ISSN: 2437-1874 Vol. 4 (2), pp. 159-168, August, 2016. Copyright ©2016 Author(s) retain the copyright of this article. http://www.globalscienceresearchjournals.org/

**Global Journal of Pests, Diseases and Crop Protection** 

Full Length Research Paper

# An evaluation on presence of some prominent wheat powdery mildew resistance genes in selected wheat cultivars

Orton Malewezi<sup>1,2</sup>\*, Xhang Lee<sup>2</sup> and Chakufwa Kondowe<sup>1</sup>

<sup>1</sup>Lilongwe University of Agriculture and Natural Resources (LUANAR), Bunda College Campus, P.O. Box 219, Lilongwe, Malawi.

<sup>2</sup>State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of P lant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.

Accepted 03 July, 2016

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 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

\*Corresponding author: E-mail: malewezi.orton@gmail.com

Author(s) agreed that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

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 Τ. 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 w heat cultivars from different parts of China w ere identified for the study (Table 1). Ten seeds of each cultivar w ere sow n on trays in greenhouse located at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. Around 15 days after sow ing, w hen three to four leaves had been developed, seedling leaves w ere detached and their DNA w as extracted follow ing cetyltrimethylammonium bromide (CTAB) extraction method (Zheng, 2010). Chancellor, a susceptible cultivar w ith no know n *Bgt* resistance genes, w as used as negative control.

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

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

PCR protocol w as done using Bio-Gener Technology, Gene explorer PCR machine as follow s: 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 w ere, thereafter, run on 2% Agarose gel immersed in 1% TAE (Tris - Acetate-EDTA) buffer solution and the image w as captured using Gel Documentation and Image Analysis System after staining in

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		- 7402/Lv419//7415	Gansu
20	Longjian127 Tian989	9362-13-4-4/lantian1	Gansu
21		9302-13-4-4/1d111d111	Henan
	Punong1		
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 / <i>T. Spelta</i> 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	<u>-</u>	Henan
49	04zhong70	<u>-</u>	Henan
50	Zheng366	_	Henan

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

#### 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 w ith a dash (-) indicate that their information could not be traced.

Table 2. Wheat cultivars used as positive control and their know n pow dery mildew resistance genes.

Cultivar designation	Wheat cultivar	Known resistance gene present
1	Ulka/8Cc	Pm 2
2	Khapli/8Cc	Pm 4a
3	Armada	Pm 4b
4	Coker 747	Pm 6
5	Kavkaz	Pm 8
6	Yangmai 5/Sub.6V	Pm 21
7	5P27	Pm 30

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

Marker	Type of marker	GeneM	arker Sequence	Chromosomal location	Annealing Temp. (°C)	Reference
Whs350-1 Whs350-Re	STS	Pm 2	AGCTGTTTGGGTACAAGGTG GCCATCGTTTTCTACTAG	5D	58	Mohler and Jahoor 1996
Xgwm356 F Xgwm356 R	SSR	Pm 4a	AGCGTTCTTGGGAATTAGAGA CCAATCAGCCTGCAACAAC	2A,6A,7A	55	Luo et al., 2005
P1 P2	STS	Pm 4b	ACGAGTGATGCTCCAGGATATGG GATCCACCTTTTCCTTGACAAGC	2A	61	Luo et al., 2005
Pm6 L Pm6 R	STS	Pm 6	GCTCCGAAGCAAGAGAAGAA TCTGCTGGTCCTCTGATGTG	2B	58	Ji et al., 2008
Pm8 L Pm8 R	SCAR	Pm 8	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

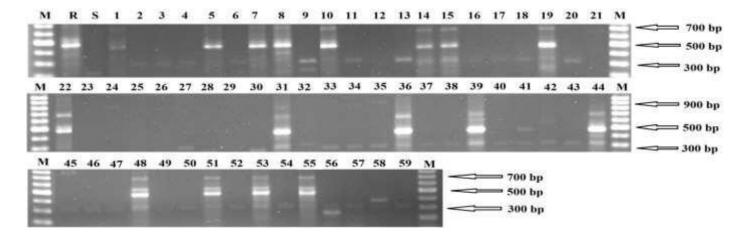
ethidium bromide solution for 15 min.

