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## GENETIC ANALYSIS AND ISSR BASED MARKERS FOR LEAF RUST RESISTANCE IN BREAD WHEAT (*Triticum aestivum* L.)

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**ABSTRACT:** The present study aimed to investigate the genetic behavior of leaf rust resistance in bread wheat (*Triticum aestivum* L.), in a population of five parents and ten F<sub>1</sub> hybrids under field conditions with artificial inoculation. Wide variations among parents and their F<sub>1</sub> hybrids were detected for the five characters under study, *i.e.* incubation period (IP), disease severity (DS), infection response (IR), average co infection (ACI) and area under disease progress curve (AUDPC). The average means of criteria related to resistant varieties showed that Giza 168 and Sakha 93 wheat cultivars have good response for resistance as long time of incubation period, as well as little amount of disease severity, the same performance of both varieties for infection response, average co infection and AUDPC=  $D [1/2 (Y_1 + Y_k) + (Y_2 + Y_3 + \dots + Y_{k-1})]$  criteria. The behaviour of F<sub>1</sub>'s for studied criteria appeared to be different among and within five criteria. Gemmiza 11 × Giza 168 possessed complete dominance of long period, high (DS) and (ACI). In contrast, it was low value of AUDPC. The analysis of variance for five criteria were recorded highly significant differences among parents and their hybrids. The regression of (Wr/Vr) was insignificantly different from unity for all criteria except disease severity (DS). Significant differences of H<sub>1</sub> and H<sub>2</sub> estimates were obtained for incubation period (IP), disease severity (DS) criteria. While, AUDPC criteria possessed significant differences of D estimate. Mean degree of dominance (H<sub>1</sub>/D)<sup>1/2</sup> indicated the presence of over dominance for all studied criteria. The proportion of genes with positive and negative effects in the parents (H<sub>2</sub>/4H<sub>1</sub>) indicated an equal frequencies of positive and negative among the parents for DS and AUDPC criteria, except IP possessed nearly equal positive and negative gene among the parents. F<sub>1</sub> graphic analysis revealed that cultivars G.168 and Sakha 93 had most dominant genes for resistance but cultivars Gemmiza 11 and Gemmiza 9 had most recessive genes for susceptibility. Bulked Segregant Analysis were used by eight ISSR primers, the results confirmed the importance of Giza 168 may be as doner of resistant gene's, as well as Gemmiza 9. These confirmed the importance of Giza 168 considered as in late genotypes for using it to transport leaf rust resistant genes to other genotypes, Gemmiza 9 followed Giza 168 in the importance of leaf rust resistance, these results confirmed also the susceptibility of Gemmiza 11 variety for leaf rust disease in bread wheat.

**Key words:** Hybridization, artificial inoculation, diallel analysis, molecular markers.

### INTRODUCTION

Leaf rust, caused by the fungal pathogen *Puccinia triticina* Eriks. The wheat leaf rust disease fungus is adapted to a range of different climates, and the disease can be found in diverse wheat growing areas throughout the world. Wheat cultivars that are susceptible to leaf rust regularly suffer yield reductions of 5–15%

according to Samborki (1985) or greater, depending on the stage of crop development when the initial rust infections occur according to Chester (1946). The nature and pre-vention of the cereal rusts as exemplified in the leaf rust of wheat. Genetic resistance is the most economical and preferable method of reducing yield losses due to leaf rust. Various wheat breeding programs throughout the world had

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multi results in producing cultivars with long-lasting, effective resistance to leaf rust. Genetic resistance to leaf rust can be most fully utilized by knowledge of the identity of resistance genes in commonly used parental germplasm and released cultivars. Depending on the severity and duration of infection, leaf rust, caused by the fungal pathogen *Puccinia triticina* Erikss., has been the most prevalent disease in wheat-producing areas (Kolmer, 2005). The greater is the area under the cultivars since ancient times, leaf rust, caused by an obligate parasitic fungus *Puccinia triticina* Erikss., has been a challenge for wheat growing and, which continues to cause annual losses and to be an important disease of wheat worldwide (Huerta-Espino *et al.*, 2011; Kolmer, 2013). Due to a wide variability of the pathogen, there are continuously emerging new virulent races which overcome resistant genes of a host. Because of the airborne nature of urediniospores, the pathogen can be transferred by wind to adjacent and distant wheat growing areas (Kolmer, 2005). Disease resistance genes, transferred from wild species, support wheat production on a global level (Davoyan A.N.E.R 2011). The present study aimed to study the genetic behavior and ISSR molecular markers for leaf rust resistance in bread wheat (*Triticum aestivum* L.) under field conditions by using artificial inoculation according to Guha *et al.* (1996).

## MATERIALS AND METHODES

The present investigation was carried out at the Experimental Farm and Molecular Genetics Lab, Faculty of Agriculture, Zagazig University, Egypt, during the three growing seasons (2014 to 2016). Five bread wheat varieties namely (Giza 168, Gemmiza 9, Gemmiza 11, Sids 13 and Sakha, 93) and their F<sub>1</sub> hybrids were used in this experiment.

Artificial inoculation used in this experiment was done by using a mixture strains of leaf rust spores, (PKDCH - IKCGC - IBHBD) during 2015-2016 and (NKTSS - PHTTT - PKTTT) during 2016-2017. These varities were obtained from Crop Research Institute, ARC, Giza, Egypt.

