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ORIGINAL ARTICLE

Pm223899, a new recessive powdery mildew resistance gene identified in Afghanistan landrace PI 223899

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Abstract

Key message **A new recessive powdery mildew resistance gene, *Pm223899*, was identified in Afghanistan wheat landrace PI 223899 and mapped to an interval of about 831 Kb in the terminal region of the short arm of chromosome 1A.**

Abstract Wheat powdery mildew, a globally important disease caused by the biotrophic fungus *Blumeria graminis* f.sp. *tritici* (*Bgt*), has occurred with increased frequency and severity in recent years, and some widely deployed resistance genes have lost effectiveness. PI 223899 is an Afghanistan landrace exhibiting high resistance to *Bgt* isolates collected from the Great Plains. An F_2 population and $F_{2:3}$ lines derived from a cross between PI 223899 and OK1059060-126135-3 were evaluated for response to *Bgt* isolate *OKS(14)-B-3-1*, and the bulked segregant analysis (BSA) approach was used to map the powdery mildew resistance gene. Genetic analysis indicated that a recessive gene, designated *Pm223899*, conferred powdery mildew resistance in PI 223899. Linkage analysis placed *Pm223899* to an interval of about 831 Kb in the terminal region of chromosome 1AS, spanning 4,504,697–5,336,062 bp of the Chinese Spring reference sequence. Eight genes were predicted in this genomic region, including *TraesCS1AG008300* encoding a putative disease resistance protein RGA4. *Pm223899* was flanked proximally by a SSR marker *STARS333* (1.4 cM) and distally by the *Pm3* locus (0.3 cM). One F_2 recombinant was identified between *Pm3* and *Pm223899* using a *Pm3b*-specific marker, indicating that *Pm223899* is most likely a new gene, rather than an allele of the *Pm3* locus. *Pm223389* confers a high level of resistance to *Bgt* isolates collected from Pennsylvania, Oklahoma, Nebraska, and Montana. Therefore, *Pm223389* can be used to enhance powdery mildew resistance in these states. *Pm3b-1* and *STARS333* have the potential to tag *Pm223389* in wheat breeding.

Introduction

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a globally important disease that occurs in most wheat-growing regions. Powdery mildew has occurred with an increased frequency in Europe, China, and many other countries in recent years (Morgounov et al. 2012). In the USA, the geographic range over which powdery mildew epidemics are sometimes or often severe is expanding from the traditional mid-Atlantic USA to southeastern states (Cowger et al. 2018), likely because of changing weather patterns and/or use of highly susceptible cultivars. A recent study indicated that there were substantial increases in the severity of powdery mildew overtime on a global level (Morgounov et al. 2012), and the predicted trend toward warmer winters in the eastern USA could increase the severity of *Bgt* epidemics by facilitating earlier epidemic onset (Cowger et al. 2018). Severe infection of powdery mildew

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Unfortunately, the caption of Figure 2 was incorrectly published in the original publication. The complete correct caption should read as follows.

Fig. 2 Graphical genotypes and phenotypes of critical F_2 plants and corresponding F_3 phenotypes. *Pm223899* was mapped to an interval flanked by *Pm3b-1* and *STARS333*. Only one plant is shown for each genotype. R, S, HR, HS, and Seg represent resistant, susceptible, homozygous resistant, homozygous susceptible, and segregating, respectively.

Also, under the "Discussion section", 3rd paragraph, the following sentence was incorrectly published and the complete correct sentence is given below.

There are 18 functional alleles at the *Pm3* locus (*Pm3a-Pm3r*) (Yahiaoui et al. 2004, 2009; Bhullar et al. 2009, 2010), and one of them, *Pm3a*, is widely used in the hard red winter wheat breeding programs in the Great Plains region (Li et al. 2016).

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can cause up to 40% yield loss, especially under humid rainfed and irrigated high-input conditions (Bennett 1984).

Cultivation of powdery mildew-resistant cultivars is an economical and environmentally friendly alternative to chemical control. A considerable number of powdery mildew resistance genes have been identified and used in wheat breeding (McIntosh et al. 2013, 2017). However, virulence shifts in *Bgt* populations lead to the rapid breakdown of powdery mildew resistance genes in mildew-prone regions. For example, *Pm17* was commercially deployed in 2004 and began to lose effectiveness in the mid-Atlantic USA in 2009 (Griffey et al. 2005a, b; Cowger et al. 2009). A recent study indicated that *Pm3f*, *Pm6*, and *Pm8* were entirely or largely defeated across the USA, whereas *Pm2*, *Pm3a*, *Pm3b*, and *Pm4a* were defeated in many regions. Moreover, widespread planting of cv. DG Shirley, believed to possess the previously widely effective *Pm1a*, has led to the emergence of *Pm1a* virulence in the North Carolina *Bgt* population in the last two years (C. Cowger, unpublished), indicating the challenge of breeding for durable resistance to powdery mildew.

