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RESEARCH ARTICLE

Molecular markers and phenotypic characterization of adult plant resistance genes *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 and their association with partial resistance to leaf rust in wheat

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Abstract. Thirty-nine wheat genotypes were studied to estimate their partial resistance levels to leaf rust at Behira governortae during three growing seasons, i.e. 2016/2017, 2017/2018 and 2018/2019. In these genotypes, partial resistance was characterized using final leaf rust severity (FRS %) and area under disease progress curve (AUDPC). Of the tested genotypes, only three wheat varieties; Giza 171, Misr 3 and Sohag 5 showed complete resistance. Further, 28 of the 39 genotypes had partial resistance as they revealed low and/or moderate values of FRS (%) and AUDPC (not exceeding 30% and 300, respectively). The other eight varieties were fast rusting, as they displayed the maximum values of FRS (%) and AUDPC. The four monogenic lines; *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 were identified in the wheat varieties using linked molecular markers; csLV34, Xgwm259, CFD71 and csGSR. Phenotypic results of the wheat varieties were confirmed by molecular marker analysis.

Keywords. wheat; leaf rust; partial resistance; molecular markers.

Introduction

Leaf rust (*Puccinia triticina* Eriks.) is the most frequent and generally distributed disease than the other two wheat rusts, yellow and stem rust, as it occurs annually wherever wheat is grown (Kolmer 2013). Grain yield losses caused by leaf rust may be up to 50% in severely infected wheat cultivars (German *et al.* 2007). In Egypt, grain yield loss resulting from artificial leaf rust has reached 32% in the susceptible wheat cultivars that are cultivated under experimental field conditions favourable to disease incidence and development (Shahin and El-Orabey 2016; El-Orabey *et al.* 2017).

Two main control strategies, i.e. genetic resistance and chemical control have been used for wheat rust diseases, especially for leaf rust. Until now, host-genetic resistance remains the most efficient, economic and environmentally safe approach, as it eliminates the use of the synthetic fungicides and decrease the production of the cost. It is achieved by the development or release and new wheat cultivars having either race-specific or race nonspecific resistance are grown. Race-specific genes for resistance allow an effective protection against only a few pathotypes of the pathogen and interact according to the gene-for-gene theory (Flor 1956). These resistance genes often rapidly overcome and/or breakdown because of the sudden evolution of new virulent pathotypes. In contrast, race nonspecific resistance also called as slow rusting resistance or partial resistance (PR) is uniformly effective to almost all pathotypes of the pathogen and mainly are inherited quantitatively. Therefore, resistance governed by these kinds of genes are more durable than others (Herrera-Foessel *et al.* 2012). This type of resistance are determined by slower disease progress rates in the field in spite of a susceptible host reaction (Broers 1989).

To date, 77 leaf rust monogenic lines have been identified in wheat, with 76 formally designated ones (McIntosh *et al.* 2017). Most of these monogenic lines are race-specific, while few of them are adult plant resistance (APR) genes. Until now, four of the previously designated resistance

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genes; *Lr* 34/*Yr* 18, *Lr* 46/*Yr* 29, *Lr* 67/*Yr* 46 and *Lr* 68 are responsible for partial resistance or slow rusting resistance to leaf rust pathogen in wheat (Herrera-Foessel *et al.* 2012).

The first slow-rusting resistance gene, *Lr* 34, located on 7DS chromosome (Dyck 1987) has provided APR to leaf rust pathogen and linked with yellow rust resistance gene, *Yr* 18. Also, it is closely associated with stem rust gene, *Sr* 57 (Lagudah *et al.* 2009); powdery mildew gene, *Pm* 38 (Pinto da Silva *et al.* 2018); and gene *Bdy* 1 that conditioning tolerance to barley yellow dwarf virus (Pinto da Silva *et al.* 2018). Moreover, it is closely linked to *Ltn* 1 gene that conferred leaf tip necrosis on the leaves, and characterized by necrosis on the leaf tips extending to a few centimetres of leaf edges (Pinto da Silva *et al.* 2018).

The second resistance gene *Lr* 46 is found on chromosome 1BL and is tightly associated with stripe rust gene, *Yr* 29, stem rust gene; *Sr* 58, powdery mildew gene; *Pm* 39 and leaf tip necrosis gene *Ltn* 2 (William *et al.* 2003).

The third slow-rusting resistance gene, *Lr* 67 is located on chromosome 4DL with a tight linkage between them and each of stripe rust resistance gene; *Yr* 46, stem rust resistance gene; *Sr* 55, powdery mildew resistance gene; *Pm* 46 and leaf tip necrosis gene; *Ltn* 3 (Moore *et al.* 2015).

Finally, *Lr* 68 is located on chromosome 7BL and also linked with leaf tip necrosis gene (Herrera-Foessel *et al.* 2012). The previous reports reveled in general, that pyramiding of *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 in different combinations within a particular wheat genotype confers high and/or sustainable level of resistance to wheat leaf rust and also expected to be long-lasting or more durable (Pinto da Silva *et al.* 2018).

Therefore, the main objectives of the current study were therefore to evaluate the response of 35 local wheat varieties and four monogenic lines against leaf rust at adult plant stage under field conditions. Also, to characterize the partial resistance and to determine gene(s) responsible for the expression of this kind of resistance in the tested wheat varieties. Eventually, validation by molecular markers linked with the four leaf rust resistance genes *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 in the tested varieties. The goal of this study was to designate the effects of the presence of one or more of these partial resistance genes in the level of APR of the tested wheat varieties.

Materials and methods

Plant materials

Thirty-nine wheat genotypes; 35 local wheat varieties and four leaf rust monogenic lines were used in the this study (table 1). Grains of the tested wheat varieties and leaf rust monogenic line; *Lr* 34 and *Lr* 46 were provided by Wheat Diseases Research Department, Plant Pathology Research Institute, Giza, Egypt. While, for the first time, grains of the two monogenic lines, *Lr* 67 and *Lr* 68 were provided to Egypt by International Maize and Wheat Improvement Center (CIMMYT), Mexico, through the website (http://www.cimmyt.org/seed-request/#wheat).

