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GENETICS OF ADULT-PLANT STEM RUST RESISTANCE IN SIX EGYPTIAN BREAD WHEAT CULTIVARS

Abdalla, M.E. *; A.A. Hagras; S.S. Negm*** and O.A. El-Sayed*****

***Plant Pathology Dept., Fac. of Agric., Mansoura Univ., Egypt**

****Wheat Research Department, FCRI, ARC, Egypt**

*****Wheat Disease Research Department, PPRI, ARC, Egypt**

ABSTRACT

This investigation was carried out to identify stem rust resistance genes in the six Egyptian bread wheat cultivars Sakha 93, Sids 1, Gemmeiza 10, Gemmeiza 11, Misr 1 and Misr 2. F_1 seed were developed from the crosses between the six wheat cultivars and each of the wheat monogenic lines of *Sr* 11, *Sr* 32, *Sr* 33 and *Sr* 40 in 2012/2013 season. In the next season, 2013/2014, F_2 plants were tested under field conditions after artificial inoculation with a mixture of stem rust physiological races at Sids Agricultural Research Station, ARC, Egypt. Chi-square test was used to test the fitness of segregation in F_2 plants for each cross to the expected ratios. Out of the six wheat cultivars, the two cultivars Sids 1 and Gemmeiza 10 showed no or negligible segregations in F_2 crosses between them and each of the four stem rust monogenic lines. All or majority of F_2 plants showed resistant type of reaction to stem rust under field conditions indicating that both cultivars expected to carry *Sr* 11, *Sr* 32, *Sr* 33 and *Sr* 40 adult-plant stem rust resistance genes. On the other hand, the remaining four wheat cultivars, Sakha 93, Gemmeiza 11, Misr 1 and Misr 2 showed significant segregation in their F_2 crosses with the monogenic lines. Segregations among F_2 plants were toward resistance in the crosses with the two cultivars Sakha 93 and Gemmeiza 11 while it was toward susceptibility in the crosses with the two cultivars Misr 1 and Misr 2. Resistance found to be dominant over susceptibility in the crosses between the two cultivars Sakha 93 and Gemmeiza 11 and the monogenic lines except for the cross Sakha 93 x Sr 32. Meanwhile, susceptibility was dominant over resistance in the crosses between the two cultivars Misr 1 and Misr 2 and the monogenic lines. The results of this study indicate the importance of the two Egyptian bread wheat cultivars Sids 1 and Gemmeiza 10 as a source of adult-plant stem rust resistance genes which could be utilized in wheat breeding program for improving stem rust resistance in Egypt.

Keywords: Wheat, adult-plant resistance and stem rust (*Puccinia graminis f sp. Tritici)*

INTRODUCTION

Wheat *(Triticum aestivum L.)*, as nutritive crop, considered one of the most important cereal crops in Egypt as well as in many parts of the world. In Egypt rust diseases are the most common and dangerous on wheat plants (Abd El-Hak *et al*, 1973). Wheat stem rust caused by the fungus *Puccinia graminis f. sp. tritici* is a heterocious obligate biotroph with a macrocyclie lifecycle feating five distinct spore stages (Leonard,2005).Stem rust considered as the most destructive disease of wheat (El – Sayed, 2011). The losses may reach 100% on susceptible wheat cultivars when conditions are favorable for the disease (Singh et al., 2002 , 2006 and 2011). The appearance of virulent and aggressive pathotypes of the causal organism is

