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Development of KASP and SSR Markers for *PmQ*, a Recessive Gene Conferring Powdery Mildew Resistance in Wheat Landrace Qingxinmai

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Abstract: A recessive gene *PmQ* conferring powdery mildew resistance was previously localized on the long arm of chromosome 2B in winter wheat landrace Qingxinmai. Breeder-friendly molecular markers are necessary for introgressing this gene into adapted wheat backgrounds for developing disease-resistant wheat cultivars. Three Kompetitive allele-specific PCR (KASP) markers were developed and validated based on SNP variants detected by Bulked segregant analysis-RNA-Seq (BSR-Seq) analysis using a recombinant inbred population derived from cross Qingxinmai × 041133. Two polymorphic SSR markers were also developed from the motifs in the Chinese Spring reference genome sequences of the target genomic region. Those markers were incorporated into a more saturated genetic linkage map for *PmQ*. The two flanking markers, *Xicsq405* and *Xicsk18*, are 1.1 and 0.9 cM from *PmQ*, respectively. The KASP marker *Xicsk19* produced unique amplification pattern in 158 out of 160 wheat cultivars or breeding lines. This marker with the gene-linked SSR markers *Xicsqc*, *Xicsqd* and *Xicsq405* provides an efficient means in molecular marker-assisted selection for *PmQ* in wheat breeding. The corresponding genomic region of *PmQ* in the Chinese Spring reference genome has a conserved synteny with the genomes of sequenced wheat cultivars and *Triticum durum*, *T. diccocoides*, *T. uratu*, and barley. The annotation of the two genes, *TraesCS2B01G517400.1* and *TraesCS2B01G517700.1*, associated with plant defense against pathogens placed a start for cloning *PmQ*.

Keywords: *Triticum aestivum*; *Blumeria graminis* f. sp. *tritici*; KASP; SSR

Citation: Gebremedhin, H.G.; Li, Y.; Hu, J.; Qiu, D.; Wu, Q.; Zhang, H.; Yang, L.; Zhou, Y.; Zhou, Y.; Liu, Z.; et al. Development of KASP and SSR Markers for *PmQ*, a Recessive Gene Conferring Powdery Mildew Resistance in Wheat Landrace Qingxinmai. *Agriculture* **2022**, *12*, 1344. https://doi.org/10.3390/agriculture12091344

Academic Editor: Rodomiro Ortiz

Received: 8 August 2022
Accepted: 20 August 2022
Published: 31 August 2022

1. Introduction

Humans get ca. 20% of calories from wheat (*Triticum aestivum* L.), which drives the wide spread of this cereal crop in a large area of arable lands throughout the world [1]. However, there are many pathogens that can infect wheat plants and limit the grain production [2–4]. The obligate biotrophic fungus *Blumeria graminis* (DC.) Speer f. sp. *tritici* (*Bgt*) causing wheat powdery mildew disease is almost everywhere in wheat production [5,6]. White pustules appear on infected wheat plant surface, most commonly seen on leaves [7]. Severe and early infection of the pathogen can kill developing seedlings or newly emerging tillers [8]. Grain yield loss due to powdery mildew can be up to 40% and even worse when wheat is infected early [9]. Wheat plants suffering from powdery mildew produce seeds with poor quality [10]. Changes in grain starch and protein content caused by powdery mildew infection have been reported in a proteomic analysis [11].

Wheat powdery mildew has been one of the nationwide diseases that jeopardize economic benefits of wheat in China since the 1970s [12]. High plant density, excessive use of nitrogen fertilizers, growth of susceptible cultivars, and improvement of irrigation conditions exacerbate the prevalence of powdery mildew. Current occurrence of this disease covers 6 to 8 million hectares of both winter and spring wheats. Management of powdery mildew is a routine practice in wheat fields severe occurrence of the disease. Although chemical treatment is the mostly used method for the disease control, resistant cultivars are preferred by farmers and become one of the breeding objectives that breeders pursue.

Breeding for disease resistance using powdery mildew (*Pm*) resistance genes can reduce powdery mildew severity. *Pm* genes can originate from common wheat, or its close and distant relatives. Moreover, wheat cultivars that lost their disease resistance through the co-evolutionary interaction due to cultivation of cultivars with certain resistance genes in a large-scale and the emergence of virulent pathogen pathotypes can also gain resistance genes through gene transfer [5,13]. Marker-assisted breeding on the basis of gene-linked DNA markers matching the phenotype of interest is routinely used in the selection of desired characteristics. In view of this technology, PCR-based markers, e.g., SSR and sequence-tagged site (STS) markers, are frequently used in tagging *Pm* genes in early studies [14]. The genome sequences of common wheat and its relative species provide an opportunity to detect numerous single nucleotide polymorphism (SNP) variations between cultivars. They can be converted into Kompetitive allele-specific PCR (KASP) markers and offer a high-throughput option for molecular mapping and detection of *Pm* genes, as well as other genes for agronomic traits of wheat [15–17].

Wheat landraces are historically grown but are no longer used in agriculture. Although landraces have been replaced by more productive cultivars, they are conserved because of their valuable genes, including those for disease resistance, for modern wheat improvement [18]. Wheat landraces have provided several designated *Pm* genes or alleles on loci such as *Pm3* [19], *Pm5* [20], *Pm24* [21], *Pm45* [22], *Pm47* [23], *Pm59* [24], *Pm61* [25], and *Pm63* [26].

Qingxinmai is a winter wheat landrace collected from Xinjiang, China. It carries gene *PmQ* conferring resistance to 12 *Bgt* isolates tested [27]. There is a need to transfer this *Pm* gene into more adapted genotypes to promote its application in wheat breeding. Because *PmQ* is a recessive gene that needs additional generations for phenotypic selection, a high-throughput molecular marker is required to facilitate its efficient transfer. Therefore, this study was initiated to develop breeder-friendly KASP and SSR markers specific for the target gene.

2. Materials and Methods

2.1. Plant Materials

A mapping population consisting of 176 $F_{2:8}$ recombinant inbred lines (RILs) was generated by crossing Qingxinmai with a powdery mildew susceptible wheat line 041133 using single seed decent (SSD) method. A collection of 160 modern wheat cultivars or advanced breeding lines (Supplementary Table S1) were used to verify the newly developed KASP and SSR markers. Zhongzuo 9504 was included as the susceptible control in the study.

