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Identification of leaf rust resistance genes in Egyptian wheat cultivars by multipathotypes and molecular markers

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Abstract: A total of twenty leaf rust resistance genes (*Lr* genes) were postulated in nine Egyptian wheat cultivars based on infection types (ITs) expressed on the tested cultivars by 72 *Puccinia triticina* pathotypes compared with the ITs expressed on the monogenic lines. The most carrier genes cultivars were Giza168 and Misr1 each may contain five genes *i.e. Lr*2c, 10, 18, 24, 41 and *Lr*3, 10, 19, 22b, 24, respectively. Five cultivars, Sakha94, Gemmeiza9, Gemmeiza10, Sids12 and Misr2 each probably contain four genes *i.e. Lr*9, 19, 29, 37; *Lr*18, 21, 24, 41; *Lr*3, 9, 19, 29; *Lr*9, 19, 26, 29 and *Lr*3, 10, 19, 26, respectively. Gemmeiza11 was the least cultivar carrying genes; it probably carries just two genes *i.e. Lr*24 and *Lr*41. The most postulated genes were *Lr*19 and *Lr*24, each postulated within five cultivars followed by *Lr*41 within four cultivars. Five *Lr* genes, *Lr*3, *Lr*9, *Lr*10, *Lr*26 and *Lr*29 each within three cultivars. The lowest postulated genes were *Lr*2c, *Lr*21, *Lr*22b and *Lr*37 each of them was postulated within only one cultivar. Five *Lr* genes, *Lr*9, *Lr*10, *Lr*19, *Lr*24 and *Lr*26 were identified by PCR-based molecular marker. The *Lr*9 gene was identified in cultivar Sids12 while, *Lr*10 was identified in cultivar Misr1. The *Lr*19 was present in two cultivars, Misr1 and Misr2. The *Lr*24 and *Lr*26 were absent in all the screened Egyptian cultivars. The obtained results for *Lr*9, *Lr*10, *Lr*19, *Lr*24 and *Lr*26 marker were in agreement with and confirm their identification by gene postulation. Markers for *Lr*9, *Lr*10 and *Lr*19 may be useful in marker-assisted breeding. Our findings showed the usefulness of the molecular marker in identifying leaf rust resistance genes in wheat cultivars, especially when used in conjunction with multipathotypes test at the pre-breeding stage. This approach may help understanding the wheat - *P. triticina* interaction and provide information to build an effective management program for leaf rust disease.

Keywords: Leaf Rust, *Puccinia triticina*, Multipathotypes, Gene Postulation, Wheat *Lr*-Genes, *Lr*-Genes Marker, Molecular Analysis

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important nutritive cereal crops in Egypt. The cultivated area of 3.04 million feddan yield 8.8 million ton of wheat grains [1]. Breeding of disease resistance is one of the important methods for increasing wheat production world-wide. Wheat is liable host three rust diseases, stripe, leaf and stem rust. Leaf rust disease is widely distributed of the three wheat rusts and has become more serious diseases [2].

Leaf rust of wheat is caused by the fungus *Puccinia triticina* Eriks. (syn. *P. recondita* Rob. Ex Desm. f. sp. *tritici* Eriks. and Henn.). The fungus primarily attacks the leaf blades, although it can also infect the leaf sheath and glumes in highly susceptible cultivars. Yield losses caused by leaf rust over a large area may reach 20%, while individual fields can be destroyed when the disease is severe prior to heading. Yield losses in wheat from *P. triticina* infections are usually the result of decreased numbers of kernels per head and lower kernel weights [3-6]. In Egypt, yield losses of wheat due to leaf rust infection could reach up to 50% [7]. Leaf rust disease has eliminated many wheat cultivars *i.e.* Giza139, Chenab70, SuperX, Giza158 and Giza160 from the cultivated area in Egypt [8].

Most of the 60 leaf rust resistance genes confer race-specific resistance in a gene-for-gene manner [9]. However, wheat cultivars relying on race-specific resistance often lose effectiveness within a few years by imposing selection for virulent leaf rust races. In addition, the cultivation of a large area of susceptible wheat cultivars allows a very large leaf rust population to proliferate, creating a reservoir for mutation and selection [6].