The short-lived nature of powdery mildew resistance genes necessitates a continuous search for new resistance sources and pyramiding of multiple genes into a single cultivar. Although slow mildewing genes, such as *Pm38* and *Pm39* (Lillemo et al. 2008), are expected to offer durable powdery mildew resistance because of their race non-specific nature, they provide only partial resistance. Therefore, combination of slow mildewing genes with race-specific, highly resistant powdery mildew resistance genes is preferred for adequate and durable resistance. An alternative approach is to combine multiple race-specific genes in a single cultivar, which makes it difficult for *Bgt* strains to infect because mutations at multiple loci in the pathogen are required.

A prerequisite of gene pyramiding is to identify molecular markers closely linked to the genes of interest. Simple sequence repeat (SSR) markers have been widely used in wheat linkage mapping, and many SSR markers have been used to tag powdery mildew resistance genes in wheat breeding and wheat genetic studies. Although there is increased interest in using single nucleotide polymorphism (SNP) markers, SSR markers still play a unique role in genotyping segregating populations, because the co-dominant nature of SSR markers allows for unambiguous genotyping. The recent release of the Chinese Spring reference genome sequence makes it feasible to reveal all SSR loci in any region, permitting development of adequate SSR markers for precise mapping of target genes.

Wheat landraces are important resistance sources, and at least 18 powdery mildew resistance genes have been identified in landraces, including *Pm2c* on chromosome 5DS (Xu et al. 2015), *Pm3b* on *1AS* (Yahiaoui et al. 2004), *Pm5d* and *Pm5e* on *7BL* (Hsam et al. 2001; Huang et al. 2003), *Pm24a* and *Pm24b* on 1DS (Huang et al. 2000a; Xue et al. 2012), *Pm45* on 6DS (Ma et al. 2011), *Pm47* on *7BS* (Xiao et al. 2013), *Pm59* on 7AL (Tan et al. 2018), and *Pm61* on 4AL (Sun et al. 2018). In addition, the temporarily named genes *MlHLT* and *PmX* were mapped to chromosomes 1DS and 2AL (Wang et al. 2015; Fu et al. 2013), respectively, and another six genes, *PmTm4* (Hu et al. 2008), *MlXBD* (Huang et al. 2000b), *Mlmz* (Zhai et al. 2008), *pmHYM* (Fu et al. 2017), *PmBYYT* (Xu et al. 2018a), and *PmSGD* (Xu et al. 2018b), were mapped to a region near the *Pm5* locus on chromosome 7BL. Compared with genes originating from wild species, powdery mildew resistance genes identified in landraces can be more easily used in wheat breeding because of the lack of linkage drag.

Li et al. (2016) reported that PI 223899 (formerly Gandom), a landrace collected from Badakhshan in Afghanistan, exhibited resistance to *Bgt* pathotypes collected in Oklahoma and suggested that PI 223899 has potential for use in wheat improvement. The objectives of this study were to determine the genomic location of the powdery mildew resistance gene in PI 223899 and develop linked markers for wheat breeding.

Materials and methods

Plant materials

An F_2 population of 221 plants developed from the cross PI 223899 × OK1059060-126135-3 was evaluated for powdery mildew response, and all plants were then transferred to a greenhouse after being vernalized for 6 weeks at 4 °C. The F_3 families were evaluated in the following season. In addition, a set of control lines carrying *Pm3a*, *Pm3b*, *Pm17*, and *Pm8* were also tested.

