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# Virulence of *Puccina graminis* f.sp. *tritici* and Postulated Resistance Genes for Stem Rust in Ten Wheat Varieties in Egypt

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Authors' contributions

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

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### **ABSTRACT**

Stem rust (*Puccina graminis* f. sp. *tritici*) samples were collected from different areas of Egypt during two season 2008/2009 and 2009/2010 growing seasons. The single pustule method of isolation was followed, and the type of infection was recorded as well for each rust samples. The identification of physiologic races revealed the presence of forty fifth and fifty fifth race groups of stem rust races. The race identification evinced the presence of race groups TT--- and TK--- as well, which run to 11.59 and 10.14 percents of samples in 2008/2009, respectively. However in season 2009/2010 race groups TT--- was the most frequent one (21.50 %), The frequency of virulence of isolates on lines possessing the resistance genes *Srs* 24, 31, 26, 6, 9e, 29, 35, 9a and 9g were the lowest among the tested lines and should be considered in breeding for resistance. The most effective resistance genes at seedling stage were *Srs* 24, 31, 29, 6, 9a, 26+9g and 9e. The postulation of genes revealed that the vars. Giza 164 and Sohag 3 probably have (tow genes, each), Sakha 94 (four genes), Sids 1 and Sakha 93 (five genes each), Sids 13 (nine genes), Giza 168 (ten genes); Gemmeiza 7 (thirteen genes) on the other hand, Giza 160 and Sakha 8 did not have any genes.

Keywords: Wheat; stem rust; infection type; physiologic races; race groups; resistance genes.

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#### 1. INTRODUCTION

Stem rust caused by Puccinia graminis f.sp. tritici, Eriks. And Henn is the most destructive disease to wheat. Under favorable conditions, stem rust may cause yield losses up to 100% to the susceptible varieties [1,2]. The new stem rust race which was designated as Ug99 in Uganda in 1999 has threatened wheat production globally [3,4] using the letter code of stem rustnomenclature system it was designated as TTKS race [5]. A fifth set of differential lines was added, thus Pgt-Ug99 is race TTKSK and variants with added virulence to Sr24 and Sr36 are TTKST and TTTSK, respectively [6,7]. Recently, four other variants of the Ug99 lineage (TTKSF, TTKSP, PTKSK, and PTKST) are present in different parts of Africa [8]. Race TTKSK and its variants are virulent to about 90% of the world's wheat cultivars [9,10]. Race Ug99 is virulent to a number of stem rust resistance genes, most notably Sr31for which Ug99 was the first reported virulent race. Also, Ug99 is highly damaging which was reported to cause yield losses of more than 71% in experimental fields [11]. Host resistance is the effective control method for stem rust and has been used worldwide for over 50 years. but TTKSK is virulent to most Sr genes [12]. Among 56 designated and few undesignated stem rust resistance genes in wheat, only eight designated genes in the primary gene pool (Sr13, Sr14, Sr22, Sr28, Sr33, Sr35, Sr42, and Sr45) confer resistance to TTKSK [3,12,13].

Nevertheless, this problem has been partially solved by the production and release of new cultivars having effective field resistance *i.e.*the first resistant cultivars are Giza 135 and Giza 139. Many wheat cultivars derived from these two cultivars possessing the same resistance were selected between 1950-1990, which can be characterized by their seedling susceptibility to most of the common physiologic races of stem rust, but in the same time they showed high levels of adult plant resistance under field conditions [14].

An international system of nomenclature for *P. g. f. sp. tritici* permits an evaluation of cultures in the area of origin and a complete phenotype description of the type cultures submitted to the Cereal Rust Laboratory according to [5].

The present work focused a spot light on race identification taking in a count the modern concepts, virulence frequency, gene efficacy and postulate seedling stem rust resistance gene(s) in 10 wheat verities in Egypt.

