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Identification of *Pm*24, *Pm*35 and *Pm*37 in thirteen Egyptian bread wheat cultivars using SSR markers

Identificação de *Pm*24, *Pm*35 e *Pm*37 em treze egípcios cultivares de trigo utilizando marcadores microssatélites

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ABSTRACT

Powdery mildew of wheat (*Triticum* spp.) caused by *Blumeria graminis* f.sp. *tritici* (DC) E.O. Speer Em. Marchal is one of the most important bread wheat diseases in Egypt. All the Egyptian common bread wheat cultivars are susceptible to that disease at seedling and adult stages. Breeding of resistant cultivars is the most economical and environmentally safe method to eliminate the disease and reduce crop losses. Combinations of two or more effective resistance genes may lead to better, more durable resistance to that disease. Eight *Pm* genes i.e. *Pm*2, *Pm*6, *Pm*12, *Pm*16, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 out of 21 powdery mildew monogenic wheat lines (*Pm*) were resistant to 42 individual isolates of powdery mildew collected from different governorates in the Nile Delta area, Egypt, at seedling and adult stages. Only four DNA specific SSR markers (*Xgwm*337, *Xcfd*7 linked to *Pm*24, *Pm*35 and *Xgwm*332, *Xwmc790*) linked to *Pm*37 resistance genes were selected to detect these genes in 13 Egyptian common bread wheat cultivars. This study reveals the absence of *Pm*24, *Pm*35 and *Pm*37 in all the 13 Egyptian bread wheat cultivars. These results gave evidence that the Egyptian cultivars are not having resistance genes and need to further incorporate one, two or more resistant genes in a single genotype as all commercial cultivars defeated by the pathogen.

Index terms: Powdery mildew; genetic control; DNA; molecular markers.

RESUMO

O oídio causado por *Blumeria graminis* f.sp. *tritici* (DC) E.O. Speer Em. Marchal é uma das doenças mais importantes do trigo (*Triticum spp.*) no Egito. Todos os cultivares de trigo egípcios são suscetíveis a essa doença tanto na fase jovem quanto em plantas adultas. O melhoramento genético de cultivares resistentes é o método mais econômico e ambientalmente seguro para eliminar a doença e reduzir as perdas de colheita. As combinações de dois ou mais genes de resistência podem conduzir a uma maior resistência a essa doença. Oito *Pm* genes (*Pm*2, *Pm*6, *Pm*12, *Pm*16, *Pm*24, *Pm*35, *Pm*36 e *Pm*37) entre 21 linhagens monogênicas de trigo foram resistentes a 42 isolados individuais de oídio coletados em diferentes províncias na área do Delta do Nilo, no Egito, em plantas jovens e adultas. Quatro marcadores microssatélites de DNA específicos, (*Xgwm*337, *Xcfd*7 ligada a *Pm*24, *Pm*35 e Xgwm332, *Xwmc*790) ligados a genes de resistência *Pm*37 foram selecionados para detectar esses genes em 13 cultivares de trigo egípcios. Nosso estudo revela a ausência de *Pm*24, *Pm*35 e *Pm*37 em todos os 13 cultivares de trigo egípcios. Estes resultados demonstram que os cultivares egípcios não possuem genes de resistência e necessitam incorporar um, dois ou mais genes resistentes num genótipo e nos cultivares comerciais suscetíveis ao patógeno.

Termos para indexação: Oídio; controle genético; DNA; marcadores moleculares.