Evaluation of powdery mildew resistance

The F_1 plants, F_2 population, and $F_{2:3}$ families were evaluated for powdery mildew response at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit at Stillwater using a previously described procedure (Tan et al. 2018). In brief, the F_2 population was evaluated with *Bgt* isolate *OKS(14)-B-3-1* in 2016. Each tested plant was grown in a single cell of 135-cell growing trays containing Sunshine Redi-earth growing mix (Sun Gro Horticulture Canada Ltd.) and inoculated at the two-leaf stage as described by Li et al. (2016). 'TAM110' and 'Jagalene' were planted in each growing tray as the resistant and susceptible checks, respectively. The inoculated plants were grown under natural light at 20 ± 2 °C in a greenhouse, and powdery mildew infection types (IT) were recorded 7–10 days after inoculation using a 0–4 scale, in which 0, 0;, and 1 represented highly resistant

responses, while 2, 3, and 4 indicated moderately resistant, moderately susceptible, and highly susceptible, respectively. Each plant was reexamined 2 days after the initial investigation. The criteria for each IT score were described earlier (Tan et al. 2018). A total of 221 $F_{2:3}$ lines were evaluated for response to *OKS(14)-B-3-1* in 2017 using a randomized complete block design with two replicates. For each $F_{2:3}$ line, 16 plants were planted in two cells of a 73-cell growing tray containing Sunshine Redi-earth growing mix in each replicate, and the protocol described above was implemented. The genotype of each F_2 plant was inferred from the corresponding $F_{2:3}$ phenotypic data.

PI 223899, together with Jagalene and four testers carrying *Pm3a*, *Pm3b*, *Pm17*, and *Pm8*, was evaluated for response to 18 *Bgt* isolates collected from different regions of the USA and maintained by the USDA-ARS Plant Science Research Unit at Raleigh, North Carolina. The detached-leaf approach described by Cowger et al. (2018) was used to assess powdery mildew responses on a 0–9 scale, which distinguished resistant (0–4), intermediate (5–6), and susceptible (7–9) reactions.

Analysis of SSR markers and genic markers

Genomic DNA was extracted from 2-week-old leaves using a previously described method (Dubcovsky et al. 1994). For each SSR assay, about 50 ng of genomic DNA was used in a volume of 10 µl containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1X PCR buffer, 0.25 unit of Taq DNA polymerase, and 0.2 mM of each primer. The reaction mixtures were denatured at 95 °C for 5 min, followed by 39 cycles of 95 °C for 30 s, 50–60 °C (depending on the annealing temperature) for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were separated in 6–10% non-denaturing polyacrylamide gels (29:1 acrylamide/bisacrylamide ratio) and visualized under UV light after stained with ethidium bromide.

Two sequence-tagged site (STS) markers developed from the *Pm3b* sequence, *Pm3b-1* (forward, 5′-TGCCTAGAA GATCTATGCTTATCAG; and reverse, 5′-CATGCCAGC ACAGTTCAG) and *Pm3b-2* (forward, 5′-TGTTCAGTT GTGGTACATCCT; and reverse, 5′-GACTGTACCAAC CTATAACCTC) (Xu et al. 2006), were used to genotype the mapping population. For a 10-µl PCR, 50 ng of genomic DNA was added to a PCR mixture containing 0.2 mM dNTP, 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.25 units of *Taq* polymerase, 0.2 mM of each pair of STS primers. The PCR cycles consisted of an initial step of 94 °C for 5 min, followed by 36 cycles of 30 s at 94 °C, 30 s at 48 °C, and 40 s at 72 °C, with a final step of 7 min at 72 °C. PCR products were separated in 1.5% agarose gels and visualized under UV light after stained with ethidium bromide.

Bulked segregant analysis

Based on F_2 genotypes inferred from $F_{2:3}$ progeny's phenotypes, DNA from each of 10 homozygous resistant and 10 homozygous susceptible F_2 plants were pooled to construct resistant and susceptible bulks, respectively. The contrasting bulks and parental DNA samples were screened with more than 600 SSR markers that are evenly distributed across all wheat chromosomes to find informative markers exhibiting polymorphism between the bulks and parents.

A single informative marker was used to genotype the F_2 population, leading to identification of a SSR marker associated with powdery mildew response. Additional SSR and genic markers previously mapped in the target region were also used to genotype the mapping population.

Development of SSR markers in the target region of the wheat genome

Based on Chinese Spring reference sequence IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr), all SSR loci in the genomic region around the powdery mildew resistance gene were identified, and primers were designed for a set of 36 SSR loci located in non-transposon regions using the GMATA software (Wang and Wang 2016). These new SSR markers, designated with prefix 'STARS' (representing Stillwater ARS) and a consecutive number (Table 1), were employed to genotype the mapping population.

Data analysis

Chi-squared tests were conducted to test the goodness of fit of observed phenotypic data to expected Mendelian ratios for a single gene. Mapmaker 3.0b (Lincoln et al. 1993) was employed to construct the genetic linkage map using the Kosambi function (Kosambi 1943), and a logarithm of the odds score of 3.0 was used as the threshold. MapDraw software (Liu and Meng 2003) was used to draw the linkage map.