## 2. MATERIALS AND METHODS

Wheat stem samples with symptoms of stem rust disease caused by *Puccinia graminis* Pers. *f.sp. tritici* collected from numerous wheat fields and Egyptian wheat trap nursery during annual survey were used for the identification of physiologic races and pathotypes in Egypt during 2008/2009 and 2009/2010 growing seasons. The collected samples were kept in glassine envelopes (8 x 15 cm). The stem pieces with rust were left at room temperature for 24 hours to remove the excess of humidity. The desiccated samples were preserved in fridge till usage. The rust of specimens was transferred on the highly susceptible wheat cultivar i.e. Morocco following the method proposed by USDA-ARS [15]. Eight daysold seedlings were moisted with atomizer in the inoculation chambers than inoculated by shaking and brushing rusted materials over the plants and sprayed gently again with water in order to induce "dew" on the plants, then the pots were kept in damp chambers for 24 hours to allow the rust spore to germinate and cause infection. The plants were transferred and placed on benches in the greenhouse and kept for 14 days. After developing the rust, three single pustules were separately isolated from each sample for reproduction on the very

susceptible wheat cultivars "Morocco" seedlings to obtain enough urediospores for inoculation. Seedling reaction was recorded as low or high depending on the infection type produced according to adopted by [5]. The differential fast series consisted of wheat lines will resistance genes i.e. 1-Srs 21, 9e, 76, 2-Srs 11, 6, 8, 9g 3-Srs 36, 9b, 30, 17. An additional differential set consisting of lines Sr 9a, 9d, 10 and Tmp was added to Table (1) according to adopted by [16]. Additional differential sets consisting of lines Sr 9a, 9d, 10 and Tmp according to USDA-ARS [5] as well as lines Sr,s 24, 31, 38 and McN according to Jin et al. [6] were added to Table 1.

Table 1. Pgt-Code for the 20 North American differentials hosts for Puccinia graminis f.sp. Tritici.

Pgt Code	Subs	et*	S	ir's	
•	1	5	21	9e	7b
	2	11	6	8a	9g
	3	36	9b	3	17
	4	9a	9d	10	Tmp
	5	24	31	38	McN
В		L	L	L	L
С		L	L	L	Н
D		L	L	Н	L
F		L	L	Н	Н
G		L	Н	L	L
Н		L	Н	L	Н
J		L	Н	Н	L
K		L	Н	Н	Н
L		Н	L	L	L
M		Н	L	L	Н
N		Н	L	Н	L
Р		Н	L	Н	Н
Q		Н	Н	L	L
R		Н	Н	L	Н
S		Н	Н	Н	L
T		Н	Н	Н	Н

<sup>\*</sup>Pgt-code consist of the designation for subset 1 followed by that for subset2, etc

The following criteria were determined:

- For pathotypes identification, the *Pgt*-code nomenclature for *Puccinia graminis* f.sp. *tritic* was followed, using a set of 20 single wheat lines, each with a single stem rust resistance gene, as differential arranged in five subset of four (Table-1).
- For frequency of virulence and gene efficacy as if consisting of 32 near isogenic lines were used for virulence analyses, the frequency of Virulence was estimated of the *Pgt* of virulence isolates to the total number of isolates for each genotype.
- Postulation of resistance gene(s) Near Isogenic Lines (Sr's) known host A and Egyptian wheat varieties unknown host B both were tested at seedling stage aginest15 isolates of *Puccinia graminis pers*. f.sp. *tritici* according to the method adopted by [17,18].

### 3. RESULTS

# 3.1 Raceldentification

Isolates of *Puccinia graminis* f. sp. *Tritici* Eriks. & E. Henn. Obtained from collections of wheat stem rust were identified based on infection types on the North American stem rust differential series used for race identification.

The presence of 45 race groups was established in 69 isolates of the first growing season (2008/09), where the most frequent race groups were TT--- and TK--- (11.59 and 10.14%, respectively) followed by TR--- and KT--- (5.79 and 4.35%, respectively). On the other hand, the frequency of race groups BB---, BH---, DK---, PF---, TF--- and TS--- was 2.89% of each, whereas, the remaining race groups were represented at 1.44% of each (Table 2).

