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# DETECTION OF THE LEAF RUST RESISTANCE GENE *LR* 9 IN SOME EGYPTIAN WHEAT VARIETIES

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**ABSTRACT:** *The objective of this research was to detect the leaf rust resistance gene Lr 9 in 12 Egyptian wheat varieties i.e. Giza 162, Giza 163, Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Gemmeiza 9, Sakha 8, Sakha 69 and Sakha 93. Segregation of $F_2$ plants of the crosses between those varieties and monogenic line having the leaf rust resistance gene Lr 9 were evaluated at adult stage under field conditions with a mixture of physiologic races of Puccinia triticina. The leaf rust resistance gene Lr 9 was found in the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8, while this gene was absent in the other tested varieties i.e. Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Sakha 69 and Sakha 93. Moreover, the strategy of sequence characterized amplified region technique (SCAR) using two specific primers for Lr 9 revealed that these primers were closely linked to the leaf rust resistance gene Lr 9.*

**Key words:** *Wheat, leaf rust, resistance genes, major genes, SCAR marker.*

## INTRODUCTION

Leaf rust of wheat (*Triticum aestivum* L.) caused by *Puccinia triticina* Eriks., is considered one of the most economic diseases on wheat in Egypt and worldwide. It annually causes severe losses in grain yield which may reach more than 20 % on some cultivars depending on the environmental conditions, the level of resistance and the dominant physiologic races (Nazim *et al*., 1983). The disease can be controlled by several methods, however, genetic resistance is the most safe and economic method. Genetic resistance can be controlled by major gene (s) which so called vertical resistance, or minor genes which so called horizontal resistance (Simons *et al*., 1978). Resistance can be fully controlled by single major gene or oligo genes. Previous research work in this field showed the effectiveness of many major genes in the Egyptian varieties (Negm, 2004; Shahin, 2004; and El-Orabey, 2008). The monogenic lines *Lr* 9, *Lr* 13, *Lr* 19, *Lr* 24, *Lr* 36 and *Lr* 46 proved their resistance to a wide range of leaf rust pathotypes (Negm, 2004; Shahin, 2004 and El-Orabey, 2008).

The development of molecular markers for specific *Lr* gene allows the detection of such gene independently in a phenotype. Also, molecular markers can be used as marker assisted selection for an effective combination of *Lr* genes in a pyramiding strategy to create a more durable resistance (Roelfs *et al*., 1992). Therefore, developing markers of such genes is very important for a successful breeding program for leaf rust resistance to determine which gene can be transferred to the high yielding varieties.

The research work aimed to detect one of the most effective genes *Lr* 9 by genetic analysis and SCARs technique in some of the local varieties.

## MATERIALS AND METHODS

### Evaluation of the wheat varieties against leaf rust:-

### Seedling stage test:

The tested wheat varieties and *Lr* 9 were planted in 10 cm diameter pots. Seedlings at 7-days old were artificially inoculated (Stakman *et. al*., 1962) with uredospores of individual pathotypes of leaf rust (*Puccinia triticina*) i.e. TTTST, TTTTT, TKTST and

PKTST, the main aggressive dominant races of leaf rust under Egyptian conditions during season 2012/13 (Unpublished data). Inoculated seedlings were placed for 24 hours under humidity conditions to allow rust spores to germinate and cause infection, then transferred to the benches in the greenhouse (Wheat Dis. Res. Dept., Agric. Res. Center, Giza). After 10-12 days from inoculation the rust reactions were recorded using the standard disease scoring scale 0 - 4 (Stakman *et. al*., 1962). The rust reactions 0, 0;, 1, and 2 were considered resistant (R), while 3 and 4 were considered susceptible (S) (Stakman *et. al*., 1962). The method used to identify leaf rust races was adopted by (Long and Kolmer, 1989).

## Adult stage tests:

This experiment was carried out under environmental field conditions at three locations i.e. the farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom, Nubariya Agric. Res. Station and Itay Elbaroud Agric. Res. Station for two successive seasons i.e. 2011/12 and 2012/13. The tested wheat varieties (Table 1) and the monogenic line having the resistance gene *Lr* 9 were planted in one replicate with 2 meter length single row, each 30 cm apart. The experiment was surrounded by spreader rows planted with mixtures of the highly susceptible varieties i.e. Morocco, Thatcher and *Triticum spelta* Saharinsis. Randomization was not used in planting these lines, since it seemed to be unnecessary (Broers, 1987) because of the high proportion of the infection reaching the tested genotypes from the spreader rows.

The rust response was recorded after the heading stage by combining severity from 0 to 100 % (percent of infection) according to the modified Cobb scale (Peterson *et al*., 1948) and reaction (type of reaction) (Johnson and Mains, 1932).

## Genetic analysis:

Twelve wheat varieties i.e. Giza 162, Giza 163, Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Gemmeiza 9, Sakha 8, Sakha 69 and Sakha 93 were crossed with the leaf rust monogenic line having the resistance gene *Lr* 9 (Table 1).

