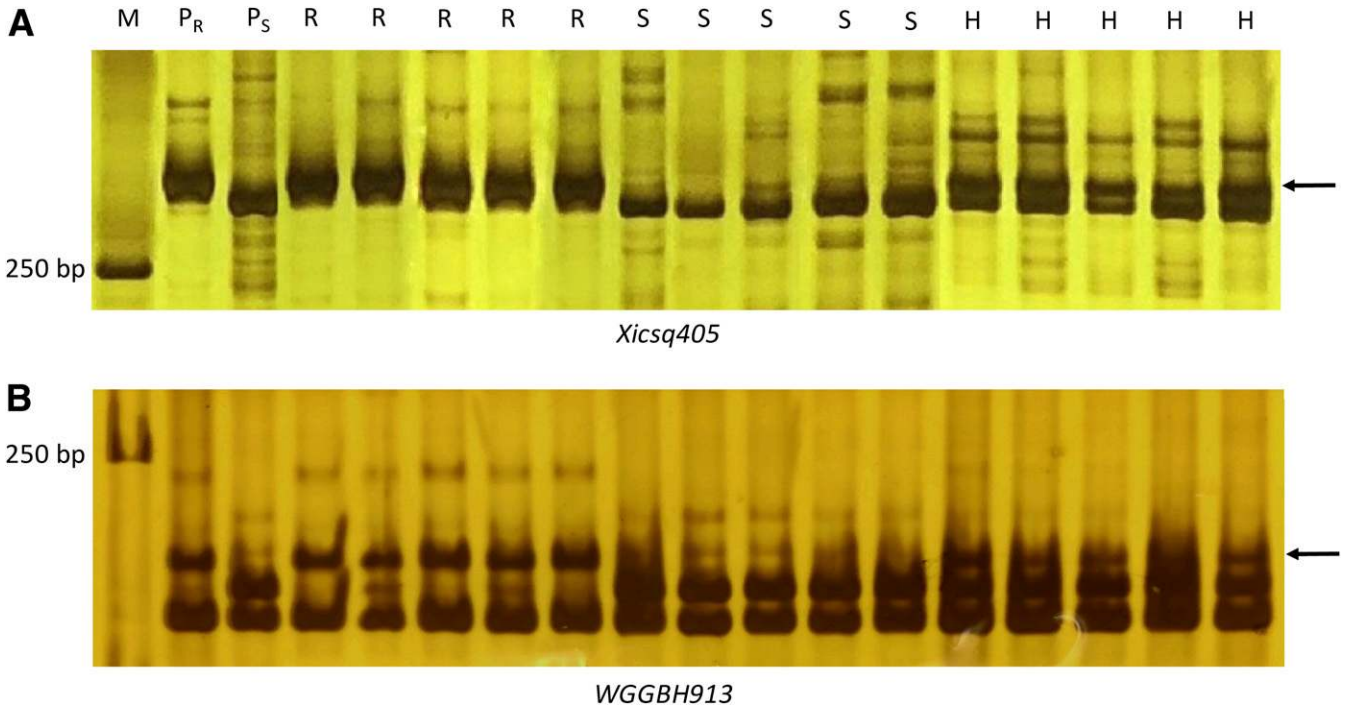

Fig. 3. Amplification patterns of *PmQ*-flanking SSR markers *Xicsq405* (**A**) and *WGGBH913* (**B**) developed from the Chinese Spring genomic sequence in the parents and selected $F_{2:3}$ families of Qingxinmai × 041133 cross in 8% silver-stained non-denaturing polyacrylamide gels. M: DL2000 DNA ladder (Tiangen Biotech Co., Beijing, China); lane 1: Qingxinmai; lane 2: 041133; lanes 3–7: homozygous resistant $F_{2:3}$ families; lanes 8–12: homozygous susceptible $F_{2:3}$ families; and lanes 13–17: heterozygous $F_{2:3}$ families. Arrows indicate the polymorphic bands that are specific for *PmQ*.