one of the most dangerous factors in the occurrence of any disease epidemic (Diab, Hoda 1994, Abdel Malik, Nagwa 2003) . The appearance of race 15B of stem rust in the USA in 1960's (Zadoks, 1971) and the appearance of race Ug99 in Uganda in 1999 were clear examples (Wanyera et al., 2006 and 2009). Breeding for adult-plant resistance is still the most economic and desirable method for controlling the disease (Mcintosh, *et al.*, 2008). Nature of wheat resistance to stem rust disease dependent on genetic agents is controlled by major or minor genes or both together (Simons, *et al.*, 1978). The effectiveness of resistance in wheat cultivars to any rust disease depends on its level, stability and durability. Therefore, the effectiveness of stem rust resistance genes (*Sr*s) at adult-plant stage against stem rust were evaluated under field conditions. Identification of stem rust resistance genes (designated "*Sr*" genes) presents in each variety enable wheat breeders to achieve their objectives more quickly and reduce the time for testing under filed conditions (Anderson et al, 1971). The characterization of specific stem rust resistance genes is very useful to determine exactly which resistance genes are present in commercial wheat varieties. Little is known about the adult plant stem rust resistance genes present in the Egyptian wheat cultivars. The main objective of the present study were to identify the genes governing adult-plant resistance to stem rust in the six Egyptian bread wheat cultivars Sakha 93, Sids 1, Gemmeiza 10, Gemmeiza 11, Misr 1 and Misr 2.

MATERIALS AND METHODS

To Identify gene (s) for stem rust resistance in the six Egyptian bread wheat cultivars Sakha 93, Sids 1, Gemmeiza 10, Gemmeiza 11, Misr 1 and Misr 2, crosses were conducted between them and each of wheat stem rust monogenic lines *Sr* 11, *Sr* 32, *Sr* 33 and *Sr* 40. Table 1 demonstrates name, pedigree and stem rust reaction of the four monogenic lines and six Egyptian bread wheat cultivars used in the study. Seeds of wheat cultivars were provided by Wheat Res. Dept., and the stem rust monogenic lines were provided by Wheat Disease Res. Dept., ARC, Egypt. The parental varieties and monogenic lines were grown in 2011/2012 growing season at Sids Agricultural Research Station in three successive sowing dates with 15 days intervals to overcome differences in the time of flowering. The Monogenic lines under the study were used as male parents for crosses with each of the wheat cultivars. Any doubtful of F_1 plants were discarded and each was harvested separately. The F_1 seeds were grown at the following season (2012 /2013) in rows of 4 m long and 30 cm apart and spaced 30 cm in order to facilitate production of F_2 seeds. In 2013/2014 growing season, parents and F_2 seeds were grown in plots, each plot of the F_2 contained 10 rows, each 4 m long and spaced 30 cm between rows and 15 cm between plants. A mixture of the two stem rust highly susceptible wheat varieties; Morocco and *Triticum spelta saharinsis* were grown as spreader around all plots. F_2 plants were tested at adult stage under artificial inoculation conditions (Stakman *et al* 1962) . Plant response to rust infection at the adult plant stage was termed infection response ($IR,^{S}$). According to the size of pustules and

associated with necrosis or chlorosis, IR,^{s} were classified into four discrete categories : R = resistant, MR = Moderately Resistant, MS = Moderately Susceptible and S = Susceptible (Roelfs *et al.*, 1992). Infection response overlapping between any particular two categories were denoted using a dash for instance " MR-MS" denoted an infection response class overlapped between the MR and MS categories. Entries were evaluated for IR,s two to three times between plant heading and maturity. The infection responses at the soft-dough stage of plant growth were used to represent the final disease scores in this study (Jin *et al.*, 2007). IR,^{s} was recorded for each F_2 plant for each cross grouped and into several categories depending on the IR, ^{s} under field conditions. The first four categories were considered resistant phenotype and the late three categories were considered as susceptible phenotypes. For identification of the adult-plant stem rust resistance genes in each cross, the chi-square test (X^2) was used to test the significance of difference between observed and expected ratios in F_2 populations for stem rust reaction according to Steel and Torrie (1960).

Table (1). Name, pedigree and stem rust reaction of the four stem rust monogenic lines and six Egyptian bread wheat cultivars used in the study.