**Table (1): List of the tested local wheat varieties, pedigree and year of release.**

| No. | Variety | Pedigree | Year of release |
|---|---|---|---|
| 1 | Giza 162 | Vcm//Cno 67/7C/3/Kal/Bb CM8399-D-4M-3Y-1M-1Y-1M-0Y | 1987 |
| 2 | Giza 163 | T. aestivum /Bon//Cno /7C CM33009-F-15M-4Y-2M-1M-1M-1Y-0M | 1987 |
| 3 | Giza 164 | KVZ/Buha "s"//Kal/Bb CM33027-F-15M-500y-0M | 1987 |
| 4 | Giza 165 | 0MCno/Mfd//Mon "S" CM43339-C-1Y-1M-2Y-1M-2Y-0B | 1991 |
| 5 | Giza 167 | Au/UP301//G11/SX/Pew"S"/4/Mai"S"/May"S"//Pew"S" CM67245-C-1M-2Y-1M-7Y-1M-0Y | 1995 |
| 6 | Giza 168 | MRL/BUC//Seri. CM93046-8M-0Y-0M-2Y-0B-0GZ | 1999 |
| 7 | Giza 170 | MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B | 1999 |
| 8 | Sids 1 | HD2172/Pavon "S"//1158.57/Maya74 "S" SD46-4Sd-2SD-1SD-0SD | 1996 |
| 9 | Gemmeiza 9 | Ald "S"/Huac "S"//CMH74A. 630/5x CGM4583-5GM-1GM-0GM | 2000 |
| 10 | Sakha 8 | Indus 66 x Norteno "S"-Pk 348 | 1979 |
| 11 | Sakha 69 | Inia/RL 4220//7C/Yr "S" CM 15430-25-65-0S-0S | 1980 |
| 12 | Sakha 93 | Sakha 92/TR 810328 S 8871-1S-2S-1S-0S | 1999 |

The experiments of this study were carried out in the farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom through three successive seasons i.e. 2010/11, 2011/12 and 2012/13.

The parental varieties and the monogenic line *Lr* 9 were grown on three successive dates at 15 days intervals to overcome differences in the time of flowering. The monogenic line *Lr* 9 was used as male parent for crosses with each of the wheat varieties to obtain the $F_1$ seeds. Any doubtful of $F_1$ plants were discarded and the other were harvested separately. The $F_1$ seeds were grown in the following season in rows 4 m long and 30 cm apart and spaced 30 cm. in order to facilitate production of $F_2$ seeds. Parents, $F_1$ and $F_2$ plants were also grown in plots, each plot of the parents and $F_1$ contained six rows 3.5 m long and 20 cm between rows. Each plot of the $F_2$ contained eight rows 4 m long spaced 30 cm and seeds were 25 cm apart, therefore, each row contained 15 plants. All plots were surrounded by a spreader area sown with a mixture of two highly susceptible wheat varieties i.e. Morocco and Thatcher.

## Inoculation and disease assessment:

For field inoculation the spreader plants were mist with water and dusted with mixture of uredospores of the prevalent races mixed with talcum powder at a rate of 1 (spores):20 (talcum powder). Dusting was carried out in the early evening (at sunset) before dew formation. The inoculation of all plants was carried out at booting stage according to the method of Tervet and Cassell (1951). Data of leaf rust severity were recorded at the adult stage of the tested plants using the modified Cobb's scale (Peterson *et al*., 1948).

Plants of the $F_2$ for each cross were grouped into six categories depending on their percentage of disease severity under field conditions i.e. very resistant (VR) with no rust visible infection, resistant (R) with low infection type up to 20 % rust severity, moderately resistant (MR) with 21 % up to 40 % rust severity, moderately susceptible (MS) with 41 % up to 60 % rust severity, susceptible (S) with 61 % up to 80 % rust severity and very susceptible (VS) with 81 % up to 100 % rust severity (Figure 1). The first three categories were considered resistant phenotypes and the other three groups (41 % to 100 %) were considered as susceptible phenotypes.

For detection of the leaf rust resistance gene *Lr* 9 in each cross, goodness of fit of the observed to the expected ratio of the phenotypic classes was tested using Chi-square ($\chi^2$) analysis according to Steel and Torrie (1960).

## Detection of *Lr* 9 by SCAR technique:

The 12 wheat varieties, 12 monogenic lines i.e. *Lr* 1, *Lr* 2a, *Lr* 3a, *Lr* 3ka, *Lr* 9, *Lr* 12, *Lr* 13, *Lr* 18, *Lr* 19, *Lr* 21, *Lr* 24, *Lr* 29, *Lr* 32, *Lr* 34, *Lr* 35, *Lr* 36, *Lr* 46 and *Lr* 47, $F_1$ of the crosses of *Lr* 9 X Gemmeiza 9 and *Lr* 9 X Giza 164, 36 plants of $F_2$ population of the cross of *Lr* 9 X Giza 164 and ten plants of $F_2$ population of the cross *Lr* 9 X Gemmeiza 9 were used as plant materials. About 10 cm long fresh leaves were collected from two weeks old plants and immediately kept in liquid nitrogen.

A potassium acetate method based on Dellaporta *et al*. (1983), the protocol was used for isolation of total genomic DNA. SCAR technique based on polymerase chain reaction (PCR) was conducted to detect specific fragment using two specific primers; J 13/1 and J 13/2. The sequence of the forward primer of 20 bp J 13/1 is 5` TCCT TTTATTCCGCACGCCGG 3` and the reverse primer is 5` CCACACTACCCCAAA GAGACG 3`. Ready-To-Go™ PCR beads (Amersham Pharmacia Biotech) were used for PCR reactions. 10 ng of genomic DNA, 13 pmol of each primer and sterile distilled water were added to a total volume of 25 µl to the bead.