Gene annotation

Genes were predicted, but were not annotated in Chinese Spring IWGSC RefSeq v1.0 (https://urgi.versailles.inra.fr). High-confidence genes in the target region were annotated using BlastX searches against the NCBI and Pfam databases for function prediction.

Table 1 Primer sequences, genomic locations, product sizes, and SSR motifs of 36 newly developed SSR markers. The Tm values of all SSR markers are 60 °C

Marker name	Forward primer sequence	Reverse primer sequence	Position	Product size (bp)	SSR motif	Mapped
STARS305	CTCATTGCAACTTGGATGTACG	TTCAGGCCCACAAAAGGTAT	2994428:2994469	195	(GT)21	
STARS306	CGCACGTAACTCAGCCTCCTC	TCTCACCCTTCACCACTCCT	3127584:3127627	189	(GT)22	Yes
STARS307	CGCATTGCTGCACAGATGATT	TGGCTGTTCAATGTGGATGT	3756360:3756403	165	(TG)22	
STARS308	CGTCATGTCGTAGGGCGTCTT	GCCTATCGGCCAGCAGTA	3778239:3778294	210	(TC)28	Yes
STARS309	CGTCGACATCATGCAGCAAAC	TGTAGAGGAGACGCAAACACC	4056759:4056802	301	(TG)22	Yes
STARS310	CCTCCATCATCACCCAGCTTC	TAAAGCAACCACCAAGCACA	4719427:4719470	310	(GT)22	
STARS311	CGGAGGCACATGTTTGCTCTT	CAAGGTCACTCCCACCTCAT	5519840:5519921	390	(AT)41	
STARS312	CAGTGTTCGGTCCTTTGTTGG	GGTTGCCGCTGATATTGTTT	6717570:6717613	340	(TA)22	Yes
STARS313	CTCGTGAAGTTGCAAAGAACG	ATTTGTTCGGGCGATAGTCA	7017534:7017593	261	(TA)30	Yes
STARS314	CTGCACCAGCACATTTAGGTC	TGGGAAGATTGCTCTCCATT	7113142:7113193	385	(TC)26	Yes
STARS315	CTTCATCGAATCGCAAAACTG	GTGTGTGTGTGTGCGTGTGT	7199808:7199847	212	(AC)20	
STARS316	CACGTGATTCCCTTGAGATGC	GTGCATGATGGCTTCAATCT	7226720:7226767	383	(AG)24	Yes
STARS320	CCAACGCATGCTCAAGAGGTA	ATGCACCCATTACCGAGAAG	3155836:3155859	388	(AC)12	
STARS321	CCTCCGATTTCCAGAATCCAA	CAGACTCCTCCTCGTCGTTC	3391551:3391582	239	(AT)16	
STARS322	CTGTCTGTGAGCTGGGTTCAG	GTATAGTGGCCGCTCGTTGT	3429822:3429860	324	(TAT)13	Yes
STARS323	CCTTCCAATTGTCACCGTGCT	TGGTAACACCAACCGGTACT	3753967:3753990	390	(TA)12	
STARS324	CGCTGGTTGTTGAGGTTGGAT	TCAATGATCTTGCCACGAAG	3845984:3846028	248	(TTG)15	Yes
STARS325	CCACTCCATTTTCCCTGCTGT	GATTTGCCAGGGATCTGAAA	4054698:4054712	280	(ATC)5	
STARS326	CCTATCTGGTGTGGCTGCAAA	CAGCAACTAAACCCATGCAA	4096006:4096029	376	(TTG)8	Yes
STARS327	CTGTTGTGAAAACGGTGGTTG	GTCTTTTCCCTCCTCGCTCT	4096615:4096629	236	(GTT)5	
STARS328	CTGGGGTTGTTGTTGCTGATA	AGCAACAACAACAACCACCA	4202495:4202512	191	(TGT)6	
STARS329	CATGGATCAGTGGGGTTAGCA	GCCGCTCTTCTTCTTCCTCT	4233820:4233839	315	(GT)10	
STARS330	CTCCCAACTCCCGTTTATCAG	GAGCTCGGGATCTGTTCTTG	4497491:4497508	333	(CTG)6	
STARS331	CTGGTCCGAATGTTTAGCACA	GGGCGCACAAATAAGTTCAC	4783229:4783246	295	(CT)9	
STARS332	CTTTATGGGCCGTTAATCTGG	TGGAAAAGATTGCGGAGAAC	5033327:5033336	501	(TC)5	
STARS333	CACACTTTGCAGCATGGATCA	GCGCACAACTATCTCCTAAGC	5336062:5336085	257	(GA)12	Yes
STARS334	CACACTTTGCAGCATGGATCA	GGAGGCAAGGACCTCATGTA	5371429:5371456	299	(GA)14	Yes
STARS335	CTCATTAAGCACACGGACTCG	CATCGCTCATGCTAAGGTCA	5610958:5610979	400	(TC)11	
STARS336	CGCTCTTGTGCTCTTCCTTGG	AAGCAGCTGGATTTGATGCT	6048693:6048722	353	(GT)15	
STARS337	CTGAAGGCGTTGTTGTTGAAG	TAGGATGGACACAGCCAACA	6223040:6223055	233	(TG)8	
STARS338	CGCAAATGCACATCGCTTATG	CTATCCGTGGTCGTGTCCTT	6402851:6402860	347	(TA)5	
STARS339	CGCAACCTGGAAAAGCAGAGT	GCCACATTTCTTGCTTAATGG	6472108:6472147	348	(ATAC)10	
STARS340	CCAAGCATCAAAACCAAGCAA	AAATGGTGGTCCCTGTGGTA	6517580:6517603	306	(AAC)8	
STARS341	CATGGGAAGCATCTCAACCTC	AGAAAGAGTTGCTCGCAAGG	6668294:6668319	376	(GA)13	
STARS342	CCAGAATTCACGGGTGCATAA	CGCACGTAGGAAACAAACAA	6871179:6871188	396	(TC)5	
STARS343	CTCGTTGCTTCTGTGGTTGAG	CTATTGCAACCGTCTCGTCA	6907436:6907445	180	(CT)5	Yes

