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ORIGINAL ARTICLE

Genetic mapping of powdery mildew resistance genes in wheat landrace Guizi 1 via genotyping by sequencing

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Abstract

Background Wheat (*Triticum aestivum* L.) powdery mildew (*Pm*), which caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a destructive disease worldwide that causes severe yield losses in wheat. Resistant wheat cultivars easily lose their ability to effectively resist newly emerged *Bgt* strains; therefore, identifying new resistance genes is necessary for breeding resistant cultivars.

Methods and Results Guizi 1 (GZ1) is a Chinese wheat cultivar with moderate and stable resistance to *Pm*. Genetic analysis indicated that the *Pm* resistance of GZ1 was controlled by a single dominant gene, designated *PmGZ1*. In total, 110 F_2 individual plants and their 2 parents were subjected to genotyping by sequencing (GBS), which yielded 23,134 high-quality single-nucleotide polymorphisms (SNPs). The SNP distributions across the 21 chromosomes ranged from 134 on chromosome 6D to 6288 on chromosome 3B. Chromosome 6A has 1866 SNPs, among which 16 are physically located between positions 307,802,221 and 309,885,836 in an approximate 2.3-cM region; this region also had the greatest SNP density. The average map distance between SNP markers was 0.1 cM. A quantitative trait locus (QTL) with a significant epistatic effect on *Pm* resistance was mapped to chromosome 6A. The logarithm of odds (LOD) value of *PmGZ1* was 34.8, and *PmGZ1* was located within the confidence interval marked by chr6a-307802221 and chr6a-309885836. Moreover, 74.7% of the phenotypic variance was explained by *PmGZ1*. Four candidate genes (which encoded two TaAP2-A and two actin proteins) were annotated maybe as resistance genes.

Conclusions The present results provide valuable information for wheat genetic improvement, QTL fine mapping, and candidate gene validation.

Keywords Wheat powdery mildew · Genotyping by sequencing · Single-nucleotide polymorphisms · Quantitative trait loci · Resistance

Introduction

Wheat (*Triticum aestivum* L.), which plays an important role in fulfilling the food demand of humans, is a widely cultivated crop species worldwide [1, 2]. Wheat powdery mildew (*Pm*), which is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a destructive wheat disease worldwide that causes severe yield losses, particularly under humid, rainfed conditions [3]. The increased use of nitrogen fertilizers has resulted in *Pm* becoming a progressively more important problem in wheat production [3, 4]. Breeding resistant cultivars is the most economical, effective, and environmentally safe strategy to control *Pm* [5, 6]. However, resistant wheat cultivars easily lose their ability to effective resist newly emerged *Bgt* strains [6, 7]. Therefore, it is necessary to identify new resistance genes to continue breeding resistant cultivars.

The first *Pm* resistance gene, named *Pm1*, was discovered in 1953 [8]. To date, more than 70 *Pm* resistance genes/alleles (*Pm1* to *Pm68*, with *Pm8* being allelic to *Pm17*; *Pm16* and *Pm30* being the same; and with *Pm18* = *Pm1c*,

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Pm22 = *Pm1e*, *Pm23* = *Pm4c*, *Pm31* = *Pm21*, and *Pm46* = *Pm48*) have been identified at 63 loci, and new resistance genes are continually discovered in common wheat and its relatives [9–14]. Among these *Pm* resistance genes/alleles, only two temporarily designated *Pm* resistance genes (*Pm21* and *Pm56*) have been reported on chromosome 6A [15, 16]. *Pm21*, which results from a 6AL·6VS translocation in *Haynaldia villosa*, was identified in 1995 [15]. Afterward, varieties carrying *Pm21* were developed and have been widely cultivated in China. Many are still resistant against prevailing *Bgt* isolates, whereas some *Pm* resistance genes (including *Pm2* and *Pm4*) have gradually lost their resistance to *Bgt* [17, 18]. Recently, the *Pm21* gene was cloned and confirmed to encode a single coiled-coil, nucleotide-binding site-containing, leucine-rich repeat protein, in which the coiled-coil domain's activity was closely related to *Pm21* resistance to *Pm* [19–21]. *Pm56*, which results from the 6AL·6RS translocation of *Secale cereale* L., was mapped to the sub-telomeric region of the arm [16].

