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Molecular Identification of some Powdery Mildew Resistance Genes in Ten Egyptian Durum Wheat Cultivars

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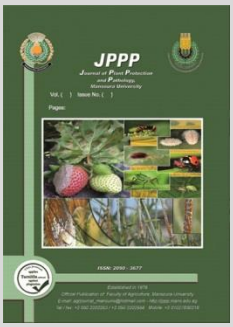

ABSTRACT

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most damaging foliar diseases of wheat world-wide. Nineteen powdery mildew differential monogenic lines (*Pm*) and ten durum wheat cultivars were evaluated for powdery mildew reaction at the seedling stage in a control-conditioned glasshouse and at adult stage under field conditions during 2018/2019 and 2019/2020 growing seasons in Gemmeiza Agriculture Research Station, ARC, Egypt. At seedling stage, *Pm*13, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 were completely effective against 78 tested isolates of powdery mildew followed by *Pm*16, *Pm*32, *Pm*34, *Pm*29 and *Pm*43 according to their descending order. At adult stage, all the *Pm* genes were resistant except *Pm*8 and *Pm*9, which showed susceptibility to the disease. Although, the durum wheat cultivars were susceptible to powdery mildew isolates at seedling stage, they ranged from intermediate resistant to resistant at the adult stage. To confirm the presence of resistant genes in 10 Egyptian durum wheat cultivars, five specific molecular markers i.e. *KSUG53*, *Xgwm*337, *Xcfd*7, *Bj*261635, and *Xgwm*332 linked to *Pm*13, *Pm*24., *Pm*35., *Pm*36 and *Pm*37 resistance genes were selected. The linked markers used in this study assured the presence of *Pm*13, *Pm*36 and *Pm*37 in all tested durum cultivars. However, *Pm*35 was present in BeniSweif1, BeniSweif3, BeniSweif5 and BeniSweif6. Moreover, data showed *that Pm*24 was absent in all tested cultivars.

Keywords: powdery mildew, durum wheat, *Pm* genes, SSR

INTRODUCTION

Durum wheat (*Triticum turgidum* L.) is cultivated tetraploid wheat species in the world (Chen *et al*. 2014; Rinaldo *et al*. 2017) used in food production such as pasta, puffed cereals, desserts and noodles (Gonzalez-Segura *et al*. 2014). Durum wheat is mainly cultivated under warm weather conditions in Upper Egypt. Durum wheat has affected annually by biotic and abiotic stresses (Singh *et al*. 2013). *Blumeria graminis* f. sp. *tritici* (*Bgt*), the causal agent for powdery mildew in bread/durum wheat, is very well known by farmers growing cereals. In Egypt, wheat powdery mildew has increased annually due to recurrent planting the same wheat area, increased planting density, climate change in recent years and increasing of nitrogen fertilization. No much data published about the effect of these factors on yield losses due to powdery mildew. In hexaploid wheat it caused over 34 % losses of the yield (Alam *et al*. 2013; Pearce *et al*.1996; El-shamy *et al*. 2012) and more over 45% (Brown *et al*. 2001). Developing the wheat introgression lines with resistance genes is the effective and environmentally efficient strategy to control powdery mildew disease. So far, 82 *Pm* resistance genes and alleles have been formally identified on 54 loci (McIntosh *et al*. 2013 and McIntosh *et al*. 2017), but most of them are race specific and are easily overcome by new *Bgt* isolates (Li *et al*. 2014). The implement of adult plant resistance (APR) to powdery mildew is more desirable for breeders than race-specific resistance where lines or cultivars showed susceptible reaction at seedling stage and being resistant at adult stage (Wang *et al*. 2005; Dieguez *et al*. 2014; Kumar *et al*. 2019). To provide an efficient breeding strategy for durable resistance to powdery mildew, it is essential to understand the genetics behavior of APR in powdery mildew. In Egypt, little studies have done on identification of *Pm* resistant genes and its efficacy in bread and durum wheat cultivars (Elshamy *et al*. 2016; Emara *et al*. 2016; Abdelrhim *et al*. 2018). So, the aims of this work are (i) evaluation of 19 powdery mildew monogenic lines and 10 durum wheat cultivars at seedling and adult stages to powdery mildew (ii) molecular identification of the most resistantnce genes in the durum Egyptian wheat.