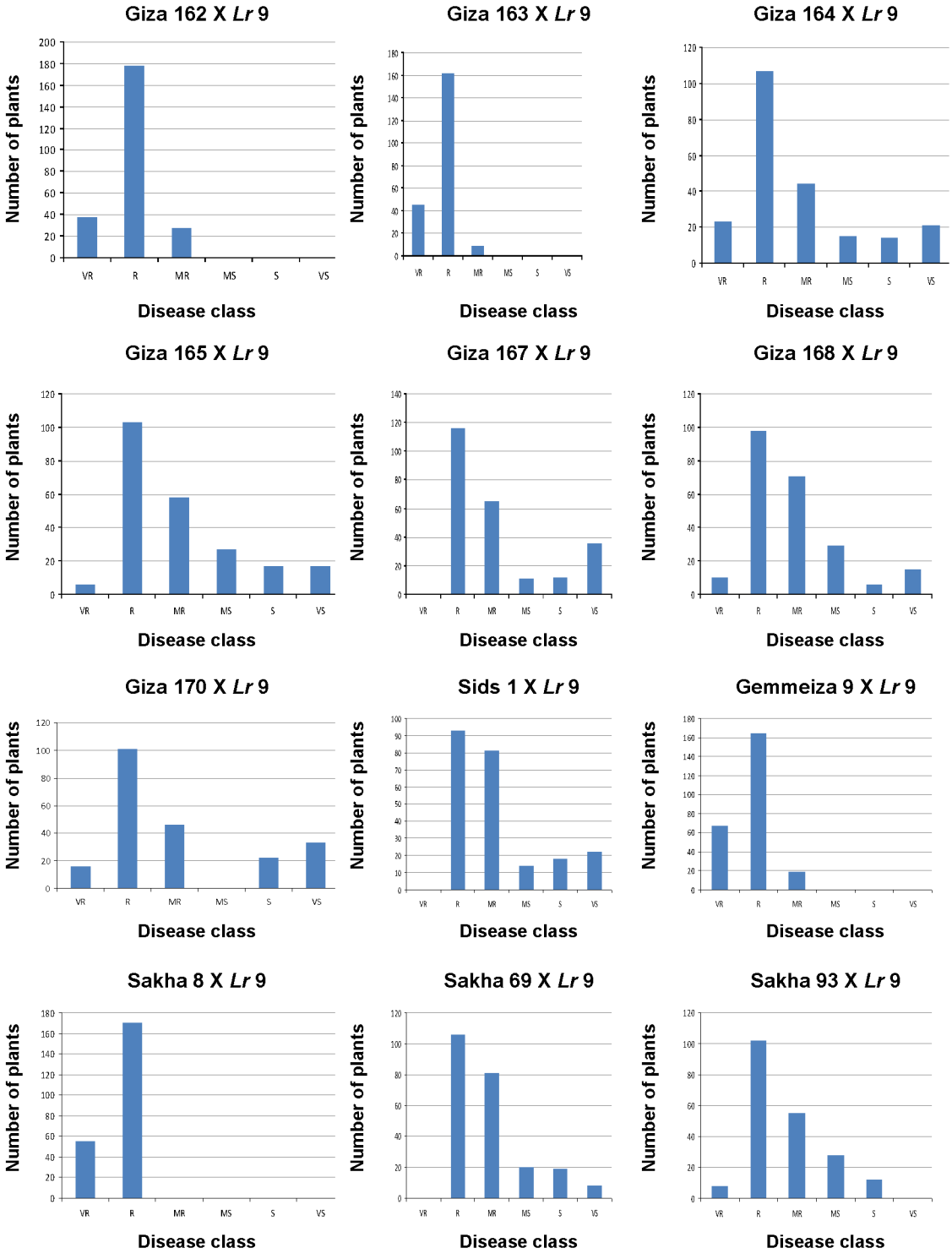


**Fig. (1): Distribution of $F_2$ plants of the crosses between the monogenic line having leaf rust resistance gene *Lr* 9 and 12 wheat varieties for disease classes under field conditions.**

Amplification was carried out in DNA thermocycler (Progen 30). The thermocycler was programmed by an initial strand separation cycle at 94 °C for 6 minutes. The next 45 cycles were composed of a denaturation step at 92°C for 1 min. an annealing step at 62 °C for 1 min. and polymerization step at 72 °C for 2 min. The final cycle was a polymerization cycle performed at 72 °C for 4 min. The PCR products of each reaction were analyzed by electrophoretic separation at 5 V/cm for about 3 h in 1.8 % agarose gel. DNA marker of Gibco BRL (100 bp DNA ladder marker) was added on one side of the gel to determine the size of the DNA pattern. Gel was stained with ethidium bromide, visualized under UV light and photographed.

## RESULTS

### Evaluation of the wheat varieties against leaf rust:-

### Seedling stage test:

The reaction of 12 wheat varieties at seedling stage against the most frequent pathotypes of leaf rust is shown in Table (2). The wheat varieties Giza 168 and Gemmeiza 9 were resistant to all pathotypes. The wheat varieties Sids 1 and Sakha 69 were susceptible to all pathotypes. While, the monogenic line having resistance gene *Lr* 9 and the wheat varieties Giza 162, Giza 163, Giza 164, Giza 165, Giza 167, Giza 170, Sakha 8 and Sakha 93 showed variable reactions between resistant to susceptible against the four pathotypes.

**Table (2): Evaluation of 12 wheat varieties against the most frequent pathotypes of leaf rust (*Puccinia triticina*) at seedling stage under greenhouse condition.**

| No. | Variety / Line | Leaf rust pathotypes / Infection type* | | | |
|---|---|---|---|---|---|
| | | TTTST | TTTTT | TKTST | PKTST |
| 1 | Giza 162 | 4 | 3 | 1 | 4 |
| 2 | Giza 163 | 3 | 2 | 3 | 0 |
| 3 | Giza 164 | 3 | 4 | 0 | 3 |
| 4 | Giza 165 | 4 | 2 | 4 | 4 |
| 5 | Giza 167 | 4 | 1 | 2 | 4 |
| 6 | Giza 168 | 0; | 2 | 1 | 1 |
| 7 | Giza 170 | 3 | 1 | 0; | 0 |
| 8 | Sids 1 | 4 | 4 | 3 | 4 |
| 9 | Gemmeiza 9 | 1 | 0; | 1 | 0 |
| 10 | Sakha 8 | 3 | 4 | 0 | 1 |
| 11 | Sakha 69 | 4 | 4 | 3 | 4 |
| 12 | Sakha 93 | 3 | 4 | 2 | 1 |
| 13 | *Lr* 9 | 0, | 3 | 0, | 0 |

* Infection types follow 0 - 4 scale (Stakman *et al.*, 1962): 0 = No uredinia or other macroscopic sign of infection, ; No uredinia but hypersensitive necrotic or chlorotic flecks present, 1 = Small uredinia surrounded by necrosis, 2 = Small to medium uredinia surrounded by chlorosis or necrosis, 3 = Medium-sized uredinia that may be associated with chlorosis, 4 = Large uredinia without chlorosis and X = Random distribution of variable sized uredinia on single leaf.