INTRODUCTION

Powdery mildew of wheat, caused by the fungus (*Blumeria graminis* f. sp. *tritici* Marchal), is a common disease that spread all over the world. In Egypt, the disease appeared in the last few years with high disease severities on most of the common wheat cultivars especially in the Gharbia governorate, Nile Delta, Egypt. Powdery mildew can lead to reduction of wheat spike and grain number resulting in a yield loss e.g. 10% - 62% in Brazil **(**Costamilan, 2005), 48% in Canada (Everts; Leath, 1992; Maxwell et al., 2009), 10-18% in Egypt (El-Shamy et al., 2012) and 10-30% in China (Huang et al., 2013**)**. Utilization of resistant cultivars is the most economical and environmental-friendly approach to control the disease, enabling reductions in fungicide use. A diversified and effective resistant gene sources must be the basis of breeding wheat cultivars with powdery mildew resistance. Development of resistant germplasm to powdery mildew is based on interspecific hybridization and backcrossing (Murphy; Navarro; Leath, 2002; Navarro et

al., 2000). To date, 61 powdery mildew resistance genes, mapped to 43 loci (McIntosh; Yamazaki; Dubcovsky, 2008; He et al., 2009; Hua et al., 2009; Li; Fang; Zhang, 2009; Luo et al., 2009) have been identified and formally catalogued in wheat. However, some of the genes, derived from wild relatives, are difficult to use in wheat breeding because of linkage drag (Hospital, 2001). Consequently, there are only a limited numbers of effective race-specific resistance genes available for use by breeders. The following genes have been primarily used in development of powdery mildew resistant cultivars i.e. *Pm*2, *Pm*3, *Pm*4a, *Pm*4b, *Pm*6 and *Pm*8. More recently described resistance genes including *Pm*13, *Pm*21, and *Pm*24 were introduced in breeding programs in order to provide more diversity (Cheng et al., 2003; Sang et al., 2006). Molecular identification of specific DNA sequences can be used to identify the presence or absence of wheat powdery mildew (Pm) genes in wheat cultivars, their chromosomal location, the number of genes, and the way in which they are transmitted to progeny (Chen and Chelkowski, 1999).

The availability of molecular markers, used for identifying, mapping, and cloning powdery mildew resistance genes, has greatly enhanced the development of molecular breeding. Currently, microsatellites [simple sequence repeats (SSR)] are the preferred type of molecular marker for marker-assisted selection (MAS) in wheat breeding. Molecular markers are known to be useful in the process of detection of the disease resistance genes, especially in genotypes where the genetic back ground has not been clarified as is the case for most commercial cultivars. The genes *Pm*24, *Pm*35, and *Pm*37 were approved to be effective genes against the disease in the regions with similar ecological condition as Egypt in the world (Huang et al., 2000; Langridge et al., 2001; Miranda et al., 2007; Perugini et al., 2008). Therefore, this study was conducted to identify those powdery mildew resistance genes i.e. *Pm*24, *Pm*35 and *Pm*37 in 13 registered Egyptian bread wheat cultivars using SSR markers under the Egyptian conditions.

MATERIAL AND METHODS

Plant materials

Thirteen Egyptian bread wheat cultivars common in Egypt obtained from the National Wheat Program, Crop Research Institute, ARC, Giza (Table 1) and twenty-one powdery mildew host differentials resistant to the powdery mildew and the susceptible check cultivar chancellor were used in this study (Table 2). The monogenic wheat lines were kindly provided by Dr. Christina Cowger (USDA-ARS, North Carolina State University). Seeds of each line were sown in individual plastic pots (10 cm diameter) containing soil mixed with peat moss (1:1 w:w) under greenhouse conditions.

Table 1: Bread wheat cultivars and their pedigree used in this study.

