Genetic Mapping of Powdery Mildew Resistance Genes in Wheat Landrace 'Guizi 1' Using Genotyping-By-Sequencing

Luhua Li

Guizhou University

Xicui Yang

Guizhou Agricultural Technology Extension Station

Zhongni Wang

Guizhou Provincial Academy of Agricultural Sciences

Mingjian Ren

Guizhou University

Chang An

Guizhou University

Susong Zhu

Guizhou Provincial Academy of Agricultural Sciences

Ruhong Xu (✉ xrhgz@163.com)

Guizhou University

Research Article

Keywords: wheat powdery mildew, genotyping-by-sequencing, single-nucleotide polymorphisms, quantitative trait loci, resistance

Posted Date: November 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1031772/v1>

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Abstract

Wheat powdery mildew (*Pm*), caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a destructive disease of wheat (*Triticum aestivum* L.) worldwide that causes severe yield losses. Resistant wheat cultivars easily lose effective resistance against newly emerged *Bgt* strains; therefore, identifying new resistance genes is necessary for breeding resistant cultivars. 'Guizi 1' is a Chinese wheat cultivar with effective moderate and stable resistance against powdery mildew. A genetic analysis indicated that powdery mildew resistance in 'Guizi 1' was controlled by a single dominant gene, designated *PmGZ1*. In total, 110 F_2 individual plants and the 2 parents were used for genotyping-by-sequencing, which produced 23,134 high-quality single-nucleotide polymorphisms (SNPs). The SNP distributions on the 21 chromosomes ranged from 134 on chromosome 6D to 6,288 on chromosome 3B. Chromosome 6A has 1,866 SNPs, among which 16 are located in a physical region between positions 307,802,221 and 309,885,836 in an approximate 2.3-cM region, which possessed the greatest SNP density. The average map distance between SNP markers was 0.1 cM. A quantitative trait locus with a significant epistatic effect on powdery mildew resistance was mapped to Chromosome 6A. The LOD value of *PmGZ1* reached 34.8, and *PmGZ1* was located within the confidence interval marked by chr6a-307802221 and chr6a-309885836. The phenotypic variance explained by *PmGZ1* was 74.7%. Four candidate genes (two each encoding TaAP2-A and actin proteins) were annotated as resistance genes. The present results provide valuable information for wheat genetic improvement, quantitative trait loci fine mapping, and candidate gene validation.

Introduction

Wheat (*Triticum aestivum* L.), which plays an important role in fulfilling the food demand of humans, is a widely cultivated crop in the world [1, 2]. Wheat powdery mildew (*Pm*), 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 powdery mildew becoming progressively a more important problem in wheat production [3, 4]. Breeding resistant cultivars is the most economical, effective, and environmentally safe strategy to control powdery mildew [5, 6]. Resistant wheat cultivars easily lose effective resistance against newly emerged *Bgt* strains [6, 7]. Therefore, it is necessary to explore new resistance genes to continue breeding resistant cultivars.

The first powdery mildew resistance gene, named *Pm1*, was discovered in 1953 [8]. To data, over 70 *Pm* genes/alleles (*Pm1* to *Pm65*, with *Pm8* being allelic to *Pm17*, *Pm16* and *Pm30* may be the same, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, *Pm31* = *Pm21*, and *Pm46* = *Pm48*) have been designated at 61 loci, and new genes are continually discovered from common wheat and its relatives [9–12]. Among these *Pm* genes/alleles, only two temporarily designated *Pm* genes (*Pm21* and *Pm56*) have been reported on chromosome 6A [13, 14]. *Pm21*, which results from the 6AL·6VS translocation on *Haynaldia villosa*, was designated in 1995 [13]. Afterwards, varieties carrying *Pm21* were developed and widely cultivated in China. Many are still resistant against prevailing *Bgt* isolates, whereas some *Pm* genes (including *Pm2* and *Pm4*) have gradually lost their resistance to *Bgt* [15, 16]. Serine and threonine protein kinase genes (including *Stpk-V*, *DvMPK1*, *DvMLPK*, *DvUPK*, and *DvPSYR1*) have been located to the *Pm21* locus and appear to be required for *Pm21*-mediated resistance to wheat powdery mildew [17, 18]. Recently, the *Pm21* gene was cloned and