Name	Pedigree	Stem rust reaction
Sr 11	Lee/6*LMPG-6 DK 37	Moderately resistant
Sr 32	ER5155 S-203 (1995) Roelfs	Moderately resistant
Sr 33	RL 5405 (1192) Kerber	Moderately resistant
Sr 40	RL.6087 Dyck	Moderately resistant
Sakha 93	Sakha 92 / TR810328 S.8871-1S-2S-1S-0S	Susceptible
Sids 1	HD2172/Pavon"S"//1158.57/Maya74"S" SD46-4SD-2SD-1SD-0SD	Moderately resistant
Gemmeiza 10	MAYA47"S"/ON//II60-147/3/BB/GLU/4/CHAT"S"/5/ CROW"S" CGM7892-2GM-1GM-2GM-1GM-0GM	Resistant
Gemmeiza 11	BOW"S"/KVZ"S"//7C/SER182/3/GIZA168/SAKHA 61 CGM5820-3GM-1GM-2GM-0GM	Susceptible
Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	Susceptible
Misr 2	SKAUZ/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S	Susceptible

RESULTS

To identify genes governing wheat adult-plant resistance to stem rust, 21 crosses between the six bread wheat cultivars, Sakha 93, Sids 1, Gemmeiza 10, Gemmeiza 11, Misr 1 and Misr 2, and each of the four wheat monogenic lines *Sr* 11, *Sr* 32, *Sr* 33 and *Sr* 40 were used. The F_2 populations of these crosses were tested for adult plant reaction for stem rust under field condition. The detailed results are showed in Table 2 and summarized in Table 3. F_2 populations from all crosses showed a range of stem rust infection response from complete resistance to segregation to susceptible and resistance with different ratios. The plants with infection

responses R, R-MR,MR and MR-MS were grouped into the resistance category (R) and MS, MS-S and S were grouped into the susceptible category (S).

Sr 11

All of the 224 and 222 F_2 plants of the crosses between the monogenic lines *Sr* 11 and each of the two wheat cultivars Sids 1 and Gemmeiza 10, respectively had resistant response and showed no segregation (Table 2). These results indicate that both cultivars are expected to carry the adult-plant resistance gene *Sr* 11. On the other hand, F_2 plants of the crosses between the monogenic line *Sr* 11 and each of the wheat cultivars Sakha 93, Gemmeiza 11, Misr 1 and Misr 2 segregated to 210 R :20 S, 180 R : 48 S, 160 R : 63 S and 70 R : 175 S with expected segregation ratios of 57:7, 13:3, 3:1 and 1:3, respectively (Table 2). These results indicate the absence of *Sr* 11 in the above four cultivars.

Sr 32

Of the 219 F_2 plants there were only 6 susceptible plants to stem rust recorded in the cross between the monogenic line *Sr* 32 and the cultivar Sids 1 (Table 2). Probability of chi-square test was very low (0.01-0.05) indicating insignificance of the segregation in F_2 population in this cross. In addition, all F_2 plants of the cross between the monogenic line *Sr* 32 and the cultivar Gemmeiza 10 showed resistance reaction for stem rust. These results indicated that both wheat cultivars Sids 1 and Gemmeiza 10 are expected to have the same stem rust resistant gene as the monogenic line *Sr* 32. On the other hand, the segregations ratios of F_2 plants confirmed that the three cultivars Sakha 93, Misr 1 and Misr 2 did not have the stem rust resistance gene *Sr* 32 (Table 2), Segregation ratios were 1R: 3S, 7R:57S and 7R:57S, respectively. F_2 plants of the crosses between the monogenic line *Sr* 32 and the cultivar Gemmeiza 11 segregated to 162 R : 68 S. The segregation fit the ratio 3 R : 1 S.

Sr 33

Only 7 susceptible plants were recorded from the 240 F_2 plants from the crossing between the monogenic line *Sr* 33 and the cultivar Sids 1. Probability of chi-square test was very low (0.01-0.05) indicating insignificance of the segregation in F_2 population of this cross. In addition, all F_2 plants (225 plants) of the cross between the monogenic line *Sr* 33 and the cultivar Gemmeiza 10 showed resistance reaction for stem rust. These results indicate that both wheat cultivars Sids 1 and Gemmeiza 10 expected to have the same adult-plant stem rust resistant gene as the monogenic line *Sr* 33. Meanwhile, F_2 plants of the crosses between the same monogenic line (*Sr* 33) and the wheat cultivars Sakha 93, Misr 1 and Misr 2 segregated to 166 R : 38 S, 41 R : 179 S and 22 R : 182 S, respectively. The segregations of F_2 plants indicate that the wheat cultivars Sakha 93, Misr 1 and Misr 2 did not have the stem rust resistance gene *Sr* 33. Probability of chi-squares was 0.90-0.95 for the expected segregation ratios 13R : 3S, 3R : 13S and 7R : 57S for the crosses with the wheat cultivars Sakha 93, Misr 1 and Misr 2, respectively.