Table 4. Newly developed simple sequence repeat (SSR) markers linked to *PmQ*

Marker	Forward primer	Reverse primer	Physical location (Mb)	Product size (bp)
Xicsq405	GAGGGAGTGTTGTGGATCCAT	ACATCTTGCTGGCATAGTTGT	710.7	267
Xicsq347	CTTTGGTGAGCGAACGGAGG	AGCTCGGTCCAAAGATCATCT	720.9	420
Xicsq253	CGGAGACATAAACAAGGCGC	CAACCAACCAACCAACGCTG	730.6	382
Xicsq453	TGTTGGATCCTGCTTATACCCA	CGCCCTAAGTCTGCTGTATTTG	730.8	408
Xicsq129	TCCTATGGCGCCCTTGATTAC	ACGTGTACTGTGTGTGTGTGT	740.3	199
Xicsq10	CGAAATTTGATAACTGCTTGGGT	TGTACATAGAAAGTCGGTGTCCT	750.0	440

The physical positions of *Pm51* and *Pm63* were close to *PmQ* (Fig. 4). *Pm51* was believed to be located on a *Th. ponticum* chromosome segment that was introgressed into wheat chromosome arm 2BL (Zhan et al. 2014). *Pm63* was detected in an Iranian common wheat landrace PI 628024 (Tan et al. 2019). The genomic intervals of these genes were 29.6 Mb (709.8–739.4 Mb) for *Pm51* and 13.1 Mb (710.3–723.4 Mb) for *Pm63*, respectively, which overlap the physical interval of *PmQ* (710.7–715.0 Mb) in the Chinese Spring reference genome. *PmQ* differs from *Pm51* in its source of origin. The banding patterns amplified by one of the flanking markers of *PmQ*, *WGGBH913*, were different from the wheat accessions carrying *PmQ*, *Pm63*, and *Pm51*; and the other flanking marker of *PmQ*, *Xicsq405*, produced bands identical in size and the nucleotide sequences. This indicates that the genomic regions of *PmQ*, *Pm63*, and *Pm51* may differ from each other. The possibility that *PmQ* is allelic with *Pm63* and *Pm51* cannot be excluded. In the 4.9 Mb genomic region of *PmQ*, there were six genes annotated as disease resistance-related genes (data not shown). Considering the large genomic intervals of *Pm51* and *Pm63*, there is a possibility that *PmQ* may not

Table 5. Polymorphic and linkage analyses of the markers linked to the powdery mildew resistance genes located on chromosome arm 2BL using the mapping populations derived from the cross of Qingxinmai × 041133

