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Identification of some Wheat Monogenic Lines Resistant to Stem Rust Disease Using Molecular Markers

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ABSTRACT

Ten stem rust monogenic lines and 14 commercial wheat cultivars were estimated under natural infection of stem rust disease for adult plant resistance (APR) during the 2019 – 2021 growing seasons at Gemmeiza Agricultural Research Station, ARC. Obtained results showed that *Sr*46, *Sr*47, and *Sr*51 were completely resistant along the three seasons, however, *Sr*50 showed zero to trace % resistance. The rest of the *Srs* tended to have a susceptibility ranging between 20% and 80%. The genes *Sr*54 and *Sr*45 had the highest mean values of area under disease progress curve (AUDPC) and rate of disease increases (r-value) followed by *Sr*49, *Sr*52, *Sr*48, and *Sr*53, respectively. Local wheat cultivars Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Giza-171, Sakha-94, Sakha-95, Sids-14, and Shandweel-1 showed different resistance values ranging from (immune) zero to 10 moderately resistance (MR). While, Giza-168, Sids-12, Misr-3, Misr-2, and Misr-1 cultivars respectively displayed susceptibility ranging between moderately susceptible Tr-MS to susceptible 60S. Six simple sequence repeat (SSR) markers linked to stem rust resistance genes i.e. *Sr*45, *Sr*46, *Sr*47, *Sr*49, *Sr*50, and *Sr*52, respectively were selected to test their presence/absence in fourteen Egyptian wheat cultivars. The SSR results indicated the presence of *Sr*45, *Sr*46, and *Sr*49 in the tested Egyptian cultivars. *Sr*47 was positive in all cultivars except in Misr-2, while *Sr*50 was present only in Gemmeiza-12, Gemmeiza-9, Gemmeiza-11, Gemmeiza-10, Shandweel-1, and Sakha-94 cultivars. However, *Sr*52 is present in all tested cultivars except Gemmeiza-11. *Sr*49, *Sr*50, and *Sr*52 displayed high levels of polymorphism (75, 100, and 100%, respectively) as analyzed the iMEC software.

Keywords: stem rust, monogenic lines, SSR, polymorphism.

INTRODUCTION

Wheat stem rust (*Puccinia graminis* f. sp. *tritici*) is a devastating destructive wheat disease that develops mainly in regions with warm weather and results in 100% losses (Leonard and Szabo, 2005). Epidemics of stem rust increased during the last decades due to climatic changes, emergence of Ug99 new variants, susceptible wheat cultivars, and excessive use of fungicides. The variants of stem rust Ug99 race render about 90% of total wheat cultivars developed around the world (Singh *et al*., 2011, 2015; Soko *et al*., 2018). TTKSK variant of the stem rust Ug99 was detected from samples collected during late-maturing trials in three research stations in Delta Egypt (BGRI 2015; Patpour *et al*., 2016). These results considered Egypt the 13th country in which one of the Ug99 races has been detected. Several *Sr* genes are derived from wild relatives of wheat and all have been introgressed into hexaploid bread wheat (Liu *et al*., 2011a, b, 2013; Niu *et al*., 2011, 2014; Qi *et al*., 2011; Klindworth *et al*., 2012; Mago *et al*., 2013; McIntosh *et al*., 2014; Periyannan *et al*., 2014). Currently about sixty-five numerically designated stem rust resistance genes and alleles, however, not all of them show resistance to stem rust (Yu *et al*., 2014). Numerous of these genes have been incorporated into cultivated wheat or have involved into breeding programs. APR offers protection against fungal diseases of cereals and is expressed between tillering and booting stages (Lagudah, 2011). Molecular technologies have become critical in plant sciences for recognizing species and finding plants relationships. Genetic linkages are crucial for germplasm selection for plant breeding, as well as the evolutionary and conservation research (Henry, 2012). Development of many markers closely linked to the resistant genes allows identification and introgression of the desirable genes. The combination of evaluation at the adult stage and molecular markers provides accurate identification of resistance genes for further use in a breeding program. In Egypt, some studies were carried out to identify effective stem rust genes at the adult stage in the Egyptian bread wheat cultivars, i.e. *Sr*13, *Sr*25, *Sr*26, and *Sr*31(El-Shamy and Omar, 2014); *Sr*2, *Sr*24, *Sr*26, and *Sr*31 (Abu Aly *et al*., 2014); *Sr*2, *Sr*13, *Sr*22 and *Sr*24 (Elkot *et al*., 2020). This work aims to evaluate 10 stem rust monogenic lines for APR to stem rust disease in natural field infection and molecular detection of the available six genes in 14 Egyptian bread wheat cultivars.

