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Molecular detection of disease resistance genes to powdery mildew (*Blumeria graminis* f. sp. *tritici)* in wheat (*Triticum aestivum*) cultivars

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A study was conducted to detect the presence of disease resistance genes to infection of wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) in selected wheat cultivars from China using molecular markers. Genomic DNA of sixty cultivars was extracted and tested for the presence of selected prominent resistance genes to the fungal disease using molecular markers linked to *Pm* genes. Results showed that 17 cultivars were detected with *Pm2* gene, 24 cultivars were detected with *Pm4b* gene, two cultivars were detected with *Pm6* gene while 24 wheat cultivars were detected with *Pm8* gene. Multiple genes were also detected in the study. Cultivars Xinxuan2039, Lankao008 and Zhengmai366 were detected with possible multiple *Pm2+Pm4b+Pm8* genes while Yumai368 was detected with possible multiple genes *Pm2+Pm4b+Pm6*. The results of this study provide a significant contribution to breeding for resistance to wheat powdery mildew disease since the identified cultivars detected with *Pm* genes will contribute to further studies on improving wheat resistance to the disease. Also, the continued resistance of cultivars with designated resistance genes demonstrates that the responsible *Pm* genes are still effective in overcoming powdery mildew infections.

Key words: *Blumeria graminis* f. sp. *tritici*, wheat powdery mildew, disease resistance, *Pm* genes, *Triticum aestivum*, molecular marker.

INTRODUCTION

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici (Bgt)*, is one of the most damaging foliar diseases of wheat in most parts of the world, especially in cool and humid areas (Cao et al., 2013; Li et al., 2013; Peng et al., 2014; Mandal et al., 2015; Wang et al., 2015). Countries such as China, United Kingdom, Germany, Japan, Russia, South and West Asia, North and East Africa and the Southeastern United States are

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highly affected by the damage caused by the disease (Bennet, 1984; Alam et al., 2011). The disease causes devastating effects both in grain yield and quality (Everts et al., 2001; Conner and Kuzyk, 2003; Asad et al., 2014). The affected area for powdery mildew from 2001 to 2006 in China was 5.9 to 9.4 million hectares resulting in 0.26 to 0.32 billion kilograms reduction in grain yield (Xue et al., 2009).

Currently, the yield losses range from 13 to 34% under high infestation in the field but during epidemic seasons, the disease can cause up to 50% yield loss (Yao et al., 2007; Zhang et al., 2008; Li et al., 2011; Alam et al., 2013; Quijano et al., 2015; Yu et al., 2015). The shift in pathogenic virulence structures range from the southwest and the southeast coastal regions of China to almost all the wheat growing areas throughout the country and the damage exceeds any other known wheat disease (Liu and Shao, 1994; Luo et al., 2009; Cao et al., 2013; Zhao et al., 2013; Shen et al., 2015).

In an attempt to control the diseases, use of resistant or tolerant cultivars has been one of the effective methods widely used by scientists as it is economical as well as environmentally friendly (Song et al., 2009; Ben-David et al., 2010). Several wheat cultivars are being developed and tested for resistance to wheat powdery mildew but their resistance is easily broken down due to continuous development of new and more virulent strains through genetic recombination (Piarulli et al., 2012; Hurni et al., 2014). Some of the developed cultivars carrying resistance genes to wheat powdery mildew lose their resistance even before they are made available for commercial production (Hao et al., 2015; Ma et al., 2015). It is, therefore, important that the development of resistant cultivars should target multiple resistances to disease isolates so that effective and lasting control can be attained. Assessment of disease resistance on new wheat cultivars can be effective when the cultivars are subjected to existing as well as new strains of powdery mildew isolates over a considerable period of time (Li et al., 2012).

In developing resistant cultivars, several powdery mildew resistance genes have been identified and mapped in wheat. The mapping has helped in locating and subsequent introgression of the genes into susceptible cultivars carrying other desirable traits (Huang and Roder, 2004; Xiao et al., 2013; Li et al., 2014). To date, about 78 formally designated *Pm* genes (*Pm1–Pm54, Pm1c = Pm18*, *Pm1e = Pm22*, *Pm4c = Pm23*, *Pm21 = Pm31*) have been catalogued at 50 loci (Hao et al., 2015; Petersen et al., 2015). Among them, loci *Pm1*, *Pm3*, *Pm4*, *Pm5* and *Pm24* have 5, 17, 4, 5 and 2 alleles, respectively (Xie et al., 2012; Mohler et al., 2013; McIntosh et al., 2013, 2014).

