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MARKER-ASSISTED IDENTIFICATION OF STEM RUST RESISTANCE GENES *SR2, SR13, SR22* AND *SR24* IN EGYPTIAN WHEAT CULTIVARS

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ABSTRACT

Wheat stem rust caused by Puccinia graminis Pers. f. sp. tritici Eriks. and E. Henn. (Pgt), is one of the most destructive wheat diseases. It can cause up to 90 % yield loss in wheat production but has been effectively under control due to the successful deployment of resistant genes in wheat cultivars since the 1950s. The identification of molecular markers of flanking disease resistance genes simplifies the identification of stem rust resistance genes. The objective of this work was to identify the stem rust resistance gens Sr2, Sr13, Sr22 and Sr24 in some Egyptian wheat cultivars. Four SSR markers Xgwm533, Xwmc580, Xcfa2123 and Xbarc71 linked to stem rust resistance genes Sr2, Sr13, Sr22 and Sr24, respectively were used to identify these four genes in 38 Egyptian wheat cultivars. The analysis of 38 Egyptian wheat cultivars for markers linked to stem rust resistance genes indicates that Sr2 was present in 32 cultivars, while Sr13 was detected in 18 cultivars, Sr22 was also detected in 7 cultivars and Sr24 wasn't detected in any cultivar. These markers should be useful in marker-assisted pyramiding of stem rust resistance genes to develop new cultivars with multiple genes resistance against stem rust races in Egypt.

Key words: ***Stem rust, Wheat, SSR markers, Resistance genes.***

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is a stable food for approximately one-third of the world population. Globally, wheat is grown on more than 215 million hectares with an annual production of 700 million tons (FAOSTAT, 2018). With the world population expected to reach 9.0 billion in 2050 compared to 7.0 billion currently, 70 percent more wheat will be required to meet the demands and ensuring yield increases of wheat to meet the future needs has become a focus of agricultural research. Wheat is the most important cereal crop in Egypt where it provides more than 30% of the population's calorie intake. Wheat production is constantly being threatened by both biotic and abiotic stresses. Among the biotic stresses, foliar diseases like rusts and mildews are of paramount importance and these cause heavy yield losses if not controlled.

Stem (black) rust caused by *Puccinia graminis* Pers. f. sp. *tritic*i Eriks. and E. Henn. (Pgt), is one of the most destructive wheat diseases. It can cause up to 90 % yield loss in wheat production. In Egypt, grain yield loss due to stem rust ranged from 1.96 % to 8.21 % in the susceptible wheat cultivars that are cultivated under experimental field conditions favorable to disease incidence and development (Ashmawy et al., 2013). This disease has been effectively under control due to the successful deployment of

resistance genes in wheat cultivars since the 1950s (McIntosh et al., 1995). However, the outbreak of a new stem rust race in Uganda named Ug99 (race TTKSK; Pretorius *et al* 2000), spread throughout much of Africa, the Middle East and Iran, poses an imminent threat to wheat production worldwide (Singh *et al.*, 2006, Sharma *et al* 2013 and Yu *et al* 2014). The global effort to identify new sources of resistance to wheat stem rust, race group Ug99 has resulted in numerous studies reporting both qualitative genes and quantitative trait loci (Yu *et al* 2014).

To improve the efficiency of wheat breeding for durable resistance to stem rust, it is essential to understand the genetic basis in the new released wheat cultivars. To date, 82 stem rust resistance (*Sr*) genes have been numerically designated in wheat as part of the International Wheat Genetics Symposium Gene Catalog (McIntosh *et al* 2017,). Several alleles conferring unique race specificities have been identified for many of these genes resulting in a total of 65 numerically designated resistance genes and alleles. Of these genes and alleles, phenotypic data have been published indicating that at least 27 are effective or partially effective to the Ug99 race group (Yu *et al* 2014), namely *Sr2 (Yr30), Sr13, Sr21, Sr22, Sr24, Sr25, Sr26, Sr27, Sr28, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr40, Sr42, Sr44, Sr45, Sr46, Sr47, Sr51, Sr52, Sr53, Sr55 (Lr67/Yr46/Pm46), Sr57 (Lr34/Yr18/Pm38), Sr58* (*Lr46/Yr29/Pm39*) (Faris *et al* 2008; Ghazvini *et al* 2012; Kolmer *et al* 2011; Jin and Singh 2006; Jin *et al* 2007; Liu *et al.* 2011a, b; McIntosh *et al* 2012; Rouse *et al* 2011; Rouse and Jin 2011; Singh *et al* 2013 and Jin *et al* 2008, 2009).

Triticum turgidum has been a good source of new stem rust resistance genes including *Sr2, Sr9d, Sr9e, Sr9g, Sr11, Sr12, Sr13, Sr14,* and *Sr17* (Singh *et al* 2011). Genes conferring resistance to race TTKSK are *Sr2, Sr13* (McIntosh 1988; Simons *et al* 2011; Singh *et al* 2006, 2011). *Sr2* confers slow rusting adult plant resistance and is linked with the pseudo-black chaff (PBC) phenotype (Singh and Rajaram, 2002). It confers partial resistance to race TTKSK when homozygous and under low to moderate disease pressure (Mago *et al* 2010 and Singh *et al* 2006). The recessive resistance gene *Sr2* is the primary component of the highly effective "*Sr2* complex" of several minor genes (Hare and McIntosh 1979). Resistance gene *Sr13* confers resistance to race TTKSK (Jin *et al* 2007).

