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Adult Plant Resistance to Stem Rust and Molecular Marker Analysis of some Egyptian and Exotic Bread Wheat Genotypes

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ABSTRACT

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is the most dangerous and common disease among global wheat cultivars. Twelve local Egyptian and four exotic genotypes of bread wheat (*Triticum aestivum* L.) obtained from the International Maize and Wheat Improvement Center (CIMMYT) were evaluated under Egyptian field conditions for adult plant resistance (APR) levels to stem rust at Sakha location during the two successive growing seasons (2016 and 2017). Also, DNA characterization for genotypes by specific markers were tested to determine the presence of the effective resistance genes (*Sr's*); *Sr22*, *Sr24*, *Sr25* and *Sr26*. Out of the tested genotypes, four Egyptian bread wheat cultivars; (Misr3, Sakha94, Gemmeiza9 and Gemmeiza11), and four of CIMMYT lines (Line6043, Line6091, Line6107 and Line6197) show high levels of adult plant resistance to stem rust. Among the tested genotypes, *Sr22* found to be the most frequent gene, present in most of them. While, *Sr24* was present only in one local wheat cultivar and two exotic lines. *Sr25* was present in five local wheat cultivars and two exotic lines. In addition, a resistance gene *Sr26* was present in three exotic lines. The obtained results in this study evince that a combination of two or more resistance genes in one cultivar confer high adult plant resistance level in this cultivar, under field condition.

Keywords: Wheat cultivars, stem rust, specific marker, resistance genes

INTRODUCTION

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), continues to threat most of the commercial wheat cultivars in Egypt and worldwide. In the recent decades, the sudden appearance, as well as rapid and wide spread of a highly aggressive pathotype; Ug99 (TTKSK), and it's subsequent derivatives or variants in Africa, however, made the majority of the current wheat cultivars vulnerable to stem rust (Wanyera *et al*., 2006; Jin *et al*., 2007, Pretorius *et al*., 2010). Wheat researchers have, therefore intensified their efforts to incorporate and/or deployment of the effective *Sr* gene(s) into commercial wheat cultivars, to enhance their genetic resistance and to face the threat of this aggressive race. The previous reports classified a host-genetic resistance into two main types: seedling and adult-plant resistance.

Adult-plant resistance is a kind of genetic resistance that, quantitatively inherited, race nonspecific (general), partial field resistance, slow rusting resistance and/or field resistance. This type of resistance hope to be long lasting, or more durable (Johnson 1984; Borner *et al.,* 2000). Meanwhile, seedling resistance has been monogenic resistance that qualitatively inherited, and/or race-specific resistance. Therefore, it is readily to overcome by the sudden emergency of new races for stem rust pathogen (Johnson 1981).

Durable resistance has been early defined as "a resistance that has been remained effective against the disease for a long period of time, over a wide range of environments and against the broad spectrum pathogen races" (Johnson 1978). The emergency and rapid spread of this aggressive race and it's variants with virulence to most of the widely used resistance genes, have focused attention on the continuous search for new sources of stem rust resistance. This race has virulence to *Sr* genes; *Sr31* and *Sr38* that widely utilized in most of the commercial wheat cultivars in worldwide.

Breeding strategies for wheat stem rust resistance aimed to utilize the most effective *Sr* genes which virulence had not been previously reported *i.e. Sr22*, *Sr24*, *Sr25* and *Sr26*. Several wheat stem rust resistance genes have been deployed and incorporated into the commercial wheat cultivars, such as; *Sr22* that derived from (*Triticum monococcum* L.), as well as the two *Sr* genes ; *Sr24* and *Sr26* derived from *Agropyron elongatum* (syn. *Thinopyrum ponticum*) (McIntosh *et al.*, 1977; Li *et al*., 2003). Also, stem rust resistance gene; *Sr25* has been transferred into wheat from *Thinopyrum ponticum* (Host); Barkworth and Dewey. The four *Sr* genes have remained effective against the Ug99 race group. Likewise stem rust resistance gene; *Sr26* is one of the few known major genes that displayed an effective resistance against *Sr31*-virulent race; Ug99 (TTKSK). In addition, it has been proved to be effective against *Sr24*-virulent derivative (TTKST). The first Australian variety released carrying *Sr26* was Eagle (Martin 1971).

This study was therefore, carried out to determine and characterize adult plant resistance level to stem rust in some local and exotic bread wheat genotypes, under Egyptian field conditions. Also, to detect and identify the presence of the four stem rust resistance genes; *Sr22*, *Sr24*, *Sr25* and *Sr26* in the tested Egyptian and CIMMYT wheat genotypes, using DNA characterization by specific markers. In order to facilitate the future use and incorporation of these important genes in the national breeding materials.