Sources of these genes have been the cultivated or wild relatives of *Triticum* species and then they got transferred to common wheat. For example, resistance gene *Pm2* originated from the wild species *Ae. tauschii* that got introgressed into common wheat *T. aestivum* (Lutz et al., 1995). Powdery mildew resistance gene *Pm4a* originated from *T. diccocum* while *Pm4b* originated from *T. carthlicum* (Briggle, 1969; Law and Wolfe, 1966). Resistance gene *Pm6* was transferred from *T. timopheevii* (Jorgensen and Jensen, 1972). Another distant wild cultivar, *S. cereale*, is where resistance gene *Pm8* came from (Hsam and Zeller, 1997) while gene *Pm21* and *Pm30* came from wild relatives *H. villosa* and *T. dicoccoides*, respectively (Chen et al., 1995; Liu et al., 2002). These genes are common in cultivars grown in Asian region and gene *Pm21* has been very effective against a broad-spectrum of wheat powdery mildew isolates found in China (Song et al., 2009). Currently, over 56 cultivars being grown in China possess *Pm4b* and *Pm8* genes (Wang et al., 2005; Zeng et al., 2014), while most red soft winter cultivars carrying gene *Pm8* are widely grown in southeastern USA (Cowger et al., 2009; Hao et al., 2012).

This study, therefore, was aimed at detecting the presence of some prominent wheat powdery mildew resistance genes in selected wheat cultivars using molecular markers linked to the genes.

MATERIALS AND METHODS

Sixty wheat cultivars from different parts of China were identified for the study (Table 1). Ten seeds of each cultivar were sown on trays in greenhouse located at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. Around 15 days after sowing, when three to four leaves had been developed, seedling leaves were detached and their DNA was extracted following cetyltrimethylammonium bromide (CTAB) extraction method (Zheng, 2010). Chancellor, a susceptible cultivar with no known *Bgt* resistance genes, was used as negative control.

Seven additional cultivars with known wheat powdery mildew resistant genes were selected and used as positive controls (Table 2). Hence, a total of 67 cultivars were selected for the molecular study using various molecular markers linked to wheat powdery mildew resistance genes. To test their purity, the extracted DNA was run on 2% Agarose gel of 1% TAE (Tris-Acetate-EDTA) buffer solution and the image was captured using Gel Documentation and Image Analysis System after staining in ethidium bromide solution for 10 min.

Following extraction and dilution to a concentration ranging between 50 and 80 ng/µl, the genomic DNA samples were amplified in a polymerase chain reaction (PCR) machine using the following mixture: 5 µl containing PCR Master mix, 2 µl of double distilled water, 1 µl reverse primer and 1 µl of forward primer for SSR markers (or as described in Table 3 for non-SSR markers). Genomic DNA amounting to 1 µl was added, making up a total of 10 µl of the PCR reaction mixture. A drop of paraffin oil was, thereafter, added to prevent the reaction mixture from evaporation during the amplification.

PCR protocol was done using Bio-Gener Technology, Gene explorer PCR machine as follows: 94°C for 3 min, 35 cycles of 94°C for 40 s, a range of 55 to 61°C depending on primer annealing temperature (Table 3) for 30 s, 72°C for 40 s and a final extension of 72°C for 10 min before soaking at 4°C. The PCR products were, thereafter, run on 2% Agarose gel immersed in 1% TAE (Tris-Acetate-EDTA) buffer solution and the image was captured using Gel Documentation and Image Analysis System after staining in

Table 1. Names of wheat cultivars, their pedigree information and origin.

