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Molecular and Genetic Analysis of Leaf Rust Resistance Genes in Two New Egyptian Wheat Cultivars

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Leaf rust caused by *Puccinia triticina* f. sp. *tritici* is the most common and wide-spread rust disease attacking many wheat cultivars in Egypt. Three main methods were used to identify leaf rust resistance genes; gene postulation, genetic analysis and molecular markers. The two resistant wheat cultivars *i.e.*, Giza-171 and Sids-14, as well as the ten monogenic lines for leaf rust resistance; *Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47*, were selected to carry out the present study. Out of the three methods used, genetic analysis and molecular markers were the best to identify the resistance genes in the two studied cultivars. Ten specific primers were used for the identification of 10 resistance genes in the two new Egyptian wheat cultivars *i.e.*, Giza-171 and Sids-14. Six leaf rust resistance genes, *Lr9, Lr25, Lr28, Lr29, Lr46* and *Lr47* were identified in Giza-171, but only three genes, *Lr29, Lr46* and *Lr47*, were detected in Sids-14. Each of these two new wheat cultivars proved to have an adequate and high level of genetic resistance to leaf rust. The tested wheat cultivars should be used as a good source of leaf rust resistance in breeding programs for rust resistance. Knowledge of the leaf rust resistance genes which has not been designated yet, will help to narrow the gap and throw light on the future objectives for the researchers interested in the full utilization of these genes in breeding materials.

Keywords: Cultivars, Genetic analysis, Gene postulation, Leaf rust, Molecular markers, Monogenic lines, Wheat.

Wheat leaf rust (*Puccinia triticina* f. sp. *tritici*) is the most common rust disease that causes a considerable annualy grain yield loss in many commercial cultivars in Egypt and worldwide (Ali *et al*., 2016). Host-genetic resistance is still the most effective and ecologically sustainable control method. Accordingly, incorporating genetic resistance to this pathogen into adapted and high yielding wheat germplasms is a major goal in most wheat breeding programs, worldwide (Huerta-Espino *et al.*, 2011). Deployment rust resistance genes in the new released wheat cultivars minimizes the need for a wide application of synthetic fungicides, thus reducing environmental contamination risks and decreasing production costs (Mebrate *et al*., 2008). To date, more than 74 leaf rust resistance genes (Lr's) have been identified; most of them are mapped on different chromosomes through marker assistant selection (McIntosh et *al*., 2013). However, the sudden appearances of new virulent races of the target pathogen in its population, combined by virulence shifts in these populations, have reduced the effectiveness of a significant number of the leaf rust

resistance genes (Johnson, 2000). Thus, stacking different leaf rust resistance genes in a given cultivar, a process also called as gene pyramiding helps to avoid rapidly breakdown of its genetic resistance and consequently, achieved a durability of such resistance (Mebrate *et al.*, 2008). Generally, there are three main methods widely used for detecting different host resistance genes to rust fungi, especially leaf rust in wheat genotypes; gene postulation, genetic analysis and molecular markers. Gene postulation is the most common method, that rapidly determines the presence of the probable leaf rust resistance genes (Lr genes), in a host cultivar at seedling stage. Many researchers have previously used this method for easily postulating Lr genes in several commercial wheat cultivars in short time (Kolmer, 2003 and Mebrate *et al.*, 2008). Meanwhile, genetic analysis was used to detect the rust resistance genes, particularly leaf rust resistance genes in a majority of wheat germplasms, worldwide (Riar *et al.*, 2012). In addition to these two methods, presence of resistance genes can be determined by testing host cultivars with specific molecular markers linked to each of these resistance genes (Samsampour *et al.*, 2010). This approach overcomes some of the problems associated with traditional gene postulation, such as gene interactions in different plant stages. Recently, mapping and development of specific molecular markers for several leaf rust resistance genes have several advances (Bipinraj *et al*., 2011 and Singh *et al*., 2012). Once these genetic factors are mapped, they can be controlled by molecular markers and the corresponding genotypes of individuals can be assessed easily. Consequently, the identification of cultivars carrying favorable alleles at their loci will facilitate the use of these promising genotypes as valuable genetic materials in wheat breeding program for disease resistance. Furthermore, the identity and detection of the effective leaf rust resistance genes in the tested wheat cultivars will be useful and have a great importance in the fully understanding their variations in disease response under field conditions, in relation to the changes in pathogen populations. This knowledge can be also used for making a good decision in the future and anticipatory wheat breeding program for rust resistance. Therefore, the current investigation aimed to detect and identify the most effective *Lr* genes present in the adapted and high yielding wheat cultivars.

Materials and Methods

The present investigation was conducted at the experimental farm of Sakha Agricultural Research Station (Kafr El-Sheikh governorate), during 2014/2015, 2015/2016 and 2016/2017 growing seasons, and the leaf rust greenhouse in Wheat Dis. Res. Dep., Plant Pathol. Res. Institute, Agricultural Research Center (ARC), Giza, Egypt. In addition, the molecular analysis was carried out at (EPCRS) Excellence Center (certified according to ISO 9001, ISO 14001 and OHSAS 18001) and Plant Pathology & Biotechnology Lab. (certified according to ISO 17025), Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Egypt.

1. Evaluation of 21 Egyptian wheat cultivars and 35 leaf rust resistance genes under field conditions:

Evaluation of 21 Egyptian wheat cultivars and 35 leaf rust resistance genes against leaf rust infection under field conditions was conducted at Kafr El-Sheikh governorate, during 2014/15 and 2015/16 growing seasons. Wheat cultivars and monogenic lines were sown in the experimental unit consisted of 3 rows (3m long and 30cm apart), each row was sown with 5g of a given tested wheat cultivar and monogenic line in randomized complete block design with three replicates. The recommended agricultural practices were applied. Disease severity (%) was scored according to a standard scale (Peterson *et al.,* 1948).