2.2. Assessment of Responses to Powdery Mildew

Wheat seedlings at the two-leaf-stage were inoculated by dusting conidiospores of isolate *BgtA44*, incubated in a dew and dark plastic bag for 24 h, and grown in a greenhouse set at 18–20 °C, 60–90% relative humidity, and a photoperiod of 12 h light/12 h dark. Infection type (IT) of the primary leaves was scored on a 0–4 scale 10–15 d after inoculation [27]. Lines were categorized into either resistant or susceptible groups when plants had ITs 0–2 or 3–4, respectively. The phenotypic tests of powdery mildew were conducted twice in separate tests in November 2021 and March 2022, respectively. A Chi-squared test (χ^2) was used to examine the goodness of fit for the observed phenotypes and the expected

segregation ratio of the RILs using the SAS statistical package (version 8.01; SAS Institute Inc., Cary, NC, USA).

2.3. Development of KASP and SSR Primers

RNA sequencing data of the phenotypically contrasting wheat plants used for developing KASP markers were reported previously [27]. In brief, the RNA samples were constructed by separately pooling 50 homozygous resistant and susceptible $F_{2:3}$ families and sequenced on the Illumina HiSeq 4000 platform (Illumina, Inc., San Diego, CA, USA). High-quality reads were aligned against the Chinese Spring (CS) reference genome RefSeq v1.0. [28]. SNP variants potentially associated with *PmQ* were selected for developing SNP and KASP markers. Sequences (200 bp in length) flanking the SNP variants were used to design KASP primers with PolyMarker [29].

The genomic sequences in the physical interval (710 to 715 Mb) between markers *Xicsq405* and *WGGBH913* where locus *PmQ* resides on the chromosome arm 2BL were extracted from the CS RefSeq v.1.0 reference sequence through JBrowse in the Triticeae Multi-Omics Center (http://202.194.139.32, accessed on 15 May 2020) [30]. SSR primer pairs were designed on the web-based software Primerserver using the SSR motifs contained in these sequences as templates.

2.4. Identification Polymorphic KASP and SSR Markers

DNA was isolated from leaves using the cetyl trimethylammonium bromide (CTAB) method [31]. Equal amounts of DNA samples from 10 resistant and susceptible RILs each were separately mixed to construct the resistant and susceptible DNA bulks. The KASP and SSR markers that were polymorphic between the two parents as well as between the contrasting DNA bulks were regarded as the *PmQ*-linked markers.

KASP markers: Each reaction mixture (10 µL) consisting of 4 µL genomic DNA (50 ng/µL), 5 µL 2× KASP master mix, 0.7 µL primer mix (12 mM of each allele-specific primer and 30 mM of the common primer) and 0.3 µL ddH_2O was amplified in the ABI 7500 device (Applied Biosystems, Foster City, CA, USA). The reaction mixtures were initially denatured at 94 °C for 15 min prior to 35 cycles of 94 °C for 20 s and 60 °C for 1 min. Fluorescence signals in green (521 nm) and pink (556 nm) colors were read at 25 °C for 2 min in a FLUOstar Omega microplate reader (BMG Labtech, Durham, NC, USA), and were transformed into FAM homozygote, HEX homozygote, and FAM/HEX heterozygote genotypes with Klustering Caller (http://www.lgcgroup.com/, 7 August 2022).

SSR markers: The reaction mixture (10 µL) was prepared by mixing 5 µL PCR mixture (consisting of Taq polymerase, dNTPs, and 10× PCR buffer with Mg^{2+}), 1 µL DNA 50 ng/µL, 2 µL (10 mM) of the primer pairs, and 2 µL ddH_2O. Amplification of DNA was performed in a Biometra T300 Thermocyler (ABI, New York, NY, USA). The amplification was initiated by denaturing the reaction mixture for 3 min at 98 °C. Thirty-five cycles, i.e., denaturing (98 °C, 10 s), annealing (54–62 °C, 10 s), and extension (72 °C, 25 s), were performed. The reaction was terminated after an extension for 10 min at 72 °C. The banding patterns of amplicons were examined by electrophoresis on nondenaturing polyacrylamide gels (8%, Acr: Bis = 19:1 or 39:1) after silver staining.

2.5. Genetic Linkage Map Construction and Statistical Analysis

The polymorphic SSR and KASP markers identified were used to genotype the entire set of RIL population of cross Qingxinmai × 041133 for constructing a genetic linkage map for *PmQ* using MapdrawV2.1 [32]. Genetic distances between the markers and *PmQ* were determined by the Kosambi function [33]. The logarithm of the odds (LOD) ratio and the maximum genetic distance allowed were set to be 3.0 and 50 cM, respectively, when establishing the linkage relationship between markers and *PmQ* with Mapmaker/Exp V3.0b [34].

2.6. Analysis of Synteny for the Target Genomic Region

Gene annotation information was obtained on the JBrowse in the Triticeae Multi-Omics Center (http://202.194.139.32) [30] through the physical location of gene mapping. Orthologous genes were analyzed with the aid of databases for the genomes of wild emmer (*T. turgidum* ssp. *dicoccoides* (Körnicke ex Asch. & Graebn.) Thell.) accession Zavitan [35], durum wheat (*T. turgidum* subsp. *durum* (Desf.) Husnot) accession Svevo [36], *T. uratu* Thumanjan ex Gandilyan accession G1812 [37], and barley (*Hordeum vulgare* L.) cultivar Morex [38], as well as the 10+ wheat genome sequences [39].

3. Results and Discussion

Powdery mildew resistant Qingxinmai was used as the maternal parent to cross with a highly susceptible wheat line 041133 for mapping *PmQ* on the chromosome arm 2BL. *PmQ* was characterized as a recessive gene based on the genetic analysis with the populations of F_1, F_2, and $F_{2:3}$ generations of this cross [27]. This cross was advanced to an $F_{2:8}$ RIL population by SSD method. Isolate *BgtA44*, collected from Shanxi province, produced distinct infection types on Qingxinmai (IT 1) and line 041133 (IT 3+) (Figure 1a). Therefore, this isolate was used to assess responses of the entire RIL population from cross Qingxinmai × 041133. The population produced 85 homozygous resistant lines (ITs 0–2) and 91 homozygous susceptible lines (ITs 3+), fitting a 1:1 segregation ratio ($\chi^2 = 0.1420$ and $p = 0.7063$) (Figure 2). This result further confirmed the monogenic inheritance of resistance to powdery mildew in Qingxinmai.