For this study, the wheat genotypes were sown at Itay El-Baroud Agricultural Research Station, Behira governorate, Egypt (30.88′55′98°N; 30.65′84′32°E). The field experiments were carried out during three growing seasons, i.e. 2016–17, 2017–18 and 2018–19, in a randomized complete block design (RCB) with three replications. Each wheat genotypes were sown in two rows of three metre plot length spaced at 30 cm apart. All plots were ringed by one metre width, planted with a mixture of the highly susceptible wheat genotypes, i.e. Morocco and Thatcher as a spreader to create high and uniform disease pressure.

To enhance the progress of leaf rust epidemic under field conditions, an experiment was irrigated and inoculated by dusting a mixture of urediniospores of the dominant and more aggressive leaf rust pathotypes: FTSSS, KTSPT, NTTJT, NTTKT, NTTTT, PTTCT, PTTGS, PTTNS, STTTK and TTTBT (El-Orabey *et al.* 2018) mixed with talcum powder, at a rate of one volume of fresh urediniospores to 20 volumes of talcum powder according to Roelfs *et al.* (1992).

Disease assessment

Rust response of the tested wheat genotypes was estimated using the two parameters; final rust severity (FRS %) and area under disease progress curve (AUDPC). The leaf rust severity (%) was estimated as a percentage of leaf area covered by leaf rust pustules (0–100%) (Peterson *et al.* 1948). Rust response for each genotype was assessed thrice at 15 days intervals. The adult plant reaction (infection type) was also recorded using the method of Roelfs *et al.* (1992).

FRS (%) was recorded for each genotype as disease severity (%) when the highly susceptible (check) variety was severely rusted and the disease rate reached its maximum level of severity (Das *et al.* 1993). AUDPC was calculated by using the equation of Pandey *et al.* (1989).

$$\text{AUDPC} = \text{D}[\tfrac{1}{2}(\text{Y}_1+\text{Y}_\text{k})+(\text{Y}_2+\text{Y}_3+\cdots\cdots+\text{Y}_{\text{k}-1})],$$

where D is the days between two consecutive records (time intervals); $\text{Y}_1 + \text{Y}_\text{k}$ is the sum of the first and last disease scores; $\text{Y}_2 + \text{Y}_3 + \cdots + \text{Y}_{\text{k}-1}$ is the sum of all in between disease scores.

Statistical analysis

Combined analysis of variance was carried out for data obtained by the tested genotypes over the three seasons at Itay El-Baroud (table 2). Significance of difference among the studied cultivars was tested by the analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1967). Mean comparisons were made for variables among genotypes using least significant differences (LSD at 5%) tests.

Table 1. Pedigree and year of release of the wheat genotypes under study.

Wheat genotype	Pedigree	Year of release
Bread wheat varieties		
Giza 139 (check)	HINDI90/KENYA256G.	1947
Sakha 8	Indus 66 × Norteno "S"-Pk 348	1979
Sakha 69	Inia/RL 4220//7C/Yr "S" CM 15430-25-65-0S-0S	1980
Giza 160	CHENAB/GIZA155.	1982
Giza 163	F-61-70/Bon//Cno /7C CM33009-F-15M-4Y-2M-1M-1M-1Y-0M	1988
Giza 164	KVZ/Buha "s"//Kal/Bb CM33027-F-15M-500y-0M	1988
Giza 165	0MCno/Mfd//Mon "S" CM43339-C-1Y-1M-2Y-1M-2Y-0B	1991
Giza 167	Au/UP301//G11/SX/Pew"S"/4/Mai"S"/May"S"//Pew"S" CM67245-C-1M-2Y-1M-7Y-1M-0Y	1995
Giza 171	Sakha 93 / Gemmeiza 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	2013
Sids 1	HD2172/PAVON"S"//1158.574"S". SD46-4SD-2SD-1SD-0SD.	1996
Gemmeiza 5	VEE"S"/SWM6525. GM4017-1GM-6GM-3GM-0GM.	1998
Giza 168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B	1999
Gemmeiza 7	CMH74A.630/SX//SER182/3/AGENT. GM4611-2GM-3GM-1GM-0GM.	1999
Gemmeiza 9	ALD"S"/HUAC"S"//CMH74A.630/SX. GM4583-5GM-1GM-0GM.	1999
Sakha 93	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S	1999
Gemmeiza 10	MAYA74"S"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/CROW"S". GM5820-3GM-1GM-2GM-0GM.	2004
Sakha 94	OPATA/RAYON//KAUZ. CMBW90Y3280-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.	2004
Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-4SD-1SD-1SD-0SD.	2007
Sids 13	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	2010
Sids 14	SW8488*2/ KUKUNACGSS01Y00081T-099M-099Y-099M-099B-9Y-0B-0SD.	2018
Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOYO1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S.	2010
Misr 2	SKAUZ/BAV92. CMSS96M0361S-1M-010SY-010M-010SY-8M-0Y-0S.	2011
Misr 3	ATTILA*2/ABW65*2/KACHU CMSS06Y00258 2T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY	2018
Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	2011
Gemmeiza 12	OTUS/3/SARA/THB//VEE .CCMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	2017
Shandaweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH	2011
Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA(TAUS)//BCN /4/WBLL1CMSA01Y00158S-040P0Y-040M-030ZTM-040SY-26M-0Y-0SY-0S	2018
Durum wheat varieties		
Sohag 1	GDOVZ469/JOS"S"//61.130.LSD.	1977
Sohag 3	MEXI"S"/MGHA/51792//DURUM6. CD21831-25H-1SH-0SH.	1991
Sohag 4	Ajaia-16//Hora/Jro/3/Gan/4/Zar/5/Suok-7/6/Stot//Altar84/Ald CDSS99B00778S-OTOPY-0M-0Y-129Y-0M-0Y-1B-0SH	2016
Sohag 5	TRN//21563/AA/3/BD2080/4/BD2339/5/Rascon 37// Tarro 2// Rascon 3/6/Auk/Gull//GreenCDSS00B00364T-0T0PB-0B-2Y-0M-0Y-1B-0Y-0SH	2016
Beni Sweif 1	JO"S"/AA"S"//FG"S". CD9799-126M-1M-5Y-0M-0SD.	1987
Beni Sweif 4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/W*2. ICD88-1120-ABL-0TR-1BR-0TR-6AP-OSD.	2007
Beni Sweif 5	DIPPERZ/BUSHEN3. CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD.	2007
Beni Sweif 6	BOOMER-21/BUSCA-3. CDSS95Y001185-8Y-0M-0Y-0B-1Y-0B0SD	2010
Monogenic lines (*Lr*'s):		
Lr 34	Thatcher^{6} × Lageadinho	1982
Lr 46	*T. aestivum* 'Pavon 76'	1998
Lr 67	PI 250413	2010
Lr 68	Parula	2012

DNA isolation and PCR protocol

DNA isolation and PCR protocol were conducted in ICARDA Biotechnology Lab, AGERI, Egypt. DNA was extracted from green leaves of five to seven-day-old seedlings following the method of Rogers and Bendich (1994). The PCR reaction mixture (15 μL) contained 5 ng DNA template, 10 pmol of forward primer, 10 pmol of reverse primer, 0.1 U of *Taq* DNA polymerase (Bioline GmbH, Germany), 25 mM of $MgCl_2$, 2 mM dNTPs

Table 2. Analysis of variance for the effects of seasons, genotypes and their interactions on FRS (%), and AUDPC for 39 wheat genotypes grown at Itay El-Baroud during three growing seasons.

