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Molecular Markers and Postulation Study of Leaf Rust Resistance Genes in Various Egyptian Wheat Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current investigation was carried out at seedling stage under greenhouse condition to: (1) postulate leaf rust resistance genes in ten Egyptian spring wheat cultivars at seedling stage and (2) Verify the presence of resistance genes in the selected cultivars using specific molecular markers linked to leaf rust resistance genes. The ten Egyptian spring wheat cultivars i.e. Misr 1, Misr 2, Sids 12, Sids 13, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sakha 93, Sakha 94 and Giza 168 as well as eight monogenic lines carrying single gene for leaf rust resistance i.e. *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47, and *Lr* 51 were tested at seedling stage using 15 leaf rust pathotypes i.e. BDGGK, CPPTB, DMKRT, FKRNK, FTFNB, KTSQG, LDDLD, LFGLL, NRRLS, NTTSR, PTKLN, STRTQ, TJKPR, TQTMJ and TTJBC. Eight specific molecular markers were known to be linked with the *Lr*s resistance genes, i.e. *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47, and *Lr* 51 were used to verify the presence of these genes in the tested Egyptian wheat cultivars. The postulation test

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theorized the presence of *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37 and *Lr* 47 in Sids 12. Moreover, the wheat cultivar Misr 1 may have four genes i.e. *Lr* 25, *Lr* 35, *Lr* 39 and *Lr* 51. The wheat cultivars Misr 2, Sids 13, Sakha 94 and Giza 168 may have two genes i.e. *Lr* 35 and *Lr* 39 in the wheat cultivar Misr 2 and the same two genes *Lr* 35 and *Lr* 37 in the three wheat cultivars Sids 13, Sakha 94 and Giza 168. On the other hand, the wheat cultivars Gemmeiza 9 and Sakha 93 may have only one gene i.e. *Lr* 35 and *Lr* 32, respectively. While, the wheat cultivars Gemmeiza 10 and Gemmeiza 11 did not have any of the tested *Lr* genes under study using the fifteen tested pathotypes of *P. triticina* but it may be carrying some other genes. In the same way, all of the tested wheat genotypes may also carries additional resistant gene (s).The molecular markers survey with specific markers revealed that, the specific markers for *Lr* 21 and *Lr* 47 were detected in all tested cultivars. The marker for *Lr* 32 was recognized in four cultivars i.e. Gemmeiza10, Sakha 93 Sakha 94 and Giza168. The marker for *Lr* 39 was identified in all cultivars except Sakha 94. The markers for *Lr* 25, *Lr* 35, *Lr* 37 and *Lr* 51 were not found in any the tested cultivars. This study may add valuable information about resistance genes in the Egyptian wheat cultivars which may be used in pyramiding more genes in each cultivar or used as donor parents in different wheat genetic improving programs.

Keywords: Leaf rust; molecular markers; Egyptian wheat cultivars; postulation study.

1. INTRODUCTION

Wheat is one of the most widely cultivated cereal crops in the world. It belongs to the genus *Triticum*. This genus contains 27 wild and domesticated species [1]. Wheat is one of the most important cereals grown successfully in Egypt. Täckholm *et al*. [2] and Boulos [3] mentioned that five species of *Triticum* were distinguished in Egypt, namely: *Triticum aestivum*, *Triticum dicoccum, Triticum turgidum*, *Triticum durum* and *Triticum pyramidale*. Dorofeev et al. [1] mentioned that *Triticum turanicum* is also distributed in Egypt.

Wheat leaf rust disease caused by *Puccinia triticina* Eriks. is considered to be one of the common and oldest diseases of wheat (*Triticum aestivum* L.) in Egypt and worldwide and it causes a significant yield reduction [4,5,6]. Most of the cultivated wheat varieties in many field across Egypt, are effected by leaf rust causing loss in yield reaching up to 23% among the susceptible wheat cultivars under the favorable environmental conditions for the fungus, mainly in the northern parts of the Delta region [6,7]. The seriousness of the disease is due to its ability of reducing of the wheat production as a result of the early death of infected leaves and the acquisition of fungus on nutrients, and the infection can lead to shrinkage of grains. It is considered as the most dangerous disease of wheat, attacking leaves and causing high and destructive loss of yield. Yield of susceptible leaf rust cultivars of wheat may drop of up to 15% or higher [8,9]. In Egypt, yield damage of wheat due to leaf rust infection could be reach up to 50% [10,11]. Leaf rust has excluded many wheat cultivars from being grown in Egypt commercially such as Giza 139, Chenab 70, Super X, Giza 158 and Giza 160 [7]. Plant breeders used a race specific resistance genes or major genes in their breeding programs and also looking for more durable type of resistance. Using resistant cultivars enriched with major and minor genes is the most preferable method among plant breeders because of its low cost in comparisons of other methods advised to control leaf rust disease [12].

