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INHERETANCE NATURE OF LEAF RUST RESISTANCE AND SOME AGRONOMIC CHARACTERS IN BREAD WHEAT

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ABSTRACT

Wheat leaf rust caused by *Puccinia triticina* is very destructive to the susceptible wheat (*Triticum aestivum* L.) cultivars. Breeding for resistance is still the most economic and desirable method for controlling the disease. The objective of this investigation was to: 1) study the nature of inheritance of leaf rust disease resistance as well as grain yield and its related characters, 2) detect leaf rust resistant genes in the studied cultivars under field condition, 3) evaluate using Sequence Characterized Amplified Regions technique (SCAR) for the leaf rust resistance gene *Lr37* and 4) use SCAR as a tool for selecting and pyramiding different *Lr*'s resistance genes in Egyptian cultivars. Combining ability effects were estimated using line × tester matting design. Four high yielding cultivars Giza 168, Sakha 93, Sakha 94 and Gemmeiza 9 as females (lines) and four leaf rust monogenic lines. i.e., *Lr10, Lr19, Lr37* and *Lr39* as males (testers). Broad sense heritability (h^2b) was computed. The Chi-square test (χ^2) was used to test the significant of difference between observed and expected ratios in F_2 populations for leaf rust reaction. PCR reaction for SCAR primers was applied. The study included four characters; plant height (PH), leaf rust resistance as average coefficient of infection (ACI), number of spikes per plant (S/P) and grain yield per plant (GY/P). The obtained results can be summarized as follow; Sakha 93 was the best general combiner for PH and Sakha 94 for leaf rust resistance and GY/P. The best combinations for PH were Giza 168 × *Lr39*, Sakha93 × *Lr19* and Sakha 94 × *Lr37*; for leaf rust resistance were Sakha 93 × *Lr37,* Sakha 93 × *Lr39*, Sakha 94 × *Lr10* and Sakha94 × *Lr19* and for yield and its components were Sakha 94 × *Lr37* and Gemmeiza 9 × *Lr19*. Giza 168, Sakha 94 and Gemmeiza 9 had one or two genes conferring resistance to leaf rust. In addition, *Lr39* leaf rust resistant gene was present in Giza 168 and Sakha 94. The laboratory studies showed that, the SCAR marker has the potential of detecting *Lr37* in the studied Egyptian cultivars as well as the monogenic lines. Moreover, Giza 168, line 1 and line 3 had the leaf rust resistance gene *Lr37*.

Keywords: *Triticum aestivum*, leaf rust , combining ability , SCAR.

INTRODUCTION

Wheat, as a nutritive crop, is considered one of the most important cereal crops in Egypt as well as in many parts of the world. Wheat leaf rust caused by *Puccinia triticina* is very destructive to the susceptible cultivars. It was the main causal of eliminating many wheat cultivars i.e. Giza 139, Chenab 70, Super X, and Giza 160. It depending on the level of rust incidence and the stage of crop development when initial infection occurs (Nazime *et al*., 1993). Breeding for resistance is still the most economic and desirable method for controlling the disease (Shehab EL-Din *et al*.,1991). Identification of the leaf rust resistance genes presented in each cultivar enable wheat breeders to achieve their objectives more quickly. Many

authors proved that disease resistance is controlled by major or minor genes or both together, however complimentary effect between major genes may enhance the response of a cultivar and give higher levels of resistance (Simons *et al*., 1978). The characterization of specific leaf rust resistance gene are very useful to determine exactly which resistance genes are presented in commercial wheat cultivars. Three methods are used to identify leaf rust resistance genes in wheat cultivars. These methods are gene postulation (Statler, 1984), using molecular markers (Dellaporta *et al*., 1985) and genetic analysis (Kolmer, 1996). Pyramiding several major rust resistance genes into one adapted cultivar is one strategy for obtaining more durable resistance. The DNA marker helps to identify desirable genes more precisely and facilitates transfer of R genes into wheat.

The choice of the parents is a very important task in a breeding program. Combining ability studies are used by plant breeders to select parents with maximum potential of transferring desirable genes to the progenies. Therefore, the main target of this study was to 1)Study the inheritance mode of leaf rust disease resistance and three agronomic characters. 2) Detect number of leaf rust resistant genes in the studied cultivars under field conditions. 3) Evaluate using Sequence Characterized Amplified Regions technique (SCAR) for identifying leaf rust resistance gene *Lr37*. 4) use SCAR technique as a tool for identifying this gene in nine Egyptian cultivars and lines.

MATERIALS AND METHODS

This investigation was carried out at the Experimental Farm of Sakha Agricultural Research Station, Agricultural Research Center (ARC), Kafrelsheikh Governorate-Egypt. The experiments were conducted during three successive seasons from 2005/2006 to 2007/2008. The laboratory work was done in Biotechnology Laboratory - Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt.

