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ORIGINAL ARTICLE

Characterization of *Pm63*, a powdery mildew resistance gene in Iranian landrace PI 628024

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Abstract

Key message **A new powdery mildew resistance gene conferring a wide spectrum of resistance to *Bgt* isolates in the USA, *Pm63*, was identified in Iranian wheat landrace PI 628024 and mapped to the terminal region of the long arm of chromosome 2B.**

Abstract Powdery mildew is a globally important wheat disease causing severe yield losses, and host resistance is the preferred strategy for managing this disease. The objective of this study was to characterize a powdery mildew resistance gene in Iranian landrace PI 628024, which exhibited a wide spectrum of resistance to representative *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates collected from different regions of the USA. An F_2 population and $F_{2:3}$ lines derived from the cross PI 628024 × CItr 11349 were used in this study, and genetic analysis indicated that a single dominant gene, designated *Pm63*, conferred resistance to *Bgt* isolate *OKS(14)-B-3-1*. Linkage analysis located *Pm63* to an interval of about 13.1 Mb on the long arm of chromosome 2B, spanning 710.3–723.4 Mb in the Chinese Spring reference sequence. Bin mapping assigned *Pm63* to the terminal bin 2BL6-0.89-1.0, 1.1 cM proximal to STS marker *Xbcd135-2* and 0.6 cM distal to SSR marker *Xstars419*. Allelism tests indicated that *Pm63* is a new powdery mildew resistance gene, which differs from other genes in the terminal bin by origin, genomic location, and responses to a set of 16 representative US *Bgt* isolates. *Pm63* can be widely used to enhance powdery mildew resistance in the Great Plains, western, and southeastern regions of the USA.

Abbreviations

Bgt	*Blumeria graminis* f. sp. *tritici*
cM	Centimorgan
RFLP	Restrict fragment length polymorphism
QTL	Quantitative trait locus
STS	Sequence tag site
SSR	Simple sequence repeat

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Introduction

Wheat is the second most important staple food crop after rice and is cultivated on approximately 220 million ha in diverse geographical regions, environments, and production systems (Singh et al. 2016). With the global population projected to exceed 9 billion by 2050, a 1.6% annual increase in wheat production was estimated to satisfy the demands of this increase in global population, such that wheat yields should be increased from the current 3 tons/ha to 5 tons/ha in 2050 (Tilman et al. 2002; Singh et al. 2016). Powdery mildew, a serious wheat disease caused by the biotrophic fungus *Blumeria graminis* f. sp. *tritici,* looms as a logical threat to this yield goal, as powdery mildew occurs globally in wheat-growing regions, especially in highly productive regions where modern wheat cultivation technologies, characterized by the use of semidwarf and high-yielding cultivars, irrigation, and high levels of nitrogen fertilizer, are utilized. A typical example is Hungary, where there were moderate or severe powdery mildew epidemics in 11 of the 14 years between 1986 and 1999, and yield losses of 5 to 8% were estimated for years of average infection and up to 30%

in years of severe epidemics (Szunics et al. 2001). In the USA, yield losses inflicted by powdery mildew ranged from 5 to 34% (Conner et al. 2003; Griffey et al. 1993), mainly due to reductions in tiller number, grain number, and kernel weight, while grain protein content was also reduced (Parry 1990; Bowen et al. 1991).

The serious yield losses caused by powdery mildew have stimulated breeding of powdery mildew-resistant cultivars. A previous survey suggested that powdery mildew was one of the top four disease priorities in 115 winter and facultative wheat-breeding programs worldwide (Braun et al. 1997). A considerable number of powdery mildew resistance genes, including 62 permanently designated genes and over 20 temporarily named genes or QTL, have been identified (McIntosh et al. 2013, 2017), and some of them, especially those identified in bread wheat, have been widely used in wheat breeding. However, most of these genes are race-specific and confer immunity or high resistance to powdery mildew, but thereby exert strong selection on *Bgt* populations and subsequently lead to the buildup of virulent pathotypes with matching virulence genes (McDonald and Linde 2002). Recent studies indicated that *Pm2*, *Pm3a*, *Pm3b*, *Pm3f*, *Pm4a*, *Pm6*, *Pm8*, and *Pm17* have been defeated in part or all of the USA, while *Pm1a* was defeated in Australia, China, and Egypt (Parks et al. 2008; Cowger et al. 2018). The ability of *Bgt* to overcome deployed race-specific resistance genes necessitates a continuous search for new resistance genes.