MATERIALS AND METHODS

Plant materials

The current study was conducted at Sakha Agriculture Research Station, during the two successive growing seasons (2016 and 2017). Twelve Egyptian wheat cultivars (*T. aestivum* L.) were received from Wheat Research Section, Field Crops Institute, Egypt, and four exotic lines from the International Maize and Wheat Improvement Center (CIMMYT); Mexico, were used in this study (Table 1). The following wheat genotypes with identified resistance genes were used as compare: (*Sr22*; Mq*6//Stewart*3/RL 5244), (*Sr24*; LcSr24AG), (*Sr25*; Agatha (CI 14048)/9*LMPG-6 DK16) and (*Sr26*; Eagle *Sr26* McIntosh).

Field testing

Sixteen genotypes of spring wheat were evaluated for adult plant resistance levels against *Puccinia graminis* f. sp. *tritici* (*Pgt*) under field conditions. 20 seeds of each genotype were sown in a single-row within plots of 1 m length with 30 cm row spacing in a randomized complete block design

(RCBD), with three replications. One row of the highly susceptible wheat variety; Morocco was also planted at every 20th entries and along the border as disease spreader rows. They were later inoculated with stem rust urediospores, when the plants were at almost booting stage by use of a syringe in the evenings to create an artificial infection and ensure uniform inoculum dissemination. The plants were repeatedly irrigated to enhance stem rust infection. Where, other cultural practices such as fertilization, and other managements were applied according to the recommended agricultural practices.

Table 1. Twelve Egyptian bread wheat cultivars and four exotic lines from CIMMYT, used in this study with their pedigree.

No.	Genotypes	Pedigree
1	Misr1	OASIS/SKAUZ//4*BCN1312*PASTOR
2	Misr2	SKAUZ/BAV92
3	Misr3	ATTILA*2/PBW65*2/KACHU
4	Giza168	MRL/BUE//SERI CM93046-8M-0Y-0M-2Y-0B-0SH
5	Giza171	Sakha93/Gemmeiza9 S 6-1GZ-2GZ-2GZ-0S
6	Sakha94	Opata/Rayon//Kauz CMBW9043180-OTOPM-3Y-010M…
7	Sakha95	POSTOR//SITE/MO/3/CHEN/AEGILOPS/SQUARROSA(TAUS) …
8	Gemmeiza9	Ald‘‘S’’/Huas//CMH74A.630/SxCG4583-5G-1G-0G
9	Gemmeiza10	AMYA74 "S "/ON//1160-147/3/BB/GLL/4/CHAT"S"/5/CROW"S"
10	Gemmeiza11	BOW"S"/KVZ"S"//7C/SERI82/3/GIZA168/SKHA61.
11	Sids-12	Buc//7c/ald/5/maya74/on//1160-147/3/bb/gll/4/chat"s"'
12	Sids-13	AMAZ19=KAUZ"S"//TSI/SNB"S"
13	Line6043	CMSS08Y00611T-099TOPM-099Y-099M-099Y-2M-0WGY
14	Line6091	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/…
15	Line6107	CMSS08B00684T-099TOPY-099M-099NJ-099NJ-9WGY-0B
16	Line6197	C80.1/3*BATAVIA//2*WBLL1/4/MN02072-7/KBIRD//…

Disease assessment

Disease severity (%) at adult stage were recorded at 10-day intervals. The disease severity (%) was recorded in the tested wheat plants based on the percentage of leaf area infected or rusted, on the basis of modified Cobb's Scale, (Peterson *et al.,* 1948). Final rust severity (FRS%) was recorded for each of the tested genotypes when the susceptible check variety; Morocco reached it's maximum and final level of disease severity (%), during the season according to Das *et al.,* (1993).

Different disease severity scores were used for calculating area under the disease progress curve (AUDPC), following an equation of Pandey *et al.,* (1989), as follows:

$$AUDPC = D\left[\tfrac{1}{2}(Y_1 + Y_k) + Y_2 + Y_3 + \ldots + Y_{k-1}\right]$$

Where, D = Time interval (days between each two successive readings), (Y_1+Y_k) = Sum of first and last disease scores. $(Y_2+Y_3+\ldots+Y_{k-1})$ = Sum of all in-between disease scores.