Guizhou Province, located in southwestern China, has a complex and dynamic climate that is favorable to the pathogenesis of *Pm*. Therefore, wheat *Pm* is a very serious problem, and an epidemic occurs every year. On the basis of many years of field observations, wheat landrace Guizi 1 (GZ1) is highly resistant to *Pm* [22]. In this study, genotyping-by-sequencing (GBS) technology was used to identify and map the *Pm* resistance genes in GZ1. A resistance gene, *PmGZ1*, was located on chromosome 6A, and a high-density genetic linkage map was produced for GZ1, which will be of great value to molecular breeding and gene cloning in wheat.

Materials and methods

Plant materials and sample preparation

Triticum aestivum L. cv. Guizi 1 (Certificate No. Qian2015003) [23] was developed from complex wide-crossing hybrids of *Triticum dicoccoides*/*Triticum durum*//*Aegilops ventricosa* Tausch/*Aegilops tauschii* Coss. Both GZ1 and Zhongyang 96-3 (ZY96-3) were grown and housed at the Guizhou Sub-Center of the National Wheat Improvement Center at the College of Agriculture in Guizhou University. GZ1 showed moderate and stable resistance to *Pm* for many years according to field observations that began in 2010, whereas ZY96-3 was susceptible to *Pm*. Both varieties were planted at an experimental farm in accordance with the protocol of Li et al. [23], and field management practices (including watering, weeding, and fertilizing) were carried out in a unified manner. In total, 206 F_2 and $F_{2:3}$ plants were obtained from GZ1/ZY96-3 crosses.

Evaluation of powdery mildew reactions

The resistance of the F_2 and $F_{2:3}$ plants to *Pm* were assessed by inoculation with a mixture of Bgt isolates (which are prevalent in Guizhou Province) and *Bgt* E20 independently. The mixture of *Bgt* isolates was inoculated onto wheat plants at the tilling stage. The E20 isolate was inoculated onto wheat plants at the one-leaf stage, and then the inoculated plants were grown under a photoperiod of 16 h of light and 8 h of darkness at 20 ± 2 °C with 75% relative humidity in a greenhouse. When the susceptible controls were fully infected, the infection types (ITs) were scored in accordance with the methods of Xue et al. [24]. There were six IT scores (0, 0;, 1, 2, 3, and 4): "0" indicates immune, with no lesions on the plants; "0;" indicates nearly immune, with hypersensitive necrotic flecks being on the leaves; "1" indicates highly resistant, with small colonies less than 1 mm in diameter being present and few conidia on the leaves; "2" indicates moderately resistant, with the leaves having moderately developed hyphae, the diameters of colonies being less than 1 mm, and with some conidia being present; "3" indicates moderately susceptible, with separate non-joined colonies with well-developed hyphae and abundant conidia being present; and "4" indicates highly susceptible, with mostly joined colonies with well-developed hyphae and abundant conidia being present [24, 25].

DNA extraction and GBS analysis

Leaf tissue (0.5–1.0 g) was collected from F_2 plants and the two parents and immediately frozen in liquid nitrogen. Genomic DNA was extracted using the cetyl-trimethylammonium bromide (CTAB) method [26]. The DNA quality was checked via electrophoresis involving 1% agarose gels and quantified using a Genova Nano microvolume spectrophotometer (Jenway, England). Then, the DNA samples were normalized to 30 ng/μL for GBS library construction.

The GBS libraries from 110 F_2 plants and the 2 parents were generated in accordance with the Elshire et al. method [27]. The DNA was processed for GBS through the Illumina HiSeq™ platform. The clean reads, adapter reads with > 10% N content, and reads of low quality (in which the base number of mass value Q ≤ 10 accounted for more than 50% of the whole read) were deleted. The second two read types were filtered to obtain high-quality clean reads for subsequent analyses. The high-quality clean reads were subjected to a BLAST search against the Chinese Spring genomic database (IWGSC RefSeq v1.0 assembly) via BWA-MEM BLAST software [28], and then, the detection and selection of single-nucleotide polymorphisms (SNPs) were carried out using SAMtools MPileup in accordance with the methods of Li et al. [29]. SNPs with separation type

"aaxbb" were retained, and SNPs with partial segregation *p* values less than 0.0001, deletion percentages greater than 30%, or heterozygosity percentages greater than 75% were deleted. Additionally, genotype correction was performed using the SMOOTH statistical method [30].

Linkage map construction and quantitative trait locus (QTL) analysis

The Kosambi mapping function of the quickEst function in ASMap software was used to calculate genetic distances in accordance with the analysis method of Taylor et al. [31], and then, a genetic map was constructed using R/qtl software. Composite interval mapping (CIM) was performed to detect QTLs using WinQTLCart software v2.5 [32]. QTLs were scanned within a 1-cM window and considered to be significant when the logarithm of odds (LOD) score was at least 7.07.

