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COMPONENTS OF SLOW RUSTING IN CERTAIN EGYPTIAN WHEAT CULTIVARS AGAINST STEM RUST

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ABSTRACT

Slow rusting resistance in 14 wheat cultivars against stem rust was studied in seedling stage under circumstances of conditioned greenhouse and in adult stage under the stress of field conditions. Inoculation was accomplished using two physiologic races of *Puccinia graminis* f. sp. *tritici* Eriks & Henn. viz. no 11 and no. 15 in seedling stage and a mixture of races under field conditions. Slow rusting components *i.e.* number of pustules per cm^2 of leaf area, number of erupted pustules/cm^2, pustule size and colony size were taken into consideration in seedling stage, while rust severity and area under disease progressive curves were considered in adult stage. The reaction of cvs. in seedling resulted in the distinction of cvs. *i.e*. Giza 168, Sakha 93, Gemmeiza-9, Gemmeiza-7 and Sakha 202 since they were significantly differentiated from the rest of the tested cvs. The reverse was true, with cvs. such as Little Club, Sakha 8, Giza 160. The obtained results also indicated that race 11 was virulent to either of the tested cvs. However, race 15 was virulent to 10 ones.

The trend of cvs. response toward the disease tend to be similar to those observed in seedling, since cvs. viz. Giza 168, Gemmeiza-9 and Sakha 93 showed moderate resistance, Giza 164 showed moderate susceptibility against either of the physiologic races. The two cultivars *i.e.* Sakha 61 and Sakha 202 proved to have slow rusting since they ceased the disease progress at (5S) and exhibited the lowest area under disease progressive curves. From such perspective, it could be concluded that slow rusting phenomenon is closely related with fewer number of uredia/cm^2, fewer erupted pustules/cm^2, fewer colony size, fewer pustule size and longer latent period or incubation period at seedling and lower disease severity and lower AUDPC at adult stage. These data could serve as an effective tool in the field of breeding disease resistance in Egypt.

Keywords: *Triticum aestivum*, stem rust, partial resistance.

INTRODUCTION

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* Eriks & Henn. is one of the three major rust diseases of wheat (*Triticum aestivum* L.) in many regions of the world and also in Egypt. Using of host resistance is an effective approach for controlling such diseases. In the last decade, some Egyptian wheat varieties showed a slower rusting (partial resistance) than others, it is well known that the longer latent period, smaller colony, pustule (uredium) size, and few number of pustules per unit leaf area all play strong roles in retarding the rust disease development. The partial resistance is characterized by a reduced rate of epidemic development despite susceptible infection type (IT). This type of resistance or slow rusting acts through a polygenic genes (Zadoks 1972; Ohm and Shaner, 1976; Parlevliet and Zadoks, 1977).

The main objectives of the present investigation were to determine the components of slow-rusting in 13 Egyptian wheat cultivars and Little Club as a standard susceptible one against virulent isolates of stem rust under greenhouse and field conditions to be exploited in the promising lines in the breeding program of wheat for rust resistance.

MATERIALS AND METHODS

The present experiments were conducted in the greenhouse and at the experimental field of Sakha Agriculture Research Station during 2005-2007.

I. The seedling stage experiment:

Thirteen Egyptian wheat cultivars showing different levels of rust severity *i.e.* Gemmeiza-1, Gemmeiza-5, Gemmeiza-7, Gemmeiza-9, Giza 160, Giza 163, Giza 164, Giza 167, Giza 168, Sakha 8, Sakha 61, Sakha 93, Sakha 202 and Little Club (as a standard susceptible check) were used in such study. Two physiologic races of *P. graminis* f. sp. *tritici* viz. races 11 and 15 were kindly supported from the Wheat Disease Res. Section, Agriculture Research Center, Giza, Egypt, and were used for inoculation of such cultivars in seedling stage under greenhouse conditions.

Seedlings of the above mentioned cultivars were sown in 10 cm diameter pots, at 7-8 days old, they were individually inoculated with either race 11 or race 15 of stem rust and incubated in a moist chamber for 24 hr thereafter. They were transferred to greenhouse benches. The inoculated seedlings were then kept under observation until disease reaction was initiated and infection types (IT) were recorded.

