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INHERITANCE OF STRIPE RUST RESISTANCE IN THE WHEAT CULTIVAR SAKHA 61.

BY

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ABSTRACT

Increased range of stripe rust (*Pucciniaa striifromis tritici*) virulence on wheat (*Triticum aestivum* L.) in Egypt at last decades has required assemblage of a broad genetic base of resistance. The objective of this study was to identify inheritance of Sakha 61 resistance to wheat stripe rust..

Six crosses between Sakha 61 and each of Sakha 8, Giza 162, Gemmiza 3, Sids 4, Sids 6 and Sids 9, were performed .Results indicated that all materials tested at seedling stage exhibited susceptible reaction against stripe rust. Breeding against stripe rust at adult stage under field condition proved that plant segregation indicated that F_1 plants of the six testers having Sakha 61 were resistant and exhibited low stripe rust severity ranged between 0 and 10MR. The result of F_2 plants reaction exhibited wide range of stripe rust severity ranged between 0 and 90S but the direction was in the side resistance and this confirmed the results of F_1. This study indicated that c.v. Sakha 61 also contains the stripe rust resistance gene at adult stage such as the tester proved to have.

The produced random analysis on amplified DNA polymorphism of tested wheat individuals using the primer (GAAACGGGTG) clearly showed that resistance gene was present in F_2 of Sakha61 /Gemmiza 3 and did not present in susceptible individuals.

INTRODUCTION

Stripe rust, caused by *Puccinia striiformis* f.sp. tritici, is one of the most important diseases of wheat in the world. In Egypt, stripe rust attacked most of the commercial wheat cultivars during 1968 to 1995, causing severe infection in North Delta area (El-Daoudi *et al.*, 1996). Recently, stripe rust caused high loss in the production of most Egyptian wheat cultivars in the Delta area during 1996/1997 growing season (El- Daoudi, 1998).

The stripe rust disease can be controlled using fungicide application. However, breeding for resistance is considered to be the most economical and environmentally suitable to reduce the degrease of pollutions. It is considered the

traditional way for transferring one or more resistance genes to a single wheat cultivar depending on filed or greenhouse screening with different races is better comparing with fungicide application, which is a very laborious and time consuming process. In recent years DNA based markers have shown great promise in clarifying the resistance genes (Robert *et al.*, 2000).

Also, the objective of this study was to identify the stripe rust resistance genes in crosses of certain wheat population.

MATERIALS AND METHODS

Six wheat cultivars i.e. Sakha 8, Giza 162. Gemmeiza 3, Sids 4, Sids 6 and Sids 9 exhibited a wide range of variability in their susceptibility to stripe rust, while the cultivar Sakha 61 exhibited high level of resistance to stripe rust at adult stage. (Aly. 1999). These parents were sown at Sakha Agric. Res. Stn. during 2001/ 2002 growing season in six rows each. All possible crosses among the six cultivars and Sakha 61 were conducted to produce the hybrid seed of the six crosses. The resulted F_1 plants are represented as follow: Sakha 61 x Sakha 8, Sakha 61 x Giza 162, Sakha 61x Gemmiza 3, Sakha 61x Sids 4, Sakha 61 x Sids 6 and Sakha 61x Sids 9, during 2002 / 2003 growing season, part of the six F_1 hybrid seed was sown to produce the F_2 seed. The rest were left for the final experiment in the next season (2003 / 2004). A cooperative experiment was conducted in a randomized complete block design with three replicates each contained two rows for each parent and F_1 well as 10 cm. for each F_2 . This performance was carried out to create uniform environmental conditions. The rows were 3m . long, 30 Cm . apart and seeds were sown 10 apart within rows, therefor, each row contained 30 plants. Mixture of highly susceptible wheat cultivars were sown around the experiments as a spreader to disseminate the stripe rust uredinispores of the pathogen (*Puccinia striiformis* f.sp. tritici) .All regular cultural practices were precisely applied during the growing seasons.