More than one hundred leaf rust resistance genes have been identified in wheat, but most of them are not effective against the existing races of *P. triticina* [13]. The frequently appearance of virulent pathotypes can limit the stability and use of rust resistance genes. Novel sources of resistance are needed to be detected and identified [14].

DNA markers known to be linked with appropriate resistance genes are effective approach and are routinely used by plant breeders and pathologist for the identification of new rust genes. A number of DNA markers linked to many leaf rust resistance genes in wheat have been analyzed and developed. Currently, characterized specific genetic markers for leaf rust resistance genes (*Lr* genes) offer an appreciated tool as a marker assisted selection (MAS) and are widely adopted in many plant breeding programs for pyramiding many important resistance genes in the elite and high yielding wheat cultivars [15,16]. Moreover, several resistance genes can be tracked in plant selection materials simultaneously by analyzing for the presence of multiple molecular DNA

markers and these MAS markers can be used at both seedling and adult stages of growth. The study of the genetic basis of plant resistance, the search for effective genes and their introduction into the culture of wheat significantly could be the effective strategy for preventing the distribution of leaf rust disease and ensure the sustainability of the grain yield capacity [17]. The objectives of this research work are to: (1) Postulate leaf rust resistance genes in ten Egyptian wheat cultivars at seedling stage and (2) Verify the presence of resistance genes in the selected cultivars using specific molecular markers known to be linked to the tested leaf rust resistant genes.

2. MATERIALS AND METHODS

Ten commercial Egyptian wheat cultivars as well as eight wheat leaf rust monogenic lines were used in this investigation to study their resistance against leaf rust disease (Table 1).

2.1 Seedling Test

Evaluation of the tested wheat genotypes against leaf rust and postulation of leaf rust resistance genes were carried out at seedling stage in the greenhouse of Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt during 2016/2017 growing season.

2.2 Plant Materials and *Puccinia triticina* Pathotypes

Seeds of ten wheat cultivars i.e. Misr 1, Misr 2, Sids 12, Sids 13, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sakha 93, Sakha 94 and Giza 168 and eight wheat leaf rust monogenic lines i.e. *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47 and *Lr* 51 (Table 1) were sown in 6 cm square plastic pots. Five seeds of each of the tested wheat materials were sown singly in each corner in clockwise order. Seven days old seedlings of the tested wheat genotypes, when first leaf full emerged, were inoculated with each of the 15 identified pathotypes kindly provided by the Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt. These pathotypes were i.e. BDGGK, CPPTB, DMKRT, FKRNK, FTFNB, KTSQG, LDDLD, LFGLL, NRRLS, NTTSR, PTKLN, STRTQ, TJKPR, TQTMJ and TTJBC. The method of inoculation was carried out as described by Stakman et al. [18], in which wheat seedling leaves (7 days old) were rubbed gently between misted fingers with tap water, sprayed with water then inoculated by shaking or brushing with each of the 15 tested pathotypes over the plant leaves and sprayed gently again with water in order to form initial film of free water on the plants which is essential for spore germination and establishment of infection. The inoculated seedlings were incubated in humid chambers for 24 hours to allow spore germination and cause infection. The inoculated plants were moved onto the benches in the greenhouse where daily temperature varied between 18-20ºC and 100% RH.

2.3 Seedling Disease Assessment

Infection types (IT) data for each tested wheat genotype were recorded 12 days after inoculation using standard infection type scoring scale 0-4 [18]. Monogenic differentials which showed low infection types (scores = 0, 0;, 1, and 2) were considered as resistant or low infection types (LITs). While, those with scores = 3 and 4 were susceptible or high infection types (HITs) [18].