Source of variation	Degree of freedom (DF)	Variables			
		FRS (%)		AUDPC	
		MS	*F* value	MS	*F* value
Replication	2	166.899	10.9221^{NS}	17544.042	9.0941^{NS}
Season (S)	2	3669.62	240.1456*	414606.799	214.9152*
Genotype (G)	38	6384.467	417.8088*	865441.640	448.6095*
S × G	76	136.921	8.9603*	25352.232	13.1416*
Error	466	15.281		1929.165	

NS, nonsignificant.
*Significant at $P \leq 0.05$.

Table 3. PCR primers used to identify the four leaf rust resistance genes in wheat varieties.

Gene	Marker	Sequence of primers 5′- 3′	Fragment size	Annealing temperature	References
Lr 34	csLV34F	GTT GGT TAA GAC TGG TGA TGG	150	55	Lagudah *et al.* (2006)
	csLV34R	TGC TTG CTA TTG CTG AAT AGT			
Lr 46	Xgwm259F	AGG GAA AAG ACA TCT TTT TTT TC	105	60	William *et al.* (2003)
	Xgwm259R	CGA CCG ACT TCG GGT TC			
Lr 67	CFD71F	CAA TAA GTA GGC CGG GAC AA	198	60	Hiebert *et al.* (2010)
	CFD71R	TGT GCC AGT TGA GTT TGC TC			
Lr 68	csGSF	AAG ATT GTT CAC AGA TCC ATG TCA	385	48	Herrera-Foessel *et al.* (2012)
	csGSR	GAG TAT TCC GGC TCA AAA AGG			

and 10× PCR buffer in 96 well thermal cycler (Applied Biosystem Thermal Cycler, Singapore). The reaction conditions were as follows: initial denaturation was for 5 min at 94°C, followed by initial 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 48°C for *Lr* 34 and *Lr* 68 and 60°C for *Lr* 46 and *Lr* 67 and extension at 72. Subsequently, 7 min final extension at 72°C was done. PCR products of SSR markers were checked for amplification on 2.5% agarose gel. The primer sequences used to identify the four leaf rust resistance genes are presented in table 3.

Results

Field evaluation and characterization of leaf rust partial resistance in the tested wheat genotypes

***FRS (%)*:** Large variations in FRS% between the tested wheat genotypes reached from 0 to 70% during the three seasons of the study at Itay El-Baroud. The 39 tested wheat genotypes were classified into three categories based on the leaf rust response (rust severity (%) and infection type). The first category is the varieties having race specific resistance, which contained the wheat varieties displayed immune and varieties with infection type resistance and moderately resistance. This category contained the three wheat varieties; Giza 171, Misr 3 and Sohag 5, which displayed field response from 0 to 5 MR during the three seasons (table 4).

The second category is the genotypes having partial resistance (slow rusting resistance) which contained wheat genotypes with infection types susceptible (S), moderately susceptible (MS) and rust severity to 30%. This category contained 28 wheat genotypes: Sakha 8, Giza 165, Gemmeiza 5, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sakha 94, Sids 12, Sids 13, Sids 14, Misr 1, Misr 2, Gemmeiza 12, Shandaweel 1, Sakha 95, Sohag 1, Sohag 3, Sohag 4, Beni Sweif 1, Beni Sweif 4, Beni Sweif 5, Beni Sweif 6, *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 which showed field response ranging from Tr S to 30 S at Itay El-Baroud location during the three seasons (table 4).

The last category is the fast rusting varieties which contained wheat varieties having infection type susceptible (S) and rust severity above 30%. This category contained eight wheat varieties; Sakha 69, Giza 160, Giza 163, Giza 164, Giza 167, Sids 1, Sakha 93 and Giza 139, which displayed field response from 40 S to 70 S at Itay El-Baroud. The check variety Giza 139 displayed the highest FRS(%), i.e. 70% during the three seasons (table 4).

***AUDPC*:** Large variations in AUDPC between the tested wheat genotypes reached from 0 to 840 during the three seasons. The 39 tested wheat genotypes were classified into three categories based on AUDPC values. The first

Table 4. FRS% and infection type (IT) of 39 wheat genotypes grown at Itay El-Baroud during 2016–17, 2017–18 and 2018–19 growing seasons.

Genotype	Season/FRS (%)		
	2016–17	2017–18	2018–19
Varieties with race specific resistance (complete resistance)			
Giza 171	0	Tr MR[a]	5 MR
Misr 3	0	0	5 R
Sohag 5	0	Tr MR	Tr MR
Genotypes with partial resistance (slow rusting resistance)			
Sakha 8	10 S	10 S	10 S
Giza 165	10 S	5 S	10 S
Gemmeiza 5	10 S	10 S	20 S
Giza 168	5 S	5 S	5 S
Gemmeiza 7	30 S	20 S	30 S
Gemmeiza 9	10 S	5 S	10 S
Gemmeiza 10	30 S	10 S	20 S
Gemmeiza 11	10 S	30 S	30 S
Sakha 94	10 S	5 S	10 S
Sids 12	10 S	10 S	10 S
Sids 13	Tr S	Tr S	5 S
Sids 14	5 S	Tr S	5 S
Misr 1	5 MS	5 MS	5 MS
Misr 2	5 MS	5 MS	5 MS
Gemmeiza 12	10 S	Tr S	10 S
Shandaweel 1	20 S	10 S	5 S
Sakha 95	Tr S	Tr S	10 S
Sohag 1	5 S	5 S	10 S
Sohag 3	5 S	5 S	10 S
Sohag 4	5 S	5 S	10 S
Beni Sweif 1	5 S	Tr S	20 S
Beni Sweif 4	10 S	Tr S	10 S
Beni Sweif 5	10 S	5 S	20 S
Beni Sweif 6	10 S	10 S	10 S
Lr 34	Tr S	Tr S	Tr S
Lr 46	10 S	5 S	10 S
Lr 67	5 S	Tr S	5 S
Lr 68	5 S	Tr S	5 S
Fast rusting varieties			
Sakha 69	50 S	50 S	60 S
Giza 160	40 S	50 S	70 S
Giza 163	50 S	30 S	60 S
Giza 164	50 S	40 S	60 S
Giza 167	40 S	40 S	60 S
Sids 1	70 S	50 S	60 S
Sakha 93	60 S	50 S	70 S
Giza 139 (check)	70 S	70 S	70 S
L.S.D at 5% of season (S)		0.5798	
L.S.D at 5% of genotypes (G)		2.561	
L.S.D at 5% of S × G		4.435	