Mixed linear CIM was performed in QTLNetwork v2.1 software to determine epistatic effects among identified QTLs [33, 34]. Multiple linear regression (with $p = 0.05$) was used to select parameters in the model with a window size of 10 cM [35]. A threshold calculated after 1000 permutations with a genome-wide error rate of 0.10 was considered a significant QTL interaction [36].

Results

Genetic characteristics of *Pm* resistance in wheat GZ1

We carried out field observations of wheat resistance to *Pm* caused *Bgt* for many years. The wheat cultivar GZ1 showed moderate and stable resistance (IT = 1) to a mixture of *Bgt* in field observations; however, ZY96-3 was completely susceptible (IT = 4). Moreover, GZ1 was highly resistant (IT = 0) to E20 in incubator observations; however, ZY96-3 was completely susceptible (IT = 4) (Fig. 1). Then, wheat GZ1 was crossed with ZY96-3; all F_1 plants showed high resistance (IT = 0) to mixed strains prevalent in Guizhou Province, and the F_2 individual plants showed resistance or susceptibility at the IT = 0–4 levels to the mixture of strains. Among the 206 F_2 plants, the segregation ratio of the resistant (150) and susceptible (56) individuals fit the 3:1 theoretical Mendelian segregation ratio ($\chi^2 = 0.4143$, $p = 3.84$) (Table 1). Furthermore, the resistance of $F_{2:3}$ individual plants to E20 *Bgt* were measured, and there were 57 homozygous resistant plants, 103 segregating plants, and 46 homozygous susceptible plants, which fit the theoretical 1:2:1 ratio ($\chi^2 = 1.1748$, $p = 5.99$) (Table 2). Our results therefore demonstrated that the *Pm* resistance of GZ1 was controlled by a single dominant gene.

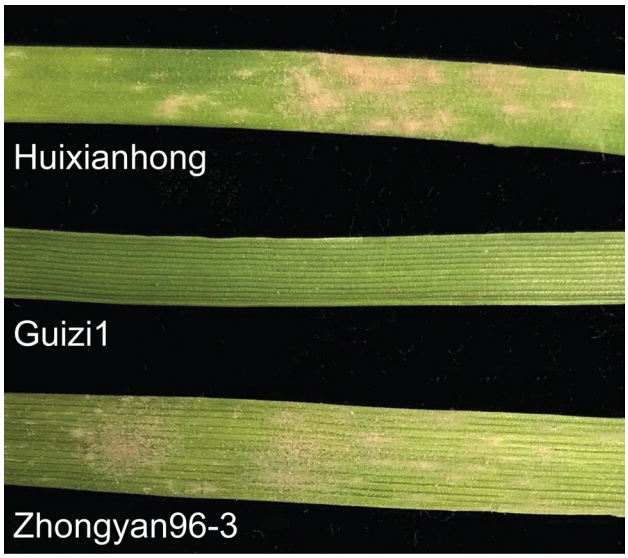

Fig. 1 Resistance of GZ1 and ZY96-3 to *Bgt* E20, with Huixianhong used as a susceptible control. Wheat GZ1, ZY96-3, and Huixianhong plants at the single-leaf stage were inoculated with *Bgt* E20. Representative leaves were taken and imaged when Huixianhong showed complete susceptibility

GBS analysis of *Pm* resistance genes in wheat GZ1

The 110 individual F_2 plants and parents were subjected to GBS, and 1,684,236,264 total clean reads were obtained. After strict filtering, we obtained 1,673,889,294 high-quality clean reads, which were mapped to the genome of Chinese Spring Wheat (IWGSC RefSeq v1.0 assembly), and 311,065 SNPs were identified. The SMOOTH statistical method was used for genotype correction, which yielded 23,134 high-quality SNPs that covered a genetic linkage map of the 21 chromosomes (5402.12 cM in total) (Fig. S1). The number of SNPs per chromosome ranged from 134 on chromosome 6D to 6288 on chromosome 3B, and 1866 SNPs were located on chromosome 6A (8.1% of the total) (Fig. 2A). Among these SNPs, 16 were located in a physical region between positions 307,802,221 and 309,885,836 in an approximately 2.3-cM region (58.6–60.9 cM) (Figs. S1, 2B). In addition, we found that chromosome 6A possessed the greatest SNP density, and the average map distance between SNP markers was 0.1 cM.