2. *Identification of leaf rust resistance genes in the two new Egyptian wheat cultivars:*

2.1. Gene postulation method:

Two new Egyptian wheat cultivars; Giza-171 and Sids-14 and ten monogenic lines; *Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47* were tested at the seedling stage using 10 isolates of *P. triticinia* obtained from collected samples during 2015/2016 growing season. All plant materials were grown in 10 cm plastic pots. Each pot was planted by four wheat genotypes, one in each corner in clockwise order. Inoculation and incubation procedures were carried out according to the methods adopted by Stakman *et al.* (1962). Rust reaction was recorded on the first leaf, 12 days after sowing. Rust data were scored as infection type (IT's), *i.e.* R= (0, 0, 1 and 2) and S= (3 and 4), which were designated as L; low infection type and H; high infection type (Johnosen, 1961). Leaf rust resistance genes (Lr's) were postulated using the methods adopted by Statler (1984), in which the absence of L:H or H:L reaction between the tested cultivar (cultivar B) and the known host (monogenic line A), indicated the presence of such gene in the tested cultivar exhibited the symbol (-0). On the other hand, when cultivar B proved to have H (high infection type) versus L (low infection type) in monogenic line A, this behavior indicated the absence of such gene in the tested cultivar = (-). The presence of L (in the cultivar B) : H (in the monogenic line A) indicated the presence of such gene in cultivar B and it may have another ones = 0. The presence of pathotypes having H:L and L:H in the comparison indicates that either of hosts did not have the same gene = (+). It must be remembered that the entries or genotypes that proved to have completely high infection type or completely low infection type must be omitted from matching (Statler, 1984).

2.2. Genetic analysis method:

To identify Lr genes in the wheat cultivars *i.e.,* Giza-171 and Sids-14, crosses were conducted among them and the ten monogenic lines *i.e., Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47*. The parental cultivars and monogenic lines were grown at Kafr El-Sheikh governorate in four successive sowing dates at 15 days intervals to overcome differences in

the time of flowering during the growing season. All monogenic lines under study were used as male parents for crosses with each of the two cultivars under study to obtain the F_1 seeds (2014/2015).

The F_1 seeds were sown in the following season 2015/2016 in rows of 3 m long and 30 cm apart and spaced 20 cm in order to allow production of F_2 seeds. In 2016/2017 growing season, the F_2 seeds were sown in plots, each consisted of 6 rows (3m long for each) spaced 30 cm and seeds were sown 15cm apart. All plots were surrounded by a spreader area of a mixture of the two highly susceptible wheat varieties *i.e.*, *Triticum spelta saharensis* and Morocco. For inoculation in the field, the spreader wheat plants were moistened and dusted with spore-powder mixtures of the most prevalent leaf rust pathotypes (PTTCT, PTTGS, PTTTT, TTTBT and TTTTT) in the area. Inoculation of all plants was carried out at late tellering and late elongation stages according to the method suggested by Tervet and Cassel (1951). Leaf rust severity (%) was recorded for each wheat plant of F_2 generation at the first appearance of leaf rust pustule. F_2 plants were grouped into ten classes depending on leaf rust severity (%), under field conditions. The classes were; 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100. The first three classes were considered as the low disease severity (resistant), while other classes (more than 30%) were considered as the high disease severity (susceptible). For the identification of leaf rust resistance genes (Lr's), in each cross, the observed and expected ratios of the phenotypic classes concerning leaf rust severity (%), were genetically analyzed by chi-square ($\chi 2$) analysis for F_2 plants (Steel and Torrie, 1960).

2.3. *Molecular markers procedure:*

This part of the investigation was carried out at Plant Pathology and Biotechnology Lab., Faculty of Agriculture, Kafr El-Sheikh Univ.

2.3.1. Plant materials:

Two new Egyptian wheat cultivars *i.e.*, Giza-171 and Sids-14 and ten Lr genes; *Lr9*, *Lr19*, *Lr21*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr46* and *Lr47* were used to detect Lr genes in the tested cultivars.

2.3.2. DNA extraction:

A modified method based on the protocol of Dellaporta *et al.* (1983) was conducted for extraction of total genomic DNA.

2.3.3. PCR Amplification:

Polymerase chain reaction was performed in thermocycler (Rocorbett-Research, CG1-96) in 25µl reaction volume containing: 2.5µl 50ng/µl of genomic DNA, lµl each primer (10 pmol, F&R) and 8µl MQ H_2O (Devos and Gale, 1992). The specific SSR primers were used to verify the presence of *Lr9*, *Lr19*, *Lr21*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr46* and *Lr47* genes are listed in Table (1). Annealing temperatures of these genes were 62, 55, 57, 58, 57, 50, 65, 58, 64, 60ºC, respectively.

Amplification products were electrophoresed at 100V/1h. After electrophoresis, the gel was stained with ethidium bromide and bands were visualized using UV light and photographed with a Syngen UV visualizer (gel documentation system, G:BOX). The Mid-Range DNA Ladder 100bp-3kbp linear sale (Jena Bioscience) was used to detect the molecular weight of the tested samples.

Table 1. Names, sequences and references of specific primers linked to the tested Lr genes used in this study

Gene	Name	Primer sequences (5'-3')	Reference
Lr9	J 13/1 J 13/2	TCC TTT TAT TCC GCA CGC CGG CCA CAC TAC CCC AAA GAG ACG	Schachermayr *et al*., 1994
Lr19	SCS73719-1 SCS73719-2	TCG TCC AGA TCA GAA TGT G CTC GTCGATTAGCAGTGAG	Prins *et al.*, 2001
Lr21	F R	CCA AAG AGC ATC CAT GGT GT CGC TTTT ACC GAG ATT GGT C	Huang and Gill, 2001
Lr24	J9/1 J9/2	TCT AGT CTG TAC ATG GGG GC TGG CAC ATG AAC TCC ATA CG	Schachermayr *et al*., 1995
Lr25	Lr25F20 Lr25R19	CCA CCC AGA GTA TAC CAG AG CCA CCC AGA GCT CAT AGA A	Procunier *et al*., 1995
Lr28	Lr 28-01 Lr 28-02	CCC GGC ATA AGT CTA TGG TT CAA TGA ATG AGA TAC GTG AA	Naik *et al*., 1998
Lr29	Lr29F24 Lr29R24	GTG ACC TCA GGC AAT GCA CAC AGT GTG ACC TCA GAA CCG ATG TCC ATC	Procunier *et al*., 1995
Lr34	L R	AGC TCT GCT TCA CGA GGA AG CTC CTC TTT ATA TCG CGT CCC	Suenaga *et al*., 2003
Lr46	F R	GGT CTT CTG GGC TTT GAT CCT GTT GCT AGG GAC CCG TAG TGG	Paillard *et al*., 2003
Lr47	PS10L PS10L2	TCT TCA TGC CCG GTC GGG T GGG CAG GCG TTT ATT CCA G	Helguera *et al*., 2000