MATERIALS AND METHODS

Wheat materials

Ten stem rust monogenic differentials with known resistance genes (*Sr* genes) were kindly supplied by Dr. Thomas (Tom) Fetch Jr., Research Scientiste, Canada. Gene name, source, and chromosomal location are given in Table (1). Also, this study includes fourteen Egyptian wheat cultivars whose names and pedigree were reported by Elshamy *et al*. (2016).

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Table 1. Gene's designation, location on chromosome, source, line and references of stem rust resistance genes.

Gene designation	Location on chromosome	Source	Line	Reference
*Sr*45	1DS	*Aegilops tauschii*	RL 5406	Singh *et al*., 2011, Sambasivam *et al*., 2008
*Sr*46	2DS	*Aegilops tauschii*	Aus 18913	Yu *et al*., 2015, Rouse *et al*., 2011, Singh *et al*., 2011
*Sr*47	2BL	*Aegilops speltoides*	DAS 15	Faris *et al*., 2008, Klindworth *et al*., 2012
*Sr*48	2AL	*T. aestivum*	Arina	Bansal *et al*., 2009
*Sr*49	5BL	Landrace AUS28011 (Mahmoudi)	AUS 28011	Bansal *et al*., 2015
*Sr*50	1DS	Rye cultivar Imperial	T6-1	Mago *et al*., 2015
*Sr*51	3A	*Aegilops searsii*	TA5622	Liu *et al*., 2011
*Sr*52	6AL	*Dasypyrum villosum*	T6AS6V3L	Qi *et al*., 2011
*Sr*53	5DL	*Aegilops geniculate*	TA5630	Liu *et al*., 2011
*Sr*54	2DL	Norin40	Norin 40	Ghazvini *et al*., 2013

Disease assessment.

APR of the 10 *Sr* monogenic lines and the 14 local wheat cultivars was evaluated under field natural infection of stem rust disease at Gemmeiza Agricultural Research Station from the 2018/2019- 2020/2021 three growing seasons. The materials were sown in rows, 1.2 m long, and 30 cm apart in three replicates. Twenty seeds were sown per row for each line and cultivar, with three replicates. Morocco, a highly susceptible variety was served as a check and as a border surrounding the experiment, 1m wide to promote homogeneous disease spread. The modified Cobb scale (0 -100) was used to calculate disease severity% (Peterson *et al*., 1948) and type of infection (Stakman *et al*., 1962) which, stem rust was classified as, 0 (immune), R (resistant), MR (moderately resistant), M (Intermediate), MS (moderately susceptible) or S (susceptible). Disease severity was scored four times until final rust severity% to estimate the AUDPC for each line (Pandy *et al*., 1989) using the following equation:

$$\mathbf{AUDPC = T\{1/2\,(Y_1 + Y_k) + (Y_2 + Y_3 + Y_{k-1})\}}$$

T = Interval time between scores

Y_1 = The first score of disease severity

Y_K =The last score of disease (final rust severity)

To calculate AUDPC values, the disease severity is multiplied by a constant value for each infection type of stem rust to calculate (ACI) according to (Saari and Wilcoxson, 1974), where: R= 0.2 MR = 0.4 MS = 0.8 S = 1.00.

Also, the r-value for each *Sr* gene was calculated as reported by Van der Plank (1963).

Molecular detection of *Sr* genes.

This work was carried out in Plant Pathology and Biotechnology and Lab EPCRS Excellence Center (accredited according to ISO 17025, ISO 9001, ISO 14001, and OHSAS 18001), Department of Agricultural Botany, Faculty of Agriculture, Kafr ElSheikh University, Egypt.

DNA Extraction.

From 200 mg of the 15-day-old seedlings' leaf tissue, the entire DNA of each stem rust monogenic line and the 14 bread wheat cultivars was extracted. Invisorb® Spin Plant Mini Kit (STRATEC Molecular, Germany) was used to extract DNA after the fresh tissues were digested in liquid nitrogen using a mortar and pestle, in according to instructions of the manufacturer.

PCR amplification conditions.

The Sr45, Sr46, Sr47, Sr49, Sr50, and Sr52 genes were detected using six unique SSR markers. Amplification for Sr regions was carried in an automated thermal cycler (C1000TM Thermal Cycler, Bio-RAD) applying the primers and conditions shown in Table 2 with one pre-denaturation cycle for 3 min at 94ºC.

Table 2. Stem rust genes, marker name, sequence, annealing temperature, and band size were used in this study.