[a]Infection types based on Roelfs *et al.* (1992); R, resistant without sporulation; MR, moderately resistant, small pustules surrounded by necrotic areas; MS, moderately susceptible, medium-sized pustules, no necrosis, but some chlorosis possible. S, susceptible, large pustules, no necrosis or chlorosis.

category is the varieties having race specific resistance which contained the wheat varieties that displayed the lowest AUDPC values. This category contained three wheat varieties: Giza 171, Misr 3 and Sohag 5 which displayed AUDPC values ranged from 0 to 49 during the three seasons (table 5).

The second category is genotypes having partial resistance, which displayed moderate AUDP values less than 300. This category contained 28 wheat genotypes: Sakha 8, Giza 165, Gemmeiza 5, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sakha 94, Sids 12, Sids 13, Sids 14, Misr 1, Misr 2, Gemmeiza 12, Shandaweel 1, Sakha 95, Sohag 1, Sohag 3, Sohag 4, Beni Sweif 1, Beni Sweif 4, Beni Sweif 5, Beni Sweif 6, *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 which displayed AUDPC values ranged from 42 to 280 during the three seasons (table 5).

The last category is fast rusting varieties which contained wheat varieties displayed high AUDP values

Table 5. AUDPC of 39 wheat genotypes grown at Itay El-Baroud during 2016–17, 2017–18 and 2018–19 growing seasons.

Genotype	Season/AUDPC		
	2016–17	2017–18	2018–19
Varieties with race specific resistance (complete resistance)			
Giza 171	0	42.0	49.0
Misr 3	0	0	49.0
Sohag 5	0	42.0	42.0
Genotypes with partial resistance (slow rusting resistance)			
Sakha 8	80.5	80.5	80.5
Giza 165	80.5	49.0	80.5
Gemmeiza 5	80.5	80.5	157.5
Giza 168	49.0	49.0	49.0
Gemmeiza 7	280.0	157.5	280.0
Gemmeiza 9	87.5	49.0	80.5
Gemmeiza 10	280.0	80.5	157.5
Gemmeiza 11	80.5	280.0	280.0
Sakha 94	80.5	49.0	80.5
Sids 12	80.5	80.5	80.5
Sids 13	42.0	42.0	49.0
Sids 14	49.0	42.0	49.0
Misr 1	49.0	49.0	49.0
Misr 2	49.0	49.0	49.0
Gemmeiza 12	80.5	42.0	80.5
Shandaweel 1	157.5	80.5	49.0
Sakha 95	42.0	42.0	80.5
Sohag 1	49.0	49.0	80.5
Sohag 3	49.0	49.0	80.5
Sohag 4	49.0	49.0	80.5
Beni Sweif 1	49.0	42.0	157.5
Beni Sweif 4	80.5	42.0	80.5
Beni Sweif 5	80.5	49.0	157.5
Beni Sweif 6	80.5	80.5	80.5
Lr 34	42.0	42.0	42.0
Lr 46	80.5	49.0	80.5
Lr 67	49.0	42.0	49.0
Lr 68	49.0	42.0	49.0
Fast rusting varieties			
Sakha 69	560.0	560.0	700.0
Giza 160	420.0	560.0	840.0
Giza 163	560.0	280.0	700.0
Giza 164	560.0	420.0	700.0
Giza 167	420.0	420.0	700.0
Sids 1	840.0	560.0	700.0
Sakha 93	700.0	560.0	840.0
Giza 139 (check)	840.0	840.0	840.0
L.S.D of season (S) at 5%		7.979	
L.S.D of genotype (G) at 5%		28.770	
L.S.D of S × G at 5%		49.830	

(more than 300). This category contained the eight wheat varieties; Sakha 69, Giza 160, Giza 163, Giza 164, Giza 167, Sids 1, Sakha 93 and Giza 139 which displayed AUDPC values ranged from 420 to 840 during the three seasons (table 5).

Molecular detection of the four leaf rust resistance genes; Lr 34, Lr 46, Lr 67 and Lr 68

Four specific markers linked with resistance genes; *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 were validated and used for the molecular detection of these four genes in the tested wheat genotypes (figures 1–4; table 6).

Amplification of Lr 34

Only 10 wheat varieties, i.e. Gemmeiza 12, Giza 171, Sids 14, Giza 167, Misr 3, Beni Sweif 1, Beni Sweif 4, Beni Sweif 5, Beni Sweif 6 and Sohag, of the 35 tested were screened for the first time with the csLV34 specific marker for *Lr* 34, since the other tested wheat varieties were previously screened with this marker (Fahmi *et al.* 2015). The

Table 6. Presence and absence of the four leaf rust resistance genes *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 in 35 tested wheat varieties using molecular marker analysis.

