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Identification of the Resistance Gene *Sr*2 in some Egyptian Wheat Hybrids

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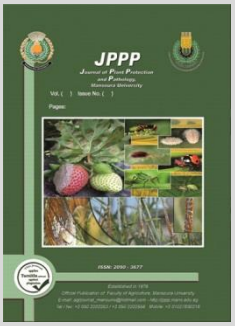

ABSTRACT

Seven Egyptian wheat cultivars were crossed with *Sr*2 (the stem rust resistant gene), and their parents, F_1, F_2 crosses were tested to natural stem rust disease in the field of Gemmeiza Agricultural Station, ARC, during the 2019 to 2021 growing seasons. The *Sr*2 gene was completely resistant to stem rust, while the Egyptian cultivars showed different responses to the disease. Sakha-95 was completely resistant, Misr-1 and Misr-2 showed high susceptibility. Gemmeiza-12, Giza-171, and Sakha-94 showed moderate resistance (MR), while a trace of moderate susceptibility (Tr-MS) was recorded for the cultivar Misr-3. All the F_1 crosses were resistant, indicating the dominance of resistance to stem rust disease. Chi-square goodness of fit was used to assess the independence of *Sr*2 resistance gene in the tested cultivars and F2 crosses. The crosses *Sr*2XGemmeiza-12, *Sr*2X Sakha-94, *Sr*2XSakha-95 and *Sr*2XGiza-171 have the expected segregation ratio of 15:1 for a duplicate gene. However, the crosses *Sr*2XMisr-1 and *Sr*2XMisr-3 fit a 3:1 ratio conditioned by one dominant gene. The resistance of the cross *Sr*2XMisr-2 fits complementary gene action (9:7). Three specific SSR markers (xgwm533, stm559tgag, and stm598cac) were used to identify *Sr*2 in the Egyptian cultivars and their F1 populations. The three markers confirmed presence of *Sr*2 gene in the Egyptian cultivars and their F1 populations. However, the stm598cac marker could differentiate between the tested cultivars and their F1 populations by amplifying an additional band with a molecular weight of 200bp. Molecular analysis confirmed the results of chi-square test and could be serving in developing wheat programs.

***Keywords*:** wheat, stem rust, segregating population

INTRODUCTION

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is among the fungal diseases that significantly affect wheat production around the world (Bhavani *et al*., 2022; Guo *et al*., 2022). To combat stem rust, breeding for resistant cultivars remains the most efficient and reliable method to control this disease (Kumar *et al*., 2022b). There are different sources of resistance to stem rust, such as wheat wild relatives. *Triticum turgidum* (Yaroslav emmer) was transferred to the Hope cultivar (Mago *et al*., 2011). Slow rusting genes like *Sr*2, *Sr*55, *Sr*56, *Sr*57, and *Sr*58 confer resistance at the adult plant stage under high disease pressure (Herrera-Foessel *et al*., 2014; Singh *et al*., 2015; Kosgey *et al*., 2021). Previously, *Sr*2 was used as a parent in several breeding programs around the world and used frequently in CIMMYT breeding materials (Singh *et al*., 2011a). There are several sources for *Sr*2 gene, including Pavon, Arthur, Lancer, and Hope. When present alone, the *Sr*2 gene showed slow rusting under intense disease pressure, but when combined with other minor genes to form the "*Sr*2 complex," it provides an appropriate level of resistance (Singh *et al*., 2011a; Singh *et al*., 2015). This gene tends to be non-specific and is currently effective against all isolates of *Puccinia graminis* f. sp. *tritici* throughout the global regions that grow wheat. In Egypt, *Sr*2 (Pavon76 cultivar) has shown stable adult-plant resistance (APR) to stem rust in the field from 2016 –2020 seasons (El-Shamy *et al*., 2019; Elkot *et al*., 2020). Shahin *et al*. (2020) evaluated *Sr*2, *Sr*24, and *Sr*26 under field conditions during the 2014 and 2015 seasons in Kafr elsheikh, Sharqia, and Nubaria governorates and found that *Sr*2 was highly effective for resistance in both seasons. El-Shamy *et al*. (2019) found that Pavon showed complete resistance to stem rust under field conditions at recommended and late dates of cultivation. Molecular genetic analysis has been used for the evaluation of both major and minor genes in various crops. Moreover, in wheat, molecular markers linked to race-specific genes for rust resistance have been identified and utilized for marker-assisted selection (Suenaga *et al*., 2003; Javadi *et al*., 2021). Molecular markers have been developed for numerous stem rust resistance genes, including *Sr*2 (Spielmeyer *et al*., 2003; Hayden *et al*., 2004; Mago *et al*., 2011). The aims of this study are to detect gene actions of resistance against stem rust disease in seven crosses with *Sr*2 gene and to detect the presence of *Sr*2 gene in the seven Egyptian bread wheat cultivars using molecular marker technique.