Marker	Physical location (Mb)	Product size (bp)	Resistance gene	Polymorphism[z]		Linkage to *PmQ*	Reference
				Parents	$F_{2:3}$ Bulks		
NAU/STSBCD135-2	738.6	230	*Pm6* and *Pm63*	–	–	–	Qin et al. 2011; Tan et al. 2018
CINAU123	631.7	274	*Pm6*	–	–	–	Qin et al. 2011
CINAU124	657.8	493	*Pm6*	–	–	–	Qin et al. 2011
Xwmc441	598.1	-	*Pm52* and *Pm63*	+	+	+	Zhao et al. 2013; Tan et al. 2018
Xgwm120	615.8	-	*Pm52* and *Pm63*	+	+	+	Zhao et al. 2013; Tan et al. 2018
Xwmc175	670.6	-	*Pm52* and *Pm63*	–	–	–	Zhao et al. 2013; Tan et al. 2018
Xgwm47	685.8	128	*Pm52*, *PmJM22*, *Pm51* and *MIzec1*	+	–	–	Mohler et al. 2005; Zhao et al. 2013; Yin et al. 2009; Zhan et al. 2014
Xicscl1795	585.0	-	*Pm52*	–	–	–	Wu et al. 2018b
Xicscl1326	581.0	-	*Pm52*	–	–	–	Wu et al. 2018b
WGGBK458	698.2	226	*Pm64*	–	–	–	Zhang et al. 2019
WGGBK552	703.9	524	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH1364	695.4	160	*Pm64*	+	–	–	Zhang et al. 2019
WGGBGB47-2	696.4	213	*Pm64*	+	+	+	Zhang et al. 2019
WGGBH218	699.2	166	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH1099	705.5	482	*Pm64*	+	+	+	Zhang et al. 2019
WGGBH913	715.0	157	*Pm64*	+	+	+	Zhang et al. 2019
WGGBK462	704.0	276	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH252	732.3	272	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH1212	656.6	241	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH612-5	710.3	369	*Pm64*	+	–	–	Zhang et al. 2019
WGGBH134	670.6	168	*Pm64*	–	–	–	Zhang et al. 2019
WGGBH686	680.0	154	*Pm64*	+	+	+	Zhang et al. 2019
WGGBH1260	695.1	220	*Pm64*	+	–	–	Zhang et al. 2019
Xstars419	710.3	376	*Pm63*	+	+	+	Tan et al. 2018
Xstars382	-	-	*Pm63*	+	–	–	Tan et al. 2018
Xgpw3090	612.9	216	*Pm63*	–	–	–	Tan et al. 2018
Xcinau130	696.7	645	*Pm63*	–	–	–	Tan et al. 2018
Xcinau139	753.1	500	*Pm63*	–	–	–	Tan et al. 2018
BQ246670	709.8	943	*Pm51*	+	+	+	Zhan et al. 2014
Xwmc332	739.4	208	*Pm51* and *Pm63*	+	+	+	Zhan et al. 2014; Tan et al. 2018
NRM31	727.7		*Pm51*	+	+	+	Zhan et al. 2014
NRM79	794.6		*Pm51*	+	+	+	Zhan et al. 2014
BE444894	765.3	893	*Pm51*	–	–	–	Zhan et al. 2014
BE500840	768.5	290	*Pm51* and *PmJM22*	+	–	–	Yin et al. 2009; Zhan et al. 2014
BE405017	767.1	402	*Pm51*	–	–	–	Zhan et al. 2014
Cos66	747.2	399	*Pm51*	–	–	–	Zhan et al. 2014
BCD135-1	723.4	234	*Pm51*	+	–	–	Zhan et al. 2014
Cos55	715.0	571	*Pm51*	+	–	–	Zhan et al. 2014
Cos65	677.3	429	*Pm51*	+	–	–	Zhan et al. 2014
BI479701	741.0	452	*Pm51*	–	–	–	Zhan et al. 2014
Xwmc817	789.4	121	*Pm51* and *MlAB10*	–	–	–	Zhan et al. 2014; Maxwell et al. 2010
P79	715.0	646	*Pm51*	–	–	–	Zhan et al. 2014
Xbarc159	793.0	235	*Pm51*	–	–	–	Zhan et al. 2014
Xwmc356	796.7	466	*PmJM22* and *MlZec1*	–	–	–	Yin et al. 2009
Xwmc445	780.0	229	*MlZec1* and *MlAB10*	–	–	–	Mohler et al. 2005; Maxwell et al. 2010
Xwmc317	784.3	529	*Pm33*, *PmJM22* and *MlAB10*	+	–	–	Zhu et al. 2005; Yin et al. 2009, Maxwell et al. 2010
Xgwm526	774.1	154	*Pm33* and *MlAB10*	+	–	–	Zhu et al. 2005; Maxwell et al. 2010
Xwmc149	779.1	232	*PmJM22* and, *MlAB10*	–	–	–	Yin et al. 2009; Maxwell et al. 2010

[z] +, polymorphic or linked; –, nonpolymorphic or unlinked. Parent, polymorphic between the parents Qingxinmai and 041133; $F_{2:3}$ population, polymorphic between the resistant and susceptible $F_{2:3}$ population.

be identical to them. It will be necessary to compare the sequences of the genes in the *Pm51* and *Pm63* genetic stocks to clarify their differences. An ongoing project is underway to finely map *PmQ* using a larger mapping population of Qingxinmai × 041133.