3. Statistical analysis:

The analysis of variance (ANOVA) of the obtained data was performed with statistical package MSTAT-C (version 2.1). The least significant difference (L.S.D.) at 5% level of significant was used to compare treatment means.

Results and Discussion

Analysis of variance:

To assess the level of leaf rust resistance of the tested genotypes; 21 Egyptian wheat cultivars and 35 monogenic lines (Lr's), combined analysis of variance, during the two seasons; 2014/2015 and 2015/2016 was carried out. Data presented in Table 2 show that significant difference in final rust severity (FRS %), was found among the tested wheat cultivars (C) and years (Y). While, highly significant difference was

also detected with regard to the interaction between years (Y) and the tested wheat cultivars (C). Also, there was a significant difference between the tested leaf rust monogenic lines (L) and years (Y), as well as the interaction between them (Table 3). The significant interactions were due to the differences in the magnitude of genotype means within each year. Due to the highly significance of the interaction between years and cultivars (Y x C), and between years and monogenic lines (Y x L), L.S.D. values were used to compare the differences in FRS (%) between any two cultivars and any pair of monogenic lines under study within each environment (years). Generally, most of the tested wheat genotypes; cultivars and monogenic lines showed diverse disease response (different levels of leaf rust resistance). Since, they recorded different values of FRS (%), during the two years of the study, as they affected by the slight changes in environmental conditions, in each growing season.

Table 2. Combined analysis of variance over the two years for final rust severity (%), expressed on 21 wheat cultivars to leaf rust, during 2014/2015 and 2015/2016 growing seasons.

S.O.V.	DF	Mean square	F prob
Years (Y)	1	1897.087**	0.0049
Error	4	59.938	-
Cultivars (C)	20	4573.777**	0.0000
Y × C	20	103.870**	0.0000
Error	80	14.968	-

Table 3. Combined analysis of variance over the two years for final rust severity (%), expressed on 35 monogenic lines to leaf rust, during 2014/2015 and 2015/2016 growing seasons.

S.O.V.	DF	Mean square	F prob
S.O.V.	1	4416.043**	0.0027
Years (Y)	4	101.581	-
Error	34	3074.002**	0.0000
Monogenic lines (L)	34	182.886**	0.0000
Y × L	136	39.581	-

Disease response of 21 commercial wheat cultivars to leaf rust was studied at adult stage under field conditions, to build up data on the regional performance and disease effects due to leaf rust at Kafr El-Sheikh governorate, Egypt, during 2014/2015 and 2015/2016 growing seasons (Table 4). In general, data presented in Table 4 reveal that the two wheat cultivars *i.e.*, Giza-171 (0.00) and Sids-14 (0.00) were completely resistant, since no symptoms could be detected in leaves of their wheat plants, during the two seasons of the study. Also, wheat cultivars; Sakha-94, Sakha-95, Giza-168, Sids-13, Misr-1, Misr-2 and Misr-3, showed high and adequate levels of leaf rust resistance, where they recorded the lowest percentages of FRS (%), ranged from 3.66 to 10.33%. On the other hand, the rest of the tested cultivars recorded the highest percentages of final rust severity (%) (reached up to 86.70%),

during the two seasons of the present study and therefore, they considered to be the highly susceptible group of cultivars. Similar results were previously reported by Abdelbacki *et al.* (2015) who revealed that the wheat cultivars Giza-168, Sakha-94, Misr-2, Misr-1, Sakha-95, Sids-13, Gemmeiza-9, Sids-12, Gemmeiza-10 and Gemmeiza-11 showed high resistance.

Table 4. Final leaf rust severity (%) of 21 commercial wheat cultivars at Kafr El-Sheikh governorate, during 2014/2015 and 2015/2016 growing seasons.

No.	Wheat cultivar	Seasons / Final rust severity (%)	
		2014/2015	2015/2016
1	**Sakha-61**	86.70	80.00
2	**Sakha-69**	13.30	8.33
3	**Sakha-93**	73.30	50.00
4	**Sakha-94**	5.00	6.66
5	**Sakha-95**	6.66	5.00
6	**Giza-160**	63.33	43.33
7	**Giza-163**	56.66	40.00
8	**Giza-164**	33.33	26.66
9	**Giza-167**	50.00	30.00
10	**Giza-168**	10.00	8.33
11	**Giza-171**	0.00	0.00
12	**Sids-1**	73.33	66.66
13	**Sids-4**	50.00	43.33
14	**Sids-8**	23.33	13.33
15	**Sids-9**	60.00	46.66
16	**Sids-12**	13.33	6.66
17	**Sids-13**	10.33	5.00
18	**Sids-14**	0.00	0.00
19	**Misr-1**	6.66	3.00
20	**Misr-2**	5.00	3.66
21	**Misr-3**	6.67	5.00

