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Leaf Rust Resistance and Molecular Identification of *Lr 34* Gene in Egyptian Wheat

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Abstract

Twenty six wheat genotypes were evaluated for their levels of adult plant resistance to leaf rust at two different locations i.e. Shibin El-Kom and Itay El-Baroud during two successive growing seasons, i.e., 2011/12 and 2012/13. The wheat varieties Sids 12, Sids 13, Misr 1 and Misr 2, Shandweel 1, Beni Sweif 4 and Beni Sweif 5 have race-specific resistance. While, the wheat genotypes *Lr* 34, Giza 165, Giza 168, Sakha 8, Sakha 94, Sakha 95, Gemmeiza 5, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11 and Sohag 3 showed high levels of slow rusting resistance. On the other hand, the other tested varieties were highly susceptible or fast rusting ones i.e. Giza 160, Giza 163, Giza 164, Sakha 69, Sakha 93, Sids 1 and Giza 139. These varieties showed higher values of final rust severity (%) and AUDPC compared to the other varieties under the same field conditions. SSR marker i.e. cslv34b allele (150 bp) for *Lr* 34 was used to confirm the presence of the adult plant resistance gene *Lr* 34 in the tested genotypes.

Keywords: Wheat; *Puccinia triticina*; AUDPC; Partial resistance; Molecular markers

Introduction

Within the last twenty years, wheat has become the most important crop in Egypt. Egypt seeks to increase productivity and yields in order to meet the target of producing 75% of its own wheat needs [1]. Leaf rust or brown rust caused by *Puccinia triticina* (formerly known as *Puccinia recondita* f. sp. *tritici*) has been the most frequent disease in wheat producing areas [2]. Studies in Egypt estimated crop losses of up to 50% due to leaf rust infection [3].

The cultivation of resistant varieties remains the most economic and environmentally preferable method to manage this disease. To date, 80 genes and alleles of leaf rust resistance genes in wheat have been mapped to chromosome location and given gene designations [4]. Some of the resistance genes are effective at seedling stage and they are race specific [5]. Several of these genes may become ineffective due to the emergence of new virulent races and also because of rapid evolution and adaptation of pathogen [6]. In contrast, others are effective through the adult plant stage and are referred to as slow rusting genes and they are race non-specific provide durable resistance or a broad spectrum of races. Therefore, a cultivar that only has slow rusting resistance to leaf rust will display susceptible infection type response throughout the entire lifecycle of the plant [7]. Slow rusting resistance can be measured in the field by recording disease severity at weekly intervals and then calculating the area under disease progress curve (AUDPC) [8].

One of these important race-nonspecific resistance genes is *Lr* 34. It is located on the short arm of chromosome 7D and it encodes ATP binding cassette (ABC) transporter [9]. Also, it is associated with leaf tip necrosis, adult plant resistance to stem rust, adult plant resistance gene *Yr* 18 to stripe rust, and tolerance to barley yellow dwarf virus [10].

Efficient incorporation of *Lr* 34 in adapted germplasm using traditional methods was difficult because of its quantitative inheritance nature. Thus, using of molecular marker technique is the best alternative methodology to identify and consequently to incorporate this important gene in economically important genotypes. Available information about *Lr* 34 gene sequence provided good tool to develop and to track its introgression in different genotypes and consequently its pyramiding in commercial varieties [9]. Therefore, development of molecular marker for *Lr* 34/7DS region has been a major objective for marker assist selection (MAS) for this important gene. [11] were able to utilize the available knowledge about this locus to develop a specific codominant marker namely; *csLV*34. This marker had the ability to diagnose the *Lr* 34 gene in diverse cultivar backgrounds [12]. It revealed a bi-allelic nature where 79 bp deletions in an intron sequence were accompanied by the presence of *Lr* 34 gene resistance. Several other markers that differentiate among the alleles of *Lr* 34 have been described [9,13].