Source of freshly collected urediospores of *Puccinia triticina*. Strains were kindly provided by wheat, Dis. Res. Dept., Pl. Pathol. Res. Inst., ARC, were used as artificial inoculation.

Moreover Morocco, *Triticum spelta* saharensis and thatcher wheat cvs, were used as a surrounding spreeder border.

To study the inheritance of wheat leaf rust resistance some wheat cultivars were chosen as fowllos, two resistant parents (Giza 168, Sakha 93) and three susceptible ones (Gemmiza 11, Gemmiza 9 and Sids 13) Each entry was sown in a plot of ten rows, it's area was 2m<sup>2</sup>, distance between each two rows was 20 cm, each row was planted by 5 gm of seeds. Leaf rust resistance components were calculated as follows:

- 1-Incubation period (IP).
- 2- Disease severity (DS) according to (Peterson *et al.*, 1948).
- 3-Infection response (IR).
- 4-Average Co Infection as outlined by Das *et al.* (1993).
- 5-Area under disease progress curve (AUDPC), as calculated by Pandey *et al.* (1989).

In the first winter season 2014 - 2015, the five cultivars were sown for hybridization between them, 10 F<sub>1</sub> hybrids were obtained as diallel cross.

In the second winter season 2015-2016, five cultivars and their F<sub>1</sub> hybrids were sown in experimental farm in 20/11/2015. Artificial inoculation for leaf rust disease was carried out with uredospores of leaf rust mixed with baby powder in the late day while there was water film on the leaves blades to allow spores germination, grow and appear the leaf rust postules (Artificial inoculation) according to Tervet and Cassel (1951).

In the third winter season 2016-2017, G.168, Gemmiza 9, Gemmiza 11, F<sub>1</sub> and F<sub>2</sub> hybrids of these cultivars were sown in experimental farm in 20/11/2016, artificial inoculation for leaf rust disease was carried out as previously mentioned for application of Bulked segregant analysis (BSA) according to Guha *et al.* (1996).

## Molecular Genetics

Bulked segregant analysis (BSA) was used in the present study. Three parents, two F<sub>1</sub> hybrids and four F<sub>2</sub> generations were investigated by using ISSR markers technique (Tables 2 and 3).

**Table 1. List of five different bread wheat cultivars and their pedigree which were evaluated throughout the present study**

Cultivar	Pedigree
- Gemmiza 9	-Ald "S"/Huac "S"/CMH74A.630/5Xcgm4583-5GM-OGM.
- Gemmiza 11	-BOW"S"/KVZ"S"/7C/SER182/3/GIZA168/SKHA61.
- Giza 168	-MIL/BUC//SeriCM93046-8M-OY-OM-2Y-OB.
- Sids 13	-AMAZ19=KAUZ"S".
- Sakha 93	-Sakha92/TR810328 S8871-IS-25-OS.

**Table 2. The nine genotypes that were used in bulked segregant analysis (BSA) by ISSR technique with eight primers**

Parents	F <sub>1</sub> hybrids	F <sub>2</sub> hybrids
1- Giza 168	4-Giza 168xGemmiza 11	6-30 (R) Giza 168xGemmiza 11*
2- Gemmiza 9	5-Gemmiza 9xGemmiza 11	7-30 (S)Giza 168xGemmiza 11
3- Gemmiza 11		8-30 (R) Gemmiza 9xGemmiza 11
		9-30 (S)Gemmiza 9xGemmiza 11

\* The thirty plant for F<sub>2</sub> resistants (R) and suscibtability (S) were collected for F<sub>2</sub> generation.

**Table 3. Sequence and codes of ISSR primers used for leaf rust resistance in bread wheat (*Triticum aestivum* L.)**

Sr. No.	Primer	Sequance (5'-3')	Primer code
1	844A	5' CTC TCT CTC TCT CTC TGC 3'	RSW-1000
2	844B	5' CTC TCT CTC TCT CTA 3'	RSW-1001
3	HB12	5' CAG CAG CAG GC 3'	RSW-1002
4	HBS10	5' GAG AGA GAG AGA CC 3'	RSW-1003
5	HBS11	5' GTG TGT GTG TGT CC 3'	RSW-1004
6	17889A	5' CAC ACA CAC ACA AC 3'	RSW-1005
7	17889B	5' CAC ACA CAC ACA GT 3'	RSW-1006
8	17899A	5' CAC ACA CAC ACA AG 3'	RSW-1007

## Statistical Analysis

Means and their least significant differences for each studied character were calculated according to **Gomez and Gomez (1984)**. Diallel F<sub>1</sub> analysis was applied to estimate the genetic parameters as described by **Hayman (1954 a and b)**. Illustration of **Singh and Chaudhary (1977)** was adopted to estimate the components of genetic variance and heritability in broad and narrow sense.

## RESULTS AND DISCUSSION

Mean performance of studied wheat genotypes for leaf rust criteria is presented in Table 4. Wide variations among parents and their F<sub>1</sub> hybrids were detected for the five characters under study, *i.e.*, 8.65 -11 for (IP), 2.4-8.26 for (DS), S-MR for (IR), 0.96-8.26 for (ACI) and 43.33-86.3 for AUDPC. The average means of criteria related to varieties resistance showed that the two varieties Giza 168 and Sakha 93 had a good response for leaf rust resistance. In contrast Gemmiza 9, Gemmiza 11 and seds 13 can consider as a susceptible varieties for leaf rust disease. Similar results show. These results may the importance of the four criteria, IP, DS, IR and ACI than the genetic mechanism of area under disease progress curve (AUDPC). These results agreed with the findings obtained by **Penthus (1959) and Oury *et al.* (1993)**.