Stem rust resistance gene *Sr22* was transferred from *T. boeoticum* and confers resistance to *Pgt* race TTKSK (also known as Ug99) but could be deployed in a limited number of cultivars due to poor agronomic performance of lines carrying the resistance gene (Olson *et al* 2010). Through lines with shortened introgressed segment have now been generated in hexaploid wheat background and markers closely linked to *Sr22* was identified (Periyannan *et al* 2011). Moreover, *Sr22* was transferred from *T. boeoticum* to hexaploid wheat (Elkot *et al* 2015). The stem rust resistance gene *Sr24* has been introgressed into wheat from *Agropyron elongatum.* Smith *et al* (1968) described the stem rust-resistant variety Agent that carries a spontaneous translocation between chromosome 3Ag of *A. elongatum* and chromosome 3DL of bread wheat. Developed robust PCR markers for *Sr24* (Mago *et al* 2005) will facilitate the incorporation of more of *Sr24*, into wheat lines.

The identification of molecular markers of flanking disease resistance genes simplifies breeding activities such as cultivar development (Bonnett *et al* 2005), near-isogenic line development (Zhou *et al* 2005), and pyramiding resistance genes into single genotypes by marker-assisted selection (MAS) (Elkot *et al* 2015). The aim of the cuurent study was to identify four important stem rust resistance genes (*Sr2*, *Sr13*, *Sr22* and *Sr24*) in 38 Egyptian wheat cultivars using different linked molecular markers.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study comprised thirty-eight Egyptian wheat commercial cultivars and four monogenic lines carrying the stem rust resistance genes *Sr2*, *Sr13*, *Sr22* and *Sr24*. Details of these cultivars and their pedigree are presented in Table (1).

Molecular marker analyses

DNA isolation

Fresh leaf samples from 10 to 15-day old seedlings were ground into fine powder in liquid nitrogen and 20 - 50 mg of powdered tissue was used for isolation of total genomic DNA using the following a Cetyl Trimethyl Ammonium Bromide (CTAB) method as modified by Allen et al (2006). The DNA was diluted to a final concentration of 10 ng/µl and quantified in 1% agarose gel for marker analysis.

Table 1. Cross/pedigree of the tested Egyptian wheat cultivars.

No.	Cultivar	Cross/Pedigree & Selection history
1	Sids 1	HD2172/PAVON//1158.57/MAYA74 SD46-4SD-2SD-1SD-0SD
2	Sids 3	SAKHA69/GIZA155 SD723-7SD-1SD-1SD-0SD
3	Sids 4	MAYA/MON//CMH74A.592/3/GIZA157*2 SD10001-2SD-3SD-2SD-0SD
4	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-47/3/BB/GLL/4/ CHAT/6/ MAYA/VUL// CMH74A.630/4*SX SD7096-4SD-1SD-1SD-0SD
5	Sids 13	KAUZ//TSI/TSI/SNB ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-SD
6	Sids 14	BOW"s"/vee"s"//BOW"S"/TSI/BANI SEWEF1 AND SD293-1SD-2SD-4SD-0SD
7	Giza 155	REGENT/2*GIZA139//MIDACADET/2*HINDI62
8	Giza 164	VEERY CM33027-F-15M-500Y- 0GZ
9	Giza 168	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B-0GZ
10	Giza 170	Kouz//Altra/AosCM111 633-6M-020y-010y-010M-2y-OM
11	Giza 171	Sakha 93/Gemmeiza 9 Gz 2003-101-1Gz-4Gz-1Gz-2Gz-0Gz
12	Sakah 8	INDS/NORTENO PK3418-65-0S-0S
13	Sakha 61	INIA/RL4220//7C/YR CM15430-2S-5S-0S-0S
14	Sakha 69	INIA/RL4220//7C/YR CM15430-2S-6S-3S-0S
15	Sakha 92	NAP063/INA66//WEAN S.1551-1S-1S-1S-0S
16	Sakha 93	SAKHA92/TR810328 S.8871-1S-2S-1S-0S
17	Sakha 94	OPATA/RAYON//KAUZ CMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S
18	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/4/WELL1 CMA01Y00158S-040POY-040M-030ZIM-040SY26M- 0Y-0B-0ET

Table 1. Cont.

No.	Cultivar	Cross/Pedigree & Selection history
19	Gemmeiza 7	CMH74A.630/SX//SERI82/3/AGENT GM4611-2GM-3GM-1GM-0GM
20	Gemmeiza 9	ALD/HUAC//CMH74A.630/SX GM4583-5GM-1GM-0GM
21	Gemmeiza 10	MAYA74/0N//160-147//3/BB/GLL/4/CHAT/5/CROW GM5820-3GM-1GM-2GM-0GM
22	Gemmeiza 11	B0W/KVZ//7C/SERI82/3/GIZA168/SAKHA61 GM7892-2GM-1GM-2GM-1GM-0GM
23	Gemmeiza 12	OTUS/3/SARA/THB//VEE CMSS97Y00227 S-5Y-010M-010Y- 010M-2Y – 1M-0Y- OGM
24	Shandweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/MIRLO/BUC CMSS03B00567S-72Y-010M-010Y-010M-0HTY-0SH
25	Nubaria 1	OASIS/5*BOR95/4/CNDO/R143//ENTE/MEX175/3/CNDO/R143
26	Bani Sweif 1	JO/AA//FG CD9799-126M-1M-5Y-0M-0SD.
27	Bani Sweif 3	CROM/RUF0 CD4893-10Y-1M-1Y-0M-0SD.
28	Bani Sweif 4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/w*2 ICD88-1120-ABL-0TR-1BR-0TR-6AP-0AP-OSD
29	Bani Sweif 5	DIPPERZ/BUSHEN3 CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD
30	Bani Sweif 6	BOOMER-21/BUSCA-3 CDSS95Y001185-8Y-0M-0Y-0B-1Y-0B0SD
31	Sohag 1	GDOVZ469/JOS//61 130-LSD.
32	Sohag 2	CR/PELICANO//CR/G SH19-1SH-1SH-0SH.
33	Sohag 3	MEXI/MGHA/51792//DURUM6 CD21831-25H-1SH-0SH
34	Sohag 4	AJAIA-16//HORA/JRO/3/GAN/4/ZAR/5/SUOK-7/6/STOT//ALTAR84/ALD CDSS99B00778B-0SHS-OTOPY-0M-0Y-129Y-0M-0Y-1
35	Sohag 5	TRN//21563/AA/3/BD2080/4/BD2339/S/RASCON37//TARRO2//RASCON3/6/AUK//GULL//GREEN CDSS00B00364T-0T0PB-0B-2Y-0M-0Y-1B-0SH
36	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S
37	Misr 2	SKAUZ/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S
38	Misr 3	ATTILA*2/ABW65*2/KACHU CMSS06Y00258 2T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY

PCR amplification and marker analysis

Four SSR markers linked to stem rust resistance genes (Table 2) were used for detecting the presence of stem rust resistance genes in Egyptian wheat.

Table 2. Primer sequence and annealing temperature of the SSR markers used for identification of stem rust resistance(*Sr*) genes in wheat cultivars.

Sr gene	SSR marker	Primer Sequence		Annealing
Sr2	*Xgwm533*	F	5' AAGGCGAATCAAACGGAATA3'	60°C
		R	5' GTTGCTTTAGGGGAAAAGCC 3'	
Sr13	*Xwmc580*	F	5′ AAGGCGCACAACACAATGAC3′	60°C
		R	5′GGTCTTTTGTGCAGTGAACTGAAG3′	
Sr22	*Xcfa2123*	F	5' CGGTCTTTGTTTGCTCTAAACC 3'	60°C
		R	5' ACCGGCCATCTATGATGAAG 3'	
Sr24	*Xbarc71*	F	5' GCGCTTGTTCCTCACCTGCTCATA 3'	55°C
		R	5' GCGTATATTCTCTCGTCTTCTTGTTGGTT 3'	

The PCR reaction was carried out in a 25 µl reaction volume containing 3.0 µl of template DNA (10ng/µl stock), 3.0 µl of 5X PCR buffer (Promega, USA), 1.5 µl of 25 mM MgCl2 (total 1.5 to 2.5 mM $MgCl_2$ per reaction), 3.0 µl of each dNTP (Promega, USA), 0.2 µl of Taq DNA polymerase (GoTaq® Flexi DNA Polymerase, Promega, USA), 1.5 µl of each SSR marker (5mM) stock and 6.3 µl distilled H_2O. Amplification was carried out in a PTC-200 Peltier thermal cycler programmed at 1 cycle of 4 min at 94°C, 94°C for 1 min, 50-61°C (depending on marker) for 1 min and 72°C for 1 min (35 cycles) and a final extension step of 72°C for 7 min (1 cycle). PCR products were resolved on 2 to 3% agarose (SIGMA, USA) gel at 100v for 3 to 4h. Gels were stained in ethidium bromide and photographed on a digital gel documentation system (ChemiDoc MP System, BIO-RAD, USA). The DNA ladder (100 bp DNA) was used (3 µl) for determining the molecular size of the DNA bands.

Evaluation of the tested wheat genotypes for stem rust at adult plant stage under field conditions:

The adult plant evaluation of the tested Egyptian wheat cultivars for stem rust reaction was carried out at the Experimental Farm of Nubaria

Agricultural Research Station during the growing season 2018/2019. A field trial was performed in a complete randomized block with three replicates. Sowing date was in mid-November. Cultivars seeds were sown in 3 m long rows (3 rows/cultivar) with 30 cm apart. Spreader rows of susceptible varieties i.e. Moroccon and Max were planted around the experiment to establish a uniform disease pressure and at every 15-20 m distance in breeding nurseries where 38 Egyptian wheat cultivars were grown. Spreader plants were inoculated artificially with the prevailing *Pgt* races in Egypt to initiate epidemics. The inoculation process was carried out at booting stage according to Tervet and Cassell (1951). All cultural practices recommended for wheat crop were applied.

Adult plant reaction to wheat stem rust was assessed at the early dough stage (Large, 1954) when rust symptoms fully developed. Based primarily on the size of pustules and the associated necrosis or chlorosis, infection responses were classified into five discrete categories (Roelfs *et al* 1992) *i.e.* immune (0), no uredinia or other symptoms of disease infection; resistant (R), miniature uredinia surrounded by necrosis; moderately resistant (MR), small uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia possibly surrounded by chlorotic areas; and susceptible (S), large uredinia without chlorosis and necrosis. Infection responses overlapping between any particular two categories are denoted using a dash. For instance, `MR-MS' indicates an infection response class that overlaps between MR and MS categories. Stem rust severity is evaluated as a percentage infected area following the Modified Cobb's scale (Peterson *et al* 1948). Entries were evaluated for stem rust severity two to three times between the heading and physiological maturity of plants.