$L.S.D._{0.05}$ for interaction (cultivars × years) = 6.28

Thirty-five monogenic lines (35 Lr genes) were evaluated against leaf rust to study their efficiency under field conditions at Kafr El-Sheikh, governorate, during 2014/15 and 2015/16 growing seasons (Table 5). The ten Lr genes; *Lr9*, *Lr19*, *Lr21*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr46* and *Lr47* showed high and adequate levels of resistance and considered to be the most effective Lr genes under field conditions, during the two seasons. While, other tested Lr genes were not effective against leaf

rust, where they recorded the highest percentages of final rust severity (reached to 83.33%) during the two seasons. *Lr19* that exhibited complete resistance to leaf rust in the current study under Egyptian field conditions is also effective in most countries of Asia, Australia and Europe and linked with the desirable genes for grain yield enhancement, which is favorable and preferable for wheat breeding (Gupta *et al.*, 2006). In addition, *Lr25* is a very important gene for South East Asian cultivars. It was transferred from *Secale cereale* L. on 4BL and conferring resistance to all pathotypes of South East Asia (Singh *et al.*, 2012). While, *Lr28* having an adequate level of leaf rust resistance to all the prevalent pathotypes in India, it is not linked with any undesirable genes that reduce the yield (Bipinraj *et al.*, 2011). Meanwhile, the adult plant resistance gene; *Lr34* confers partial resistance (PR) in a majority of wheat cultivars, worldwide (Suenaga *et al*., 2003). Also, *Lr46* is considered to be slow rusting or PR gene, as it is remained effective for a long period of time (many years), against most of the leaf rust pathotypes in a wide range of environmental conditions (Rosewarne *et al*., 2006). Based on the obtained results in this part of the study, the two new Egyptian wheat cultivars; Giza-171 and Sids-14, as well as the ten leaf rust resistant monogenic lines; *Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47* were chosen as plant materials to detect the resistance genes in these two cultivars using the three widely used methods.

Table 5. Disease response of 35 wheat monogenic lines (Lr genes) against leaf rust infection in adult stage at Kafr El-Sheikh governorate, during 2014 /2015 and 2015/2016 growing seasons.

No.	Lr gene	Seasons/Final rust severity(%)		No.	Lr gene	Seasons/Final rust severity(%)	
		2014/2015	2015/2016			2014/2015	2015/2016
1	***Lr1***	76.67	53.33	**19**	***Lr21***	10.00	8.33
2	***Lr2a***	26.67	16.67	**20**	***Lr 22b***	53.33	26.67
3	***Lr2b***	53.33	26.67	**21**	***Lr23***	56.67	53.33
4	***Lr2c***	66.67	53.33	**22**	***Lr24***	23.33	16.67
5	***Lr3***	63.33	46.67	**23**	***Lr25***	8.333	6.67
6	***Lr3ka***	53.33	50.00	**24**	***Lr28***	0.00	0.00
7	***Lr3bg***	33.33	26.67	**25**	***Lr29***	6.67	5.00
8	***Lr9***	13.33	20.00	**26**	***Lr30***	23.33	16.67
9	***Lr11***	83.33	63.33	**27**	***Lr34***	13.33	6.67
10	***Lr12***	70.00	53.33	**28**	***Lr35***	73.33	63.33
11	***Lr14a***	73.33	63.33	**29**	***Lr36***	33.33	20.67
12	***Lr14b***	76.67	43.33	**30**	***Lr37***	73.33	56.67
13	***Lr15***	66.67	53.33	**31**	***Lr38***	53.33	26.67
14	***Lr16***	73.33	63.33	**32**	***Lr39***	66.67	53.33
15	***Lr17***	60.00	33.33	**33**	***Lr40***	83.33	73.33
16	***Lr18***	33.33	23.33	**34**	***Lr46***	8.33	8.33
17	***Lr19***	0.00	0.00	**35**	***Lr47***	16.67	8.33
18	***Lr20***	26.67	13.33				

L.S.D.$_{0.05}$ for interaction (monogenic lines × years) =10.07

Identification of leaf rust resistance genes in the two new Egyptian wheat cultivars:

To identify the responsible genes for leaf rust resistance in the two wheat cultivars; Giza-171 and Sids-14, three main methods; gene postulation, genetic analysis and molecular markers, were used.

1. Gene postulation:

Infection type's data were used successfully to postulate genes for leaf rust resistance in the two wheat cultivars; Giza-171 and Sids-14. Seedlings of these two cultivars with unknown genes for resistance, along with monogenic lines possessing designated leaf rust resistance genes were tested against 10 *P. triticina* isolates. To postulate resistance gene(s), infection type of each tested cultivar, was compared with those of the designated genotypes (Lr's) across all pathogen isolates used (Kolmer, 2003). Data in Table 6 show the seedling reaction of 12 wheat genotypes (cultivars and Lr genes) as affected by the inoculation with 10 isolates of leaf rust pathogen. Data indicate also that Giza-171 and Sids-14, *Lr9, Lr19* and *Lr25* exhibited the highest levels of leaf rust resistance against the tested isolates, as they showed low infection types (L) against most of the tested isolates (Table 6). However, the lowest levels of resistance were recorded with *Lr21, Lr46* and *Lr47*, where, they showed high infection types (H) against most of the used isolates. On the other hand, isolates 5 and 8 were the most aggressive, while, 6 and 10 were the less aggressive isolates to the tested wheat genotypes.

The matching between both wheat cultivars; Giza-171 and Sids-14 and each of Lr genes; *Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47* against the tested isolates of leaf rust showed that eight genes have been postulated in Giza-171; *Lr9, Lr19, Lr21, Lr25, Lr28, Lr29, Lr46* and *Lr47* (Table 7). On the other hand, it was found that this cultivar did not have any of the two genes; *Lr24* and *Lr34*. Other wheat cultivar under study; Sids-14 probably possesses five Lr genes; *Lr21, Lr28, Lr29, Lr46* and *Lr47*. While, other Lr genes; *Lr9, Lr19, Lr24, Lr25* and *Lr34* did not postulated in Sids-14 (Table 7). The most common resistance genes, being found in the two wheat cultivars, under study (100% frequency) were; *Lr21, Lr28, Lr29, Lr46 and Lr47*. Meanwhile, other Lr genes *i.e., Lr9, Lr19* and *Lr25* have been postulated in only one cultivar, thus they exhibited 50% frequency. However, the two Lr's (*Lr24* and *Lr34*) could not postulate in any of the tested wheat cultivars (Table 7).