Variety	Leaf rust resistance gene				Number of genes*
	Lr 34	*Lr* 46	*Lr* 67	*Lr* 68	
Bread wheat					
Giza 139 (check)	−	−	−	−	0
Sakha 8	+	−	−	−	1
Sakha 69	−	−	−	−	0
Giza 160	−	−	−	−	0
Giza 163	−	−	−	−	0
Giza 164	−	−	−	−	0
Giza 165	−	+	−	−	1
Giza 167	−	−	−	−	0
Giza 171	−	+	−	−	1
Sids 1	−	−	−	−	0
Gemmeiza 5	−	−	+	−	1
Giza 168	−	+	+	+	3
Gemmeiza 7	−	−	+	−	1
Gemmeiza 9	−	+	−	−	1
Sakha 93	−	−	−	−	0
Gemmeiza 10	−	+	−	−	1
Sakha 94	+	−	−	−	1
Sids 12	−	+	−	−	1
Sids 13	+	−	−	−	1
Sids 14	−	−	−	−	0
Misr 1	−	−	+	+	2
Misr 2	−	−	+	+	2
Misr 3	+	−	+	+	3
Gemmeiza 11	−	+	−	−	1
Gemmeiza 12	−	−	+	−	1
Shandaweel 1	+	−	−	−	1
Sakha 95	+	−	−	−	1
Durum wheat					
Sohag 1	−	+	−	−	1
Sohag 3	−	+	−	−	1
Sohag 4	−	+	−	−	1
Sohag 5	−	−	−	−	0
Beni Sweif 1	−	+	−	−	1
Beni Sweif 4	−	−	−	−	0
Beni Sweif 5	−	−	−	−	0
Beni Sweif 6	−	−	−	−	0

(+) Fragment is amplified and the gene is present; (−) no specific fragment is amplified and the gene is absent.
*Number of genes detected in that particular variety.

reason for screening all the 35 tested wheat varieties with this marker was to confirm the molecular markers results for this gene. This marker amplified two fragments of 150 and 229 bp in positive and negative controls, respectively. The positive 150 bp fragment was amplified on *Lr* 34 (lane 1) and the six wheat varieties, i.e. Sakha 8, Sakha 94, Sids 13, Misr 3, Shandaweel 1 and Sakha 95, indicated that these varieties have leaf rust resistance gene *Lr* 34. While, the other tested varieties showed the 229 bp fragment, indicating the absence of *Lr* 34 in these wheat varieties (figure 1; table 6).

Amplification of Lr 46

The Xgwm259 marker linked to *Lr* 46 showed one specific fragment with PCR product size 105 bp. Of the 35 tested wheat varieties, only 11, i.e. Giza 165, Giza 171, Giza 168, Gemmeiza 9, Gemmeiza 10, Sids 12, Gemmeiza 11, Sohag 1, Sohag 3, Sohag 4 and Beni Sweif 1, showed the 105 bp fragment, this confirmed that these varieties have *Lr* 46. While, the other tested wheat varieties did not show 105 bp fragment, indicating absence of *Lr* 46 in these varieties (figure 2; table 6).

Amplification of Lr 67

The CFD71 marker for *Lr* 67 showed one specific fragment with PCR product size 198 bp. Only seven wheat varieties, i.e. Gemmeiza 5, Giza 168, Gemmeiza 7, Misr 1, Misr 2, Misr 3 and Gemmeiza 11, of the tested varieties showed 198 bp fragment. This result indicated that these seven varieties proved to have *Lr* 67. In contrast, the

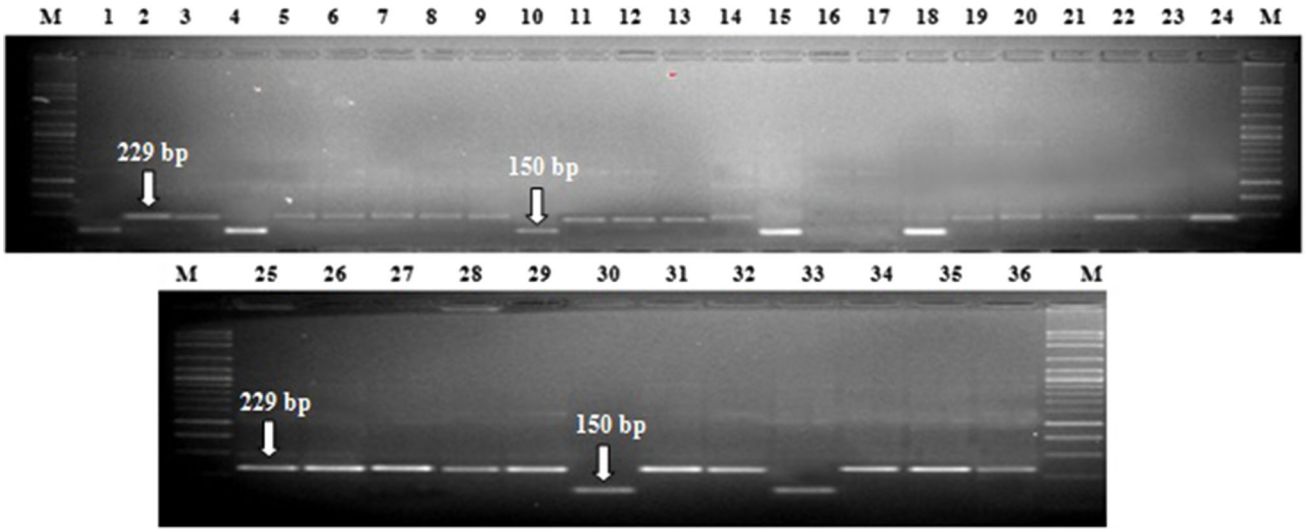

Figure 1. Amplification products using specific marker for *Lr* 34 (*csLv34*) in the 35 wheat varieties. M, 100-bp ladder; 1, *Lr* 34; 2, Sids 1; 3, Sids 12; 4, Sids 13; 5, Gemmeiza 9; 6, Gemmeiza 10; 7, Gemmeiza 11; 8, Gemmeiza 12; 9, Sakha 93; 10, Sakha 94; 11, Misr 1; 12, Misr 2; 13, Giza 168; 14, Giza 171; 15, Shandweel 1; 16, Gemmeiza 7; 17, Sids 14; 18, Sakha 95; 19, Beni Sweif 1; 20, Beni Sweif 4; 21, Beni Sweif 5; 22, Beni Sweif 6; 23, Sohag 3; 24, Giza 139; 25, Giza 160; 26, Giza 163; 27, Giza 164; 28, Giza 165; 29, Giza 167; 30, Sakha 8; 31, Sakha 69; 32, Gemmeiza 5; 33, Misr 3; 34, Sohag 1; 35, Sohag 4; 36, Sohag 5.