Sr 40

All of the 235 and 214 F_2 plants of the crosses between the monogenic line *Sr* 40 and each of the wheat cultivars Sids 1 and Gemmeiza 10, respectively were resistant to stem rust (Table 2). Absence of segregation in F_2 plants of the two crosses indicated that the two wheat cultivars Sids 1 and Gemmeiza 10 expected to have the adult-plant stem rust resistance gene as the monogenic line, *Sr* 40. On the other hand, F_2 plants of the crosses between the monogenic line and the wheat cultivars Misr 1 and Misr 2 segregated to 21R : 190S and 22R : 200S, respectively. The presence of segregation in the crosses indicates the absence of *Sr* 40 in the two cultivars.

DISCUSSION

The most economical and preferable method for controlling wheat stem rust is the utilization of host genetic resistance. Therefore, special focusing has been concentrated on the adult-plant resistance genes to stem rust, which is very important to protect wheat plants during the flowering and seed filling stage.

Gene *Sr* 11 was isolated from *Triticum turgidum var. durum* cv. Gaza (Watson and Stewart, 1956) and it was mapped to chromosome 6 B L (Loegering, and Sears,. 1966). Results of this study showed the expression of this gene in the two cultivars Sids 1 and Gemmeiza 10 and no expression in the other four cultivars Sakha 93, Gemmeiza 11, Misr 1 and Misr 2 indicating the absence of *Sr* 11 in the last four cultivars (Table 3). In addition, the monogenic *Sr* 11 and each of the four cultivars differ in at least two pair of genes. Resistance to stem rust is dominant over susceptibility in the three cultivars Sakha 93, Gemmeiza 11 and Misr 1.

Gene *Sr* 32 was isolated from *Triticum speltoides* and was allocated on chromosome 2 A (Mcintosh *et al.,* 1974). F_2 results demonstrated its expression in Sids 1 and Gemmeiza 10 but not in the three cultivars Sakha 93, Misr 1 and Misr 2 as the presence of segregations in F_2 confirmed that these cultivars did not have the stem rust resistance gene *Sr* 32 (Table 3). The results indicate that the three cultivars have at least two recessive resistance genes against stem rust differ from Sr *32*. Meanwhile, the wheat cultivars Gemmeiza 11 has one dominant gene for stem rust resistance different from the tested gene *Sr* 32.

Gene *Sr* 33 was found from *Triticum tauschii RL.5288* and was allocated on chromosome I DL(Kerber and Dyck, 1979). In the present study six Egyptian bread wheat cultivars were tested for the presence of the gene *Sr* 33. Results showed that all of F_2 plants of the crosses between the monogenic line carrying the gene *Sr* 33 and each of the cultivars Sids 1, Gemmeiza 10 showed resistance responses of infection to stem rust under field conditions. These results confirmed the expression of the adult-plant resistance gene *Sr* 33 in the two wheat cultivars. On the other hand, the segregation of F_2 plants indicated that the cultivars Sakha 93, Misr 1 and Misr 2 did not have the stem rust resistance gene *Sr* 33. Resistant are dominant over susceptibility in the cross with Sakha 93 while the opposite was in the other two crosses.

Table (2). Stem rust infection response, observed ratio, expected ratio, chi-square and probability of F_2 plants from the crosses between four stem rust monogenic lines and six Egyptian bread wheat cultivars under artificial inoculation in field condition in the season 2013/2014.