MATERIALS AND METHODS

1. Plant materials

Seven of the recent Egyptian bread wheat cultivars i.e. Gemmeiza-12, Sakha-94, Sakha-95, Giza-171, Misr-2, Misr-3 and Shandweel-1 were crossed as females with the resistant cultivar Pavon76 carrying stem rust gene *Sr*2 as a male parent in 2018/ 2019 growing season at Gemmeiza Agricultural Station, ARC. The names and pedigree of tested materials were reported by El-Shamy *et al*. (2019). Seeds of Pavon (*Sr*2) stock were obtained from ICARDA, while the bread wheat cultivars derived from The National Wheat Dept., Crop Research Institute. In 2019/2020 growing season, the hybrid seeds were sown in 1row, 2m long, 40 cm apart and 20 cm within rows for each cross to have F_1 seeds. In 2020/2021growing season, the parents and F_1 hybrid seeds

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were sown in 1row, 2m long, 40 cm apart and 20 cm within rows while about 100 to 150 grains of F2 seeds of each cross were drilled in plots consisted of 6 rows, 7m. long, 20cm apart and 10 cm in-between seeds for evaluation to stem rust disease. The experiment was surrounded by border 1 m of a highly susceptible cultivar i.e. Morocco and left to natural stem rust infection. Fertilizer application followed recommended cultural practices 70 kg N and 100 kg P/fed.

2. Disease assessment

Disease severity was scored for the parents, F1 and F2 plants of each cross when Morocco plants showed maximum rust severity using the modified Cobb scale (Peterson *et al*., 1948) and the host response was classified as either 0 (immune), R (resistant), MR (moderately resistant), M (intermediate), MS (moderately susceptible) or S (susceptible) according to Roelfs (1992). 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.

3. Chi-square analysis

Chi-square analysis was used to evaluate the goodness of fit of observed segregation with expected ratios and to estimate the number of genes responsible for stem rust resistance of the F2 population (Snedecor and Cochran 1989).

4. Molecular study

DNA extraction

The total DNA of *Sr*2, wheat cultivars, and their F1 populations were extracted from 200 mg of 10 days seedlings. The extraction method was carried out according to Emara *et al*. (2016).

PCR amplification conditions

Three specific microsatellites (SSR) markers linked to *Sr*2 were used to detect its presence in the 7 bread wheat cultivars and their F1 population. The markers' names, sequences, and annealing temperatures were listed in Table 1. The PCR was conducted following Hayden *et al*. (2004). Each PCR mixture has the following composition; Promega Nuclease free water (9.5 µl), 25ng nucleic acid (1µl), each primer (10) pmol (1µl), and GoTag® Colorless Master Mix 12.5 µl, PCR products (15 µl) were analyzed by electrophoresis in a 2% agarose gel, stained with ethidium bromide (7.0 µg/50 ml) and DNA bands were visualized using a UV trans-illuminator and photographed.

Table 1. *Sr* gene, Markers, expected PCR fragment size, and annealing temperature.

Sr gene	Markers	Expected fragment size (bp)	Primer sequence	Annealing temperature	References
Sr 2	xgwm533	120bp	GTTGCTTTAGGGGAAAAGCC AAGGCGAATCAAACGGAATA	48.3°C	Hayden *et al* ., 2004
	stm559tgag	from 79 to 85 bp	AAGGCGAATCAAACGGAATA TGTGTGTGTGTGTGAGAGAGAG	50°C	
	stm598cac	from 61 to 67 bp	GTTGCTTTAGGGGAAAAGCC TCTCTCTCTC TCTCACACACAC	49.3°C	

RESULTS AND DISCUSSION

Results

Evaluation at the adult stage

The *Sr*2 showed completely resistance to stem rust under field conditions at the adult stage during the years of the study (Fig.1). The Egyptian wheat cultivars showed varied reactions to stem rust disease under field conditions ranging from zero to susceptible reaction (30S). The wheat cultivars divided into two groups, the resistant group was Sakha-95(0), Gemmeiza-12(Tr- MR), Giza-171(Tr-MR), and Sakha-94 (5MR). While the susceptible group included Misr-3 (Tr-MS), Misr-2(20S), and Misr-1(30-S), respectively.