The relative area under disease progress curve (rAUDPC) for each entry was calculated by using the equation of Akello *et al.,* (2017) as follows:

$$rAUDPC = \frac{AUDPC \text{ of the tested genotype}}{AUDPC \text{ of the susceptible (check) variety}} \times 100$$

Assessment of variance components and heritability (h^2%)

The genetic components of variance were computed using mean squares to partition phenotypic variance into it's two components, *i.e.* environmental variance (VE) and genotypic variance (VG). Also, heritability estimates in it's broad sense (h2%) was calculated for the studied disease parameters, using the equation of Miller *et al.,* (1958) as follows:

$$\%\ Heritability\ (h^2) = \frac{Genotypic\ variance\ (\sigma^2 g)}{Phenotypic\ variance\ (\sigma^2 ph)} \times 100$$

Where: $\sigma^2 g = [\sigma^2 e + r\sigma^2 g) - \sigma^2 e] / r$, $\sigma^2 ph = (\sigma^2 e + r\sigma^2 g)/r$

Molecular analysis

DNA extraction

Four specific primers were used for an identification of stem rust resistance genes *i.e. Sr22*, *Sr24*, *Sr25* and *Sr26*. Total genomic DNA of each genotype was extracted from leaves following the protocol described by Mago *et al.,* (2005). Samples of 60 mg leaf tissue were digested in liquid nitrogen with a mortar and pestle using i-genomic plant DNA Extraction, Mini Kit (iNtRON Biotechnology, Inc., Cat. No. 17371), following to manufacturer’s instructions. The DNA was stored at -20 ^{o}C to use as DNA template in PCR assays.

Marker analysis

PCR reaction was conducted in reaction volume of 25µl. Each PCR mixture (25µl) contains 1µl of 25 ng nucleic acid, 1 µl of each primer (10pmol), 12.5µl of GoTag (R) Colorless Master Mix (Promega Corporation, USA) and 9.5 µl of Nuclease free water (Promega). 15µl of all PCR products were analyzed by electrophoresis through a 1.5% agarose gel, stained with ethidium bromide. DNA bands were visualized using a UV Tran illuminator. Sequences of primers (Table 2).

Table 2. Stem rust resistance genes *(Sr's)* sequences of the four tested primers.

Gene	marker	Primer sequence	Reference
Sr22	WMC633	WMC633-F:ACACCAGCGGGGATATTTGTTAC -3’ WMC633-R:GTGCACAAGACATGAGGTGGATT -3’	Olson *et al*., (2010)
Sr24	Sr24#12	Sr24#12-F 5'- CAC CCG TGA CAT GCT CGT A -3' Sr24#12-R 5'- AAC AGG AAA TGA GCA ACG ATG T -3'	Mago *et al*., (2005)
Sr25	Gb	Gb-F: CAT CCT TGG GGA CCT C -3’ Gb-R: CCA GCT CGC ATA CAT CCA -3’	Liu *et al*., (2010)
Sr26	Sr26#43	Sr26#43-F 5'- AAT CGT CCA CAT TGG CTT CT -3' Sr26#43-R 5'- CGC AAC AAA ATC ATG CAC TA -3'	Mago *et al*., (2005)

Statistical analysis

Analysis of variance (ANOVA) test of the data was carried out using statistical software package (SPPS13). The least significant difference (LSD) at 1% and 5% levels of significance was used to compare between genotype means.

RESULTS

To characterize, more carefully, the adult-plant resistance level in the tested wheat genotypes, three disease parameters *i.e.* final rust severity (FRS%), the area under the disease progress curve (AUDPC), and relative area under disease progress curve (rAUDPC), were estimated for each genotype during the two growing seasons *i.e.* 2016 and 2017. Analysis of variance (ANOVA) showed highly significant differences at $P \leq 0.01$ between the tested wheat genotypes, for all the disease parameters, under study (Table, 3).

Table 3. Analysis of variance (ANOVA) for the obtained data of the stem rust disease parameters *i.e.* FRS (%)[a], AUDPC[b], and rAUDPC[c], expressed on seventeen wheat genotypes across the two years; (2016 and 2017)

Source of variance (SOV)	Df	MS		
		2016 growing season		
		FRS (%)[a]	AUDPC[b]	rAUDPC[c]
Replicates	2	0.13	487.29	4.319
Genotypes (G)	16	1311.18**	224985.98**	1949.24**
Error	32	0.66	270.17	0.65
Total	50	-	-	-
		2017 growing season		
Replicates	2	2.43	1554.11	11.73
Cultivars (G)	16	1471.15**	276058.82**	1868.92**
Error	32	0.79	924.20	1.57
Total	50	-	-	-

[a]FRS%, Final rust severity%; [b]AUDPC, Area under disease progress curve; [c]rAUDPC, Relative area under disease progress curve.
**Significant at 0.01 level of probability.

Characterization of adult plant resistance (APR) in the tested genotypes

Sixteen selected bread wheat genotypes as well as the check variety; Morocco, were evaluated for stem rust resistance under field conditions at Sakha location.