Seven molecular markers of the follow ing types: Three Sequence Tagged Sites (STS) markers, tw o Simple Sequence Repeats (SSR) markers and tw o Sequenced Characterized Amplified Region (SCAR) Markers w ere used for the study (Table 3).

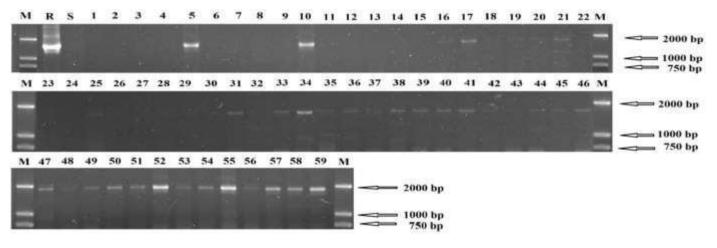
## RESULTS

Among the sixty wheat cultivars, the results showed that

17 cultivars contained possible *Pm2* gene (Figure 1). The cultivars included Tian0015, Yujia00338, Tian00127, Zhengnong01059, Xinyumai836, Pu02056, Tian01-104, Lantian095, Punong1, Zhoumai19, Yumai368, Guomai301, Xinxuan2039, Zhongyu885, Lanka0008, 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 pow dery mildew gene *Pm2* as identified in the w heat 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 pow dery mildew gene *Pm4b* as identified in the w heat 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, Zhoumai32. Zhonaxin01. 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, Taikong06, Lantian20, 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*,

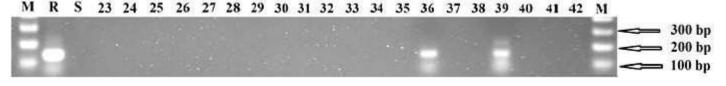
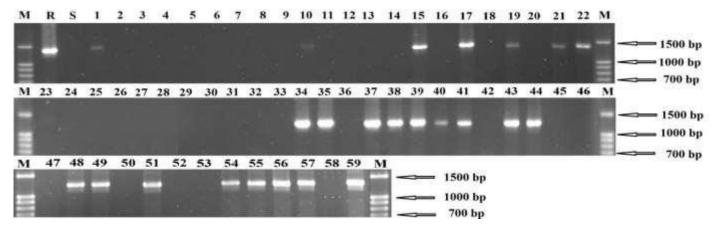


Figure 3. Pow dery mildew gene *Pm*6 as identified in the tested cultivars at 140 bp. Lanes 23-42 represent cultivars designation. R is resistant cultivar Coker 747, S is susceptible cultivar Chancellor. M is a 100 bp DNA ladder.



**Figure 4.** Pow dery 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

Cultivar designation	Name of cultivar	Detected Bgt resistance genes using molecular markers
S	Chancellor	Susceptible (no gene)
1	Tian0015	Pm 2+Pm 8
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	<u>.</u>
13	AvocetYrA	
14	Pu02056	Pm2
15		
	Tian01-104	Pm2+Pm8
16	Tian02-195	- Dm 4b · Dm 0
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
38	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. Bgt resistance genes detected using molecular markers.

51	Lankao008	Pm 2+Pm 4b +Pm 8	
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	



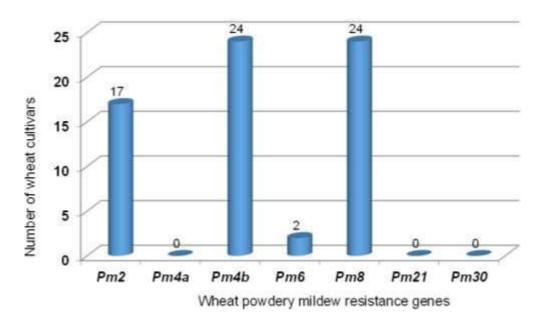


Figure 5. Total number of w heat cultivars detected w ith w heat pow dery mildew resistance genes.