2.4 Postulation of Leaf Rust Resistance Genes

Ten Egyptian wheat cultivars and eight monogenic genotypes carrying single gene for leaf rust resistance were tested at seedling stage using 15 pathotypes of leaf rust i.e. BDGGK, CPPTB, DMKRT, FKRNK, FTFNB, KTSQG, LDDLD, LFGLL, NRRLS, NTTSR, PTKLN, STRTQ, TJKPR, TQTMJ and TTJBC. All plant materials were grown in plastic pots, as previously mentioned. Seedlings were inoculated by urediniospores of the selected identified pathotypes during 2016/17 growing season. The inoculated seedlings were incubated also as previously mentioned and transferred onto benches of the greenhouse. Both inoculation and incubation procedures were done according to the method described by Tervet and Cassel [19]. Leaf rust disease infection types (IT) data were recorded for the wheat tested genotypes as mentioned before in disease assessment previously suggested by Stakman et al. [18]. Genes were postulated according to the methods of Browder & Eversmyer [20] and Statler [21].

2.5 Molecular Markers

The specific molecular marker were used to verify the presence of *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47 and *Lr* 51 genes in the ten Egyptian wheat cultivars (Table 1). This part of the investigation was carried out in

Table 1. List of the tested plant materials and their pedigree

Wheat cultivar	Pedigree	Monogenic lines	Pedigree
Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOYO1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S.	*Lr* 21	Karl//TAM200/KS86WGRC2
Misr 2	SKAUZ/BAV92. CMSS96M0361S-1M-010SY-010M-010SY-8M-0Y-0S.	*Lr* 25	Thatcher*6/Rosen (rye)
Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-4SD-1SD-1SD-0SD.	*Lr* 32	Marquis-K/RL5713 (synthetic hexaploid -Tetra Canthatch/RL5497-1 *T. tauschii*)
Sids 13	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	*Lr* 35	Thatcher^{6} × *Triticum speltoides*
Gemmeiza 9	ALD"S"/HUAC"S"//CMH74A.630/SX. GM4583-5GM-1GM-0GM.	*Lr* 37	Thatcher^{6} × VPM1
Gemmeiza 10	MAYA74"S"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/CROW"S". GM5820-3GM-1GM-2GM-0GM.	*Lr* 39	Wild material *Aegilops speltoides*
Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	*Lr* 47	Pavon *8// T7AS-7S # 1S-7S # 1S / ph1b
Sakha 93	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S	*Lr* 51	TAM 107 *4/ TA 870.
Sakha 94	OPATA/RAYON//KAUZ. CMBW90Y3280-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.		
Giza 168	MRL/BUC//Seri. CM93046-8M-0Y-0M-2Y-0B-0GZ		

Table 2. Leaf rust resistance genes specific primer sequences

Lr genes	Primer sequences (5`-3`)	References
Lr 21	**F** 5'- CGC TTT TAC CGA GAT TGG TC -3'	[23]
	R 5'- CCA AAG AGC ATC CAT GGT GT -3'	
Lr 25	F5'- GTG ACC TCA GGC AAT GCA -3'	[24]
	R 5'- GTG ACC TCA GAA CCG ATG -3'	
Lr 32	**F** 5'- ATC GCC ATC TCC TCT ACC A -3'	[25]
	R 5'- GCG AAC CCA TGT GCT AAG T -3'	
Lr 35	**F** 5'- AGA GAG AGT AGA AGA GCT GC -3'	[26]
	R 5'- AGA GAG AGA GCA TCC ACC -3'	
Lr 37	F 5'- AGG GGC TAC TGA CCA AGG CT -3	[27]
	R 5'- TGC AGC TAC AGC AGT ATG TAC ACA AAA -3'	
Lr 39	F 5-L 5'- CCT GCT CTG CCC TAG ATA CG -3'	[28]
	R 5'- ATG TGA ATG TGA TGC ATG CA -3'	
Lr 47	**F** 5' GCT GAT GAC CCT GAC CGG T 3'	[29]
	R 5' TCT TCA TGC CCG GTC GGG T 3'	
Lr 51	F 5'**-** GCA TCA ACA AGA TAT TCG TTA TGA CC-3'	[30]
	R 5'- TGG CTG CTC AGA AAA CTG GAC C -3'	

the laboratory of Agronomy department, University of Nebraska Lincoln, USA during (2014-2015).