Sr genes	Markers	Primer sequence	Annealing temperature	Bands size (bp)	References
*Sr*45	cssu45	5′CGAGTTTCAATACTTCGCCC3′ 5′GATTACTATGCAATAGGGCCC3	48.5°C	220-228 bp	Periyannan *et al*. (2014)
*Sr*46	Xgwm210	5'TGCATCAAGAATAGTGTGGAAG3' 5' TGAGAGGAAGGCTCACACCT 3'	60°C	172 -178bp	Yu *et al*. (2015)
*Sr*47	Xgpw4043	5'- ACATATGCACGCACGCAC 3' 5'- CATTGACACCCCTGACACTC 3'	57.5°C	128,152, 297, and 379 bp	Klindworth *et al*. (2017)
*Sr*49	sun209	5'AGACTATGAGCTTCGCTATTG3' 5'GTGATTGGTTCGGATTACTTA3'	60°C	140–158 bp	Bansal *et al*. (2015)
*Sr*50	IB-267	5'GCAAGTAAGCAGCTTGATTTAGC3' 5'-AATGGATGTCCCGGTGAGTGG3'	55°C	200- 300bp	Mago *et al*. (2002)
*Sr*52	WMS570	5'- TCG CCT TTT ACA GTC GGC 3' 5'- ATG GGT AGC TGA GAG CCAAA 3'	60°C	100 -200 bp	Qi *et al*. (2011)

The PCR mixture was as follows, 1 µl of each primer (10 pmol), (1 µl) of 25 ng nucleic acid, 9.5 µl of Nuclease free water (Promega), and (12.5 µl) of Colorless Master Mix (GoTag®). PCR products were resolved on 2 to 3% agarose (SIGMA, USA) gel at 100v for 3 to 4h. Ethidium bromide was used to stain the gels, which were then photographed using a digital gel documenting 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. The amplified bands of SSRs were recorded as present (1) or absent (0).

Genetic analysis of DNA bands.

According to Amiryousefi *et al*., (2018), the Marker Efficiency Calculator Online software (iMEC) is used to calculate polymorphism information content (PIC), expected heterozygosity (H), effective multiplex ratio (E), arithmetic mean heterozygosity(Havp), discriminating power(D), Marker Index (MI) and resolving power (R).

RESULTS AND DISSCUTIONS

Results

Adult plant response to stem rust disease.

Data in Table 3 showed that the stem rust monogenic lines; *Sr*46, *Sr*47, *Sr*50, and *Sr*51 were completely resistant during the three seasons of study. The remained *Sr*s lines showed susceptibility ranging from the 20S to 80S. In general, disease severity of stem rust was very high in the 2020 season than in the 2019 and 2021 seasons. The calculated AUDPC of *Sr* monogenic lines runs parallel to the high rust severity and vice versa with the low severity of each *Sr* line along with the seasons of study (Table 4 and Fig.1).

Table 3. Final disease severity of 10 monogenic lines to stem rust disease under natural field infection during 2019 -2021 three growing seasons.

Sr genes	Final disease severity in three growing seasons		
	2018/2019	2019/2020	2020/2021
*Sr*45	60S	80S	50S
*Sr*46	0.00	0.00	0.00
*Sr*47	0.00	Tr-R	0.00
*Sr*48	20 S	40S	30-S
*Sr*49	40S	50S	40S
*Sr*50	0.00	Tr-R	0.00
*Sr*51	0.00	0.00	0.00
*Sr*52	30S	50S	40S
*Sr*53	30S	50S	40S
*Sr*54	70S	80S	50S

R= Resistance S= Susceptible Tr = trace 0= Immune

Table 4. AUDPC and r- value for 10 *Sr* genes during 2019 – 2021 three growing seasons.

Sr genes	AUDPC			Mean of AUDPC	r-value
	2019	2020	2021		
*Sr*45	900	1225	750.00	958.33	0.418
*Sr*46	0.00	0.00	0.00	0.00	0.000
*Sr*47	0.00	1.00	0.00	0.33	0.0008
*Sr*48	250	750	600.00	533.33	0.198
*Sr*49	600	775	600.00	658.33	0.286
*Sr*50	0.00	1.00	0.00	0.33	0.0008
*Sr*51	0.00	0.00	0.00	0.00	0.000
*Sr*52	450	660	600.00	570.00	0.264
*Sr*53	250	850	250	450.00	0.253
*Sr*54	1050	1325	850	1075.00	0.441

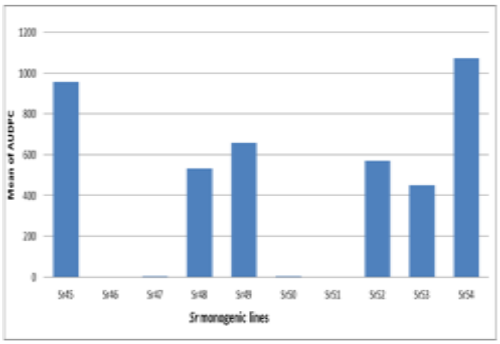

Fig. 1. Mean Area Under Disease Progress Curve (AUDPC) of 10 *Sr* monogenic lines during 2019 – 2021 growing seasons.