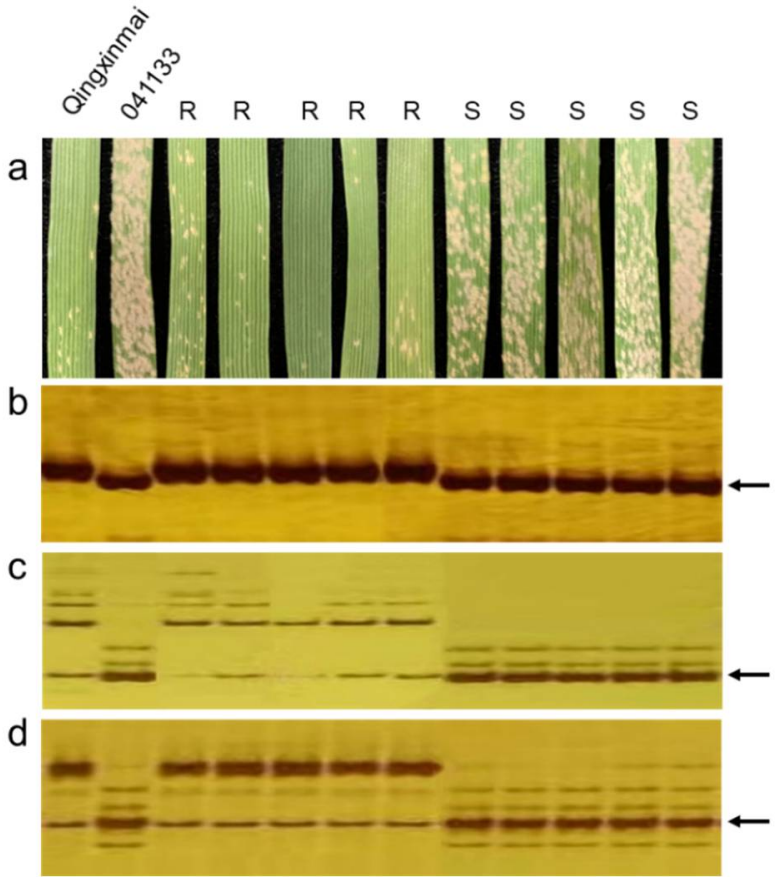

Figure 1. Powdery mildew disease responses infected with isolate *BgtA44* (**a**), and amplification patterns of SSR markers *Xicsq405* (**b**), *Xicsqc* (**c**), and *Xicsqd* (**d**) for Qingxinmai, 041133, and selected lines from the RIL population of cross Qingxinmai × 041133. R: lines resistant to powdery mildew; S: lines susceptible to powdery mildew. Arrows indicate the polymorphic bands amplified by the SSR markers.

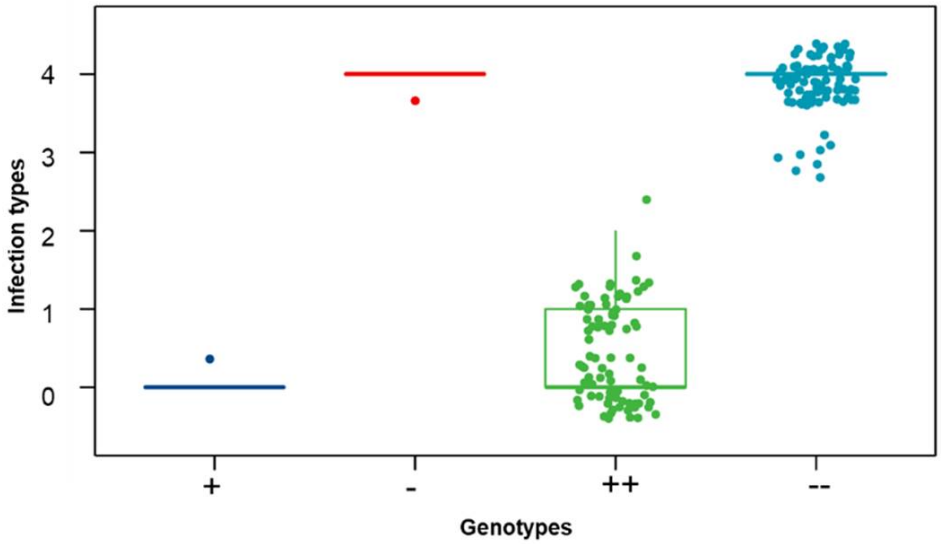

Figure 2. Box plots of infection types for the RIL population of cross Qingxinmai × 041133 when challenged with isolate *BgtA44*. Lines were grouped based on the amplification patterns of the *PmQ*-flanking markers *Xicsq405* and *Xicsk18*. +: Qingxinmai; −: 041133; ++: lines with the same genotype as Qingxinmai (n = 85); −−: lines with the same genotype as 041133 (n = 91).

KASP makers are frequently used to tag many genes or QTL conferring disease resistance and other agronomic traits of wheat. Bulked segregant analysis-RNA-Seq (BSR-Seq) analysis is powerful to detect SNPs associated with the traits of interest [40]. We have identified 53 candidate SNPs potentially associated with *PmQ* in a 40 Mb (710.1–750.3 Mb) genomic region on the chromosome arm 2BL [27]. Sequences flanking these candidate SNPs were used to search against the CS RefSeq v.1.0. A total of 34 KASP markers were developed based on the 33 corresponding homologous scaffolds identified. Three of them, *Xicsk18* (G/C), *Xicsk19* (C/T), and *Xicsk29* (T/C) (Table 1), were polymorphic between the resistant and the susceptible DNA bulks (Figure 3a–c), indicating their potential linkage to the target gene *PmQ*.