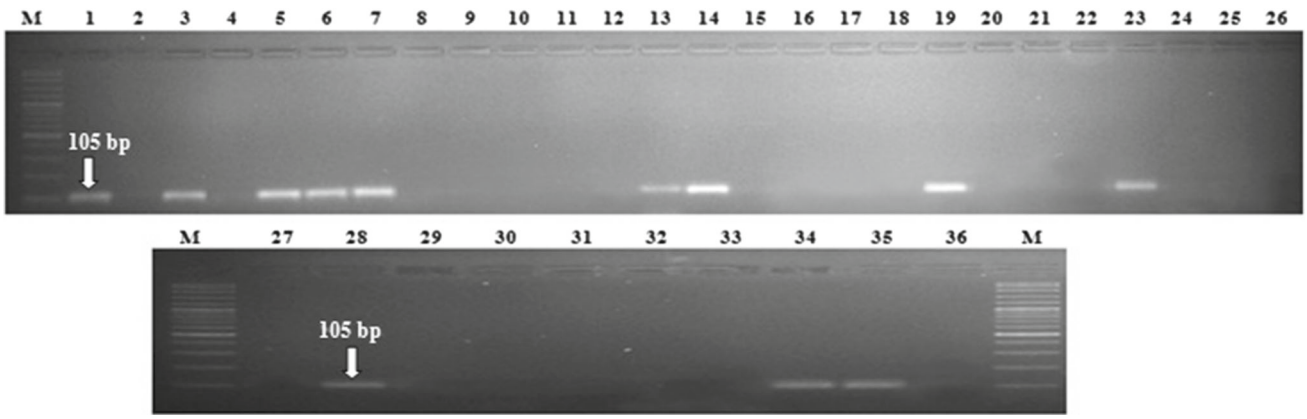

Figure 2. Amplification products using specific marker for *Lr* 46 (*Xgwm259*) in the 35 wheat varieties. M, 100-bp ladder; 1, *Lr* 34; 2, Sids 1; 3, Sids 12; 4, Sids 13; 5, Gemmeiza 9; 6, Gemmeiza 10; 7, Gemmeiza 11; 8, Gemmeiza 12; 9, Sakha 93; 10, Sakha 94; 11, Misr 1; 12, Misr 2; 13, Giza 168; 14, Giza 171; 15, Shandweel 1; 16, Gemmeiza 7; 17, Sids 14; 18, Sakha 95; 19, Beni Sweif 1; 20, Beni Sweif 4; 21, Beni Sweif 5; 22, Beni Sweif 6; 23, Sohag 3; 24, Giza 139; 25, Giza 160; 26, Giza 163; 27, Giza 164; 28, Giza 165; 29, Giza 167; 30, Sakha 8; 31, Sakha 69; 32, Gemmeiza 5; 33, Misr 3; 34, Sohag 1; 35, Sohag 4; 36, Sohag 5.

other wheat varieties in this study did not show 198 bp fragment, revealing that the resistance gene Lr 67 could not be detected in these varieties (figure 3; table 6).

Amplification of Lr 68

Molecular marker analysis indicated that the csGS specific marker for *Lr* 68 showed one specific fragment with PCR product size 385 bp. Of the 35 tested wheat varieties, only four, i.e. Misr 1, Misr 2, Misr 3 and Giza 168 showed 385 bp fragment. This indicated that these varieties have *Lr* 68. While, the other tested wheat varieties did not have this fragment, indicating the absence of *Lr* 68 in these varieties (figure 4; table 6).

Discussion

Host-genetic resistance and/or release of wheat cultivars with sustainable resistance to rust diseases, especially leaf rust are still the viable strategy to successfully control these dangerous diseases. However, two main kinds of genetic resistance to rust pathogens have been usually applied and utilized in most of the wheat breeding programmes world-wide. The first kind is the complete resistance, race-specific

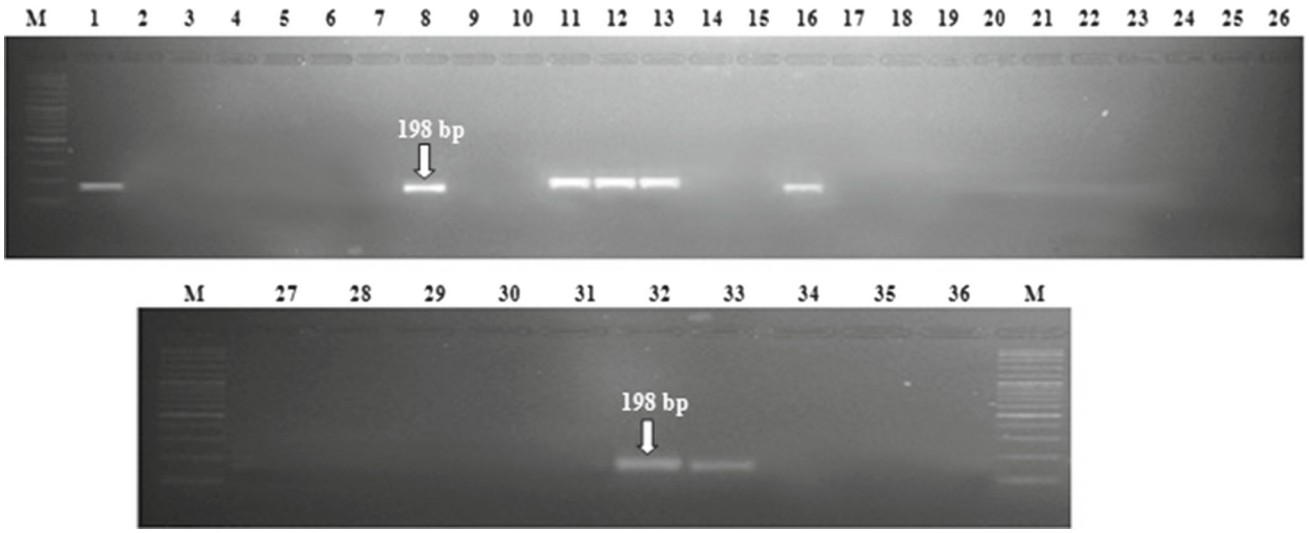

Figure 3. Amplification products using specific marker for *Lr* 67 (*CFD71*) in the 35 wheat varieties. M, 100-bp ladder; 1, *Lr* 34; 2, Sids 1; 3, Sids 12; 4, Sids 13; 5, Gemmeiza 9; 6, Gemmeiza 10; 7, Gemmeiza 11; 8, Gemmeiza 12; 9, Sakha 93; 10, Sakha 94; 11, Misr 1; 12, Misr 2; 13, Giza 168; 14, Giza 171; 15, Shandweel 1; 16, Gemmeiza 7; 17, Sids 14; 18, Sakha 95; 19, Beni Sweif 1; 20, Beni Sweif 4; 21, Beni Sweif 5; 22, Beni Sweif 6; 23, Sohag 3; 24, Giza 139; 25, Giza 160; 26, Giza 163; 27, Giza 164; 28, Giza 165; 29, Giza 167; 30, Sakha 8; 31, Sakha 69; 32, Gemmeiza 5; 33, Misr 3; 34, Sohag 1; 35, Sohag 4; 36, Sohag 5.