The genes *Sr*54 and *Sr*45 had the highest mean values of AUDPC (1075.00 and 958.33), followed by *Sr*49 (658.33), *Sr*52 (570.00), *Sr*48 (533.33), and *Sr*53 (450.00). Also, the r-value of the tested genes revealed that *Sr*54 and *Sr*45 ranked first and second (0.441 and 0.418), respectively (Table 4). The genes *Sr*47, *Sr*50, and *Sr*48 were the least in this respect (0.0008, 0.0008, and 0.198) respectively.

The Egyptian wheat cultivars showed different responses to natural stem rust infection. The local wheat cultivars Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Giza-171, Sakha-94, Sakha-95, Sids-14, and shandweel-1 showed resistance responses ranging from zero to 10MR. While, the cultivars Giza-168, Sids-12, Misr-3, Misr-2, and Misr-1, respectively showed susceptible reactions ranging between Tr-MS to 60S through the three seasons (Table 5).

Table 5. Final disease severity of Adult plant reaction of 14 Egyptian bread wheat cultivars to natural infection of stem rust disease during 2019 – 2021 seasons.

Cultivars	Final disease severity		
	2018/2019	2019/2020	2020/2021
Gemmeiza-9	5MR	5MR	TrR
Gemmeiza-10	0	0	0
Gemmeiza-11	0	0	0
Gemmeiza-12	10MR	10MR	Tr-MR
Giza-168	5-MS	5-MS	Tr-MS
Giza-171	Tr-MR	Tr-MR	Tr-MR
Sakha-94	10MR	10MR	5MR
Sakha-95	0	0	0
Sids-12	10S	10S	Tr-S
Sids-14	0	0	0
Shandweel-1	Tr-MR	Tr-MR	Tr-R
Misr-1	60-S	40-S	30-S
Misr-2	40S	30S	20S
Misr-3	5-MS	5-MS	Tr-MS

R= Resistance S= Susceptible Tr = trace M = Moderately 0= Immune

Molecular detection of stem rust genes in the local wheat cultivars.

The available six specific SSR markers cssu45, Xgwm210, Xgpw4043, sun209, IB-267, and WMS570 were used to detect the *Sr* genes *Sr*45, *Sr*46, *Sr*47, *Sr*49, *Sr*50, and *Sr*52 in fourteen Egyptian bread wheat cultivars.

***Sr*45**

The microsatellite SSR marker, cssu45, linked to the *Sr* gene *Sr*45, amplified a band with 220 bp in the monogenic *Sr* gene and all the used bread wheat cultivars showing its presence in all wheat cultivars (Fig. 2).

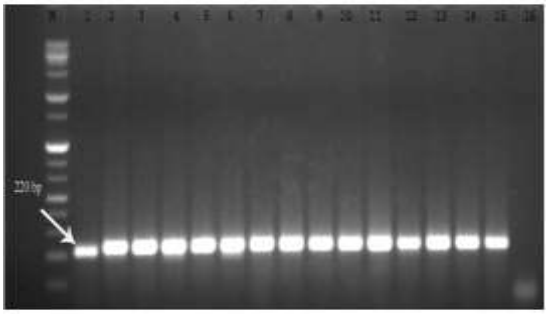

Fig. 2. Electrophoregram of SSR Cssu45 in *Sr*45 and 14 wheat cultivars. M: 100bp DNA ladder RTU (Gene Direx). 1: *Sr*45 gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

***Sr*46**

The microsatellite SSR marker, Xgwm210 linked to the *Sr* genes *Sr*46, amplified a band with 178 bp in the monogenic *Sr* gene and all the used bread wheat cultivars indicating the presence of *Sr*46 in all wheat cultivars (Fig. 3).