Knowledge of the identity of the leaf rust resistance genes in wheat cultivars is essential for incorporation of new effective genes for resistance into high yielding wheat genotypes through breeding programs and maintenance of genetic diversity for resistance. Recently, the use of molecular marker technique in identifying leaf rust resistance genes have increased attention as a promising useful and faster tool than the traditional methods ones. Molecular marker technique is very useful to determine exactly which resistance genes are present in Egyptian wheat cultivars. It is an important method and useful in marker-assisted breeding and has become critical to continue the development of improved wheat cultivars, especially when used in conjunction with multi-pathotypes testing with leaf rust isolates at the pre-breeding stage [10]. The present work aimed to identify leaf rust resistance genes in Egyptian wheat cultivars using multipathotypes and PCR-based molecular marker techniques.

2. Materials and Methods

2.1. Multipathotypes Test

Multipathotypes test of *P. triticina* isolates on wheat (also called gene postulation) was used to postulate leaf rust resistance genes in Egyptian wheat cultivars according to the method of Browder & Eversmeyer [11] and Statler [12]. A total of nine Egyptian wheat cultivars *i.e.* Sakha94, Giza168, Gemmiza9, Gemmiza10, Gemmiza11, Sids12, Sids13, Misr1 and Misr2 and 20 monogenic lines carrying leaf rust resistance genes *i.e. Lr*2c, *Lr*3, *Lr*9, *Lr*10, *Lr*18, *Lr*19, *Lr*21, *Lr*22a, *Lr*22b, *Lr*24, *Lr*26, *Lr*27, *Lr*29, *Lr*30, *Lr*32, *Lr*34, *Lr*37, *Lr*41, *Lr*42 and *Lr*44 were used and inoculated at seedling stage using 72 pathotypes (physiologic races) of *Puccinia triticina* obtained from Egypt during 2010 - 2012.

All plant materials (five grains per entry) were grown in plastic pots (10 cm. diam.). Each pot contained four entries clockwise in each corner. Inoculation was carried out as described by Stakman *et al*. [13], in which the 8-days-old seedling leaves were rubbed gently between moisted fingers with tap water, sprayed in the incubation chambers with water, then inoculated by shaking or brushing collected urediniospores over the plant leaves then re-sprayed gently with water in order to induce thin film of free water on the plants which is essential for spore germination and establishment of infection. The inoculated plants were then incubated in a dark dew chamber overnight at 18ºC and 95% relative humidity to allow the rust spores to germinate and cause infection. The inoculated plants were then moved to the benches in the greenhouse and maintained at 19-22ºC and 95-100% relative humidity and kept under observation until the rust pustules are developed. Light intensity was supplied at about 7600 lux in a photoperiod of 16 hours light and 8 hours dark [14]. Infection types data against the obtained races were scored two weeks after inoculation. The infection types 0, 0;, 1, 2, 3, 4, and X (Table 1) were the same as described by Johnston & Browder [15]. Infection types 0, 0;, 1 and 2 reflect resistance reaction, while infection types 3, 4 and X reflect susceptibility. These data were transformed into low infection type (0, 0;, 1 and 2) and high infection type (3, 4 and X).

Table 1. *Infection types of wheat leaf rust used in disease assessment*

Infection type		Host response	Symptoms
Resistance (LIT)	0	Immune	No uredia or other macroscopic sign of infection
	0;	Nearly immune	No uredia, but hypersensitive necrotic or chlorotic flecks present
	1	Very resistant	Small uredia surrounded by necrosis
	2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
Susceptibility (HIT)	3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
	4	Very susceptible	Large uredia without chlorosis or necrosis
Mesothetic (HIT)	X	Heterogeneous	Random distribution of variable-sized uredia on single leaf