The analysis of variance for the five criteria showed high significant differences among parents and their hybrids (Table 5). The major assumption postulated for diallel analysis (**Hayman, 1954a**) was found to be valid as the  $t^2$  value was insignificant for all characters. The regression of (W<sub>r</sub>/V<sub>r</sub>) was insignificant different from unity for all criteria except disease severity (DS).

Estimates of the genetic components of variations and their proportions are presented in Table 6. Significant differences of H<sub>1</sub> and H<sub>2</sub> estimates were obtained for incubation period (IP), disease severity (DS) criteria indicating the importance of dominance gene effects in the genetic control of them. While, AUDPC criteria possessed significant differences of D estimate,

indicating the importance of additive gene effects in its genetic control. These results confirm with the previous conclusion about the behavior of F<sub>1</sub> hybrids for these criteria. Mean degree of dominance (H<sub>1</sub>/D)<sup>1/2</sup> indicated the presence of over dominance for all studied criteria. The proportion of genes with positive and negative effective effects in the parents (H<sub>2</sub>/4H<sub>1</sub>) indicated unequal frequencies of positive and negative among the parents for DS and AUDPC criteria, except IP possessed nearly equal positive and negative gene among the parents. Also unequal frequencies of dominance and recessive genes were observed between parents for all the three criteria with more dominant than recessive genes. By comparing (W<sub>r</sub>+V<sub>r</sub>) values for each array with the mean of common parents, comparing (W<sub>r</sub>i+V<sub>r</sub>i) correlation coefficient (r) between them is negative, it means that parents containing the most increasing genes and having the lowest values of (W<sub>r</sub>i+V<sub>r</sub>i) and thus containing most dominant genes, these are conclude whether or not the increasing or decreasing genes are the dominant over (**Singh and Chaudhary, 1977**). Long period dominant over short period for incubation period for negative disease severity, the lower values dominant over higher value of disease severity. As well as, lower AUDPC value dominant over higher value. These results agree with the findings from W<sub>r</sub>/V<sub>r</sub> graph, whereas the parent Seds 13 possess most dominant genes. In contrast, Gemmiza 11 possess most recessive genes. Disease severity from W<sub>r</sub>/V<sub>r</sub> graph, indicated that the seds 13 possess most dominant genes and sakha 93 has most recessive genes, these results explained the susceptibility of dominant over resistance. In the same trend for AUDPC-Y<sub>D</sub> Y<sub>R</sub>, values indicate the previous conclusion in the direction of dominance. Whereas the heritability in broad and narrow sense indicate the lower values of narrow sense and the broad sense. These results confirm the importance of non additive gene effects which play an important role for the inheritance of leaf rust disease resistance, which be selected at late generations to improve the resistance of leaf rust.

**Table 4. Average mean of incubation period (IP), disease severity (DS), infection responses (IR) and area under disease progress curve (AUDPC) of wheat genotypes to leaf rust disease under field conditions with artificial inoculation**

Genotype	Incubation period (IP)	Disease severity (%)	Infection response (IR)	Average co Infection (ACI)	AUDPC
Gem.9	9.33	5.866	S	5.866	66.5
Gem.11	8.65	5.866	S	5.867	71.166
G.168	11	2.4	MR	0.96	52.5
Sids 13	11	8.266	S	8.267	72.33
Sakha 93	10	2.4	MR	0.96	43.33
P1×P2	8.	5.866	S	5.867	71.16
P1×P3	10	3.466	MS	2.733	61.83
P1×P4	10	6.933	S	6.933	73.5
P1×P5	11	7.6	S	7.6	58.33
P2×P3	8	5.866	S	5.866	38.5
P2×P4	10	6.933	S	6.933	58.33
P2×P5	8	2.933	MR	1.173	70
P3×P4	9	6.933	S	6.933	77
P3×P5	10	8.266	S	8.266	78.16
P4×P5	11	6.933	S	6.933	86.33

**Table 5. Analysis of variance,  $t^2$  values, regression coefficients for validity and their test of significant for incubation period (IP), disease severity (DS) and area under disease progress curve (AUDPC)**

Source of variation	MS			
	df	IP	DS	AUDPS
Replication	2	38.6**	33.60**	281.10**
Genotypes	14	3.86*	12.285*	520.2**
Error	28	0.123	1.451	148.51
$T^2$		2.494	1.209	0.118
<b>b</b>		0.410	0.745	0.052
$\pm$ SE ( <b>b</b> )		0.544	0.068	0.964
H0: <b>b</b> =0		0.754	10.92**	0.053
H0: <b>b</b> =1		1.083	3.480*	0.981

**Table 6. Mean estimates of components of genetic variation and their proportions of the studied criteria under artificial inoculation with leaf rust strains**