RESULTS AND DISCUSSION

Molecular markers analysis

Microsatellites or simple sequence repeats (SSRs) markers show co-dominant expression and multiallelism, highly polymorphic, genome-specific, abundantly distributed throughout the genome and have become important genetic markers in wheat breeding and because of these characteristics, simple sequence repeats markers/microsatellite markers exhibit high PIC values (Polymorphism information content). These

markers display high gene diversity scores which make them useful in distinguishing closely related genotypes. Somers *et al* (2004). Four markers were used to identify stem rust resistance genes in some Egyptian wheat cultivars.

Sr2

Sr2 is located on the short arm of chromosome 3B and confers partial resistance only in the homozygous state (recessive resistance gene). It was originally transferred from Yaroslav emmer wheat into hexaploid wheat. This gene is very effective under Egyptian field conditions during 2016/17, 2017/18 and 2018/19 growing seasons (El-Orabey et al., 2019). The SSR marker namely *Xgwm533* was tightly linked to *Sr2* (Spielmeyer *et al* 2003). The microsatellite marker *Xgwm533* was used to identify *Sr2* introgression in thirty-eight Egyptian wheat cultivars. Out of the total 38 Egyptian wheat cultivars, 32 cultivars were positive with the *Xgwm533*, while 6 cultivars were negative for the *Xgwm533* linked to *Sr2* and did not show any introgression (Fig. 1, Table 3).

Sr13

Likewise, the microsatellite marker *Xwmc580* the closer common marker to *Sr13* (Table 2) was used to identify *Sr13* in Egyptian wheat. The location of *Sr13* in tetraploid wheat cultivars is on the long arm of chromosome 6A. *Sr13* is the only known gene effective against the TTKS complex of *P. graminis* sp. *tritici*; the TTKSK (Ug99) race and its variants, TTKST and TTTSK. Currently, this gene is the only one effective against the TTKS complex. Out of the total 38 cultivars, 18 cultivars were positive for markers flanking the *Sr13* and 20 cultivars did not show any introgression for markers linked to the *Sr13* (Fig. 2, Table 3).

Sr22

The microsatellite marker *Xcfa2123* mapped on the long arm of chromosome 7A and linked to stem rust resistance gene *Sr22* was used to identify the *Sr22* introgression in Egyptian wheat cultivars. Out of the total 38 cultivars, 7 cultivars (Sakha 94, Bani Swief 1, 3, 4, 6 and Sohag 2, 3) were positive for marker *Xcfa2123* linked to *Sr22* and 31 cultivars didn't show introgression for markers linked *Sr22* (Fig. 3, Table 3).

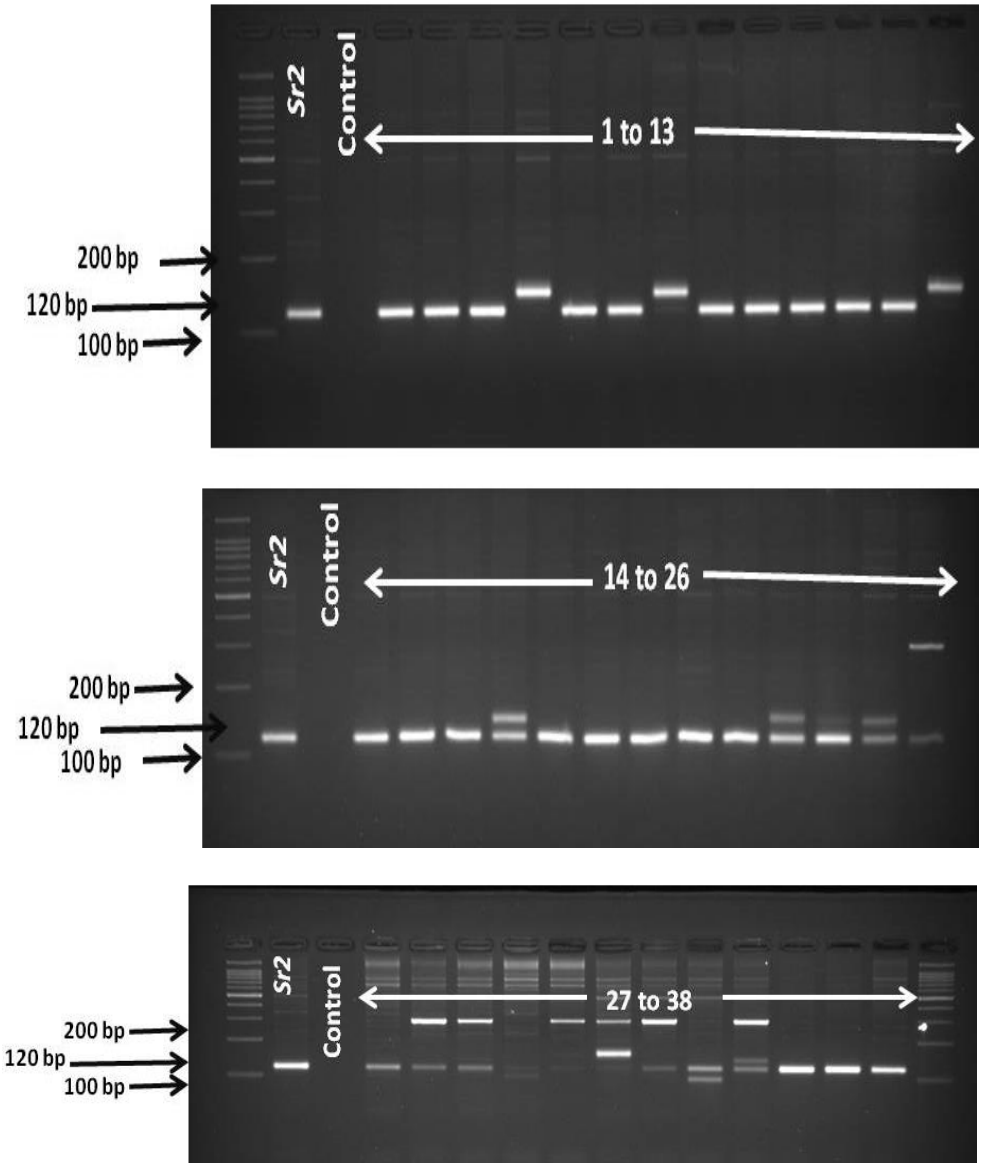

Fig. 1. PCR amplification profile of *Xgwm533* marker linked to *Sr2* with thirty-eight Egyptian wheat cultivars.

