

# Postulation and identification of resistance genes against *Puccinia triticina* in new wheat cultivars in Egypt using molecular markers

Ashraf Abdelbacki, Nor Soliman, Mohamed Najeeb, Reda Omara.

**Abstract**— Knowledge of the geographical distribution for physiologic races of *Puccinia triticina* and identification of leaf rust resistance genes (Lr's) in the recent Egyptian wheat cultivars are essential for maximizing resistance in future-bred cultivars. The aim of this study was to know the status of resistance in Egyptian wheat cultivars against wheat leaf rust and the most frequent race distributed. Infected samples were collected from five Governorates, i.e., Dakahlia, Kafr el-Sheikh, Beheira, Sharqia and Sohag comprised the wheat growing area in Egypt. These samples were isolated, purified and identified on the differential sets. Gene postulation was done using fifteen identified races on Egyptian wheat cultivars correlated with Lr genes. Thirty three races identified during three seasons 2009/2010, 2010/2011 and 2011/2012. The most frequent race was TK (10%) followed by race BB (7.58%), PK (6.55%), TT (4.82%), PT (3.79%) and MT (3.44%). Moreover, races; BB, TT and PT were present during three seasons while these races appeared in some Governorates and disappeared in other Governorates. On the other hand, the most frequently occurring gene in ten Egyptian wheat cultivars was *Lr35* (70%), followed by *Lr22* (60%), *Lr27* (40%), *Lr34* (30%), *Lr19* (30%), *Lr18* (10%), *Lr36* (10%) and *Lr46* (10%), eight out of sixteen Lr genes were not present in the tested cultivars. Four genes; *Lr28*, *Lr24*, *Lr34* and *Lr19* were confirmed using molecular marker. It is concluded that there was a good variation in Lr genes carried by wheat cultivars commercially grown in Egypt. Therefore, strategies for deploying resistance genes to prolong effective disease resistance are suggested to control wheat leaf rust disease.

**Keywords**— Gene postulation, Resistance genes, *Puccinia triticina*, Wheat.

## I. INTRODUCTION

WHEAT leaf rust, caused by *P. triticina*, is one of the most common diseases of wheat worldwide. It probably results in higher total annual losses worldwide because of its more frequent and widespread occurrence [1]. In Egypt, leaf rust is the most common and important wheat disease. It caused severe losses in grain yield which reached 23% on some varieties

depending on the level of rust incidence and the stage of crop development when initial infection occurs [2], [3].

The use of resistant cultivars is the most efficient, economical and environmentally safe method to control leaf rust disease. Although there are about 60 known genes involved in the resistance of wheat cultivars to *P. triticina*, a majority of these are not effective against current races of *P. triticina* [4], [5]. *P. triticina* has diverse virulence and is able to overcome resistance genes. The emergence of virulent pathotypes can restrict the durability and use of rust resistance genes. Accordingly, there is an ongoing need to identify, characterize and deploy new sources of resistance [6].

For assessing the vulnerability of the crop to leaf rust, knowledge of the major resistance genes present in the predominant wheat cultivars is a prerequisite. In turn, when this information is combined with data on virulence features of the *P. triticina* population in Egypt, it is possible to make informed decisions for improving the leaf rust resistance in the Egyptian wheat cultivars. The presence of race-specific resistance gene(s) in a cultivar is postulated based on the gene-for-gene relationship [7], provided that an array of pathogen cultures with diverse combinations of avirulence and virulence genes is used. In wheat, the presence of a specific resistance gene for *Puccinia spp.* can be ascertained by an interaction with the *Puccinia spp.* culture that lacks the corresponding gene for virulence. Leaf rust isolates that produce distinct low infection type (IT) on specific Lr genes, will also produce low ITs on those cultivars that have the same resistance genes [8]. A great deal of information on postulated leaf rust resistance genes has been collected from countries (including Australia, US, Canada, China, India, Pakistan and South Africa) where wheat is a major crop [9], [8], [10], [11], [12]. Little information is available on Lr genes present in Egyptian wheat cultivars. The objective of this study was to study the geographic distribution of *Puccinia triticina* in five wheat growing Governorates in Egypt during three successive seasons (2009-2012) and postulation of resistance genes in the Egyptian wheat cultivars.

Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e.: base pair deletions, substitutions, additions or patterns) [13]. There are various methods to detect and amplify these polymorphisms so that they can be used for breeding analysis and these techniques will be focused in this thesis review. Molecular markers are superior to other forms of MAS because they are relatively simple to detect, abundant throughout the genome even in highly bred cultivars, completely independent of

Ashraf Abdelbacki is with the Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt. (Corresponding author's phone: 0020111269405; fax: 0020235717355; e-mail: [amaeg@hotmail.com](mailto:amaeg@hotmail.com)).

Nor Soliman is with the Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt.

Mohamed Najeeb is with Wheat Disease Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

Reda Omara is with Wheat Disease Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

environmental conditions and can be detected at virtually any stage of plant development.

Knowledge of the number and identity of the leaf rust resistance genes in these cultivars will be useful in understanding their field reaction to changing *P. triticina* populations and it can be used as parents for improving future wheat cultivars.

## II. MATERIALS AND METHODS

Samples were collected from wheat Egypt farmers and wheat Trap Rust Nurseries (EWTRN), which incorporate monogenic lines of leaf rust (*Lr*'s), certain local cvs. and highly susceptible check varieties viz. "Morocco and *Triticum spleta saharences*" from five Governorates, i.e., Dakahlia (NE), Kafr el-Sheikh (N), Beheira (W), Sharqia (E) and Sohag (S). These samples were purified and multiplied by picking 3-5 pustules on the highly susceptible variety Morocco in greenhouse and laboratory of wheat Diseases Department, Plant Pathology Research Institute (PPRI), A.R.C., Giza during the period (2009-2012). The multiplied isolates were used for inoculating the differential sets.

### *Isolation and purification*

The urediniospores of the infected specimens were transferred on the seedlings of the susceptible wheat variety Morocco. The method of inoculation was carried out as described by [14], in which the seedling leaves were rubbed gently between moisten fingers with tap water, sprayed in the incubation chambers with water, then inoculated by shaking or brushing rusted materials over the plant leaves and sprayed gently again with water in order to induce initial a film of free water on the plants which is essential for spore germination and the establishment of infection. The inoculated plants were then incubated in moist chambers for 24 hours to allow the rust spores to germinate and cause infection. The inoculated plants were then moved onto the benches in the greenhouse and kept under observation until the rust pustules are developed. After developing the pustules, 3-5 single pustules were isolated separately from each sample for rust reproduction on the highly susceptible wheat variety Morocco seedlings to obtain enough urediniospores for inoculation.

### *Race identification*

The method used to identify leaf rust races was adopted by [15] based on inoculation of isogenic lines (*Lr*'s) with *P. triticina* (uredia spores) that we had modified. According to this system the plant reaction is determined on 20 lines divided into 5 groups of four lines. The first group includes isogenic *Lr*-lines 1, 2a, 2c,3; the second- 9, 16, 24, 26; the third group- 3Ka, 11, 17 30; the fourth- 10, 18, 21, 2b; the fifth was the set of lines *Lr14b*, *Lr15*, *Lr35* and *Lr42* (addition set for Egypt by [16]). According to combination of responses of low infection type (L) and High infection type (H) plants each rust agent isolate was coded in letters. As a result each pathotype has a code including 5 letters consonants of English alphabet from B through T.

### *Disease assessment*

The infection types for all the isogenic lines were recorded after 12 days on appearance of pustules on near-isogenic lines, the infection types for all the near-isogenic lines were recorded using standard disease scoring scale 0-4 [14]. The virulence patterns on differential sets were assessed on the basis of low infection types produced by each line in response to infection (infection type 0, 1 and 2 represented avirulent while 3 and 4 represent virulent [14]).

### *Gene postulation*

Ten Egyptian wheat cvs.; Sakha-94, Sakha-95, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Sids-12, Sids-13, Giza-168, Misr-1 and Misr-2 and sixteen resistance monogenic lines; *Lr2a*, *Lr9*, *Lr18*, *Lr19*, *Lr22a*, *Lr21*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr36*, *Lr43*, *Lr45*, *Lr46* and *Lr47* were tested at the seedling stage using 15 pathotypes of *P. triticinia* obtained from collected samples during 2010/2011 growing season.