QTL analysis

QTL analysis was used to map the resistance genes via CIM, and one *Pm*-related QTL was detected on chromosome 6A (Fig. 3A). This QTL was designated as *PmGZ1*. The LOD value of *PmGZ1* reached 34.8, and *PmGZ1* was located within the confidence interval marked by chr6a-307802221

Table 1 Genetic analysis of *Pm* resistance of parents and different populations at the seedling stage to a mixture of *Bgt*

Parents	Generation	Total number	Phenotype and number of tested plants			Expected ratio	χ^2
			Resistant	Segregating	Susceptible		
Guizi1	Resistant parent	30	30				
Zhongyan96-3	Susceptible parent	30			30		
	F_1	15	15				
	F_2	206	150		56	3:1	0.4143

χ^2 represents the chi-square value of 3:1, $x^2_0 = 0.4143 < x^2_{0.05,1} = 3.84$

Table 2 Genetic analysis of *Pm* mildew resistance of parents and an $F_{2:3}$ population at the seedling stage to *Bgt* E20

Parents	Generation	Total number	Phenotype and number of tested plants			Expected ratio	χ^2
			Resistant	Segregating	Susceptible		
Guizi1	Resistant parent	30	30				
Zhongyan96-3	Susceptible parent	30			30		
	$F_{2:3}$	206	57	103	46	1:2:1	1.1748

χ^2 represents the chi-square value of 1:2:1, $x^2_0 = 1.1748 < x^2_{0.05,2} = 5.99$

and chr6a-309885836 (Table S1), which corresponded to the genetic position of 58.6–60.9 cM (2.3 cM) in the Chinese Spring reference genome. *PmGZ1* accounted for 74.7% of the phenotypic variance (Fig. 3B). Thus, the *Pm* resistance gene *PmGZ1* in GZ1 was mapped to chromosome 6A.

Then, 27 putatively annotated genes within the 2.3 cM candidate interval (chr6a-307802221 to chr6a-309885836) of *PmGZ1* were identified via comparisons with IWGSC RefSeq v1.0 (IWGSC et al. 2018) (Table S2). Among the candidate genes, four were annotated as resistance genes, including two genes encoding a TaAP2-A protein and two encoding an actin (ACT-1) protein (Table S2).

Discussion

Wheat *Pm* resistance genes mainly provide resistance to specific *Bgt* races; however, different *Bgt* races can easily generate novel viral *Bgt* isolates through virulent mutations to escape recognition of resistance genes, resulting in the *Pm* resistance genes losing their ability to generate resistance to *Pm* [5, 37, 38]. Therefore, there is a vital need to discover, identify, and utilize new and effective *Pm* resistance genes for wheat production [39]. GZ1, which is highly resistant to *Bgt*, was determined to be controlled by a single dominant gene (Fig. 1, Tables 1 and 2), and GZ1 wheat has exhibited a stable resistant phenotype in field observations since 2010.

Here, GBS was used for genetic analysis and mapping of *Pm* resistance genes of GZ1 wheat. SNPs, which are preferred over other marker systems, are the most commonly used DNA markers for genetic studies in wheat [40, 41]. Compared with that via GBS, genotyping populations via SNPs may produce less accurate and biased results, which is possible because of the identification of high-quality population-specific SNPs [42, 43]. The GBS protocol involves the use of two restriction enzymes (PstI/MspI) for targeting and reducing complex genomes, thereby achieving a more unified sequencing library [27, 44]. GBS has been used for genotyping wheat to identify high-quality SNPs. In total, 133,039 and 24,767 SNPs were identified after sequencing 369 Iranian hexaploid wheat accessions and 180 common wheat accessions originating from Asia and Europe, respectively [45, 46]. Recently, 1576 high-quality SNPs were obtained for the precise mapping of Hessian fly resistance genes in wheat through GBS, and two QTLs (*QHf.hwwg-3B* and *QHf.hwwg-7A*) were mapped [35]. For wheat *Pm* resistance genes, Wiersma et al. mapped *Pm58* to chromosome 2DS [47], Li et al. developed 165 new *Thinopyrum elongatum*-specific markers [48], and Pang et al. identified one QTL (*qPm6A.3*) associated with *Pm* resistance [49]. Here, 23,134 high-quality SNPs, which covered 21 chromosomes, were identified after individual F_2 plants and parents (wheat GZ1 and ZY96-3) were sequenced via GBS (Figs. S1, 2). In addition, *PmGZ1* was detected on chromosome 6A (with a