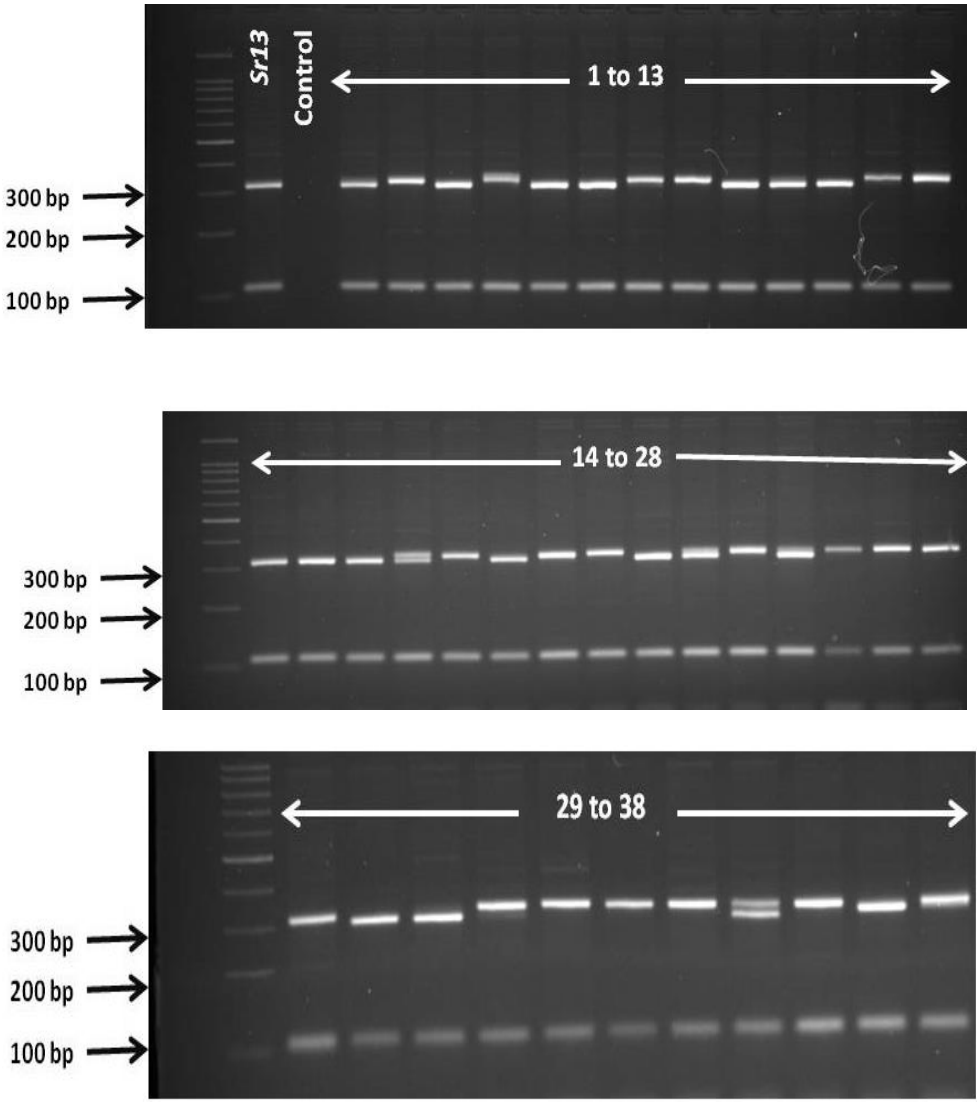

Fig. 2. PCR amplification profile of *Xwmc580* marker linked to *Sr13* with thirty-eight Egyptian wheat cultivars.

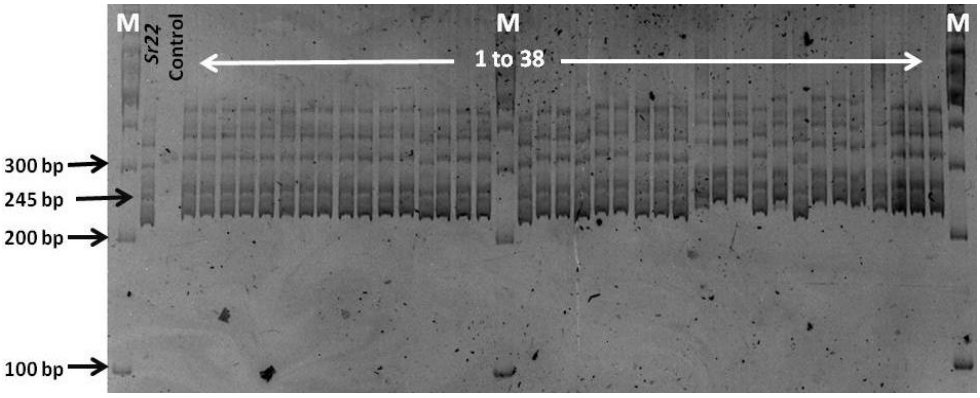

Fig. 3. PCR amplification profile of *XCfa2123* marker linked to *Sr22* with thirty-eight Egyptian wheat cultivars.