Pathogenicity test:

A- At seedling stage

Eight pots for each of the parents and F_1 as well as 20 pots for each of F_2 plants were sown . Each pot contained 10 seed. Seedling (7-8 days-old) of the parents, F_1 and F_2 were uniformly inoculated with the urediniospres of P. s. f.sp. tritici which was used for inoculating all of the tested materials at seedling stage in the greenhouse using the technique described by Johnson *et al.*, (1972). Infection type data against the pathogen were recorded after approximately 15 days of inoculation according to the scale described by McNeal *et al.*, (1971). The infection types i.e. 0, 1,and 2 were considered resistant, 3,4 and 5 types, moderate resistant or (intermediate sporulation), 6 and 7 moderate susceptible and 8 and 9 high susceptible.

B- At adult stage:

In the adult tests under field condetion, inoculation was restricted in the spreader plants which were moistened and dusted with spore mixture using the most prevalent stripe rust races in the area. The inoculum was mixed at the rate of

1:20 (urediniospores to talcum powder) (w:w)..All materials were inoculated at booting stage according to the method adopted by Tervet and Cassel (1951). Data of stripe rust severity % were recorded on adult plants according to Peterson *et al.* (1948). To study inheritance of resistance, the F_2 plants were grouped into 10 categories depending on the percentage of the disease severity and infection type under field conditions. The disease severity (%) i.e. 0, 10R, 10MR, 30 MR were considered as the resistant phenotypes, while 20MS, 10S, 30S, 40S, 60S, and 80S were considered as the susceptible ones. Statistical and genetic analysis, frequency distribution values, were estimated for each of parents, F_1 and F_2 populations for infection type in all of the tested crosses in respect. To clarify, mode of inheritance of the expected ratio of the phenotype classes of the stripe rust, infection types were determined using X^2 analysis according to the method of Steel and torrire, (1960).Molecular markers assigned for detection of stripe rust resistance genes in wheat were applied .

Plant material:

RAPD marker linked to Sakha 61 was identified when Sakha 61 (resistant to stripe rust) at adult stage was crossed to Gemmiza 3 (susceptible) and gave resistant in F_2 segregating plant. during 2003/2004 F_2 plants were grown at adult stage in the greenhouse under controlled temperature, light and humidity, which enhanced the disease progression and help in the distinction of disease reaction .

The specific primer was chosen according to the findings of Motawi, *et al.* (2003) who tested 21 RAPD primers assigned for wheat stripe rust and found that only two, (GAAACGGGTG) and (GACCGCTTGT) gave additional band to the resistance of Sakha 61. Only one of them was chosen herein viz (GAAACGGGTG).

Statistical analysis and goodness of fit to a 3:1 ratio was calculated for RAPD marker using Chi-square (x^2) test.

RESULTS

The infection type, frequency distribution and the disease severity classes of the parents, F_1 and F_2 populations of each of the six crosses were performed. Inoculation at seedling was accomplished by using race 134E158 and a mixture of the most prevalent races in the area at adult stage Data presented in Table (1) showed that, all of parents F1 and f_2 plants tested at seedling exhibited susceptible reaction against the physiologic race 134E158. While, the crosses between cultivar Sakha 61 and the wheat cultivars i.e. Sakha 8, Gemmeiza 3, Giza 162, Sids 4, Sids 6 and Sids 9 showed no segregation. This result indicated that, these cultivars do not have the stripe rust resistance gene at seedling stage. The results of crosses between the six wheat cultivars i.e. Sakha 8, Gemmeiza 3, Giza 162, Sids 4 Sids 6 and Sids 9 and the stripe rust resistant cultivar Sakha 61 at adult stage are shown in Table (2). All of six parents exhibited high susceptibility, where, stripe rust severity (%) ranged between (60s – 90s). Meanwhile, Sakha 61 was highly resistant. As for F_1 plants of the six tested crosses exhibited high resistance, where their stripe rust severity (%) ranged between 0 and 5R. These results revealed that resistance was dominant over susceptibility in these crosses in F_1 at adult stage.