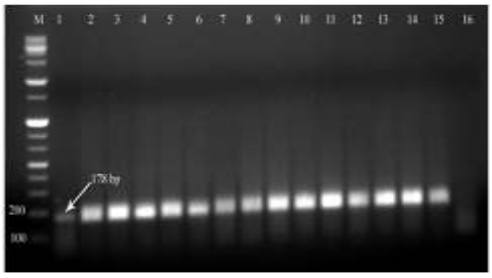

Fig. 3. Electrophoregram of Xgwm210 in *Sr*46 gene and 14 wheat cultivars. M: DNA ladder. 1: *Sr*46 gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

***Sr*47**

Data in Fig. 4. Illustrate that the microsatellite SSR marker, Xgwm4043 linked to the gene *Sr*47 amplified 4 fragments at 128,152, 297, and 379 bp in the monogenic *Sr*47 gene. *Sr*47 was absent from Misr-2. Three fragments with molecular sizes 128,152, and 297bp were present in all cultivars, and a fragment with molecular size 379bp was present in most cultivars and absent from Gemmeiza-9 and Gemmeiza-11.

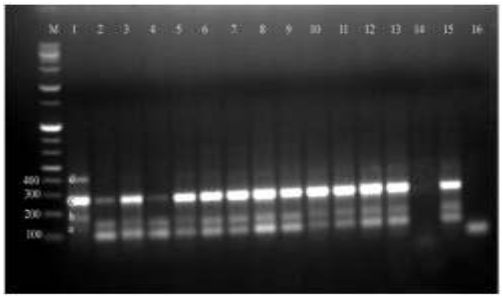

Fig.4. Electrophoregram of Xgpw4043 in *Sr*47 and the 14 wheat cultivars. M: DNA ladder. 1: *Sr*47 gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

***Sr*49**

The microsatellites SSR marker, sun209 linked to the *Sr*49 gene amplified a band at 158 bp in the monogenic line *Sr*49 and all the bread wheat cultivars showing it presence in all wheat cultivars (Fig.5).

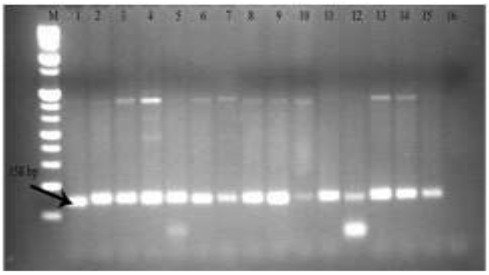

Fig. 5. Electrophoregram of Sun209 in *Sr*49 and 14 wheat cultivars. M: DNA ladder. 1: *Sr*49 gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

Other bands with molecular sizes 450, 600, and 750bp were amplified in some cultivars and absent from others. Band with molecular size 750bp was present in all the cultivars except with Gemmeiza-12, Sids-14, Shandweel-1and Misr-3, it was absent.

***Sr*50**

Fig.6. illustrates that the marker IB-267 amplified a band at 200 bp in the monogenic *Sr*50 and six wheat cultivars e.g. Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Sakha-94, and Shandweel-1 only. Also, the marker amplified three additional bands with molecular sizes 180, 400, and 650 bp. Bands with molecular weight 400 and 650 present in Gemmeiza-9, Gemmeiza-10, Giza-168, Sakha-95, Sids-12, Misr-1, Misr-2, and Misr-3.While a band with molecular size 180bp is present in Giza-168, Sakha-95, Misr-1, Misr-2, Misr-3 and Shandweel-1.

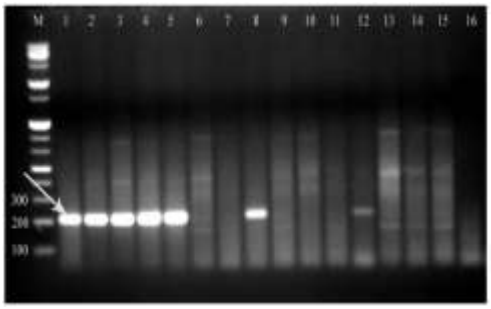

Fig. 6. Electrophoregram of IB267 in *Sr*50 and 14 wheat cultivars. M: DNA ladder. 1: *Sr*50 gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

***Sr*52**

The SSR marker WMS570 amplified a fragment at 100 bp in the monogenic *Sr*52 and all the tested cultivars except in Gemmeiza-11. Another allele (150bp) was amplified by WMS570 marker in all of the tested cultivars except in Gemmeiza-11(Fig. 7).

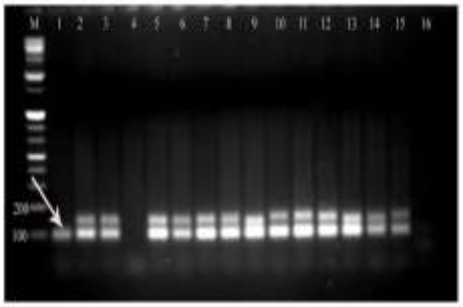

Fig. 7. Electrophoregram of WMS570 in *Sr52* and 14 wheat cultivars. M: DNA ladder. 1: *Sr52* gene, 2: Gemmeiza-9, 3: Gemmeiza-10, 4: Gemmeiza-11, 5: Gemmeiza-12, 6: Giza-168, 7: Giza-171, 8: Sakha-94, 9: Sakha-95, 10: Sids-12, 11: Sids-14, 12: Shandweel-1, 13: Misr-1, 14: Misr-2, 15: Misr-3, 16: negative control (water).