Cultivars/ Pm genes	Pedigree
Gemmeiza-7	CMH74A.360/5x//Seri82/3/Agent CGM4611-2GM-3GM-1GM-0GM.
Gemmeiza-10	Maya 74"S"/ On// 1160-174/3/ Bb/4/ Chal "S"/5/Ctow.
Gemmeiza-11	BUC"S"/ Kvz"S"// 7c/ Seri 82 /3/Giza 168/ Sakha 61 GM7892-2GM-1GM- 0 GM.
Gemmeizas-12	OTS/3/ SARA/ THB// vee-CMss97Y0027S-5Y-010M-010Y-010M-2Y-1M-0Y-06M-06M-0GM.
Sids-12	BUC// 7c/ Ald/5/ Maya 74/ On/ 1160.147/3/ BB/ G11/4/ Chat"S" /6/ Maya/ vu1 // Cmh 74A.630/4* sx, SD7096- 4SD- 1SD-0SD.
Sids-13	ALmaz 19= Kauz "S" // Tsi /snb"S" ICW 94-0375- 4AP- 2AP-030AP-) APS- 2AP- 0APS- 050AP- 0AP- 0SD.
Sids-14	SW8488*2/ KUKUNA- CGSS01Y00081T-099M-099Y-099M-099B-9Y-0B-0SD.
Giza-168	MIL/BUC// Seri CM93046-8M-0Y-0M-2Y-0M
Giza-171	Gemmeiza-9 / Sakha-93
Misr-1	Oasis/skauz//4* BCN/3/2* Pastor CMss 00Y01881T-050M-030Y-030M-030WGY-33M- 0Y-0S.
Misr-2	SKAUZ/ BAV 92. CMss 96 M03611S- 1M- 0105Y-010M-010SY- 8M- 0Y-0S.
Sahka-93	Sakha 92TR81032 S8871-1S-2S-1S-0S.
Gemmeiza-9	Ald"S"/ Huac"S" // CMH 74A.630/ 5x CGM 4583-5GM- 1GM-0GM.

Table 2: Designated names, source, and chromosomal positions of identified resistance genes to powdery mildew used in this study.

Host differential	*Accession number*	*Resistance gene*	*Position*	*Source*
Axminster / 8*Ccy	CI 14114	*Pm*1a	*7AL*	*T. aestivum*
Ulka / 8* Cc	CI 14118	*Pm*2	*5DS*	*T.aestivum/ A. tauschii*
Asosan / 8* Cc	CI 14120	*Pm*3a	*1AS*	-
Chull / 8* Cc	CI 14121	*Pm*3b	1AS	*T. aestivum*
Sonora / 8* Cc	CI14122	*Pm*3c	1AS	*T. aestivum*
Ralle	None	*Pm*3d	1AS	*T. aestivum*
Pm3f	CI15888	*Pm*3f	1AS	*T. aestivum*
Pm4a	CI14124	*Pm*4a	*2AL*	*T. dicoccum*
Ronos	None	*Pm*4b	*2AL*	*T. carthlicum*
Pm5a	CI14125	*Pm*5a	*7BL*	*T. aestivum*
Coker747	None	*Pm*6	*2BL*	*T. timopheevii*
Transec	None	*Pm*7	*4BS.4BL-2Rl*	*S. cereale*
Kavkaz	PI 361879	*Pm*8	*1RS.1BL*	*S. cereale*
N14	None	*Pm*9	*7A*	*T. aestivum*
Wembley	None	*Pm*12	*6Bs*	*A. speltoides*
Pm 16	None	*Pm*16	*4A*	*T. dicoccoides*
Amigo	None	*Pm*17	1RS.1AL	*S. cereale*
Pm 24	None	*Pm*24	1DS	*T. aestivum*
NC96BGTD3	None	*Pm*35	5DL	*A. tauschii*
5-BIL29(durum)	None	*Pm*36	5BL	*T. dicoccoides*
NC99BGTAG11	None	*Pm*37	7AL	*T. timopheevii*
Chancellor	CI12333	None		

Disease assessments

Seedlings of each cultivar per line were inoculated 10 days after emergence by shaking conidia of the fungus from infected plants onto their leaves. The inoculum source originated from field grown plants infected with the fungus collected from different locations and multiplied on Morocco plants under controlled green house at Gemmeiza Research Station, Gharbya Governorate in 2013 and 2014 growing seasons. Disease response was recorded 8 days after inoculation according the scale used by Leath and Heun (1990). In this scale; 0 = immune, 1 - 3 = resistant reaction, 4 - 6 = intermediate reaction, 7 - 9 susceptible reaction. Efficacy % of Pm gene resistance was calculated according to the following according to Samborsky and Dyck (1976) as follow:

$$\text{Efficacy \% of Pm-gene} = \frac{\text{No. of resistant genes}}{\text{Total No. of the tested genes}} \text{ x } 100$$

DNA extraction

Total DNA of each wheat cultivar and 3 monogenic line designated in Table 3 was extracted from 200 mg leaf fresh leaf tissue was ground in liquid nitrogen with a mortar and pestle and subsequently DNA extraction accomplished using the Invisorb® Spin Plant Mini Kit (STRATEC Molecular, Germany) for purification for the DNA, according to manufacturer's instructions.