Table 1. Newly designed KASP and SSR markers on the chromosome arm 2B.

Marker	Forward Primer-1	Forward Primer-2	Reverse Primer	Marker Type	R/S Allele and SSR Motif	Product Size (bp)	Physical Position (Mb)
Xicsk18	TCCAAGGACTG ATGGTACTGC	TCCAAGGACTG ATGGTACTGG	TGGTTCAACAG AAGAGGCGT	KASP	G/C	56	712.8
Xicsk19	GGTTAGCAACATC ACCATTCAGC	GGTTAGCAAC ATCACCATTCAGT	GCCGAGAAAG ATACCCATGTTT	KASP	C/T	103	723.5
Xicsk29	GGCCATCATCTC TTTGTTTCCA	GGCCATCATCTC TTTGTTTCCG	GACAGGAAGG AGTAGCAGTC	KASP	T/C	80	731.9
Xicsqa	CGAGCATATATA CTCCGAGCCG	-	AGCATGTGTATT CTTCAGACCGA	SSR	$(AT)_6$	273	714.9
Xicsqc	AGGAACGGG AAAGATGTCACA	-	CAGGACT GGCAGCACTCT	SSR	$(CT)_5$	292	712.7
Xicsqd	CATGAGACAA GGTGGTATGCC	-	AACGGCTGAA GTATTCCAGG	SSR	$(CT)_5$	294	712.7
Xicsqg	GCCACCGTAAA TCTTTAGTCCG	-	TCACAAGAATC AAGCCCTACCT	SSR	$(TTGTT)_5$	283	712.6

Note: R, resistance allele; S, susceptible allele.

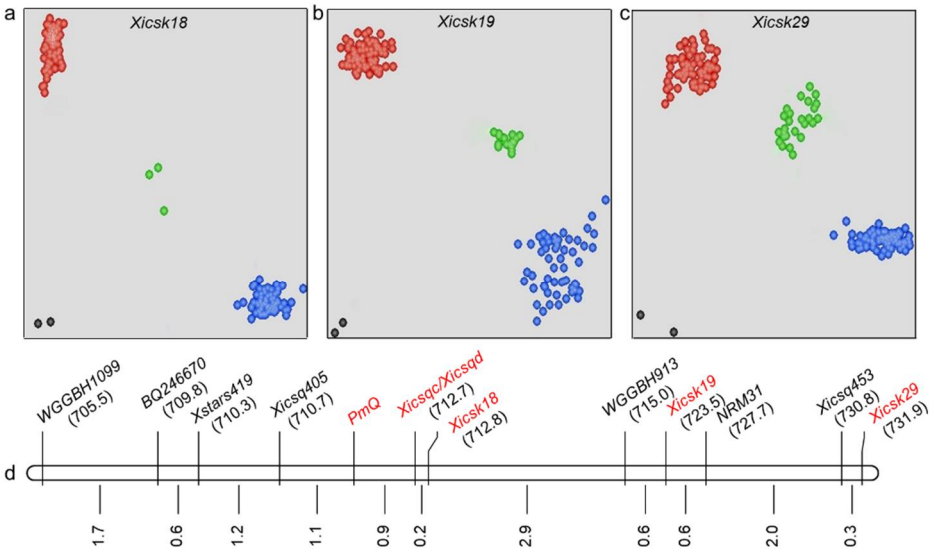

Figure 3. Genotypes of *Xicsk18* (**a**), *Xicsk19* (**b**) and *Xicsk29* (**c**) by Kompetitive Allele Specific PCR (KASP) assay and genetic linkage map (**d**) for *PmQ*. The red, green, and blue dots represent the homozygous susceptible, heterozygous, and homozygous resistant RILs of cross Qingxinmai × 041133 cross, respectively. The newly developed KASP and SSR markers *Xicsqc* and *Xicsqd* in the genetic linkage map (**d**) are indicated by red fonts. Numbers in brackets indicate the physical positions of the markers (Mb) in the CS RefSeq v.1.0.

To develop more molecular markers for *PmQ*, the SSR motifs within the target genomic region between previously identified markers *Xicsq405* and *WGGBH913* (710.1–715.1 Mb) [27] were extracted from the CS RefSeq v1.0. Four out of nine primer pairs designed were polymorphic and potentially linked to *PmQ* as determined by amplifying the two parents and the contrasting DNA bulks (Table 1). Markers *Xicsqc* and *Xicsqd* (Figure 1c,d) were selected to genotype the mapping population because they are co-dominant, and the other two polymorphic markers *Xicsqa* and *Xicsqg* were not used further because they are dominant. In addition, 10 SSR markers, *WGGBH686*, *WGGBH1099*, *BQ246670*, *Xstars419*, *Xicsq405*, *WGGBH913*, *NRM31*, *Xicsq253*, *Xicsq453*, and *Xwmc332* previously linked to *PmQ* [27], also produced polymorphic banding patterns between the resistant and susceptible DNA bulks.

The three KASP markers and two SSR developed in this study, together with the 10 SSR markers previously identified, were chosen to genotype the entire RIL population. A genetic linkage map of *PmQ*, which spanned a genetic region of 12.1 cM, was established. The newly developed polymorphic KASP and SSR markers were localized in the distal side of *PmQ*. The KASP marker *Xicsk18* (0.9 cM) was closer to *PmQ* than the other two KASP markers *Xicsk19* (4.6 cM) and *Xicsk29* (7.5 cM). The two SSR markers, *Xicsqc* and *Xicsqd*, were co-segregated and 1.1 cM distant from the target gene. *PmQ* was thus placed in a 2.0 cM genetic interval between the SSR marker *Xicsq405* and the KASP marker *Xicsk18* (Figure 3d). The groups of the RILs based on these flanking markers *PmQ* agree well with the phenotypic performance (Figure 2).