Table 6 summarises the molecular detection of six *Sr* genes in Egyptian bread wheat cultivars.

Table 6. Presence/absence of 6 stem rust genes /alleles within 14 Egyptian bread wheat cultivars.

	cssu45	Xgwm210	Xgwm4043				sun209				IB267				WMS570	
Cultivar	**220 bp**	**178 bp**	**128 bp**	**152 bp**	**297 bp**	**379 bp**	**158 bp**	**450 bp**	**600 bp**	**750 bp**	**180 bp**	**200 bp**	**400 bp**	**650 bp**	**100 bp**	**200 bp**
Gemmeiza-9	1	1	1	1	1	0	1	0	0	1	0	1	1	1	1	1
Gemmeiza-10	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1
Gemmeiza-11	1	1	1	1	1	0	1	1	1	1	0	1	0	0	0	0
Gemmeiza-12	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
Giza-168	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1
Giza-171	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
Sakha-94	1	1	1	1	1	1	1	0	0	1	0	1	0	0	1	1
Sakha-95	1	1	1	1	1	0	1	0	0	1	1	0	1	1	1	1
Sids-12	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1
Sids-14	1	1	1	1	0	1	1	0	0	0	0	0	0	0	1	1
Shandweel-1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	1	1
Misr-1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1
Misr-2	1	1	1	0	0	0	1	0	0	0	1	0	1	1	1	1
Misr-3	1	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1

1= present 0= absent

Using the iMEC software, the primers linked to *Sr*49, *Sr*50, and *Sr*52 linked showed a high percentage of polymorphism (75, 100, and 100%) respectively. The expected heterozygosity values (H) showed a narrow range between 0.124 and 0.56. Also, the polymorphism information content (PIC) exhibited values ranging from 0.116 to 0.461. The values of effective multiplex ratio (E) were more varied compared to H and PIC; their values ranged from 0.93 to 1.00. On the other hand, the mean heterozygosity and the marker index exhibited very low values 0.0083 and 0.0077, for *Sr*45 and *Sr*46, respectively while the other *Srs* showed higher values reached to 0.53 (*Sr*47) and 0.56 (*Sr*50) for both parameters. Additionally, the discriminating power values ranged from 0.133(*Sr*45 and *Sr*46) to 0.59 (*Sr*50). Also, values of the resolving power were very low, 0.133 for both *Sr*45 and *Sr*46 (Table 7).

Table 7. Marker efficiency parameters of stem rust resistance genes include polymorphism information content, expected heterozygosity, effective multiplex ratio, mean heterozygosity, discriminating power, Marker Index and resolving power.

Sr	**NPB***	**NMB***	**%polymorphism**	**PIC_0**	**H_0**	**Hav_0**	**E_0**	**D_0**	**MI_0**	**R_0**
*Sr*45	1	1	0	0.1167	0.1244	0.0083	0.9333	0.1333	0.00774	0.1333
*Sr*46	1	1	0	0.1167	0.1244	0.0083	0.9333	0.1333	0.00774	0.1333
*Sr*47	1	3	25	0.4453	0.53287	0.5328	1	0.3167	0.53278	
*Sr*49	3	1	75	0.3563	0.39944	0.3994	1	0.30234	0.39944	
*Sr*50	4	0	100	0.4612	0.56	0.56	1	0.5905	0.56	
*Sr*52	2	0	100	0.2266	0.24	0.24	1	0.2571	0.24	