Cultivar designation	Name of cultivar	Pedigree information*	Origin
S	Chancellor	Carina/Mediterranean//Dietz/ Carina/3/ P-1068/3×Purplestraw	-
1	Tian0015	-	Gansu
2	Longjian101	8487/85-173-12-2	Gansu
3	Tian96-86	863-13/8560-2-2-1	Gansu
4	Tian03-160	0037-1-2 / 9938-2-2-1	Gansu
5	Yujiao0338	-	Henan
6	Tian00296	9362-13-3-4/8748-0-2-1	Gansu
7	Zhengnong01059	-	Henan
8	Xinyumai836	-	Henan
9	Tian03-142	9589-8-1-2-1/Qing 95-111	Gansu
10	Tian00127	(Baidatou/C184-3-4-1)F2//85-173-4	Gansu
11	Tian9681	863-13/87148-1-1-2-2-2	Gansu
12	Lantian093	Lantian23/Zhou92031	Gansu
13	AvocetYrA	Avocet	USA
14	Pu02056	Zhoumai16/ Yumai24	Henan
15	Tian01-104	93R177 / 912-2-1-2	Gansu
16	Tian02-195	Wenmai8/Tian96-1c1	Gansu
17	05bao1-1	Zhongliang22+ gDNA of oil sunflower	Gansu
18	Tian02-204-1	Wenmai8/ 9157-3-2-2-1	Gansu
19	Lantian095	-	Gansu
20	Longjian127	7402/Lv419//7415	Gansu
21	Tian989	9362-13-4-4/lantian1	Gansu
22	Punong1	-	Henan
23	Longjian102	Lin87-4535/81168-4-3//Longyuan932	Gansu
24	Tian98101	9362-13-4-4/Tian94-3	Gansu
25	N. Strampelli	LIBERO//S.Pastou/C.Jrometh.lig	Italy
26	Zhongliang 27	90293///Zhongliang12/Zhongsi// Bulgaria10/Xiannong4	Gansu
27	03bao1-1	Lantian10+ DNA of oil sunflower	Gansu
28	Zhongzhi4	Mianyou2/Zhongzhi1	Beijing
29	Lantian097	92R137/87-121 //Shan167	Gansu
30	Zhongzhi1	Shan167/C591	Beijing
31	Zhoumai19	Neixiang185 / Zhoumai9	Henan
32	Kenya Kongoni	C18154/2×Fr/2/Romm/3/WIS.245-II-50-7/C8154/2/2×Fr	USA
33	Keyuan5	-	Henan
34	Xinmai19	(C5/xinxiang3577) F3d1s/Xinmai9	Henan
35	Lantian20	CappelleDesprez/Lantian10	Gansu
36	Yumai368	-	Henan
37	Taikong06	Space-flight mutation from Yumai49	Henan
38	Guoan368	-	Henan
39	Guomai301	G883/ Pumai9	Henan
40	Yangao03710	-	Henan
41	Zhou99233	-	Henan
42	Zhongzhi2	Shan167/ Guinong22 / *T. Spelta* album	Beijing
43	Longchun26	Yong3263/Gaoyuan448	Gansu
44	Xinxuan2039	-	Henan
45	Lantian23	SXAF4-7/87-121	Gansu
46	Zhoumai32	Zhoumai12/ Wenmai6 // Zhoumai13	Henan
47	Zhongxin01	-	Henan
48	Zhongyu885	-	Henan
49	04zhong70	-	Henan
50	Zheng366	-	Henan

Table 1. Contd.

51	Lankao008	-	Henan
52	Tianmin198	R81/Bainong64//Yanzhan4110	Henan
53	Zhengyumai9989	Benyumai21/Yumai2//Yumai57	Henan
54	Zhengmai9023	[Xiaoyan6/Xinong65//83(2)33/84(14)43] F3/3Shan213	Henan
55	Zhengmai366	Yumai47/PH82-2-2	Henan
56	Zhoumai16	Zhoumai9/Zhou8425B	Henan
57	Yanzhan4110	[(C39/Xibei78(6)9-2) /(FR81-3/ Aizao781-4)] /Aizao781-4	Henan
58	Bainong160	Duokang893/Wenmai6//Bainong64/Wenmai6	Henan
59	Lantian15	Lantian10 /Ibis	Gansu

*Cultivars with a dash (-) indicate that their information could not be traced.

Table 2. Wheat cultivars used as positive control and their known powdery mildew resistance genes.

Cultivar designation	Wheat cultivar	Known resistance gene present
1	Ulka/8Cc	*Pm2*
2	Khapli/8Cc	*Pm4a*
3	Armada	*Pm4b*
4	Coker 747	*Pm6*
5	Kavkaz	*Pm8*
6	Yangmai 5/Sub.6V	*Pm21*
7	5P27	*Pm30*

Table 3. Description of molecular markers used for *Bgt* resistance gene identification.