Table 2. Frequency % of *Puccinia graminis* f.sp. *tritici* pathotypes in Egypt during 2008/09 growing season

No.	Pgt	No. of isolates	Freq.(%)	No.	Pgt	No. of isolates	Freq.(%)
1	BB	2	2.89	24	PG	1	1.44
2	BH	2	2.89	25	PF	2	2.89
3	BK	1	1.44	26	PJ	1	1.44
4	BT	1	1.44	27	PT	1	1.44
5	DF	1	1.44	28	QF	1	1.44
6	DK	2	2.89	29	QK	1	1.44
7	FD	1	1.44	30	QS	1	1.44
8	FG	1	1.44	31	QT	1	1.44
9	FJ	1	1.44	32	RG	1	1.44
10	FT	1	1.44	33	RR	1	1.44
11	JH	1	1.44	34	SC	1	1.44
12	JK	1	1.44	35	SJ	1	1.44
13	KC	1	1.44	36	SK	1	1.44
14	KH	1	1.44	37	SL	1	1.44
15	KK	1	1.44	38	SR	1	1.44
16	KT	3	4.35	39	ST	1	1.44
17	LC	1	1.44	40	TF	2	2.89
18	MF	1	1.44	41	TH	1	1.44
19	MS	1	1.44	42	TK	7	10.14
20	MT	1	1.44	43	TR	4	5.79
21	NC	1	1.44	44	TS	2	2.89
22	NJ	1	1.44	45	TT	8	11.59
23	NK	1	1.44	Total		69	100.00

During the second growing season (2009/10) 55 race groups were indentified on 93 isolates, and TT--- (21.50%) was the most frequent among them. The race groups DH---, TK--- and TS--- occupied the second rank (4.30%, each), followed by the race group PT--- (3.22%), meanwhile the race groups BB---, BK---, DK---, DT---, KT---, PS---, SR--- and TP--- revealed at 2.15% of each. Each remaining race groups was repesented in 1.07% (Table 3).

Table 3. Frequency % of *Puccinia graminis* f. sp. *tritici* pathotypes in Egypt during 2009/10 growing season

No.	Pgt	No. of isolates	Freq.(%)	No.	Pgt	No. of isolates	Freq.(%)
1	BB	2	2.15	29	NG	1	1.07
2	BC	1	1.07	30	NR	1	1.07
3	BK	2	2.15	31	NS	1	1.07
4	BT	1	1.07	32	NT	1	1.07
5	CQ	1	1.07	33	PJ	1	1.07
6	CT	1	1.07	34	PL	1	1.07
7	DF	1	1.07	35	PR	1	1.07
8	DG	1	1.07	36	PS	2	2.15
9	DH	4	4.30	37	PT	3	3.22
10	DK	2	2.15	38	QK	1	1.07
11	DT	2	2.15	39	QR	1	1.07
12	FH	1	1.07	40	RT	1	1.07
13	FT	1	1.07	41	SG	1	1.07
14	GH	1	1.07	42	SH	1	1.07
15	GT	1	1.07	43	SM	1	1.07
16	HK	1	1.07	44	SR	2	2.15
17	HT	1	1.07	45	ST	1	1.07
18	JG	1	1.07	46	TB	1	1.07
19	JT	1	1.07	47	TH	1	1.07
20	JT	1	1.07	48	TK	4	4.30
21	KF	1	1.07	49	TN	1	1.07
22	KG	1	1.07	50	TP	1	1.07
23	KJ	1	1.07	51	TP	2	2.15
24	KK	1	1.07	52	TQ	1	1.07
25	KT	2	2.15	53	TR	1	1.07
26	LC	2	2.15	54	TS	4	4.30
27	LK	1	1.07	55	TT	20	21.50
28	LT	1	1.07	Total		93	100.00

# 3.2 Frequency of Virulence and Gene Efficacy

Different frequencies of virulence to *S*r genes in terms of infection types. They were showed during 2008/2009 low frequencies of virulence of *S*r 24, *S*r 31 and *S*r 26+9g were 0%, 0% and 11.67%, respectively. On the other hand, *S*r *PI* and *S*r9g showed the highest frequencies of virulence 90.0% and 85.0%, respectively. While the rest of *S*r genes showed different frequencies of virulence ranging from 20.0% to 83.33% (Table 4).

Ten stem rust resistance genes (Srs) had high efficacy more than (51.67%) i.e. Sr 24 (100.00), Sr31 (100.00), Sr26+9g (88.33), Sr33+5 (80.00), Sr7a (75.00), Sr35 (70.00), Sr29 (66.67), Sr16 (65.00), Sr32 (58.33), Sr11 (51.67) and Sr22 (51.67).On the other hand Srs genes 8b, 6, Wld, 9g and PL had the lowest gene efficacy (21.67, 16.67, 16.67, 15.00 and 10.00%, respectively) (Table 4).