2.6 DNA Extraction

A modified method based on the protocol of Dellaporta et al. [22] was conducted for extraction of total genomic DNA. PCR amplification (polymerase chain reaction) was performed in thermocycler (Rocorbett-Research, CG1-96) in 25 uL reaction volume containing: 2.5 uL of genomic DNA, 1 uL of each primer (10 p mol, F and R) and 8 uL MQ H2O [23]. Amplification products were electrophoresed at 100 V/1 h. 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 100 bp-3kbp linear scale (Jena Bioscience) was used as standard marker for molecular weight. The PCR conditions (Techne, PROGENE Thermocycler) for all primers sets were optimized in initial studies (Table 2).

3. RESULTS AND DISCUSSION

Ten wheat cultivars were tested against 15 leaf rust pathotypes at seedling stage during 2016/2017 growing season (Table 3). The wheat cultivars Misr 1 and Misr 2 are the highly resistant cultivars to 14 pathotypes, followed by the wheat cultivars Sids 12 (resistant to 13 pathotypes), Sids 13 (resistant to 12 pathotypes), Gemmeiza 11 (resistant to 11 pathotypes), Giza 168 (resistant to 10 pathotypes), Sakha 93 and Sakha 94 (each was resistant to 9 pathotypes) and Gemmeiza 9 (resistant to 8 pathotypes). Gemmeiza 10 was susceptible to 10 pathotypes, hence more susceptible than the other. The Egyptian wheat breeders through their continuous efforts had released the two cultivars Sakha 93 and Giza 168 which were characterized by their high resistance to stripe rust and high yielding ability [31]. In addition, the wheat cultivar Gemmeiza 9 proved its superiority as compared with the commercial wheat cultivars Sakha 93, Sids1 and Gemmeiza 5 as well as its highly resistance to the three rust diseases [32,33].

3.1 Postulation of Leaf Rust Resistance Genes (*Lr*s)

Low and high infection types displayed by the ten tested wheat genotypes (Table 3) compared with the infection types of eight known *Lr* genes (Table 4) against fifteen identified tested pathotypes of *Puccinia triticina* under greenhouse condition. Data obtained in Tables (3 and 4) were summarized in Table (5) in which different genes could be postulated as follows:

The wheat genotype Sids 12 probably possessed five genes i.e. *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, and *Lr* 47. Moreover the wheat cultivar Misr 1 may have four genes i.e. *Lr* 25, *Lr* 35, *Lr* 39 and *Lr* 51. While, the wheat cultivars Misr 2, Sids 13, Sakha 94 and Giza 168 may have two genes i.e. *Lr* 35 and *Lr* 39 in the wheat cultivar Misr 1 and the same two genes *Lr* 35 and *Lr* 37 in the three wheat cultivars Sids 13, Sakha 94 and Giza 168. On the other hand, the wheat cultivars

Table 3. Infection types of ten Egyptian wheat genotypes against fifteen pathotypes of *Puccinia triticina* during 2016/17 growing season at seedling stage under greenhouse condition

Cultivars	Leaf rust pathotype / Leaf rust infection type*														
	NTTSR	TJKPR	DMKRT	TQTMJ	FTFNB	TTJBC	PTKLN	BDGGK	NRRLS	LDDLD	LFGLL	CPPTB	KTSQG	STRTQ	FKRNK
Gemmeiza 9	H***	H	H	L**	L	H	H	L	H	L	L	H	L	L	L
Gemmeiza 10	H	H	H	H	H	L	H	L	L	H	L	L	H	H	H
Gemmeiza 11	L	L	L	L	L	L	H	L	H	L	H	L	L	H	L
Sakha 93	H	H	L	L	L	L	H	L	H	L	H	H	L	L	L
Sakha 94	H	H	L	L	L	L	H	L	H	L	L	H	L	H	L
Giza 168	H	H	H	L	L	L	H	L	H	L	L	L	L	L	L
Sids 12	L	L	L	L	L	L	H	L	L	L	L	H	L	L	L
Sids 13	H	L	L	L	L	L	H	L	H	L	L	L	L	H	L
Misr 1	L	L	L	L	L	H	L	L	L	L	L	L	L	L	L
Misr 2	L	L	L	L	L	L	L	H	L	L	L	L	L	L	L

** Infection type as described by Stakman et al. (1962) [18].*
*** L= Low infection type (0,0; ,1 and 2).*** H= High infection type (3 and 4).*

Table 4. Infection types of eight monogenic lines (*Lr*s) against fifteen pathotypes of *Puccinia triticina* under greenhouse condition during 2016/17 growing season at seedling stage