Many researchers have previously used this method for easily detecting Lr genes in several commercial wheat cultivars (Kolmer, 2003 and Mebrate *et al.*, 2008). This method could be facilitated the use of such genes in wheat anticipatory breeding program, aimed to release new wheat varieties with an acceptable level of leaf rust resistance. A relatively little variation in Lr genes, that found in the two wheat cultivars, during this study was due to the strong similarity between the pedigree and narrow base of genetic background for these two cultivars. To avoid and decrease selection pressure imposed by the host cultivar on the target pathogen races, it could be cultivated or regionally deployed several wheat cultivars having different effective resistance genes.

Table 6. **Seedling reaction of twelve wheat genotypes, against ten isolates of *Puccinia triticina* in terms of low infection types (L) and high infection types (H), under greenhouse conditions.**

Wheat genotype		*Puccinia triticina* isolates/Infection types:									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
a.cultivars	**Giza-171**	H	L	L	L	H	L	L	H	L	L
	Sids-14	H	L	L	L	L	L	L	H	L	L
b. Lr genes	***Lr9***	L	L	L	L	H	L	L	H	L	L
	Lr19	L	L	L	L	H	L	L	H	L	L
	Lr21	H	H	H	H	H	L	L	H	H	H
	Lr24	L	H	L	H	L	H	H	L	L	H
	Lr25	L	L	L	L	H	L	L	H	L	L
	Lr28	H	H	H	L	H	L	H	H	H	L
	Lr29	H	H	H	H	H	L	H	H	L	L
	Lr34	H	L	H	L	L	L	H	L	H	L
	Lr46	H	H	H	H	H	L	H	H	H	H
	Lr47	H	H	H	H	H	H	H	H	L	L

L = low infection types (0, 0, 1 and 2) and H = high infection types (3 and 4)

Table 7. **leaf rust resistance that probably present in the two Egyptian wheat cultivars; Giza-171 and Sids-14 at seedling stage, under greenhouse conditions.**

Monogenic line (Lr's)	Wheat cultivar		Gene frequency (%)
	Giza-171	**Sids-14**	
Lr9	0	+	50.0
Lr19	0	+	50.0
Lr21	0	0	100.0
Lr24	+	+	0.0
Lr25	0	+	50.0
Lr28	0	0	100.0
Lr29	0	0	100.0
Lr34	+	+	0.0
Lr46	0	0	100.0
Lr47	0	0	100.0
Postulated gene	**(8 genes); *Lr9, Lr19, Lr21, Lr25, Lr28 Lr29, Lr46, Lr47***	**(5 genes); *Lr21, Lr28, Lr29, Lr46, Lr47***	-

(0) = presence of such gene in the cultivar and it probably possesses another one and (+) = the cultivar did not have the same gene

2. Genetic analysis:

Most of the adult plant resistance (APL) genes considered to be slow-rusting or partial resistance (PR) with quantitative nature of inheritance. Therefore, they play an important role in the durability of leaf rust resistance in most cultivated wheats. To identify, more accurately, Lr genes in the two wheat cultivars under study, 20 crosses were carried out among these two wheat cultivars and each of the ten wheat monogenic lines *i.e., Lr9, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr46* and *Lr47* (Table 8). The observed and expected ratios of the phenotypic classes concerning leaf rust severity (%), were determined by chi-square ($\chi 2$) analysis for F_2 plants (Steel and Torrie 1960). The obtained results indicated that F_2 plants of the cross between *Lr9* and Giza-171 showed no segregation. These results confirmed that Giza-171 possesses the leaf rust resistance gene; *Lr9.* While, F_2 cross between *Lr9* and Sids-14 were segregated to ratio (166 L: 49 H). This ratio fitted the expected ratio; 3:1, indicating that this cultivar did not possess *Lr9* and the prevailing situation was low rust severity (Table 8). Also, wheat plants of F_2 crosses between *Lr19* and the two cultivars *i.e.*, Giza-171 and Sids-14, were segregated to (157 L: 44 H) and (172 L: 61 H), respectively. The segregations fit the ratio 3:1. Likewise, F_2 plants of the crosses between *Lr21* and the same two cultivars, were segregated according to the ratios (136 L : 99 H) and (195 L : 16 H), respectively. These segregations fit the theoretical ratios; 9:7 and 15:1, respectively. F_2 plants obtained from the crosses between *Lr24* and the two cultivars; Giza-171 and Sids-14 were found to be segregated to ratios (178 L: 35 H) and (119 L: 85 H), respectively. These segregations fit the expected ratios; 13:3 and 9:7, respectively, indicated that the wheat cultivars under study did not have the three resistance genes; *Lr19, Lr21* and *Lr24.* Till now *Lr19* proved to display a high efficacy (complete resistance) to leaf rust under Egyptian field conditions, in most wheat growing areas (Abdelbacki *et al.,* 2015). It is also effective in many countries of Asia, Australia and Europe (Gupta *et al*., 2006). Also, it is present in numerous wheat cultivars in CIMMYT in combination with other resistance genes which continues to give excellent rust protection (Huerta-Espino *et al*., 2011). Although, this study could not detect the presence of an important and effective Lr gene (*Lr19*) in the two new wheat cultivars, it may be found in other Egyptian wheat cultivars. Due to the high efficacy of this gene against most of the pathogen races under a wide range of field conditions in Egypt, it should be taken into a consideration to make a good decision.

Data presented in Table 8 indicate also that all of F_2 plants resulted from the crosses between the two Lr genes; *Lr25* and *Lr28* and wheat cultivar; Giza-171 were found to be resistant. This result confirmed the presence of these two genes in the tested cultivar. While, F_2 plants of the crosses between the same two genes and Sids-14 were segregated to 177 L:66H and 162 L: 49H, respectively, revealing the absence of these two genes in the cultivar; Sids-14. On the other hand, all of F_2 plants of the crosses among the three Lr genes; *Lr29, Lr46* and *Lr47* and the two cultivars *i.e.*, Giza-171 and Sids-14, were resistant and showed no segregations. These results indicate that each of the two cultivars have the resistance genes; *Lr29, Lr46* and *Lr47*. In contrast, F_2 plants of the crosses between *Lr34* and the same cultivars of the study showed the observed ratios (210 L: 53H) and (174 L: 48H), respectively.