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.

#### ACKNOWLEDGEMENT

The authors would like to thank the Chinese Government

through the Chinese Scholarship Council (CSC) for funding the research.

#### **REFERENC ES**

- Alam MA, Mandal MSN, Wang C, Ji W (2013). Chromosomal location and SSR markers of a pow dery mildew resistance gene in common w heat line N0308. Afr. J. Microbiol. Res. 7(6):477-482.
- Alam MA, Xue F, Wang C, Ji W (2011). Pow dery Mildew Resistance Genes in Wheat: Identification and Genetic Analysis. J. Mol. Biol. Res. 1(1):20-39.
- Asad MA, Bai B, Lan C, Yan J, Xia X, Zhang Y, He Z (2014). Identification of QTL for adult-plant resistance to pow dery mildew in Chinese w heat landrace Pingyuan 50. Crop J. 2(5):308-314.
- Ben-David R, Xie W, Peleg Z, Saranga Y, Dinoor A, Fahima T (2010). Identification and mapping of *PmG16*, a pow dery mildew resistance gene derived from w ild emmer w heat. Theor. Appl. Genet. 121:499-510.
- Bennett FGA (1984). Resistance to pow dery mildew in w heat: a review of its use in agriculture and breeding programmes. Plant Pathol. 33(3):279-300.
- Briggle LW (1969). Near-isogenic lines of w heat w ith genes for resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci. 9:70-72.
- Cai SB, Cheng SH, Wu JZ, Yan W (2005). Evaluation, improvement and utilization of introduced w heat reserve resource resistant to pow dery mildew. Acta Tritical Crops 25:116-120.
- Cao X, Luo Y, Zhou Y, Duan X, Cheng D (2013). Detection of pow dery mildew in two w inter w heat cultivars using canopy hyperspectral reflectance. Crop Prot. 45:124-131.
- Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ (1995). Development and molecular cytogenetic analysis of w heat-Haynaldia villosa 6VS/6AL translocation lines specifying resistance to pow dery mildew. Theor. Appl. Genet. 91:1125-1129.
- Conner RL, Kuzyk AD, Su H (2003). Impact of pow dery mildew on the yield of soft w hite spring w heat cultivars. Can J. Plant Sci. 83:725-728.
- Costamilan LM (2005). Variability of the w heat pow dery mildew pathogen Blumeria graminis f. sp. tritici in the 2003 crop season. Fitopatol. Bras. 30:420-422.
- Cow ger C, Parks R, Marshall D (2009). Appearance of powdery mildew of w heat caused by *Blumeria graminis* f. sp. *tritici* on *Pm17*-bearing cultivars in North Carolina. Plant Dis. 93:1219.
- Everts KL, Leath S, Finney PL (2001). Impact of pow dery mildew and leaf rust on milling and baking quality of soft red w inter w heat. Plant Dis. 85:423-429.
- Gao H, Zhu F, Jiang Y, Wu J, Yan W, Zhang Q, Jacobi A, Cai S (2012). Genetic analysis and molecular mapping of a new pow dery mildew resistant gene *Pm46* in common w heat. Theor. Appl. Genet. 125(5):967-973.
- Graybosch RA (2001). Uneasy unions: quality effects of rye chromatin transfers to w heat. J. Cereal Sci. 33:3-16.
- Hao Y, Chen Z, Wang Y, Bland D, Parks R, Cow ger C, Johnson J (2012). Identification of *Pm8* suppressor at the *Pm3* locus in soft red w inter w heat. Crop Sci. 52:2438-2445.
- Hao Y, Parks R, Cow ger C, Chen Z, Wang Y, Bland D, Murphy JP, Guedira M, Brow n-Guedira G, Johnson J (2015). Molecular characterization of a new pow dery mildew resistance gene *Pm54* in soft red w inter w heat. Theor. Appl. Genet. 128:465-476.
- Hsam SLK, Zeller FJ (1997). Evidence of allelism betw een genes *Pm8* and *Pm17* and chromosomal location of pow dery mildew and leaf rust resistance genes in the common w heat cultivar 'Amigo'. Plant Breed. 116:119-122.
- Huang XQ, Röder MS (2004). Molecular mapping of pow dery mildew resistance genes in w heat: a review . Euphytica 137:203-223.
- Hurni S, Brunner S, Stirnw eis D, Herren G, Peditto D, McIntosh MA, Keller B (2014). The pow dery mildew resistance gene Pm8 derived from rye is suppressed by its w heat ortholog *Pm3*. Plant J. 79:904-913.
- Ji X, Xie C, Ni Z, Yang T, Nevo E, Fahima T, Liu Z, Sun Q (2008). Identification and genetic mapping of a pow dery mildew resistance