Prior to the release of the wheat genome sequence, isolation of a gene from the supersized (~17 Gb) and complex genome of hexaploid wheat was difficult (Keller et al. 2005). Besides the traditional method of map-based cloning, several strategies have been proposed for rapidly cloning disease resistance genes from common wheat (Bettgenhaeuser and Krattinger 2018), which include mutant map (MutMap) (Abe et al. 2012), mutant resistance gene enrichment sequencing (MutRenSeq) (Steuernagel et al. 2016), mutant chromosome sequencing (MutChromSeq) (Sánchez-Martín et al. 2016), targeted chromosome-based cloning via long-range assembly (TACCA) (Thind et al. 2017), and association genetics with R gene enrichment sequencing (AgRenSeq) (Arora et al. 2018). These technologies incorporate ethylmethanesulfonate (EMS) mutation, chromosome sorting, chromosome sequencing, and target gene enrichment. Gene *Pm2* was isolated using the MutChromSeq strategy (Sánchez-Martín et al. 2016). These newly emerged technologies will facilitate the isolation of the disease resistance genes on chromosome arm 2BL, and the eventual discrimination between *PmQ* and other *Pm* genes on this chromosome arm.

Qingxinmai is resistant to powdery mildew and produces more tillers that are needed for the improvements in grain yield. However, the combination of tall plant architecture with the weak culm indicates that Qingxinmai needs to be improved by crossing with semidwarf high yielding modern cultivars. Gene *PmQ* also should be stacked with other *Pm* genes to broaden spectrum of resistance and to increase its durability. Markers *Xicsq405* and *WGGBH913* could be used in molecular marker-assisted selection for the transfer of *PmQ*.

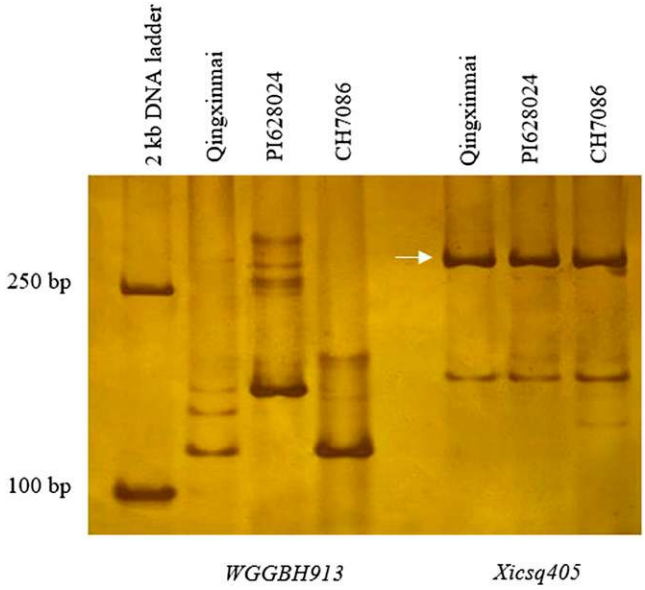

Fig. 5. Amplification profiles of *PmQ*-linked markers *WGGBH913* and *Xicsq405* in Qingxinmai (*PmQ*), PI628024 (*Pm63*), and CH7086 (*Pm51*). M: DL2000 DNA ladder (Tiangen Biotech Co., Beijing, China).