The method of inoculation, incubation and recording of the IT were performed according to the methods of Stakman *et al*., (1962). The components of slow rusting *i.e.* number of infection sites per square centimeter, (number of erupted and non-erupted pustules per square centimeter), latent period (the period at which 50% of the formed pustules become erupted), colony size and uredium size were measured for each tested entry with each physiologic race according to (Hooker, 1967; Ohm and Shaner, 1976; Samborski *et al.*, 1977 and Zwatz, 1980).

To determine the colony and uredium size, segments with 1-3 cm. long were cut-off from the middle part of inoculated leaf blades at the end of sporulation period. The segments were cleared, stained and prepared for microscopic examination using the whole-leaf-clearing technique as adopted by (Shipton and Brown, 1962). In which, the infected leaf segments were immersed in 10-15 ml of alcoholic lactophenol cotton blue (2:1 v:v), immediately after cutting and simmered for one minute. After the leaf segments were sank, the solution was boiled again for ½ minute. Leaf segments were allowed to remain in the stain for approximately 48 hr. at room temperature. Then removed, rinsed in water and placed in chloral hydrate for 30-50 minutes.

The cleared leaf segments were then mounted on microscope slides in 50% glycerin for the examination using ordinary light microscope. The mycelium was stained with cotton blue and were distinguished by its blue purple color, while other leaf tissues were cleared by chloral hydrate. Colony diameter was measured by using an equilibrium ocular micrometer of light microscope and its size was estimated as an ellipse, using the following formula According to Tomerline *et al.,* (1984):

Colony size =¼(length×width)×π

The colony size was measured for each entry 15 days after inoculation (at sporulation over) until 50% of pustules were erupted *i.e*. latent period.

II. Field experiments:

The previous 14 cultivars were sown during the two successive seasons of 2005/006 and 2006/007 in a complete block randomized design with three replications. Each of the 14 wheat cultivars was sown in 2 rows with 1.5 m. long and 3 g. seed rate using the broadcasting method. The experiment was surrounded with a belt of susceptible cv. mixture *i.e* Little Club, Giza 160 and Sakha 8 to serve as a spreader. Irrigation, fertilization and weed control …etc. were carried out according to the technical recommendations of the crop. Artificial inoculation was carried out on the spreader with a mixture of stem rust isolates using the method adopted by (Tervet and Cassel, 1951) in which the mixture of stem rust isolates was added to talcum powder at rate of 1:20 (w.w.). The inoculation was performed between late tillering and booting stages. Plants of the spreader were moistened and rubbed to remove the waxy layer, then rust inoculum was sprayed just before sunset and irrigated thereafter. Disease severity was determined 10 days after inoculation and continued every 8 days intervals thereafter, in terms of IT (infection type) according to Stakman, *et al*., (1962).

Data were transformed to ACI (Average Coefficient of Infection) according to the Cobb scale adopted by Peterson, *et al*., (1948) to be easy for statistical analysis. While AUDPC was estimated using the equation proposed by Pandy, *et al*., (1989).

$$AUDPC = D[½(Y_1+Y_k)+Y_2+Y_3+…+Y_{k-1}]$$

where**:**

D = time interval.

(Y_1+Y_k) = Sum of first and last disease scores.

$(Y_2+Y_3+…+Y_{k-1})$ = Sum of all in-between disease scores.

All data obtained were statistically analyzed for each season individually and combined analysis of variance over the two seasons was also carried out. Significant differences among entries were tested by the analysis of variance test of a split plot design with 3 replicates as outlined by (Snedecor and Cochran, 1967). The difference among the means of the entries tested was compared by using Dunkank's new Multiple Range Test.

RESULTS

I. The seedling stage experiments:

The data indicate that the infection types of races 11 and 15 were different on the 14 tested cultivars (Table 1). out of 14 cultivars, 8 showed susceptible reaction (IT 4) with race 11, while the rest of cultivars exhibited moderate susceptibility (IT 3). On the other hand, race 15 was less virulent than race 11 on the same cultivars. Only, 3 were susceptible (IT 4), 6 were moderate susceptible (IT 3) and 5 ones were moderate resistant (IT 2).