gene in wild emmer (*Triticum dicoccoides*) accession IW72 from Israel. Euphytica 159:385-390.

- Jorgensen JH, Jensen CI (1972). Genes for resistance to w heat pow dery mildew in derivatives of *Triticum timopheevii* and *Triticum carthlicum*. Euphytica 21:121-128.
- Law C N, Wolfe M S (1966). Location for genetic factors for mildew resistance and ear emergence time on chromosome 7B of w heat. Can. J. Genet. Cytol. 8:462-470.
- Li B, Cao X, Chen L, Zhou Y, Duan X, Luo Y, Fitt BDL, Xu X, Song Y, Wang B, Cao S (2013). Application of geographic information systems to identify the oversummering regions of *Blumeria* graminis f. sp. tritici in China. Plant Dis. 97:1168-1174.
- Li N, Jia S, Wang X, Duan X, Zhou Y, Wang Z, Lu G (2012). The effect of w heat mixtures on the pow dery mildew disease and some yield components. J. Integr. Agric. 11(4):611-620.
- Li N, Wen Z, Wang J, Fu B, Liu J, Xu H, Kong Z, Zhang L, Jia H, Ma Z (2014). Transfer and mapping of a gene conferring latergrowth-stage pow dery mildew resistance in a tetraploid w heat accession. Mol. Breed. 33:669-677.
- Liu J, Liu D, Tao W, Li W, Wang S, Chen P, Cheng S, Gao D (2000). Molecular marker-facilitated pyramiding of different genes for pow dery mildew resistance in w heat. Plant Breed. 119:21-24.
- Liu WC, Shao ZR (1994). Epidemiology, occurrence and analysis of w heat pow dery mildew in recent years. Plant Prot. Technol. Ext. 6:17-20.
- Liu Z, Sun Q, Ni Z, Nevo E, Yang T (2002). Molecular characterization of a novel pow dery mildew resistance gene *Pm30* in w heat originating from w ild emmer. Euphytica 123:21-29.
- Luo PG, Luo HY, Chang ZJ, Zhang HY, Zhang M, Ren ZL (2009). Characterization and chromosomal location of *Pm40* in common w heat: a new gene for resistance to pow dery mildew derived from *Elytrigia intermedium*. Theor. Appl. Genet. 118:1059-1064.
- Luo Y, Chen X, Xia L, Chen X, He Z, Ren Z (2005). Molecular Marker - Assisted selection of DH plants conferring genes resistant to powdery mildew in w heat (*Triticum aestivum* L). Acta Agron. Sin. 31(5):565-570.
- Lutz J, Hsam SLK, Limpert E, Zeller FJ (1995). Chromosomal location of pow dery mildew resistance genes in *Triticum* aestivum L. (common w heat) Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. Heredity 74:152-156.
- Ma P, Xu H, Xu Y, Li L, Qie Y, Luo Q, Zhang X, Li X, Zhou Y, An D (2015). Molecular mapping of a new powdery mildew resistance gene *Pm2b* in Chinese breeding line KM2939. Theor. Appl. Genet. 128:613-622.
- Mandal MDN, Fu Y, Zhang S, Ji W (2015). Proteomic Analysis of the Defense Response of Wheat to the Pow dery Mildew Fungus, *Blumeria graminis* f. sp. *tritici.* Protein J. 33:513-524.
- McIntosh RA, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Xia XC (2014). Catalogue of gene symbols for w heat: 2013–2014 supplement. Ann Wheat New sl. 60:153-175.
- McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2013). Catalogue of gene symbols for w heat. In. Ogihara Y (ed) Proceeding of the 12th international w heat genetics symposium, Yokohama, Japan, 8–13 Sept, 2013.
- Mohler V, Bauer C, Schw eizer G, Kempf H, Hartl L (2013). *Pm50*: a new powdery mildew resistance gene in common w heat derived from cultivated emmer. J. Appl. Genet. 54(3):259-263.
- Mohler V, Hsam SLK, Zeller FJ, Wenzel G (2001). An STS marker distinguishing the rye-derived pow dery mildew resistance alleles at the Pm8/Pm17 locus of common w heat. Plant Breed. 120:448-450.
- Mohler V, Jahoor A (1996). Allele-specific amplification of polymorphic sites for the detection of pow dery mildew resistance loci in cereals. Theor. Appl. Genet. 93:1078-1082.
- Parks R, Carbone I, Murphy J, Marshall D, Cow ger C (2008). Virulence structure of the eastern US w heat pow dery mildew population. Plant Dis. 92:1074-1082.
- Peng F, Song N, Shen H, Wu H, Dong H, Zhang J, Li Y, Peng H, Ni Z, Liu Z, Yang T, Li B, Xie C, Sun Q (2014). Molecular mapping of a recessive pow dery mildew resistance gene in spelt w heat cultivar Hubel. Mol. Breed. 34:491-500.
- Petersen S, Lyerly JH, Worthington ML, Parks WR, Cow ger C, Marshall DS, Brow n-Guedira G, Murphy JP (2015). Mapping of pow dery

mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red w inter w heat. Theor. Appl. Genet. 128:303-312.

- Piarulli L, Gadaletaa A, Manginia G, Signorilea MA, Pasquinib M, Blancoa A, Simeone R (2012). Molecular identification of a new pow dery mildew resistance gene on chromosome 2BS from *Triticum turgidum* ssp. *dicoccum*. Plant Sci. 196:101-106.
- Purnhauser L, Bo'na L, La'ng L (2011). Occurrence of 1BL.1RS w heat-rye chromosome translocation and of Sr36/Pm6 resistance gene cluster in w heat cultivars registered in Hungary. Euphytica 179:287-295.
- Qin B, Cao A, Wang H, Chen T, You FM, Liu Y, Ji J, Liu D, Chen P, Wang X (2011). Collinearity-based marker mining for the fine mapping of *Pm6*, a pow dery mildew resistance gene in w heat. Theor. Appl. Genet. 123:207-218.
- Quijano CD, Brunner S, Keller B, Gruissem W, Sautter C (2015). The environment exerts a greater influence than the transgene on the transcriptome of field-grow n w heat expressing the *Pm3b* allele Transgenic Res. 24:87-97.
- Ryabchenko AS, Serezhkina GV, Mishina GN, Andreev LN (2003). Morphological variability of w heat powdery mildew in the context of its parasitic adaptation to w heat-*Aegilops* lines w ith different resistance. Biol. Bull. Acad. Sci. 30:255-261.
- Shen XK, Ma LX, Zhong SF, Liu N, Zhang M, Chen WQ, Zhou YL, Li HJ, Chang ZJ, Li X, Bai GH, Zhang HY, Tan FQ, Ren ZL, Luo PG (2015). Identification and genetic mapping of the putative *Thinopyrum intermedium*-derived dominant pow dery mildew resistance gene *PmL962* on w heat chromosome arm 2BS. Theor. Appl. Genet. 128:517-528.
- Shi YQ, Wang BT, Qiang L, Wu XY, Fang W, Heng L, Tian YE, Liu QR (2009). Analysis on the virulent genes of *Erysiphe graminis* f.sp. *tritici* and the resistance genes of w heat commercial cultivars in Shaanxi Province. J. Triticeae Crops 29:706-711.
- Song W, Xie C, Du J, Xie H, Liu Q, Ni Z, Yang T, Sun Q, Liu Z (2009). A "one-marker-for-tw o-genes" approach for efficient molecular discrimination of *Pm12* and *Pm21* conferring resistance to pow dery mildew in w heat. Mol. Breed. 23:357-363.
- Tang X, Shi D, Xu J, Li Y, Li W, Ren Z, Fu T (2014). Molecular cytogenetic characteristics of a translocation line betw een common w heat and *Thinopyrum intermedium* w ith resistance to pow dery mildew . Euphytica 197:201-210.
- Wang Z L, Li L H, He Z H, Duan X Y, Zhou Y L, Chen X M, Lillemo M, Singh R P, Wang H, Xia X C (2005). Seedling and adult plant resistance to pow dery mildew in Chinese bread w heat cultivars and lines. Plant Dis. 89:457-463.