Wheat landraces are not used directly in agriculture and breeding programs due to their inferior yield potential and other undesirable traits compared to the current cultivar. Pre-breeding is usually required to improve agronomic performances of landraces. Many powdery mildew resistance genes provided by wheat landraces have recessive mode of inheritance [41]. Additional generations are needed to transfer such genes into adapted genotypes compared to dominant genes. Molecular marker-assisted selection (MAS) will be useful during gene transfer. Gene *PmQ* in Qingxinmai is such a recessive gene. Several KASP markers linked to this gene were developed. The genotypes of the KASP marker *Xicsk19* in Qingxinmai (CC) is different from the genotype of 158 out of 160 wheat cultivars or advanced breeding lines (TT) in breeding programs, with exception of Jimai 22 and Zhoumai 11 that are identical to Qingxinmai (Figure 4a). In addition, the amplification

banding patterns of *PmQ*-linked SSR markers *Xicsqc*, *Xicsqd*, and *Xicsq405* in Qingxinmai were different from most of the cultivars analyzed, except Jimai 22. The KASP marker *Xicsk18* produced the same genotype as Qingxinmai (GG) in 67 out of the 160 wheat accessions (Figure 4b). Therefore, this marker is not informative despite of closer linkage with *PmQ*. These results demonstrate that the KASP marker *Xicsk19* and the SSR markers *Xicsqc*, *Xicsqd*, and *Xicsq405*, are effective tools in breeding programs for MAS of *PmQ*.

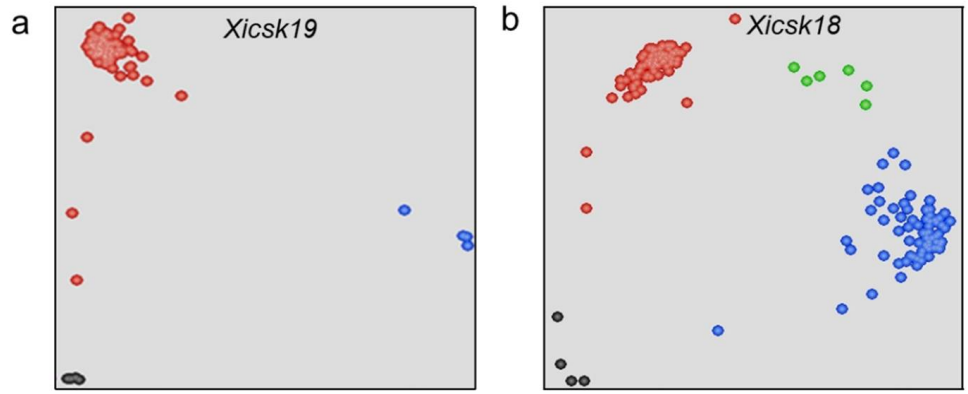

Figure 4. Genotypic detection of 160 wheat accessions by the *PmQ*-linked KASP markers *Xicsk19* marker (**a**) and *Xicsk18* (**b**). The red and blue dots represent the wheat cultivars or advanced breeding lines that are different from or identical as Qingxinmai, respectively. The green dots represent the wheat cultivars or breeding lines that are heterozogous in the loci detected.

Because Jimai 22 and Zhoumai 11 produced identical amplification patterns with the KASP marker *Xicsk19*, more information is needed to discriminate the genes conferring their powdery mildew resistance. The *Pm* gene in Jimai 22, *PmJM22*, was originally detected on the chromosome arm 2BL [42], which was likely to be identical to *Pm52* detected in Liangxing 99 [43]. The physical location of *PmQ* has proven to be different from *Pm52* [27]. Yumai 51 was developed from cross Zhou8425B × Yumai 17 [44]. Previously, QTL conferring powdery mildew resistance at the adult plant stage have been observed on chromosomes 1B, 3B, 4B, and 7D in Zhou8425B [45]. There is a possibility that Yumai 51 inherits certain loci controlling resistance to powdery mildew from Zhou8425B. In such a case, molecular markers, in combination with pedigree information, will facilitate accurate detection of target genes.

The genetic interval of *PmQ* between the flanking markers *Xicsq405* and *Xicsk18* corresponded to a ~2.0 Mb (710.7–712.7 Mb) genomic region on the chromosome arm 2BL of Chinese Spring. Twenty-two high confidence genes were annotated in this genomic region (Supplementary Table S2). A synteny of this genomic region was detected with those of the *T. turgidum* ssp. *dicoccoides* accession Zavitan 2BL (1.77 Mb, 706,469,440–708,241,117 bp), *T. durum* cultivar Svevo 7BL (1.84 Mb, 698,334,686–700,178,115 bp), and the *T. urartu* accession G1812 2AL (1.31 Mb, 701,203,016–702,509,302). The micro-collinearity analysis in the 10+ wheat genomes also revealed a good synteny of the annotated genes in this genomic region among those wheat cultivars (Figure 5). Based on the annotated functions, genes *TraesCS2B01G517400.1* and *TraesCS2B01G517700.1*, encoding for the WRKY family transcription factor and Leucine-rich repeat receptor-like protein kinase family protein, respectively, were associated with plant defense against pathogens.