Monogenic line (*Lr*s)	Leaf rust pathotypes / Leaf rust infection type*														
	NTTSR	TJKPR	DMKRT	TQTMJ	FTFNB	TTJBC	PTKLN	BDGGK	NRRLS	LDDLD	LFGLL	CPPTB	KTSQG	STRTQ	FKRNK
21	H**	H	L	L	H	L	L	L	L	H	L	H	L	H	H
25	L***	L	H	L	L	H	H	H	H	H	H	L	H	L	H
32	L	L	H	H	H	L	L	L	H	H	H	L	L	H	L
35	L	L	H	H	L	L	H	H	H	H	L	L	L	L	H
37	H	H	L	L	H	L	H	L	H	L	L	H	H	H	H
39	L	L	L	H	H	H	L	L	L	L	L	L	L	H	H
47	H	L	H	L	H	L	H	L	L	H	H	H	L	H	H
51	L	L	L	L	L	H	L	H	H	L	L	L	L	L	L

** Infection type as described by Stakman et al. (1962) [18].*
*** H= High infection type (3 and 4).** L= Low infection type (0,0; ,1 and 2).*

Gemmeiza 9 and Sakha 93 may have only one gene i.e. *Lr* 35 and *Lr* 32, respectively. While, the wheat cultivars Gemmeiza 10 and Gemmeiza 11 did not has any of the tested *Lr* genes under study using the fifteen tested pathotypes of *P. triticina* but it may be carry some additional genes. Moreover, all of the tested wheat genotypes may be carries some additional gene (s).

Identification of leaf rust resistance genes is very useful to determine which resistance genes are present in Egyptian wheat cultivars. Several methods for detection of wheat rust resistance genes is based on the classical method of obtaining population from the crosses between the tested cultivars and lines with known resistance genes and analysis of the F_2 generation. Another preliminary and faster method is a gene postulation test which is based on the comparison of reactions of the tested cultivar to a set of different pathotypes with reactions of lines owning known resistance genes.

Gene postulation applies the principles of gene-for-gene theory to theorize which *Lr* genes possibly have been presented in host plant. The effective advantage of gene postulation test is to predict the probability of the presence of likely *Lr* genes in a short duration or within few weeks using the primary leaves of seedling plants. Using this approach facilitate screening a large numbers of breeding genotypes in a relatively short period of time [34,35]. The development of molecular biology has enabled the detection of resistance genes by molecular markers.

In this study, eight wheat leaf rust monogenic lines i.e. *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47 and *Lr* 51 and their reaction to 15 leaf rust races were used to postulate possible genes in ten wheat cultivars i.e. Misr 1, Misr 2, Sids 12, Sids 13, Gemmeiza 9, Gemmeiza10, Gemmeiza 11, Sakha 93, Sakha 94 and Giza 168 based on the infection types (ITs) produced on the tested wheat cultivars by 15 *P. triticina* pathotypes compared with ITs produced on the tested monogenic lines. The results indicated that *Lr* 35 and *Lr* 37 proved to be the most postulated genes in the tested cultivars which were postulated in seven cultivars and four cultivars, respectively. Sallam et al. [33] found that in their postulated study concerning leaf rust resistance genes in some selected Egyptian wheat cultivars that the wheat cultivars Sakha 95 and Sids 12 may have seven resistance genes. Also, Gemmeiza 10 may have five genes and Misr 1 may have three genes. While, Giza 168, Sids 1, Misr 2 and Shandweel 1 may have two genes. The wheat genotypes Gemmeiza 11 and Gemmeiza 12 may have only single gene. Also, all the tested wheat genotypes may contain additional genes. In contrast, the wheat genotype Sakha 94, Gemmeiza 7, Gemmeiza 9 and Sids 13 did not have any of the tested genes.

The matching between using gene postulation and molecular markers in detection of leaf rust resistance genes in this study (table 5) permitted gene postulation of eight seedling resistance genes these pathotypes and differential lines may not sufficient to postulate and determine the identity of all known resistance genes [36,37,38].