Table (1): Evaluation of crosses of the six wheat *cvs.* having Sakha 61 against stripe rust infection using race 134 E 158 at seedling stage, under controlled conditions at Sakha during 2003/2004.

Crosses and parents	Plant No.	Infection types (IIs)											Phenotypes		Expected ratio	X^2
		0	0	1	2	3	4	5	6	7	8	9	R	S		
Sakha 61 × Sakha 8	60										60					
P_1	60											60				
P_2																
F1	30										30				-	-
F2	120									30	30	60		120		
Sakha 61× Gemmeiza	60										60					
P_1	60											60				
P2																
F1	30											30	-		-	-
F_2	150									20	30	100		150		
Sakha 61 ×Giza 162p1	60										60					
P_1	60											60				
P2																
F1	30											30			-	
F2	130									20	30	80		130		
Sakha 61x Sids	60										60					
p1	60											60				
P2																
F1	30											30				
F2	160									50	50	60		160	-	-
Sakha 61 x Sids 6	60										60					
p1	60											60				
P2																
F1	30										30					
F2	130										100	30		130		
Sakha 61 x Sids 9	60											60				
p1	60											60				
P2																
F1	30											30				
F2	120									40		80				

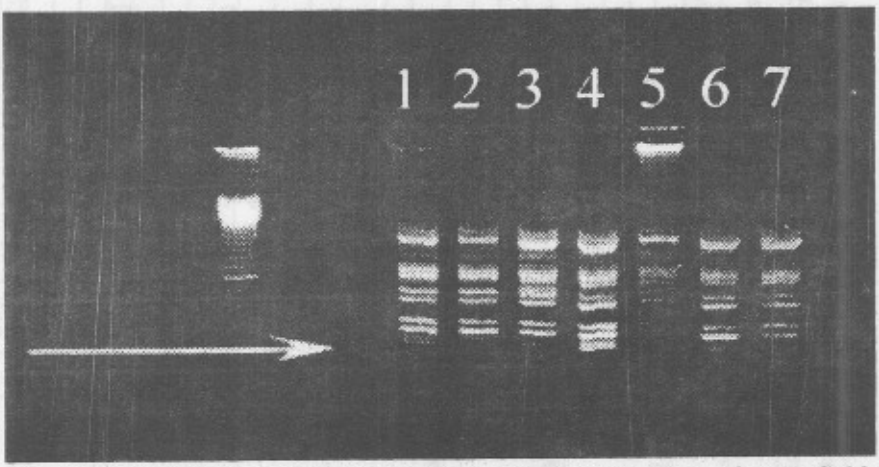
Table (2) : Evaluation of crosses of the six wheat *cvs.* having Sakha 61 against stripe rust infection using a mixture races at adult stage, at the experimental farm (Sakha) during 2003/2004.

Crosses and parents		Plant No.	Infection types (IIs)											Phenotypes		Expected ratio	X^2
			0	5 R	20 r	10 Mr	20 Mr	5Ms	20 Ms	10 s	30 s	60 s	90 s	R	S		
Sakha 61× Sakha 8	P_1	60	60														
	P_2	60											60				
	F_1	40		40													
	F2	120	23	25	18	17		20			13	4		83	37	3:1	2:1
Sakha 61× Gemmeiza 3	P_1	60	60														
	P_2	60										60					
	F_1	48		48													
	F2	180	37	40	11	20	32	8	12		10	10		140	40	3:1	0.59
Sakha 61× Giza 162	P_1	60	60														
	P_2	65										65					
	F_1	45	45														
	F2	163	20	15	26	10	47		5	15	10		15	118	45	3:1	0.75
Sakha 61× Sids 4	P_1	60	60														
	P_2	60											60				
	F_1	41		41													
	F2	198	20	13	30	30	60		10		15	20		153	45	3:1	0.54
Sakha 61× Sids 6	P_1	60	60														
	P_2	50											50				
	F_1	40		40													
	F2	180	27	40	31	20	12	10		10	22	8		130	50	3:1	0.75
Sakha 61× Sids 9	P_1	60	60														
	P_2	60											60				
	F_1	38		38													
	F2	220	40	15	40	48	20	25		15	10		7	163	57	3:1	0.096