HIT High infection type, LIT Low infection type

The tested leaf rust resistance genes were postulated in the tested wheat cultivars based on infection types (ITs) expressed on the wheat cultivars by 72 *P. triticina* pathotypes compared with the ITs expressed on monogenic lines. The HIT: LIT and LIT: HIT are most critical to determining postulated resistance genes. The four categories were based on whether or not each of these infection types occurred. For each pair of hosts, the infection types expressed were classified into categories according to the following scheme: Category (0), absence of LIT: HIT reactions indicating that host B has the same gene (s) as in host A , however host B may have additional resistance genes. Category (-), no HIT: LIT reactions but some LIT: HIT reactions indicating that host B dose not contain the resistance gene in host A. Category (-0), no HIT: LIT reactions and no LIT: HIT reactions indicating that both hosts carry the same resistance genes at least for resistance to the used races. Category (+), some HIT: LIT reactions and some LIT: HIT reactions indicating that the hosts do not carry the same resistance gene(s).

2.2. Molecular Approach

The objective of this experiment was to detect leaf rust resistance genes in Egyptian wheat cultivars using DNA marker technique to be compared with multipathotypes test.

2.2.1. Plant Material

Nine Egyptian wheat cultivars *i.e.* Sakha94, Giza168, Gemmiza9, Gemmiza10, Gemmiza11, Sids12, Sids13, Misr1 and Misr2 as well as five leaf rust resistance genes *i.e. Lr9*, *Lr10*, *Lr19*, *Lr24* and *Lr26* were tested by PCR-based DNA marker using specific primers (Table 2). The susceptible cultivar Gemmiza7 was supplemented as a check control.

2.2.2. Extraction of Genomic DNA

The total genomic DNA was extracted from 7-day-old seedling leaves by a modified cetyl trimethyl ammonium bromide (CTAB) extraction method according to Doohan *et al.* [16]. Briefly, 0.25 g of fresh leaves was harvested then 1 ml of CTAB buffer (2% CTAB, 100 Mm EDTA and 1.4 M NaCl) was added to the detached leaves and grinded with a pestle then samples were collected to an Eppendorf tube. The samples were vortexes and incubated at 60ºC for 30 min. then supplemented by cold chloroform-isoamyl alcohol (24,1/vol.) mixture, shaken vigorously and centrifuged by Hermle, 2230M, BHG at 3622 g for 15 min. After centrifugation, the supernatant was transferred to a new Eppendorf tube and supplemented with cold isopropanol, mixed and left in the refrigerator at –20ºC for 30 min. to precipitate the DNA. Samples were centrifuged at 8765 g for 15 min., the supernatant was removed and the precipitated DNA was washed carefully with 0.5 ml of cold ethanol (70%) and air dried then dissolved again in 20 µl of TE buffer (10Mm Tris-HCL, pH 8.0 and 1 mM EDTA). Purity and quantity of DNA were determined via UV spectrophotometer measurement (Pharmacia, Biotech., Ultrospec 1000). The DNA samples were diluted for final concentration of 10 ng/µl and stored at -20°C until use.

2.2.3. PCR Amplification and Gel Electrophoresis

Five different DNA markers for leaf rust resistance genes *i.e. Lr*9, *Lr*10, *Lr*19, *Lr*24, and *Lr*26 were used by specific primer each (Table 2). Polymerase chain reaction Master Mix (Dream Taq Green PCR Master Mix (2X), Thermo Scientific) was used for PCR reaction, containing all PCR reagents (dNTPs 0.4 mM each, 2X Taq DNA polymerase and 4 mM $MgCl_2$) except primers and DNA template. A total volume of 25 µl PCR reaction contained 12.5µl of Master Mix., 0.8µl of forward/reverse primers (Table 2), 2µl of DNA template and 9.7 µl sterile distilled water. The PCR conditions (Techne, PROGENE Thermocycler) for all primers sets were optimized in initial studies (Table 2).

Amplification products of PCR (10 µl each sample) were electrophoreses at 100 V for about 20 min. in 1.5% agarose gel stained with ethidium bromide. The DNA ladder (100 bp DNA ladder H3 RTU, Nippon Genetics Europe GmbH) was used (5 µl) to determine the molecular size of the DNA bands. The DNA patterns were visualized using UV-transilluminator (Herolab UVT 2020, Kurzwellig) and photographed.