High disease pressure on the tested genotypes was created in the field, by the application of artificial infection, during both seasons of the study. Where, the susceptible (check) variety Morocco displayed high percentages of final rust severity %, (reached to 84.67% and 88.33%) in the first and second growing seasons, respectively. A diverse field reaction or a wide variation in the stem rust severity (%) was observed among the tested genotypes within each season. The obtained results revealed that intensity of stem rust epidemic during the first season; 2016 was relatively high, as compared to the second season; 2017 .

No disease symptoms (stem rust pustules) could be detected or noticed in the wheat plants of the two exotic lines; Line6091 and Line6197, during the two seasons of the study (Tables 4 & 5). They therefore, should be characterized as the promising or advanced lines, as they revealed the highest adult plant resistance levels to stem rust.

Therefore, these advanced wheat germplasm were classified as the completely resistant wheat genotypes.

In 2016 season

During this growing season, a wide variation in the stem rust disease reaction among the tested wheat genotypes (Table, 4). These genotypes were grouped into two groups. The first group included wheat genotypes with high adult plant resistance (APR) levels, as they showed lowest of FRS% (ranging from 1.87% to 20.00%), lowest AUDPC values (less than 250) and least rAUDPC ratios (from 2.05 to 22.33). This group include wheat genotypes; Misr3, Giza168, Giza171, Sakha94, Gemmeiza9, Gemmeiza10, Gemmeiza 11, Sids-12, Sids-13, Line6043 and Line6107. In contrast, the second group contains the highly susceptible or fast rusting wheat genotypes i.e. Misr1, Misr2 and Sakha95, as well as the check variety; Morocco. These genotypes displayed high levels of FRS% (reached to 84.67.00%), high estimates of AUDPC (ranging from 440 to 1075) and high values of rAUDPC (reached to 100) (Table, 4).

Table 4. Adult-plant response of the tested wheat entries against stem rust, expressed as the three disease parameters; FRS%[a], AUDPC[b], and rAUDPC[c], under field conditions during 2016 growing season.

Wheat genotypes	Disease parameters		
	FRS%[a]	AUDPC[b]	rAUDPC[c]
Misr1	50.00	440.00	41.03
Misr2	52.00	461.67	43.02
Misr3	6.00	23.33	2.18
Giza168	20.00	240.00	22.33
Giza171	13.67	55.33	5.16
Sakha94	1.87	22.00	2.05
Sakha95	55.00	550.00	51.16
Gemmeiza9	7.67	29.33	2.73
Gemmeiza10	4.67	26.67	2.48
Gemmeiza11	13.00	79.67	7.43
Sids-12	18.00	160.00	14.92
Sids-13	12.33	72.00	6.71
Line6043	2.00	24.00	2.23
Line6091	0.00	0.00	0.00
Line6107	2.33	39.00	3.64
Line6197	0.00	0.00	0.00
Morocco (check)	84.67	1075.00	100.00
LSD at 0.01	1.82	36.75	1.81
0.05	1.35	27.34	1.34

[a]FRS% = Final rust severity%; [b]AUDPC = Area under disease progress curve; [c]rAUDPC = Relative area under disease progress curve.

In 2017 season

Despite the heavy stem rust disease pressure during this season, five Egyptian wheat cultivars (Misr3, Giza171, Sakha94, Gemmeiza9, and Gemmeiza10) and two exotic lines (Line6043 and Line6107) remained in the first group, as they showed high adult plant resistance (APR) levels. These resistance genotypes exhibited the lowest FRS (ranging from 2.67 to 25.00%), least AUDPC values (less than 250) and least rAUDPC ratios (ranging from 2.25 to 20.13). On the other hand, the second group of genotypes includes the highly susceptible cultivars; Misr1, Misr2 and Sakha95 as well as the check variety; Morocco which displayed the highest percentages of final rust severity (reached to 88.33%), highest AUDPC values (up to 1200) and highest rAUDPC values (reached to 100) (Table, 5).

Variance components and heritability estimates

Phenotypic variance (σ^2ph) and it's two components; genotypic and environmental variances, as well as heritability estimates in it's broad-sense for all three disease parameters,

were estimated and presented in Table (6). As indicated from the obtained results in this table, each of phenotypic variance (σ^2ph) and genotypic variance (σ^2g) components was very high in it's magnitude. However, their values were higher than 436 for all the three disease parameters in the two growing seasons, under study. Meanwhile, environmental variance component (σ^2e) was sharply decrease as it was found in the three disease parameters, compared with the other two variances under study.

The obtained data (Table, 6) in the same table also indicated that, all disease parameters have high heritability estimates (up to 99%). The results implied that all the studied disease parameters are less influenced by environmental effects and mainly controlled by genetic structure of the tested wheat genotypes.

Molecular characterization of tested genotypes

Most of the tested wheat genotypes, previously evaluated in the first part of this study against stem rust under field conditions, were selected for molecular characterization.