Because of the superiority of molecular markers in MAS for genes in different genetic background even in highly bred cultivar under any environmental conditions, different types of molecular markers based on genetic variations have been developed in Egyptian wheat cultivars [14-17]. Also, haplotype polymorphism among Egyptian wheat varieties for *Lr* 34/7DS region had been studied using microsatellite markers [16]. Therefore, the objectives of the present investigations were: (1) to evaluate Egyptian wheat varieties to leaf rust at adult plant stage under field conditions and to identify the presence of *Lr* 34 gene with *csLV*34 specific marker in Egyptian wheat varieties.

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Materials and Methods

Plant materials

Wheat varieties (Table 1) and *Lr* 34 line were evaluated under field conditions at two locations namely; farm of the Faculty of Agriculture, Menoufiya University, Shibin El-Kom and Itay El-Baroud Agricultural Research Station, Egypt, for two successive seasons (2011/12 and 2012/13).

Field inoculation of wheat genotypes and disease assessment

The tested wheat genotypes were sown in rows and were surrounded by spreader plants (Morocco and Thatcher) which were moisture by a fine spray with water and dusted with a mixture of leaf rust urediniospores and talcum powder at the ratio of. 1:20 (v/v). The inoculation of all plants was carried out at botting stage [18]. Adult plant response was scored as rust severity (%) for each genotype after disease on set till the early dough stage according to the scale proposed by [19]. Rust severity of each genotype was recorded every seven days after the appearance of initial infection, using the modified Cobbs scale [20]. Final rust severity (FRS) was recorded as outlined by [21], as disease severity (%) when the highly susceptible check variety was severely rusted and the disease rate reached the highest severity. Also, area under disease progress curve (AUDPC) was estimated to compare different responses of the tested genotypes using the following equation:

AUDPC= D [1/2 (Y1+Yk) + Y2 + Y3 + ……..Y(k-1)] as described by [22], where;

D = days between reading, Y1=First disease recording and Yk=Last disease recording

DNA extraction and PCR reaction

Young leaves were collected from two-week old plants of all genotypes and were subjected to CTAB protocol for genomic DNA Extraction, which is based on method of [23]. DNA concentration was estimated and used as PCR template. DNA samples were visualized on 1-2% agarose. Polymerase chain reaction (PCR) was conducted to detect specific *Lr* 34 gene fragment using specific primer namely; *Lr*csLV34 as described in [11]. The sequence of the forward primer is 5`GTTGGTTAAGACTGGTGATGG3`and the reverse primer is 5`TGCTTGCTATTGCTGAATAGT3`. Polymerase Chain Reaction (PCR) was undertaken in 50 µL total volume containing 5 µL of 10X PCR buffer, 4 µL (25 mM $MgCl_2$), 1 µL (10 ng) of DNA, 1 µL (100 ng, 125 picomole) of primer (forward and reverse), 1 unite of Taq DNA polymerase. PCR amplification conditions were initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, annealing at 55°C for 30 s for 35 cycles, extension at 72°C 1 min, and final extension at 72°C for 5 min. The PCR products were analyzed by electrophoretic separation in a 1-2% agarose gel. DNA marker of 100 bp DNA ladder marker was added on one side of the gel to determine the size of the DNA pattern. Gel was stained with ethidium bromide.

Microsoft Excel 2010 (Microsoft Corporation, USA) computer program was used to draw the standard curve and to estimate fragment size.

Variety	Pedigree	Year of release
Bread wheat varieties		
Giza 139 (check)	HINDI90/KENYA256G.	1947
Sakha 8	Indus 66 x 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
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
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
Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	2011
Shandaweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH	2011
Sakha 95	SKAUZ*2_SRMA-CMBW91MO2694P-0T0PY-7M-010Y -010M-010Y-5	
Variety	Pedigree	Year of release
Durum wheat varieties		
Sohag 3	MEXI"S"/MGHA/51792//DURUM6. CD21831-25H-1SH-0SH.	1991
Beni Swear 4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/W*2. ICD88-1120-ABL-0TR-1BR-0TR-6AP-OSD.	2007
Beni Swear 5	DIPPERZ/BUSHEN3. CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD.	2007
Monogenic line		
Lr 34	Thatcher[6] x Lageadinho	1982

Table 1: List of the local wheat varieties that were used, pedigree and year of release.

Results

To assess leaf rust disease resistance of some Egyptian wheat varieties, final rust severity (FRS %) and area under disease progress curve (AUDCP) were determined.