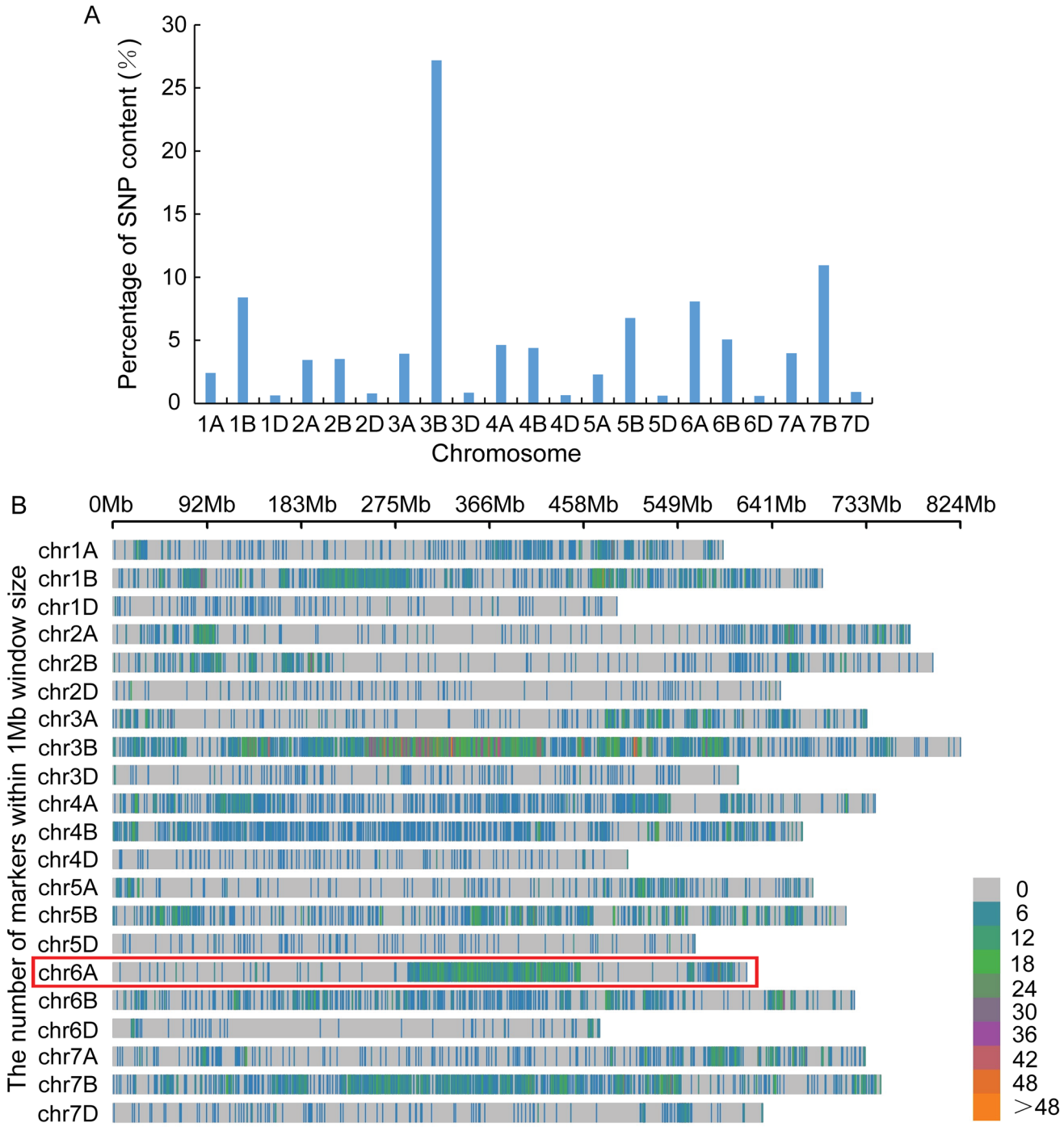

Fig. 2 GBS. **A** Percentage of candidate SNPs per chromosome. **B** Distribution the 23,134 high-quality SNPs per chromosome

high LOD value of 34.8) through CIM, which demonstrated that *PmGZ1* was located on chromosome 6A (Fig. 3).