Plant Materials

Eight bread wheat parents were used in this investigation; four commercial cultivars namely Giza 168, Sakha 93, Sakha 94 and Gemmeiza 9 as lines (Females) and four leaf rust monogenic lines. i.e., *Lr10, Lr19, Lr37* and *Lr39* as testers(males) for line × tester analysis. In addition, nine bread wheat cultivars and lines were used for detection of the leaf rust resistance gene *Lr37* (Table 1).

Table 1: Bread wheat genotypes name and pedigree as well as their reaction to leaf rust disease.

Genotypes	Pedigree	Leaf rust reaction*
Lines		
Giza 168	**MAL / BUC // SERI** CM93046-8M-0Y-OM-2Y-0P	**R**
Sakha 93	**Sakha 92 / TR810328** S.8871-1S-2S-1S-0S	**S**
Sakha 94	**OPATA / RAYON // KAUZ** CMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S	**R**
Gemmeiza 9	**ALD''S'' / Huac''s'' // CMH 74A.630/SX** CGM4583-5GM-1GM-0GM	**R**
Testers		
Lr10	**TC*6 / EXCHANGE (RL6004)**	**S**
Lr19	**TC*7 / TR (RL6040)**	**S**
Lr37	**TC*7 / VPM (RL6081)**	**MS**
Lr39	***T.tauschii* (KS86NGRCO2)**	**R**
Cultivars and lines for *Lr37* detection		
GEMMIZA 10	**MAYA47"S"/ON//II60-147 /3/ BB/GLL/4/CHAT"S" /5/ CROW"S"** CGM7892-2GM-1GM-2GM-1GM-0GM	**S**
GEMMIZA 11	**BOW"S"/KVZ"S"//7C/SER182/3 /GIZA168/SAKHA 61** GM5820-3GM-1GM-2GM-0GM	**S**
SIDS 12	**BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//C MH74A.630/4*SX** SD7096-4SD-1SD-1SD-0SD	**R**
SIDS 13	**KAUZ'S" / TSI / SNP"S"** ICW 94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD	**MS**
CHANDWEEL 1	**SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC** CMSS93B00567S-72Y-010M-010Y-010M-3Y-0M-0HTY-0SH	**MS**
Line 1	**GEMMIZA 1 / GIZA 168** S. 15647-8S-0SY-1S -0S	**MR**
Line 2	**PFAU/SERI.1B//AMAD/3/WAXWING** CGSS02Y00153S-099M-099Y-099M-46Y-0B	**S**
Line 3	**F6031478/MRL//CNO79/3/KA/NAC/4/STAR** CMSS92Y01017T-32Y-010M-010Y-010Y-010Y-9M-0Y	**MS**
Line 4	**SAKHA 93 / GEMMEIZA 9** S.6-1GZ-4GZ-1GZ-2GZ-0S	**MS**

*** R, resistance. MR, moderatly resistance. MS, moderatly susceptable. S, susceptiple.**

Experimental Design

In 2005/2006 season, top crosses were made by using line × tester matting design to produce the 16 F_1's hybrid seeds. In 2006/2007 season, the parents and the 16 obtained F_1 s were grown to produce the F_2 seeds and the same crosses were repeated to obtain fresh F_1's hybrid seeds. In 2007/2008 season, The 16 F_1's, F_2's and their eight parents were arranged in the randomized complete block design with three replicates. The experiment was surrounded by mixed of wheat cultivars, highly susceptible to leaf rust, as a spreader. All recommended culture practices were applied at the proper time. Data were recorded on 30, 30, 30 and 300 guarded plants of P_1, P_2, F_1 and F_2 rows, respectively. The study covered four characters plant height (PH), leaf rust reaction as average coefficient of infection (ACI), number of spikes per plant (S/P) and grain yield per plant (GY/P).

Statistical Analysis

The statistical procedure was used according to the regular analysis of variance of randomized complete block design as outlined by Cochran and Cox (1957). Combining ability analysis was computed based on the

procedure developed by Singh and Chaudhary (1977). Moreover, broad sense heritability (h^2b) was computed according to Allard (1960). The Chi-square test (χ^2) was also used to test the significant difference between observed and expected ratios in F_2 populations for leaf rust reaction according to Steel and Torrie (1960).