Season 2011/12

Data presented in Table 2 showed that, the tested genotypes could be classified into three main groups on the basis of FRS (%) and AUDPC values. The first group included the wheat varieties with race-specific resistance which displayed the lowest values of FRS (%) and AUDPC. This group included the wheat varieties Sids 12, Misr 1, Misr 2, Shandaweel 1, Beni Sweif 4 and Beni Sweif 5 which were immune and showed zero percent rust severity and Sids 13 (Tr MR) at Shibin El-Kom location. Meanwhile, at Itay El-Baroud location the wheat varieties Shandaweel 1 and Beni Sweif 5 showed zero percent rust severity, Sids 12, Sids 13, Misr 1 and Beni Sweif 4 (each with Tr MR) and Misr 2 (5 MR) showed the lowest values of FRS (%). Moreover, these varieties showed the lowest values of AUDPC ranged from 0 to 49 at the two locations. The second group included the wheat genotypes which displayed low values of FRS (%) and AUDPC (less than 300). Therefore, they were characterized as slow rusting varieties or partially resistant varieties. This group included the wheat genotypes *Lr* 34 and Sohag 3 (each with Tr MS), Sakha 94 (Tr S), Sakha 8, Sakha 95, Gemmeiza 9, Gemmeiza 10 and Gemmeiza 11 (each with 5 S), Giza 168 (10 S), Giza 165 and Gemmeiza 5 (each with 20 S) and Gemmeiza 7 (30 S) at Shibin El-Kom location. While, at Itay El-Baroud location *Lr* 34 (5 MS), Sakha 94 (5 S), Gemmeiza 10 (5 S), Sohag 3 (5 S), Giza 168 (10 S), Sakha 8 (10 S), Sakha 95 (10 S), Gemmeiza 9 (10 S), Gemmeiza 11 (10 S), Gemmeiza 7 (20 S), Giza 165 (30 S) and Gemmeiza 5 (30 S). Moreover, the wheat varieties in this group showed the lowest values of AUDPC at the two locations Shibin El-Kom and Itay El-Baroud ranged from 49 to 192.5. The third group included the wheat varieties which revealed the highest values of FRS (%) and AUDPC than partially resistant ones (more than 300) and were identified as the fast rusters. This group included the wheat varieties Giza 160 (30 S), Giza 163 (30 S), Giza 164 (30 S), Sakha 93 (50 S), Sakha 69 (60 S), Giza 139 (60 S) and Sids 1 (70 S) at Shibin El-Kom location. While, at Itay El-Baroud location vars. Giza 164 (30 S), Sakha 69 (30 S), Giza 160 (40 S), Giza 163 (40 S), Sakha 93 (60 S), Sids 1 (80 S) and Giza 139 (90 S) showed the highest values of FRS (%). Moreover, the wheat varieties Giza 160 (315), Giza 164 (315), Giza 163 (385), Sakha 69 (385), Sakha 93 (560), Sids 1 (560) and Giza 139 (700) showed high values of AUDPC at Shibin El-Kom location. While, at Itay El-Baroud these wheat varieties showed highest values of AUDPC i.e. 315, 315, 420, 420, 560, 630 and 840, respectively.