### *Molecular markers*

Four specific primers were used for identification of four resistance genes *Lr28*, *Lr24*, *Lr34* and *Lr19*:

*Lr9-1* (TCC TTT TAT TCC GCA CGC CGG), *Lr9-2* (CCA CAC TAC CCC AAA GAG ACG), *Lr24-1* (TCT AGT CTG TAC ATG GGG GC), *Lr24-2* (TGG CAC ATG AAC TCC ATA CG), *Lr34L* (AGC TCT GCT TCA CGA GGA AG), *Lr34R* (CTC CTC TTT ATA TCG CGT CCC), *Lr28-01* (CCC GGC ATA AGT CTA TGG TT) and *Lr28-02* (CAA TGA ATG AGA TAC GTG AA).

All plant materials were grown in (10 cm) plastic pots. Each contained four varieties, one in each corner clockwise. Inoculation procedures, genes were postulated and rust data were carried out according to the methods adopted by [14], [17], [18]. DNA extraction and manipulation was done according to [13].

## III. RESULTS

During the three seasons 2009/2010, 2010/ 2011 and 2011/2012. The highest mean in collected leaf rust samples was Kafr el-Sheikh followed by Dakahlia and Sharqia while Sohag was the lowest one. On the other hand, Sharqia was the highest successive in uredial cultural followed by Kafr el-Sheikh and Dakahlia while Sohag was the lowest one in uredial cultural during three seasons. Moreover, the highest number of collected samples were during season 2009/2010 followed by season 2010/2011 and season 2011/2012 (Table 1).

Thirty three physiological races identified during three seasons 2009/2010, 2010/ 2011 and 2011/2012. Therefore, race TT was the most common virulence race while, BB was the lowest one. On the other hand, race TK was the most frequent race (10%) followed by race BB (7.58%), PK (6.55%), TT (4.82%), PT (3.79%) and MT (3.44%). Whereas, the proportion of rest races were ranged between (0.68 – 2.41) of the total isolates during the three seasons. Moreover, races; BB, TT and PT were present during three seasons but these races appeared in some Governorates and disappeared in other Governorates.

### *Gene postulation*

Data presented in Table 2. the matching between both local wheat cultivars and Lr genes against the tested physiological races of leaf rust at seedling stage under greenhouse condition indicated that postulated genes in cultivar Misr-2 were Lr19, Lr22a, Lr27, Lr34, Lr35. Likewise, two cultivars Sids-12 and Giza 168 probably have a single Lr gene, four cultivars Sakha-94, Sakha-95, Gemm.-10 and Sids-13 have two Lr genes, one cultivar Misr-1 has three Lr genes and two cultivar Gemm.-9 and Gemm.-11 have four Lr genes. On the other hand, Lr 19 and Lr34 were the most likely contributing genes for resistance against leaf rust in which Lr 19 present in cultivars Sakha-95, Misr-1 and Misr-2 and Lr34 in Gemm.-11, Giza-168 and Misr-2. Therefore, these cultivars are considered as resistance to leaf rust disease.

Data in Table 2. also clear that *Lr35* was the most common leaf rust resistance gene, being postulated in seven wheat cultivars, i.e., Sakha-94, Gemm.-9, Gemm.-10, Gemm.-11, Sids-12, Sids-13 and Misr-2 among ten Egyptian wheat cultivars and this gene exhibited frequency 70%. Others common Lr genes include *Lr22a* (six cultivars) exhibited frequency 60%, *Lr27* (four cultivars) have frequency 40%, *Lr19* and *Lr34* (three cultivars) have frequency 30% and *Lr18*, *Lr36* and *Lr46* (one cultivar) exhibited frequency 10%. However, eight genes (*Lr2a*, *Lr9*, *Lr25*, *Lr28*, *Lr29*, *Lr43*, *Lr45* and *Lr47*) out of sixteen Lr genes were not present in tested cultivars.

The common leaf rust resistance gene(s) may be or probably present between the ten tested wheat cultivars. These data indicated that Single common gene was probably present between each of (Sakha-94, Sids-13) and (Gemm.-9, Gemm.-10). Likewise, two common genes probably present between Gemm.-11 and each of (Sakha-94, Sids-13) and between Misr-1 and each of (Sakha-94, Sakha-95). While, three common genes probably present between Misr-2 and each of (Sakha-94, Gemm.-11 and Sids-13) (Table 3).