In the second season 2009/10, data presented in Table 4 showed different frequencies of virulence to the tested Sr genes in terms of infection types. The least frequencies of virulence were found to Sr 24, Sr 31, Sr 6, Sr9e, Sr29, Sr 35, Sr 9a and Sr 9g showing 0%, 0%, 11.95%, 16.12%, 18.00%, 18.36%, 19.14% and 19.38%, respectively. The virulence against Sr30 and Sr17 showed the highest frequencies 87.36% and 82.97%. While the rest of Sr genes showed the different frequencies of virulence ranging from 20.0% to 73.68%.

Table 4. Frequency of virulence of *Puccinia graminis* f.sp. *tritici* isolates and *Srs* seedling efficacy during 2008/09 and 2009/10 growing season

	<u> </u>	2008/2009			2009/2010		
No.	Srgene (s)	No. of virulent	Virulence	Gene efficacy	No. of virulent	Virulence	Gene
- `,		isolates	frequency (%)	(%)	isolates	frequency (%)	efficacy (%)
1	5	44	73.33	26.67	67	28.87	71.27
2	6	50	83.33	16.67	81	11.95	88.04
2	7a	15	25.00	75.00	66	31.25	68.75
4	7b	37	61.67	38.33	64	30.43	69.56
5	8a	40	66.67	33.33	70	34.06	76.92
6	8b	47	78.33	21.67	75	21.87	78.12
7	9a	32	53.33	46.67	76	19.14	80.85
8	9b	41	68.33	31.67	75	21.87	78.12
9	9d	31	51.67	48.33	61	35.10	64.89
10	9e	44	73.33	26.67	78	16.12	83.87
11	9g	51	85.00	15.00	79	19.38	80.61
12	10	33	55.00	45.00	63	34.37	65.62
13	11	29	48.33	51.67	58	35.55	64.44
14	13	42	30.00	70.00	67	33.00	67.00
15	15	40	66.67	33.33	71	27.55	72.44
16	16	21	35.00	65.00	28	70.52	29.47
17	17	44	73.33	26.67	78	82.97	17.02
18	21	42	70.00	30.00	61	64.21	27.36
19	22	29	48.33	51.67	51	56.04	43.95
20	23	45	75.00	25.00	61	67.03	32.96
21	24	0	0.00	100.00	0	0	100.00
22	26+9g	7	11.67	88.33	56	61.53	38.46
23	29	20	33.33	66.67	18	18.00	82.00
24	30	39	65.00	35.00	83	87.36	12.63
25	31	0	0	100.00	0	0	100.00
26	32	25	41.67	58.33	35	38.88	61.11
27	33+5	12	20.00	80.00	24	26.66	73.33
28	35	18	30.00	70.00	18	18.36	71.42
29	Tmp	38	63.33	36.67	49	51.04	48.95
30	PI	54	90.00	10.00	62	71.26	28.73
31	Wld	50	83.33	16.67	70	73.68	26.31
32	TT3+10	44	73.33	26.67	64	69.56	30.43

Generally the level of gene efficacy was higher than the previous season where, stem rust resistance genes (*Srs*) had more than 71.27% in the second season efficacy *i.e. Sr 24* (100.00), *Sr31* (100.00), *Sr6* (88.04), *Sr9e* (83.87), *Sr29* (82.00), *Sr9a* (80.85), *Sr9g* (80.61), *Sr9b* (78.12), *Sr8b* (78.12), *Sr8a* (76.92), *Sr33+5* (73.33), *Sr15* (72.44), *Sr35* (71.42) and *Sr5* (71.27). Meanwhile, *Sr.s* genes 30, 17, Wld, 21 and PL had the lowest gene efficacy (12.63, 17.02, 26.31, 27.36 and 28.73%, respectively).