Wheat powdery mildew resistance gene pools include bread wheat and wheat relatives. A considerable number of these resistance genes originated from cultivated and wild relatives. Some of them, such as *Pm6* from *Triticum timopheevii* and *Pm8* from rye (*Secale cereale* L.) (Helmsjørgensen and Jensen 1973; McIntosh et al. 2011), have been widely used in wheat improvement. However, it is a daunting task to eliminate linkage drag associated with alien genes. For example, *Pm21* was transferred to wheat in the early 1990s (Qi et al. 1996), but elite breeding lines with *Pm21* were not released in the most important wheat-growing region in China, the Huang-Huai River Valley, until recently (Cao et al. 2015). On the other hand, powdery mildew resistance genes identified in landraces, which have experienced extreme environmental challenges, can be more easily introgressed and deployed in new cultivars. Several powdery mildew resistance genes, such as *Pm2c, Pm3b*, *Pm5d, Pm5e*, *Pm24a, Pm24b*, *Pm45*, *Pm47*, *Pm59*, and *Pm61,* have been identified in landraces and used in germplasm enhancement and wheat breeding (Huang et al. 2000, 2003; Hsam et al. 2001; Yahiaoui et al. 2004; Ma et al. 2011; Xue et al. 2012; Xiao et al. 2013; Xu et al. 2015; Sun et al. 2018; Tan et al. 2018).

Recently, a set of landraces exhibiting high resistance to *Bgt* isolates from the Great Plains of the USA were identified; many of them had been collected from Middle East, a major center of origin for cereal species (Li et al. 2016). Of these, PI 628024, a landrace collected from Iran, showed a wide spectrum of resistance to *Bgt* isolates collected in the USA. The objectives of this study were to characterize the resistance gene in PI 628024 using molecular markers and to determine its resistance spectrum.

Materials and methods

Plant materials

PI 628024, an Iranian landrace, was highly resistant to *Bgt* isolates collected from Oklahoma, including *OKS (14)-B-3-1* [infection type (IT) = 0] and *Bgt2015* (IT = 0; Li et al. 2016). An F_2 population and a set of $F_{2:3}$ lines derived from PI 628024 × CItr 11349 were used to map the powdery mildew resistance gene in PI 628024. CItr 11349, obtained from Bulgaria in 1929, is highly susceptible to both *OKS (14)-B-3-1* (IT = 4) and *Bgt2015* (IT = 4). The F_2 population consisted of 243 plants, 212 of which produced sufficient F_3 seeds for phenotypic analysis. PI 628024 and CItr 11349 were provided by the USDA-ARS National Small Grains Collection at Aberdeen, Idaho.

Evaluation of powdery mildew resistance

A protocol described previously by Tan et al. (2018) was used to determine powdery mildew responses. In brief, F_2 plants were inoculated with *Bgt* isolate *OKS(14)-B-3-1* at the two-leaf stage, and the inoculated plants were grown under natural light at 20 ± 2 °C in a greenhouse at the USDA-ARS Wheat, Peanut, and other Field Crops Research Unit in Stillwater, OK. ITs were recorded 7–10 days after inoculation when the susceptible check, Jagalene, was severely infected and re-assessed for confirmation after 2 days. A 0-to-4 infection type scale, representing highly resistant (IT = 0, 0, and 1), moderately resistant (IT = 2), moderately susceptible (IT = 3), and highly susceptible (IT = 4) responses, was employed (Tan et al. 2018).

After evaluation, all F_2 plants were vernalized at 5 °C for 6 weeks and then transplanted to a greenhouse. The $F_{2:3}$ lines were evaluated for powdery mildew responses in spring 2017 using a randomized complete block design with two replicates. For each replicate, 16 plants of each $F_{2:3}$ lines were evaluated using the protocol described above. Assuming variation at a single locus, the genotype of each F_2 plant was inferred from corresponding F_3 phenotypic data.