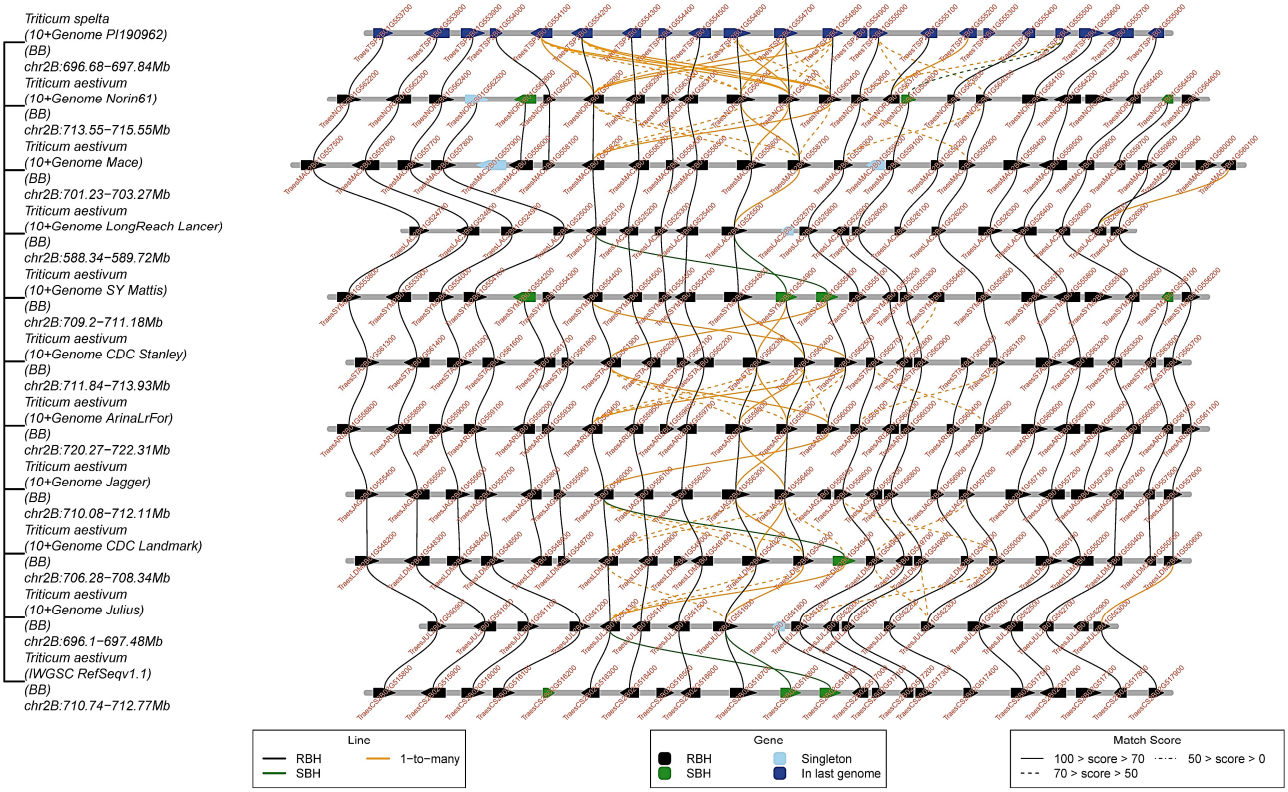

Figure 5. Micro-collinearity of the *PmQ* genomic region based on the projection of the 2 Mb genomic region on the Triticeae-Gene Tribe website (http://wheat.cau.edu.cn/TGT/, accessed on 11 December 2020). The homologous genes are divided into four types: RBH, Reciprocal Best Hit; SBH, Single-side Best Hit; singleton, and 1-to-many (all putative homologous genes).

4. Conclusions

We developed three KASP and two SSR markers to saturate the genetic linkage map of *PmQ* in wheat landrace Qingxinmai. The linkage between the newly developed molecular markers and *PmQ* was confirmed by genotyping a RIL population. Since gene *PmQ* has not been widely applied in wheat breeding, it can be transferred into other wheat genetic backgrounds for developing more adapted lines with powdery mildew resistance. The KASP marker *Xicsk19*, together with SSR markers *Xicsqc*, *Xicsqd*, and *Xicsq405*, can be useful in MAS during this pre-breeding process.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091344/s1, Table S1: Comparison of amplification patterns and genotypes of the three *PmQ* linked makers, *Xicsq405*, *XicsK18*, and *XicsK19* in 160 wheat cultivars or advanced breeding lines; Table S2: Comparative genomic analysis of the high-confidence genes in the 2 Mb-target genomic region of *PmQ* in the Chinese Spring, wild emmer, durum wheat, *T. urartu*, and barley.