MATERIALS AND METHODS

Wheat materials

Nineteen powdery mildew differentials monogenic lines were provided by Dr. Christina Cowger (USDA, ARS, North Carolina State University), Table (1) and ten Egyptian durum wheat cultivars common in Egypt obtained from the National Wheat Program, Field Crops Research Institute, ARC, Giza (Table 2) were used in this Study. The highly susceptible cultivar Chancellor was used in this study as susceptible check.

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Table1. Designated name, source, and chromosomal position of 19 identified resistance genes to powdery mildew according to Cowger *et al* (2012).

***Pm* genes**	**Position**	**Cultivar/line**	**Source**
Pm2	5DS	Ulka/*Cc	*Triticum aestivum/Aegilops*
Pm6	2DL	Coker747	*T.timopheevii*
Pm7	4BS.4BL-2Rl	Transec	*Secale cereal*
Pm8	1RL.1BL	Kavkas	*Secale cereal*
Pm9	7A	N14	*T.aestivum*
Pm12	6BS	Rembley	*A.speltoides*
Pm13	1DS	Pm13	*Aegilops. Longissima*
Pm16	4A	Norman rec. line	*T. dicoccoides*
Pm17	1RS.1AL	Amigo	*Secale cereal*
Pm20	6BS.6RL	Tam W-104	*Secale cereal*
Pm21/Pm31	6VS.6AL	DH2	*Haynaldia villosa*
Pm24	1DS	Chiyacao	*T. aestivum*
Pm29	7DL	Pova	*A. ovate*
Pm32	1BL.1SS	L501	*Ae. Speltoides*
Pm34	5DL	NC97BGTD7	*Ae. Tauschii*
*Pm*35	5DL	NC96NGTD3	*Ae. Tauschii*
*Pm*36	5BL	5-BIL29 (durum)	*T.dicoccoides*
*Pm*37	7AL	NC96NGTAG11	*T.timopheevii*
Pm43	2DL	NC96NGTAD8-CH5025	*T. intermedium*

Table 2. Durum wheat cultivars used in this study and their pedigree.

Cultivar	**Cross/pedigree and selection history**
BeniSweif1	JO/AA//FG CD9799-126M-1M-5Y-0M-0SD.
BeniSweif3	CROM/RUF0 CD4893-10Y-1M-1Y-0M-0SD.
BeniSweif4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/w*2 ICD88-1120-ABL-0TR-1BR-0TR-6AP-0AP-OSD
BeniSweif5	DIPPERZ/BUSHEN3 CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD
BeniSweif6	BOOMER-21/BUSCA-3 CDSS95Y001185-8Y-0M-0Y-0B-1Y-0B0S
Sohag1	GDOVZ469/JOS//61130-LSD.
Sohag2	CR/PELICANO//CR/GSH19-1SH-1SH-0SH.
Sohag3	MEXI/MGHA/51792//DURUM6 CD21831-25H-1SH-0SH
Sohag4	AJAIA-16//HORA/JRO/3/GAN/4/ZAR/5/SUOK-7/6/STOT//ALTAR84/ALD CDSS99B00778B-0SHS-OTOPY-0M-0Y-129Y-0M-0Y-1
Sohag5	CBC509CHILE//SOOTY-9/RASCON-37/9/USDA595/3/ D67.3/RABI//GRA/4/ALO/5/HUI/YAV1/6/ARDENTE/7/HUI/YAV79/8/POD-9DSS02Y01233T-0TOPB-0Y-0M-26Y-0Y-0SD

Disease assessment

At seedling stage.

The durum wheat cultivars and 19 powdery mildew monogenic lines (*Pm*) were tested at seeding stage in the controlled glasshouse, Wheat Diseases Res., Dept. at Gemmeiza Agric. Res. Station, ARC during 2018/2019 and 2019/2020 seasons. The inoculum source is 78 samples obtained from commercial wheat fields infected with the fungus from different locations in Delta provinces and multiplied on highly susceptible cultivar Chancellor.