## Adult stage test:

The aim of this work was to study the response of 12 local wheat varieties to leaf rust at different location of Egypt. Therefore, all materials were inoculated and grown in three locations i.e. Shibin El-Kom, Itay Elbaroud and Nubariya for two growing seasons (2011/12 and 2012/13).

Data in Table (3) revealed that the tested wheat varieties showed different levels of rust severity ranged from 0 to 80 S, according to the wheat variety and location. The monogenic line having resistance gene *Lr* 9 and the wheat varieties Gemmeiza 9, Sakha 8, Giza 168, Giza 170, Giza 162 and Giza 164 showed low values of final rust severity less than (40 %). While, the wheat varieties Sids 1, Sakha 93, Sakha 69, Giza 163, Giza 167 and Giza 165 showed high values of final rust severity. Concerning the mean final rust severity on 12 wheat varieties grown at three locations i.e. Shibin El-Kom, Itay Elbaroud and Nubariya for two seasons (2011/12 and 2012/13), data obtained in Table (3) showed that the wheat varieties could be classified into two main groups depending on their values of final rust severity (%). The first group included varieties exhibited low values of final rust severity (Less than 30.00 %). The mean values of final rust severity (%) were 2.67 %, 5.83 %, 7.50 %, 9.17 %, 15.83 %, 21.67 %, 26.67 % and 28.33 % for *Lr* 9 and the wheat varieties Gemmeiza 9, Sakha 8, Giza 168, Giza 170, Giza 162, Giza 164 and Giza 165. The second group included wheat varieties with high values of final rust severity (more than 30.00 %) i.e. Sids 1 (68.33 %), Sakha 93 (53.33 %), Sakha 69 (41.67 %), Giza 163 (33.33 %) and Giza 167 (30.00 %).

## Detection of *Lr* 9 by genetic analysis:

The detailed results of the crosses between the monogenic line having leaf rust resistance gene *Lr* 9 and the 12 Egyptian wheat varieties are shown in Table (4) and Figure (1). All of the 244, 216, 250 and 225 $F_2$ plants of the crosses between *Lr* 9 and the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8, respectively, were resistant and showed no segregation. These results indicated that the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8 carry the leaf rust resistance gene *Lr* 9.

$F_2$ plants of the crosses between this monogenic line having *Lr* 9 and the wheat varieties Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Sakha 69 and Sakha 93 segregated to (174 R: 50 S), (167 R: 61 S), (181 R: 59 S), (179 R: 50 S), (163 R: 55 S) (174 R: 54 S), (187 R: 47 S) and (165 R: 40 S), respectively. These segregations fit the ratio 3 R : 1 S, indicated that the wheat varieties Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Sakha 69 and Sakha 93 do not have the leaf rust resistance gene *Lr* 9.

## Detection of *Lr* 9 by SCARs technique:

The development of molecular markers for specific *Lr* gene allows the detection of independent gene in any phenotype. In this study, a molecular marker method was demonstrated to be used for identifying the *Lr* 9 gene.

To confirm the relationship of *Lr* 9 and the detected band using the two specific primers J13/1 and J13/2 for *Lr* 9, 18 *Lr* genes were tested. These genes were *Lr* 1, *Lr* 2a, *Lr* 3a, *Lr* 3ka, *Lr* 9, *Lr* 12, *Lr* 13, *Lr* 18, *Lr* 19, *Lr* 21, *Lr* 24, *Lr* 29, *Lr* 32, *Lr* 34, *Lr* 35, *Lr* 36, *Lr* 46 and *Lr* 47. The results of SCAR experiment using the two specific primers J13/1 and J13/2 for *Lr* 9 with the leaf rust monogenic lines are presented in Figure (2). Only one band of 100 bp was amplified in the monogenic line *Lr* 9. Whereas, no band was detected in the other monogenic lines. The results obtained revealed that the 100 bp band was the distinct marker for *Lr* 9.

The SCAR primers were also used to detect *Lr* 9 in 12 wheat varieties i.e. Giza 162, Giza 163, Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Gemmeiza 9, Sakha 8, Sakha 69 and Sakha 93. The results showed the same fragment size, which produced with *Lr* 9 and the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8 (Figure 3). These results are in agreement with those obtained by the genetic analysis.