#### 3.3 Gene Postulation

The reactions of the tested wheat cultivars were compared to the reactions of 20 wheat monogenic lines (Sr, s) against 15 cultures of stem rust (Puccinia graminis f.sp. tritici). Difference and similarity of the reaction in terms of infection types (IT) between the monogenic lines and the tested cultivars were used to postulate gene(s) for resistance. The results given in Tables (5 and 6) show that no a virulent isolates of the casual organism against cvs. Giza 160 and Sakha 8 were detected. This means that the cvs. Giza 160 and Sakha 8 have gene (s) that were not encountered in the tested Sr lines set. Also, few isolates were a virulent to cv. Sohag 3, so data obtained could not accurately postulated all genes in this cultivar. On the other hand, virulent and a virulent isolates to the rest of the tested cultivars were available. Thus genes for stem rust resistance in these cultivars could be postulated. Comparison between the different Sr monogenic lines and the local cultivars revealed the probability of genes for resistance in these cultivars; i.e. cv. Gemmeiza7.was resistant to 10 isolates, however, it probably carries genes; Sr 5, Sr 9e, Sr 9g, Sr 10, Sr 13, Sr 15, Sr 16, Sr 22, Sr 29, Sr 30, Sr 17, Sr 34 and SrTmp. Also, from the comparison between its reaction and Sr genes reactions, it seemed that this cultivars probably has additional genes for stem rust resistance; cv. Giza 168. showed LIT to 9 cultures, thus; it probably carries ten tested Sr genes; Sr 5, Sr 9e, Sr 9g, Sr 30, Sr 16, Sr 17, Sr 7a, Sr 26, SrTmp and Sr Tt3 and it has addition to other resistance genes(s);cv. Sids 13.showed LIT to 8 cultures, thus; it probably carries nine tested Sr genes; Sr 5, Sr 9e, Sr 16, Sr 30, Sr 17, Sr 7a, Sr 26, SrTmp and Sr Tt3 and it has addition to other resistant genes(s).

Sids 1.cultivars gave low infection type (LIT) to 7 cultures thus; it probably carries five tested *Sr* genes; *Sr* 5, *Sr* 9e, *Sr* 9g, *Sr* 16, and *Sr* 17 and it have addition to other resistant genes (s); cv. Sakha 93. gave low infection type (LIT) to 7 cultures thus; it probably carries five tested *Sr* genes; *Sr* 5, *Sr* 9e, *Sr* 9g, *Sr* 16, and *Sr* 17 and it have addition to other resistant genes (s); cv. Sakha 94. gave low infection type (LIT) to 5 cultures thus; it probably carries four tested *Sr* genes; *Sr* 5, *Sr* 9e, *Sr* 16, and *Sr* 17 and it has addition to other resistance genes (s); cv. Giza 164. Was resistant to 5 isolates; however, it probably carries two genes; *Sr* 16 and *SrTmp*. Also, from the comparison between its reaction and *Sr* genes reactions, it seemed that this variety probably has additional genes for stem rust resistance; cv. Sohag 3. This variety was resistant to 5 isolates; however, it probably carries genes; *Sr* 16 and *SrTmp*. Also, from the comparison between its reaction and *Sr* genes reactions, it seemed that this variety probably has additional genes for stem rust resistance; the check wheat varieties Giza 160 and Sakha 8 showed high infection types against all the tested rust isolates and none of the tested genes were postulated (Table 7).

Table 5. Seedling reactions of twenty wheat monogenic lines carrying single gene for stem rust resistance against 15 isolates of *Puccinia graminis* f.sp. *tritici* under greenhouse conditions during 2010/2011 growing season

No	Monogenic	W	heat	tster	n rus	st iso	late/	Stem	rus	t infe	ction	type				,
	line (Srs)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	5			L	L	L		L								
2	9e				L											
3	9g	L			L	L	L							L	L	
4	9d	L		L						L		L	L	L		
5	10					L										L
6	13					L					L					
7	15		L	L		L					L	L				
8	16					L										
9	22	L	L	L	L	L	L	L		L	L	L	L	L	L	L
10	29				L							L				
11	30	L	L		L											
12	17			L	L	L										
13	7a				L	L			L							
14	26		L		L											
15	34				L		L									L
16	35		L	L										L		L
17	Tmp		L			L		L		L			L			
18	PI .			L		L									L	L
19	TT3			L	L	L	L	L					L			
20	Wld			L	L	L		L				L				

L: Low infection type (Blank) High infection type

Table 6. Seedling reaction of ten Egyptian wheat varieties against 15 isolates of *Puccinia graminis* f.sp. *tritici* under greenhouse conditions during 2010/2011 growing season