Results

Inheritance of powdery mildew resistance in PI 223899

PI 223899 was highly resistant to *Bgt* isolate *OKS(14)-B-3-1* with IT 0;, and OK105960-126135-3 was susceptible with IT 4. F_1 plants were susceptible, and the F_2 population segregated with 47 resistant and 174 susceptible plants, suggesting that resistance was conferred by a single recessive allele ($\chi^2_{1:3} = 1.62$, df = 1, $p = 0.2$).

The F_3 progeny tests confirmed the single locus segregation; 47 and 58 lines were classified homogenous resistant and homogeneous susceptible, respectively, and the remaining 116 lines segregated, confirming that PI 223899 carries a recessive powdery mildew resistance gene ($\chi^2_{1:2:1} = 1.64$, $df = 2$, $p = 0.44$) that was designated *Pm223899*.

Mapping of the powdery mildew resistance gene in PI 223899

BSA using more than 600 SSR primer pairs detected a single marker, CFA2153, on chromosome 1AS that distinguished the resistant and susceptible bulks and parents. After genotyping the entire F_2 population for the marker, genetic distance between *CFA2153* and *Pm223899* was estimated to be 8.2 cM. A set of SSR markers mapped to the terminal region of 1AS was then screened for polymorphism between the two parents, leading to identification of *PSP2999*. *PSP2999* was 6.1 cM distal to *Pm223899*.

Given that *PSP2999* was closely linked to the *Pm3* locus (Xu et al. 2006), positioned at approximately 4.5 Mb of the Chinese Spring reference assembly, we identified all SSR loci in the genomic region possibly harboring *Pm223899*; this ranged from 2.99 to 7.23 Mb in the reference sequence. A total of 526 SSR loci were identified in the region, and 36 loci located in non-transposon regions were chosen for marker development. Of these, 13 markers were polymorphic between PI 223899 and OK105960-126135-3, and the remaining 23 SSR markers were monomorphic (Table 1). The polymorphic markers were subsequently used to genotype the F_2 population.

Based on F_2 genotypic data, a linkage map of 25 cM was constructed. The 13 newly developed SSR markers, located from 3.12 to 7.22 Mb on the Chinese Spring physical map, covered 23.2 cM. The orders of newly developed SSRs on the linkage map were consistent with their physical positions on the Chinese Spring reference assembly (Fig. 1). Linkage analysis placed *Pm223899* to an interval of 3 cM, flanked by *STARS326* and *STARS333* (Fig. 1).