**Table (3): Evaluation of 12 wheat varieties against leaf rust (*Puccinia triticina*) at adult stage under field conditions at Shibin El-Kom, Nubariya and Itay Elbaroud locations during two growing seasons (2011/12 and 2012/13).**

| No. | Variety / Line | Location / Season / Final rust severity* (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | | Shibin El-Kom | | Nubariya | | Itay Elbaroud | | |
| | | 2011/1* | 2012/13 | 2011/12 | 2012/13 | 2011/12 | 2012/13 | |
| 1 | Giza 162 | 20 S | 30 S | 30 S | 20 S | 20 S | 10 S | 21.67 |
| 2 | Giza 163 | 30 S | 40 S | 40 S | 40 S | 30 S | 20 S | 33.33 |
| 3 | Giza 164 | 10 S | 30 S | 30 S | 30 S | 20 S | 30 S | 26.67 |
| 4 | Giza 165 | 20S | 10 S | 30 S | 50 S | 20 S | 40 S | 28.33 |
| 5 | Giza 167 | 40 S | 20 S | 20 S | 40 S | 30 S | 30 S | 30.00 |
| 6 | Giza 168 | 10 S | 5 S | 10 S | 10 S | 10 S | 10 S | 9.17 |
| 7 | Giza 170 | 5 S | 20 S | 10 S | 20 S | 20 S | 20 S | 15.83 |
| 8 | Sids 1 | 70 S | 60 S | 80 S | 70 S | 70 S | 60 S | 68.33 |
| 9 | Gemmeiza 9 | 5 S | 5 S | 10 S | 5 S | 5 S | 5 S | 5.83 |
| 10 | Sakha 8 | 5 S | 5 S | 10 S | 10 S | 5 S | 10 S | 7.50 |
| 11 | Sakha 69 | 60 S | 40 S | 30 S | 40 S | 50 S | 30 S | 41.67 |
| 12 | Sakha 93 | 50 S | 60 S | 60 S | 60 S | 40 S | 50 S | 53.33 |
| 13 | *Lr* 9 | 0 | Tr MR | Tr MR | 5 MR | 0 | 5 MR | 2.67 |

* Rating includes two components: disease severity based on modified Cobb scale (Peterson *et al.*, 1948), where 5 = 5 % up to 100 = 100 %, and host response based on scale described by Roelfs *et al.* (1992), where MR = moderately resistant and S = susceptible.

**Table (4): Segregation of $F_2$ populations of the crosses between *Lr* 9 and 12 wheat varieties at adult stage under field conditions in 2012/13 growing season.**

| Cross | No. of plants | | Total | Expected ratio | $\chi^2$ | *P*[b] |
|---|---|---|---|---|---|---|
| | Resistant | Susceptible | | | | |
| Giza 162 X *Lr* 9 | 244 | 0 | 244 | No segregation | - | - |
| Giza 163 X *Lr* 9 | 216 | 0 | 216 | No segregation | - | - |
| Giza 164 X *Lr* 9 | 174 | 50 | 224 | 3:1 | 0.857 | 0.355 |
| Giza 165 X *Lr* 9 | 167 | 61 | 228 | 3:1 | 0.374 | 0.541 |
| Giza 167 X *Lr* 9 | 181 | 59 | 240 | 3:1 | 0.022 | 0.881 |
| Giza 168 X *Lr* 9 | 179 | 50 | 229 | 3:1 | 1.224 | 0.269 |
| Giza 170 X *Lr* 9 | 163 | 55 | 218 | 3:1 | 0.006 | 0.938 |
| Sids 1 X *Lr* 9 | 174 | 54 | 228 | 3:1 | 0.211 | 0.646 |
| Gemmeiza 9 X *Lr* 9 | 250 | 0 | 250 | No segregation | - | - |
| Sakha 8 X *Lr* 9 | 225 | 0 | 225 | No segregation | - | - |
| Sakha 69 X *Lr* 9 | 187 | 47 | 234 | 3:1 | 3.014 | 0.083 |
| Sakha 93 X *Lr* 9 | 165 | 40 | 205 | 3:1 | 3.293 | 0.070 |

*Pb* values higher than 0.05 indicate non-significant of $\chi 2$

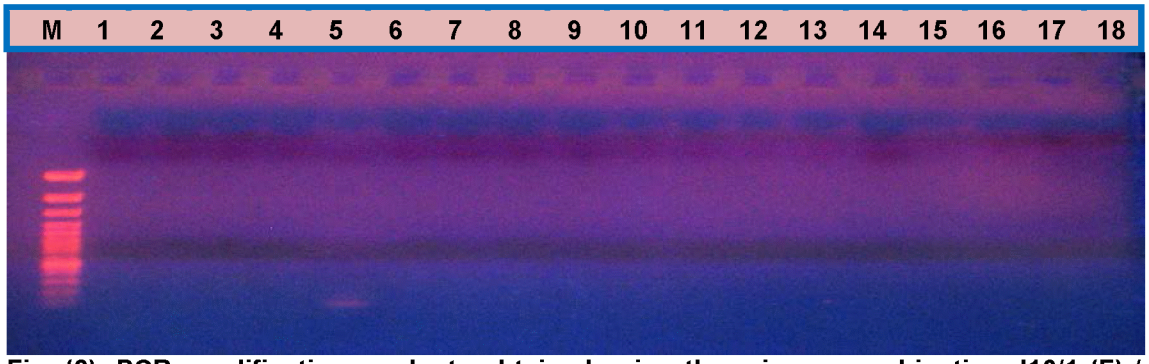


**Fig. (2): PCR amplification products obtained using the primers combination J13/1 (F) / J13/2 (R) showing a polymorphic band only in resistant line possessing *Lr* 9. 1= *Lr* 1, 2= *Lr* 2a, 3= *Lr* 3a, 4= *Lr* 3ka, 5= *Lr* 9, 6= *Lr* 12, 7= *Lr* 13, 8= *Lr* 18, 9= *Lr* 19, 10= *Lr* 21, 11= *Lr* 24, 12= *Lr* 29, 13= *Lr* 32, 14= *Lr* 34, 15= *Lr* 35, 16= *Lr* 36, 17= *Lr* 46, 18= *Lr* 47 and M= 100 bp ladder size marker.**