No.	Wheat	WI	neat	ster	n ru	t isol	ate/S	Stem	rust	infec	tion	ype				
	cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Giza160															
2	Giza 164		L			L	L		L							L
3	Giza168	L	L	L	L	L	L	L	L			L				
4	Gemmeiza 7	L	L	L	L	L	L	L		L	L	L	L	L		L
5	Sids 13	L	L	L	L	L	L	L	L							
6	Sakha 8															
7	Sakha 93	L		L	L	L	L				L			L		
8	Sakha 94		L	L	L		L							L		
9	Sids 1	L		L	L		L	L			L			L		
10	Sohag 3		L			L	L		L							L

L: Low infection type (Blank) High infection type

The postulated genes for stem rust resistance that were detected in the ten cultivars revealed the presence of 16 stem rust resistance genes (Table 8). Gene *Sr* 16 proved to be the most frequent gene that was postulated in 8 out of 10 Egyptian wheat genotypes under study (80.00% frequency) followed by *Sr* 5, *Sr* 9e and *Sr* 17 (60.00% each), *SrTmp* (50.00%

frequency), Sr 9g (40.00% frequency), Sr 30 (30.00% frequency), Sr 57a, 26 and Sr 10, 13, 15, 29 and 34 (10.00% each).

Table 7. Probable resistance genes for stem rust (Srs) in ten Egyptian wheat varieties

No.	Wheat cultivars	Probable Sr,s genes for stem rust infection type					
1	Giza160	Not found					
2	Giza 164	16, Tmp?*					
3	Giza168	5, 9e, 9g, 16, 30, 17, 7a, 26, Tmp, Tt3?					
4	Gemmeiza7	5, 9e, 9g, 10, 13, 15, 16, 22, 29, 30, 17, 34, Tmp?					
5	Sids 1	5, 9e, 9g, 16, 17					
6	Sids 13	5, 9e, 16, 30, 17, 7a, 26, Tmp, Tt3?					
7	Sakha 8	Not found					
8	Sakha 93	5, 9e, 9g, 16, 17?					
9	Sakha 94	5, 9e, 16, 17?					
10	Sohag 3	16, Tmp?					

<sup>\*? =</sup> additional genes may be found

Table 8. Frequency (%) of probable genes for stem rust resistance (Srs genes) detected in ten Egyptian wheat cultivars

No.	Monogenic line (Sr,s)	No. of varieties caring Sr,s genes	Gene frequency (%)
1	5	6	60.00
2	9e	6	60.00
3	9g	4	40.00
4	9d	0	00.00
5	10	1	10.00
6	13	1	10.00
7	15	1	10.00
8	16	8	80.00
9	22	0	0.00
10	29	1	10.00
11	30	3	30.00
12	17	6	60.00
13	7a	2	20.00
14	26	2	20.00
15	34	1	10.00
16	35	0	0.00
17	Tmp	5	50.00
18	PI	0	0.00
19	TT3	2	20.00
20	Wld	0	0.00

#### 4. DISCUSSION

One of the most important steps in breeding programs for rust resistance in wheat is the identification of the prevailing physiological races in the region. Such program will be successful if all physiological isolates of the disease are included [19,20].

The identification of virulent phenotypes has been a very important part of the program to breed resistant host cultivars. The study of both alterations in virulence and regional spread of pathogen are prerequisits of combatting yield losses [21]. The evolutionary changes that occur in population of pathogen such as P g. f. sp. tiritici necessitate changes in differential host to maintain relevancy of race determination as well. Experience has indicated that a review of the test sortiment used is required at least every 10 years. The system for race determination is designed to assign the virulence combinations directly, i.e., eliminate the need to contact with a central source before publishing data. The differential host genes were selected on the following basis: a) stability and ease of classification under the range of environmental conditions, generally available for testing at most laboratories, b) both low and high infection type can be detected in most areas of the world. c) the phenotypic difference detected is important in breeding for resistance or in studies of evolution and epidemiology. Additionally, they attempted to maintain a connection with the Stakman series [5,17]. The identification of the genes for rust resistance in the resistance selection was based largely on infection data. Most of the stem rust resistance selection had more than one gene. Before the individual genes possessed by each selection could be identified from infection type data, they had to be separated into lines each with a single gene. The use of these lines for genes identification should be reliable [22]. The virulence combination was designated by a four letter code [16].