A single colony for each isolate was transferred, using the spatula method on 10-day-old 'Chancellor'plants for multiplication. Five seeds of each entry were sown in individual plastic pots (10 cm diameter) containing mixed soli with coco peat (1:1 w: w) in three replicates as well as the susceptible cultivar Chancellor as control check. Infection types were recorded 8 days post inoculation using the 0-9 scale (Leath and Heun 1990) when the check showed complete infection with powdery mildew. Infection type (IT) from 0 to 3 was considered resistant (R), 4 to 6 moderately resistant (MR), and 7 to 9 susceptible (S). Gene efficacy was calculated according to the following equation (Green, 1966):

Gene efficacy % = No. of times the gene is resistance / Total no. of isolates x100

Evaluation of the tested materials under field conditions

Each genotype of the *Pm* genes and the durum wheat seeds were sown in one row, 2m length, 40 cm apart, 10 cm distance plant to plant and 20 seeds/row during 2019- 2020 seasons. Randomized complete block design with three replicates was followed. The experiment was surrounded by border rows of highly susceptible cultivar Chancellor and left to natural powdery mildew infection. Disease severity was scored according to Leath and Heun (1990) scale, when Chancellor showed maximum disease severity.

Molecular detection of *Pm* genes.

DNA extraction.

Fresh healthy leaf tissue (200 mg) of each wheat cultivar and monogenic line was used for extraction of total DNA. Leaves were ground in liquid nitrogen using tissuelyserand subsequently DNA extraction was accomplished using the CTAB method and (Cetyl trimethyl ammonium bromide) method as modified by Allen *et al* (2006). The DNA was diluted to a final concentration of 10 ng/µl and quantified in 1% agarose gel.

PCR amplification conditions.

PCR was carried out for each resistance gene using linked markers listed in (Table3).The PCR reaction was carried out in a 10 ml reaction volume containing 3.0 µl of template DNA (10ng/µl stock), 4.0 µl of 5X master mix PCR buffer (GeneDirex), 1.5 µl of each SSR marker (5mM) stock , the details of PCR amplification and product analysis were used as described by Elkot *et al* (2015)

Table 3. Powdery mildew genes, primers, their sequence and PCR conditions.

Gene	Primer	Sequence	Annealing temperature
*Pm*13	*KSUG 53*	5GCTGGCAGAGAGAGATTGAG-3 5CCAAATGACACAAACAACAT 3	42°C
*Pm*24	*Xgwm* 337	5 CCTCTTCCTCCCTCACTTAGC 3 5 T CTAACTGGCCTTTGCC 3	55°C
*Pm*35	*Cfd7*	5 AGCTACCAGCCTAGCAGCAG 3 5 TCAGACACGTCTCCTGAAAA 3	55°C
*Pm*36	*Bj* 261635	5 TAGCCTGGTACCATTCTGCC 5 CATTACACCAGAAGCCTAG	51.5°C
*Pm*37	*Xgwm* 332	5 AGCCAGCAAGTCACCAAAAC 3 5 AGTGCTGGAAAGAGTGAAGC 3	54°C

RESTULTS AND DISCUSSION

Results

Disease assessment at seedling and adult stages.

Data in Table (4) showed the differential monogenic lines; *Pm13*, *Pm24*, *Pm*35, *Pm*36 and *Pm*37 were completely resistant to all isolates at seedling stage followed by *Pm*16, *Pm*32, *Pm*34 (98.71% efficacy for each), *Pm*29 (97.43%efficacy) then Pm43 (91.02 % efficacy). *Pm*8, *Pm*9, and *Pm*17 genes showed the lowest efficacy percentage (from 15.38% to 20.51%), however, the rest genes i.e. *Pm*2, Pm6, *Pm*7, *Pm*12 and *Pm*21 showed efficacies ranged between 61.53 to 79.42% during 2018-2019 growing season. At adult stage, all the tested *Pm* genes showed reaction ranged from resistance to intermediate resistance (0 to 6 IT), while, *Pm*8 and *Pm*9 were susceptible (7 and 8 IT) as well as the Chancellor check (9 IT).

Table 4. Mean efficacy percentage of 19 *Pm* genes to 78 powdery mildew isolates in two growing seasons (2018/2019 and 2019/2020)

Pm gene	Disease reaction At seedling stage		Efficacy %	Disease reaction At adult stage
	S	R		
2	12	58	74.35	0
6	6	62	79.48	0
7	22	56	71.79	6
8	66	12	15.38	7
9	62	16	20.51	8
12	12	66	84.61	1
13	0	78	100.00	0
16	1	77	98.71	0
17	52	16	20.51	3
20	48	30	38.46	5
21	32	48	61.53	6
24	0	78	100.0	0
29	2	76	97.43	0
32	1	77	98.71	0
34	1	77	98.71	0
35	0	78	100.00	0
36	0	78	100.00	0
37	0	78	100.00	0
43	7	71	91.02	3
Chancellor	78	0	0.00	9

The data in Table (5) revealed that all the durum cultivars were highly susceptible at seedling stage (9 infection type).However, all the cultivars showed resistance to intermediate resistance responses to powdery mildew at adult stage ranged between 1 to 5 infection types. Beni Sweif cultivars showed resistant reaction types among 1 and 2. However, Sohag cultivars were intermediate resistant to powdery mildew with infection types 4 or 5.