Author Contributions: Conceptualization, H.L., P.Z. and Z.L.; investigation, H.G.G., Y.L., J.H. and D.Q.; validation, H.Z., L.Y. and Y.Z. (Yang Zhou), software, Q.W.; writing—original draft preparation, H.L. and H.G.G.; writing—review and editing, H.L., P.Z., Z.L. and Y.Z. (Yijun Zhou); supervision, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 32172001 and 31871621), the Agricultural Science and Technology Innovation Program, grant number CAAS-ZDRW202002, and the Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shiferaw, B.; Smale, M.; Braun, H.-J.; Duveiller, E.; Reynolds, M.; Muricho, G. Crops that Feed the World 10. Past Successes and Future Challenges to the Role Played by Wheat in Global Food Security. *Food Secur.* **2013**, *5*, 291–317. [CrossRef]
2. Tolmay, V.L. Resistance to Biotic and Abiotic Stress in the Triticeae. *Hereditas* **2004**, *135*, 239–242. [CrossRef] [PubMed]
3. Afzal, F.; Chaudhari, S.K.; Gul, A.; Farooq, A.; Ali, H.; Nisar, S.; Sarfraz, B.; Shehzadi, K.J.; Mujeeb-Kazi, A. Bread Wheat (*Triticum Aestivum* L.) Under Biotic and Abiotic Stresses: An Overview. In *Crop Production and Global Environmental Issues*; Hakeem, K., Ed.; Springer: Cham, Switzerland, 2015; pp. 293–317.
4. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The Global Burden of Pathogens and Pests on Major Food Crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. [CrossRef] [PubMed]
5. Wicker, T.; Oberhaensli, S.; Parlange, F.; Buchmann, J.P.; Shatalina, M.; Roffler, S.; Ben-David, R.; Doležel, J.; Šimková, H.; Schulze-Lefert, P.; et al. The Wheat Powdery Mildew Genome Shows the Unique Evolution of An Obligate Biotroph. *Nat. Genet.* **2013**, *45*, 1092–1096. [CrossRef]
6. Sotiropoulos, A.G.; Arango-Isaza, E.; Ban, T.; Barbieri, C.; Bourras, S.; Cowger, C.; Czembor, P.C.; Ben-David, R.; Dinoor, A.; Ellwood, S.R.; et al. Global Genomic Analyses of Wheat Powdery Mildew Reveal Association of Pathogen Spread with Historical Human Migration and Trade. *Nat. Commun.* **2022**, *13*, 4315. [CrossRef]
7. Agrios, G.N. *Plant Pathology*, 5th ed.; Elsevier Academic Press Inc.: San Diego, CA, USA, 2005.
8. Cunfer, B.M. Powdery mildew. In *Bread Wheat: Improvement and Production*; Curtis, B.C., Rajaram, S., Gómez Macpherson, H., Curtis, B.C., Rajaram, S., Gómez Macpherson, H., Eds.; Plant Production and Protection Series; FAO: Rome, Italy, 2002; Volume 30, pp. 317–330.
9. Draz, I.S.; Esmail, S.M.; Abou-Zeid, M.A.E.-H.; Essa, T.A.E.-M. Powdery mildew susceptibility of spring wheat cultivars as a major constraint on grain yield. *Ann. Agric. Sci.* **2019**, *64*, 39–45. [CrossRef]
10. Cao, X.-R.; Yao, D.-M.; Duan, X.-Y.; Liu, W.; Fan, J.-R.; Ding, K.-J.; Zhou, Y.-L. Effects of Powdery Mildew on 1000-Kernel Weight, Crude Protein Content and Yield of Winter Wheat in Three Consecutive Growing Seasons. *J. Integr. Agric.* **2014**, *13*, 1530–1537. [CrossRef]
11. Gao, H.; Niu, J.; Yang, X.; He, D.; Wang, C. Impacts of Powdery Mildew on Wheat Grain Sugar Metabolism and Starch Accumulation in Developing Grains. *Starch-Stärke* **2014**, *66*, 947–958. [CrossRef]
12. Huo, Z.G.; Chen, L.; Liu, W.C.; Xue, C.Y.; Zhao, S.J.; Zhuang, L.W. Climatic Zonation of Wheat Powdery Mildew in China. *Acta Ecol. Sin.* **2002**, *22*, 1873–1881.
13. Ben-David, R.; Dinoor, A.; Peleg, Z.; Fahima, T. Reciprocal Hosts' Responses to Powdery Mildew Isolates Originating from Domesticated Wheats and Their Wild Progenitor. *Front. Plant Sci.* **2018**, *9*, 75. [CrossRef]
14. Huang, X.-Q.; Röder, M.S. Molecular Mapping of Powdery Mildew Resistance Genes in Wheat: A Review. *Euphytica* **2004**, *137*, 203–223. [CrossRef]
15. Rasheed, A.; Wen, W.; Gao, F.; Zhai, S.; Jin, H.; Liu, J.; Guo, Q.; Zhang, Y.; Dreisigacker, S.; Xia, X.; et al. Development and Validation of KASP Assays for Genes Underpinning Key Economic Traits in Bread Wheat. *Theor. Appl. Genet.* **2016**, *129*, 1843–1860. [CrossRef] [PubMed]
16. Majeed, U.; Darwish, E.; Rehman, S.U.; Zhang, X. Kompetitive Allele Specific PCR (KASP): A Singleplex Genotyping Platform and Its Application. *J. Agric. Sci.* **2018**, *11*, p11. [CrossRef]
17. Makhoul, M.; Rambla, C.; Voss-Fels, K.P.; Hickey, L.; Snowdon, R.J.; Obermeier, C. Overcoming polyploidy pitfalls: A user guide for effective SNP conversion into KASP markers in wheat. *Theor. Appl. Genet.* **2020**, *133*, 2413–2430. [CrossRef] [PubMed]
18. Marone, D.; Russo, M.; Mores, A.; Ficco, D.; Laidò, G.; Mastrangelo, A.; Borrelli, G. Importance of Landraces in Cereal Breeding for Stress Tolerance. *Plants* **2021**, *10*, 1267. [CrossRef] [PubMed]
19. Bhullar, N.K.; Street, K.; Mackay, M.; Yahiaoui, N.; Keller, B. Unlocking Wheat Genetic Resources for the Molecular Identification of Previously Undescribed Functional Alleles at the *Pm3* Resistance Locus. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 9519–9524. [CrossRef]
20. Xie, J.; Guo, G.; Wang, Y.; Hu, T.; Wang, L.; Li, J.; Qiu, D.; Li, Y.; Wu, Q.; Lu, P.; et al. A Rare Single Nucleotide Variant in *Pm5e* Confers Powdery Mildew Resistance in Common Wheat. *New Phytol.* **2020**, *228*, 1011–1026. [CrossRef]
21. Lu, P.; Guo, L.; Wang, Z.; Li, B.; Li, J.; Li, Y.; Qiu, D.; Shi, W.; Yang, L.; Wang, N.; et al. A Rare Gain of Function Mutation in A Wheat Tandem Kinase Confers Resistance to Powdery Mildew. *Nat. Commun.* **2020**, *11*, 1–11. [CrossRef]
22. Ma, H.; Kong, Z.; Fu, B.; Li, N.; Zhang, L.; Jia, H.; Ma, Z. Identification and Mapping of a New Powdery Mildew Resistance Gene on Chromosome 6D of Common Wheat. *Theor. Appl. Genet.* **2011**, *123*, 1099–1106. [CrossRef]
23. Xiao, M.; Song, F.; Jiao, J.; Wang, X.; Xu, H.; Li, H. Identification of The Gene Pm47 on Chromosome 7BS Conferring Resistance to Powdery Mildew in the Chinese Wheat Landrace Hongyanglazi. *Theor. Appl. Genet.* **2013**, *126*, 1397–1403. [CrossRef]