Regarding the components of slow rusting (partial resistance) *i.e*., number of uredio-pustules formed in one cm^2 of leaf area, latent period (LP) and the size of colony and pustule formed. The data indicated that there were significant differences among cultivars in the number of pustules and the erupted ones per/cm^2 of leaf area. The highest number of pustules formed with race 11 and the erupted ones on L. Club were (32.2 and 31.0 pustules/cm^2) followed by Sakha 8 (30.2 28.0 pustules/cm^2). While the least number of pustules were recorded with Giza 168 (19.3 and 18.0 pustules/cm^2) followed by Gemmeiza-5 (20.3 and 18.0 pustules/cm^2) and Gemmeiza-1 (20.3 and 18.7 pustules/cm^2). The least percentage of the erupted pustules were recorded with Gemmeiza-7 (76.00%) and Gemmeiza-5 (88.67%).

As regard to the evaluation against race 15, the trend was a little bet similar to that of race 11, since most of the tested entries were significantly different regarding the number of pustules and the erupted pustules/cm^2 of leaf area. Giza 164, Little Club, Sakha 8, Giza 160 and Sakha 93 showed the highest number of the formed uredio pustules as being 22.00, 21.00, 20.30, 20.02, and 20.0 putules/cm^2 of the leaf area, respectively and the number of the erupted pustules were 18.0, 19.7, 19.0, 19.0 and 15.7/cm^2 of the leaf area, respectively.

On the other hand, Gemmeiza-7 and Gemmeiza-5 exhibited relatively lower number of uredio pustules/cm^2 of the leaf area were *i.e.* 13.8 and 14.3, and the number of the erupted pustules exhibited were 12.0 and 13.0/cm^2, respectively. The highest percentages of the erupted pustules were recorded with Giza 160 (94.60%), Little Club (93.81%), Sakha 8 (93.59%) and Giza 167 (93.26%), while the lowest percentages were recorded with Gemmeiza-9 and Giza 168 *i.e.* 70.5% and 72.43%, respectively.

The LP of race 11 was longer with entries Gemmeiza-9 and Giza 164 that recorded 14 and 13 days, respectively, while it was shorter in Giza 160, Giza168, Sakha 8 and Little Club that recorded 7 days. Concerning the LP with race 15, it was longer (14 days) in Gemmeiza-9, Giza 164 and Sakha 93 and it was shorter in L. club and both of Giza 160 and Sakha 8, as recorded 7 and 8 days, respectively.

Concerning the colony and pustule sizes, data showed that there were significant differences among the tested cultivars. The most restricted colony size were determined with Giza 164, Sakha 202, Sakha 93 and Gemmeiza-9 as being 0.014, 0.014, 0.015 and 0.017 mm^2 with race 11 and 0.011, 0.012, 0.013 and 0.015 mm^2 with race 15, respectively, while the greatest colony size were recorded with Little Club (0.073 mm^2) and Gemmeiza-5 (0.041 mm^2). The least urediopustule size (0.012 mm^2) was determines with race 11 on Giza 168 and on Sakha 202 and with race 15 on Gemmeiza-7 and Gemmeiza-9 as being 0.004 and 0.008 mm^2, respectively. In contrast, the greatest pus5tules size of race 11 was recorded with L. club, Gemmeiza-7 and Gemmeiza-9 *i.e.* 0.032, 0.028 and 0.027 mm^2, respectively and that of race 15 was found on L. club and Giza 160 as being 0.030 and 0.020 mm^2, respectively.

II. Field experiments:

Evaluation of certain wheat entries to stem rust disease in terms of diseases severity and AUDPC during 2005/2006 and 2006/2007 growing season:

Data presented in (Table 2) revealed that out of the 14 tested entries; 3 were moderate resistant, 1 was moderate susceptible and 10 were susceptible. The least disease severity (5MR) was recorded with both of Giza 168 and Gemmeiza-9. The highest disease severity was recorded with Little club (check) (70S) follo5wed by Giza 160 (40S) and Sakha 8 (30S). During the growing season of 2006/07 stem rust severities of the tested entries showed the same trend as the previous growing season with slight increase in disease severity.