Marker	Type of marker	Gene	Marker Sequence	Chromosomal location	Annealing Temp. (°C)	Reference
Whs350-1 Whs350-Re	STS	*Pm2*	AGCTGTTTGGGTACAAGGTG GCCATCGTTTTCTACTAG	5D	58	Mohler and Jahoor 1996
Xgwm356 F Xgwm356 R	SSR	*Pm4a*	AGCGTTCTTGGGAATTAGAGA CCAATCAGCCTGCAACAAC	2A,6A,7A	55	Luo et al., 2005
P1 P2	STS	*Pm4b*	ACGAGTGATGCTCCAGGATATGG GATCCACCTTTTCCTTGACAAGC	2A	61	Luo et al., 2005
Pm6 L Pm6 R	STS	*Pm6*	GCTCCGAAGCAAGAGAAGAA TCTGCTGGTCCTCTGATGTG	2B	58	Ji et al., 2008
Pm8 L Pm8 R	SCAR	*Pm8*	GGAGACATCATGAAACATTTG CTGTTGTTGGGCAGAAAG	1B	55	Mohler et al., 2001
Pm21 C Pm21 D	SCAR	*Pm21*	CACTCTCCTCAAACCTTGCAAG CACTCTCCTCCACTAACAGAGG	6A	61	Luo et al., 2005
Xgwm159 F Xgwm159 R	SSR	*Pm30*	GGGCCAACACTGGAACAC GCAGAAGCTTGTTGGTAGGC	5B, 5D	60	Liu et al., 2002

ethidium bromide solution for 15 min.

Seven molecular markers of the following types: Three Sequence Tagged Sites (STS) markers, two Simple Sequence Repeats (SSR) markers and two Sequenced Characterized Amplified Region (SCAR) Markers were used for the study (Table 3).

RESULTS

Among the sixty wheat cultivars, the results showed that 17 cultivars contained possible *Pm2* gene (Figure 1). The cultivars included Tian0015, Yujiao0338, Tian00127, Zhengnong01059, Xinyumai836, Pu02056, Tian01-104, Lantian095, Punong1, Zhoumai19, Yumai368, Guomai301, Xinxuan2039, Zhongyu885, Lankao008, Zhengyumai9989 and Zhengmai366. Positive control wheat cultivar, Ulka/8Cc was used to determine the size of the gene and it showed that the resistance *Pm2* gene was detected with a molecular weight of 480 bp (Figure

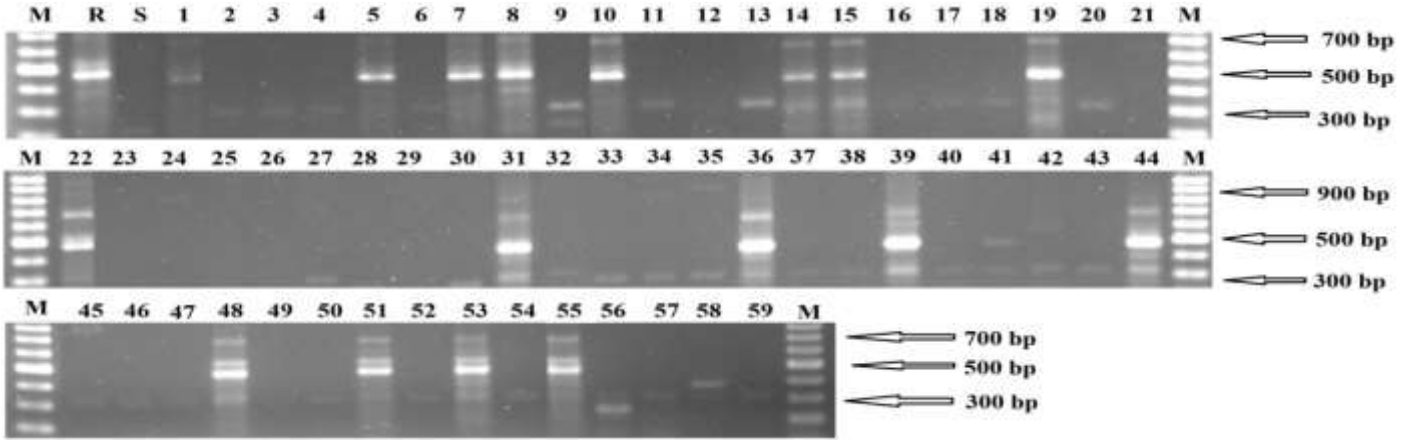

Figure 1. Wheat powdery mildew gene *Pm2* as identified in the wheat cultivars at 480 bp. Lanes 1-59 represent cultivars designation. R is resistant cultivar Ulka/8Cc, S is susceptible cultivar Chancellor. M is a 100 bp DNA ladder.