Four simple sequence repeat (SSR) markers were evaluated for an efficacy in detecting the stem rust resistance genes *i.e. Sr22*, *Sr24*, *Sr25* and *Sr26*. The SSR markers showed polymorphism for *Sr* genes in wheat genotypes under study. Data obtained from Figures 1, 2, 3, and 4 were summarized in Table (7).

Table 5. Adult-plant response of the tested wheat entries against stem rust, expressed as the three disease parameters; FRS%[a], AUDPC[b], and rAUDPC[c] under field condition during 2017 .

Wheat genotypes	Epidemiological parameters		
	FRS%[a]	AUDPC[b]	rAUDPC[c]
Misr1	51.67	458.33	37.94
Misr2	52.00	456.67	37.77
Misr3	7.00	28.33	2.34
Giza168	25.00	245.00	20.13
Giza171	16.67	68.33	5.61
Sakha94	3.00	28.00	2.32
Sakha95	60.70	675.00	55.48
Gemmeiza9	9.67	36.00	2.97
Gemmeiza10	5.67	29.00	2.40
Gemmeiza11	15.67	93.33	7.71
Sids-12	24.00	218.33	18.03
Sids-13	16.00	79.97	6.62
Line6043	3.33	27.33	2.25
Line6091	0.00	0.00	0.00
Line6107	2.67	41.67	3.43
Line6197	0.00	0.00	0.00
Morocco (check)	88.33	1216.67	100.00
LSD at 0.01	19.38	189.74	15.65
0.05	1.98	67.97	2.80

[a]FRS% = Final rust severity%; [b]AUDPC = Area under disease progress curve; [c]rAUDPC = Relative area under disease progress curve.

Table 6. Phenotypic (σ^2ph), genotypic (σ^2g) and environmental variances (σ^2_e) and broad sense heritability (h^2) for the disease parameters; FRS% [a], AUDPC[b] and rAUDPC[c], during 2016 and 2017 growing seasons.

Genetic components	Disease parameters		
	2016 growing season		
	FRS (%)[a]	AUDPC[b]	rAUDPC[c]
Phenotypic variance (σ^2ph)	437.50	75175.44	650.18
Genotypic variance (σ^2g)	436.84	74905.27	649.53
Environmental variance (σ^2e)	0.66	270.17	0.65
Heritability (h^2 %)	99.85	99.64	99.90
	2017 growing season		
Phenotypic variance (σ^2ph)	490.91	92635.74	624.02
Genotypic variance (σ^2g)	490.12	91711.54	622.45
Environmental variance (σ^2e)	0.79	924.20	1.57
Heritability (h^2 %)	99.84	99.00	99.75

[a]FRS%, Final rust severity%; [b]AUDPC, Area under the disease progress curve; [c]rAUDPC, Relative area under disease progress curve.

***Sr22* screening**

The *Sr22* gene was introgressed from *T. monococcum* L. ssp. *Aegilopoides* (synonym of *T. boeoticum Boiss.*). The amplicon with a size of 215 bp, described by Yu *et al.*, (2010) as a diagnostic fragment. Marker, *Sr22#*, was used in the current study to detect *Sr22* in the tested wheat cultivars and lines. As indicated from (Fig. 1 and Table 7), *Sr22* gene is present in 10 genotypes; Misr1, Misr2, Sakha94, Giza168, Giza171, Gemmeiza9, Gemmeiza10, Sids-12, Sids-13 and Line6197.

***Sr24* screening**

The marker analysis indicated that *Sr24* was identified as a fragment of 500bp, Sr24 located on chromosome 3DL in Agent-or 1BS in Amigo-derived lines (Mago *et al.*, 2005). The obtained results PCR fragment was amplified in the tested genotypes; Misr2, Line6091 and Line6197, but it was not detected in other wheat genotypes under study (Fig. 2 and Table 7).

***Sr25* screening**

A dominant marker *Gb* was developed for haplotyping, the important stem rust resistance gene *Sr25*, that showed a high efficacy against the new race Ug99 and it's variants, (Liu *et al*., 2010). The presence of the *Sr25* marker was confirmed by the detection of a 130-bp fragment. Marker analyses indicated that *Sr25* was present in the five Egyptian wheat cultivars (Misr1, Misr2, Gemmeiza9, Gemmeiza10 and Gemmeiza11), as well as the two of CIMMYT genotypes; Line6091 and Line6197 (Fig. 3 and Table 7).