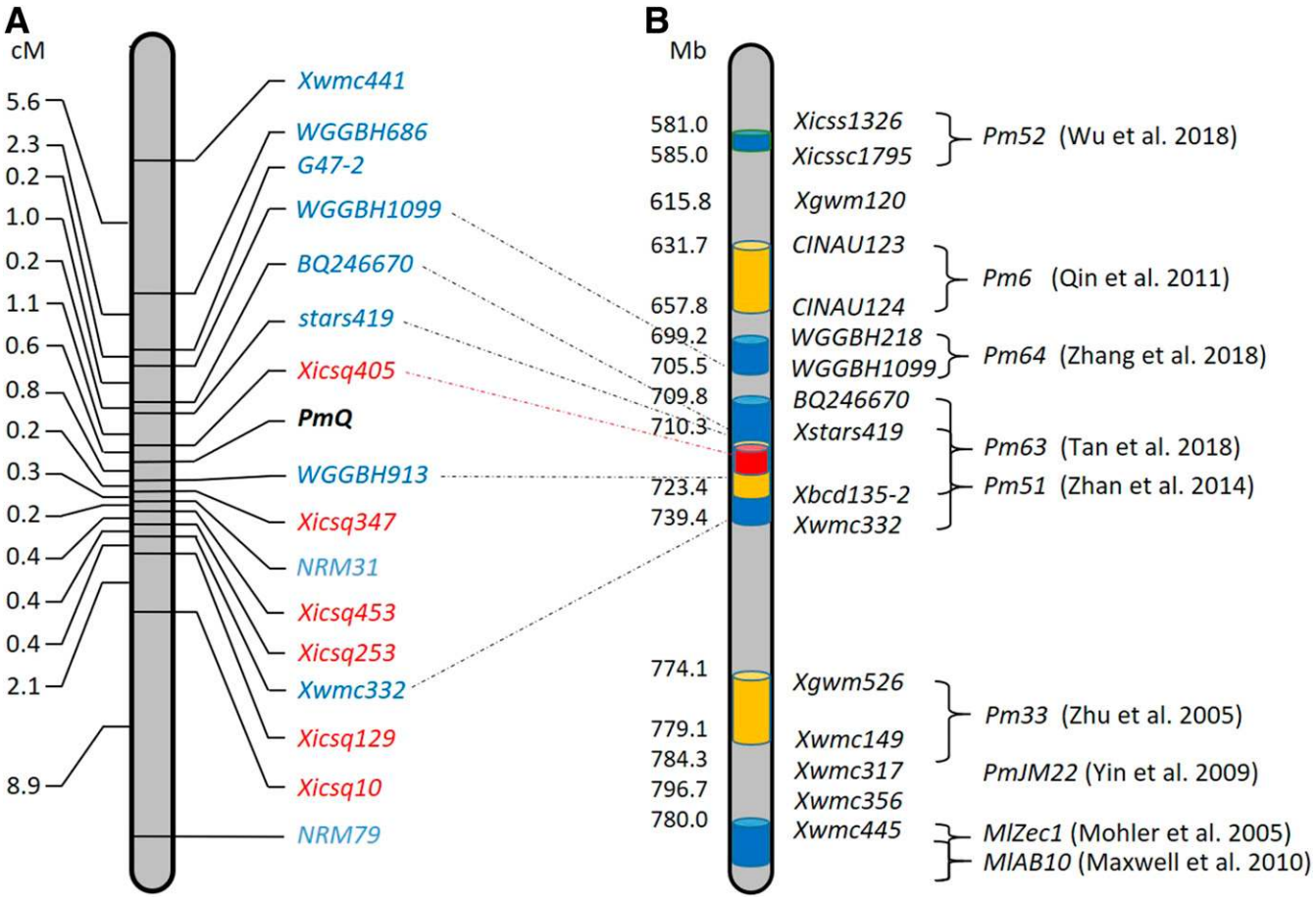

Fig. 4. A genetic linkage map of *PmQ* (**A**) and the physical locations of known genes on chromosome arm 2BL and the relative positions of the linked markers in the Chinese Spring reference genome (**B**). The newly developed SSR markers are indicated by red fonts.

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