PI 628024, CItr 11349, Coker747, Jimai 22, and Jagalene were evaluated for responses to a set of 16 *Bgt* isolates collected from different regions of the USA and maintained as pure cultures on detached leaves at the USDA-ARS Plant

Table 1 Responses of F_2 plants and $F_{2:3}$ families to the *Bgt* isolate *OKS(14)-B-3-1*

F_2 plants[a]		$F_{2:3}$ lines			Losses[b]
Response	Plants	Homozygous HR	Segregating	Homozygous HS	
HR	186	47	116	–	23
HS	57	–	–	49	8
Total	243		212		31

[a]*HR* highly resistant, *HS* highly susceptible

[b]Losses were treated as random

Science Research Unit at Raleigh, North Carolina (Table 1). Coker747 and Jimai 22 carry *Pm6* and *PmJM22*, respectively, and Jagalene was used as a susceptible control. The detached-leaf method described previously by Cowger et al. (2018) was followed, and disease severities were assessed 10 days after inoculation using a 0-to-9 scale, which distinguished resistant (0–4), intermediate (5–6), and susceptible (7–9) reactions. The 0-to-9 scale was designed to differentiate phenotypes conferred by different powdery mildew resistance genes and to obtain accurate phenotypic data comparable to that based on the 0-to-4 scale. The 0-to-4 scale is suitable for evaluation of mapping or breeding populations.

Bulked segregant analysis

Bulked segregant analysis (BSA) (Michelmore et al. 1991) was used to map the powdery mildew resistance gene in PI 628024. In brief, the F_2 genotypes were inferred from F_3 phenotypic data, and a protocol described by Dubcovsky et al. (1994) was used to extract genomic DNA from two-week-old leaves. Equal amounts of genomic DNA from 10 highly resistant (IT = 0) F_2 plants and 10 highly susceptible (IT = 4) F_2 plants were pooled to construct the resistant and susceptible bulks, respectively. A set of over 400 simple sequence repeat (SSR) markers evenly distributed across the wheat chromosomes were initially surveyed for polymorphism between the two parents and the two contrasting bulks. Polymerase chain reaction (PCR) amplifications were performed on Applied Biosystems 2720 thermal cyclers (Applied Biosystems Inc., CA) using a previously described protocol (Xu et al. 2006), and denatured PCR products were separated in 6.5% polyacrylamide gels running in a Li-Cor DNA Analyzer. Electrophoresis conditions were set at 1500 V, 40 W, 35 mA, and 50 °C for 3 h in 1X Tris–borate-EDTA (TBE) buffer.

Informative SSR markers exhibiting polymorphism between the two contrasting bulks, as well as the parents, were used to genotype the F_2 population, leading to the identification of SSR markers closely linked to the single powdery mildew resistance gene. Based on their genomic locations, molecular markers previously mapped in the target region were further used to genotype the population. In addition, two SSR loci, including *STARS382* (forward primer: TGGGATGGAGGGAGTACTTG; reverse primer: TCATATCCATGGTGGGGAAC) and *STARS419* (forward primer: GCCCTTGTCAGTTTCAGTCC; reverse primer: GTCGATCGCTCCACCTCTAC), were identified in the target region, and primers were designed to genotype the F_2 population.

Date analysis

MAPMAKER 3.0 software (Lander et al.1987) was used to construct the genetic linkage map, and the Kosambi mapping function was used to convert recombination values to map distances (Kosambi 1943). A logarithm of the odds (LOD) score of 3.0 was set as the threshold for linkage. Chi-squared (χ^2) tests were performed to test the hypothesis that a dominant powdery mildew resistance allele conferred resistance in the F_2 and F_3 populations.

Bin mapping

Based on linkage mapping results, molecular markers flanking the powdery mildew resistance gene in PI 628024 were used to genotype Chinese Spring nulli-tetrasomic lines, N2AT2B, N2BT2A, and N2DT2A, as well as five 2BL deletion lines, 2BL-3, 2BL-4, 2BL-5, 2BL-1, and 2BL-6, to determine the physical locations of markers close to the powdery mildew resistance gene. All aneuploid lines were provided by the Kansas State University Wheat Genetics Resource Center (Manhattan, Kansas).

Allelism tests

An F_2 population derived from PI 628024 × Jimai 22 was used to determine the allelic relationships between gene *PmJM22* and the gene in PI 628024. The protocol described above was used to determine responses of each F_2 plant to *Bgt* isolate *OKS(14)-B-3-1*, which is avirulent to PI 628024 and Jimai 22. The recombination fraction was estimated, and genetic distance in centiMorgans (cM) was calculated (Kosambi 1943). A Chi-squared (χ^2) test was conducted to test the hypothesis that two independent powdery mildew resistance genes segregated in the F_2 population.