PCR amplification conditions

Four specific microsatellite (SSR) markers were screened in order to detect the *Pm*24, *Pm*35 and *Pm*37 genes in 13 common bread wheat cultivars grown in Egypt. These markers were Xgwm 337 SSR (*Pm*24), Xcfd7 SSR (*Pm*35) and Xgwm332, *Xwmc 790* (*Pm*37) listed in Table 4. Amplification of powdery mildew

monogenic lines region was conducted in an automated thermal cycler (C1000™ Thermal Cycler, Bio-RAD) using the primers and conditions listed in Tables 4 and 5 with one pre-denaturation cycle at 94 ºC for 3 min. Each PCR mixture was 25 µl with the following composition; 1 µl of 25 ng nucleic acid, 1 µl of each primer (10 pmol), 12.5 µl of GoTag® Colorless Master Mix (Promega Corporation, USA) and 9.5 µl of Nuclease free water (Promega). PCR products (15 µl) were analyzed by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide (7.0 µg/50 ml) and DNA bands were visualized using a UV trans-illuminator and photographed.

RESULTS AND DISCUSSION

Disease assessment of powdery mildew

Data in Table 6 reveal the efficacy percentage of 21 *Pm*-genes for resistance against 42 isolates of pathotypes of *B. graminis tritici*. However, the genes *Pm*2, Pm6, *Pm*12, *Pm*16, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 have remained completely effective for all isolates (100% efficacy). However, the genes *Pm*3b, *Pm*4b and *Pm*17 were completely effective in 2013 only and showed intermediate efficacy in 2014. The genes *Pm*1a, *Pm*3a, *Pm*3c, *Pm*3d, *Pm*3f, *Pm*4a, *Pm*5a, *Pm*7, *Pm*8 and *Pm*9 showed fluctuated efficacy, which ranged from 25.00 to 87.50% in 2013 season and from 38.23 to 97.05% in 2014 season. It could be recorded that the variety Chancellor showed 0.00 efficacy over the two seasons. All the Egyptian bread wheat cultivars showed susceptible responses ranged from 7 - 8 when tested to the 52 isolates of powdery mildew.

Microsatellite markers

Four SSR diagnostic markers which linked with resistance genes of *Pm*24, 35 and 37 were used in this study. The diagnostic marker *Xgwm*337 linked with *Pm*24 amplified fragment of 200 bp in the control *Pm*24 and was absent in all 13 Egyptian cultivars tested (Figure 1). Data in Figure 2 shows the fragment profile of the PCR amplifications of the SSR locus *Xcfd*7 (251 bp) in the control *Pm*35 and indicates the absence of *Pm*35 in all 13 Egyptian bread wheat cultivars. For detection of *Pm*37, two primers *Xgwm*332 and *Xwmc790* linked to resistance gene were used. The two primers amplified fragments of 193 bp (*Xgwm*332) and 76 bp for (*Xwmc790*) in the control *Pm*37 and were absent in the Egyptian bread wheat cultivars (Figures 3 and 4).

Molecular identification of specific DNA sequences can be used to identify the presence or absence of wheat powdery mildew (*Pm*) genes in wheat cultivars, (Chen and Chelkowski, 1999). Molecular marker techniques used for identification

Table 3: Designated names, source, and chromosomal positions of identified resistance genes to powdery mildew used in this study.

Pm genes	Cultivar/line	Source	Position	Reference
*Pm*24	*Pm*24	*T. aestivum*	1DS	Huang et al., 2000
*Pm*35	NC96NGTD3-Ab	*A. tauschii*	5DL	Miranda et al., 2007
*Pm*37	NC96NGTAG11-Ab	*T. timopheevii*	7AL	Perugini et al., 2008

Table 4: Powdery mildew genes, primers, their sequence and size of amplified fragment used in this study.