#### Molecular markers

Four specific primers were used for identification of four resistance genes *Lr28*, *Lr24*, *Lr34* and *Lr19*:

Lr9-F (TCC TTT TAT TCC GCA CGC CGG), Lr9-R (CCA CAC TAC CCC AAA GAG ACG), Lr24-F (TCT AGT CTG TAC ATG GGG GC), Lr24-R (TGG CAC ATG AAC TCC ATA CG), Lr34L (AGC TCT GCT TCA CGA GGA AG), Lr34R (CTC CTC TTT ATA TCG CGT CCC), Lr28-F (CCC GGC ATA AGT CTA TGG TT) and Lr28-R (CAA TGA ATG AGA TAC GTG AA).

Two genes were present in the ten tested cultivars, i.e. *Lr34* and *Lr19*. *Lr28* were present in five out of ten cultivar tested while *Lr24* were absent in all cultivar tested figure 1 and 2.

#### IV. DISCUSSION

One of the most important steps in breeding programs for rust resistance in wheat is identification of the prevailing physiological races in the region. Such program will be successful if all physiological isolates of the disease are included [19], [20]. Generally, new races of leaf rust developed by mutation [21]; heterokaryosis [19]; recombination [4], [22]; migration [23] and natural selection

of virulence race against resistance of growing wheat varieties in the region [24].

Survey for wheat leaf rust in Egypt during three growing seasons 2009-2012, indicated the presence of the disease incited by *P. triticina* in different governorates i.e., Dakahlia, Kafr el-Sheikh, Beheira, Sharqia and Sohag.. Most of diseased samples were collected from farmer fields and trap nurseries. The three annual surveys, 2009/01, 2010/11 and 2011/12 resulted in the presence of 33 physiologic races. Race TT was the most common virulence race while, BB was the lowest one. This result was in accordance with those of [25], [26] who cleared that race TT was the most virulent one. Similar results were reported by [3], [27].

The most frequent race was TK through the three seasons followed by races; BB, PK, TT, PT and MT. Regardless of the presence of these races during the three seasons, but they appeared in some Governorates and disappeared in other Governorates. This result may be due to climate changes i.e., temperature and rainfall in different Governorates which are necessary for the disease occurrence [28], [29] and ability of *P. triticina* to form new races that can attack resistant varieties and their potential to develop rapidly under optimal environmental conditions and cause serious losses [30]. For example in the present study, Sohag was the lowest Governorate in collected samples and physiological races. This may be due to high temperature and lack of rain in this Governorate and therefore we advise to grow high-yield susceptible varieties in Upper Egypt because the sever infection will be very low in this area due to the environmental conditions.

The wheat leaf rust population in Egypt is made up of a great diversity of races and that the most common races reappear year after year. The similar levels of diversity of leaf rust races in Egypt and the United States, particularly in the Southern Plains, may be cause to reinvestigate the possibility of oversummering of leaf rust in Egypt. Leaf rust inoculum arrives in Egypt from external sources each year and transferred from area to area in the same year [16].

Resistance gene expression is dependent on the genetics of host-parasite interaction, temperature conditions, plant developmental stage, and interaction between resistance genes with suppressors or other resistance genes in the wheat genomes [31]. Gene postulation is the most frequent method to determine the presence of the probable race-specific seedling resistance genes (Lr genes) in a host cultivar, many researchers have used this method for identifying Lr genes in a group of wheat genotypes [8], [11], [32], [33]. Therefore, in this study we identified eight known genes and several unidentified genes for seedling leaf rust resistance in a range of wheat cultivars grown in Egypt. Genes masked by suppression in the seedling stage or under the given environment conditions could remain undetected, although they may still have an effect on resistance in the field [8].

The gene *Lr35* (70%) was the most commonly postulated gene in the Egyptian wheat cultivars followed by *Lr22* (60%), *Lr27* (40%), *Lr34* (30%), *Lr19* (30%), *Lr18* (10%), *Lr36* (10%) and *Lr46* (10%). On a global scale, *Lr19* is probably the most widely distributed gene for resistance to *P. triticina* [4], [34]. Therefore, it is still considered important gene

Table I. Number of leaf rust samples collected and the succeeded cultures during the three seasons 2009/2010, 2010/ 2011 and 2011/2012.