Environmental conditions suitable for spreading out of leaf rust in Egypt is found to be more favorable in the north and west Delta parts. Also, the fungus of leaf rust is mainly varying in the west Delta region and frequently the new emerged pathotypes are often first started there. Thus wheat cultivars known to be of high levels of resistance are recommended for the north and west Delta, while high yielding cultivars with adequate resistance or susceptibility can be specified for southern and middle Egypt because of its dry condition which are not conductive to the development of the leaf rust disease. Breeding programmers aiming to screen wheat genotypes in different parts in Egypt help in following the strategy of site-specific cultivar recommendation procedure. Highly resistant cultivars, such as Giza 168, Sakha 94 and Gemmeiza 9, are recommended for the west and north Delta. Gemmeiza 7 is a high yielding cultivar with slow leaf rusting genes, and is therefore grown in the north and west Delta. Cultivar Sakha 93 is widely adapted to all regions in the country and is moderately susceptible to leaf rust, but tolerant with no or minor losses. It is therefore recommended for all ecological zones. This policy in breeding and screening wheat genotypes for resistance to leaf rust has abridged yield losses to lowest levels.

3.2 Specific Molecular Markers

The specific molecular markers known to be linked to leaf rust resistant genes were used to verify the presence of *Lr* 21, *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37, *Lr* 39, *Lr* 47 and *Lr* 51 genes in the ten Egyptian cultivars (Table 1). This part of the investigation was carried out in the laboratory of Agronomy department, University of Nebraska

Lincoln, USA during (2014-2015). Data presented in Table (6) and figures (1, 2, 3 and 4) illustrate leaf rust resistance genes identified in the used selected cultivars using molecular markers. The polymorphic survey revealed that out of the 10 cultivars, the markers for *Lr* 21 and *Lr* 47 were identified in all the studied Egyptian wheat cultivars at 200 and 500 bp respectively (Table 3). These results were agreed with Abdelbacki *et al*. [39] and Fahmi et al. [40]. Different studies concerning the origin of *Lr* 21 gene showed that *Aegilops tauschi,* the D-genome originator of bread wheat *(Triticum aestivum)* is a valuable source of some leaf rust resistant genes including *Lr* 21 [41]. The *Lr* 21 gene seems to be common in *Aegilops tauschi,* as it has been transmitted from a number of diverse accessions to *T. aestivum.* The results from Huang and Gill [42] showed that *Lr* 21 and *Lr* 40 are allelic. The markers in our study confirmed the presence of this gene (s) in all of the studied wheat cultivars (Fig. 1) at 200 bp. On the other hand the *Lr 47* gene confers resistance to a wide range of leaf rust pathotypes. This gene was known to be transferred from chromosome 7S of *T. speltoides* to chromosome 7A of *T. aestivum.* The used molecular marker *Xabc465* in our study detected the presence of *Lr* 47 gene in all of the studied cultivars (Fig. 4).

The marker for *Lr* 32 was identified in four cultivars i.e. Gemmeiza 10, Sakha 93, Sakha 94 and Giza168 at 270 bp (Table 3 and Fig. 2). *Lr* 32 is a gene transferred from bread wheat ancestor *Aegilops tauschi* to chromosome arm 3DS of *T. aestivum* that confers resistance to leaf rust

Table 5. Leaf rust resistance genes (*Lr*s) probably present in the ten Egyptian wheat genotypes

Genotype	Probable *Lr* gene	*Lr* gene identified by specific marker
Misr 1	25, 35, 39, 51 +?*	21, 39, 47
Misr 2	35, 39 +?	21, 39, 47
Sids 12	25, 32, 35, 37, 47 +?	21, 39, 47
Sids 13	35, 37 +?	21, 39, 47
Gemmeiza 9	35 +?	21, 39, 47
Gemmeiza 10	+?	21, 32, 39, 47
Gemmeiza 11	+?	21, 39, 47,
Sakha 93	32 +?	21, 32, 39, 47,
Sakha 94	35, 37 +?	21, 32, 47,
Giza 168	35, 37 +?	21, 32, 39, 47.

** +? = Means that the concerned genotype may have additional gene (s) that were not detected using the tested Lr genes and isolates of the study.*

Table 6. Presence or absence of leaf rust resistance genes among Egyptian wheat cultivars at seedling stage

Cultivars	Leaf rust resistance genes							
	***Lr* 21**	***Lr* 25**	***Lr* 32**	***Lr* 35**	***Lr* 37**	***Lr* 39**	**Lr 47**	**Lr 51**
Misr 1	+	-	-	-	-	+	+	-
Misr 2	+	-	-	-	-	+	+	-
Sids 12	+	-	-	-	-	+	+	-
Sids 13	+	-	-	-	-	+	+	-
Gemmeiza 9	+	-	-	-	-	+	+	-
Gemmeiza 10	+	-	+	-	-	+	+	-
Gemmeiza 11	+	-	-	-	-	+	+	-
Sakha 93	+	-	+	-	-	+	+	-
Sakha 94	+	-	+	-	-	-	+	-
Giza 168	+	-	+	-	-	+	+	-
Gene frequency (%)	100	0	40	0	0	90	100	0