confirmed to encode a single coiled-coil, nucleotide-binding site, leucine-rich repeat protein, in which the coiled-coil domain's activity was closely related to *Pm21* resistance to powdery mildew [19–21]. *Pm56*, which resulted from the 6AL-6RS translocation of *Secale cereale* L. was mapped to the subtelomere region of the arm [14].

Guizhou Province, located in southwestern China, has a complex and changeable climate that is favorable to the pathogenesis of powdery mildew. Therefore, wheat powdery mildew is a very serious problem and an epidemic occurs every year. Wheat landrace 'Guizi 1' (GZ1) is highly resistant to powdery mildew according to many years of field observations [22]. In this study, genotyping-by-sequencing (GBS) technology was used to identify and map the powdery mildew resistant genes in GZ1. A resistant gene, *PmGZ1*, was located on chromosome 6A and the high-density genetic linkage map produced for GZ1 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] is cultivated from complex wide-crossing hybrids of *Triticum dicoccoides*/ *Triticum durum*// *Aegilops ventricosa* Tausch/ *Aegilops tauschii* Coss. GZ1 and Zhongyang 96-3 (ZY96-3) were cultivated and stored in the Guizhou Sub-Center of National Wheat Improvement Center at the College of Agriculture in Guizhou University. GZ1 showed a moderate and stable resistance to powdery mildew for many years according to field observations that began in 2010, whereas ZY96-3 showed a susceptibility to powdery mildew. Both varieties were planted on the experimental farm in accordance with the protocol of Li et al. [23], and the field management (including watering, weeding, and fertilization) was carried out in a unified manner. In total, 206 F_2 and the derived $F_{2:3}$ plants were obtained from a GZ1/ZY96-3 cross.

Evaluation of powdery mildew reactions.

The reactions of the F_2 and $F_{2:3}$ plants to powdery mildew were assessed by inoculation with mixed Bgt isolates (prevalent in Guizhou Province) and Bgt E20, independently. The mixture of *Bgt* isolates was inoculated into wheat plants at the tillering stage. The E20 isolate was inoculated into wheat plants at the one-leaf stages, and then inoculated plants were grown under a daily cycle of 16 h of light and 8 h of darkness at $20 \pm 2^\circ\text{C}$ with 75% relative humidity in a greenhouse. When the susceptible controls were fully infected, the infection types (ITs) were scored in accordance with Xue et al. [24]. There were six IT scores (0, 0₁, 1, 2, 3, and 4): "0" indicates immune, no lesions on plants; "0₁" indicates nearly immune, hypersensitive necrotic flecks on leaves; "1" indicates highly resistant, small colonies of less than 1-mm diameter and having few conidia on leaves; "2" indicates mildly resistant, the leaves have moderately developed hyphae, the diameters of colonies are less than 1 mm, and some conidia; "3" indicates moderately susceptible, separate non-joined colonies with well-developed hyphae and abundant conidia; "4" indicates highly susceptible, mostly joined colonies with well-developed hyphae and abundant conidia [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 CTAB method [26]. The DNA quality was checked by electrophoresis using 1% agarose gels and quantified using a Genova Nano micro-volume spectrophotometer. Then, the DNA samples were normalized to 30 ng/ μ L 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 (2011). The DNA was processed for GBS through the Illumina HiSeq™ platform. The clean reads, adapter reads with > 10% N content ratios, and low-quality reads (in which base number of mass value $Q \leq 10$ accounted for more than 50% of the whole read) were deleted. The latter two read types were filtered to obtain high-quality clean reads for the following analyses. The high-quality clean reads were subjected to a BLAST search against the Chinese Spring genomic database (IWGSC Refseq v1.0 assembly) using BWA-MEM blast software [27], and then, the detection and selection of single-nucleotide polymorphisms (SNPs) were carried out using Samtools mpileup in accordance with Li et al [28]. The SNPs with separation type “aaxbb” were kept, and SNPs with partial segregation p values less than 0.0001, deletion ratios greater than 30%, or heterozygosity ratios greater than 75% were deleted. Additionally, genotype correction was performed using the Smooth statistical method [29].