The obtained results derived from F_2 of the six tested crosses having resistance gene exhibited a wide range of reaction to stripe rust severity ranged between 0-90 s. The segregated phenotypes were as follow, 83 R: 37 S, 140 R: 40 S, 118R: 45 S, 153R: 45 S, 130 R: 50 S and 163 R: 57 S respectively, with expected ratio 3: 1. This 3:1 ratio verified that single dominant gene pair control resistance and supported the fact that cultivar Sakha 61 carried the adult plant resistance gene and showed gene expression of resistance to stripe rust in all tested crosses at adult stage.

In respect to the detection of resistance genes in wheat crosses using the melucular markers, data in table (3) and illustrated in Fig (1) revealed that, the produced DNA bands of tested wheat individuals, clearly showed that the bands of Sakha 61 are present in lane (the consequence of bands) and the four individuals of F_2 plants are only linked with the primer and rendering as specific clear bands at (900 pb) with the exception of Gemmiza 3 (lane 5) and one susceptible individual of F_2 dived from Sakha 61x Gemmiza 3 (lane 7). Meanwhile, Gemmiza 3 did not link with the primer at (900pb) and could not be detected .

In this respect,the analysis of this polymorphism revealed that only 28 out of 40 individuals of F_2 have linked with the primer where the rest 11 individuals did not. This result revealed that, the resistant: susceptible individuals are 28: 12 with expected ratio 3: 1, which verified by X^2. This result confirmed the presence of resistance gene in the segregation of the resulted crosses and verified that a single dominant gene pair controls resistance .



.**Fig (1): RAPD-PCR analysis of parents and F_2 segregates, having cv. Sakha 61as resistance gene and linked with primer (GAAACGGGTG) (Lane 1 Sakha 61),(Lane 5, Gemmeiza 3), (Lanes 2,3,4,6; resistance F_2 Lane 7, while, susceptible F_2)** the arrowhead pointed to the bands at (900 pb) which differentiated between susceptible and resistance cross.

Table (3): RAPD marker linked with c.v. .Sakha 61 in resistant wheat crosses segregation of F_2 .

RAPD markers	Tested cross	Phenotypes R	Phenotypes S	Expected ratio	X^2
900pb	Sakha61 X Gemmeiza 3	28	12	3:1	0.533

DISCUSSION

Wheat (*Triticum aestivum* L.) is one of the most important food crops in Egypt and allover the world. Stripe rust incited by Puccinia striiformis f.sp. tritici in particular, was a sporadic disease in Egypt before (1990). .Most of the Egyptian cultivars exhibit considerable level of susceptibility, with few exception ElDauodi *et al.* (1998).