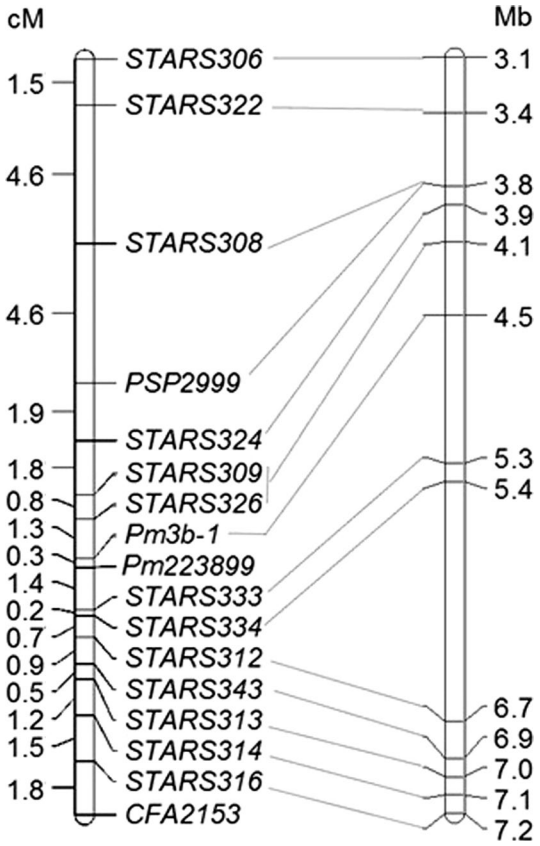

Fig. 1 Linkage (left) and physical map (right) of *Pm223899*. Marker names are shown at the right of the linkage map, and genetic distances in cM on the left. The physical positions of molecular markers are given at the far right of the physical map. The precise positions (in bp) of these markers are given in Table 1

Responses of lines containing *Pm223899*, *Pm3a*, *Pm3b*, *Pm17*, and *Pm8* to *Bgt* isolates from different regions of the USA

Lines possessing *Pm223899* and other genes located on 1AS were phenotyped with 18 *Bgt* isolates collected from different regions of the USA (Table 2). PI 223899 with *Pm223899* showed resistance to isolates collected from Pennsylvania, Oklahoma, Nebraska, and Montana, indicating that *Pm223899* can be used to enhance powdery mildew resistance in these states. However, the representative *Bgt* isolates from Georgia, Mississippi, North Carolina, New York, and Michigan were virulent.

Two alleles at the *Pm3* locus, *Pm3a* and *Pm3b*, were also tested for responses to these differential isolates (Table 2). Both alleles were overcome by the isolates from the southeast and mid-Atlantic regions, but showed resistance to three isolates from Oklahoma and two from Montana. *Pm3a* exhibited resistance to a Nebraska isolate, and *Pm3b* conferred resistance to two Michigan isolates and one Pennsylvania isolate. In addition, *Pm17*, located on a wheat-rye 1AL/1RS translocation segment, exhibited either susceptible or intermediate reactions to all isolates in the panel, further confirming that *Pm17* has been largely defeated in the USA. Similar results were observed with the rye-derived *Pm8* gene, located on a wheat-rye 1BL/1RS translocation segment. *Pm8* conferred resistance to only one isolate collected from New York, NYA-E-3-3, and showed either susceptible or intermediate reactions to the other 17 isolates.

Recombination between *Pm3* and *Pm223899*

Pm223899 was mapped to a genomic region near *Pm3*. Therefore, it was essential to determine its relationship with *Pm3*. Xu et al. (2006) developed *Pm3b*-specific markers *Pm3b-1* and *Pm3b-2* from the *Pm3b* sequence (Yahiaoui et al. 2004). We used these markers to genotype two parents. *Pm3b-1* was polymorphic and was used to genotype the F_2 population. One recombination was identified between *Pm3* and *Pm223899* in plant 69, which was homozygous susceptible (*pm223899 pm223899*) and heterozygous at the *Pm3b-1*

Table 2 Responses of lines containing *Pm223899*, *Pm3a*, *Pm3b*, *Pm8*, and *Pm17* to *Bgt* isolates collected from different regions of the USA