NPB* = Polymorphic bands number, NMB* = Monomorphic bands number

Discussion

Wheat production has been impacted by many pathogens, such as *Puccinia graminis* f. sp. *tritici* (*Pgt*) that cause stem rust disease (Pardey *et al.,* 2013; Singh *et al.,* 2015). Stem rust disease continues to be the major threat facing wheat production as it frequently develops new virulent races. Our study clearly that the genes *Sr*46, *Sr*47, *Sr*50, and *Sr*51 showed resistant responses to natural infection of stem rust for the three seasons of study. These results have been supported by other authors. Rouse *et al.* (2011) found that the two accessions of *Aegilops tauschii* (AUS 18913 and TA 1703) included *Sr46* displayed multiple consistent resistance to TTKSK, TRTTF, TTTTF, and TPMKC races of stem rust however, their reactions varied to QTHJC and RKQQC races. Moreover, *Sr*46 is temperature- sensitive as it is barely effective at low temperatures (Yu *et al*., 2015). Additionally, *Sr*47 gene carried in the DAS15 wheat line and was reported as highly effective against race TTKSK (Ug99) and the broad range of worldwide *Pgt* races (Faris *et al*., 2008; Klindworth *et al*., 2012 and 2017). *Sr*50, introgressed from chromosome 1R of rye, shows resistance to all worldwide *Pgt* races, including Ug99. It was the first wheat *Sr* resistance gene cloned from the tertiary gene pool. This development will enable *Sr*50 introgression into new wheat cultivars without detrimental effect of rye (Mago *et al*., 2015; Yadav *et al*., 2015).

The genes *Sr*45, *Sr*48, *Sr*49, *Sr*52, *Sr*53, and *Sr*54 showed susceptibility ranging from 20S – 80S along the three seasons of study. These results are contradicted by other authors. Virulence isolates were reported for *Sr*45 at the adult stage while it was effective against Ug99 (TTKS) of stem rust at the seedling stage (Sambasivam *et al*., 2008). *Sr*52 showed a temperature-sensitive resistance pattern to stem rust race Ug99 (TTKSK), it was most effective at 16°C, partially effective at 24°C, and ineffective at 28°C (Qi *et al*., 2011). Also, *Sr*54 gene identified in Norin40 cultivar did not confer resistance to Ug99 (Ghazvini *et al*., 2013). In contrast to the susceptibility of these genes under the Egyptian condition, the gene *Sr*49 showed resistance to Ug99 in seedling and adult plant stages (Eric *et al*., 2014). *Sr*49 was effective to all commercially significant Australian *Pgt* races and showed a R to MR reaction in field conditions (Bansal *et al*., 2015). The highest mean of AUDPC values were recorded for the genes, *Sr*54 and *Sr*45 (1075.00 and 958.33) followed by *Sr*49 (658.33), *Sr*52 (570.00), *Sr*48 (533.33), and *Sr*53 (450.00). The local wheat cultivars Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Giza-171, Sakha-94, Sakha-95, Sids-14, and

Shandweel-1 showed resistance responses ranged from zero – 10MR.While, the cultivars Giza-168, Sids-12, Misr-3, Misr-2, and Misr-1, respectively showed susceptibility ranging between Tr-MS to 60S. The susceptibility of the local Egyptian cultivars was lower than the *Sr* lines. This is due to they have multiple genes in their background.

Marker-assisted selection (MAS) technologies have been facilitated by the rapid advancement of molecular marker technology and gene identification, particularly in wheat breeding. Those MAS technologies were successfully developed because of PCR-based markers (Aktar *et al*., 2017). Molecular markers have been deployed for several *Sr* resistance genes, like *Sr*45 (Periyannan, *et al*., 2014), *Sr*46 (Yu *et al*., 2015), *Sr*50 (Yadav *et al*., 2015), *Sr*51 (Liu *et al*., 2011a), and *Sr*52 (Qi *et al*., 2011). The objective of the present study was to detect the Sr genes; *Sr*45, *Sr*46, *Sr*47, *Sr*49, *Sr*50, and *Sr*52 in 14 commonly widely grown cultivars in Egypt using 6 simple sequence repeat (SSR) markers. Our results indicated the presence of *Sr*45, *Sr*46, and *Sr*49 in all the tested Egyptian cultivars. *Sr*47 was positive in all varieties except in Misr-2, while *Sr*50 was present only in Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Gemmeiza-12, Sakha-94, and Shandweel-1 cultivars. However, *Sr*52 is present in the tested cultivars at 178pb except for Gemmeiza-11. Moreover, *Sr*49 and *Sr*50 amplified additional alleles in some tested cultivars, while the *Sr*52 gene was present in all of the tested cultivars. Periyannan, *et al.* (2014) stated that primer *cssu45* linked to *Sr*45 amplified products with 238bp and 220 bp in susceptible and resistant plants, respectively. According to Guotai *et al.* (2015), Xgwm210 was linked to an *Sr*46 amplified band with varied sizes from different wheat cultivars and lines, while in CIae 25 amplified a band with 178-bp. *Sr*47 was detected in Joppa cultivar at168 and 185 bp using the marker *Xrwgsnp4* (Klindworth *et al*., 2017). Bansal *et al.* (2015) stated that marker *sun209* produce a fragment with a 158 bp in Yitpi and a 148 bp in Mahmoudi wheat cultivars. *Sr*50 was predicted at 200–300 bp when using the marker IB-267(Mago *et al*., 2002). Also, Yadav *et al.* (2015) selected the IB-267 marker to detect *Sr*50 that generated amplicon of 200–300 bp. Finally, *Sr*52 was detected by Qi *et al.* (2011) using the microsatellite marker WMS570 which amplified a fragment of between 100 and 200 bp in lines containing an intact 5AL. The results show that there is an agreement between our detection of the genes understudy in the Egyptian wheat cultivars with those obtained by the mentioned authors at the same base pair. Also, our results indicate the usefulness of marker-assisted selection (SSR) of these genes in breeding program backgrounds. Our data reveal that the PIC values for markers (*Sr*47, *Sr*49, *Sr*50 and *Sr*52) were 0.445, 0.356, 0.461 and 0.226, respectively. The percentage of polymorphism was from 25 to 100 %. PIC values for dominant markers are higher for markers with multiple alleles. Botstein *et al*. (1980) reported that the PIC of the fragment polymorphism is based on allele numbers and their frequency distribution. The marker index (MI) ranged from 0.007 to 0.56.) MI is a statistical metrics utilized to calculate the maker system's total utility (Powell *et al*. 1996; Nagaraju *et al*. 2001). Also, the high level of polymorphism indicates that the SSR markers are more useful for evaluating wheat genotypes' genetic diversity. iMEC can handle a variety of data types, including DNA produced by microsatellites, and help to accurate estimates of primer efficacy.