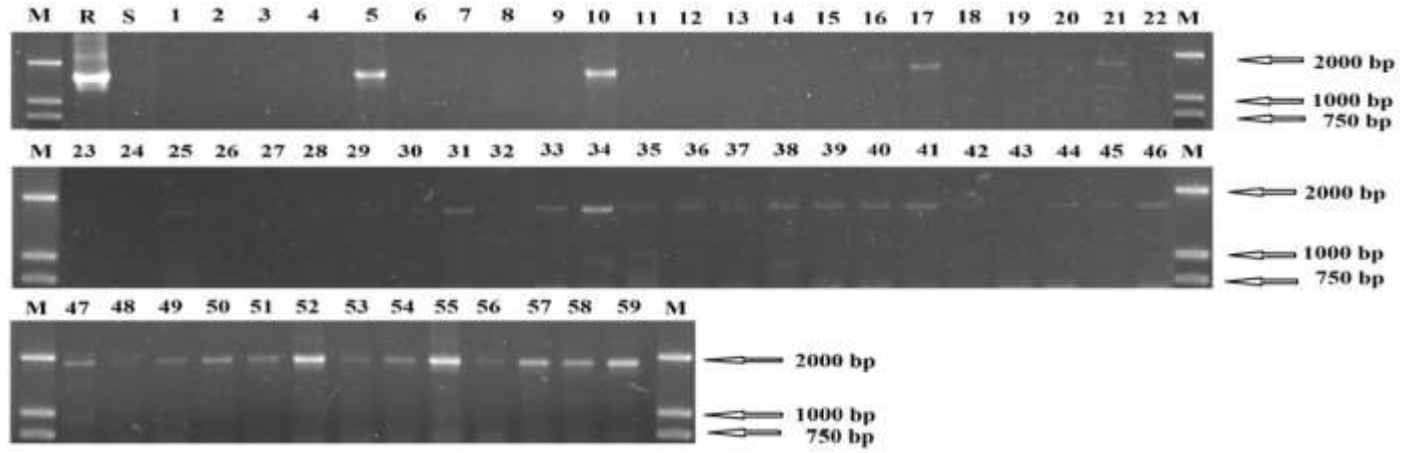

Figure 2. Wheat powdery mildew gene *Pm4b* as identified in the wheat cultivars at 1800 bp. Lanes 1-59 represent cultivars designation. R is resistant cultivar Armada, S is susceptible cultivar Chancellor. M is a D2000 DNA ladder.

1). This was confirmed due to the absence of the alleles in the susceptible cultivar Chancellor.

A total of 24 wheat cultivars were detected with possible wheat powdery mildew *Pm4b* gene. The cultivars were; Yujiao0338, Tian00127, 05bao1-1, Zhoumai19, Keyuan5, Xinmai19, Yumai368, Guoan368, Guomai301, Yangao03710, Zhou99233, Xinxuan2039, Zhongxin01, Zhoumai32, 04zhong70, Zheng366, Lankao008, Tianmin198, Zhengyumai9989, Zhengmai9023, Zhengmai366, Yanzhan4110, Bainong160 and Lantian15 (Figure 2). Positive control cultivar, Armada detected the gene as having a molecular size of 1800 bp when a D2000 DNA ladder was used (Figure 2).

Only two wheat cultivars; Yumai368 and Guomai301 were detected with possible *Pm6* gene. The gene had a molecular size of 140 bp detected in cultivar Coker747, which was used as positive control (Figure 3).

A total of 24 wheat cultivars were detected with possible *Pm8* gene. Among them were cultivars Tian0015 and Tian00127, which both had faint but positive alleles for *Pm8* gene. Other wheat cultivars were 05bao1-1, Tian01-104, Lantian095, Tian989, Punong1, Xinmai19, Lantian20, Taikong06, Guoan368, Guomai301, Yangao03710, Zhou99233, Longchun26, Xinxuan2039, Zhongyu885, 04zhong70, Lankao008, Zhengmai9023, Zhengmai366, Zhoumai16, Yanzhan4110 and Lantian15 (Figure 4). The gene was detected using a positive control cultivar Kavkaz and it had a molecular size of 1300 bp.