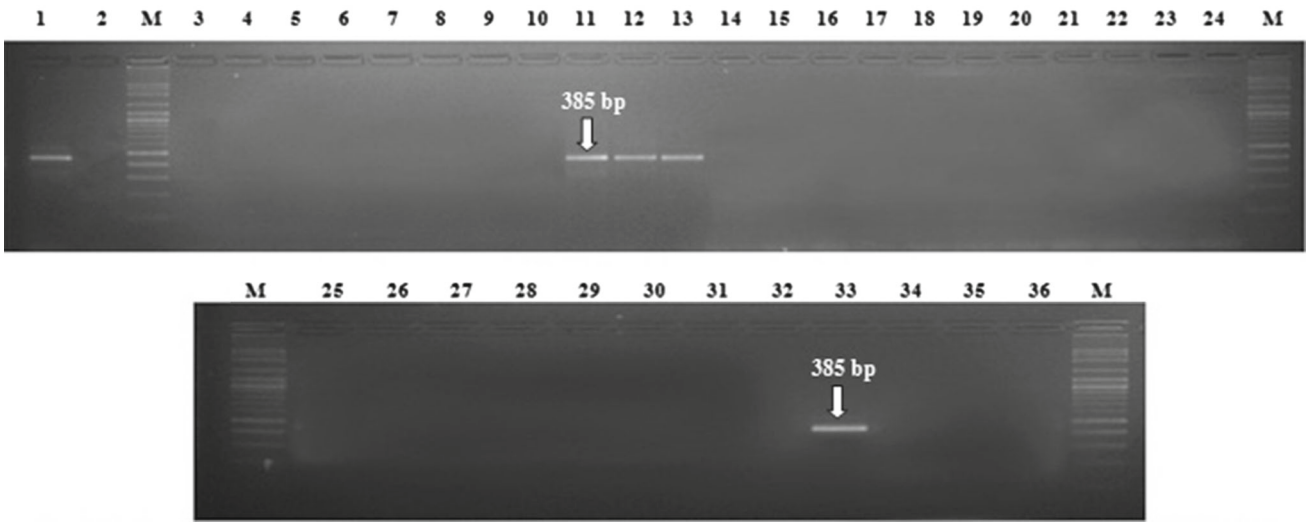

Figure 4. Amplification products using specific marker for *Lr* 68 (*csGS*) in the 35 wheat varieties. M, 100-bp ladder; 1, *Lr* 34; 2, Sids 1; 3, Sids 12; 4, Sids 13; 5, Gemmeiza 9; 6, Gemmeiza 10; 7, Gemmeiza 11; 8, Gemmeiza 12; 9, Sakha 93; 10, Sakha 94; 11, Misr 1; 12, Misr 2; 13, Giza 168; 14, Giza 171; 15, Shandweel 1; 16, Gemmeiza 7; 17, Sids 14; 18, Sakha 95; 19, Beni Sweif 1; 20, Beni Sweif 4; 21, Beni Sweif 5; 22, Beni Sweif 6; 23, Sohag 3; 24, Giza 139; 25, Giza 160; 26, Giza 163; 27, Giza 164; 28, Giza 165; 29, Giza 167; 30, Sakha 8; 31, Sakha 69; 32, Gemmeiza 5; 33, Misr 3; 34, Sohag 1; 35, Sohag 4; 36, Sohag 5.

resistance or monogenic resistance that is qualitatively inherited and is usually conferred by a major dominant gene, which renders the host incompatible with the causal pathogen. While, the other kind is partial or incomplete resistance (PR), race nonspecific resistance or polygenic resistance that is conferred by multiple genes of resistance with a minor and additive effect, and is therefore, inherited in a quantitative style (Parlevliet 1979). However, the levels of such resistance are usually less distinct and progress of disease on wheat plants usually are measured quantitatively (parameters) to characterize more accurately this kind of resistance under field conditions.

PR to wheat leaf rust has been previously identified by decreasing rate of an epidemic progress under field conditions despite the susceptible infection type of the host plant (Broers 1989). Moreover, PR resulted from a longer latent period, lower receptivity, smaller pustule size, and lower spore production and also, can be characterized under

field conditions by lower FRS (%) and smaller AUDPC compared with a highly susceptible check variety (Parlevliet 1979). El-Orabey (2018) classified the wheat cultivars into two different sets depending on the values of AUDPC. In the first group, cultivars showed AUDPC values up to 30%, and in the second one, cultivars showed AUDPC values more than 30%. The cultivars in the first group were seen as expressing good levels of slow rusting and in the second group had fast rusting cultivars. Also, Parlevliet (1979) showed that wheat lines with variable field infection responses of MS to S are expected to possess genes that confer partial resistance.

In the present study, 35 wheat varieties and four monogenic lines were tested under field conditions for three growing seasons; 2016–17, 2017–18 and 2018–19 at Itay El-Baroud and using two parameters, FRS(%) and AUDPC. The wheat genotypes under study were divided into three main categories. The first category included wheat varieties having race-specific resistance (complete resistance), the second category is genotypes with partial resistance (slow rusting resistance) and the last group is fast rusting varieties. The Three wheat varieties, Giza 171, Misr 3 and Sohag 5 were in the first category, i.e. race-specific resistance (complete resistance) and exhibited least values of FRS (%) (0–5%) and least values of AUDPC (0–49). Resistance of these three wheat varieties were mainly because of the presence of major gene(s). Niks and Dekens (1991) reported that complete resistance (race-specific resistance) is usually governed by major gene(s) of resistance. Moreover, German and Kolmer (1992) revealed that association of leaf rust major genes resistance in a wheat cultivar provided a great level of resistance and enhanced the resistance of this cultivar.