***Sr26* screening**

Stem rust resistance gene; *Sr26* was transferred into the long arm of wheat chromosome 6A from *Thinopyrum ponticum* (Mago *et al*., 2005). A dominant SSR marker *Sr26#43* was developed for detecting this wheat stem rust resistance gene, and a 207-bp band was amplified in wheat genotypes with *Sr26*. Marker *Sr26#43* was used to detect this fragment in the tested genotypes. The marker for *Sr26* was identified in only three genotypes; Misr2, Line6091 and Line6197, No any visible band was detected in remaining tested wheat genotypes, suggesting that these varieties do not carry *Sr26* (Fig. 4 and Table 7).

Some wheat genotypes carrying multiple stem rust resistance genes were identified during this study. The combination of two and/or three stem rust genes was the most

frequent, being present in seven genotypes. Marker analyses indicated that *Sr22* and *Sr25* are present together in four genotypes.

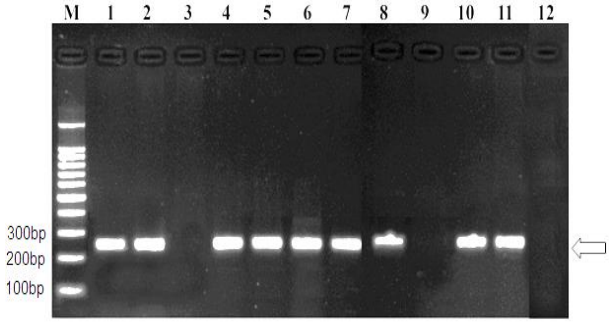

Fig. 1. DNA amplification products of wheat samples using primers to the *WMC633* locus linked with the *Sr22* resistance gene. M: 215 bp ladder 1. Monogenic *Sr22*, 2. Gemmeiza9, 3. Gemmiza11, 4. Giza168, 5. Giza171, 6. Sids-12, 7. Sids-13, 8. Sakha94, 9. Sakha95, 10.Misr1, 11. Misr2 and 12.Misr3. The arrow shows the fragment, which is associated with *Sr22*.

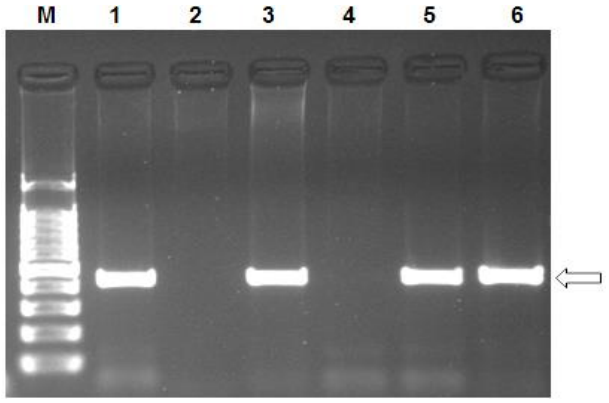

Fig. 2. DNA amplification products of wheat samples using primers to the *Sr24*# locus linked with the *Sr24* resistance gene. M: 500bp ladder 1. Monogenic *Sr24*, 2.Misr1, 3. Misr2, 4. *Sr26*, 5. Line6091 and 6.Line6197. The arrow shows the fragment, which is associated with *Sr24*.

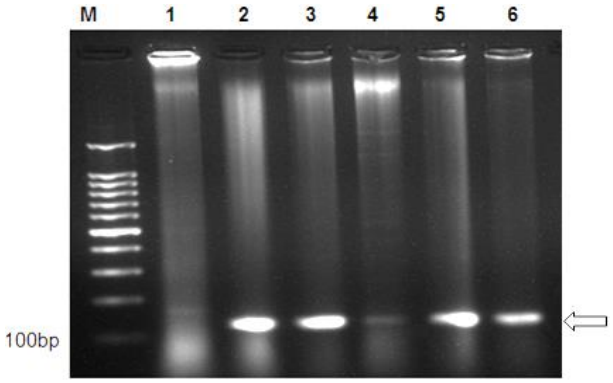

Fig. 3. DNA amplification products of wheat samples using primers to the (*Sr25*) (Ruler, 130bp DNA Ladder); locus linked with the *Sr25* resistance gene.1. Monogenic *Sr25*, 2.Misr1, 3. Misr2, 4. *Sr26*, 5. Line6091 and 6.Line6197.The arrow shows the fragment, which is associated with *Sr25*.