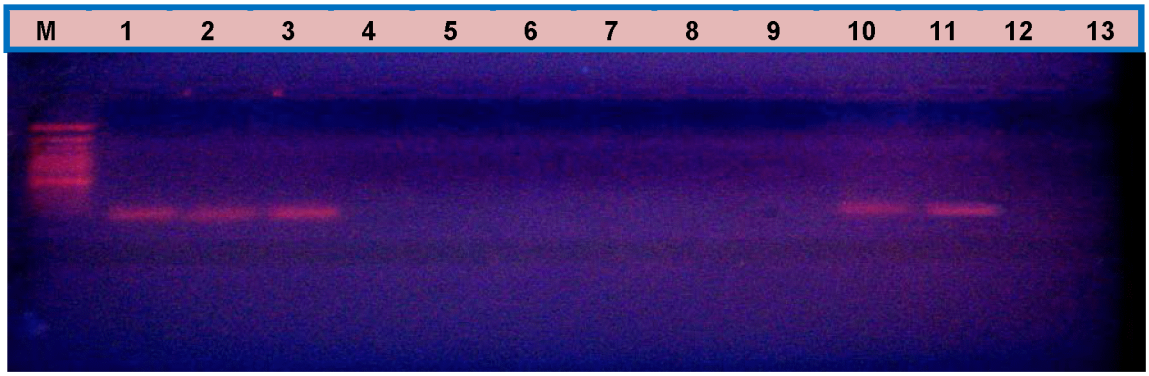


**Fig. (3): Detection of the resistance gene *Lr* 9 in 12 wheat varieties. PCR products obtained from the same wheat varieties with the primers combination J13/1 (F) / J13/2 (R). 1= *Lr* 9, 2= Giza 162, 3= Giza 163, 4= Giza 164, 5= Giza 165, 6= Giza 167, 7= Giza 168, 8= Giza 170, 9= Sids 1, 10= Gemmeiza 9, 11= Sakha 8, 12= Sakha 69, 13= Sakha 93 and M= 100 bp ladder size marker.**

Moreover, the SCAR of random plants of $F_1$ and some plants of $F_2$ populations of the cross between *Lr* 9 and Gemmeiza 9 using the same SCAR primers were conducted. The results indicated that all $F_2$ populations of the cross between *Lr* 9 and Gemmeiza 9 showed the specific fragment for *Lr* 9 (Figure 4).

On the other hand, the $F_2$ populations of the crosses between *Lr* 9 and the wheat variety Giza 164 exhibited 19 out of 36 SCAR band with a good fit with the ratio 3 R : 1 S (Figures 5, 6 and 7).

## DISCUSSION

Leaf rust is one of the most serious diseases of wheat. Specific resistance genes are used to breed resistant varieties.

Many of these resistance genes were introgressed from wild relatives of wheat by wide crosses (Baum *et al*., 1992). The *Lr* 9 resistance gene localized on the long arm of chromosome 6B (Sears, 1961), was translocated into wheat from *Aegilops umbellulata* (Sears, 1956). In addition, no undesirable traits have been associated with the leaf rust resistance of *Aegilops umbellulata* (Soliman *et al*., 1963).However, the presence of *Lr* 9 in combination with other resistance genes is desirable in new cultivars to be released. In order to determine the presence of *Lr* 9 in a complex genetic background, a genetic marker for *Lr* 9 is needed.

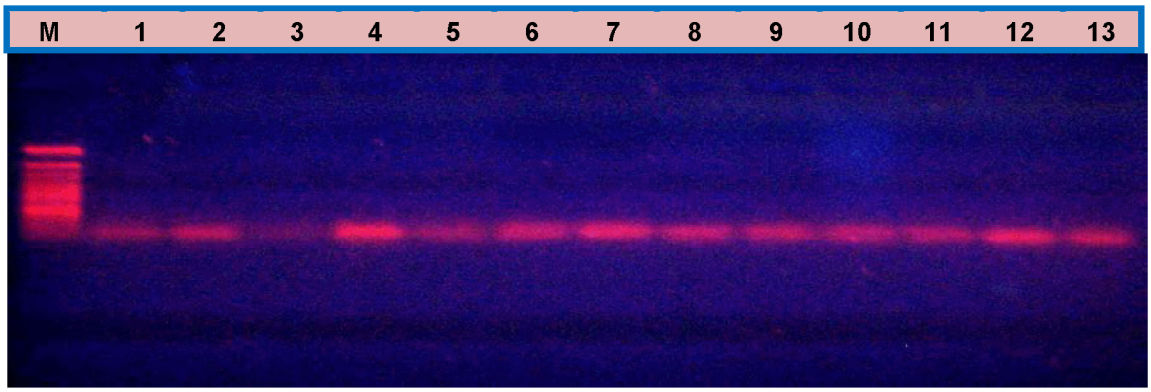


**Fig. (4): Segregation of PCR amplification products that is completely linked to the *Lr* 9 resistance gene. Amplification was performed with the primers combination J13/1 (F) / J13/2 (R). 1= Gemmeiza 9, 2= *Lr* 9, 3= $F_1$ plants of the cross between *Lr* 9 and Gemmeiza 9, 4 - 13 = $F_2$ plants of the cross between *Lr* 9 and Gemmeiza 9 and M= 100 bp ladder size marker.**