Results

Inheritance of powdery mildew resistance in PI 628024

The parental lines and F_2 population derived from PI 628024 × CItr 11349 were evaluated for responses to *Bgt*

isolate *OKS(14)-B-3-1*. PI 628024 and CItr 11349 were highly resistant (IT = 0) and highly susceptible (IT = 4), respectively. Among F_2 plants, 186 were resistant, and 57 were susceptible (Table 1). The χ^2 test indicated a single dominant allele conferred resistance ($\chi^2_{3:1} = 0.31$, $df = 1$, $p = 0.58$).

Of the 212 $F_{2:3}$ lines evaluated for response to *OKS(14)-B-3-1*, 47 and 49 were rated as homozygous resistant and homozygous susceptible, respectively, and 116 segregated, confirming that PI 628024 carries a dominant resistance allele ($\chi^2_{1:2:1} = 1.92$, $df = 2$, $p = 0.38$) (Table 1).

Linkage mapping

BSA identified four SSR markers that distinguished the two parents and two contrasting bulks, *Xgwm148*, *Xgwm374*, *Xwmc441*, and *Xwmc332*. All these markers were previously mapped to the long arm of chromosome 2B. Therefore, we used them to genotype the F_2 population. Linkage analysis indicated that the powdery mildew resistance gene in PI 628024, originally designated *Pm628024*, was 7.7 cM distal to *Xwmc441*.

Xwmc441 was located at approximately 598.06 Mb in the Chinese Spring reference sequence IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr). Therefore, an additional 43 markers previously mapped in this region were tested for polymorphism, and 10 polymorphic markers were subsequently used to genotype the mapping population. Linkage analysis placed *Pm628024* in an interval of 1.7 cM, flanked distally by STS marker *Xbcd135-2* with a genetic distance of 1.1 cM and proximally by SSR marker *Xstars419* with a genetic distance of 0.6 cM (Fig. 1).

Physical location of *Pm628024*

Using the genomic sequence of the restriction fragment length polymorphism (RFLP) probe *BCD135* (https://wheat.pw.usda.gov/GG3/), we located *Xbcd135-2* to approximately 723.4 Mb in the Chinese Spring reference sequence IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr). *Xstars419* was located at approximately 710.3 Mb. Therefore, *Pm628024* resides in a genomic region of about 13.1 Mb, spanning 710.3–723.4 Mb of the Chinese Spring reference sequence.

We further located *Pm628024* on the Chinese Spring bin map (Fig. 1). Five markers flanking *Pm628024* (*Xwmc175*, *Xstars419*, *Xcinau130*, *Xwmc332,* and *Xcinau139*) were used to genotype three homoeologous group 2 nulli-tetrasomic lines. All target bands were amplified from N2AT2B and N2DT2A, but not from N2BT2A, confirming that all markers were located on chromosome 2B. We further used these markers to genotype deletion lines 2B-1, 2BL-3, 2BL-4, 2BL-5, and 2BL-6. All except *Xwmc175* amplified