Leaf Rust Assessment

The evaluation experiment was sown on a late sowing date at 12 December, to have good chance to be subject to the pathotypes (*Puccinia triticina*) at the proper time. The artificial inoculation was done with powder mixture of the fresh leaf rust urediniospores of most prevalent leaf rust pathotypes (one volume of fresh urediniospores : 20 volume of talcum powder) at booting stage (Tervet and Cassell, 1951). Leaf rust data were recorded according to the scale of Stubbes *et al*. (1986). In this method resistance, moderately resistance, medium, moderately susceptible and susceptible field responses were symbolized as R, MR, M, MS and S, respectively. For the quantitative analysis, field response was converted into an average coefficient of the infection (ACI) according to the method of Stubbes *et al*. (1986) and modified by Shehab El-Din and Abd-El-Latiff (1996). In this method, an average coefficient of infection (ACI) could be calculated by multiplying infection severity by assigned constant values namely, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 for 0, 0;, R, MR, M, MS and S infection types, respectively.

DNA Extraction and SCAR Procedure

Total genomic DNA was isolated according to CTAB (hexadecyl trimethyl ammonium bromide) method described by Murray and Thompson (1980). SCAR technique based on polymerase chain reaction (PCR) was conducted to detect specific DNA fragment Linked to *Lr37* leaf rust resistant gene using two specific primers. The two primers (Ventriup and LN2) developed by Helguera *et al*. (2003) were used to detect the 2NS fragment (contained *Yr17-Lr37-Sr38* cluster) transferred from *Ae.Ventricosa*, that yields a 259-bp band. The sequence of the two primers are 5' AGG GGC TAC TGA CCA AGG CT-3' (Ventriup) and 5' TGC AGC TAC AGC AGT ATG TAC ACA AAA-3' (LN2). PCR reaction for SCAR primers was applied according to Helguera *et al*.2003 with some modification. The PCR reactions were performed with Perkin Elmer GeneAmp PCR system 2400. PCR reactions used 30 ng of wheat genomic DNA and final concentrations of 1× *Taq* polymerase buffer (Fermentas),1.0 u *Taq* DNA polymerase (Fermentas), 2.0 m*M*$MgCl_2$, 50 pmole of each primer, and 200 µ*M* of each dNTP. Final volume for PCR reactions was 10 µl. PCR reactions were performed using the following profile: 95 ^{0}C for 5 min (initial denaturation), 94 ^{0}C for 1min, 63 ^{0} C for 1min (primer annealing), 72 ^{0}C for 2min and for 30 cycles with final extension 72 ^{0}C for 7min and stored the last temperature was 4C^0. Samples were separated by electrophoresis in (w/v) 1.2% agarose gel, 50 bp DNA ladders (Fermentas) were used as molecular weight markers. Gels were photographed using BioDoc analysis software.,

RESULTS AND DISCUSSION

Analysis of Variance

Mean squares for plant height (PH), leaf rust reaction (ACI), number of spikes per plant (S/P) and grain yield per plant (GY/P) in F_1 and F_2 generations are presented in Table 2. Mean squares of genotypes, parents and crosses were highly significant indicating presence of true genetic variation among studied genotype (Fida *et al*. 2004). Mean squares of lines (females), testers (males) and lines × testers were significant for most studied characters (Akbar *et al*. 2009 and Esmail 2007). Broad sense heritability estimates were high for all studied characters with values ranged from 99.46 for PH to 80.9% for S/P, indicating that, the main portion of the total variance was due to the genetic variance (Menshawy and Youssef 2004, Aglan 2003, Hendawy *et al*., 2009 and Zhang *et al*., 2001).

Table 2: Analysis of variance and broad since heritability (h^2b) for the studied characters in both F_1 and F_2 generations of wheat.

sov	df	MS							
		PH		LR		S/P		GY/P	
		F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Replications	2	23.6	11.9	0.2	0.1	3.7	4.8	28.0	1.7
Genotypes	23	1509.0**	1106.4**	17.2**	9.8**	35.8**	22.6**	103.1**	57.2**
Parents	7	2658.6**	2658.6**	11.6**	11.6**	35.7**	35.7**	131.4**	131.4**
Crosses	15	951.0**	434.8**	20.7**	17.9**	36.6**	17.9**	71.5**	25.7 **
parentsvs crosses	1	1834.0**	314.9**	3.6**	2.1*	24.2	1.7	379.0**	10.1
line (female)	3	390.4**	576.2**	26.3*	12.1*	25.7**	15.5*	79.8**	20.1
tester(male)	3	4274.8**	1163.8**	56.6**	25.6**	48.5**	43.2**	73.2**	56.2**
line x tester	9	29.9**	144.7**	6.8**	3.2**	36.3**	10.3	68.1**	17.4
error	46	8.2	11.6	0.4	0.4	6.4	5.3	15.8	9.2
h^2b		99.5	99.0	97.8	96.4	84.8	80.9	86.8	86.1
CV%		2.1	2.6	19.7	20.1	12.8	11.1	13.2	11.6

*** and ** significant at 0.05 and 0.01 levels of probability, respectively. (CV%) coefficient of variation, (PH) plant height, (S/P) number of spikes per plant, (LR) leaf rust reaction and (GY/P) grain yield per plant.**

Genotype Means

Mean values of lines, testers and their sixteen F_1's and F_2's for all studied characters are presented in Tables 3 and 4. The desirable mean values for PH were obtained from Sakha 93 and the cross Sakha 93 × *Lr39*, while the desirable mean values for Leaf rust as well as grain yield were obtained from the crosses Giza 168 × *Lr39*, Sakha 93 × *Lr39*, Sakha 94 × *Lr37* and Sakha 94 × *Lr39* .