Gene	Primer	Sequence	Size of amplified marker fragment (bp)	Reference
*Pm*24	*Xgwm* 337 SSR	F 5′ CCTCTTCCTCCCTCACTTAGC 3′ R 5′ T CTAACTGGCCTTTGCC 3′	200 bp	Huang et al., 2000
*Pm*35	*Xcfd*7 SSR	F 5′ AGCTACCAGCCTAGCAGCAG 3′ R 5′ TCAGACACGTCTCCTGAAAA 3′	251 bp	Miranda et al., 2007
*Pm*37	*Xgwm*332 SSR	F 5′ AGCCAGCAAGTCACCAAAAC 3′ R5′ AGTGCTGGAAAGAGTGAAGC 3′	193 b	Perugini et al., 2008
	Xwmc 790 SSR	F5′AATTAAGATAGACCGTCCATATCATCCA3′ R 5′ CGACAACGTACGCGCC 3′	76 bp	

and confirmation of *Pm* genes to powdery mildew include Simple Sequence Repeats (SSR) known as microsatellite. It remains one of the most popular markers to-date and the latest designated powdery mildew resistant genes; *Pm*46 (Gao et al., 2012), *Pm*47 (Xiao et al., 2013), *Pm*49 (Piarulli et al., 2012) and *Pm*50 (Mohler et al., 2013) have been identified and mapped using this technique. Several other genes including *Pm* 1e, *Pm*5e, *Pm*24a, *Pm*24b, *Pm*27, *Pm*30, *Pm*31, *Pm*36, *Pm*40, *Pm*42, *Pm*43 and *Pm*45 were also located and mapped using microsatellite or Simple Sequence Repeat (SSR) markers.

Table 5: Cycle conditions of the used primers.

Pm gene	Conditions
*Pm*24	1 cycle of 3 min at 95 °C, followed by 35 cycles of 30 sec. at 95 °C, 20 sec at 55 °C, and 30 sec. at 72 °C, and finishing with 10 min incubation at 72 °C.
*Pm*35	1 cycle of 5 min at 94 °C, followed by 34 cycles of 1 min at 94 °C, 30 sec. at 56 °C, and 1 min at 72 °C, and finishing with 10 min incubation at 72 °C.
*Pm*37	1 cycle of 3 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 54 °C, and 2 min at 72 °C, and finishing with 10 min incubation at 72 °C.
*Pm*37	1 cycle of 3 min at 95 °C, followed by 40 cycles of 45 sec at 94 °C, 45 sec at 55 °C, and 1 min at 72 °C, and finishing with 10 min incubation at 72 °C.

Table 6: Gene efficacy percentage of 21 *Pm* genes to 8 and 34 isolates of powdery mildew at seedling stage and mean disease response of 13 Egyptian bread wheat cultivars in 2013 and 2014 seasons.

Pm gene	Gene efficacy percentage in						Mean disease response	
	2013			2014				
	*S	**R	Efficacy%	*S	*R	Efficacy%	Cultivar	Disease response
1a	5	3	37.50	22	12	35.29	Gemmeiza-7	8***
2	0	8	100	0	34	100	Gemmeiza-10	7
3a	1	7	87.50	19	15	44.11	Gemmeiza-11	8
3b	0	8	100	13	21	61.76	Gemmeiza-12	8
3c	1	7	87.50	22	12	35.29	Sids-12	6
3d	1	7	87.50	1	33	97.05	Sids-13	9
3f	5	3	37.50	13	21	61.76	Sids-14	9
4a	3	5	62.50	10	24	70.58	Giza-168	8
4b	0	8	100	6	28	82.35	Giza-171	8
5a	1	7	87.50	21	13	38.23	Misr-1	8
6	0	8	100	0	34	100	Misr-2	8
7	4	4	50	21	13	38.23	Sahka-93	7
8	6	2	25	18	16	47.58		
9	6	2	25	19	15	44.11		
12	0	8	100	0	34	100		
16	0	8	100	0	34	100		
17	0	8	100	21	13	38.23		
24	0	8	100	0	34	100		

To be continued...