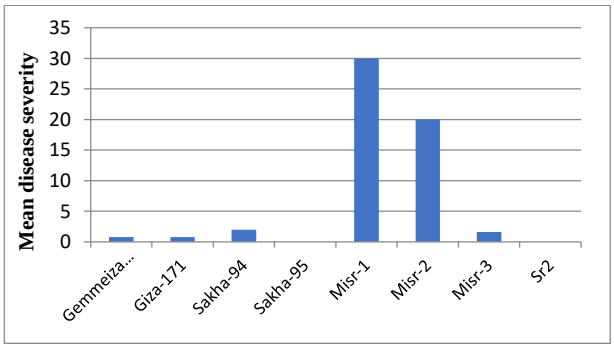

Fig. 1. Mean disease severity of 7 Egyptian bread wheat cultivars and *Sr*2 to stem rust disease during the growing season 2020/2021.

The Chi-square test

An analysis of adult plants resistance of F1 and F2 populations to stem rust disease is shown in Table (2). The results clear that all the F1 populations of the seven crosses showed adult plant resistance responses to natural infection of stem rust. The F2 populations showed segregation in its responses as follow. The crosses *Sr*2XGemmeiza-12, *Sr*2X Sakha-94, *Sr*2XSakha-95 and *Sr*2XGiza-171 showed 15R:1S ratio, which segregated in 150R:10S, 220R:20S, 200R:30S, and 230R:10S, respectively. However, F2 plants of the crosses *Sr*2XMisr-1 and *Sr*2XMisr-3 showed 3:1 ratio which segregated in 110R:30S and 160R:50S, respectively. The F2 plants of the cross *Sr*2XMisr-2 showed 9R:7S which segregated in 80R:60S.

The molecular detection of *Sr*2

The markers xgwm533, stm559tgag, and stm598cac were used to determine the presence of *Sr*2 in the Egyptian cultivars and the F1 population. Amplification with the xgwm533 primer developed for *Sr*2 produced two PCR amplicon with a size of 120 bp and 65 bp (Fig. 2). The amplified fragment with 120 bp was present in all of the tested cultivars and the F1 populations. While, the additional band with molecular weight from 65 bp appeared in all the Egyptian cultivars, and absent from their F1. Amplification with the stm559tgag marker developed for *Sr*2 produced a single PCR amplicon with a size about 80 bp in the *Sr*2 parent but in the Egyptian cultivars and their F1 hybrids with the molecular size 100 p (Fig. 3). Amplification with the stm598cac primer produced two amplicons with size from 61 to 67 bp in the *Sr*2, the Egyptian cultivars as parents and their F1 populations (Fig. 4). Another fragment was amplified with molecular weight 200bp in Misr-1, Misr-3 and Giza-171 and their F1 populations. Moreover, this band appears in Sakha-95 cultivar and absent from its F1 population. However, it was absent in Gemmeiza- 12, Sakha -94, and Misr-2 cultivars and present in their F1 population (Table 3).

Table 2. Segregation of resistance to stem rust pathogen in the F2 populations derived from the cross *Sr*2 and 7 Egyptian wheat cultivars.

Cross name		No. of tested plants	Response			Segregation		Expected ratio		X^2	p-value
			*R	**I	***S	R	S	R	S		
*Sr*2XGemmeiza 12	F_1	54	38	16							
	F_2	160	130	20	10	150	10	15	1	0.00	1.000
*Sr*2X Sakha-94	F_1	65	44	21							
	F_2	240	200	20	20	220	20	15	1	1.778	0.1824
*Sr*2XSakha- 95	F_1	49	40	9							
	F_2	230	190	10	30	200	30	15	1	18.116	0.0001
*Sr*2XMisr-1	F_1	52	33	19							
	F_2	140	80	30	30	110	30	3	1	0.952	0.3291
*Sr*2XMisr-2	F_1	44	30	14							
	F_2	140	40	40	60	80	60	9	7	0.045	0.8314
*Sr*2XMisr-3	F_1	47	34	13							
	F_2	210	110	50	50	160	50	3	1	0.159	0.6903
*Sr*2XGiza-171	F_1	50	39	11							
	F_2	240	130	100	10	230	10	15	1	1.778	0.1824