Table 5. Mean of infection type of 10 Egyptian durum wheat cultivars to powdery mildew at seedling and adult stages in two growing seasons (2018/2019 and 2019/2020)

Cultivar	Infection type at	
	Seedling stage	Adult stage
BeniSweif1	9	1
BeniSweif3	9	2
BeniSweif4	9	2
BeniSweif5	9	2
BeniSweif6	9	2
Sohag1	9	4
Sohag 2	9	5
Sohag 3	9	4
Sohag 4	9	5
Sohag 5	9	5

Molecular identification of *Pm* genes.

Five molecular markers linked with the resistance genes *Pm*13, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 were used in this study.

Pm13

Figure (1) illustrates that the gene specific marker *KSUG 53* linked with *Pm*13 amplified product of 1000 bp in the control *Pm*13 and it was present in all 10 durum cultivars

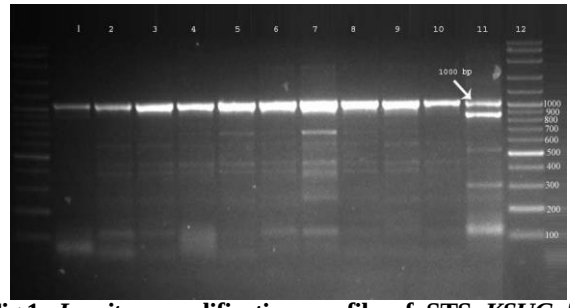

Fig.1. *In vitro* amplification profile of STS *KSUG 53* (1000 bp) in *Pm*13 and 10 Egyptian cultivars. 1: BeniSweif1, 2: BeniSweif3, 3: BeniSweif4, 4: BeniSweif5, 5: BeniSweif6, 6: Sohag1, 7: Sohag2, 8: Sohag3, 9: Sohag4, 10: Sohag5, 11: *Pm*13, 12:100bp DNA ladder .

Pm24

Figure (2) illustrates that the SSR marker *Xgwm*337 linked to *Pm*24 amplified fragment of 200 bp in the monogenic *Pm24* line. It was absent in all the ten durum cultivars.

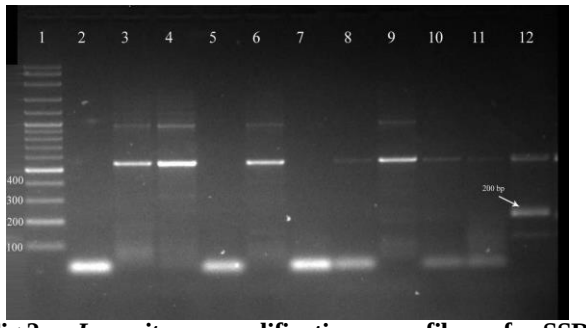

Fig.2. *In vitro* amplification profile of SSR *Xgwm337*(200 bp) in *Pm*24 and 10 Egyptian cultivars. 1: 100bp DNA ladder, 2: BeniSweif1, 3: BeniSweif3, 4: Beni Sweif4, 5: BeniSweif-5, 6: BeniSweif6, 7: Sohag1, 8: Sohag2, 9: Sohag3, 10: Sohag4, 11: Sohag5, 12: *Pm* 24.

Pm35

For powdery mildew resistance gene *Pm35*, the SSR the *Xcfd7* linked to *Pm35* amplified fragment of 251 bp in the control *Pm*35 .The data shown the presence of

*Pm*35 in BeniSweif1, BeniSweif3, BeniSweif5 and BeniSweif6 while it was absent in BeniSweif4 and all Sohag durum cultivars (Fig.3).