Concerning the evaluation of the 14 wheat entries against stem rust in terms of 5 by Sakha 93 (21.33) and Gemmeiza-9 (22.30). On the other hand, The highest value was recorded with Little club (303.33) followed by Sakha 8 (208.00) and Giza 160 (168.70). The rest of tested entries showed intermediate response lied between the two limits.

On the other hand during 2006/07, the least (AUDPC) value was recorded with Sakha 93 (25.00), Gemmeiza-9 (27.38), and Giza 168 (37.65), however, the highest value was recorded with Little club (400.30) followed by Sakha 8 (248.32) and Giza 160 (205.00).

Table 2. Evaluation of 14 wheat entries against a mixture of stem rust (*Puccinia graminis* f. sp. *tritici*) under field conditions during two growing seasons *i.e.* 2005/2006 and 2006/2007 at adult stage.

Cultivars	2005/2006		2006/2007	
	Diseases severity	AUDPC	Diseases severity	AUDPC
Giza 160	40 (S)	168.70	50 (S)	205.00
Giza 163	10 (S)	81.00	20 (S)	93.00
Giza 164	10 (S)	37.65	20 (S)	49.67
Giza 167	5 (S)	48.00	10 (S)	47.00
Giza 168	5 (MR)	21.32	10 (MR)	37.65
Sakha 8	30 (S)	208.00	40 (S)	248.32
Sakha 61	5 (S)	25.33	20 (S)	45.00
Sakha 93	10 (MR)	21.33	20 (MR)	25.00
Sakha 202	5 (S)	25.33	10 (S)	52.33
Gemmeiza-1	30 (MS)	81.68	40 (MS)	95.35
Gemmeiza-5	5 (S)	46.33	10 (S)	32.00
Gemmeiza-7	10 (S)	46.33	20 (S)	47.00
Gemmeiza-9	5 (MR)	22.30	10 (MR)	27.38
Little club (check)	70 (S)	303.33	80 (S)	400.30

DISCUSSION

The present study threw high lights on the components of slow rusting resistance against stem rust disease in 13 Egyptian wheat cultivars and L. Club in both seedling and adult stages. The tested cultivars were selected because they had a low severity of stem rust coupled with a compatible infection type.

The results revealed that cultivars such as Gemmeiza-9, Giza 168, Sakha 93 have the high level of slow rusting characters as they showed low numbers of pustules, erupted pustules, and the smallest colony and pustule sizes at the seedling stage. On contrary, Little Club, Giza 160 and Sakha 8 proved to be more fast rusters as they have the highs numbers of pustules and erupted pustules, the shorter LP and the greatest colony and pustule sizes. These results were supported by those of (Vander Plank, 1963; Hooker, 1967; Ohm and Shaner, 1976; El-Daoudi *et al*., 1985, Parlevliet, 1986; Simmonds and Rajaram, 1988 and Kapoor and Joshi, 1986), who confirmed that cultivars proved to have slow rusting resistance exhibited lower pustule density, small colony and/or pustule size, prolonged latent period and reduced spore production.

The previous cultivars were evaluated in field trials to confirm their slow rusting characters in the adult stage. The cultivars Gemmeiza-9, Giza 168 and Sakha 93 which showed the highest level of slow rusting resistance in seedling stage also had the lowest severity of rust and the smallest AUDPC at the adult stage during the two successive seasons. Also, the cultivars Little Club, Giza 160 and Sakha 8 which were fast rusters at the seedling stage exhibited high levels of rust severity and AUDPC at the adult stage. Similar results were reported by (Rowell, 1982; Khalifa, 1986) who confirmed the same conclusion.