No.	Location	Season/Number of samples and cultures per location						Total	
		2009-2010		2010-2011		2011-2012			
		samples	cultures	Samples	cultures	samples	cultures	samples	cultures
1	Dakahlia	17	44	9	17	7	12	33	73
2	Kafr el-Sheikh	27	28	9	19	10	25	46	72
3	Sharqia	11	21	9	22	11	51	31	94
4	Beheira	2	5	10	27	5	9	17	41
5	Sohag	2	3	3	4	3	3	8	10
Total		59	101	40	89	36	100	135	290

because it is present in several bred cultivars in CIMMYT in combination with other adult plant resistance genes which continue to give excellent leaf rust protection [1]. In Egypt, this gene is important gene of resistance and postulated in cultivars Sakha-94, Misr-1 and Misr-2 (present study) which are considered resistance to leaf rust disease.

Pyramiding genes has been suggested as a method to achieve more durable resistance against pathogens with low genetic diversity, high gene flow and asexual mating systems [35], [33]. The combination of several effective resistance genes into a single cultivar should extend the period of resistance i.e., Misr-2 (*Lr19*, *Lr22a*, *Lr27*, *Lr34*, and *Lr35*), Gemm.-9 (*Lr22a*, *Lr35*, *Lr36* and *Lr46*) and Gemm-11 (*Lr22a*, *Lr27*, *Lr34*, and *Lr35*). Slow rusting or partial resistance has been reported to be a more durable resistance than single seedling resistance genes [36]. The pyramiding of such differently functioning genes is simplified by the use of molecular markers that have been developed for most genes for leaf rust resistance. These markers already have been developed for slow rusting resistance genes *Lr34* and *Lr46* [37], [6]. In this study, these genes were postulated in cultivars Gemm-11, Giza-168 and Misr-2. Similar results were

Concerning the matching within commercial cvs, the obtained results gave evidence to the presence of common gene (s) between Sakha-94 and Sids-13, Gemm.-9 and Gemm.-10, Gemm.-11 and each of Sakha-94 and Sids-13, Misr-2 and each of Sakha-94, Gemm.-11 and Sids-13. These common genes are *Lr22a* or *Lr35* which were already postulated in the tested cultivars (Table 3). Therefore, this result gives evidence to the presence of these genes in these cultivars.

The knowledge of which leaf rust seedling resistance genes are present facilitates future studies and the use of adult plant resistance genes in the wheat cultivars. The genes *Lr22*, *Lr35*, *Lr27*, *Lr34*, *Lr19*, *Lr18*, *Lr36* and *Lr46* were the most frequent seedling leaf rust resistance genes postulated to be present in Egyptian wheat cultivars. Therefore, there is relatively inadequate variation in Lr genes carried by wheat cultivars commercially grown in Egypt. Two genes were present in the ten tested cultivars, i.e. *Lr34* and *Lr19*. *Lr28* were present in five out of ten cultivar tested while *Lr24* were absent in all cultivar tested which means that some resistance genes were

Table II. The postulated Lr genes that may be or probably present within ten wheat cultivars at seedling stage during season 2010/2011.

Genes Cultivars	Lr genes															Postulated resistance genes	
	<i>Lr2a</i>	<i>Lr9</i>	<i>Lr18</i>	<i>Lr19</i>	<i>Lr22a</i>	<i>Lr25</i>	<i>Lr27</i>	<i>Lr28</i>	<i>Lr29</i>	<i>Lr34</i>	<i>Lr35</i>	<i>Lr36</i>	<i>Lr43</i>	<i>Lr45</i>	<i>Lr46</i>		<i>Lr47</i>
Sakha-94	+	+	+	+	0	+	+	-	+	+	0	+	+	+	+	-	22a, 35
Sakha-95	+	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	19,27
Gemmeiza-9	+	+	+	+	0	+	+	+	+	+	0	0	+	+	0	-	22a,35,36,46
Gemmeiza-10	-	+	+	+	0	+	+	-	+	+	0	-	+	-	+	-	22a,35
Gemmeiza-11	+	+	+	+	0	+	0	-	+	0	0	+	+	+	+	-	22a,27,34,35
Sids-12	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	35
Sids-13	+	+	+	+	0	+	+	-	+	+	0	+	-	+	+	-	22a,35
Giza-168	-	+	+	+	+	-	+	-	+	0	+	+	+	+	+	+	34
Misr-1	+	+	0	0	+	+	0	+	+	+	+	+	+	+	+	+	18,19,27
Misr-2	+	+	+	0	0	+	0	+	+	0	0	+	+	+	+	-	19,22a,27,34,35
No. of cultivars carrying Lr genes	0	0	1	3	6	0	4	0	0	3	7	1	0	0	1	0	
Frequency %	0	0	10	30	60	0	40	0	0	30	70	10	0	0	10	0	