Table 2. *Sequences of primers, PCR conditions and references for Lr marker used to identify leaf rust resistance genes in wheat cultivars*

Lr	Sequence of primer 5¯to 3¯	PCR programme	Reference
*Lr*9	fwd, TCC TTT TAT TCC GCA CGC CGG rev, CCA CAC TAC CCC AAAGAG ACG	94°C 6 min, 45 cycles (92°C 1 min, 62°C 1 min, 72°C 2 min), 72°C 4 min.	[17]
*Lr*10	fwd, GTG TAA TGC ATG CAG GTT CC rev, AGG TGT GAG TGA GTT ATG TT	94°C 3 min., 35 cycles (94°C 45 s, 57°C 45 s, 72°C 30 s), 72°C 3 min.	[18]
*Lr*19	fwd, CAT CCT TGG GGA CCT C rev, CCA GCT CGC ATA CAT CCA	94°C 5 min, 30 cycles (94°C 1.30 min, 55°C 2 min, 72°C 1.30 min), 72°C 5 min.	[19]
*Lr*24	fwd, TCT AGT CTG TAC ATG GGG GC rev, TGG CAC ATG AAC TCC ATA CG	94°C 4 min, 40 cycles (92°C 1 min, 60°C 1 min, 72°C 2 min), 72°C 5 min.	[20]
*Lr*26	fwd, CAT CCT TGG GGA CCT C rev, CCA GCT CGC ATA CAT CCA	94°C 2 min, 35 cycles (94°C 30 s, 63°C 1 min, 72°C 2 min), 72°C 5min.	[10]

3. Results

3.1. Multipathotypes Test

Twenty genes for leaf rust resistance were postulated in nine resistant Egyptian wheat cultivars using 20 wheat lines each carrying single gene for leaf rust resistance. Data in Table (3) revealed the results of matching between 9 wheat cultivars and 20 monogenic lines based on infection types produced by the inoculation with 72 pathotypes of *P. triticina* fungus. For the validity of the comparison, the completely virulent or avirulent isolates were omitted. Like wise, the completely resistant or susceptible cultivars and monogenic lines were also omitted [11, 12]. These data clarified that among 20 *Lr* genes, thirteen *Lr* genes, *Lr2c, Lr3, Lr9, Lr10, Lr18, Lr19, Lr21, Lr22b, Lr24, Lr26, Lr29, Lr37* and *Lr41* probably present within cultivars, Sakha94, Giza168, Gemmiza9, Gemmiza10, Gemmiza11, Sids12, Sids13, Misr1 and Misr2 which may have additional genes as indicated by the symbol (0). Most of the tested cultivars probably included genes other than those tested in the comparison (+). Finally, each of *Lr24* and *Lr41* may be present within Gemmiza11 in respect (-0).

As revealed from data in Table (3), the detail of postulated genes included within 9 Egyptian wheat cultivars. The obtained results showed that the most carrier genes cultivars were Giza168 and Misr1 each may containing five genes *i.e. Lr2c, 10, 18, 24, 41* and *Lr3, 10, 19, 22b, 24,* respectively and may have additional genes. Five cultivars, Sakha94, Gemmeiza9, Gemmeiza10, Sids12 and Misr2 each probably

containing four genes *i.e. Lr9, 19, 29, 37*; *Lr18, 21, 24, 41*; *Lr3, 9, 19, 29*; *Lr9, 19, 26, 29* and *Lr3, 10 , 19, 26*, respectively and may have additional genes. The least carrier genes cultivars was Gemmeiza11, it probably carries just two genes *i.e. Lr24* and *Lr41* followed by Sids13 probably had three genes *i.e. Lr24*, *26* and *41* and may have additional genes.