Table 3: Mean values of the studied characters for bread wheat lines and testers.

Line	PH	LR	S/P	GY/P	Tester	PH	LR	S/P	GY/P
GIZA 168	101.2	0.45	19.73	33.69	***Lr10***	149.5	5.09	23.00	16.15
SAKHA 93	91.2	5.28	16.63	19.15	***Lr19***	159.83	3.42	21.63	19.31
SAKHA 94	106.8	0.74	18.93	30.96	***Lr37***	166.33	2.5	26.27	21.33
GEMMIZA 9	113.0	1.96	16.50	31.11	***Lr39***	102.5	0.48	17.23	23.35

(PH) plant height, (S/P) number of spikes per plant, (LR) leaf rust reaction and (GY/P) grain yield per plant.

Table 4: Mean values of the studied characters for F_1 and F_2 generations of bread wheat.

Genotype	PH		LR		S/P		GY/P	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
GIZA 168 × Lr10	147.2	136.1	6.3	4.3	20.3	20.4	30.2	23.5
GIZA 168 × Lr19	143.0	136.2	3.8	3.5	18.7	22.1	27.1	23.9
GIZA 168 × Lr37	147.3	135.5	1.4	1.5	16.1	19.5	22.7	23.2
GIZA 168 × Lr39	102.3	106.2	0.5	0.8	16.8	15.7	31.4	27.9
SAKHA 93 × Lr10	136.1	124.7	8.9	6.3	26.8	25.4	32.3	31.9
SAKHA 93 × Lr19	130.8	125.4	6.6	5.2	21.6	22.9	31.0	23.4
SAKHA 93 × Lr37	138.5	126.1	2.6	3.0	18.0	19.1	24.6	23.6
SAKHA 93 × Lr39	99.7	100.6	0.5	1.1	15.4	17.0	32.3	28.9
SAKHA 94 × Lr10	146.0	136.3	1.2	1.9	21.4	21.4	34.2	25.5
SAKHA 94 × Lr19	150.0	135.7	1.3	1.6	14.4	21.2	21.8	26.2
SAKHA 94 × Lr37	145.0	136.0	1.2	1.0	23.4	22.0	36.1	23.7
SAKHA 94 × Lr39	113.0	136.3	0.5	1.9	18.6	21.4	37.9	25.5
GEMMIZA 9 × Lr10	147.5	139.1	5.2	5.2	16.3	19.7	24.2	23.4
GEMMIZA 9 × Lr19	147.8	131.8	4.5	4.2	21.5	20.8	30.9	20.6
GEMMIZA 9 × Lr37	147.8	134.8	2.7	3.2	16.4	19.7	24.0	22.8
GEMMIZA 9 × Lr39	109.8	110.9	0.5	0.9	14.4	16.8	27.6	28.9
LSD 0.05	**6.4**	**5.3**	**1.0**	**0.9**	**6.8**	**3.4**	**6.5**	**4.7**
0.01	**8.9**	**7.1**	**1.3**	**1.2**	**9.4**	**4.6**	**8.8**	**6.3**

(PH) plant height, (S/P) number of spikes per plant, (LR) leaf rust reaction and (GY/P) grain yield per plant.

General combining ability effects

The general combining ability (GCA) effects for all studied characters in F_1 and F_2 generations are presented in Table 5. Using tall cultivars causes much lodging and yield losses. So, Egyptian wheat breeder developed the semi dwarf cultivars (100-110cm) to avoid these looses. Therefore, the negative GCA effects considered the desirable estimates in this respect (Chowdhary *et al.,* 2007). Sakha 93 was the best combiner for PH. The high value of (ACI) refer to low level of resistance and vise versa, so, the negative GCA effects considered the desirable estimates for resistance. Sakha 94 was the best general for leaf rust resistance. Sakha 93 and Sakha 94 had positive GCA effects for GY/P and S/P. For testers, the best combiner for most studied characters was *Lr39* in both F_1 and F_2 generations except S/P. These genotypes had potential of transmitting desirable genes to the progenies and may be used in wheat breeding program in order to improve high yielding and leaf rust resistant cultivars.