Table 3. Adult plant stem rust response during 2018/19 grwoing season and *Sr* gene-linked molecular marker of thirty-eight Egyptian wheat cultivars.

No.	Cultivar	Stem rust response	*Sr2*	*Sr13*	*Sr22*	*Sr24*
			Xgwm533	*Xwmc580*	*Xcfa2123*	*Xbarc71*
1	Sids 1	Tr S	+	+	-	-
2	Sids 3	0	+	-	-	-
3	Sids 4	0	+	+	-	-
4	Sids12	0	-	-	-	-
5	Sids13	0	+	+	-	-
6	Sids14	*	+	+	-	-
7	Giza 155	Tr R	-	-	-	-
8	Giza 164	0	+	-	-	-
9	Giza 168	10 MS	+	+	-	-
10	Giza 170	0	+	+	-	-
11	Giza 171	10 MR	+	+	-	-
12	Sakha 8	Tr R	+	-	-	-
13	Sakha 61	5 R	-	-	-	-
14	Sakha 69	Tr R	+	+	-	-
15	Sakha 92	0	+	+	-	-
16	Sakha 93	0	+	+	-	-
17	Sakha 94	Tr R	+	+	+	-
18	Sakha 95	Tr MR	+	-	-	-

Table 3. Cont.

No.	Cultivar	Reaction	*Sr2*	*Sr13*	*Sr22*	*Sr24*
			Xgwm533	*Xwmc580*	*Xcfa2123*	*Xbarc71*
19	**Gemmeiza 7**	20 MS	+	+	-	-
20	**Gemmeiza 9**	5 MR	+	-	-	-
21	**Gemmeiza 10**	0	+	-	-	-
22	**Gemmeiza 11**	0	+	+	-	-
23	**Gemmeiza 12**	5 MR	+	+	-	-
24	**Shadaweel 1**	Tr R	+	-	-	-
25	**Nobaria 1**	5 MR	+	+	-	-
26	**Bani Sweif 1**	*	+	-	+	-
27	**Bani Sweif 3**	*	+	-	+	-
28	**Bani Sweif 4**	0	+	-	+	-
29	**Bani Sweif 5**	0	+	+	-	-
30	**Bani Sweif 6**	*	-	+	+	-
31	**Sohag 1**	*	-	-	-	-
32	**Sohag 2**	*	-	-	+	-
33	**Sohag 3**	*	+	-	+	-
34	**Sohag 4**	Tr MS	+	-	-	-
35	**Sohag 5**	*	+	+	-	-
36	**Misr 1**	60 S	+	-	-	-
37	**Misr 2**	30 MS	+	-	-	-
38	**Misr3**	Tr MS	+	-	-	-

R: Resistant, MR: Moderately Resistant, MS: Moderately Susceptible, S: Susceptible, (*): Data is not presented, (+): Presence of *Sr* genes, (-): Absence of *Sr* genes

Sr24

Sr24 was mapped on the 3DL chromosome, within a spontaneous translocation from the 3Ag chromosome of *Agropyron elongatum* (Mago *et al* 2005). There are several molecular markers available for *Sr24*, including an SSR (*Xbarc71*), 38 Egyptian wheat cultivars were analysed for marker *Xbarc71* linked to stem rust resistance gene *Sr24*. The thirty-eight Egyptian wheat cultivars were negative for linked marker *Xbarc71* and didn't show introgression for markers linked to *Sr24* (Fig. 4, Table 3).

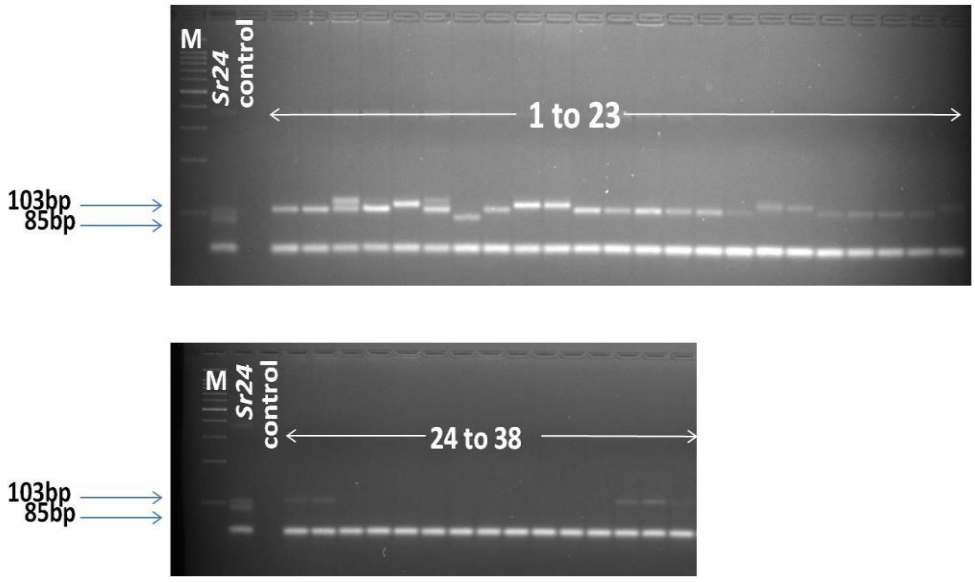

Fig. 4. PCR amplification profile of *Xbarc71* marker linked to *Sr24* with thirty-eight Egyptian wheat cultivars.