Component of variation and proportion	IP	DS	AUDPC
<b>D</b> ±SE(D)	0.470±0.537	-0.856±2.067	272.447±94.89**
<b>F</b> ±SE(F)	0.4261.341	-2.638±5.164	212.906±237.05
<b>H1</b> ±SE(H1)	3.165±0.364**	12.039±1.402**	369.190±256.28
<b>H2</b> ±SE(H2)	3.234±1.315*	8.766±5.063	301.521±232.45
<b>h<sup>2</sup></b> ±SE(h <sup>2</sup> )	1.499±0.888	0.817±3.418	6.374±156.94
<b>E</b> ±SE(E)	0.896±0.219**	1.198±0.843	52.45±38.74
<b>(H1/D)<sup>1/2</sup></b>	2.594	1.59	1.164
<b>H2/4H1</b>	0.238	0.179	0.204
<b>Dom/Res</b>	1.423	1.131	2.011
<b>r</b>	0.265	-0.019	-0.136
<b>r<sup>2</sup></b>	0.701	0.001	0.0185
<b>Y<sup>D</sup></b>	8.650	6.070	53.75
<b>Y<sup>R</sup></b>	10.88	5.940	148.36
<b>H<sup>2</sup><sub>(ns)</sub></b>	0.007	0.433	0.332
<b>H<sup>2</sup><sub>(bs)</sub></b>	0.470	0.798	0.726

**Table 7. Mean value of Wr, Vr of the arrays under study for incubation period, disease severity, average co infection and area under disease progress curve**

Array	IP			DS			AUDPC		
	Wr	Vr	Yr	Wr	Vr	Yr	Wr	Vr	Yr
<b>1</b>	0.272	1.477	9.333	0.199	6.421	5.866	13.22	161.56	66.5
<b>2</b>	1.261	1.966	8.633	0.704	2.467	5.866	14.76	44.64	71.16
<b>3</b>	-0.505	1.588	11.00	0.327	3.889	2.40	204.43	138.42	52.5
<b>4</b>	0.072	0.323	11.00	0.071	0.533	8.266	141.35	63.019	72.3
<b>5</b>	0.438	1.366	10.00	0.152	7.559	2.40	93.65	285.60	43.33
<b>Total</b>	1.538	6.710	47.333	2.353	20.87	24.798	467.43	693.26	339.5
<b>Mean</b>	0.307	1.340	9.466	0.470	4.174	4.959	93.486	138.65	67.9