Specific combining ability effects

The specific combining ability (SCA) effects of sixteen F_1's and F_2's for all studied characters are presented in Table 6. The desirable SCA estimates were obtained from the crosses Giza 168 × *Lr39*, Sakha 93 × *Lr19* and Sakha 94 × *Lr37* for PH; from crosses Sakha 93 × *Lr3*7, Sakha 93 × *Lr39*, Sakha 94 × *Lr10* and Sakha 94 × *Lr19* for leaf rust resistance and crosses Sakha 94 × *Lr37* and Gemmeiza 9 × *Lr19* for GY/P and S/P.

Table 5: Estimates of general combining ability effects for the studied characters in F_1 and F_2 generations of bread wheat.

Genotypes	PH		LR		S/P		GY/P	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Lines								
GIZA 168	0.5	0.3	0.03	-0.33	-0.8	-0.9	-1.4	-
SAKHA 93	-8.2**	-9.1**	1.7**	1.1**	1.7*	0.8	0.8	-
SAKHA 94	4.0**	7.8**	-1.9**	-1.2**	0.7	1.2	3.3**	-
GEMMIZA 9	3.7 **	0.9	0.3	0.5**	-1.6*	-1.1	-2.6*	-
Testers								
Lr10	9.7**	5.8**	2.4**	1.6 **	2.5**	1.4*	1.0	-
Lr19	8.4**	4.1**	1.1**	0.8**	0.3	1.4*	-1.6	-
Lr37	10.2**	4.9**	-1.0**	-0.7**	-0.3	-0.3	-2.5*	-
Lr39	-28.3**	-14.7**	-2.5**	-1.7**	-2.5**	-2.6	3.0*	-
LSD 0.05	**1.7**	**2.0**	**0.4**	**0.4**	**1.5**	**1.3**	**2.3**	**-**
LSD 0.01	**2.2**	**2.6**	**0.5**	**0.5**	**2.0**	**1.8**	**3.1**	**-**

*** and ** significant at 0.05 and 0.01 levels of probability, respectively. (PH) plant height, (S/P) number of spikes per plant, (LR) leaf rust reaction and (GY/P) grain yield per plant.**

Table 6: Estimates of specific combining ability effects for the studied characters in F_1 and F_2 generations of bread wheat.

CROSS	PH		LR		S/P		GY/P	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
GIZA 168 × Lr10	2.5	1.8	0.9*	0.2	-0.2	-	1.4	-
× Lr 19	-0.4	3.7	-0.3	0.2	0.5	-	0.8	-
× Lr 37	2.2	2.1	-0.6	-0.3	-1.6	-	-2.8	-
× Lr 39	-4.3*	-7.6**	-0.02	-0.1	1.3	-	0.5	-
SAKHA 93 × Lr10	0.2	-0.4	1.8 **	0.8*	3.9*	-	1.3	-
× Lr 19	-3.9*	2.2	0.9*	0.5	0.8	-	2.5	-
× Lr 37	2.1	2.0	-1.1**	-0.3	-2.2	-	-3.0	-
× Lr 39	1.7	-3.9	-1.6**	-1.1**	-2.6	-	-0.8	-
SAKHA 94 × Lr10	-2.2	-5.6**	-2.3**	-1.3**	-0.5	-	0.8	-
× Lr 19	3.1	-4.5*	-0.8*	-0.8*	-5.4**	-	-9.2**	-
× Lr 37	-3.7*	-4.9*	1.2**	0.1	4.3**	-	6.0*	-
× Lr 39	2.8	15.0 **	1.9**	2.0**	1.6	-	2.4	-
GEMMIZA 9 × Lr10	-0.5	4.2*	-0.5	0.2	-3.3*	-	-3.4	-
× Lr 19	1.2	-1.4	0.2	0.03	4.1**	-	5.8*	-
× Lr 37	-0.6	0.8	0.5	0.5	-0.5	-	-0.3	-
× Lr 39	-0.1	-3.5	-0.3	-0.8*	-0.3	-	-2.1	-
$LSD_{0.05}$	3.3	4.0	0.7	0.7	3.0	-	4.6	-
$LSD_{0.01}$	4.4	5.3	1.0	0.9	3.9	-	6.1	-

*** and ** significant at 0.05 and 0.01 levels of probability, respectively. (PH) plant height, (S/P) number of spikes per plant, (LR) leaf rust reaction and (GY/P) grain yield per plant.**

Nature of inheritance for leaf rust disease

Segregation of the F_2 plants of the crosses between Giza 168 and the monogenic lines *Lr10*, *Lr19*, *Lr37* and *Lr39* gave a good fit to the ratios 3:13, 1:3, 9:7 and 1:0 respectively,(Table 7) indicating that, Giza 168 had one or two gene conferring resistance to leaf rust and it had *Lr39* leaf rust resistant gene. Segregation of F_2 plants of the crosses between Sakha94 and the monogenic lines *Lr37* and *Lr39* gave a good fit to ratios 3:1and 1:0 respectively, indicating that, *Lr39* leaf rust resistant gene is present in Sakha 94 and *Lr37* is absent. Reversed ratios of F_2 segregation such as 3:13, 1:3,

1:15 were obtained in many cases indicating dominance or susceptibility over resistance. Although resistance was dominant over susceptipility in most studies. Many researcher reported these reversed ratios (Shain 1998, Nagaty *et al.,* 2007, El- Orabey 2008).