Our study indicated that only one cultivar (Sakha94) was positive for three markers indicating the presence of *Sr2*, *Sr13* and *Sr22* genes. Out of total 38 cultivars, 17 cultivars were positive for two markers (*Xgwm533* and *Xwmc580*), 3 cultivars were positive for another two markers (*Xgwm533* and X*cfa2123*), one cultivar was positive for two markers (*Xwmc580* and *Xcfa2123)* and 12 cultivars were positive for one marker only, while 4 cultivars didn't show the presence of any of the four markers (Table 3).

The *Sr2* gene has played an important role in conferring durable resistance to stem rust worldwide, the gene is difficult to select for in breeding programs. The partial resistance phenotype can be difficult to score in the field and is often masked by other effective resistance genes in the background. Breeders often rely on the expression of pseudo-black chaff to transfer the gene, but this trait is also an unreliable indicator due to variable levels of pigmentation observed in different *Sr2* carrying wheats. The durable *Sr2* gene is, therefore, an ideal candidate for deploying a breeder-friendly marker that will remove some of the contingencies and permit more accurate selection in breeding (Mago *et al* 2011). An accurate and robust

marker for *Sr2* would benefit the wheat breeding community worldwide because *Sr2* is an important resistance gene which is difficult to select. *Sr2* is located on the short arm of wheat chromosome B (Hare and McIntosh 1979). Spielmeyer *et al* (2003) described a tight linkage of the SSR marker *gwm533* with *Sr2* (approx. 2 cM) and showed that a 120 bp product was associated with the presence of the gene in most lines tested. Our result showed that, the cultivars carrying *Sr2* introgression amplified at a 120 bp product (Fig. 1). Our molecular analysis of 38 cultivars showed that 32 cultivars were carrying *Sr2* introgression and the cultivars carrying *Sr2* introgression showed a good level of resistance under field condition. That can be possible because *Sr2* is a stem rust resistance gene that has been used in breeding for around 60 years as a source of durable and broad-spectrum adult plant resistance, which includes resistance to Ug99 and its related isolates.

Sr13 is a stem rust resistance gene present in several *Triticum turgidum* ssp. Durum cultivars. Its main source is the Ethiopian landrace ST464 and the *T. turgidum* ssp. *dicoccum* L. (emmer wheat) germplasm Khapli (Knott 1962). Several resistance genes are still effective against the three TTKS lineages in both tetraploid (*T. turgidum* ssp. *durum* L.) and hexaploid wheat (*T. aestivum* L.) cultivars (Jin *et al* 2007). Among them, *Sr13* is the only known gene with effective resistance to the TTKS races. The Ethiopian landrace ST464, and the domesticated emmer wheat (*T. turgidum* ssp. *dicoccum* L.) Khapli are the two major sources of *Sr13* in durum (Knott 1962 and Klindworth *et al* 2007). The *Sr13* resistance gene from Khapli was transferred to the common wheat variety Khapstein from the cross Steinwedel 9 Khapli and was subsequently mapped on the distal region of the long arm of chromosome 6A by McIntosh (1972). The moderate resistance of *Sr13* to TTKS makes it a good candidate for gene pyramiding with other stem rust resistance genes. Our findings revealed that 18 Egyptian cultivars were positive for marker linked to *Sr13* and most of these cultivars showed a good level of resistance in the field from immune reaction to trace moderately resistance and some of these cultivars showed 20 MS, that can be due to the presence of new races of Ug99 in Egypt last two years. The tightly linked marker used in this study (*Xwmc580*) would be

useful for marker-assisted selection efforts for *Sr13* only in targeted populations generated from parental lines with known *Sr13* alleles.

The *Sr22* gene was originally identified in the diploid wheat species *Triticum monococcum* ssp. *boeoticum* accession G-21 (Gerechter-Amitai *et al* 1971) and *T. monococcum* L. accession RL5244 (Kerber and Dyck 1973). It was then transferred to tetraploid and hexaploid wheat through interspecific hybridizations. *Sr22* was mapped on the long arm of chromosome 7A. Among three reliable linked markers, *cfa2019*, *cfa2123*, and *Xbarc121* (Miranda *et al* 2007), we used *cfa2123* linked marker to monitor the presence of *Sr22* into 38 Egyptian wheat cultivars. Our results indicated the presence of *Sr22* in 7 Egyptian wheat cultivars. However, only the 245-bp fragment was amplified in *Sr22*, indicating that only the 245-bp fragment was specific to *Sr22*. *Sr22* transferred from *T. boeoticum* to hexaploid wheat has not been used widely because of linkage drag associated with it. As this gene confers resistance to the stem rust race TTKSK (known as Ug99), renewed interest in its deployment demanded shortening of the introgressed segments. DNA markers closely linked to *Sr22* were identified and used to shorten the introgressed segments (Olson *et al* 2010). The *Sr22* was transferred from *T. boeoticum* to the Indian wheat cultivars PBW343, PBW621 using marker-assisted selection (Elkot et al 2015).