Table 8. Segregation and Chi square (χ2) analysis of F_2 plants of the crosses among the ten Lr genes and the two cultivars; Giza-171 and Sids-14, under field conditions at Kafr El-Sheikh governorate, during 2016/2017 growing season.

Cross name	No. of F_2 plants		Expected ratio	χ2	P^b
	L	H			
Giza-171 x *Lr9*	209	0	No segregation	-	-
Sids-14 x *Lr9*	166	49	3:1	0.56	0.50-0.25
Giza-171 x *Lr19*	157	44	3:1	1.04	0.50-0.25
Sids-14 x *Lr19*	172	61	3:1	0.17	0.75-0.50
Giza-171 x *Lr21*	136	99	9:7	0.25	0.75-0.50
Sids-14 x *Lr21*	195	16	15:1	0.64	0.50-0.25
Giza-171 x *Lr24*	178	35	13:3	0.75	0.50-0.25
Sids-14 x *Lr24*	119	85	9:7	0.85	0.50-0.25
Giza-171 x *Lr25*	231	0	No segregation	-	-
Sids-14 x *Lr25*	177	66	3:1	0.61	0.50-0.25
Giza-171 x *Lr28*	265	0	No segregation	-	-
Sids-14 x *Lr28*	162	49	3:1	0.35	0.50-0.25
Giza-171 x *Lr29*	223	0	No segregation	-	-
Sids-14 x *Lr29*	207	0	No segregation	-	-
Giza-171 x *Lr34*	210	53	13:3	0.34	0.75-0.50
Sids-14 x *Lr34*	174	48	3:1	1.35	0.025-0.10
Giza-171 x *Lr46*	234	0	No segregation	-	-
Sids-14 x *Lr46*	217	0	No segregation	-	-
Giza-171 x *Lr47*	229	0	No segregation	-	-
Sids-14 x *Lr47*	236	0	No segregation	-	-

L= Low rust severity < 30% H= High rust severity > 30%

P^b values higher than 0.05 indicate that non-significance of χ2

These ratios fit the expected ratios; 13:3 and 3:1, respectively, indicating that these two cultivars did not have the resistance gene; *Lr34*. Similar results were previously reported by Riar *et al*. (2012) who stated that a single gene was segregated for leaf rust resistance according to the expected ratio; 3:1 in F_2 population.

3. Molecular markers:

Molecular markers have become an important and new tool in which specific molecular markers are successfully used to identify and designate, more definitely, resistance genes in wheat genotypes, where the genetic background has not yet been clarified, like most commercial wheat cultivars (Bipinraj *et al.*, 2011). Results of the present study clearly demonstrated the advantage of molecular markers for detection the presence of Lr genes in the tested wheat cultivars compared to their pedigree data, and are in accordance with numerous studies and reviews, that previously carried out (Samsampour *et al.*, 2010, Singh *et al.*, 2012 and Abdelbacki *et al.*, 2015). Ten specific primers were used for the identification of 10 resistance genes (Lr's) in the two new Egyptian wheat cultivars *i.e.*, Giza-171 and Sids-14. The polymorphic survey revealed that out of the tested Lr genes, the marker linked to *Lr9* was identified as a fragment of 300bp in Giza-171. While, Sids-14 did not show the presence of *Lr9* (Fig. 1). Likewise, the markers for *Lr19, Lr21* and *Lr24* were not identified in the two wheat cultivars under study, revealing the absence of these three genes (Fig. 1). In contrast, the diagnostic PCR fragments associated with *Lr25* and *Lr28* were detected in Giza-171 cultivar, as a fragment of 250bp and 400bp, respectively and didn't detect in Sids-14 cultivar (Fig. 2). On the other hand, the marker for *Lr29* was identified as a fragment of 150bp in the two cultivars; Giza-171 and Sids-14 (Fig. 2). Whilst, the marker for *Lr34* was not detected in the two cultivars under study. However, the markers for *Lr46* and *Lr47* were also identified as a fragment of 310bp and 224bp in Giza-171 and Sids-14, respectively (Fig. 3). Similar results were previously reported by Vida *et al.* (2010), who recorded that the wheat genotypes having the three leaf rust resistance genes*; Lr*9, *Lr*19 and *Lr*28, showed excellent and high levels of leaf rust resistance at adult stage.

On the basis of the obtained results of the present investigation and according to the previous studies, the best methods for identification of leaf rust resistance genes in the wheat cultivars were the genetic analysis and molecular markers technique because the results were completely identical (Samsampour *et al.*, 2010). Where, the six Lr genes; *Lr9, Lr25, Lr28, Lr29, Lr46* and *Lr47* were identified in Giza-171 and three of them; *Lr29, Lr46* and *Lr47* were also identified in Sids-14 (Table 9). On the other hand, the obtained results from gene postulation method were differed from the other two methods, under study. This may be due to the tested genotypes (cultivars and Lr genes) proved to have completely high infection types or completely low infection types and must be omitted from matching (Statler 1984).

In the present study, the two wheat cultivars; Giza-171 and Sids-14 showed good and high levels of adult plant resistance, under field conditions. This result was confirmed by the detecting of more than one gene for leaf rust resistance in these cultivars, which enhance the resistance response of the cultivar giving high level of resistance. This knowledge can be also used for making an adequate decision in the

future and anticipatory wheat breeding program for rust resistance. Moreover, identification of the most effective Lr genes present in the adapted and high yielding wheat cultivars could facilitate the use of these genotypes as a good source of resistance in wheat breeding program.