Linkage map construction and quantitative trait loci (QTLs) analysis.

The Kasombi 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. [30], and then, a genetic map was constructed using R/qtl software. Composite interval mapping (CIM) was performed to detect QTLs using the WinQTLCart software v2.5 [31]. QTLs were scanned at a 1-cM window and claimed to be significant at an LOD score of 7.07.

Mixed linear composite interval mapping was performed in the software QTLNetwork 2.1 to determine epistatic effects among identified QTLs [32, 33]. The multiple linear regression at $p = 0.05$ for selecting parameters in the model with a window size of 10 cM [34]. A threshold, which calculated by 1,000 permutations at a genome-wide error rate of 0.10, was noted as a significant QTL interaction [35].

Results

Genetic characteristics of powdery mildew resistance in wheat GZ1.

We carried out field observations of powdery mildew responses to *Bgt* for many years. Wheat variety GZ1 showed moderate and stable resistance (IT = 1); however, ZY96-3 was completely susceptible (IT = 4) to mixed *Bgt*s in field observations. GZ1 was highly resistant (IT = 0); however, ZY96-3 was completely susceptible (IT = 4) to E20 in incubator observations (Fig. 1). Then, the wheat GZ1 was crossed with ZY96-3, all the F_1 plants showed a high resistance (IT = 0) to mixed strains prevalent in Guizhou Province, and the F_2 individual plants showed resistance or susceptibility at the level of IT = 0-4 to the mixed strains, respectively. 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 responses of $F_{2:3}$ individual plants to E20 *Bgt* were detected, and they segregated into 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 demonstrated that the powdery mildew resistance of GZ1 was controlled by a single dominant gene.

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

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

GBS analysis of powdery mildew resistance genes in wheat GZ1.

The 110 F_2 individual plants and parents were used for GBS, and 1,684,236,264 total clean reads were obtained. After strict filtering, we obtained 1,673,889,294 high-quality clean reads. Then, the high-quality clean reads 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, resulting in 23,134 high-quality SNPs that covered a genetic linkage map of the 21 chromosomes (totally 5,402.12 cM) in wheat being obtained (Fig. S1). The number of SNPs per chromosome ranged from 134 on chromosome 6D to 6,288 on chromosome 3B, and 1,866 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) (Fig. S1 and Fig. 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.

A QTL analysis was used to map the resistance genes with 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 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 powdery mildew resistance gene *PmGZ1* in GZ1 was mapped to chromosome 6A.

Then, 27 putative annotated genes within the 2.3 cM candidate interval (chr6a-307802221 to chr6a-309885836) of *PmGZ1* were identified by comparison with the IWGSC RefSeq v1.0 (IWGSC et al. 2018) (Table

S2). Among the candidate genes, four were annotated as resistance genes, including two genes encoding for a TaAP2-A protein and two for an actin (ACT-1) protein (Table S2).

Discussion

Wheat *Pm* genes mainly exhibit resistance to specific *Bgt* races; however, the *Bgt* races can easily generate novel viral *Bgt* isolates through virulent mutations to escape recognition of resistance genes, resulting in the *Pm* genes losing their ability to generate resistance to powdery mildew [5, 36, 37]. Therefore, there is a vital need to discover, identify, and utilize new and effective *Pm* genes for wheat production [38]. GZ1, which is highly resistance to *Bgt*, was determined to be controlled by a single dominant gene (Fig. 1; Tables 1 and 2), and GZ1 wheat have shown a stable resistant phenotype in field observations since 2010.