Table 6: Continuation.

35	0	8	100	0	34	100
36	0	8	100	0	34	100
37	0	8	100	0	34	100
Chancellor	8	0	0.00	34	0	0.00

*S: Susceptible; **R: Resistance; ***7-9: Susceptible response.

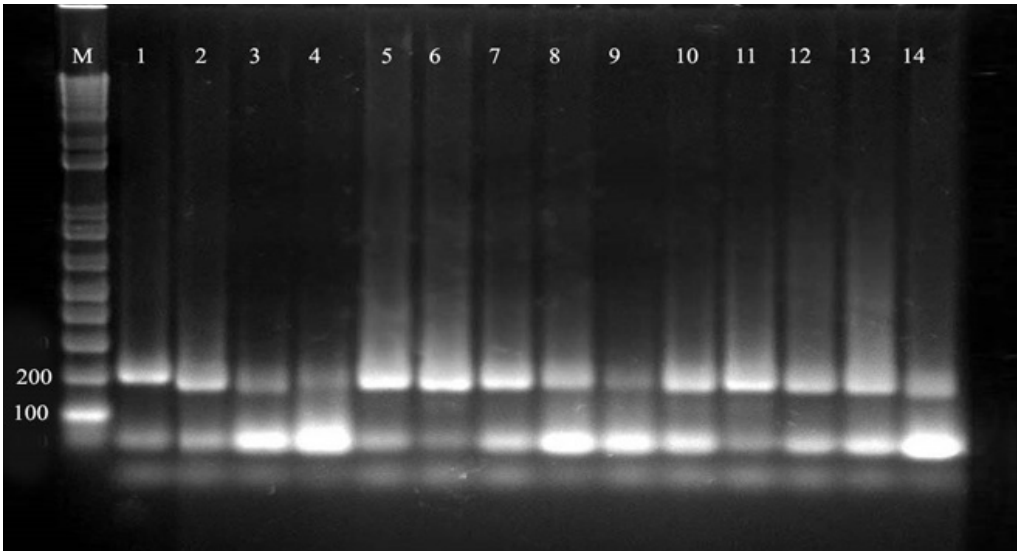

Figure 1: Fragment profile of the PCR amplifications of SSR *Xgwm*337 locus (200 bp) in *Pm*24 and 13 Egyptian cultivars. M: 100bp DNA ladder RTU (Gene Direx). 1: *Pm24* gene, 2: Gemmeiza-7, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: sids-12, 7: Sids-13, 8: Sids-14, 9: Giza-168, 10: Giza-171, 11: Misr-1, 12: Misr-2, 13: Sahka93.

The obtained data reveals that out of twenty-one *Pm* lines of wheat evaluated to 42 individual powdery mildew isolates, *Pm* genes *Pm*2, *Pm*6, *Pm*12, *Pm*16, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 were resistant at seedling stage and also, at adult stage. Only four DNA specific SSR markers i.e. *Xgwm*337, *Xcfd*7 linked to *Pm*24, *Pm*35 and *Xgwm*332, *Xwmc790* linked to *Pm*37 resistance genes were selected to detect these genes in 13 Egyptian common bread wheat cultivars, while all the tested Egyptian wheat cultivars showed susceptible responses at the both stages. This is first time to evaluate *Pm* genes in Egypt either at seedling or adult stages. The four SSR markers used in this study confirmed the absence of *Pm*24, *Pm*35 and *Pm*37 in the Egyptian bread wheat cultivars. SSR markers amplification products of the tested cultivars showed alleles to each resistance gene with different sizes. For detection of *Pm*24, *Pm*35 and *Pm*37 in Egyptian cultivars showed alleles with smaller size than in monogenic line. The results of our study are in agreement with the work of Mirinda et al. (2007). They found that when *Xcfd*7 microsatellite marker tested in the F2 progeny, a 251 bp fragment was associated with the resistant NCD3 (*Pm*35) allele and a 240 bp band was associated with the susceptible allele. Also, similar results were obtained by Blanco et al. (2008). In their study, the EST-SSR BJ261635 primer linked to *Pm*36 amplified two co-migrating bands (236 and 244bp) and one band in the susceptible plants (237bp). Two primers linked to *Pm*37 showed alleles with larger sizes than in the monogenic line. These alleles may be linked to susceptibility since the difference between resistance and susceptibility is base pair change (Lawrence et al., 1994). Also, Perugini et al. (2008) stated Simple sequence repeat (SSR) markers *Xgwm*332 and *Xwmc*790 were located 0.5 cM proximal and distal, respectively, to *Pm*37. Differences within genes or between genes in the DNA strand as long as unique sequences varying between the