Table 7: Segregation and chi square (χ^2)analysis of F_2 plants from the crosses between the four wheat cultivars and four monogenic lines under field conditions.

Lines	Testers	F_2 generation		Total	χ^2	Expected ratio	P value
		R	S				
GIZA 168	***Lr10***	54	246	300	0.111	3:13	90
GIZA 168	***Lr19***	88	212	300	3.004	1:3	10
GIZA 168	***Lr37***	173	127	300	0.245	9:7	95 - 90
GIZA 168	***Lr39***	297	3	300	No segregation	1:0	5
SAKHA 93	***Lr10***	0	250	250	No segregation	0:1	-
SAKHA 93	***Lr19***	12	288	300	2.592	1:15	10
SAKHA 93	***Lr37***	90	210	300	4.000	1:3	5
SAKHA 93	***Lr39***	248	52	300	0.395	13:3	50
SAKHA 94	***Lr10***	106	194	300	8.636	7:9	1 - 0.1
SAKHA 94	***Lr19***	156	144	300	2.202	9:7	10
SAKHA 94	***Lr37***	212	88	300	3.004	3:1	5-10
SAKHA 94	***Lr39***	292	8	300	No segregation	1:0	50
GEMMEIZA 9	***Lr10***	2	298	300	No segregation	0:1	-
GEMMEIZA 9	***Lr19***	27	273	300	3.872	1:15	5
GEMMEIZA 9	***Lr37***	35	265	300	9.880	3:13	0.1
GEMMEIZA 9	***Lr39***	251	49	300	1.150	13: 3	50

(R) resistance, (S) susceptible.

Laboratory results.

The SCAR marker linked to the leaf rust resistance gene *Lr 37* could be used easily in a practical breeding program. Robert *et al*. (1999) and Seah *et al*.(2001) developed PCR markers for the identification of the cluster of resistance genes *Sr38-Yr17-Lr37*, but these markers were not publicly available. Helguera *et al*. (2003) developed the first public PCR marker for this chromosome fragment which was used in this work. The laboratory experiments aim to, 1) Evaluate using Sequence Characterized Amplified Regions technique (SCAR) for the leaf rust resistance gene *Lr37* 2) use SCAR as a tool for selecting and pyramiding different *Lr*'s resistance gene in Egyptian cultivars.

Evaluation of the leaf rust resistance gene *Lr37* in the eight genotypes.

A total of eight genotypes, four leaf rust monogenic lines i.e., *Lr10*, *Lr19*, *Lr37* and *Lr39* and four Egyptian bread wheat cultivars namely Giza 168, Sakha 93, Sakha 94 and Gemmeiza 9 were used to evaluate the use of the SCAR system for the presence / absence of *Lr37* resistance gene. Monogenic line *Lr37* contain the leaf rust resistant gene *Lr37*, while the other monogenic lines contain the susceptible allele. Two different experiment were done to evaluate the system. First SCAR analysis were done on the eight different genotypes. The second analysis were used on parents, F_1 and F_2 of across between the genotypes, one parent contained the resistance gene while the second parent contained the susceptible allele. The system

was tested according to Helguera *et al*. (2003) using *Lr37*, however, no band appear until annealing temperature were lower from 65C ^{0} to 63C^0.

The eight genotypes (2 Positive and 6 negative) were tested for polymorphism using the primers combination VENTTRIUP and LN2. when the DNA of these genotypes were tested with the primer combination VENTTRIUP and LN2, only one band was amplified in the monogenic line *Lr37* and Giza168. Whereas, no band was detected in the other monogenic lines and the reaming cultivars (Fig.1). These results indicate that this fragment was completely linked to the *Lr37* resistant gene and the system has the potential of detecting *Lr37* in Egyptian cultivars as well as the monogenic lines.