The stem rust resistance gene *Sr24* has been introgressed into wheat from *Agropyron elongatum*. The closely linked marker *Xbarc71* was used to indicate the presence of *Sr24* introgression into Egyptian wheat cultivars. The marker *Xbarc71* amplified fragment size 103–bp and 85-bp in monogenic lines specific for *Sr24*. None of the tested thirty-eight Egyptian wheat cultivars showed the introgression of *Sr24*. Mago *et al* (2005) reported that *Xbarc71* amplified 85- and 103-bp fragments in *Sr24* containing lines and a 107-bp fragment in most but not all susceptible lines. Olson *et al* (2010) reported that *Xbarc71* amplified 83-, 88-, and 101-bp fragments from the translocated segment containing *Sr24* locus, however, *Xbarc71* also amplified wheat fragment 107 bp in length when the *Lophopyrum* translocation was on 1BS. However, Abo Aly *et al* (2014) reported the presence of *Sr24* in Egyptian wheat cultivars Sakha93 and Misr1 using AFLP marker *Sr24*#12 which is linked to *Sr24*.

Evaluation of the tested wheat genotypes to stem rust at adult plant stge under filed conditions:

Adult plant reaction data to wheat stem rust were recorded at Nubaria Agricultural Research Station, Agricultural Research Center of Egypt during the growing season 2018/2019, under artificial inoculation. Out of the tested thirty-eight Egyptian cultivars, 12 cultivars (Sids3, Sids4, Sids12, Sids13, Giza 164, Giza170, Sakha61, Sakha92, Sakha93, Gemmeiza11 and Bani Swief 4, 5) recorded null of infection type (immune) exhibiting the best performance of resistance. Five cultivars (Giza155, Sakha8, Sakha69, Sakha94 and Shandaweel1) showed full resistance response (R), and three cultivars (Giza171, Sakha95, Gemmeiza 9) showed moderate resistance (MR). However, five cultivars (Giza168, Gemmeiza7, Sohag4, Misr2 and Misr3) were moderately susceptible (MS), and only one cultivar Misr1 showed high susceptibility (60 S) (Table 3). This indicates the possibility that these varieties carry several stem rust resistance genes some effective while others ineffective against Ug99. There is also a possibility that they may have effective stem rust resistance genes but the expression level of the genes is still low to provide sufficient resistance (Table 3). This might be due to the most stem rust resistance genes have become ineffective against Ug99 new variants. For instance, the *Sr31* gene which was widely used in the majority of the world's wheat germplasm, as a result, it was broken down by the most variants of the Ug99 stem rust race.

Sr2 was also present in recently released Ug99-resistant varieties Misr1 and Misr2 in Egypt (Singh *et al* 2011). This fact was confirmed by our results obtained, however, Misr1 and Misr2 showed high level of susceptibility to stem rust races in Egypt, recording 60 S and 30 MS, respectively. Resistance gene *Sr2* was found to be ineffective against the local *Pgt* population. Thus, wheat cultivars Misr1 and Misr2 are not prone to infection by Ug99 and its variants, as both carry the *Sr2* gene. Therefore, it is important to broaden the genetic base of stem rust resistance in future wheat varieties by pyramiding multiple stem rust resistance genes, especially those effective against local *Pgt* races (Draz 2017). The pathogen is changing rapidly and seven variants are now recognized as being part of the Ug99 race lineage. All are closely related, having nearly identical DNA

fingerprints, but differ slightly in their avirulence/virulence profiles (Szabo *et al* 2007 and Visser *et al* 2010).

In the present study, we used linked markers for four stem rust resistance genes in Egyptian wheat cultivars, which help to identify the presence of these genes in Egyptian wheat cultivars. The identification of disease resistance genes can help in pyramiding major and minor genes in different breeding materials, hence genes identified in specific background, marker-assisted selection can facilitate the transfer of these genes in new advanced breeding materials.

CONCLUSION

Molecular markers linked to effective stem rust resistance genes can be used to predict the presence of specific genes with high accuracy, thus helping with the transfer of several genes into adapted material. With the availability of next-generation sequencing platforms, more diagnostic resistance gene markers will be available for high-throughput screenings and application of MAS in breeding for stem rust resistance.

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إستخدام المعلمات الجزيئيه لتحديد جينات مقاومة صدأ الساق *Sr2*، *Sr13*، *Sr22*، *Sr24* في أصناف القمح المصري

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يعتبر صدأ الساق المتسبب عن الفطر Puccinia graminis f. sp. tritici من أهم أمراض القمح والذى قد يسبب خسائر تصل الى ٩٠% فى محصول القمح ولكن تم السيطرة عليه بشكل فعال من خلال إدخال جينات المقاومة الفعاله في أصناف القمح منذ الخمسينيات. تحديد المعلمات الجزيئية المرتبطه بجينات مقاومة الأمراض يبسط من تحديد جينات مقاومة صدأ الساق. وكان الهدف من هذا العمل هو التعرف على جينات المقاومة لصدأ الساق Sr2 ، Sr13 ، Sr22 ، Sr24 في بعض أصناف القمح المصري. تم استخدام أربعة معلمات جزيئية وهى Xgwm533 ، Xwmc580 ، Xcfa2123 ، Xbarc71 مرتبطة بجينات المقاومه لصدأ الساق Sr2، Sr13، Sr22 ، Sr24 على التوالي لتحديد هذه الجينات الأربعة في ٣٨ صنف قمح مصرى. أظهرت نتائج الدراسه أن تحليل ٣٨ صنف قمح مصري باستخدام المعلمات الجزيئية المرتبطة بجينات مقاومة صدأ الساق إلى تحديد Sr2 في ٣٢ صنفًا، Sr13 في ١٨ صنفًا، Sr22 في ٧ أصناف، بينما Sr24 لم يظهر في أي من الأصناف محل الدراسة. يجب أن تكون هذه المعلمات الجزيئية أداه جيده فى نقل جينات المقاومة الفعالة لتطوير أصناف جديدة ذات مقاومة متعددة الجينات ضد صدأ الساق فى مصر.

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