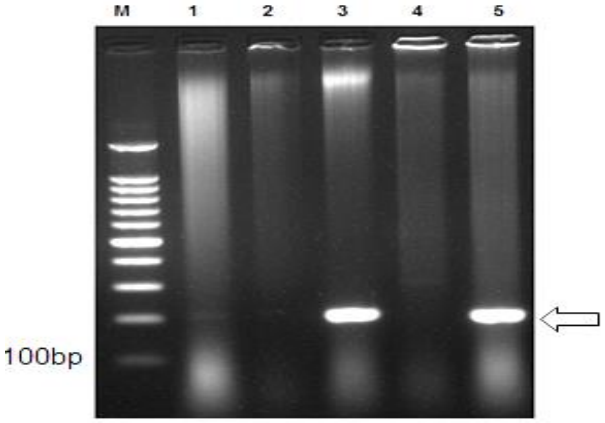

Fig. 4. DNA amplification products of wheat samples using primers to the (*Sr26*) (Ruler, 207bp DNA Ladder); locus linked with the *Sr26* resistance gene.1. Misr1, 2. Misr2, 3. Monogenic *Sr26*, 4. Line6091 and 5. Line6197.The arrow shows the fragment, which is associated with *Sr26*.

Three experimental genotype was found to have the *Sr24* and *Sr25* combination, only one genotype was found to have *Sr22* and *Sr26* combination (Line6107) and one genotype was found to have the *Sr24*, *Sr25*, and *Sr26* combination (Line6197). The results of marker analyses are largely consistent with the field evaluation of wheat genotypes. Where, Line6197 was exhibited high-level of adult plant resistance, to stem rust disease.

Table 7. Stem rust resistance genes (*Sr's*) detected by PCR based markers in twelve Egyptian wheat cultivars and four CIMMYT wheat lines.

Genotypes	*Sr22*	*Sr24*	*Sr25*	*Sr26*	No. of genes*
Misr1	+	+	+	-	3
Misr2	+	-	+	-	2
Misr3	-	-	-	-	-
Sakha94	+	-	-	-	1
Sakha95	-	-	-	-	-
Giza168	+	-	-	-	1
Giza171	+	-	-	-	1
Gemmeiza9	+	-	+	-	2
Gemmeiza10	+	-	+	-	2
Gemmeiza11	-	-	+	-	1
Sids-12	+	-	-	-	1
Sids-13	+	-	-	-	1
Line 6043	-	-	-	+	1
Line6091	-	+	+	-	2
Line 6107	+	-	-	+	2
Line6197	-	+	+	+	3

(+) =presence of *Sr* gene in wheat cultivars, (-) =absence of *Sr* gene in wheat genotype (?), =did not appear clearly and, *Number of genes detected each genotypes.

DISCUSSION

Stem rust is historically an old disease in Egypt. However, numerous numbers of efforts have been early done by breeders and pathologists to release and develop new wheat varieties having a sustainable resistance to rust diseases. During the last three decades, stem rust has been successfully controlled in Egypt by using and/or cultivation wheat varieties having an effective resistance to this disease.

As early as 1950's, wheat-breeding strategy in Egypt, aimed to enhance stem rust resistance in the new released and high yielding wheat varieties by hybridization through the crossing blocks in the national breeding programme.

However, Giza139 is the first Egyptian wheat cultivars released by this method. Since that time, stem rust has been successfully controlled in Egypt, and therefore it could be greatly avoid and circumvented sever stem rust epidemics. The threats due to stem rust disease have been re-emerged owing to the appearance of the new race; Ug99, (TTKSK) in Uganda in 1999 (Pretorius *et al.*, 2000). More recently, (in 2016), the first confirmation and detecting of Ug99 race, in Egypt was carried out by Patpour and his coworkers (2016). Egypt has been cooperated with (CIMMYT) in Mexico to release new resistant wheat varieties and stop the spread of Ug99 into other countries. Egypt being the first country that release wheat cultivars; Misr1 and Misr2 with an effective resistance to Ug99.

Durable resistance to wheat rusts, especially stem rust is the main objective of most wheat breeding programs in all wheat-growing regions of the world. The sudden emergency besides widely and rapid spread of a new virulent race of wheat stem rust TTKSK (Ug99), and its variants, has driven a search for new sources of resistance to these aggressive races. This study aimed to evaluate and characterize adult plant resistance in some wheat genotypes. Also, DNA characterization for genotypes by specific markers were tested to determine the presence of the effective resistance genes (*Sr's*); *Sr22*, *Sr24*, *Sr25*, and *Sr26*, TTKSK-effective gene (Xu *et al*., 2008; Olson *et al*., 2010).

Different disease reactions to stem rust observed between the tested genotypes suggested that these wheat genotypes had diverse genetic backgrounds. It can be inferred that the two genotypes namely Line 6091 and Line 6197, only showed high resistant response to the disease with no visible stem rust infections or pustules. They, therefore, could be characterized as the completely resistant cultivars. This type of resistance may be conferred by either a single effective major gene or a combination of those. Singh *et al*., (2005) previously reported that a combination of 4-5 minor effective genes with race non-specific responses provided near immunity reaction to leaf rust.