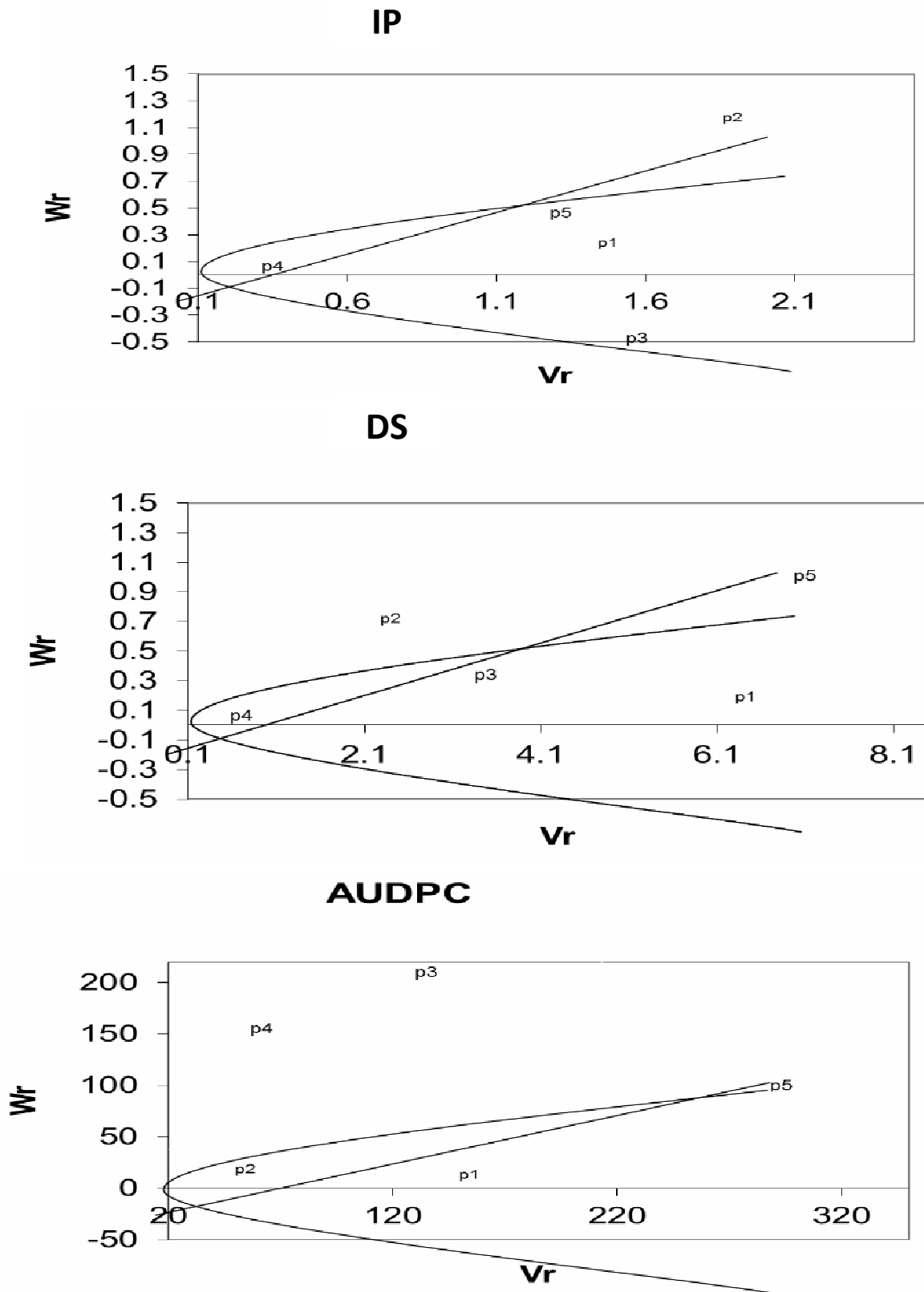


Fig. 1. Graphic analysis for  $F_1$   $W_r/V_r$  of incubation period (IP), disease severity (DS) and area under disease progress curve (AUDPC)

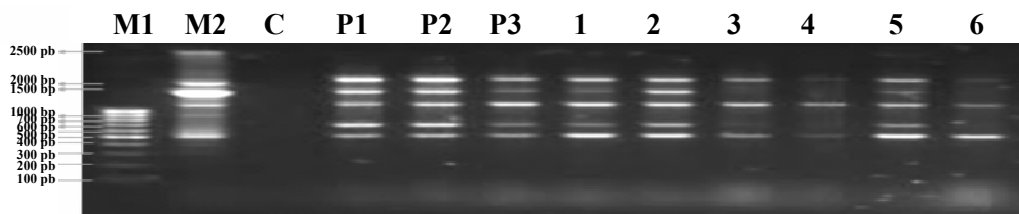
Graphic analysis of these criteria showed that these varieties have over dominance for area under disease progress curve, incubation period and disease severity. The F<sub>1</sub> graphic analysis confirmed the above results on the mean degree of dominance, in addition most dominance gene effects play an important role in genetic control of leaf rust, the regression line passed below the point of origin for disease severity (DS), incubation period (IP) and area under disease progress curve (AUDPC) suggesting over dominance. These findings confirmed the previous results from (H<sub>1</sub>/D)<sup>1/2</sup> proportions.

### Molecular Analysis

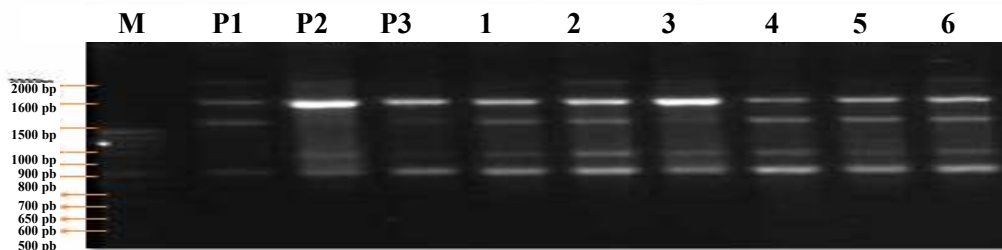
In this ISSR investigation, eight ISSR primers were used. Bulked segregant analysis technique were applied in two hybrids, each hybrid included two parents (resistant and susceptible), F<sub>1</sub>'s and two extreme of F<sub>2</sub> (plant resistant group and other plant susceptible group). The results confirmed the importance of Giza 168 cultivar, whereas possessed one band (2000bp) in primer 844A at the parent and more resistant F<sub>2</sub> group. As well as, Gemmiza 9

cultivar had one 400bp band with (844A), and their most resistant F<sub>2</sub> group. In contrast Gemmiza 11 had one band (1500bp) with (HBS10) primer and the more susceptible of their F<sub>2</sub> group, as well as, this cultivar Gemmiza 9 had also one band (1000bp) with (HBS10) primer and more susceptible for their F<sub>2</sub> group. For confirming of the important results were obtained from (17889A) primers, whereas 1500bp band appeared at Giza 168 parent, their F<sub>1</sub>'s and more resistant F<sub>2</sub> group. More remark confirmed the important of Giza 168 as a doner for leaf rust resistant gene's. This cultivar had 17889B primer, on two bands *i.e.*, 1200bp and 700bp at the mother cultivar (Giza 168), their F<sub>1</sub>'s and more resistant F<sub>2</sub> group. The conclusion of these results confirmed the importance of Giza 168 wheat cultivars which considered as illate genotype for using of it to transport it leaf rust resistant genes to other genotypes, Gemmiza 9 followed Giza 168 in the importance of leaf rust resistance, these results confirmed also the susceptibility of Gemmiza 11 cultivar for leaf rust disease.