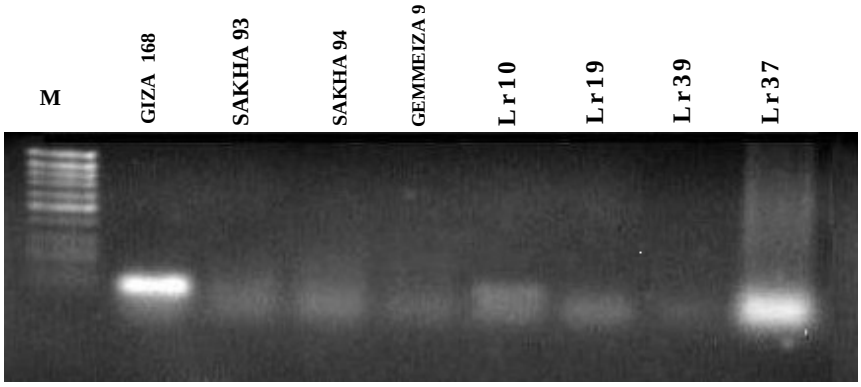

Fig.1: PCR amplification products obtained using the primers combination VENTTRIUP and LN2 for the four wheat cultivars and the four leaf rust monogenic line, (M) 50bp DNA ladder size marker.

To confirm the validity of this system, more analysis were done using a cross between a positive parent contain the resistant gene *Lr37* namely monogenic line *Lr37* with another negative parent contain the susceptible allele of *Lr37* gene namely Sakha 93. Parents, F_1 and F_2 were tested using the same system. The results are shown in Figure 2. Positive control (*Lr37*), F_1, and six individuals of F_2 progeny (4, 5, 9, 14, 20 and 27) tested were positive for presence of the resistance *Lr37* gene and gave a ratio of 21absent band : 6 present band. This ratio fit the ratio 3:1(χ^2 = 0.11, P value = 90). From these results, it could be concluded that, this system was suitable for selecting the *Lr37* gene from different wheat genotypes. Some negatively resistant individual in F_2 showed a faint corresponding band of *Lr37* resistant gene (lanes 3, 6, 7, and 12) Figure 2 using the profile as described in material and method. In spite of different annealing temperature and $MgCl_2$ concentration were tried in order to eliminate the faint band, the gel pattern did not change and the faint band papered.

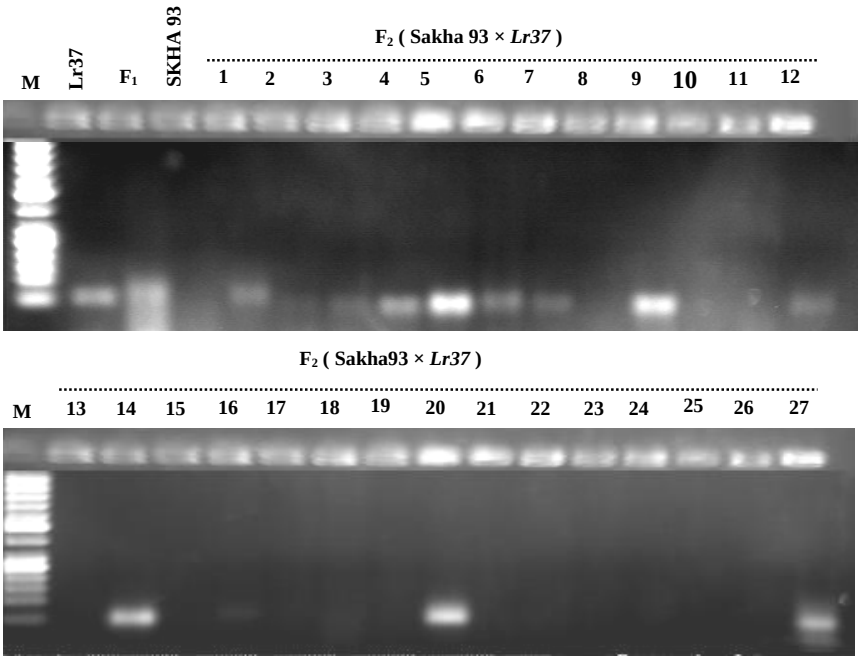

Fig.2: Segregation of PCR amplification products obtained using the primers combination VENTTRIUP and LN2 for the parents Sakha93 and Lr37 and their F_1 as well as 27 plant of F_2 generation. (M) 50bp DNA ladder size marker .

Detection of the leaf rust resistance gene *Lr37* in some new Egyptian cultivars and breeding lines.

Five new Egyptian bread wheat cultivars i.e. Gemmeiza 10, Gemmeiza 11, Sids 12, Sids 13 and Shandweel 1 and four breeding lines (Table 1) were tested for presence of *Lr37* gene using the same system. Data present in Figure 3 revealed that, only line 1 and line 3 gave the amplification PCR fragment linked to *Lr37*. While, no amplification product was detected in the other tested wheat cultivars and lines, indicating that, line 1 and line 3 posses the leaf rust resistance gene *Lr37*.