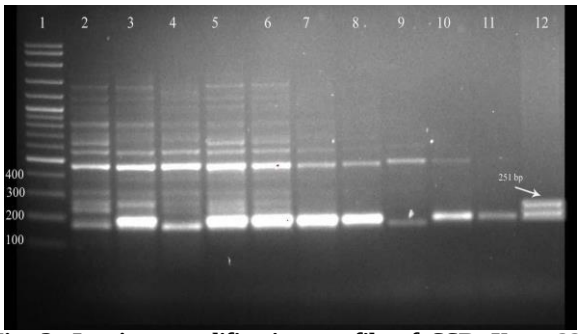

Fig. 3. *In vitro* amplification profile of SSR *Xgwm337* (251 bp) in *Pm*35 and 10 Egyptian cultivars. 1: 100bp DNA ladder. 2: BeniSweif1, 3: BeniSweif3, 4: BeniSweif-4, 5: BeniSweif5, 6: BeniSweif-6, 7: Sohag1, 8: Sohag2, 9: Sohag3, 10: Sohag4, 11: Sohag5, 12: *Pm* 35.

***Pm*36**

Genotyping with molecular marker BJ261635 linked to *Pm* 36 yielded positive fragment at 241bp and 248bp. The data indicates the presence of *Pm*36 in all the durum cultivars (Fig. 4).

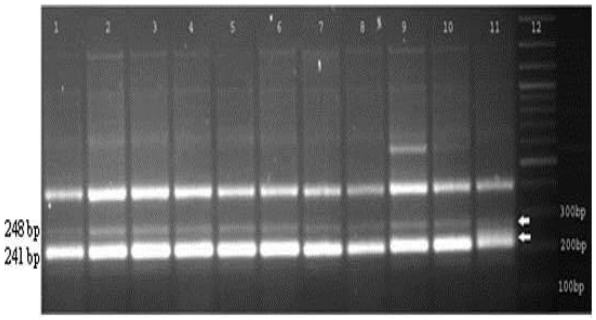

Fig.4. *In vitro* amplification profile of BJ261635 (244bp) in *Pm*36 and 10 durum cultivars. 1: BeniSweif1, 2: BeniSweif3, 3: BeniSweif4, 4: BeniSweif5, 5: BeniSweif6, 6: Sohag1, 7: Sohag2, 8: Sohag3, 9: Sohag4, 10: Sohag5, 11: *Pm* 36, 12:100bp DNA ladder RTU (Gene Direx).

Pm37

The SSR marker *Xgwm*332 linked to resistance gene *Pm37* was used to screen its presence in the tested ten durum cultivars. Obtained data revealed that the *Xgwm332* marker yielded positive product at 193 bp in all the tested durum wheat cultivars (Fig.. 5).

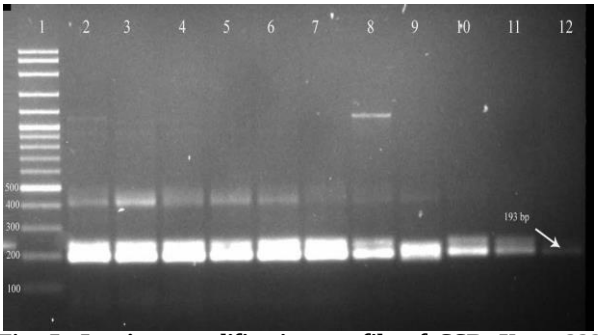

Fig. 5. *In vitro* amplification profile of SSR *Xgwm332* (193 bp) in *Pm*37 and 10 Egyptian cultivars. 1: 100bp DNA ladder., BeniSweif1, 3: BeniSweif3, 4: BeniSweif4, 5: BeniSweif5, 6: BeniSweif6, 7: Sohag1, 8: Sohag2, 9: Sohag3, 10: Sohag4, 11: Sohag5, 12: *Pm* 37.

We could summarize the obtained molecular marker data in Table (6).

Table 6. Monogenic lines linked primers, their presence/ absence in the durum wheat cultivars.

Cultivar	*KSUG 53 (Pm13)*	*Xgwm 337 (Pm24)*	*Cfd7 (Pm35)*	*Bj261635 (Pm36)*	*Xgwm332 (Pm37)*
BeniSweif1	+	-	+	+	+
BeniSweif3	+	-	+	+	+
BeniSweif4	+	-	-	+	+
BeniSweif-5	+	-	+	+	+
BeniSweif6	+	-	+	+	+
Sohag1	+	-	-	+	+
Sohag2	+	-	-	+	+
Sohag3	+	-	-	+	+
Sohag4	+	-	-	+	+
Sohag5	+	-	-	+	+