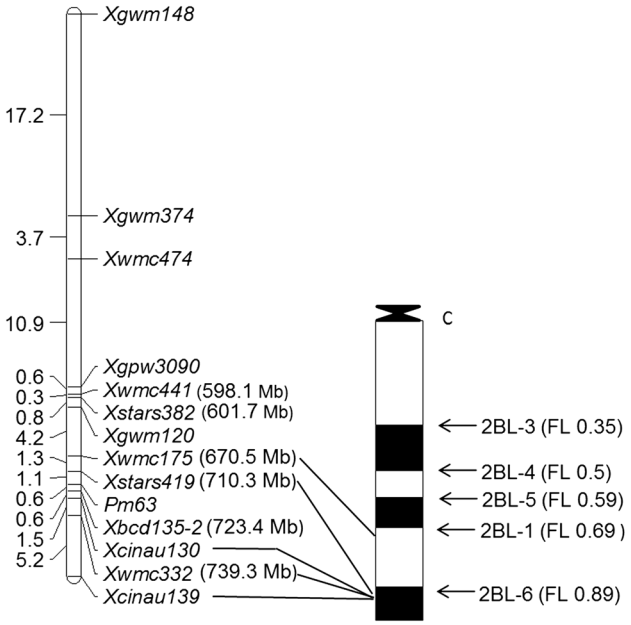

Fig. 1 Linkage (left) and physical bin map (right) of *Pm63* (originally Pm628024). Marker names are shown at the right of the linkage map and genetic distances in cM on the left. The physical positions of some markers on the Chinese Spring reference assembly IWGSC RefSeq v1.0 are given in the following parentheses. Molecular markers flanking *Pm63* are connected to their appropriate physical bins. The breakpoint of each Chinese Spring deletion line is shown with an arrow, and the corresponding fraction length (FL) value is given in the following parentheses

the target bands from Chinese Spring, but not from any of these deletion lines, indicating that *Xstars419*, *Xcinau130*, *Xwmc332*, *Xcinau139*, as well as *Pm628024*, reside in terminal bin 2BL6-0.89-1.0. *Xwmc175* amplified the target bands from Chinese Spring and 2BL-6, but not from the other four deletion lines, indicating that *Xwmc175* was present in bin 2BL1-0.69-0.89 (Fig. 1). The target band of *Xbcd135-2* was amplified from PI 628024, rather than Chinese Spring. Therefore, *Xbcd135-2* was not used in physical mapping.

Responses of *Pm628024*, *Pm6,* and *PmJM22* to *Bgt* isolates collected from different regions of the USA

A set of 16 representative *Bgt* isolates were used to determine the response spectrum of *Pm628024*. All isolates used in this study were virulent to CItr 11349 and the susceptible control Jagalene (Table 2). PI 628024 showed intermediate reactions to two isolates collected from North Carolina, *NCF-D-1-1* and *NCC-B-1-3*, and a susceptible reaction to an isolate collected from New York, *NYA-E-3-3*. The other 13 isolates were avirulent to PI 628024. *Pm6*, introgressed from *T. timopheevii* into bread wheat, was also evaluated because it was also mapped in the terminal bin of chromosome 2BL. *Pm6* was susceptible to 14 isolates and exhibited

Table 2 Responses of lines carrying *Pm63* (PI 628024), *Pm6* (Coker747), and *PmJm22* (Jimai 22) to 16 representative *Bgt* isolates collected from different regions of the USA

Bgt isolate			Wheat accession				
Name	Origin	Region	PI 628024	Coker747	Jimai 22	CItr 11349	Jagalene
GAP-B-2-2	Georgia	Southeast	R[a]	S	I	S	S
MSG-A-3-1	Mississippi	Southeast	R	S	–	S	S
MSG-C-3-4	Mississippi	Southeast	R	S	–	S	S
NCF-D-1-1	North Carolina	Mid-Atlantic	I	S	R	S	S
NCC-B-1-3	North Carolina	Mid-Atlantic	I	S	R	S	S
NYA-E-3-3	New York	Great Lakes	S	I	S	S	S
PAF-E-2-2	Pennsylvania	Great Lakes	R	S	I	S	S
MIR(14)-D-3-3	Michigan	Great Lakes	R	S	R	S	S
MIR(14)-E-1-3	Michigan	Great Lakes	R	S	R	S	S
NEI 3-1	Nebraska	Great Plains	R	S	R	S	S
NEI 5-5	Nebraska	Great Plains	R	S	I	S	S
OKH-A-2-3	Oklahoma	Great Plains	R	S	R	S	S
OKS-A-2-2	Oklahoma	Great Plains	R	S	R	S	S
OKS-B-2-2	Oklahoma	Great Plains	R	S	–	S	S
MTG1-3a	Montana	Western	R	S	I	S	S
MTG1-1a	Montana	Western	R	I	R	S	S

[a]*R*, *S*, and *I* represent resistant, susceptible, and intermediate reactions, respectively

an intermediate reaction to the remaining two isolates, *NYA-E-3*-3 and *MTG1-1a*, confirming that *Pm6* has been largely defeated in the USA (Cowger et al. 2018). Since *PmJM22* was previously identified in bread wheat cultivar Jimai 22 and mapped to the terminal bin 2BL6-0.89-1.0 (Yin et al. 2009), Jimai 22 was included in the panel. Jimai 22 was resistant to 1, 2, 2 and 3 isolates from the Western, Mid-Atlantic, Great Lakes, and Great Plains regions, respectively, and exhibited a susceptible reaction to *NYA-E-3-3*. Additionally, it showed an intermediate reaction to another four isolates (Table 1).