(+) = presence of Lr gene in wheat cultivars and (-) = absence of Lr gene in wheat cultivars.

at seedling stage [43,44]. The accession of *A. tauschi* in which *Lr* 32 was originate was RL5497-1 (2n = 14 = DD), which was transferred by crossing with the tetraploid genotype Tetra Canthatch (2n = 28 = AABB) to obtain the hexaploid synthetic line RL571 3. Leaf rust resistant gene *Lr* 32 was then transferred from RL5713 to the genotype RL6086 which had a pedigree of (Thatcher*7//RL5713/Marquis K) and to the line BW196 (Katepwa*6//RL5713/2*MarquisK). Basnet et al. [45] in their study showed that Marquis-Thatcher germplasm from North America has the highest effect on overall Egyptian cultivars. A study of the fragments amplified in wheats of various origin exhibited that each of wmc43 and barc135 were served good as markers for detecting *Lr* 32 with other largely effective leaf rust resistance genes. While, the marker for *Lr* 39 was identified in all of the used cultivars at 300 bp except the cultivar Sakha 94 (Table 3 and Fig. 3). *Lr* 25, *Lr* 35, *Lr* 37 and *Lr* 51 did not show the presence in any of the cultivars (Table 3) using the selected markers. Presence of resistance genes for leaf rust previously investigated in commercial Egyptian cultivars using specific markers, result showed that *Lr* 10 and *Lr* 19 were identified in Misr 1, while Misr 2 was identified for the presence of *Lr* 19 only and *Lr* 9 was identified in Sids12 [46]. Gemmeiza 9 and Gemmeiza 10 screened by specific molecular marker gave positive results to many leaf rust genes such as *Lr* 13, *Lr* 19, *Lr* 24, *Lr* 26, *Lr* 34, *Lr* 35, *Lr* 36, *Lr* 37and *Lr* 39 [47]. El-Orabey and Nagaty [48] found that gene *Lr* 9 was found in the wheat varieties Giza 162, Giza 163, Gemmeiza 9 and Sakha 8 using specific primers for *Lr* 9, 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.

In Egypt breeding efforts for high yielding wheat have been made to release important bread wheat cultivars, i.e. Gemmeiza 10 [49], Sakha 94 [50], Sids 12 [51] and Sids 13. When following up grain yield of the released cultivars over the past 50 years we found that the new cultivars always yielded more than the old ones. This genetic yield increase amounted 211% with 4.2% increase per year during the 50 years period since 1950 till the year of 2000 [52]. Wheat yield have increased gradually over the past 50 years from 4.95 ard/ fed in the year 1950 to 17.8 ard/fed in the year 2000 mainly due to the continuous genetic improvement of wheat germplasm in the breeding program. Molecular markers identified rust resistant genes and continuous search for these genes are of great important to enrich and pyramid the commercial high yielded wheat cultivars in the breeding programs in Egypt with effective resistant genes with the aid of marker assisted selection.

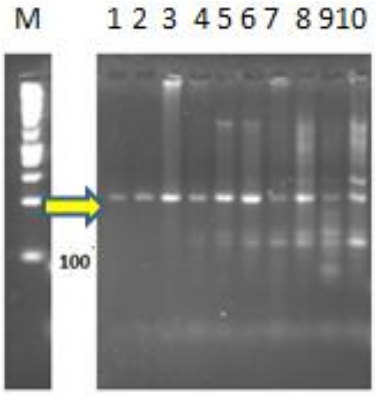

Fig. 1. Detection of the resistance gene *Lr* 21 in 10 wheat cultivars. PCR products obtained from the same wheat cultivars with the specific primer for *Lr*21. 1= Misr 1, 2= Misr 2, 3= Sids 12, 4= Sids 13, 5= Gemmeiza 9, 6= Gemmeiza 10, 7= Gemmeiza 11, 8= Sakha 93, 9= Sakha 94, 10= Giza 168 and M= 100 bp ladder size marker. The yellow arrowhead indicates the 200 bp PCR amplification product