Cross	Stem rust infection response							Observed ratio		Expected ratio	X^2	Probability
	R[a]	R-MR	MR[b]	MR-MS	MS[c]	MS-S	S[d]	R	S			
Sakha 93× *Sr*11	40	48	60	62	6	4	10	210	20	57:7	1.187	0.25-0.50
Sids 1× *Sr*11	123	14	85	2	0	0	0	224	0	No segregation	-	-
Gemmeiza 10 × *Sr*11	169	20	22	11	0	0	0	222	0	No segregation	-	-
Gemmeiza11× *Sr*11	54	33	62	31	6	13	29	180	48	13:3	0.794	0.25-0.50
Misr 1× *Sr*11	48	18	63	31	48	8	7	160	63	3:1	1.257	0.25-0.50
Misr 2× *Sr*11	22	13	25	10	30	12	133	70	175	1:3	1.667	0.10-0.25
Sakha 93× *Sr*32	20	10	35	8	72	10	80	73	162	1:3	4.609	0.01-0.05
Sids 1× *Sr*32	126	3	80	4	6	0	0	213	6	No segregation	4.605	0.01-0.05
Gemmeiza 10 × *Sr*32	73	12	140	0	0	0	0	225	0	No segregation	-	-
Gemmeiza11× *Sr*32	10	15	125	12	50	8	10	162	68	3:1	2.557	0.10-0.25
Misr 1× *Sr*32	5	2	9	4	59	9	120	20	188	7:57	0.373	0.50-0.75
Misr 2× *Sr*32	7	3	6	3	48	11	114	19	173	7:57	0.214	0.50-0.75
Sakha 93× *Sr*33	83	7	94	8	29	2	7	166	38	13:3	0.002	0.90-0.95
Sids 1× *Sr*33	140	15	68	10	3	0	4	233	7	No segregation	4.551	0.01-0.05
Gemmeiza 10 × *Sr*33	55	10	160	0	0	0	0	225	0	No segregation	-	-
Misr 1× *Sr*33	3	4	16	18	48	58	73	41	179	3:13	0.002	0.90-0.95
Misr 2× *Sr*33	6	3	8	5	52	21	109	22	182	7:57	0.005	0.90-0.95
Sids 1× *Sr*40	80	5	150	0	0	0	0	235	0	No segregation	-	-
Gemmeiza 10 × *Sr*40	42	8	164	0	0	0	0	214	6	No segregation	4.659	0.01-0.05
Misr 1× *Sr*40	9	3	4	5	25	11	154	21	190	7:57	0.210	0.50-0.75
Misr 2× *Sr*40	8	4	6	4	90	9	101	22	200	7:57	0.241	0.25-0.50

a R = Resistant; b MR = Moderately resistant; c MS = Moderately susceptible; d S = Susceptible.

Gene *Sr* 40 was originally isolated from *Triticum timophem* and allocated on 2BS (Dyck, 1992). and closley linked for Sr 36 (El- Daoudi, *et al.*, 1995 and Hassan, 2006). All of F_2 plants of the crosses between the monogenic line carrying the gene Sr 40 and each of the cultivars Sids 1 and Gemmeiza 10 found to be resistance and showed no segregation. These results indicated that the two cultivars Sids 1 and Gemmeiza 10 expected to carry the stem rust resistance gene *Sr* 40. On the other hand, the two cultivars Misr 1 and Misr 2 segregated and its segregations fit the ratio 7R : 57S indicating that these two wheat cultivars have three independent recessive genes and susceptibility was dominant over resistance (Youssef *et al.*, 2012).

In conclusion, the adult plant stem rust resistance in the six Egyptian wheat cultivars under study is controlled by a group of the genes *Sr* 11, *Sr* 32, *Sr* 33 and *Sr* 40, these genes are expected to be a part of the genetic pool of the two Egyptian bread wheat cultivars Sids 1 and Gemmiza 10 (Table 3). Moreover, these adult-plant stem rust resistance genes are conditioning effective field resistance against stem rust disease.

Table (3). Summary of genes expected to be present in the six Egyptian bread wheat cultivars under study.