* R= Resistant, **I= Intermediate, ***S= Susceptible

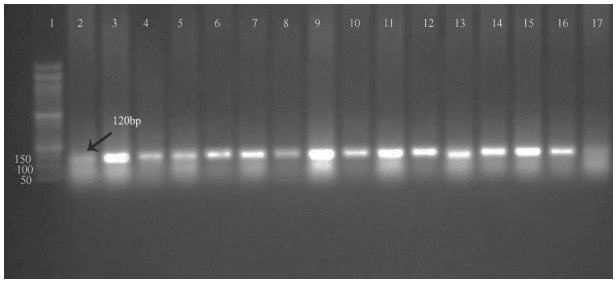

Fig. 2. xgwm533-PCR amplifications in *Sr*2 (120 bp), 7 wheat cultivars, and their F1 populations. 1: 50bp DNA ladder RTU (Gene Direx), 2: *Sr*2 3: Gemmeiza-12, 4: *Sr*2 x Gemmeiza-12, 5: Sakha-94, 6: *Sr*2 XSakha-94, 7: Sakha-95, 8: *Sr*2 X Sakha-95, 9: Misr-1, 10: *Sr*2 X Misr-1, 11: Misr-2, 12: *Sr*2 XMisr-2, 13: Misr-3, 14: *Sr*2 X Misr-3, 15: Giza-171, 16: *Sr*2X Giza-171, 17: *Sr*2.

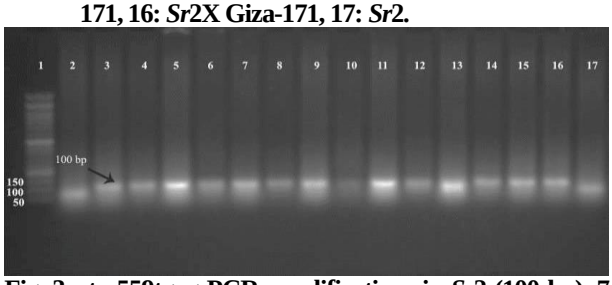

Fig. 3. stm559tgag PCR amplifications in *Sr*2 (100 bp), 7 Egyptian wheat cultivars and their F1 populations. 1: 50bp DNA ladder RTU (Gene Direx), 2: *Sr*2 3: Gemmeiza-12, 4: *Sr*2 x Gemmeiza-12, 5: Sakha-94, 6: *Sr*2 XSakha-94, 7: Sakha-95, 8: *Sr*2 X Sakha-95, 9: Misr-1, 10: *Sr*2 X Misr-1, 11: Misr-2, 12: *Sr*2 XMisr-2, 13: Misr-3, 14: *Sr*2 X Misr-3, 15: Giza-171, 16: *Sr*2X Giza-171, 17: *Sr*2.

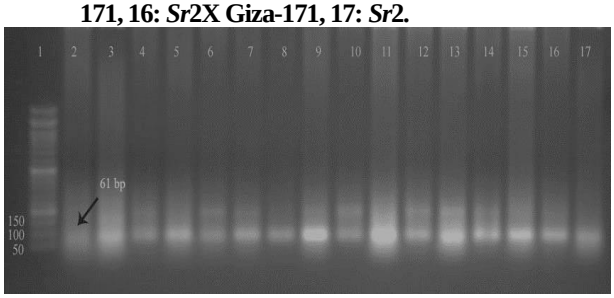

Fig. 4. stm598cac-PCR amplifications in *Sr*2 (from 61 to 67 bp), 7 Egyptian wheat cultivars, and their F1 populations. 1: 50bp DNA ladder, 2: *Sr*2 3: Gemmeiza-12, 4: *Sr*2 x Gemmeiza-12, 5: Sakha-94, 6: *Sr*2 XSakha-94, 7: Sakha-95, 8: *Sr*2 X Sakha-95, 9: Misr-1, 10: *Sr*2 X Misr-1, 11: Misr-2, 12: *Sr*2 XMisr-2, 13: Misr-3, 14: *Sr*2 X Misr-3, 15: Giza-171, 16: *Sr*2X Giza-171, 17: *Sr*2.