Multiple genes were also observed in some wheat cultivars during the study. For example, four cultivars; Tian00127, Xinxuan2039, Lankao008 and Zhengmai366 were detected with three possible multiples genes for *Pm2*, *Pm4b* and *Pm8* while one cultivar, Yumai368 had a possible combination of three multiple genes for *Pm2*,

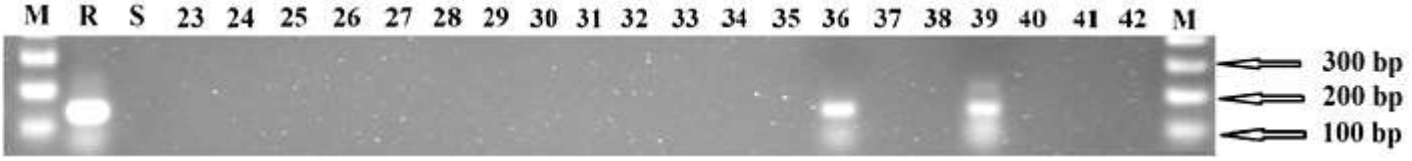

Figure 3. Powdery mildew gene *Pm6* as identified in the tested cultivars at 140 bp. Lanes 23-42 represent cultivars designation. R is resistant cultivar Coker 747, S is susceptible cultivar Chancellor. M is a 100 bp DNA ladder.

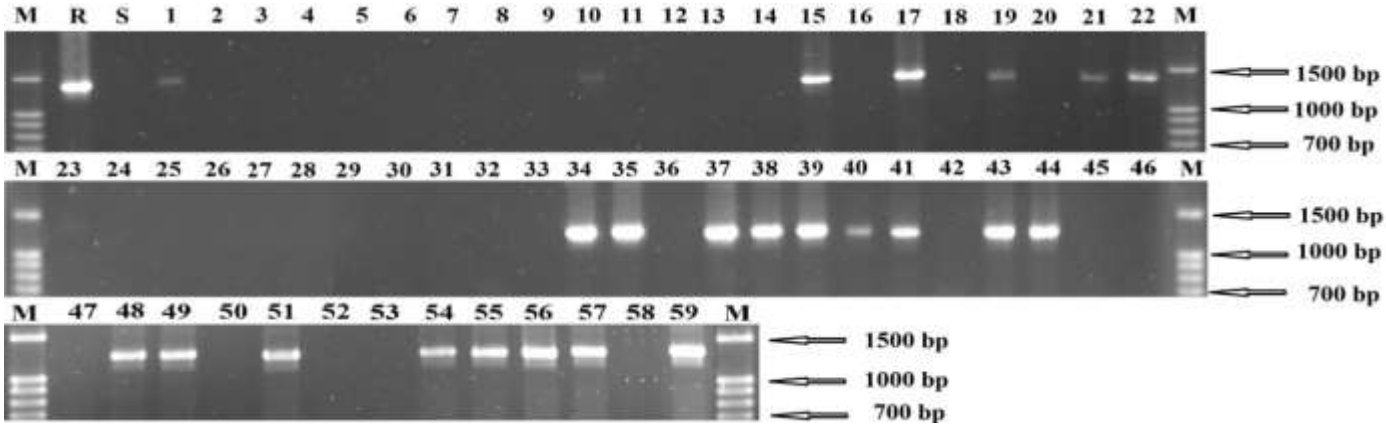

Figure 4. Powdery mildew gene *Pm8* as identified in the tested cultivars at 1300 bp. Lanes 1-59 represent cultivars designation, R is resistant cultivar Kavkaz, S is susceptible cultivar Chancellor, M is a 100 bp DNA ladder.

Pm4b and *Pm6*. Sixteen cultivars were detected with two possible multiple wheat powdery mildew resistance genes (Table 4).