24. Tan, C.; Li, G.; Cowger, C.; Carver, B.F.; Xu, X. Characterization of Pm59, a Novel Powdery Mildew Resistance Gene in Afghanistan Wheat Landrace PI 181356. *Theor. Appl. Genet.* **2018**, *131*, 1145–1152. [CrossRef] [PubMed]
25. Sun, H.; Hu, J.; Song, W.; Qiu, D.; Cui, L.; Wu, P.; Zhang, H.; Liu, H.; Yang, L.; Qu, Y.; et al. Pm61: A Recessive Gene for Resistance to Powdery Mildew in Wheat Landrace Xuxusanyuehuang Identified by Comparative Genomics Analysis. *Theor. Appl. Genet.* **2018**, *131*, 2085–2097. [CrossRef] [PubMed]
26. Tan, C.; Li, G.; Cowger, C.; Carver, B.F.; Xu, X. Characterization of Pm63, a Powdery Mildew Resistance Gene in Iranian Landrace PI 628024. *Theor. Appl. Genet.* **2018**, *132*, 1137–1144. [CrossRef] [PubMed]
27. Li, Y.; Shi, X.; Hu, J.; Wu, P.; Qiu, D.; Qu, Y.; Xie, J.; Wu, Q.; Zhang, H.; Yang, L.; et al. Identification of a Recessive Gene *PmQ* Conferring Resistance to Powdery Mildew in Wheat Landrace Qingxinmai Using BSR-Seq Analysis. *Plant Dis.* **2020**, *104*, 743–751. [CrossRef]
28. International Wheat Genome Sequencing Consortium (IWGSC). Shifting the Limits in Wheat Research and Breeding Using a Fully Annotated Reference Genome. *Science* **2018**, *361*, eaar7191. [CrossRef]
29. Ramirez-Gonzalez, R.H.; Uauy, C.; Caccamo, M. PolyMarker: A Fast Polyploid Primer Design Pipeline: Figure 1. *Bioinformatics* **2015**, *31*, 2038–2039. [CrossRef]
30. Ma, S.; Wang, M.; Wu, J.; Guo, W.; Chen, Y.; Li, G.; Wang, Y.; Shi, W.; Xia, G.; Fu, D.; et al. WheatOmics: A Platform Combining Multiple Omics Data to Accelerate Functional Genomics Studies in Wheat. *Mol. Plant* **2021**, *14*, 1965–1968. [CrossRef]
31. Allen, G.C.; Flores-Vergara, M.A.; Krasynanski, S.; Kumar, S.; Thompson, W. A Modified Protocol for Rapid DNA Isolation from Plant Tissues Using Cetyltrimethylammonium Bromide. *Nat. Protoc.* **2006**, *1*, 2320–2325. [CrossRef]
32. Liu, R.-H.; Meng, J.-L. MapDraw: A Microsoft Excel Macro for Drawing Genetic Linkage Maps Based on Given Genetic Linkage Data. *Hereditas* **2003**, *25*, 317–321.
33. Kosambi, D.D. The Estimation of Map Distances from Recombination Values. *Ann. Eugen.* **1943**, *12*, 172–175. [CrossRef]
34. Lincoln, S.E.; Daly, M.J.; Lander, E.S. *Constructing Genetic Linkage Maps with MAPMAKER/EXP Version 3.0: A Tutorial and Reference Mannual*, 3rd ed.; Whitehead Institute for Medical Research: Cambridge, MA, USA, 1993.
35. Avni, R.; Nave, M.; Barad, O.; Baruch, K.; Twardziok, S.O.; Gundlach, H.; Hale, I.; Mascher, M.; Spannagl, M.; Wiebe, K.; et al. Wild Emmer Genome Architecture and Diversity Elucidate Wheat Evolution and Domestication. *Science* **2017**, *357*, 93–97. [CrossRef] [PubMed]
36. Maccaferri, M.; Harris, N.S.; Twardziok, S.O.; Pasam, R.K.; Gundlach, H.; Spannagl, M.; Ormanbekova, D.; Lux, T.; Prade, V.M.; Milner, S.G.; et al. Durum Wheat Genome Highlights Past Domestication Signatures and Future Improvement Targets. *Nat. Genet.* **2019**, *51*, 885–895. [CrossRef] [PubMed]
37. Ling, H.-Q.; Ma, B.; Shi, X.; Liu, H.; Dong, L.; Sun, H.; Cao, Y.; Gao, Q.; Zheng, S.; Li, Y.; et al. Genome Sequence of the Progenitor of Wheat a Subgenome Triticum Urartu. *Nature* **2018**, *557*, 424–428. [CrossRef] [PubMed]
38. Mascher, M.; Gundlach, H.; Himmelbach, A.; Beier, S.; Twardziok, S.O.; Wicker, T.; Radchuk, V.; Dockter, C.; Hedley, P.E.; Russell, J.; et al. A Chromosome Conformation Capture Ordered Sequence of The Barley Genome. *Nature* **2017**, *544*, 427–433. [CrossRef] [PubMed]
39. Walkowiak, S.; Gao, L.; Monat, C.; Haberer, G.; Kassa, M.T.; Brinton, J.; Ramirez-Gonzalez, R.H.; Kolodziej, M.C.; Delorean, E.; Thambugala, D.; et al. Multiple Wheat Genomes Reveal Global Variation in Modern Breeding. *Nature* **2020**, *588*, 277–283. [CrossRef] [PubMed]
40. Li, Z.; Xu, Y. Bulk Segregation Analysis in the NGS Era: A Review of Its Teenage Years. *Plant J.* **2021**, *109*, 1355–1374. [CrossRef] [PubMed]
41. Li, J.T.; Liu, Z.J. Discovery and Mapping of Powdery Mildew Resistance Genes in Wheat Landraces in China. *Hubei Agric. Sci.* **2015**, *54*, 4113–4116.
42. Yin, G.-H.; Li, G.-Y.; He, Z.-H.; Liu, J.-J.; Wang, H.; Xia, X.-C. Molecular Mapping of Powdery Mildew Resistance Gene in Wheat Cultivar Jimai 22. *Acta Agron. Sin.* **2009**, *35*, 1425–1431. [CrossRef]
43. Qu, Y.-F.; Wu, P.-P.; Hu, J.-H.; Chen, Y.-X.; Shi, Z.-L.; Qiu, D.; Li, Y.-H.; Zhang, H.-J.; Zhou, Y.; Yang, L.; et al. Molecular Detection of the Powdery Mildew Resistance Genes in Winter Wheats DH51302 and Shimai 26. *J. Integr. Agric.* **2020**, *19*, 931–940. [CrossRef]
44. Zheng, T.C.; Li, X.P.; Yang, G.Y.; Zhang, X.D.; Sun, Y.X.; Dong, D.D. Analysis of Main Characteristics of Breeding Characteristic Level of Yumai 51. *Crops* **2000**, *5*, 35–36.
45. Jia, A.; Ren, Y.; Gao, F.; Yin, G.; Liu, J.; Guo, L.; Zheng, J.; He, Z.; Xia, X. Mapping and Validation of a New QTL For Adult-Plant Resistance to Powdery Mildew in Chinese Elite Bread Wheat Line Zhou8425B. *Theor. Appl. Genet.* **2018**, *131*, 1063–1071. [CrossRef] [PubMed]