Table 3. *Incidence of LIT – HIT comparisons of 20 leaf rust resistance genes and 9 Egyptian wheat cultivars produced by 72 P. triticina isolates*

***Lr* gene**	**Wheat cultivar**									**% Gene frequency**
	Sakha 94	**Giza 168**	**Gemmiza9**	**Gemmiza10**	**Gemmiza11**	**Sids 12**	**Sids 13**	**Misr 1**	**Misr 2**	
Lr2c	+	0	+	+	+	+	+	+	+	11.11
Lr3	+	+	+	0	+	+	+	0	0	33.33
Lr9	0	+	+	0	+	0	+	+	+	33.33
Lr10	+	0	+	+	+	+	+	0	0	33.33
Lr18	+	0	0	+	+	+	+	+	+	22.22
Lr19	0	+	+	0	+	0	+	0	0	55.56
Lr21	+	+	0	+	+	+	+	+	+	11.11
Lr22a	+	+	+	+	+	+	+	+	+	00.00
Lr22b	+	+	+	+	+	+	+	0	+	11.11
Lr24	+	0	0	+	-0	+	0	0	+	55.56
Lr26	+	+	+	+	+	0	0	+	0	33.33
Lr27	+	+	+	+	+	+	+	+	+	00.00
Lr29	0	+	+	0	+	0	+	+	+	33.33
Lr30	+	+	+	+	+	+	+	+	+	00.00
Lr32	+	+	+	+	+	+	+	+	+	00.00
Lr34	+	+	+	+	+	+	+	+	+	00.00
Lr37	0	+	+	+	+	+	+	+	+	11.11
Lr41	+	0	0	+	-0	+	0	+	+	44.44
Lr42	+	+	+	+	+	+	+	+	+	00.00
Lr44	+	+	+	+	+	+	+	+	+	00.00

LIT - HIT Low infection type - High infection type, 0 wheat cultivar has the same gene as in *Lr* gene however wheat cultivar may have additional resistance genes, -0 both hosts carry the same genes at least for resistance to the tested cultures, + the hosts do not carry the same resistance gene.

Also, data in Table (3) revealed the representation of *Lr* genes which were postulated within 9 Egyptian cultivars. These data indicated that *Lr*19 and *Lr*24 were the most postulated genes within all the tested cultivars by 55.56% gene frequency, each postulated within five cultivars *i.e.* Sakha94, Gemmeiza10, Sids12, Misr1**,** Misr2 and Giza168, Gemmiza9, Gemmiza11, Sids13, Misr2, respectively, followed by *Lr*41 giving frequency 44.44% within four cultivars *i.e.* Giza168, Gemmiza9, Gemmiza11 and Sids13. Five *Lr* genes, *Lr*3, *Lr*9, *Lr*10, *Lr*26 and *Lr*29, each giving 33.33% frequencies within three cultivars *i.e.* Gemmiza10, Misr1, Misr2; Sakha94, Gemmiza10, Sids12; Giza168, Misr1, Misr2; Sids12, Sids13, Misr2 and Sakha94, Gemmeiza10, Sids12, respectively. The lowest postulated genes were *Lr*2c, *Lr*21, *Lr*22b and *Lr*37, each postulated within only one cultivar *i.e.* Giza168, Gemmeiza9, Misr1 and Sakha94, respectively, giving frequency 11.11% followed by *Lr*18 (22.22%) within two cultivars *i.e.* Giza168 and Gemmeiza9. Seven *Lr* genes, *22a, 27, 30, 32, 34, 42* and *44* were absent within wheat cultivars tested.

3.2. Molecular Marker

Five amplification fragments corresponding to DNA markers of leaf rust resistance genes *i.e. Lr10*, *Lr19*, *Lr24*, *Lr9* and *Lr26* were amplified from DNA of nine Egyptian wheat cultivars *i.e.* Sakha94, Giza168, Gemmiza9, Gemmiza10, Gemmiza11, Sids12, Sids13, Misr1 and Misr2. The *Lr9* marker, with a DNA fragment of 1100 bp, was identified in wheat cultivar Sids12 (Fig. 1 and Table 4). However, the marker for *Lr10* was identified in the cultivar Misr1 as a fragment of 310 bp (Fig. 2 and Table 4). The amplification product of the *Lr19* marker was 130 bp and was present in two wheat cultivars *i.e.* Misr1 and Misr2 (Fig. 3 and Table 4). Markers for *Lr24* and *Lr26* were absent in all the tested cultivars (Table 4). All the five tested resistance genes were absent in the susceptible cultivar Gemmiza7.