Cultivar	Stem rust gene			
	Sr 11	*Sr* 32	*Sr* 33	*Sr* 40
Sakha 93	-[a]	-	-	NA[c]
Sids 1	+[b]	+	+	+
Gemmeiza 10	+	+	+	+
Gemmeiza 11	-	-	NA	NA
Misr 1	-	-	-	-
Misr 2	-	-	-	-

a (-) = The gene is absent; b (+) = The gene is expected to be present; c (NA) = the cross was not available

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وراثة مقاومة النبات البالغ لصدأالساق فى ستة أصناف قمح خبز مصرية

محمد السيد عبد الله* ، عادل عبد العزيز هجرس ، صبحى سيد محمد نجم***و اسامة عبد البديع السيد*****

***قسم أمراض النبات – كلية الزراعة – جامعة المنصورة**

****قسم بحوث القمح – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية - الجيزة**

*****قسم بحوث أمراض القمح – معهد بحوث أمراض النباتات – مركز البحوث الزراعية - الجيزة**

اجريت هذه الدراسة لتحديد جينات مقاومة النبات البالغ لصدأالساق فى الاصناف الستة من قمح الخبز المصرية سخا ٩٣ و سدس ١ و جميزة ١٠ وجميزة ١١ و مصر ١ ومصر ٢. ففى الموسم الزراعى ٢٠١٢/٢٠١١ تم التهجين بين الاصناف الست وكل سلالات القمح الاربع الحاملة لجينات المقاومة لصدأالساق بصورة فردية وهى Sr 11 و Sr 32 و Sr 33 و Sr 40 لانتاج حبوب الجيل الاول. فى الموسم الزراعى التالى زرعت حبوب الجيل الاول لانتاج حبوب الجيل الثانى. وفى الموسم الزراعى ٢٠١٤/٢٠١٣ زرع الاحدى وعشرون هجين للجيل الثانى المتاحة فى المزرعة البحثية لمحطة البحوث الزراعية بسدس واحيطت التجربة بداير زرع بخليط من الأصناف العالية القابلية للاصابة واجريت العدوى الصناعية لنباتات الداير بخليط من الجراثيم اليوريدية لسلالات صدأالساق. وتم تسجيل رد فعل النباتات الفردية للجيل الثانى لصدأالساق واخضعت النسب المشاهدة لحساب النسب المتوقعة باستخدام اختبار مربع كاى. دلت النتائج على انعدام الانعزال او وجوده بدرجة قليلة جدا فى جميع الهجن التى اجريت بين الصنفين سدس ١ وجميزة ١٠ و السلالات الاربع الحاملة لجينات المقاومة لصدأالساق بصورة فردية. حيث لوحظ ان جميع او الغالبية العظمى لنباتات الجيل الثانى مقاومة لصدأالساق. وبذلك يتوقع ان يكون كلا الصنفين من القمح المصرى يحملان الجينات الاربع التى تم اختبارها (Sr 11 و Sr 32 و Sr 33 و Sr 40).

اما بالنسبة للهجن التى اشتركت فيها الأصناف المصرية الاربعة الاخرى (سخا ٩٣ و جميزة ١١ و مصر ١ ومصر ٢) مع كل من السلالات الاربع الحاملة لجينات المقاومة لصدأالساق بصورة فردية فقد لوحظ فى سلوكها وجود انعزالات فى المقاومة والقابلية للاصابة فى نباتات الجيل الثانى مما يدل على عدم احتمال وجود هذه الجينات فى تلك الاصناف الاربعة المصرية. اظهرت نسب الانعزال ان المقاومة لمرض صدا الساق كانت سائدة على القابلية للاصابة فى جميع الهجن بين الصنفين سخا ٩٣ وجميزة ١١ وكل من السلالات الاربع الحاملة لجينات المقاومة لصدأالساق بصورة فردية عدا الهجين بين الصنف سخا ٩٣ والسلالة الحاملة للجين Sr 32. بينما دلت نسب الانعزال عكس ذلك فى جميع الهجن بين الصنفين مصر ١ ومصر ٢ وكل من السلالات الاربع الحاملة لجينات المقاومة لصدأالساق بصورة فردية حيث كانت القابلية للاصابة لمرض صدا الساق سائدة على المقاومة للمرض. كما تدل نتائج هذه الدراسة على اهمية الصنفين سدس ١ وجميزة ١٠ فى مقاومة صدأالساق فى القمح لاحتوائها على جينات مقاومة يمكن الاستفادة منها فى برنامج التربية لانتاج اصناف مقاومة تحت الظروف المصرية.