- Wang Z, Li H, Zhang D, Guo L, Chen J, Chen Y, Wu Q, Xie J, Zhang Y, Sun Q, Dvorak J, Luo M, Liu Z (2015). Genetic and physical mapping of pow dery mildew resistance gene *MIHLT* in Chinese w heat landrace Hulutou. Theor. Appl. Genet. 128:365-373.
- Xiao M, Song F, Jiao J, Wang X, Xu H, Li H (2013). Identification of the gene *Pm47* on chromosome 7BS conferring resistance to pow dery mildew in the Chinese w heat landrace Hongyanglazi. Theor. Appl. Genet. 126(5):1397-1403.
- Xie W, Ben-David R, Zeng B, Dinoor A, Xie C, Sun Q, Röder MS, Fahoum A, Fahima T (2012). Suppressed recombination rate in 6VS/6AL translocation region carrying the *Pm21* locus introgressed from *Haynaldia villosa* into hexaploid w heat. Mol. Breed. 29:399-412.
- Xue F, Zhai W, Duan X, Zhou Y, Ji W (2009). Microsatellite mapping of a pow dery mildew resistance gene in w heat landrace Xiaobaidong. Acta Agron. Sin. 35(10):1806-1811.
- Yao G, Zhang J, Yang L, Xu H, Jiang Y, Xiong L, Zhang C, Zhang Z, Ma Z, Sorrells ME (2007). Genetic mapping of two pow dery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions. Theor. Appl. Genet. 114:351-358.
- Yu S, Long H, Deng G, Pan Z, Liang J, Yu M (2015). Localization of the pow dery mildew resistance gene *Pm07J126* in w heat (*Triticum aestivum* L.). Euphytica 205(3):691-698.
- Zeng F, Yang L, Gong S, Shi W, Zhang X, Wang H, Xiang L, Xue M, Yu D (2014). Virulence and Diversity of *Blumeria graminis* f. sp. *tritici* Populations in China. J. Integr. Agric. 13(11):2424-2437.
- Zhang K, Zhao L, Hai Y, Chen G, Tian J (2008). QTL mapping for adult-plant resistance to pow dery mildew, lodging resistance, and internode length below spike in w heat. Acta Agron. Sin. 34(8):1350-1357.
- Zhao Z, Sun H, Song W, Lu M, Huang J, Wu L, Wang X, Li H (2013). Genetic analysis and detection of the gene *MILX99* on chromosome 2BL conferring resistance to pow dery mildew in the w heat cultivar Liangxing 99. Theor. Appl. Genet. 126:3081-3089.
- Zheng Y (2010). Detection of latent infection of w heat leaves caused by *Blumeria graminis* f. sp. *tritici* using Real-time PCR. MSc thesis (in Chinese), Chinese Academy of Agricultural Sciences, pp. 9-34.