Allelism test

PI 628024, Jimai 22, and 1557 F_2 plants derived from PI 628024 × Jimai 22 were evaluated for responses to *OKS(14) B-3*-1. Both PI 628024 and Jimai 22 were immune (IT = 0), but 77 F_2 plants were highly susceptible, indicating that *Pm628024* was not allelic to *PmJM22*. A Chi-squared test of independence indicated that the two genes were loosely linked ($\chi^2_{15:1} = 4.53$, $df = 1$, $p = 0.03$), confirming that *PmJM22* resides on chromosome 2B. The estimated recombination fraction and genetic distance between *Pm63* and *PmJM22* were 0.3145 and 37 cM, respectively.

Discussion

Pm628024, as well as *Pm6*, *Pm33*, *Pm51*, *Pm52*, *PmJM22*, *MlZec1*, and *MlAB10,* was mapped to the long arm of chromosome 2B; all but *Pm52* were mapped to the terminal bin 2BL6-0.89-1.0 (Tao et al. 2000; Mohler et al. 2005; Zhu et al. 2005; Yin et al. 2009; Maxwell et al. 2010; Zhao et al. 2013; Zhan et al. 2014). *Pm52* was identified in Lingxing99, a winter wheat cultivar widely grown in Northern China, and was mapped to bin 2BL2-0.36-0.5 (Zhao et al. 2013). Therefore, *Pm628024* is not *Pm52*.

PmJM22 was identified in the Chinese winter wheat cultivar Jimai 22. It was 7.7 cM distal to SSR marker *Xwmc149* (Yin et al. 2009), positioned at 779.1 Mb in the Chinese Spring reference assembly (https://urgi.versailles.inra.fr). *Pm628024* was mapped to an interval spanning 710.3–723.4 Mb in the reference assembly. Thus, the physical distance between *Pm628024* and *PmJM22* is over 55.7 Mb. The allelism test further confirmed that *Pm628024* and *PmJM22* were not allelic, and the estimated genetic distance between them was 37 cM. The remaining five genes in the distal bin originated from wheat wild relatives, including *Pm6* from *T. timopheevii* (Helmsjørgensen and Jensen 1973), *Pm33* from *T. carthlicum* (Zhu et al. 2005), *Pm51* from *Thinopyrum ponticum* (Zhan et al. 2014), and *MlZec1* and *MlAB10* from *T. dicoccoides* (Mohler et al. 2005; Maxwell et al. 2010). More recently, a new adult plant resistance gene, *Pm62*, was transferred from *D. villosum* into common wheat in the form of Robertsonian translocation T2BS.2VL#5 (Zhang et al. 2018). Given that PI 628024 is a landrace and should not carry any alien chromosomal segment, *Pm628024* is not any of them.

A quantitative trait locus (QTL) for adult powdery mildew resistance, *QPm.uga-2BL*, was identified in the US soft red winter wheat cultivar 26R61 and mapped to a genomic region near SSR marker *Xbarc332*, positioned at

about 576.0 Mb in the Chinese Spring reference sequence IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr) (Hao et al. 2015). *QPm.uga-2BL* is over 150 Mb away from *Pm628024*. Another minor QTL for adult powdery mildew resistance on chromosome 2B, *QPm.vt-2B*, was detected in the US winter wheat cultivar 'Massey' and was mapped to the terminal region of chromosome 2BL spanning 681.5 Mb (*Xcdo244*)–795.4 Mb (*Xbcd1231*) of the Chinese Spring reference sequence (Liu et al. 2011). Given that *QPm.vt-2B* only explained a small portion (11%) of the phenotypic variance in the population derived from a cross between Massey and 'Becker,' it is unlikely the same gene as the all-stage resistance gene *Pm628024*. Altogether, *Pm628024* is a new powdery mildew resistance gene and has been designated *Pm63*.