plants of interest are referred to as polymorphisms. The use of resistant cultivars has proven to be an effective and environmentally safe strategy for controlling wheat fungal pathogens and eliminating or reducing the use of fungicides. Molecular markers tightly linked to disease resistance genes allow selection for resistance without the need to perform disease tests and even in the absence of the pathogen and that will facilitate combining more than one effective disease resistance gene in one genotype (Blanco et al., 2008; Langridge et al., 2001; Tanksley et al., 1989).

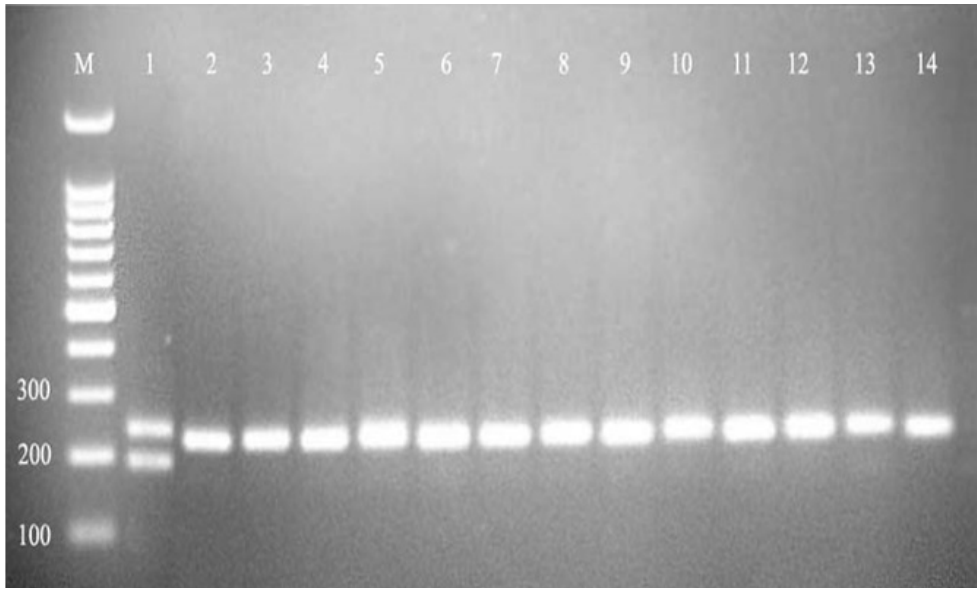

Figure 2: Fragment profile of the PCR amplification of SSR loci *Xcfd*7 (251 bp) in *Pm* 35 and 13 Egyptian cultivars. M.: 100bp DNA ladder RTU (Gene Direx). 1: *Pm35* gene, 2: Gemmeiza-7, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: sids-12, 7: Sids-13, 8: Sids-14, 9: Giza-168, 10: Giza-171, 11: Misr-1, 12: Misr-2, 13:Gemmeiza9.