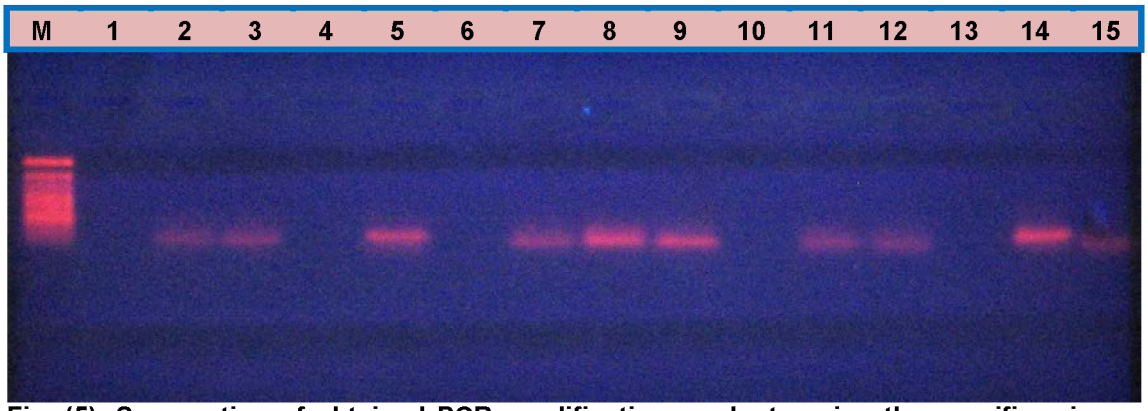


**Fig. (5): Segregation of obtained PCR amplification products using the specific primers J13/1 (F) / J13/2 (R). 1= Giza 164, 2= *Lr* 9, 3= $F_1$ plants of the cross between *Lr* 9 and Giza 164, 4 - 15 = $F_2$ plants of the cross between *Lr* 9 and Giza 164 and M= 100 bp ladder size marker.**

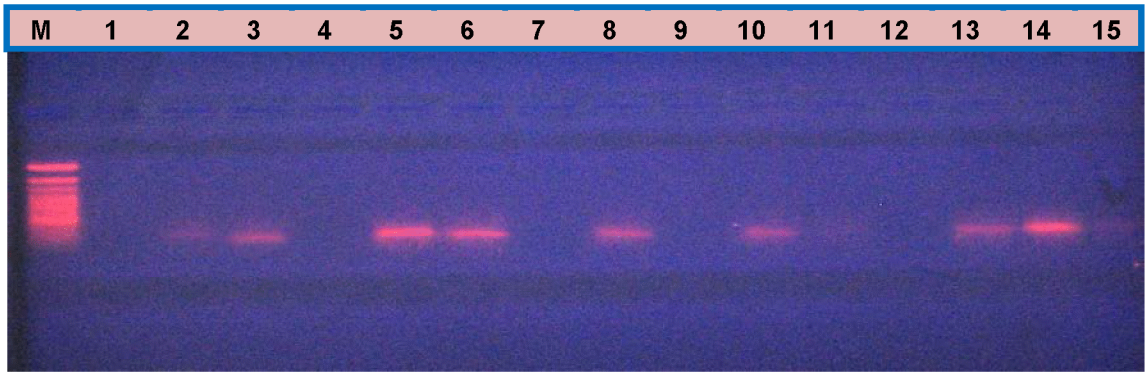


**Fig. (6): Segregation of a PCR amplification products obtained using the specific primers J13/1 (F) / J13/2 (R). 1= Giza 164, 2= *Lr* 9, 3= $F_1$ plants of the cross between *Lr* 9 and Giza 164, 4 - 15= $F_2$ plants of the cross between *Lr* 9 and Giza 164 and M= 100 bp ladder size marker.**

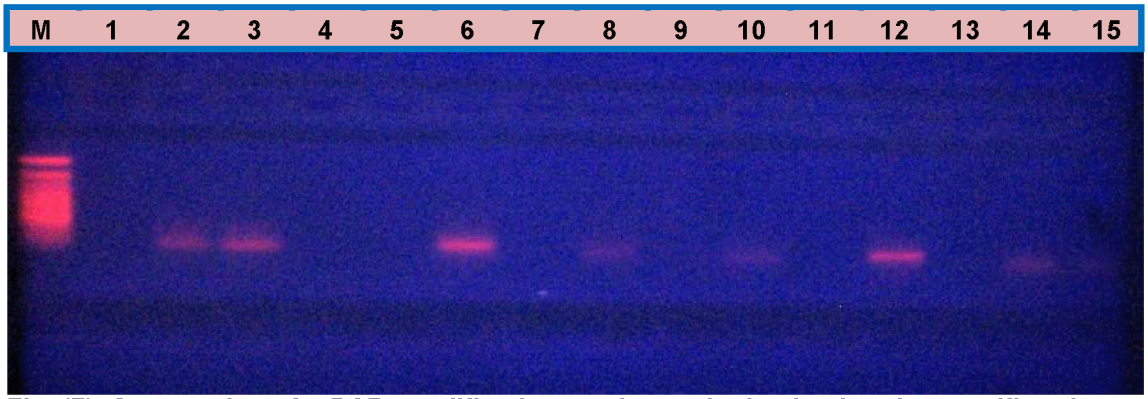


**Fig. (7): Segregation of a PCR amplification products obtained using the specific primers J13/1 (F) / J13/2 (R). 1= Giza 164, 2= *Lr* 9, 3= $F_1$ plants of the cross between *Lr* 9 and Giza 164, 4 - 15 = $F_2$ plants of the cross between *Lr* 9 and Giza 164 and M= 100 bp ladder size marker.**

No virulence for *Lr* 9 has been found in Switzerland, the Netherlands, southern France and Germany (Denissen and van der Putten 1991; Poinso and Ollivier 1988). In Egypt, the monogenic lines *Lr* 9, *Lr* 13, *Lr* 19, *Lr* 24, *Lr* 36 and *Lr* 46 proved their resistance to a wide range of virulences (Shahin, 2004; Negm, 2004 and El-Orabey, 2008). To prevent a rapid breakdown of *Lr* 9 once it is integrated into new wheat varieties, this gene should be used in combination with other leaf rust resistance genes (Roelfs *et al.*, 1992). There are several methods for identification and detection of resistance genes. The classical method is based on resistance analysis of the $F_2$ plants of the crosses between the tested cultivars and lines with known resistance genes. A faster method is based on comparison of reactions of the tested cultivar to a set of different pathotypes with reactions of lines possessing the concerned resistance gene (s). The development of molecular biology has enabled the detection of resistance genes by molecular markers.