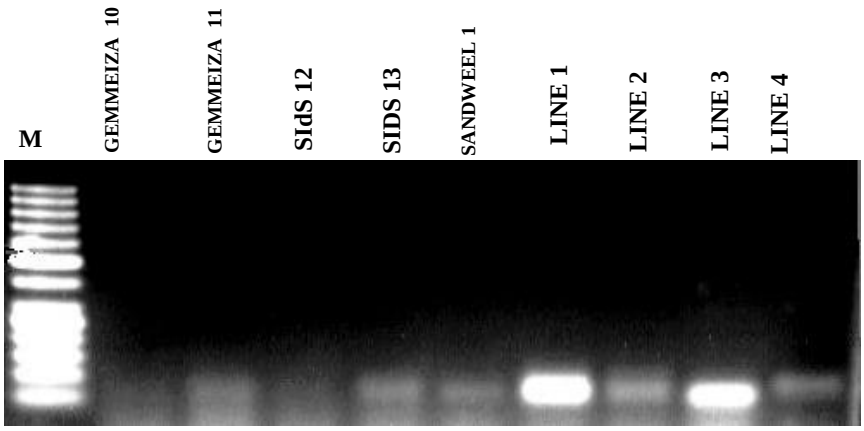

Fig. 3: PCR amplification products obtained using the primers combination VENTTRIUP and LN2 for the five new Egyptian bread wheat cultivars and the four breeding lines, (M) 50bp DNA ladder size marker.

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طبيعة توارث المقاومة لصدا الأوراق والمحصول ومكوناته في قمح الخبز

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أجريت هذه الدراسة في محطة البحوث الزراعية بالتعاون مع معمل البيوتكنولوجي -مركز بحوث الأرز بسخا- التابعين لمركز البحوث الزراعية - مصر- في المواسم ٢٠٠٥/٢٠٠٦ , ٢٠٠٦ /٢٠٠٧ , ٢٠٠٧ – ٢٠٠٨ . ويهدف هذا البحث إلي دراسة وراثة المقاومة لصدا الأوراق والمحصول وبعض مكوناته, التعرف على جينات المقاومة في الأصناف المدروسة تحت ظروف الحقل و تقييم المعلم المتخصص للجين *Lr37* بالإضافة إلى استخدامه في انتخاب هذا الجين في الأصناف المصرية . كما قدرت القدرة على الائتلاف باستخدام تحليل السلالة × الكشاف, حيث استخدمت أربعة أصناف تجارية عالية المحصول هي جيزة ١٦٨ وسخا ٩٣ وسخا ٩٤ وجميزة ٩ كأمهات(سلالات), أربعة سلالات أحادية الجين (Monogenic lines) لصدأ الأوراق هي *Lr10* و*Lr19* و*Lr37* و*Lr39* كأب مذكر (كشاف). كما حسبت الكفاءة الوراثية بمعناها الواسع, واستخدم مربع كأي لمعرفة مدى تطابق نسب الانعزال المشاهدة والمتوقعة للمقاومة لصدا الأوراق في الجيل الثاني. كما تم استخدام تكنيك الـ SCAR للمعلم الخاص بالجين*Lr37* . وتمت الدراسة على مجموعة صفات وهي ارتفاع النبات, مقاومة صدا الأوراق, عدد السنابل و محصول الحبوب لكل نبات و تلخيص النتائج كالأتي:-

سجل الصنف سخا ٩٣ أفضل قدرة عامة على الإتلاف لصفة ارتفاع النبات , والصنف سخا٩٤ لصفة محصول الحبوب والمقاومة لصدأ الأوراق. كما كانت أفضل الهجن من حيث قدرتها الخاصة علي الإتلاف هي الهجن جيزة ١٦٨ × *Lr39* , سخا ٩٣ × *Lr19* , سخا ٩٤ × *Lr37* لصفة طول النبات , والهجن سخا ٩٣ × *Lr37* , سخا ٩٣ × *Lr39* , سخا٩٤ × *Lr10* , سخا ٩٤ × *Lr19* لصفة المقاومة لصدا الأوراق , والهجن سخا ٩٤ × *Lr37* ، جميزة٩ × *Lr19* لصفة المحصول ومكوناته. وأظهرت نتائج التحليل الوراثي لانعزال المقاومة لصدا الأوراق في الجيل الثاني وجود زوج او زوجين من جينات المقاومة في الأصناف جيزة ١٦٨ وسخا ٩٤ وجميزة ٩. كما دلت نتائج المعمل أن معلم الـ SCAR المتخصص للجين *Lr37* ذو كفاءة كأداة انتخاب لهذا الجين, وان الصنف جيزة ١٦٨ و سلالتي القمح ١و ٣ تحتوي كل منهما علي هذا الجين.

قام بتحكيم البحث

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