Bgt isolate			Resistance gene					
Name	State	Region	*Pm3a*	*Pm3b*	*Pm8*	*Pm17*	*Pm223899*	Jagalene
GAP-A-2-3	Georgia	Southeast	S[#]	S	S	I	S	S
GAP-B-2-2	Georgia	Southeast	S	S	S	I	S	S
MSG-A-3-1	Mississippi	Southeast	S	S	I	I	S	S
MSG-C-3-4	Mississippi	Southeast	S	S	S	I	I-S	S
NCF-D-1-1	North Carolina	Mid-Atlantic	S	S	S	I	S	S
NCC-B-1-3	North Carolina	Mid-Atlantic	S	S	I	S	S	S
NYA-E-3-3	New York	Great Lakes	S	S	R	I	S	S
NYB-E-1-2	New York	Great Lakes	S	S	S	I	S	S
MIR(14)-D-3-3	Michigan	Great Lakes	S	R	S	I	S	S
MIR(14)-E-1-3	Michigan	Great Lakes	S	R	S	I	S	S
PAF(14)-D-1-2	Pennsylvania	Great Lakes	S	R	S	I	R	S
PAF-E-2-2	Pennsylvania	Great Lakes	S	S	I	I	R	S
OKH-A-2-3	Oklahoma	Plains	R	R	I	I	R	S
OKS-A-2-2	Oklahoma	Plains	R	R	S	S	R	S
OKS-B-2-2	Oklahoma	Plains	R	R	S	I	R	S
NEI 1-3	Nebraska	Plains	R	S	S	S	R	S
MTG1-3a	Montana	Northwest	R	R	i	I	R	S
MTG1-1a	Montana	Northwest	R	R	I	I	R	S

#R, S, and I represent resistant, susceptible, and intermediate responses, respectively

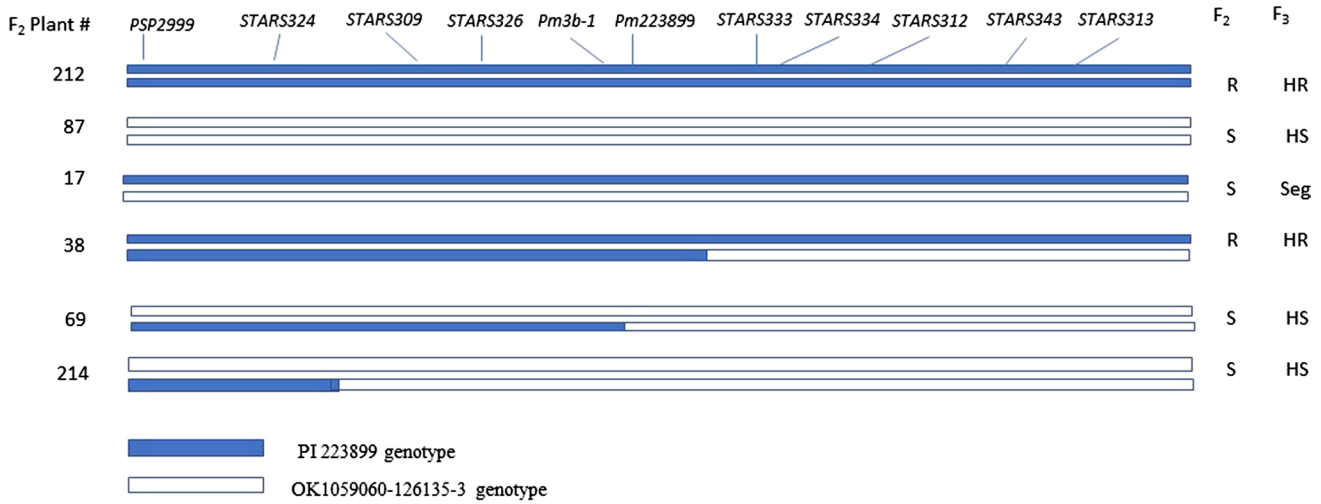

Fig. 2 Graphical genotypes and phenotypes of critical F_2 plants and corresponding F_3 phenotypes. *Pm223899* was mapped to an interval flanked by *Pm3b-1* and *STARS333*. Only one plant is shown for each genotype. R, S, HR, HS, and Seg represent resistant, susceptible, homozygous resistant, homozygous susceptible, and segregating, respectively

locus (*Pm3b-1 pm3b-1*) (Fig. 2), suggesting that *Pm223899* and *Pm3* are different loci. The genotype of plant 69 was further confirmed by genotyping and phenotyping 16 additional F_3 plants. The estimated genetic distance between *Pm3* and *Pm223899* was 0.3 cM (Fig. 1). Based on the physical locations of *Pm3* and *STARS333* on the Chinese Spring reference assembly, *Pm223899* resides in an 831-Kb genomic region from 4,504,697 to 5,336,062 bp in the Chinese Spring reference.