+ : presence - : absence

Discussion

Due to dynamic nature of *B. graminis* f. sp. *tritici*, new virulent isolates have evolved and defeated resistant wheat cultivars. Therefore, identification of resistance genes either in commercial wheat cultivars or wild relatives is crucial factor for utilizing it in breeding programs. A total of 19 powdery mildew genes and 10 durum wheat cultivars were tested for resistance against 78 *B. graminis* f. sp. *tritici* isolates in the growing season 2018-2019. Our study indicated that *Pm*13, *Pm*24, *Pm*35, *Pm*36 and *Pm*37 monogenic lines were totally effective against powdery mildew at seedling. Moreover, they were also resistant at adult stage under natural disease conditions. Similar results were obtained by several workwers like Petersen *et al*. 2015; Elshamy *et al*. 2016; Golzar *et al*. 2016. In contrast to our work, Li *et al*. (2019) found that *Pm*35 showed moderately susceptible or highly susceptible reaction while *Pm*13 and *Pm*37 conferred high or moderate resistance to powdery mildew isolate. On the other hand, durum wheat cultivars response changed from susceptible at seedling to resistant at adult stage may be due to the additive effect of existing *Pm* resistance genes.

This meaning that the cultivar showed adult plant resistance (APR) have genes becomes effective at the post-seedling stages in the field. Genes responsible for APR resistance in these tested durum wheat cultivars were not characterized before, so, specific SSR markers were used to confirm the presence of resistance genes. SSR marker is efficient and fast method for identification of resistance genes since conventional phenotypic methods are time consuming. Moreover, The microsatellite markers are easy to handle, inexpensive, highly polymorphic, reliable and used for mapping and identifying many powdery mildew resistance genes (Yua *et al*. 2018). Our SSR results revealed that *Pm*13, *Pm36* and *Pm37* were present in the evaluated durum cultivars , *Pm*35 present in some cultivars while *Pm24* was absent in all durum cultivars. Altogether, the current study provided reliable information on the presence of powdery mildew resistance genes in commercial durum wheat cultivars which can be implemented in Egypation national wheat breeding programs. Therefore, durum wheat cultivars and resistant monogenic lines are promsing source of resistance to powdery mildew disease since bread wheat cultivars are susceptible to powdery mildew. Specific hybridization will transfer one or more of resistance genes from durum to bread wheat for more durable reistance.

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التعريف الجزيئى لبعض جينات المقاومة لمرض البياض الدقيقى فى عشرة أصناف مصريه من قمح الديورم باستخدام المعلمات الوراثية

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يعتبر مرض البياض الدقيقى واحدا من أهم الأمراض المدمرة لمحصول القمح واسعة الانتشار. تم تقييم عدد تسعة عشر سلاله قمح أحادية الجين وعشرة أصناف مصرية من قمح الديورم لمرض البياض الدقيقى فى طور البادرة لعدد ٧٨ عزله من البياض الدقيقى بالصوبة الزجاجية المكيفة وفى طور النبات البالغ بمحطة البحوث الزراعية بالجميزة – مركز البحوث الزراعية خلال موسمى الزراعة ٢٠١٨/ ٢٠١٩ و ٢٠١٩ / ٢٠٢٠. أظهرت النتائج أن الجينات *Pm*13, *Pm*24, *Pm*35, *Pm*36, *Pm*37 كانت مقاومه تماما يليها الجينات *Pm*43 *Pm*29 *Pm*34 *Pm*32 *Pm*16, بينما أظهرت جميع الجينات مقاومة للمرض فى طور النبات البالغ فيما عدا الجينات *Pm*8 و *Pm*9 كانت قابلة للإصابة بالبياض الدقيقى. على الرغم من أن أصناف قمح الديورم أظهرت قابليه للإصابة فى طور البادرة إلا أنها اظهرت رد فعل يتراوح بين متوسط المقاومة إلى المقاوم للمرض فى طور النبات البالغ. للتأكيد على وجود الجينات *Pm*13, *Pm*24, *Pm*35, *Pm*36, Pm37 من عدمه فى أصناف قمح الديورم تم استخدام عدد خمس من المعلمات الوراثيه المرتبطه بهذه الجينات باستخدم تكنيك *P.C.R* أظهرت النتائج أن الجينات *Pm*13, *Pm*36, *Pm*37 موجودة باصناف القمح المختبره بينما الجين *Pm*35 كان موجودا بالأصناف بنى سوف ١ – بنى سويف٣ – بنى سويف٥ و بنى سويف٦ فقط بينما الجين *Pm24*, لم يكن موجودا بجميع الأصناف المختبرة.