Overall, 22 wheat cultivars did not show presence of any of the seven powdery mildew genes. Among them were; Longjian101, Tian96-86, Tian03-160, Tian00296, Tian03-142, Tian9681, Lantian093, AvocetYrA, Tian02-195, Longjian127, Tian02-204-1, Longjian102, Tian98101, Strampelli, Zhongliang27, 03bao1-1, Zhongzhi4, Lantian097, Zhongzhi1, Kenya kongoni, Zhongzhi2 and Lantian23 (Table 4).

The molecular study showed that *Pm4b* and *Pm8* were the powdery mildew genes present in the highest number of wheat cultivars. A total of 24 wheat cultivars carried these genes. Molecular marker detected possible *Pm2* in 17 cultivars while *Pm6* was the lowest detected in two cultivars. Three powdery mildew resistance genes *Pm4a*, *Pm21* and *Pm30* were not detected in any of the tested cultivars using the molecular markers (Figure 5).

DISCUSSION

In this study, 17 cultivars were detected with possible *Pm2* gene. This resistance gene had been widely used and had been highly effective in different parts of Europe and China years ago (Liu et al., 2000). Though the growing number of virulent strains has been reported in some parts of China, the gene occurs in high frequency in so many commercial cultivars as it is easily transferred from resistant to susceptible cultivars (Parks et al., 2008; Gao et al., 2012). Recently, Ma et al. (2015) reported the presence of a new gene *Pm2b* in the cultivar KM2939, a Chinese breeding line, which exhibits high resistance to powdery mildew at both the seedling and adult stages. This gene was reported to carry a single dominant powdery mildew resistance allele of *Pm2*, designated as *Pm2b* and was mapped on chromosome 5DS, rendering the previous allelic designation *Pm2* to be re-designated as *Pm2a*.

Resistance genes *Pm4b* and *Pm8* were genes detected in highest frequency among the cultivars used in the present molecular study. This is consistent with the finding reported by Li et al. (2012) that these genes are the most widely distributed among wheat cultivars in China. *Pm8* cultivars have been grown worldwide since the 1980s and due to wide use, their effectiveness has since declined (Parks et al., 2008; Tang et al., 2014; Zeng et al., 2014). Several virulent strains of powdery mildew have emerged rendering the gene ineffective. In fact, it is one of the major reasons that an epidemic boom of wheat powdery mildew was recorded in China in the early 1990s (Graybosch, 2001; Ryabchenko et al., 2003).

Among the 59 cultivars, only two cultivars Guomai301 and Yumai368 were detected with possible *Pm6* gene apart from other multiple genes also suspected to be present in the cultivars. *Pm6* has been widely and successfully used in breeding for wheat powdery mildew

Table 4. *Bgt* resistance genes detected using molecular markers.

Cultivar designation	Name of cultivar	Detected *Bgt* resistance genes using molecular markers
S	Chancellor	Susceptible (no gene)
1	Tian0015	*Pm2+Pm8*
2	Longjian101	-
3	TianTian96-86	-
4	Tian03-160	-
5	Yujiao0338	*Pm2+Pm4b*
6	Tian00296	-
7	Zhengnong01059	*Pm2*
8	Xinyumai836	*Pm2*
9	Tian03-142	-
10	Tian00127	*Pm2+Pm4b+Pm8*
11	TianTian9681	-
12	Lantian093	-
13	AvocetYrA	-
14	Pu02056	*Pm2*
15	Tian01-104	*Pm2+Pm8*
16	Tian02-195	-
17	05bao1-1	*Pm4b+Pm8*
18	Tian02-204-1	-
19	Lantian095	*Pm2+Pm8*
20	Longjian127	-
21	Tian989	*Pm8*
22	Punong1	*Pm2+Pm8*
23	Longjian102	-
24	Tian98101	-
25	Strampelli	-
26	Zhongliang27	-
27	03bao1-1	-
28	Zhongzhi4	-
29	Lantian097	-
30	Zhongzhi1	-
31	Zhoumai19	*Pm2+Pm4b*
32	Kenya Kongoni	-
33	Keyuan5	*Pm4b*
34	Xinmai19	*Pm4b+Pm8*
35	Lantian20	*Pm8*
36	Yumai368	*Pm2+Pm4b+Pm6*
37	Taikong06	*Pm8*
38	Guoan368	*Pm4b+Pm8*
39	Guomai301	*Pm2+Pm4b+Pm6+Pm8*
40	Yangao03710	*Pm4b+Pm8*
41	Zhou99233	*Pm4b+Pm8*
42	Zhongzhi2	-
43	Longchun26	*Pm8*
44	Xinxuan2039	*Pm2+Pm4b+Pm8*
45	Lantian23	-
46	Zhoumai32	*Pm4b*
47	Zhongxin01	*Pm4b*
48	Zhongyu885	*Pm2+Pm8*
49	04zhong70	*Pm4b+Pm8*
50	Zheng366	*Pm4b*

Table 4. Contd.