Predicted genes in the target region

Eight genes, *TraesCS1AG008200–TraesCS1AG008900*, were predicted in the genomic region spanning 4,504,697–5,336,062 bp in the Chinese Spring reference (https://urgi.versailles.inra.fr). *TraesCS1AG008300* encodes an analog of putative disease resistance protein RGA4 (resistance gene analog 4), which together with RGA5 in rice directly binds with *Magnaporthe oryzae* avirulence

proteins AVR-Pia and AVR1-CO39 to induce hypersensitive responses (Cesari et al. 2013). Another gene, *TraesCS1AG008800*, encodes a dirigent-like protein induced during disease response in plants. *TraesCS1AG008400*, *TraesCS1AG008500*, and *TraesCS1AG008600* were annotated as 2′-deoxymugineic-acid 2′-dioxygenase, uncharacterized acetyltransferase, and alpha-humulene synthase genes, respectively, and the functions of *TraesCS1AG008200*, *TraesCS1AG008700*, and *TraesCS1AG008900* are still unknown.

Discussion

Powdery mildew poses a persistent threat to wheat production worldwide. Identification and deployment of new powdery mildew resistance genes are essential for reducing large-scale yield losses caused by the breakdown of host resistance. In this study, we identified a recessive powdery mildew resistance gene, *Pm223899*, in an Afghanistan landrace and located it to the terminal region of chromosome 1AS.

Of the known powdery mildew resistance genes, *Pm3* and *Pm17* were mapped to chromosome 1AS. *Pm17* is an alien resistance gene derived from rye (Mohler et al. 2001). Given that PI 223889 is a landrace, *Pm223899* is unlikely *Pm17*. Analysis of a diagnostic marker for the *Sec-1* locus of rye (Shimizu et al. 1997) indicated the absence of chromosome 1RS in PI 223899, confirming that *Pm223899* is not *Pm17*.

Pm223899 was mapped to an interval of about 831 Kb flanked by *Pm3* and *STARS333*. A recombinant between *Pm3* and *Pm223899* was identified among the F_2 plants. The presence of the recombinant indicated that *Pm223899* is a new gene different from the *Pm3* locus. There are 18 functional alleles at the *Pm3* locus (*Pm3a-Pm3r*) (Yahiaoui et al. 2004, 2009; Bhullar et al. 2009, 2010), and one of them, *Pm3a*, is widely used in the hard red winter wheat breeding programs in the Great Plains region (Li et al. 2016). A recent study indicated that 12–14% of *Bgt* isolates collected in this region in 2013 and 2014, as well as 90–100% of isolates collected in other regions of the USA, were virulent to *Pm3a* (Cowger et al. 2018), suggesting that new powdery mildew resistance genes are required in the Great Plains. PI 223899 is highly resistant to *Bgt* isolates collected from the Great Plains, Pennsylvania, and Montana and can be used as an alternative resistance source in these regions, but needs to be combined with other resistance genes to ensure any level of durability. Molecular markers closely linked to *Pm223899*, such as *Pm3b-1* and *STARS333*, have the potential to tag *Pm223389* in wheat breeding.

A set of eight genes were predicted in the interval in which *Pm223899* was located, including *TraesCS1AG008300*, an R gene encoding an RGA4 protein. To date, five dominant powdery mildew seedling resistance genes, *Pm2*, *Pm3b*, *Pm8*, *Pm21*, and *Pm60* (Yahiaoui et al. 2004; Hurni et al. 2013; Sánchez-Martín et al. 2016; Xing et al. 2017; Zou et al. 2018), have been cloned. Of these, *Pm2* and *Pm3b* were identified in bread wheat, while *Pm8*, *Pm21*, and *Pm 60* originated from rye, *Haynaldia villosa*, and *Triticum urartu*, respectively. All of them are R genes encoding coiled-coil nucleotide binding site leucine-rich repeat (CC-NBS-LRR) domain proteins (Yahiaoui et al. 2004; Hurni et al. 2013; Xing et al. 2017; Zou et al. 2018). *Pm223899* is a recessive gene, and the underlying mechanism may be different from these dominant genes. A previous study indicated that the rice resistance protein pair RGA4/RGA5 directly binds with *Magnaporthe oryzae* avirulence proteins AVR-Pia and AVR1-CO39 to induce hypersensitive responses (Cesari et al. 2013). Thus, *TraesCS1AG008300* is likely a candidate gene for *Pm223899*. In addition, another gene in the target region, *TraesCS1AG008800*, is also involved in plant defense, and the functions of three other genes are still unknown. Further cloning of *Pm223899* is essential for understanding the mechanism of powdery mildew resistance in wheat.

Author contribution statement XX, GL, BFC, and GB designed the research; 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 The authors declare that they have no conflicts of interest.

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