Genotype	FRS (%)		AUDPC	
	Shebin El-Kom	Itay El-Baroud	Shebin El-Kom	Itay El-Baroud
Group I: Varieties with race specific resistance				
Sids 12	0	Tr MR	0	42.0
Sids 13	Tr MR	Tr MR	42.0	42.0
Misr 1	0	Tr MR	0	42.0
Misr 2	0	5 MR	0	49.0
Shandaweel 1	0	0	0	0
Beni Sweif 4	0	Tr MR	0	42.0
Beni Sweif 5	0	0	0	0
Group 2: Varieties with slow rusting resistance				
Lr 34	Tr MS	5 MS	42.0	49.0
Giza 165	20 S	30 S	157.5	192.5
Giza 168	10 S	10 S	66.5	66.5
Sakha 8	5 S	10 S	49.0	66.5
Sakha 94	Tr S	5 S	42.0	49.0
Sakha 95	5 S	10 S	56.0	66.5
Gemmeiza 5	20 S	30 S	157.5	192.5
Gemmeiza 7	30 S	20 S	192.5	157.5
Gemmeiza 9	5 S	10 S	49.0	80.5
Gemmeiza 10	5 S	5 S	49.0	49.0
Gemmeiza 11	5 S	10 S	49.0	80.5
Sohag 3	Tr MS	5 S	42.0	49.0
Group 3: Varieties with fast rusting resistance				
Giza 160	30 S	40 S	315.0	315.0
Giza 163	30 S	40 S	385.0	420.0
Giza 164	30 S	30 S	315.0	315.0
Sakha 69	60 S	30 S	385.0	420.0
Sakha 93	50 S	60 S	560.0	560.0
Sids 1	70 S	80 S	560.0	630.0
Giza 139 (check)	70 S	90 S	700.0	840.0

Table 2: Percentage final leaf rust severity (FRS %) and area under disease progress curve (AUDPC) of 26 wheat genotypes grown at Shebin El-Kom and Itay El-Baroud locations during 2011/12 growing season.

Season 2012/13

Results given in Table 3 showed that the tested wheat varieties showed different levels of final rust severity (%) ranging from 0 to 70 S at Shibin El-Kom location and from 0 to 80 S at Itay El-Baroud location. According to the response of the tested genotypes, they were divided into the same three groups of Table 2. **1.** Race-specific resistant varieties: These varieties showed FRS (%) from 0 to 5 MR which included the wheat varieties Shandaweel 1, Beni Sweif 5, Sids 12, Sids 13, Misr 1, Misr 2 and Beni Sweif 4 at the two location Shibin El-Kom location and Itay El-Baroud. Meanwhile, these wheat varieties showed lowest values of AUDPC ranged from 0 to 49 at two locations. **2.** Genotypes with slow rusting resistance which included the wheat genotypes Gemmeiza 11, *Lr* 34, Giza 168, Sakha 8, Sakha 94, Gemmeiza 9, Gemmeiza 10, Sohag 3, Giza 165, Sakha 95, Gemmeiza 7 and Gemmeiza 5 which showed low levels of FRS (%) at Shibin El- Kom location i.e. Tr S, 5 MS, 5 S, 5 S, 5 S, 5 S, 5 S, 5 S, 10 S, 10 S, 20 S and 30 S, respectively. While, at Itay El-Baroud location the wheat varieties Sakha 94 (5 S), Gemmeiza 9 (5 S), Gemmeiza 11 (5 S), Sohag 3 (5 S), *Lr* 34 (10 S), Giza 168 (10 S), Sakha 8 (10 S), Sakha 95 (10 S), Gemmeiza 10 (10 S), Giza 165 (20 S), Gemmeiza 5 (30 S) and Gemmeiza 7 (30 S) showed low values of FRS (%). Moreover, the wheat varieties in this group showed low values of AUDPC at the two locations ranged from 49 to 192.5. **3.** Fast rusting varieties including the wheat varieties Giza 160 (40 S), Giza 163 (40 S), Giza 164 (40 S), Sakha 69 (40 S), Sakha 93 (60 S), Sids 1 (60 S) and Giza 139 (70 S) which showed the highest values of FRS (%) at Shibin El-Kom location. While, at Itay El-Baroud location the wheat varieties Giza 164 (30 S), Giza 163 (40 S), Sakha 69 (40 S), Giza 160 (50 S), Sakha 93 (60 S), Sids 1 (70 S) and Giza 139 (80 S) showed the highest values of FRS (%). Moreover, the tested varieties in this group showed the highest values of AUDPC at the two locations ranged from 315 to 700 at Shibin El-Kom. While, at Itay El-Baroud the tested wheat varieties showed the highest values of AUDPC ranged from 315 to 840.