The trace reaction noted could be associated with hypersensitivity whereby fungal infection signals as a defense mechanism leading to cell collapse which restrict further disease spread (Rubiales and Nicks, 2000). The presence of effective major genes in a variety limit infection process by triggering necrosis of the host cells in the neighborhood of the infective structures (Leonard and Szabo 2005). Meanwhile, wheat cultivars; Misr3, Giza168, Giza171, Sakha94, Gemmeiza9, Gemmeiza10, Gemmeiza11, Sids-12, Sids-13, as well as the two exotic genotypes; Line6043 and Line6107, have displayed the relatively high adult plant resistance levels to stem rust, under field conditions during the two seasons against the mixture of stem rust races at adult stage. Accordingly, the four highly resistant lines could also be harboring a combination of minor effect genes. They showed the lowest percentage of FRS, the lowest estimated of AUDPC values and lowest rAUDPC values. In contrast, the second group contains the highly susceptible or fast rusting wheat genotypes *i.e.* Misr1, Misr2 and Sakha95, as well the check variety; Morocco which displayed the highest percentages of final rust severity, highest AUDPC values and highest rAUDPC values. These results run in the lines with those of (McIntosh *et al*., 1995; Roelfs 1988 and Singh *et al*., 2012)

Molecular markers are particularly useful for identifying genotype with multiple genes and for pyramiding multiple resistance genes, which is difficult and sometimes impossible to do. Among the tested genotypes, *Sr22* found to be the most frequent gene, as it was present in most of them. The marker for *Sr24* was present in three genotypes only, but it was not detected in remaining genotypes under study. *Sr25* was present in five local wheat cultivars and two exotic lines. Also, a resistance gene *Sr26* was present in three exotic lines.

Some wheat genotypes carrying multiple stem rust resistance genes were identified during this study. The combination of two and/or three stem rust genes was the most frequent, being present in seven genotypes. Marker analyses also indicated that *Sr25* and *Sr26* were present together in Line6197 that was displayed high-level adult plant resistance (APR), exhibiting near immune resistance to stem rust disease.

By matching genotypic and phenotypic data for wheat germplasm, we specified that the markers for both *Sr25* and *Sr26* foretell with high dependability the presence of these resistance genes (Liu *et al*., 2010). Markers for these genes could then aid in selecting APR, pyramiding R-genes and in combining APR genes with R-genes (Yu *et al.*, 2011).

Further examination of these entries could prove worthwhile in the search for *Sr* genes effective against highly virulent races of stem rust.

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مقاومة النبات البالغ والتحليل الجزيئي لبعض التراكيب الوراثية من القمح المصري والمستوردات لمرض صدأ الساق

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يعتبر مرض صدأ الساق في القمح والمتسبب عن الفطر بكسينيا جرامنيس ترتيساى أكثر الأمراض خطورة على معظم اصناف القمح في مصر والعالم. وقد تم دراسة السلوك المرضى لإثنى عشر تركيب وراثي مصري بالإضافة الى أربعة تراكيب وراثية مستورده من هيئة السيميت مقارنة بصنف حساس Morocco تحت ظروف الحقل بمحطة البحوث الزراعية بسخا خلال موسمي الزراعة 2016 و2017 . وقد تم أيضا اجراء التحليل الجزيئي Molecular marker باستخدام الدلائل الجزئية المتخصصة لتحديد وجود الجينات المسئولة عن مقاومة المرض وهى *Sr22*, *Sr24*, *Sr25* و *Sr26* فقد أوضحت نتائج الدراسة أن أربعة من الاصناف المصرية (مصر3 وسخا 94 وجميزة 9 والصنف جميزة11) واربعة من سلالات السيميت (السلالة 6043 و6091 و 6107 و 6197) قد اظهروا مستوى عالي من مقاومة المرض. وبفحص المدخلات الوراثية مع اربعة دلائل جزيئية للكشف عن وجود أو عدم وجود جينات المقاومة لصدا الساق وجد ان جين المقاومة *Sr22* هو أكثر تلك الجينات انتشاراً بالتراكيب الوراثية المختبرة حيث قد ثبت وجوده في معظم تلك التراكيب. بينما ظهر جين المقاومة *Sr24* في صنف واحد فقط من القمح المحلى واثنين من السلالات النباتية المستوردة والجين *Sr25* وجد في خمسة من أصناف القمح المحلية واثنين من التراكيب المستوردة أيضا. وبالنسبة للجين *Sr26* فقد وجد في ثلاثة تراكيب وراثية من المستوردات فقط. وقد أوضحت النتائج المتحصل عليها بتلك الدراسة أن وجود أكثر من جين واحد من الجينات المسئولة عن مقاومة مرض صدأ الساق بصنف معين من أصناف القمح يؤدى الى اكتسابه مستوى عالٍ وجيد من المقاومة لهذا المرض تحت الظروف الحقلية.