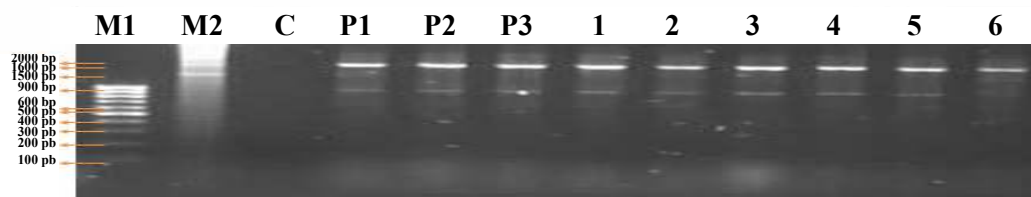
#### Primer 844A



#### Primer 844B

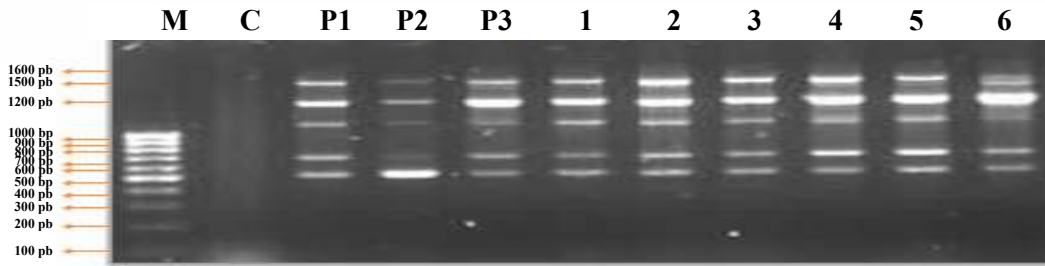


#### Primer HB12

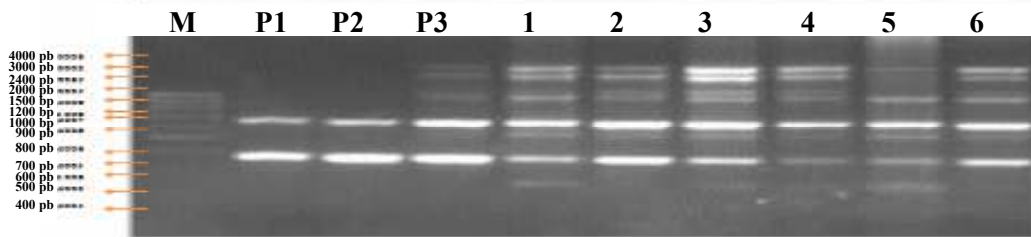




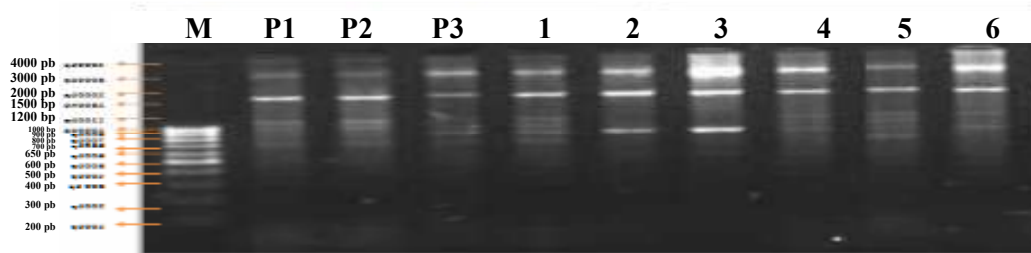
**Primer HBS10**



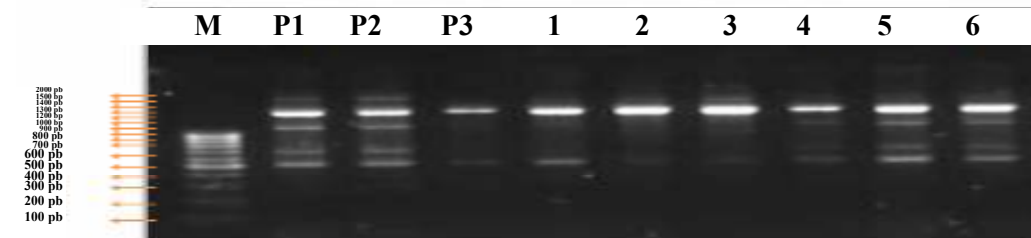
**Primer HBS11**



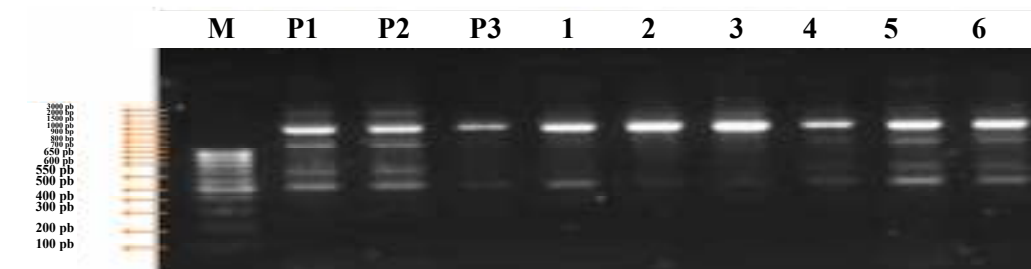
**Primer 17889A**



**Primer 17889B**



**Primer 17899A**



**Fig. 2. Photos of gel electrophoresis for nine DNA bulks from wheat genotypes by using eight primers**

**Table 7. Survey of amplified ISSR bands in the studied leaf rust resistance in bread wheat (*Triticum aestivum* L.) after used primer**