Also, analysis of the data in Table (4) proved that the obtained results for *Lr*9, *Lr*10, *Lr*19, *Lr*24 and *Lr*26 markers have confirmed their identification by gene postulation. Among 50 reactions between postulation test and molecular technique, 35 reactions were in agreement.

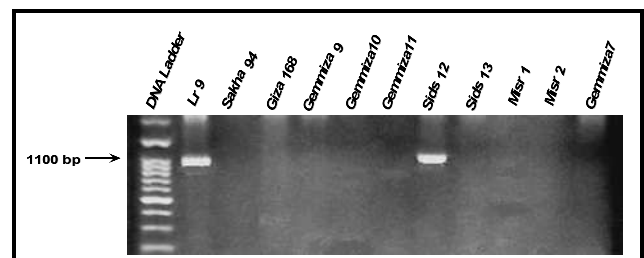

Figure 1. *Polymerase chain reaction of Lr9 marker (1100 bp) in certain Egyptian wheat cultivars.*

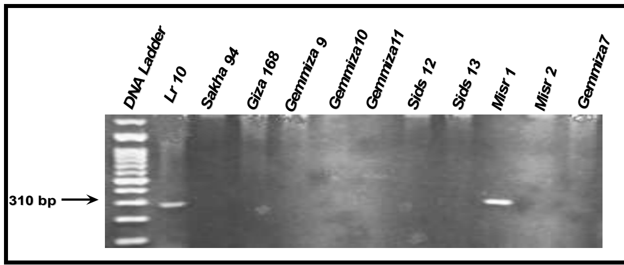

Figure 2. *Polymerase chain reaction of Lr10 marker (310 bp) in certain Egyptian wheat cultivars.*

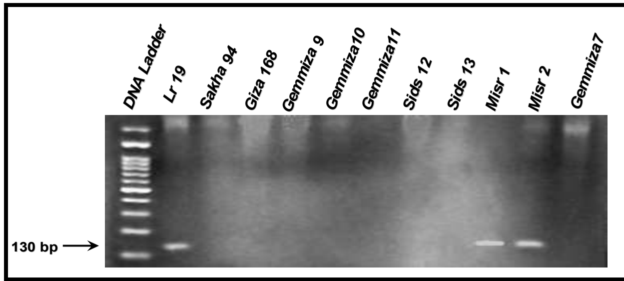

Figure 3. *Polymerase chain reaction of Lr19 marker (130 bp) in certain Egyptian wheat cultivars.*

Table 4. *Identification of five leaf rust resistance genes in Egyptian wheat cultivars by molecular marker as compared with their gene postulation*

Cultivar	Leaf rust resistance genes									
	***Lr*9**		***Lr*10**		***Lr*19**		***Lr*24**		***Lr*26**	
	P	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**
Sakha94	+	–	–	–	+	–	–	–	–	–
Giza168	–	–	+	–	–	–	+	–	–	–
Gemmiza9	–	–	–	–	–	–	+	–	–	–
Gemmiza10	+	–	–	–	+	–	–	–	–	–
Gemmiza11	–	–	–	–	–	–	+	–	–	–
Sids12	+	+	–	–	+	–	–	–	+	–
Sids13	–	–	–	–	–	–	+	–	+	–
Misr1	–	–	+	+	+	+	+	–	–	–
Misr2	–	–	+	–	+	+	–	–	+	–
Gemmiza7		–		–		–		–		–

P Postulation test, M Molecular marker, + Present, – Absent, Blank Excluded.