To date, only *Pm21* and *Pm56* have been mapped to chromosome 6A [15, 16]. SM142 and KU.962 markers that are linked to *Pm21* and *Pm56*, respectively, were used for polymorphism analyses and both markers showed no polymorphism. *PmGZ1* was located on the long arm of chromosome 6A, which indicated that *PmGZ1* is not *Pm56*. Distant hybridization of *T. dicoccoides*, *T. durum*, *A. tauschii*, and *A. ventricosa* was used for GZ1 wheat breeding. However, *Pm21* originated from the 6AL·6VS translocation of *H. villosa*. Additionally, wheat varieties carrying *Pm21* are reportedly immune and/or highly resistant to *Bgt* isolates [21, 50]. Many years of field observations have indicated that GZ1 is

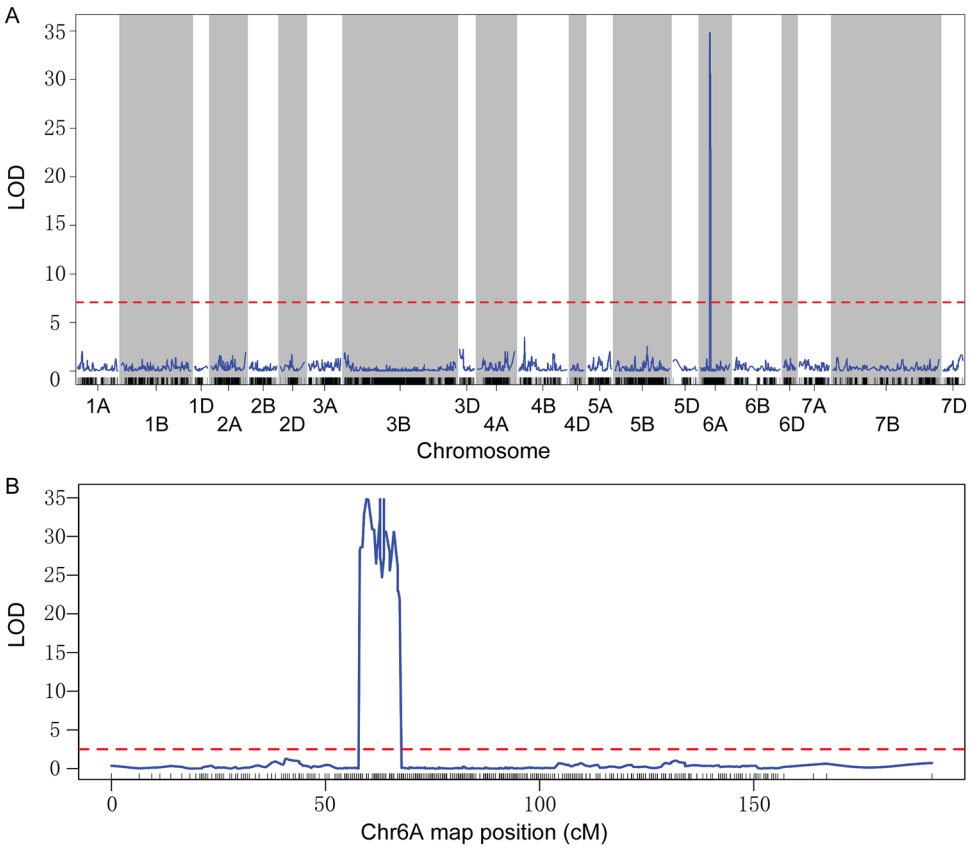

Fig. 3 CIM of the *Pm* resistance gene *PmGZ1*. **A** LOD curves of the 21 chromosomes. **B** LOD curve of chromosome 6A. *Note* The blue curve indicates the LOD values, and the red dashed line (dotted line) indicates the threshold LOD. (Color figure online)

moderately and stably resistant to *Pm*. Therefore, *PmGZ1* may be a new *Pm* resistance gene.

PmGZ1 was located in the 6A:307802221–6A:309885836 confidence interval of Chinese Spring chromosome 6A, which contained 27 putatively annotated genes, including 4 that may be associated with disease resistance (Table S2). The genes *TraesCS6A02G326500LC* and *TraesCS6A02G326600LC* are predicted to encode TaAP2-A proteins that are involved in resistance against the causal pathogen of Fusarium head blight [51]. Similarly, the genes *TraesCS6A02G326700LC* and *TraesCS6A02G327000LC* encode actin (ACT-1) proteins, which can stimulate depolymerization to increase plant resistance against pathogens [52]. Nonetheless, further studies are needed to determine the relationships between these genes and *Pm* resistance and to precisely map *PmGZ1* in GZ1 wheat.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07287-3.

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Author contributions XY performed experiments; MR did data collection; CA performed genes annotation; RX and LL designed the study. LL, RX, ZW and SZ wrote the manuscript.

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Declarations

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

Research involving human and/or animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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