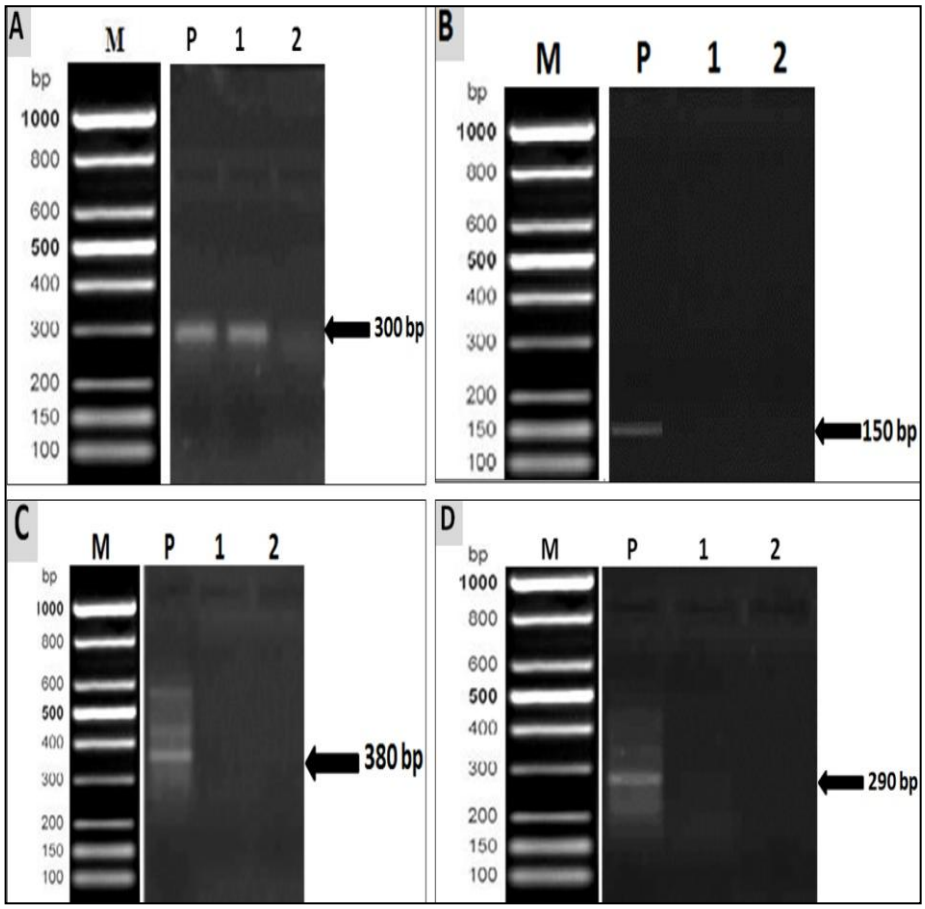

Fig. 1. Electrophoretic amplified pattern of DNA extracted from the two cultivars under study using the specific primer for *Lr9* (A), *Lr19*(B), *Lr21* (C) and *Lr24* (D). M= DNA Ladder (DNA Marker), P=Positive, Lane 1= Giza-171 and Lane 2= Sids-14.

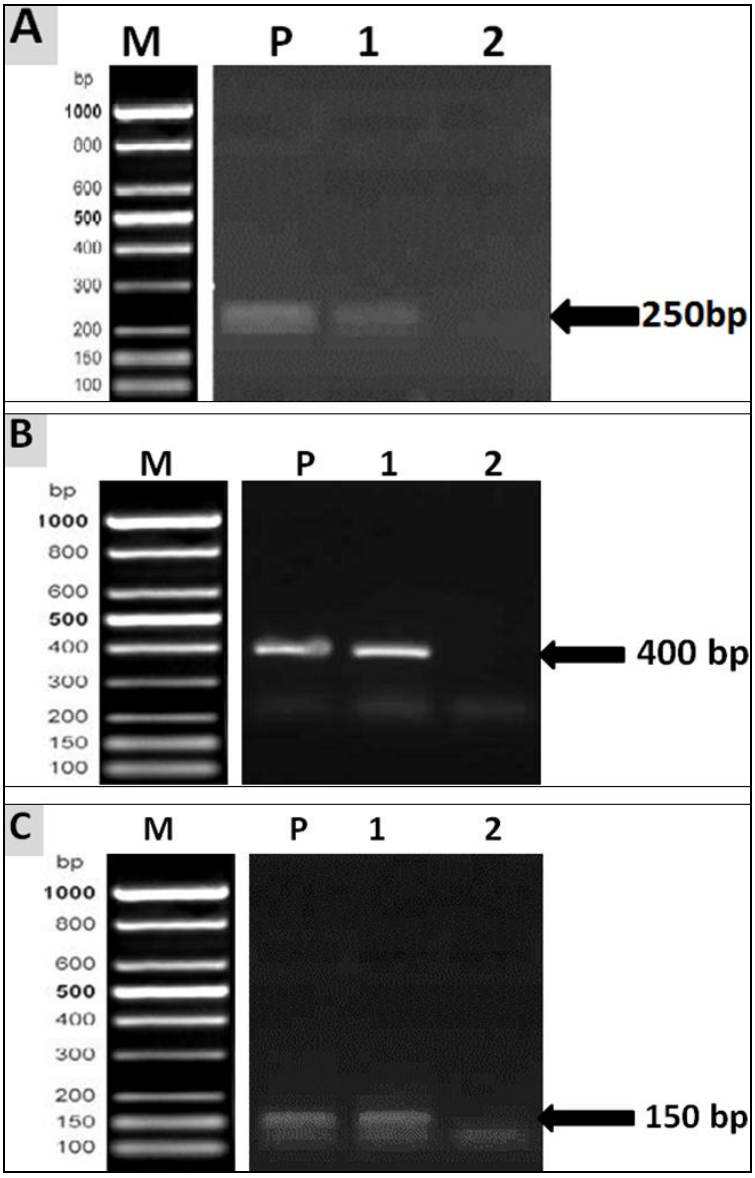

Fig. 2. Electrophoretic amplified pattern of DNA extracted from the two cultivars under study using the Specific primer for *Lr25* (A), *Lr28* (B) and *Lr29* (C). M= DNA Ladder (DNA Marker), P= Positive, Lane 1= Giza-171and Lane 2= Sids-14.