The identification of 45 races from 66 samples in first season and 55 races from 39 samples in second season was a clear indication of high virulence diversity within the *Pgt* population in Egypt. A comparison of the races identified in the present study with earlier reports [23] revealed some differences. The earlier study reported the races PTTIT, PPTTT and LTTTT to be the most widespread, whereas the current survey indicated TT---and TK--- races grope to be the dominant races.

Such variation over time is not uncommon, as the races prevalent in a specific season depend on the type of wheat cultivars grown in the season [24] and to some extent on the predominant environmental conditions, especially temperature [25]. Virulence diversities within *Pgt* populations were also reported from countries such as South Africa, Mexico, USA and Canada [26,24,27,28,29].

After analyzing 162 isolates from regions representing the major wheat-growing areas of the country, four important stem rust resistance genes, namely *Sr3*1, *Sr24* and *Sr26* were found to confer resistance to most of the races prevalent in Egypt. *Sr24* and *Sr31* are known to be genes not effective against race Ug99 TTKS, in addition to the field disease severity and infection responses, they identified a number of resistance genes that are effective against Ug99 [30].

On the basis the probability of the presence of 16 *Srs* genes in the tested cultivars except the two cvs. Sakha 8 and Giza 160. The combination of several effective resistance genes into a single cultivar should extend than single seedling genes [25,31].

Gene postulation at seedling stage and gene expression under field conditions may lead to the conclusion that if any cultivar proved to have only one single gene for stem rust resistance, it doesn't lead to durable resistance due to the rapidly development of a new physiologic race of the fungus, that may defeat and overcome this gene after its incorporation into the concerned wheat cultivars. Therefore, this cultivar will be discarded soon after it's released. [32,33,34,35].

Regarding the situation of the tested cultivars in relation to the postulated genes, our results gave an evidence to the probability of the presence of 16 *Srs* genes, *i.e.* in Gemmeiza 7 (13 *Srs* genes) followed by Giza 168 (10 *Srs* genes), Sids 13 (9 *Srs* genes), Sids 1 and Sakha 93(5 *Srs* genes each), Sakha 94 (4 *Srs* genes), Giza 164 and Sohag 3(2 *Srs* each). Whereas, the wheat varieties Giza 160 and Sakha 8 do not contain any resistance genes of the *Sr* set and cultures that were used in this study. These results are in accordance with these previously reported by [10,36]; Pyramiding of multiple stem rust resistance genes into one cultivar will be necessary for long-term control of stem rust. One of the major factors for successful control of stem rust in North America has been the development of cultivars carrying multiple *Srs* genes [37]. These results are in agreement with those previously reported by [38,39], On the other hand one of the several wheats with durable resistance to stem rust is reported to carry *Sr* 2, *Sr* 5, *Sr* 6, *Sr* 7a, *Sr* 8, *Sr* 9b, *Sr* 12 and *Sr* 13 [40]. In contrast, *Sr* 5 and *SrTmp* were effective and included in most of cultivars, respectively and are effective to all races of Uq 99 tell now which confirms the report of [9].

Pyramiding of race-specific resistance genes and the use of more durable, race nonspecific resistance genes are strategies to avoid rapid adaptation of pathogens in the field [41,42,43]. Nevertheless, classical genetic and molecular marker analyses will be needed to further validate and expand the findings of the present study regarding the *Sr* genes responsible for both seedling and adult plant resistance to stem rust in the Egyptian wheat cultivars.

### 5. CONCLUSION

In this study we found race groups TT--- and TK--- more than any race and The frequency of virulence of isolates on lines possessing the resistance genes Sr,s 24, 31, 26, 6, 9e, 29, 35, 9a and 9g were the lowest among the tested lines and should be considered in breeding for resistance. The most effective resistance genes at seedling stage were Sr,s 24, 31, 29, 6, 9a, 26+9g and 9e. About the postulation of genes revealed that the vars. Giza 164 and Sohag 3 probably lowest number of genes, In contrast Giza 168 and Gemmeiza 7 the largest number of genes on the other hand, Giza 160 and Sakha 8 did not have any genes.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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