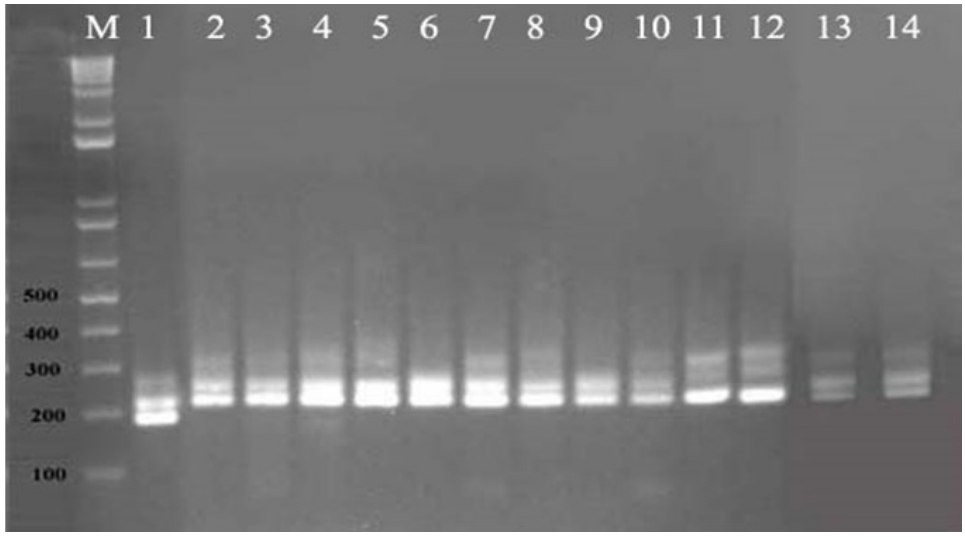

Figure 3: Fragment profile of the PCR amplification of SSR xgwm 332 locus (193 bp) in *Pm*37 and 13 Egyptian cultivars. M: 100bp DNA ladder RTU (Gene Direx). 1: *Pm*37 gene, 2: Gemmeiza-7, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: sids-12, 7: Sids-13, 8: Sids-14, 9: Giza-168, 10: Giza-171, 11: Misr-1, 12: Misr-2, 13: Sahka93.

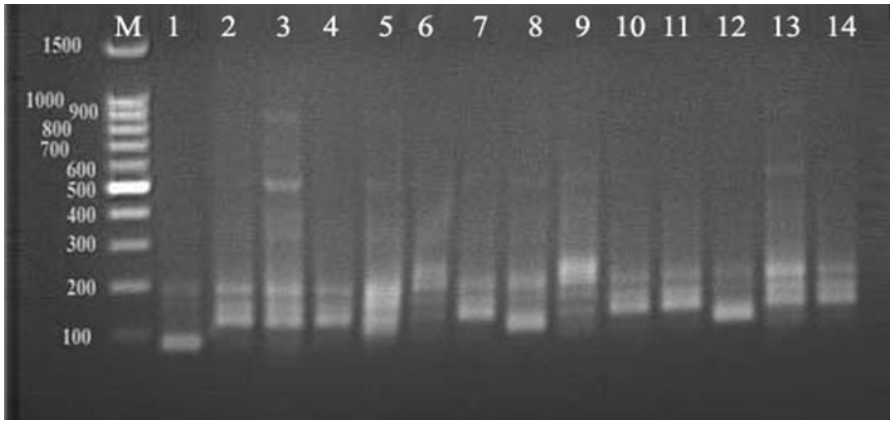

Figure 4: Shows the fragment profile of the PCR amplifications of SSR *Xwmc*790 locus (76 bp) in *Pm*37 and 13 Egyptian cultivars. M: 100bp DNA ladder RTU (Gene Direx). 1: *Pm37* gene, 2: Gemmeiza-7, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: sids-12, 7: Sids-13, 8: Sids-14, 9: Giza-168, 10: Giza-171, 11: Misr-1, 12: Misr-2, 13: Sahka93.

CONCLUSIONS

This study shows that *Pm*24, *Pm*35 and *Pm*37 are absent in the 13 tested Egyptian bread wheat cultivars. So, incorporating these resistance genes through wheat breeding program may lead to powdery mildew resistance genotypes with more durable resistance to the disease.

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