51	Lankao008	*Pm2+Pm4b+Pm8*
52	Tianmin198	*Pm4b*
53	Zhengyumai9989	*Pm2+Pm4b*
54	Zhengmai9023	*Pm4b+Pm8*
55	Zhengmai366	*Pm2+Pm4b+Pm8*
56	Zhoumai16	*Pm8*
57	Yanzhan4110	*Pm4b+Pm8*
58	Bainong160	*Pm4b*
59	Lantian15	*Pm4b+Pm8*

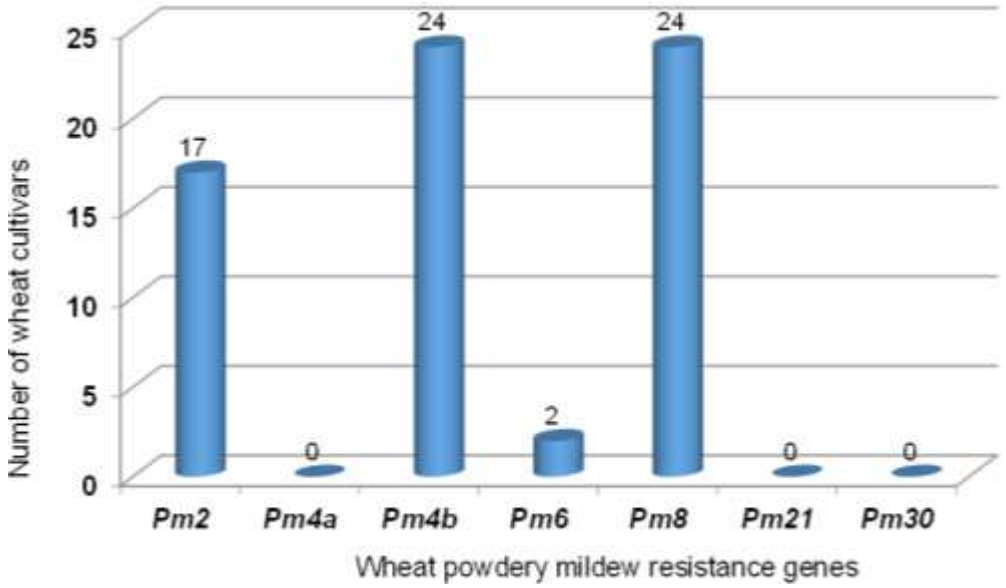

Figure 5. Total number of wheat cultivars detected with wheat powdery mildew resistance genes.

resistance for long time. The gene usually exhibits its best expression from the third leaf stage of wheat growth and thereafter, it is moderately effective, but recognizable at the seedling stage (Bennett, 1984; Qin et al., 2011; Li et al., 2014). Virulence matching the *Pm6* gene has occurred in many regions but still, the gene remains effective in some areas, especially when other genes such as *Pm2* and *Pm4b* are combined during their use (Cai et al., 2005; Costamilan, 2005; Shi et al., 2009; Purnhauser et al., 2011). This could be one of the possible reasons why the two cultivars were resistant during the study (data not presented) as the genes such as *Pm2*, *Pm4b* and *Pm8* were also possibly present in these cultivars.

Conclusion

This study detected 24 cultivars likely carrying *Pm4b* and *Pm8* while two cultivars carried likely *Pm6* gene. Despite the recent findings on these genes indicating that they are being overcome by emerging virulent isolates, their abundant availability in the currently produced wheat cultivars signifies their continued contribution to wheat production in China. The cultivars detected with these genes can still be useful in various breeding programs for disease resistance. The detection of likely multiple resistance genes in some wheat cultivars in this study showed that where multiple genes were involved in conferring resistance to powdery mildew disease, the cultivars could effectively resist infection from isolates that would have otherwise caused virulence if a single gene was involved.

Conflict of interest

The authors have not declared any conflicts of interest.

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