In this study testing $F_2$ plants of the crosses between monogenic line having leaf rust resistance gene *Lr* 9 and the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8 at adult stage under field conditions exhibited resistance reactions clearly showed the presence of *Lr* 9 in these wheat varieties. While, the results of $F_2$ plants of the crosses between *Lr* 9 and the wheat varieties Giza 164, Giza 165, Giza 167, Giza 168, Giza 170, Sids 1, Sakha 69 and Sakha 93 confirmed that resistance in these varieties is controlled by dominant gene (s) which is different from the leaf rust resistance gene *Lr* 9. Negm (2004) found that the wheat variety Sakha 8 has the leaf rust resistance genes *Lr* 12, *Lr* 13, *Lr* 24 and *Lr* 34; the wheat variety Sakha 61 has *Lr* 34 and *Lr* 36; the wheat variety Sakha 69 has *Lr* 13 and *Lr* 37; the wheat variety Sakha 92 has *Lr* 13, *Lr* 34, *Lr* 35 and *Lr* 36 and the wheat variety Sids 1 has *Lr* 13 and *Lr* 41. Also, Shahin (2004) found that the wheat variety Gemmeiza 1 has the leaf rust resistance genes *Lr* 12, *Lr* 13, *Lr* 34 and *Lr* 36; the wheat variety Gemmeiza 3 has *Lr* 13, *Lr* 24 and *Lr* 34; the wheat variety Gemmeiza 5 has *Lr* 13, *Lr* 24 and *Lr* 35; the wheat variety Gemmeiza 7 has *Lr* 37 and the wheat variety Gemmeiza 9 has *Lr* 13, *Lr* 24, *Lr* 34 and *Lr* 41. So, using the above mentioned five varieties as resistance sources for *Lr* 9 in a breeding program is an economic and effective to minimize losses caused by leaf rust.

Identification of molecular markers closely linked to resistance genes can facilitate the accumulation of other major genes into a single variety. PCR amplification using specific primers for *Lr* 9 enabled the identification of molecular markers in Thatcher near-isogenic lines containing these resistance genes. The amplification of a short unique sequence only in lines with a single *Lr* gene could

indicate that the SCAR marker is highly specific for the *Lr* gene (Schachermayr *et al*., 1994; Schachermayr *et al*., 1995 and Schachermayr *et al*., 1997). The results of amplification of marker for gene *Lr* 9 correspond with the results of original study (Schachermayr *et al*., 1997). The fragment size produced from PCR experiments was 100 bp long. This fragment is different from the fragment size produced by Schachermayr *et al*. (1994). This difference may be due to the different lab conditions. Hussain *et al*. (2011) screened 25 Pakistani wheat germplasm for the presence of leaf rust resistance gene *Lr* 10 using specific STS primer. They found that 18 genotypes have *Lr* 10 gene, while seven genotypes did not show the presence of *Lr* 10 gene. Also, Kadkhodaei *et al*. (2012) detect the leaf rust resistance genes *Lr* 9, *Lr* 26, *Lr* 28, *Lr* 34 and *Lr* 35 using STS and SCAR markers in 83 Iranian wheat genotypes. They found that *Lr* 9 and *Lr* 35 were only present in the positive controls, while *Lr* 26 was only detected in four cultivars and the gene *Lr* 34 was present in six cultivars. The *Lr* 28 primers could not be validated the presence or absence of this gene. Therefore, it was concluded that the amplification of specific PCR products is an easy and repeatable method that will be useful in the detection of resistance genes in breeding lines. Also, it avoids time-consuming pathogenic tests in breeding programs.

## CONCLUSION

The results of this experiment show that markers J 13/1 and J 13/2 for gene *Lr* 9 are highly specific to varieties with this leaf rust resistance gene. Therefore, it is recommended to use SCAR marker is usefulness for detection of the leaf rust resistance gene *Lr* 9 in various varieties and can be exploited in a wide range of genetic backgrounds, a prerequisite for breeding. However, the resistance tests done on $F_2$ plants of the same varieties confirm the efficacy of SCAR marker.

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# تحديد جين المقاومة *Lr 9* لمرض صدأ الأوراق فى بعض أصناف القمح المصرية

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**الملخص العربى**

أجريت هذه الدراسة بهدف تحديد جين المقاومة *Lr* 9 لمرض صدأ الأوراق فى 12 صنف قمح مصرى وهم جيزة 162 , جيزة 163 ، جيزة 164 ، جيزة 165 ، جيزة 167 ، جيزة 168 ، جيزة 170 ، سدس 1 ، جميزة 9 ، سخا 8 ، سخا 69 و سخا 93. كان أهم النتائج المتحصل عليها بفحص إنعزالات نباتات الجيل الثانى الناتج من التهجين بين السلالة النباتية الحاملة لهذا الجين والأصناف تحت الدراسة وذلك فى طور النبات البالغ تحت ظروف الحقل وذلك بالعدوى بخليط من سلالات فطر صدأ الأوراق. إتضح من النتائج أن الجين *Lr* 9 موجود فى الأصناف جيزة 162 , جيزة 163 ، جميزة 9 و سخا 8. بينما الأصناف جيزة 164 ، جيزة 165 ، جيزة 167 ، جيزة 168 ، سدس 1 ، سخا 69 و سخا 93 لا تحتوى على هذا الجين. أتضح أيضاً أن إستخدام تقنية SCAR والتى أستخدم فيها بادئان متخصصان لجين المقاومة *Lr* 9 أنهما مرتبطان إرتباطا وثيقا بهذا الجين.