(-) = indicated the absence of such gene in the cultivar; (0) = indicated the presence of such gene in host B and it may have another one and (+) = indicates that either of hosts did not have the same gene.

recorded by [38] who indicated the presence of gene *Lr34* in cultivar Biointa 2004.

confirmed to be present in the cultivars compared with gene postulation and genetic analysis.

Table III. Presence of common genes amongst wheat cultivars at seedling stage during 2010/2011

Cultivars	Sakha-94	Sakha-95	Gemmeiza-9	Gemmeiza-10	Gemmeiza-11	Sids-12	Sids-13	Giza-168	Misr-1	Misr-2
Sakha-94		+	+	+	-	-	0	-	+	-
Sakha-95	+		+	+	+	+	+	+	+	+
Gemmeiza-9	+	+		0	+	+	+	+	+	+
Gemmeiza-10	+	+	-		-	+	+	+	+	+
Gemmeiza-11	0	+	+	+	+	+	0	+	+	-
Sids-12	+	+	+	+	+		+	+	+	+
Sids-13	-	+	-	+	-	-		+	+	-
Giza-168	-	+	+	+	+	+	+		+	+
Misr-1	0	0	+	+	+	+	+	+		+
Misr-2	0	+	+	+	0	+	0	+	+	

(-) = indicated the absence of such gene in the cultivar; (0) = indicated the presence of such gene in host B and it may have another one and (+) = indicates that either of hosts did not have the same gene.

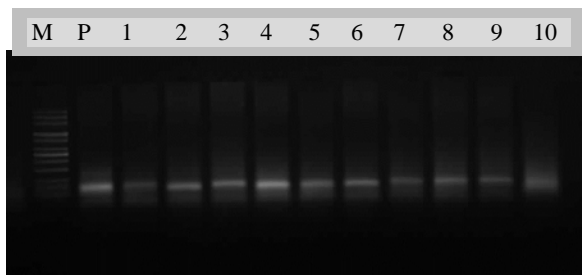


Fig.1. *Lr 34* in the ten cultivar tested (P= positive sample, 1= Saha-94, 2= Sakha-95, 3= Gemmeiza-9, 4= Gemmeiza-10, 5= Gemmeiza-11, 6= Sids-12, 7= Sids-13, 8= Giza-168, 9= Misr-1, 10= Misr-2, M= 100pb marker)

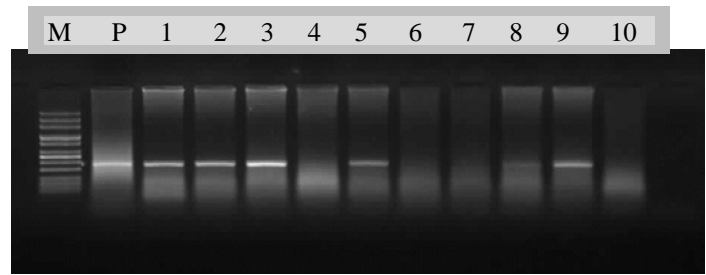


Fig.2. *Lr 28* in the ten cultivar tested (P= positive sample, 1= Saha-94, 2= Sakha-95, 3= Gemmeiza-9, 4= Gemmeiza-10, 5= Gemmeiza-11, 6= Sids-12, 7= Sids-13, 8= Giza-168, 9= Misr-1, 10= Misr-2, M= 100pb marker)

Future host selection pressure on the pathogen could be further decreased by rotating genes through time and space by mixtures or regional deployment of cultivars with different effective resistance genes. Nevertheless, classical genetic and molecular marker analyses will be needed to further validate and expand the findings of the present study regarding the *Lr* genes responsible for both seedling and adult plant resistance to leaf rust in the Egyptian wheat cultivars.

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