The present data showed that some of good and bad component (character) of slow rusting could be presented in one cultivar in the same time for example Gemmeiza-5 had little number of the formed and erupted pustules and small AUDPC with race 15 but it had (IT 3) and large colony and pustule size. Also, Gemmeiza-7 exhibited moderate resistance type (IT 2) with race 15 and the lowest number of formed and erupted pustules, and small size of pustules but it exhibited a great colony and pustule size with race 11 and high disease severity by using a mixture of races. This phenomenon was explained by (Caldwell, 1966; Kochman and brown, 1975; Parlevliet, 1975) who pointed out that resistance of a non-hypersensitive type could interfere with disease development at any of several stages of pathogenesis and thus resulted in slow rusting. Exclusion of the fungus, longer LP, and restriction of pustule size all have been known to operate one degree or another in oats, barley and wheat toward their respective rust pathogens added that as slow rusting is a polygenic resistance, therefore, it may be thought that each component of slow rusting lied under control of different certain genes of resistance. It is not necessary that each slow rusting wheat cultivar contains all of a slow rusting resistance components.

In the present study, slow rusting resistance was more recognizable at field in the first season than in the second one, therefore, it could be suggested that slow rusting resistance could be influenced by environmental conditions, this suggestion confirmed results reported by (Ohm and Shaner, 1976; Kochman and brown, 1975) suggesting that slow rusting may be less effective against pathogenesis in some years at a location or at some locations than others.

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مكونات إبطاء الصدأ فى بعض الاقماح المصرية ضد مرض صدأ الساق

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لقد درست ظاهرة إبطاء الصدأ كميكانيكية للمقاومة فى ١٤ صنف من القمح ضد مرض صدأ الساق فى مرحلة البادرة تحت ظروف الصوبة الزجاجية ومرحلة النبات البالغ تحت ظروف الحقل .

وقد تمت العدوى باستخدام سلالتين فسيولوجيتين من الفطر بكسينيا جرامينس تريتساى وهما السلالة رقم ١١ ورقم ١٥ فى ظروف البادرة كما استخدم مخلوط من السلالات الفسيولوجية تحت ظروف الحقل فى طور النبات البالغ وتشمل مكونات إبطاء الصدأ عدد البثرات فى كل سم٢ من المساحة الورقية وعدد البثرات المنفجرة فى كل سم٢ وحجم البثرة وحجم المستعمرة قد تم تسجيلهما فى طور البادرة بينما تم تسجيل الشدة المرضية والمساحة الواقعة تحت منحنى المرض فى طور النبات البالغ .

وقد نتج عن رد فعل النبات فى طور البادرة تميز الأصناف جيزة ١٦٨ وسخا ٩٣ وجميزة ٩ وجميزة ٧ وسخا ٢٠٢ حيث تباينت هذه الأصناف معنوياً عن بقية الأصناف المختبرة فى اتجاه المقاومة وبالعكس أظهرت الأصناف Little Club و سخا ٨ وجيزة ١٦٠ رد فعل قابل للإصابة ودلت النتائج أيضا ان السلالة رقم ١١ من المسبب المرضى لها ضراوة أعلى من السلالة رقم ١٥ لكل من الأصناف المختبرة .

وكان اتجاه الاستجابة فى هذه الأصناف ضد المرض فى طور البلوغ مشابهة لسلوك الأصناف فى طور البادرة حيث أظهرت الأصناف جيزة ١٦٨ وجميزة ٩ وسخا ٩٣ أظهرت مقاومة متوسطة بينما الصنف جيزة ١٦٤ اظهر قابلية متوسطة ضد كل من السلالتين موضع الاختبار وقد اظهر الصنفين سخا ٦١ وسخا ٢٠٢ اقل إبطاء فى الصدأ حيث أنهما تمكنا من وقف الشدة المرضية عند مستوى (5S) وامتلكت ايضاً اقل مساحة تحت منحنى تطور المرض ومن هذا المنظور يمكن استنباط ان ظاهرة إبطاء الصدأ ترتبط بشدة بقلة عدد البثرات اليوريدية فى سم٢ وقلة عدد البثرات المنفجرة فى سم٢ وقلة حجم المستعمرة وحجم البثرات وطول مدة الحضانة او طول فترة التحضين فى طور البادرة وقلة الشدة المرضية وقلة المساحة المرضية تحت منحنى المرض فى طور النبات البالغ ويمكن لهذه النتائج ان تخدم كأداة فعالة فى حقل التربية للمقاومة لأمراض أصداء القمح فى مصر .