Here, GBS was used for the genetic analysis and gene mapping of powdery mildew resistance genes of GZ1 wheat. SNPs, which are preferred over other marker systems, are the most common DNA markers for genetic studies in wheat [39, 40]. The genotyping of populations using SNPs may produce less accurate and biased results than GBS, which overcomes these problems by identifying high-quality population-specific SNPs [41, 42]. The GBS protocol uses two restriction enzymes (PstI/MspI) for targeting and reducing complex genomes, thereby achieving a more unified sequencing library [43, 44]. GBS has been used for genotyping wheat to identify high-quality SNPs. A total of 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, 1,576 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 in wheat [34]. For wheat *Pm* genes, Wiersma et al. (2017) mapped *Pm58* to chromosome 2DS [47], Li et al. (2019) developed 165 new *Th. elongatum*-specific markers [48], and Pang et al. (2019) identified one QTL (*qPm6A.3*) for powdery mildew resistance [49]. Here, 23,134 high-quality SNPs, which covered 21 chromosomes, were identified after sequencing *F*₂ individual plants and parents (wheat GZ1 and ZY96-3) using GBS (Fig. S1 and Fig. 2). In addition, *PmGZ1* was detected on chromosome 6A with high an 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 [13, 14]. The SM142 and KU.962 markers that are linked to *Pm21* and *Pm56*, respectively, have been used for polymorphism analyses. *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 the *Bgt* isolates [21, 50]. The many years of field observations have indicated that GZ1 has a moderate and stable resistance to powdery mildew. Therefore, *PmGZ1* may be a new powdery mildew resistance gene.

PmGZ1 was located in the 7A:307802221–7A:309885836 confidence interval of the Chinese Spring chromosome 7A, which contained 27 putative annotated genes including 4 that may be associated with disease resistance (Table S2). Genes *TraesCS6A02G326500LC* and *TraesCS6A02G326600LC* are predicted to encode TaAP2-A proteins that are involved in resistance against the causative pathogen of fusarium head blight [51]. Genes *TraesCS6A02G326700LC* and *TraesCS6A02G327000LC* encode actin (ACT-1) proteins,

which can stimulate depolymerization to increase plant resistance against pathogens [52]. Further studies are needed to determine the relationships between these genes and powdery mildew resistance and to precisely map *PmGZ1* in GZ1 wheat.

Declarations

Acknowledgments:

This project was supported by grants from the National Natural Science Foundation of China (3216150357), Guizhou Science and Technology Plan Project ([2020] 1Y101), Guizhou Science and Technology Support Project ([2021] YiBan272), Guizhou Science and Technology Plan Project ([2020] 1Z018), the Guizhou Science and Technology Plan Project ([2019] 1073), and the National Natural Science Foundation of China (31760379). We thank International Science Editing (<http://www.internationalscienceediting.com>) for editing this manuscript.

Author Contributions:

Xicui Yang performed experiments; Mingjian Ren did data collection; Chang An performed genes annotation; Ruhong Xu and Luhua Li designed the study. Luhua Li, Ruhong Xu, Zhongni Wang and Susong Zhu wrote the manuscript.

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

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Figures

Figure 1

Resistance of 'Guizi 1' and 'Zhongyan 96-3' to Bgt E20, with 'Huixianhong' as the susceptible control. One-leaf stages of wheat 'Guizi 1', 'Zhongyan 96-3', and 'Huixianhong' were inoculated with Bgt E20. Representative leaves were taken and photographed when 'Huixianhong' showed complete susceptible.

Figure 2

GBS. (A) Percentage of candidate SNPs per chromosome. (B) Distribution of the 23,134 high-quality SNPs per chromosome.

Figure 3

The composite interval mapping of the powdery mildew resistance gene PmGZ1. (A) LOD curve graph of the 21 chromosomes. (B) LOD curve graph of chromosome 6A. Note: The blue curve indicates the LOD value curve, and the red dashed line indicates the threshold LOD.

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