Molecular marker detection

For further resistance evaluation of the wheat genotypes under investigation, the presence of the *Lr* 34 was investigated. The STS marker for *Lr* 34 gene was used to identify the presence of the resistance allele in genotypes under study. The *csLV*34 is a PCR-based marker and it was mapped 0.4 cM from this gene and validated in many genotypes from different parts of the world [11]. In other words, this marker is capable of differentiating among lines with/out this gene. The *csLV*34 primer amplified two fragments of 150 and 229 bp in positive and negative controls, respectively. The *csLV*34a allele (229 bp) was detected in the check cultivar Giza 139 and *csLV*34b allele (150 bp) was detected in the near isogenic line Thatcher *Lr* 34 (Figure 1). Five wheat varieties i.e. Sakha 8, Sakha 94, Sakha 95, Sids 13 and Shandweel 1 showed *csLV*34b allele. Based on the amplification of the 150 bp fragment, these varieties were identified to carry *Lr* 34. While the wheat varieties Sakha 69, Sakha 93, Misr 1, Misr 2, Giza 160, Giza 163, Giza 164, Giza 165, Giza 168, Gemmeiza 5, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sids 1, Sids 12 and Sids 13 showed *csLV*34a allele. This result indicated the absence of *Lr* 34 in these varieties. At the same time, durum wheat varieties showed the *cslV*34a allele indicated the absence of *Lr* 34 (Figures 2 and 3) in addition of large fragment which it was unrelated to the gene *Lr* 34. However, the diploid *Aegilops tauschii* the progenitor of D genome in cultivated wheat was tested and the presence of *csLV*34a allele was demonstrated.

Genotype	FRS (%)		AUDPC	
	Shebin El-Kom	Itay El-Baroud	Shebin El-Kom	Itay El-Baroud
Group I: Varieties with race specific resistance				
Sids 12	Tr MR	Tr MR	42.0	42.0
Sids 13	Tr MR	Tr MR	42.0	42.0
Misr 1	Tr MR	5 MR	42.0	49.0
Misr 2	Tr MR	5 MR	42.0	49.0
Shandaweel 1	0	0	0	0
Beni Sweif 4	Tr MR	Tr MR	42.0	42.0
Beni Sweif 5	0	0	0	0
Group 2: Varieties with slow rusting resistance				
Lr 34	5 MS	10 S	49.0	80.5
Giza 165	10 S	20 S	80.5	157.5
Giza 168	5 S	10 S	49.0	66.5
Sakha 8	5 S	10 S	49.0	66.5
Sakha 94	5 S	5 S	49.0	49.0
Sakha 95	10 S	10 S	66.5	66.5
Gemmeiza 5	30 S	30 S	192.5	192.5
Gemmeiza 7	20 S	30 S	157.5	192.5
Gemmeiza 9	5 S	5 S	49.0	49.0
Gemmeiza 10	5 S	10 S	49.0	66.5
Gemmeiza 11	Tr S	5 S	42.0	49.0
Sohag 3	5 S	5 S	49.0	49.0
Group 3: Varieties with fast rusting resistance				
Giza 160	40 S	50 S	315.0	315.0
Giza 163	40 S	40 S	385.0	420.0
Giza 164	40 S	30 S	315.0	315.0
Sakha 69	40 S	40 S	385.0	420.0
Sakha 93	60 S	60 S	560.0	560.0
Sids 1	60 S	70 S	560.0	630.0
Giza 139 (check)	70 S	80 S	700.0	840.0

Table 3: Percentage final leaf rust severity (FRS %) and area under disease progress curve (AUDPC) of 26 wheat genotypes grown at Shebin El-Kom and Itay El-Baroud locations during 2012/13 growing season.

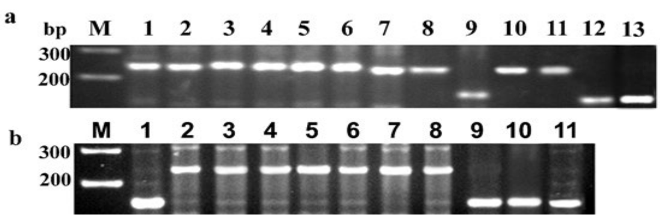

Figure 1: M: 100 bp ladder; a: 1: Misr 1, 2: Misr 2, 3: Giza 139, 4: Giza 160, 5: Giza 163, 6: Giza 164, 7: Giza 165, 8: Giza 168, 9: Sakha 8, 10: Sakha 69, 10: Sakha 93, 12: Sakha 94, 13: *Lr* 34; b: 1: Sakha 95, 2: Gemmeiza 5, 3: Gemmeiza 7, 4: Gemmeiza 9, 5: Gemmeiza 10, 6: Gemmeiza 11, 7: Sids 1, 8: Sids 12, 9: Sids 13, 10: Shandaweel 1, 11: *Lr* 34.