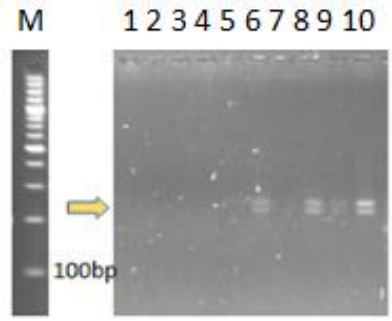

Fig. 2. Detection of the resistance gene *Lr* 32 in 10 wheat cultivars. PCR products obtained from the same wheat cultivars with the specific primer for *Lr*32. 1 = Misr 1, 2= Misr 2, 3= Sids 12, 4= Sids 13, 5= Gemmeiza 9, 6= Gemmeiza 10, 7= Gemmeiza 11, 8= Sakha 93, 9= Sakha 94, 10= Giza 168 and M= 100 bp ladder size marker. The yellow arrowhead indicates amplification of PCR product at the 270 bp

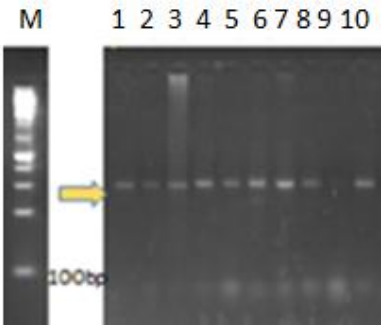

Fig. 3. Detection of the resistance gene *Lr* 39 in 10 wheat cultivars. PCR products obtained from the same wheat cultivars with the specific primer for *Lr*39. 1= Misr 1, 2= Misr 2, 3= Sids 12, 4= Sids 13, 5= Gemmeiza 9, 6= Gemmeiza 10, 7= Gemmeiza 11, 8= Sakha 93, 9= Sakha 94, 10= Giza 168 and M= 100 bp ladder size marker. The yellow arrowhead indicates the 300 bp PCR amplification product.

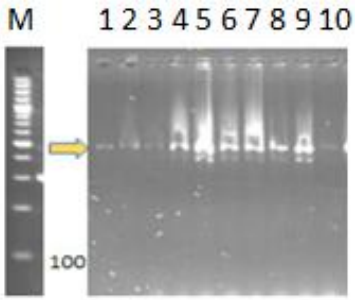

Fig. 4. Detection of the resistance gene *Lr* 47 in 10 wheat cultivars. PCR products obtained from the same wheat cultivars with the specific primer for *Lr*47. 1= Misr 1, 2= Misr 2, 3= Sids 12, 4= Sids 13, 5= Gemmeiza 9, 6= Gemmeiza 10, 7= Gemmeiza 11, 8= Sakha 93, 9= Sakha 94, 10= Giza 168 and M= 100 bp ladder size marker. The yellow arrowhead indicates the 500 bp PCR amplification product.

4. CONCLUSION

The postulation test theorized the presence of *Lr* 25, *Lr* 32, *Lr* 35, *Lr* 37 and *Lr* 47 in Sids 12. Wheat cultivar Misr 1 may have four genes i.e. *Lr* 25, *Lr* 35, *Lr* 39 and *Lr* 51. The wheat cultivars Misr 2, Sids 13, Sakha 94 and Giza 168 may have two genes i.e. *Lr* 35 and *Lr* 39 in the wheat cultivar Misr 1 and the same two genes *Lr* 35 and *Lr* 37 in the three wheat cultivars Sids 13, Sakha 94 and Giza 168. On the other hand, the wheat cultivars Gemmeiza 9 and Sakha 93 may have only one gene i.e. *Lr* 35 and *Lr* 32, respectively. While, the wheat cultivars Gemmeiza 10 and Gemmeiza 11 did not have any of the tested *Lr* genes under study using the fifteen tested pathotypes of *P. triticina* but it may be carry some additional genes. In the same way, all of the tested wheat genotypes may be carries additional resistant gene (s).The molecular markers survey with specific markers revealed that, the specific markers for *Lr* 21 and *Lr* 47 were detected in all of the studied cultivars. The marker for *Lr* 32 was recognized in four cultivars namely: Gemmeiza10, Sakha93 Sakha94 and Giza 168. The marker for *Lr* 39 was identified in all cultivars except the cultivar Sakha 94. This study may add valuable information about resistance genes in the Egyptian wheat cultivars which may be used in pyramiding more genes in each cultivar or used as donor parents in different breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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