Table 3. Presence/absence of *Sr*2 in Egyptian cultivars and their F1 population.

Cultivars	xgwm533		stm559tgag	stm598cac	
	120bp	65bp	100 bp	61-67bp	200bp
Gemmeiza-12	1	1	1	1	0
*Sr*2XGemmeiza-12	1	0	1	1	1
Giza-171	1	1	1	1	1
*Sr*2X Giza-171	1	0	1	1	1
Sakha-94	1	1	1	1	0
*Sr*2X Sakha-94	1	0	1	1	1
Sakha-95	1	1	1	1	1
*Sr*2X Sakha-95	1	0	1	1	0
Misr-1	1	1	1	1	1
*Sr*2X Misr-1	1	0	1	1	1
Misr-2	1	1	1	1	0
*Sr*2XMisr-2	1	0	1	1	1
Misr -3	1	1	1	1	1
*Sr*2X Misr -3	1	0	1	1	1

1= present 0= absent

Discussion

Wheat stem rust affects wheat production around the world as more than 90% of wheat cultivars have susceptible reactions to the Ug99 race group (Singh *et al*., 2011b). In Egypt, this pathogen causes 1.96 to 8.21% losses in the yield of most of the local wheat varieties (Shehab-Eldeen and Abou-Zeid, 2020). The deployment of diverse resistance genes especially from wild relatives is the most effective way to reduce the potential of stem rust. To date, 73 *Sr* genes/alleles have been identified and about 35 are effective against the Ug99 race lineages (Singh *et al*., 2015). Unfortunately, most of them are race specific and don't provide durable resistance to stem rust. On the other hand, adult plant resistance provides long-lasting durable resistance to multipathogenic resistance and is provided by some resistant genes including *Sr*2, *Sr*54, *Sr*55, and *Sr*57 (Kosgey *et al*., 2021). Of them, *Sr*2 has been successfully deployed in many breeding programs around the world like Australia, India, CIMMYT, and China due to its high yield and resistance to stem rust (McIntosh 1995; Malik *et al*., 2013, Bhavani *et al*., 2014; Xu *et al*., 2017). In Egypt, *Sr*2 has been evaluated under field condition and provide high resistance to stem rust (El shamy *et al.*, 2019; Shahin *et al.,* 2020; Elkot *et al*., 2016; El-Orabey *et al*., 2019). Also, Singh *et al.* (2006) reported that both Sonalika and Pavon 76 (*Sr*2) were resistant during field assessments in 2004 and 2005 in Kenya with a maximum disease score of 15MS. In our study *Sr*2 was crossed as a male parent with seven Egyptian cultivars, and their F1 and F2 were evaluated to stem rust under natural infection during the study. *Sr*2 and Sakha-95 were completely resistant to stem rust. Gemmeiza-12, Giza-171 and Sakha-94 showed moderate resistance ranged from traces to 5 MR. While cultivars Misr-3,