Discussion

A set of Egyptian wheat varieties released from 1979 to 2014 and an older variety namely Giza 139 were tested for the leaf rust resistance and for the variation in the locus *Lr* 34. Thus rust incidence as final rust severity (FRS%) was recorded for each of the tested genotypes. However, the wheat varieties Sids 12, Sids 13, Misr 1, Misr 2, Shandweel 1, Beni Sweif 4 and Beni Sweif 5 were very resistant during the two growing seasons 201/12 and 2012/13 at both locations i.e. Shibin El-Kom and Itay El-Baroud. Therefore it was concluded that the resistance in these varieties mainly due to race-specific resistance gene (s) against leaf rust.

Slow rust resistance at adult plant stage to leaf rust in the tested wheat varieties can be accurately measured by using area under disease progress curve (AUDPC) parameter, which considered the most convenient and a good reliable estimator for indicating the amount of rust infection occurred during an epidemic. Furthermore, AUDPC in particular is the result of all factors that influence disease development such as differences in environment, varieties and population of the pathogen [22,24] reported that disease development and AUDPC are the best estimators of partial resistance in wheat to leaf rust.

According to the obtained results and depending on the values of AUDPC, it could be stated that the wheat genotypes *Lr* 34, Giza 165, Giza 168, Sakha 8, Sakha 94, Sakha 95, Gemmeiza 5, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11 and Sohag 3 have high level of slow rusting resistance under field conditions through the two growing seasons at both locations. These genotypes showed the lowest AUDPC values (less than 300), therefore this group of genotypes characterized as slow rusting resistant group. On the other hand, the wheat varieties Giza 160, Giza 163, Giza 64, Sakha 69, Sakha 93, Sids 1 and Giza 139 have been severely rusted, showing the highest values

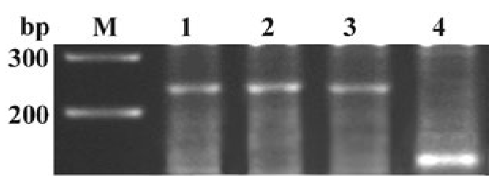

Figure 2: M: 100 bp ladder, 1: Sohag 3, 2: Beni Sweif 4, 3: Beni Sweif 5, 4: *Lr* 34.

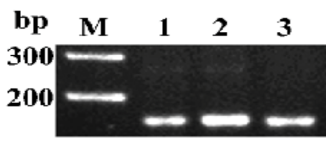

Figure 3: M: 100 bp ladder, 1: Sakha 8, 2: *Aegilops tauschii*, 3: *Lr* 34.

of AUDPC (up to 840). Consequently, these varieties classified as the highly susceptible or fast rusting varieties group [25] found that the wheat cultivar Agra Local showed the highest value of AUDPC (1300), the wheat cultivar Kundan showed least AUDPC value (217). While the wheat cultivars Trap (317), Galvez-78 (344), Mango (412), Chris (504) and PBW-348 (737) [26] reported that the wheat cultivars Chenab 70, WL 711, Pak. 81 were fast rusting cultivars, while the cultivars Pavon, FSD and INQ-91 were slow rusting cultivars [27] found that the wheat varieties Giza 168 and Gemmeiza 7 showed partial resistance which they showed lowest values of FRS (%) (did not exceed 250) and AUDPC (not more than 250). Marker-assisted selection offers the opportunity to select desirable lines on the basis of genotype rather than phenotype [28], especially in the case of combining different genes in a single genotype. Results of this study showed the usefulness of the SSR markers for identification of the leaf rust resistance gene *Lr* 34 in the tested wheat genotypes.