Studying six crosses to stripe rust infection at seedling stage under greenhouse condition revealed that all of them showed susceptibility to stripe rust infection, in the greenhouse. However F_2 populations did not fit any ratio or they show more susceptibility than resistance, which,may be due to the phenomenon of partial suppression of resistance genes. At adult stage Sakha 61 and its crosses with tested cvs. (F_1), exhibited high resistance. The rest of tested parents showed different degrees of susceptibility. Conversely F_2 segregations of crosses having Sakha 61 indicated that resistance was dominant over susceptibility . Also results indicated that crosses fitted the expected ratio 3: 1. This ratio verified that single dominant gene pair control stripe rust resistance and supported the F_1 result at adult stage. Molecular marker can be used as marker assists selection for an effective combination of genes and in a pyramiding strategy to create more durable resistance Rolefs *et al.*,(1992). Motawi *et al.*, (2003) develop molecular marker from the Sakha 61 DNA sequence which, was very specific for this cultivar resistance gene in breeding material of diverse genetic origin. The produced random amplified DNA polymorphism (PAPD) of tested wheat individuals using the primer (GAAACGGGTG),.clearly showed that cv Sakha 61 carried a gene which was successfully transferred present in F_2 of Sakha 61 x Gemmeiza 3 which showed resistance and did not present in susceptible individuals. This result confirmed the presence of resistant gene in the segregations of the resulted crosses and verified that a single dominant pair gene controls stripe rust resistance at adult stage. This work could be usefully applicable in the breeding wheat program against rust diseases in general and stripe rust in particular under Egyptian conditions.

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المقاومة الوراثية للصدأ الأصفر في صنف القمح " سخا ٦١"

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يعتبر الصدأ الأصفر في القمح المتسبب عن الفطر (بكسينا سترايفورميس طراز تريتيسای) أكثر أمراض القمح خطورة في مصر حيث تكرر ظهوره بحالة وبائية مسببا خسائر عالية في المحصول مما أدى إلى إلغاء عدة أصناف تجارية ونظرا للزيادة المضطردة في القدرة المرضية لسلالات هذا الفطر على إصابة نبات القمح فقد دعت الحاجة إلى البحث عن مصادر المقاومة لهذا المرض تحتوى على عوامل وراثية ذات تأثير واسع وفعال .

كان الهدف من هذه الدراسة هو التعرف على عوامل المقاومة الوراثية للصدأ الأصفر الموجودة في الصنف سخا ٦١ لذلك تم التهجين بين الأصناف المختارة للحصول على الهجن الآتية (سخا ٦١ X سخا ٨) ؛ (سخا ٦١ X جميزة ٣) ؛ (سخا ٦١ X جيزة ١٦٢) ؛ (سخا ٦١ X سدس ٤ و٦و٩).

دلت النتائج على أن كل المادة المختبرة في طور البادرة كانت قابلة للإصابة بينما أكدت تجارب التربية ضد الصدأ الأصفر في مرحلة الطور البالغ تحت ظروف الحقل أن نباتات الجيل الأول للهجن التي تحتوى على سخا ٦١ كانت كلها مقاومة. إذ أظهرت أقل نسبة إصابة والتي تراوحت بين الطراز المرضى (R 10- 0) وقد أظهرت هذه النتائج أيضا أن صفة المقاومة سائدة على صفة القابلية للإصابة في الجيل الأول كما أظهرت نتائج الجيل الثاني مدى واسع من رد فعل النبات لمرض الصدأ الأصفر والتي تراوحت بين الطراز المرض(90S-0) ولكن كان اتجاه المقاومة للمرض هو السائد على القابلة للإصابة في ٦ هجن ومؤكدا نتائج الجيل الأول . وهذه الدراسة توضح أن الصنف سخا ٦١ يحتوى على جين المقاومة للصدأ الأصفر في طور النبات البالغ وكذلك الهجن التي تحتوى على سخا ٦١ .

كما أدى استخدام طريقة RAPD-DNA للكشف عن وجود جين المقاومة في أفراد هجين القمح المنعزل في الجيل الثاني سخا ٦١ × جميزه ٣ باستخدام البادي الوراثي المتخصص (GAAACGGGTG) تأكد وجود جين المقاومة في الأفراد المقاومة المنعزلة ولم يكن موجودا في الأفراد القابلة للإصابة وقد أكدت هذه النتيجة نسبة ٣: ١ .ويفيد هذا البحث تطبيقيا في عمليات التربية للمقاومة للأمراض بصفة عامة ولأصداء القمح بصفة خاصة.