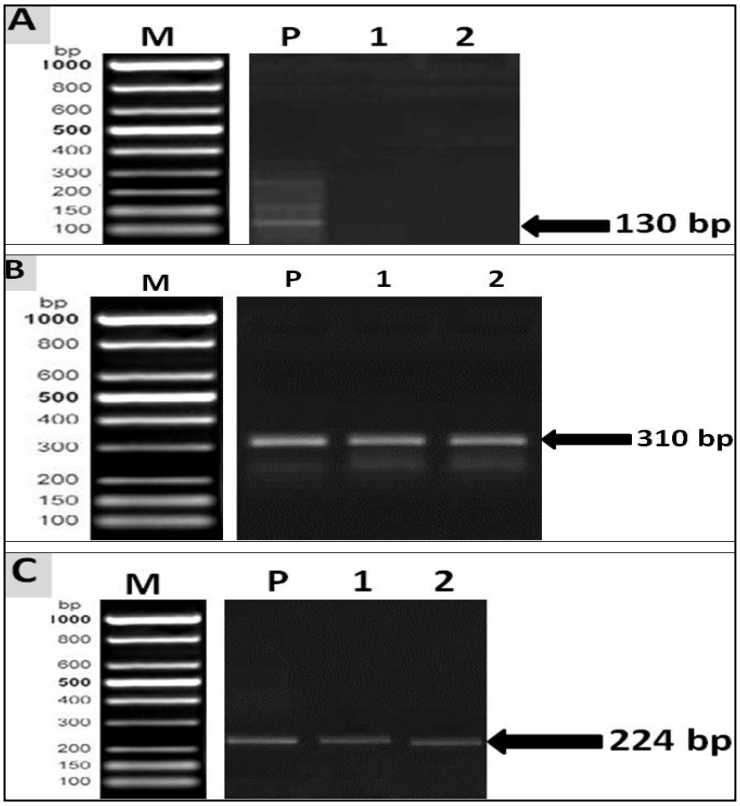

Fig. 3. Electrophoretic amplified pattern of DNA extracted from the two cultivars under study using the specific primer for *Lr34* (A), *Lr46* (B) and *Lr47* (C). M= DNA Ladder (DNA Marker), P=Positive, Lane 1 = Giza 171 and Lane 2 = Sids-14.

Table 9. Leaf rust resistance genes (Lr's) identified in the two new Egyptian wheat cultivars, using the three main methods; gene postulation, genetic analysis and molecular markers.

Wheat cultivar	Gene postulation	Genetic analysis	Molecular markers
Giza-171	*Lr9*, *Lr19*, *Lr21*, *Lr25*, *Lr28 Lr29*, *Lr46* and *Lr47*	*Lr9*, *Lr25*, *Lr28*, *Lr29*, *Lr46* and *Lr47*	*Lr9*, *Lr25*, *Lr28*, *Lr29*, *Lr46* and *Lr47*
Sids-14	*Lr21*, *Lr28*, *Lr29*, *Lr46* and *47*	*Lr29*, *Lr46* and *Lr47*	*Lr29*, *Lr46* and *Lr47*

Conclusion

It could be concluded that the two new bread wheat cultivars; Giza-171 and Sids-14 exhibited high levels of adult plant resistance to leaf rust, under Egyptian field conditions, during the current study. This result was confirmed by the identification of more than one gene responsible for such resistance by using the three certified methods. The two methods; genetic analysis and molecular markers are considered the best ones in this concern. Further studies are needed to identify and designate other new Lr genes in wheat genotypes, to facilitate the use full utilization and incorporation, of these genes into breeding materials. Also, it achieve a wide diversity or high genetic variations in the cultivated wheat varieties, having different effective resistance genes.

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التحليل الجيني والوراثي لجينات المقاومة لمرض صدأ الأوراق في صنفين من الأقماح المصرية الحديثة

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يُعتبر مرض صدأ أوراق القمح المتسبب عن الفطر *Puccinia triticina* f.sp. *tritici* من أكثر أمراض القمح انتشاراً، حيث يصيب معظم أصناف القمح المصرية. تم تعريف الجينات المسئولة عن المقاومة لهذا المرض وذلك باستخدام الثلاثة طرق القياسية الرئيسية المستخدمة في ذلك المجال وهي التوقع الجيني والتحليل الوراثي والمعلمات الجزيئية. وبناءً على ذلك، تم اختيار أفضل الأصناف مقاومة وهي جيزة ١٧١، سدس ١٤، وعشر سلالات نباتية حاملة لجينات فردية مقاومة وهم: *Lr9*، *Lr19*، *Lr21*، *Lr24*، *Lr25*، *Lr28*، *Lr29*، *Lr34*، *Lr46*، *Lr47*، وذلك لتحديد الجينات المسئولة عن المقاومة بهذين الصنفين. وكانت أفضل الطرق لتحديد جينات المقاومة في صنفي القمح تحت الاختبار هما التحليل الوراثي والمعلمات الجزيئية حيث أوضحت نتائج الدراسة وجود ست جينات مقاومة لمرض صدأ الأوراق وهم *Lr9*، *Lr25*، *Lr28*، *Lr29*، *Lr46*، *Lr47* في صنف القمح جيزة ١٧١ وثلاثة جينات مقاومة وهم *Lr29*، *Lr46*، *Lr47* في صنف القمح سدس ١٤. وبالتالي يتميز هذين الصنفين تحت ظروف الدراسة بمنطقة كفر الشيخ بمستوى عال من المقاومة لهذا المرض، مما يتيح استخدام هذين الصنفين كمصادر للمقاومة في برامج التربية المختلفة. ومن ناحية أخرى فإن هذه النتائج قد تساهم في توفير بعض المعلومات عن جينات المقاومة لمرض صدأ الأوراق التي لم يتم تعريفها أوتحديدها حتى الأن، مما يساعد في تضييق الفجوة في ذلك المجال وإلقاء الضوء علي بعض الأهداف المستقبلية للباحثين المهتمين بالاستفادة القصوى من هذه الجينات في برامج التربية للمقاومة.