Misr-2, and Misr-1 showed susceptible reaction ranged from traces to 30-S. The F1 of all crosses showed completely resistance to stem rust indicating that the resistance is dominant, but the F2 population showed segregation in their responses to the disease. A chi-square goodness-of-fit test was calculated for F2 crosses to determine the number of genes that control resistance to stem rust. The chi-square test on the observed segregation ratio was consistent with digenic Mendelian segregation for the crosses *Sr*2XGemmeiza-12, *Sr*2XSakha-94, *Sr*2XSakha-95 and *Sr*2XGiza-171 segregated in 15R:1S ratio, indicating that two dominant independent genes conditioning the resistance to stem rust in these crosses. However, the F2 plants of the crosses *Sr*2XMisr-1 and *Sr*2XMisr-3 segregated in 3:1 ratio indicating that the two crosses possess one (a single) dominant gene. The F2 plants of the cross *Sr*2XMisr-2 showed 9R:7S which segregated in 160R:50S indicating that it possess a pair of dominant complementary genes. Nzuve *et al.* (2013) crossed five resistant wheat lines (KSL-2, 3, 5, 12 and 19) with the susceptible line Cacuke stem rust to determine the stem rust resistance gene inheritance. Chi square test showed that the segregation in KSL-2 parent is conditioned by a single dominant gene of resistance. While, the stem rust inheritance in the parents KSL-5, KSL-19, KSL-12 and KSL-3 were conditioned by two genes. Previous studies conducted in Egypt showed that *Sr*2 provides resistance to stem rust under field conditions and the tested Egyptian cultivars contain *Sr*2 as confirmed by SSR markers (Elkot *et al*., 2020; Abu Aly *et al*., 2014). However, it couldn't differentiate between cultivars regardless of their response to stem rust-resistant and susceptible ones. In our study, three diagnostic-specific markers linked to *Sr*2 were used; gwm533, stm559tgag, and stm598cac to accurately determine the presence of the *Sr*2 gene in the Egyptian cultivars and their F1 population. The two additional markers stm559tgag and stm598cac were used in this study to overcome the drawbacks of the gwm533 marker. Hayden et al. (2004) stated that *Sr*2 gene closely linked microsatellite marker Xgwm533 and typically amplifies a 120-bp fragment from wheat lines known to carry *Sr*2. However, it couldn't discriminate between the *Sr*2 carrier and non-*Sr*2 carrier wheat lines. Also, Vishwakarma *et al.* (2019) suggested the existence of allelic homoplasy in *Sr*2 gene non-carriers varieties like the Aroona variety. The three tested markers indicated the presence of *Sr*2 in the tested cultivars and their crosses. The stm598cac marker is considered a diagnostic marker as it could differentiate between tested cultivars and their F1 populations by amplifying an additional band with a molecular weight of 200bp. This maker is considered as a diagnostic marker since it could make differences between the tested cultivars.

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CONCLUSION

*Sr*2 is a good source of resistance to stem rust since it confers resistance to stem rust. Moreover, the stm598cac marker is a diagnostic marker as it is the only marker that could differentiate between the tested cultivars and their F1 population by amplifying an additional band with a molecular weight of 200bp.

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تعريف جين *Sr2* المقاوم لمرض الصدأ الأسود فى بعض الهجن المصرية

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الملخص

تم تهجين سبعة أصناف من القمح المصري مع سلاله قمح أحادية الجين (*Sr2*) تحت ظل الظروف الحقلية وتقييم الأباء والجيلين الأول والتانى لمرض صدأ الساق في محطة بحوث الجميزة خلال مواسم النمو 2019 و 2021. ، وتم اختبار هذه النباتات فى مرحلة الطور البالغ تحت ظروف العدوى الطبيعية فى الحقل .أثناء الدراسة كان *Sr2* مقاومًا تمامًا لصدأ الساق ، بينما أظهرت الأصناف المصرية درجات متفاوته من ردود الفعل لمرض الصدأ الأسود.حيث كان صنف سخا -95 مقاومًا تمامًا بينما الاصناف مصر-1و مصر-2 كانت أكثر الاصناف إصابة بينما كانت الأصناف جيزة-171 و جميزة-12 ومصر-3 متوسطه المقاومة للصدأ الأسود. أظهر الجيل الاول مقاومة للصدأ الساق بينما كان هناك انعزالات فى هجن نباتات الجيل الثانى. أستخدم اختبار مربع كاى للتعرف على عدد الجينات التى تحكم المقاومة للمرض .أظهرت النتائج وجود اثنين من الجينات المستقله تحكم صفة المقاومة فى الهجن الخاصة بالجين *Sr2* مع كل من جميزة- 12و سخا-94 و سخا-95 وجيزة-171 . بينما يوجد جين واحد سائد يحكم صفة المقاومة الخاصة بالجين *Sr2* مع الأصناف مصر-1 ومصر-3 . كما يوجد زوج من الجينات المكملة المهيمنه على صفة المقاومة فى الهجين الخاص بالجين *Sr2* مع الصنف مصر-2 . تم استخدام ثلاثة من المعلمات SSR وهى (xgwm533 و stm559tgag و stm598cac) مرتبطة بالجين *Sr2* لاختبار وجوده في الأصناف المصرية كذلك في هجن الجيل الأول. أكدت المعلمات الجزيئية الثلاثة وجود الجين *Sr2* في الأصناف المصرية وكذلك فى هجن الجيل الأول. كما أظهر المعلم stm598cac كفاءة متخصصة فى التفريق بين الأصناف المختبرة وهجن الجيل الأول بوجود band متخصصة بوزن جزيئى (200bp).

الكلمات الافتتاحية: القمح ، صدأ الساق ، اجيال انعزالية