CONCLUSION

Out of 10 wheat stem rust monogenic lines tested for adult plant resistance, *Sr*46, *Sr*47, *Sr*50 and *Sr*51 showed complete resistance along 2019- 2021 three seasons. Molecular detection revealed the presence of *Sr*45, *Sr*46, and *Sr*49 in14 Egyptian bread wheat cultivars, however *Sr*47 was positive in all cultivars except in Misr-2, *Sr*50 was present in 6 cultivars, and *Sr*52 is present in 13 cultivars except Gemmeiza-11.

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تعريف بعض سلالات القمح أحادى الجين المقاومة لمرض الصدأ الأسود باستخدام المعلمات الوراثية

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الملخص

تم تقييم عشرة سلالات من القمح أحادية الجين و 14 صنفًا تجاريًا من قمح الخبز فى طورالنباتات البالغة لمقاومة مرض صدأ الساق تحت ظروف العدوى الطبيعية خلال ثلاثة مواسم 2019-2021. أظهرت النتائج أن *Sr*46 و *Sr*47 و *Sr*51 كانت مقاومة كليا خلال مواسم الدراسه الثلاثة ، بينما أظهرالجين *Sr*50 درجه عاليه من المقاومة. بينما أظهرت الجينات الباقيه قابليته للاصابة ما بين 20٪ و 80٪. أظهر الجينين *Sr*54 و *Sr*45 أعلى متوسط قيم للمساحه الواقعه تحت منحنى تقدم المرض(AUDPC) وكذلك قيم معدل تطور المرض (r-value) يليهما الجينات *Sr*49 و *Sr*52 و *Sr*48 و *Sr*53 على التوالي. أظهرت اصناف القمح جميزة9 , جميزة10, جميزة11 , جميزة12 , جيزة171 , سخا94 , سخا95 , سيدس14, شندويل1 مقاومة عالية من صفر إلى طراز متوسط المقاومة . بينما أظهرت الأصناف جيزة 168 ، سدس 12 ، مصر 3 ، مصر 2 ، مصر 1 قابلية للاصابة تتراوح بين اثار (Tr-MS) إلى درجه إصابة (60S). تم استخدام ستة من المعلمات الوراثية (SSR) المرتبطة 6جينات مختلفة لاختبار وجودها في أصناف القمح المصرية. أشارت النتائج إلى وجود الجينات *Sr*45 , *Sr*46 , *Sr49* , *Sr*47 في اصناف القمح. وكان الحين *Sr*47 موجبًا في جميع الأصناف ماعدا مصر2 ، بينما الجين *Sr*50 كان موجودًا في جميزة9 , جميزة10, جميزة11 , جميزة12 , جيزة171 , سخا94 , شندويل1. ، بينما الجين *Sr*52 موجود في الأصناف المختبرة وغائب فى جميزة11. أظهر برنامج iMEC 4 بادئات(primers) بنسبة عالية من تعدد الأشكال للجينات(polymorphism) مع *Sr*47، *Sr*49، *Sr*50 ، و *Sr*52 بنسب 25 ، 75 ، 100، 100 ٪ ، على التوالي.

الكلمات الدالة: صدأ الساق ، سلالات أحادية الجين ، SSR ، تعدد الأشكال.