Amplified DNA fragment	Giza 168	Gem. 9	Gem. 11	1×3 F1	1×3® F2	1×3(s) F2	2×3 F1	2×3® F2	2×3(s) F2	
<b>844A</b>										
2000bp	+	+	-	-	+	-	+	-	+	Polymorphic
1500bp	+	+	+	+	+	+	+	+	+	Monomorphic
1000bp	+	-	+	+	-	+	+	+	+	Polymorphic
700bp	-	-	-	-	-	-	-	+	-	Unique
600bp	-	+	+	+	+	+	+	+	+	Polymorphic
500bp	+	+	+	+	+	+	+	+	+	Monomorphic
400bp	-	+	-	-	-	-	-	+	-	Polymorphic
<b>844B</b>										
2000bp	+	+	+	+	+	+	+	+	+	Monomorphic
1600bp	-	-	-	-	+	-	-	-	-	Unique
1500bp	+	+	+	+	-	-	+	+	-	Polymorphic
1000bp	+	+	+	+	+	+	+	+	+	Monomorphic
650bp	+	+	+	+	+	-	+	+	-	Polymorphic
500bp	+	+	+	+	+	+	+	+	+	Monomorphic
<b>HB12</b>										
2000bp	+	+	+	+	+	+	+	+	+	Monomorphic
1600bp	-	-	-	-	-	-	-	-	+	Unique
1500bp	-	-	-	-	-	-	-	-	+	Unique
900bp	+	+	+	+	+	+	+	+	-	Polymorphic
<b>HBS10</b>										
1600bp	+	+	+	+	+	+	+	+	+	Monomorphic
1500bp	-	-	+	-	-	+	-	-	+	Polymorphic
1200bp	+	+	+	+	+	+	+	+	+	Monomorphic
1000bp	-	-	+	-	-	+	+	-	+	Polymorphic
900bp	+	+	+	+	+	+	+	+	+	Monomorphic
500bp	+	+	+	+	+	+	+	+	+	Monomorphic
400bp	+	+	+	+	+	+	+	+	+	Monomorphic
<b>HBS11</b>										
4000bp	-	+	+	+	+	+	+	+	+	Polymorphic
3000bp	+	+	+	+	+	+	+	+	-	Polymorphic
2400bp	-	-	-	-	-	-	-	+	-	Unique
2000bp	-	-	-	-	-	-	-	+	-	Unique
1500bp	+	+	+	+	+	+	+	+	+	Monomorphic
<b>17889A</b>										
4000bp	-	+	-	-	-	-	-	-	+	Polymorphic
3000bp	+	+	+	+	+	+	+	+	+	Monomorphic
2000bp	+	+	+	+	+	+	+	+	+	Monomorphic
1500bp	+	+	+	+	-	+	-	+	+	Polymorphic
1200bp	-	-	-	-	-	-	-	+	+	Polymorphic
1000bp	+	+	+	+	+	+	+	+	+	Monomorphic
800bp	-	-	-	+	-	-	-	-	-	Unique
700bp	-	+	-	+	-	-	-	-	-	Polymorphic
650bp	+	-	-	+	-	-	-	-	-	Polymorphic
400bp	+	-	-	-	-	-	-	-	-	Unique
300bp	+	-	-	-	-	-	-	-	-	Unique
100bp	-	-	-	+	-	-	-	-	-	Unique
<b>17889B</b>										
2000bp	+	+	-	+	+	-	+	-	-	Polymorphic
1500bp	+	+	+	+	+	+	+	+	+	Monomorphic
1300bp	+	+	-	-	-	-	-	+	+	Polymorphic
1200bp	+	+	-	-	-	+	-	+	+	Polymorphic
900bp	+	+	-	-	-	-	-	-	+	Polymorphic
700bp	+	+	-	-	-	+	-	+	+	Polymorphic
500bp	+	+	+	+	+	+	+	+	+	Monomorphic
<b>17899A</b>										
3000bp	-	-	+	+	+	+	+	+	+	Polymorphic
2000bp	-	-	-	-	+	+	+	-	+	Polymorphic
1500bp	-	+	+	+	+	+	+	+	+	Polymorphic
1000bp	+	+	+	+	+	+	+	+	+	Monomorphic
900bp	-	-	+	+	+	-	+	+	+	Polymorphic
700bp	-	-	+	-	-	-	-	-	-	Unique
500bp	+	+	+	+	+	+	+	+	+	Monomorphic
300bp	-	-	-	+	+	-	-	+	-	Polymorphic