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Table 1. The evaluation of 14 local wheat cvs. in terms of components of partial resistance *i.e.* Infection type, number of pustule/cm2, latent period (in days), colony size and pustule size against stem rust *Puccinia graminis* f. sp. *tritici* (race 11 and race 15) at seedling stage.

Cultivars	Infection type		No. of Pustules/cm^2						Latent period in days		Colony size		Pustule Size	
			Race 11			Race 15								
	Race 11	Race 15	Total N.P.	Er. Pus.	Erupted P.%	Total N.P.	Er. Pus.	Erupted P.%	Race 11	Race 15	Race 11	Race 15	Race 11	Race 15
Gemmeiza-1	4	3	20.3^{b}	18.7^{c}	92.11	16.3^{e}	15.0^{e}	92.02	9^{e}	10^{e}	0.031^{h}	0.018^{g}	0.019^{c}	0.012^{e}
Gemmeiza-5	4	3	20.3^{b}	18.0^{b}	88.67	14.3^{b}	13.0^{d}	90.91	9^{e}	12^{c}	0.041^{k}	0.020^{h}	0.018^{bc}	0.016^{g}
Gemmeiza-7	3	2	25.0^{g}	19.0^{d}	76.00	13.8^{a}	12.0^{c}	86.95	12^{c}	12^{c}	0.033^{i}	0.016^{e}	0.028^{e}	0.004^{a}
Gemmeiza-9	3	2	21.8^{c}	19.8^{e}	90.82	15.6^{d}	11.0^{a}	70.51	14^{a}	14^{a}	0.017^{c}	0.016^{e}	0.027^{e}	0.008^{b}
Giza 160	4	4	28.^{0j}	26.7^{k}	95.35	20.0^{i}	19.0^{i}	94.6	7^{f}	8^{g}	0.038^{j}	0.035^{j}	0.020^{cd}	0.020^{h}
Giza 163	3	2	27.3^{i}	25.0^{i}	91.57	15.0^{c}	13.0^{de}	86.66	9^{e}	9^{f}	0.023^{g}	0.020^{h}	0.015^{a}	0.012^{e}
Giza 164	3	2	23.4^{f}	22.0^{g}	94.02	22.0^{l}	18.0^{h}	81.82	13^{b}	14^{a}	0.014^{a}	0.011^{a}	0.018^{bc}	0.010^{c}
Giza 167	3	3	23.3^{e}	22.0^{g}	94.42	19.3^{g}	18.0^{h}	93.26	9^{e}	9^{f}	0.020^{e}	0.017^{f}	0.016^{ab}	0.014^{f}
Giza 168	3	2	19.3^{a}	18.0^{a}	93.26	15.6^{d}	11.3^{b}	72.43	7^{f}	11^{d}	0.018^{d}	0.015^{d}	0.012^{a}	0.010^{c}
Sakha 8	4	4	30.2^{l}	29.0^{l}	96.03	20.3^{j}	19.0^{i}	93.59	7^{f}	8^{g}	0.022^{f}	0.020^{h}	0.020^{cd}	0.016^{g}
Sakha 61	4	3	28.3^{k}	25.7^{j}	90.81	18.3^{f}	15.0^{f}	81.97	9^{e}	10^{e}	0.023^{g}	0.021^{i}	0.022^{d}	0.016^{g}
Sakha 93	4	3	22.9^{d}	21.3^{f}	93.01	20.0^{h}	15.7^{f}	71.36	10^{d}	14^{a}	0.015^{b}	0.013^{c}	0.014^{a}	0.012^{e}
Sakha 202	4	3	25.3^{h}	23.0^{h}	90.90	18.3^{f}	16.0^{g}	87.43	9^{e}	13^{b}	0.014^{a}	0.012^{b}	0.012^{a}	0.011^{d}
Little Club	4	4	32.2^{m}	31.0^{m}	96.27	21.0^{k}	19.7^{j}	93.81	7^{f}	7^{h}	0.073^{l}	0.070^{k}	0.032^{f}	0.030^{i}

N. P. = Number of pustules/cm^2 of leaf area, Er. Pus. = Erupted pustules.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.