4. Discussion

Leaf rust disease of wheat incited by the fungus *Puccinia triticina* Eriks., is one of the most common rust diseases of wheat, causing a significant loss in grain yield up to 50% in Egypt [7]. Knowledge of the identity of the leaf rust resistance genes in wheat cultivars is essential for incorporation of new effective genes for resistance into high yielding wheat genotypes through breeding programs and maintenance of genetic diversity for resistance. Identification of leaf rust resistance genes is very useful to determine which resistance genes are present in Egyptian wheat cultivars. Two methods were used to identify the leaf rust resistance gene(s) in the wheat cultivars. These methods are multipathotypes testing with leaf rust isolates (gene postulation) and molecular marker technique**.**

Gene postulation applies the principles of gene-for-gene specificity to hypothesize which *Lr* genes probably are present in host plant. The main advantage of gene postulation is to determine the probable *Lr* genes within few weeks using the primary leaves of seedling plants. Large numbers of cultivars and breeding lines can thus be evaluated in a relatively short period of time [21-23].

Twenty genes for leaf rust resistance genes were postulated within nine Egyptian wheat cultivars *i.e.* Sakha94, Giza168, Gemmiza9, Gemmiza10, Gemmiza11, Sids12, Sids13, Misr1 and Misr2 based on infection types (ITs) produced on the cultivars by 72 *P. triticina* pathotypes compared with the ITs produced on monogenic lines. Such cultivars have high efficacy against the used races at seedling stage and exhibited resistance response against leaf rust at adult plant stage. The results indicated that *Lr*19 and *Lr*24 proved to be the most postulated genes (55.56% each) each postulated within five cultivars, followed by *Lr*41 giving frequency 44.44% within four cultivars. Five *Lr* genes (*Lr*3, *Lr*9, *Lr*10, *Lr*26 and *Lr*29) each giving 33.33% frequencies within three cultivars. The method was previously reported as a quick method to serve breeding for rust resistance [11, 12, 24-30].

Concerning the situation of the tested Egyptian wheat cultivars, the obtained results gave evidence to the probability of the presence of 13 *Lr* genes, *Lr2c, Lr3, Lr9, Lr10, Lr18, Lr19, Lr21, Lr22b, Lr24, Lr26, Lr29, Lr37* and *Lr41* within the tested Egyptian wheat cultivars. The obtained results showed that the most carrier genes cultivars were Giza168 and Misr1 each may contain five genes. Five cultivars (Sakha94, Gemmeiza9, Gemmeiza10, Sids12 and Misr2) each probably containing four genes however, Gemmeiza11 and Sids13 were the least carrier genes cultivars they probably carries just two and three genes, respectively. These results were in accordance to those previously reported [24-31].

It could be concluded from these results that a genetic approximation is found between the tested cultivars, since most of the *Lr* genes could be detected in most of the tested cultivars proved to have more *Lr* genes. These *Lr* genes, plus the probably postulated one gave evidence that most of the tested cultivars are considered superior and excellent as resistant wheat materials against leaf rust [32-35].

The molecular markers are fast and accurate techniques to detect the leaf rust resistance genes which are present in Egyptian wheat cultivars. The results proved that three genes *i.e. Lr*9, *Lr*10, *Lr*19 were present in certain tested cultivars *i.e.* Misr1, Misr2 and Sids12. The *Lr*9 was present in Sids12 while, *Lr*10 was present in Misr1. The *Lr*19 were present in two cultivars *i.e.* Misr1 and Misr2, while *Lr*24 and *Lr*26 were absent in all tested cultivars. It could be concluded that the obtained results for *Lr*9, *Lr*10, *Lr*19, *Lr*24 and *Lr*26 marker have confirmed their identification by gene postulation and showed that markers for these genes may be useful in marker-assisted breeding for developing Egyptian wheat cultivars. These results were supported by the previously findings [10-36]. Further molecular studies on the expression profile of these resistance genes are needed.

Our findings show the usefulness of the molecular marker technique in identifying leaf rust resistance genes in wheat

cultivars, especially when used in conjunction with multipathotypes testing with leaf rust races (gene postulation) at the pre-breeding stage. However, exceptions to previous results of gene postulation tests were obtained in the present study, demonstrating the need for caution when interpreting the results of each approach which has become critical to continue the development of improved wheat cultivars. Combination of multipathotypes and molecular markers will facilitate the screening of *Lr* genes in wheat cultivars and would allow improving the breeding program.

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