The 24 wheat cultivars in the second category, which displayed a low FRS (%) values (Tr S–30 S), infection type susceptible and low AUDPC values (smaller than 300) have a high degree of PR. The wheat varieties in both first and second categories could be used as good sources for leaf rust resistance in breeding programmes. Shahin and El-Orabey (2015) reported that the two wheat cultivars Gemmeiza 9 and Giza 168 displayed partial resistance to wheat leaf rust and exhibited low values of FRS (%) and AUDPC, this was mainly because of the presence of *Lr* 46. While, the eight varieties in the last category were fast rusting varieties which showed the highest values of FRS (%) and AUDPC. Previous report by Fahmi *et al.* (2015) found that the wheat varieties Giza 139 and Sids 1 were fast rusting varieties.

Generally, three methods, i.e. gene postulation, genetic analysis and recently used molecular markers were used for the detection of resistance genes to rust diseases in wheat cultivars especially leaf rust. The two conventional methods for detecting *Lr* genes were exhausting and slightly expensive. While, the molecular markers in comparison with the two conventional methods is time saving and the results were obtained rapidly. At present, the individual *Lr* gene(s) can be detected using specific molecular markers, namely *csL*V34 marker which was used to detect *Lr* 34 (Lagudah *et al.* 2006), *Xgwm*259 marker used for detecting *Lr* 46 (William *et al.* 2003), CFD71 marker used for detecting *Lr* 67 (Hiebert *et al.* 2010), while the csGS marker was used for detecting *Lr* 68 (Herrera-Foessel *et al.* 2012).

Molecular characterization of the four leaf rust genes; *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 revealed that the presence of *Lr* 46 in the wheat cultivar Giza 171 and genes, *Lr* 34, *Lr* 67 and *Lr* 68 in Misr 3. According the concept of partial resistance by decreasing the rate of an epidemic progress in the field in spite of a susceptible infection type (Broers 1989). These two wheat varieties; Giza 171 and Misr 3 in the first category, which showed infection type resistance or moderately resistance possess partial resistance genes and the response of these cultivars are resistance and these resistance may be result from major gene (s) that covered the reaction of partial resistance. After overcoming these major genes by new leaf rust pathotypes, these slow rusting genes will express (Niks and Dekens 1991; Fahmi *et al.* 2015).

The four wheat varieties, Sids 14, Beni Sweif 4, Beni Sweif 5 and Beni Sweif 6 in the second category partial resistance varieties which displayed low FRS(%) and AUDPC values did not possess any of the four genes *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68. The resistance in these varieties may have resulted from the existence of some minor genes or one of the newly characterized slow rusting resistance genes *Lr* 75, *Lr* 77 and *Lr* 78 (Pinto da Silva *et al.* 2018). Imbaby *et al.* (2014) evaluated 15 Egyptian wheat varieties at adult plant stage and found that the leaf rust response of the tested wheat varieties ranged from 10 MR/MS to 90 S, and also they detected *Lr* 34 using a specific primer for this gene. They found that the *Lr* 34 was present in all the tested wheat varieties although some of these varieties showed high level of rust severity which reached upto 90 S. This may be due the specific primer used in this study that is not specific to *Lr* 34 or the optimization and adjustment of the PCR protocol was not followed.

Absence of *Lr* 34 and *Lr* 67 genes from the seven durum varieties, i.e. Sohag 1, Sohag 3, Sohag 4, Beni Sweif 1, Beni Sweif 4, Beni Sweif 5 and Beni Sweif 6 mostly resulted from the lack of genome D of these varieties and the two genes located on chromosome D; *Lr* 34 found on chromosome 7DS (Dyck 1987) and *Lr* 67 found on chromosome 4DL (Moore *et al.* 2015). The response of wheat varieties Misr 1, Misr 2 and Giza 168 was very low, this is because of the combinations of three genes in Giza 168, i.e. *Lr* 46, *Lr* 67 and *Lr* 68, and a combinations of two genes in each of the two varieties Misr 1 and Misr 2, i.e. *Lr* 67 and *Lr* 68. Similar results were previously obtained by Herrera-Foessel *et al.* (2012), as they found that each of the two resistance genes; *Lr* 68 and *Lr* 34 is found alone in any wheat genotype, smaller resistance will occurred than their present in combination, which suggested additive effect of

these genes. Moreover, resistance of wheat verities have *Lr* 34, i.e. Sakha 8, Sakha 94, Sakha 95, Sids 13 and Shandweel 1 is stronger than other wheat verities have each of *Lr* 46, *Lr* 67 and *Lr* 68. The response of these cultivars varied from Tr S to 10 S. Herrera-Foessel *et al.* (2012) compared the effectiveness of each of the four genes; *Lr* 34, *Lr* 46 *Lr* 67 and *Lr* 68 during three seasons, i.e. 2008–09, 2009–10, 2010–11 at Ciudad Obregon, Mexico. They reported that the *Lr* 68 effect was smaller compared to each of *Lr* 34, *Lr* 46 and *Lr* 67 effect during 2010–11. However, the effect of *Lr* 68 was a stronger resistance response than *Lr* 46. Similar results was seen in Mexico that the *Lr* 68 effect was smaller than each of *Lr* 34 and *Lr* 46 effect (Lillemo *et al.* 2011).

Finally, wheat breeders should select the wheat genotypes having slow rusting resistance in breeding programme for rust resistance because the rust pathogens can easily change their virulence by mutation, selection pressure and migration, and then overcome resistant cultivars. Therefore, in the following studies, researchers have to no longer emphasize on race-precise resistance.

In conclusion, wheat genotypes under study showed wide dissimilarities in their levels of leaf rust resistance. Most of these genotypes showed good performance at adult stage during the three growing seasons of the study compared to the susceptible check variety (Giza 139) at the Itay El-Baroud location. The three wheat varieties Giza 171, Misr 3 and Sohag 5 showed complete resistance. Twenty-four wheat genotypes of the 39 displayed the lowest FRS (%) values (<30% with moderately susceptible and susceptible infection type) and lowest AUDPC values less than 300, implies the presence of high levels of slow rusting resistance. These wheat varieties can be used in the future breeding programmes as a source of resistance to slow rusting leaf rust resistance.

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