## REFERENCES

- Chester, K.S. (1946). The Nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. Waltham, MA: Chronica Botanica, 169.
- Davoyan, A.N.E.R. (2011). Kravchenko A. forms in preservation and exploitation of the gene pool of wild common wheat relative. 480485 <http://dx.doi.org/10.1134/S2079059712060044>M., Zubanova Y.S. 2011. The use of synthetic
- Das, M.K., S. Rajaram, W.E. Kronstad, C.C. Mundt and R.P. Singh (1993). Associations and genetics of three components of slow rusting in leaf rust of wheat. *Euphytica.*, 68 (112): 99-109.
- Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Research 2<sup>nd</sup> Edition. An international Rice Research. A Wiley-Interscience publication, John Wiley Sons, New York.
- Guha, G., J.G. Bhowal and S.K. Dasgupta (1996). New sources of resistance to stem, leaf, and stripe rusts from crosses of *Triticum aestivum* L. and *T. timopheevii* Zhuk. *Annu. Wheat Newslett*, 42 : 113.
- Hayman, B.I. (1954a). The analysis of variance of diallel tables . *Biomet.*, 10: 234-244
- Hayman, B.I. (1954b). The theory and analysis of diallel crosses. *Genet.*, 39 : 789-809.
- Huerta-Espino J., R.P. Singh, S. German, B.D. McCallum, R.F. Park, W.Q. Chen, S.C. Bhardwaj and H. Goyeau (2011). Global status of wheat leaf rust caused by *puccinia triticina*. *Euphytica*, 179 (1):143-160
- Kolmer, J.A. (2005). Tracking wheat rust on a continental scale. *Current Opinion in Plant Biol.*, 8 (4): 441-449. [http://dx.doi.org/ 10.1016/j.pbi.2005.05.001](http://dx.doi.org/10.1016/j.pbi.2005.05.001)
- Kolmer, J. (2013). Leaf rust of wheat: pathogen biology, variation and host resistance. *Forests*, 4 (1): 70-812 <http://dx.doi.org/10.3390/f4010070>Davoyan R.O., Bebyakina I. V., Davoyan O. R., Zinchenko
- Oury, F.X., P. Brabant, P. Pluchard, P. Bered and M. Rousset (1993). The superiority of wheat hybrids for grain filling : Results of amultilocation experiment. *Agron.*, 13 (5): 381-393.
- Penthus, M.J. (1959). Observation on the phenology of spring wheat, barley and oats. *Bul. Res. Coun. Israel*, 7 D 71 – 80.
- Pandy, H.N., T.C.M. Menon and M.V. Rao (1989). A simple formula for calculating area under Disease progress Curve. *Rachis*, 8: (2) 38-39.
- Peterson, R.F., A.B. Campbell and A.E. Hanna (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.*, 26:496-500
- Samborki, D.J. (1985). Wheat leaf rust. See *Ruf.*, 95 (2): 39-59.
- Singh, R.K. and B.D. Chaudhary (1977). *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publisher, New Delhi-Ludhiana, India
- Tervet, I. and R.C. Cassel (1951). The use of cyclone separation in race identification of cereal rusts. *Phytopathol.*, 41:282-285.

## التحليل الوراثي ومعلومات قواعد الـISSR لمقاومة صدأ الأوراق في قمح الخبز

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الدراسة الحالية هدفها دراسة وتوضيح السلوك الوراثي لمقاومة صدأ الأوراق في قمح الخبز في مجموعة من خمسة آباء وعشرة هجن تحت ظروف الحقل عن طريق إجراء العدوى الصناعية، التباين الواسع بين الآباء وهجن الجيل الأول تم اكتشافه في خمس صفات تحت الدراسة هي فترة التحضين وشدة الإصابة والاستجابة للعدوى ومتوسط تأثير العدوى والمساحة تحت المنحنى المرضى، متوسط تلك الأصناف العائد إلى مقاومة هذه الأصناف أوضح أن جيزة ١٦٨ وسخا ٩٣ تملك استجابة عالية للمقاومة كوقت طويل لفترة التحضين بالإضافة إلى قلة شدة الإصابة المرضية ونفس المعدل لكلا الصنفين في الاستجابة للعدوى ومتوسط الإصابة والمساحة تحت المنحنى، تحليل التباين للخمس صفات سجل اختلافات غير معنوية لكل الصفات ماعدا شدة الإصابة، الاختلافات المعنوية لكل من  $H_1$ ,  $H_2$  المتحصل عليها لصفتي فترة التحضين وشدة الإصابة بينما صفة المساحة تحت المنحنى المرضى أوضحت اختلافات معنوية لتقدير الجين السائد، متوسط قيم السيادة أشارت إلى وجود السيادة الفائقة لكل الصفات المدروسة، نسبة تأثير الجينات الفعالة الموجبة والسالبة في الآباء أشارت إلى تكرار موجب وسالب غير متساوي بين الآباء لصفتي شدة الإصابة والمساحة تحت المنحنى المرضى ماعدا فترة التحضين التي تملك جينات موجبة وسالبة متقاربة ومتساوية بين الآباء، تحليل الشكل البياني أشار إلى أن الأصناف جيزة ١٦٨ وسخا ٩٣ تملك معظم جينات السيادة للمقاومة لكن الأصناف جيزة ١١ جميزة ٩ تملك معظم جينات التنحي لصفة الحساسية، المعلومات الجزيئية لتكنيك Bulk segregant analysis تم استخدامها عن طريق تكنيك ISSR الذي تم استخدامه عن طريق ثماني بادئات، أكدت نتائج التحليل الجزيئي أن جيزة ١٦٨ تم اعتباره تركيب وراثي هام جدا لمقاومة مرض صدأ الأوراق، هذا الصنف امتلك اربعة قطع للوزن الجزيئي ٢٠٠٠ في البادئ 844A و١٥٠٠ في البادئ 17889A و١٢٠٠ و٧٠٠ في البادئ 17889B هذه القطع ظهرت في الاب جيزة ١٦٨ وهجن الجيل الأول وأكثر في الجيل الثاني المقاوم، جيزة ١٦٨ ربما تملك معظم جينات المقاومة لمرض صدأ الأوراق، على العكس جميزة ١١ في الوزن الجزيئي ١٥٠٠ في البادئ HBS10 وأكثر في مجموعة الجيل الثاني الحساس، أيضاً هي أكدت مدى حساسية الصنف جميزة ١١ لمرض صدأ الأوراق في قمح الخبز.

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