However, the evaluation of the tested genotypes for two seasons at two locations gave an evidence of the present of slow rusting resistance gene (s). Therefore, using marker-assisted selection to confirm the presence of the resistance gene *Lr* 34 was significant. The wheat varieties Giza 165, Giza 168, Gemmeiza 5, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10 and Gemmeiza 11 did not show the 150 bp band but the AUDPC of these varieties showed that these varieties have slow rusting resistance gene (s). The resistance in these varieties appeared to be based on gene (s) other than *Lr* 34. This gene (s) may be the slow rusting resistance *Lr* 46 and/or *Lr* 68. [27] found that, partial resistance in the two wheat varieties Gemmeiza 9 and Giza 168 mainly due to the presence of the adult plant resistance gene *Lr* 46 which confirmed by genetic analysis. Moreover, [29] found that adult plant resistance to leaf rust (*Puccinia triticina*) in line Parula is governed by at least three independent slow rusting resistance genes i.e. *Lr* 34, *Lr* 46 and *Lr* 68 gene on 7BL. [30] found that the partial resistance in the wheat cultivar HD2009 is similar in expression to that conferred by the gene *Lr* 34, but cultivar HD2009 did not show leaf tip necrosis, a morphological marker tightly linked to the leaf rust resistance gene *Lr* 34 [31]. However, varieties Sakha 8, Sakha 94, Sakha 95, Sids 13 and Shandweel 1 showed to carry the gene *Lr* 34. Varieties Sakha 8, Sakha 94 and Sakha 95 showed slow rusting infection in AUDPC analysis. Thus the marker assist selection confirmed the presence of the important gene *Lr* 34. It can be concluded that the slow rusting phenotype may be due the *Lr* 34 gene. Slow rusting resistance for broad spectrum of pathotype due to *Lr* 34 alone had been reviewed [32]. On the other hand, the two varieties Sids 13 and Shandweel 1 showed to carry the gene *Lr*34. However they showed race specific resistance. The resistance in these two varieties may be due to resistance genes other than *Lr* 34 of slow rusting phenotype. Many leaf rust resistance genes showed race specific resistance in seedling stage and remained effective in the adult stage such as *Lr*1, *Lr*10 and *Lr*21 [33]. Since, these two varieties were recently released; therefore, they may contain one of these genes of this nature. Moreover, resistance to leaf rust in these varieties mainly due to race-specific resistance gene (s) [34] found that individual major genes for adult plant resistance to leaf rust can enhanced the effectiveness of resistance when combined in wheat cultivars. Therefore, presence of adult plant resistance gene (s) in the two varieties Sids 13 and Shandweel 1 may be masked the effect of the gene *Lr* 34.

The obtained molecular results by the *cslV*34 marker in combination with the knowledge of the origin of the varieties understudy, may be enabled the most likely the origin of the important gene *Lr* 34 in wheat Egyptian genotypes. Results of this research proved that Sakha 8 carried this gene. Also, previous results in our laboratory by [35] came with the same conclusion using genetic analysis. Sakha 8 was released in 1987. In this era 1970s, Akakomughi of Japanese origin appeared in the pedigree of all Egyptian cultivars released [36]. Akakomughi is a grandparent of spring wheat variety Frontana which was used widely as a source of *Lr* 34 [6]. Therefore, it may be concluded that the *Lr* 34 gene was first introduced to Egyptian varieties back in 1970s. Also, Sakha 8 may become the donor of this gene in subsequent derivatives of crosses which led to many recent varieties such as Sakha 94, Sakha 95, Sids 13 and Shandweel 1.

Finally, *T. aestivum* is hexaploid with a genome constitution of AABBDD, and was formed about 8,000 years ago from hybridization between *T. turgidum* (AABB) and *A. tauschii* (DD) [37]. Also, *cslV*34 marker is very specific for *T. aestivum* and D genome progenitor *A. tauschii* [11]. Therefore, it was investigated in *A. tauschii* diploid genome. The results of this research confirmed the presence of *cslV*34b allele and consequently the presence of the *Lr* 34 gene for resistance in diploid D genome progenitor. The presence of *Lr* 34 for resistance in the current *A. tauschii* suggests that this resistance gene may have arisen before hexaploid synthesis.

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