Pm63 was mapped to a genomic region near *Pm6*, which has been widely used in wheat breeding and extensively characterized with molecular markers (Tao et al. 2000; Ji et al. 2008; Qin et al. 2011). In this study, *Pm6* was susceptible to almost all representative US *Bgt* isolates, confirming that *Pm6* has been defeated in the USA because of its extensive deployment in wheat cultivars (Cowger et al. 2018). A previous study indicated that *Pm6* was 1.6 cM distal to RFLP marker *Xbcd135* (Tao et al. 2000). Although two large populations were used to fine-map *Pm6*, its precise location is still elusive because of recombination suppression caused by the alien chromosome segment harboring *Pm6* (Qin et al. 2011). *Pm63* was 0.6 cM proximal to *Xbcd135-2*, an STS marker converted from *Xbcd135*. Given that recombination suppression is common between wheat and alien chromosomes (Qin et al. 2011), we cannot use an allelism test to determine whether *Pm63* is allelic to *Pm6*.

Pm51 was identified in a wheat-*Thinopyrum ponticum* introgression line CH7086 and was 7 cM distal to *Pm6* and 8.2 cM distal to *Xbcd135* (Zhan et al. 2014). Therefore, *Pm51* is more distal to *Pm63* than *Pm6*. The remaining three genes, *Pm33*, *MlZec1*, and *MlAB10*, were mapped to the terminal region of 2BL, and they are either close or distal to *PmJM22*. *Pm33* was introduced into bread wheat from *T. carthlicum* accession PS5. A previous study suggested that *Pm33* was 1.1 cM proximal to SSR marker *Xgwm317* (Zhu et al. 2005), while *PmJM22* was 7.7 cM distal to SSR marker *Xwmc149*. Sequence alignment analysis located *Xgwm317* and *Xwmc149* to approximately 782.4 Mb and 779.1 Mb in the Chinese Spring reference assembly, respectively. Therefore, *Pm33* is close to *PmJM22*. Both *MlZec1* and *MlAB10* were mapped to the end of chromosome 2BL. *MlZec1* was 10 cM distal to *Xwmc356*, and *MlAB10* was 7 cM distal to *Xwmc445*. *Xwmc356* and *Xwmc445* were located at approximately 796.7 Mb and 800.0 Mb in the reference assembly, respectively. Given that *Pm63* is physically distant to these genes, *Pm63* is unlikely allelic to any of them.

Pm63 exhibited high resistance to all *Bgt* isolates collected from the Great Plains and from western and southeast regions of the USA. Therefore, it can be used to enhance powdery mildew resistance in these regions. This is especially important because some powdery mildew resistance genes widely used in the Great Plains, such as *Pm3a* and *Pm17*, have been overcome in most of the USA (Cowger et al. 2018), and *Pm63* is an ideal alternative to these genes in the Great Plains. PI 628024 was susceptible to one of four *Bgt* isolates collected from the Great Lakes region and exhibited an intermediate reaction to two isolates from the Mid-Atlantic region (Table 2). Thus, *Pm63* must be combined with other genes in these mildew-prone regions. Gene pyramiding is a preferred strategy for prolonging powdery mildew resistance. Advances in wheat genomics have greatly facilitated gene stacking using marker-assisted selection. STS marker *Xbcd135-2* and SSR marker *Xstars419* were 0.6 cM distal and 1.1 cM proximal to *Pm63*, respectively, and have the potential to tag *Pm63* in wheat-breeding populations.

Conclusions

Powdery mildew poses a persistent threat to wheat production worldwide, and plant host resistance is a cost-efficient and environmentally friendly alternative to chemical control. However, mutation to virulence and recombination in *Bgt* populations often lead to erosion of resistance, necessitating a continuous search for resistance genes. *Pm63* is a dominant powdery mildew resistance gene identified in Iranian landrace PI 628024 and was mapped to an interval of approximately 13.1 Mb on the long arm of chromosome 2B, spanning 710.3–723.4 Mb in the Chinese Spring reference sequence. *Pm63* was located in the terminal bin 2BL6-0.89-1.0 and is different from other genes in origin, genomic location, and response to differential *Bgt* isolates. Therefore, *Pm63* should be considered a new powdery mildew resistance gene. *Pm63* exhibited resistance to all representative *Bgt* isolates collected from the Great Plains, western, and southeastern regions of the USA and can be used to enhance powdery mildew resistance in these regions.

Author contribution statement XX, BFC, CT, and GL designed the research; CT and GL performed the research; CC evaluated responses of differential lines to *Bgt* isolates; XX wrote the paper. All authors read, revised, and approved the manuscript.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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