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RESISTANCE TO FUSARIUM WILT DISEASE IN SOME EXPERIMENTAL GENOTYPES AND COMMERCIAL CULTIVARS OF COTTON

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ABSTRACT

This study aimed to evaluate some cotton germplasm as a part of the Screening Program of Fusarium Wilt Resistance. This program has been conducted annually in the greenhouses of Cotton and Fiber Crops Diseases Research Section, Plant Pathology Research Institute. The present study included 200 families of ten experimental genotypes and 933 families of seven commercial cultivars. Healthy survival rate of families was used as a criterion to evaluate the reactions of the tested germplasm to Fusarium wilt. The experimental genotypes showed a very narrow range of reactions to Fusarium wilt with healthy survival rate of families this narrow range, ranging from 94.73 to 99.48%. Thus, all the tested genotypes were classified as very highly resistant (VHR).However, within significant differences were observed among some of the tested genotypes. Most of the families of the tested commercial cultivars were also classified as VHR.The percentages of families in this reaction class ranged from 57.82 (families of Giza 87) to 100% (families of Giza 88).The commercial cultivars were classified into three distinct groups based on the dissimilarity distances(DDs)generated from cluster analysis of the profiles of their reaction classes. Within each group, the cultivars were almost identical in the patterns of their reaction classes.

Keywords: Cotton, Fusarium wilt, resistance.

INTRODUCTION

Fusarium wilt of cotton is a serious fungal disease responsible for significant losses in yield and quality in the major cotton- producing areas in the world. *Fusarium oxysporum Schlecht.*f.sp. *vasinfectum*(ATK)Snyd and Hans (FOV) is the causal pathogen of this disease. FOV penetrate taproots of cotton behind the root tip. Wilt symptoms that appear on the infected plants are due to the occlusion of the xylem vessels. This occlusion is the consequence of the combined effects of fungal metabolites and the production of lipodial compounds by host in response to infection (Hillocks,1984).FOV has a very good survival ability in soil and hence it is very difficult to eradicate it from infested fields. Cotton is susceptible to FOV at all stages of growth showing symptoms, which include seedling death, wilting, vascular discoloration and finally plant death (Watkins,1981).Symptoms at seedling stage cane be confused with symptoms of damping- off caused by *Pythium* spp., *Rhizoctonia solani* ,and *Fusarium* spp, but the brown vascular system of the hypocotyls distinguishes Fusarium wilt from seedling disease. The death of seedlings results in uneven stands, which further contribute to production problems throughout the season. Symptoms of older plants include stunting, wilting, chlorosis and necrosis if leaves, dieback often beginning at the top of the plant, and plant death (Watkins,1981).

Outside Egypt, it is commonly associated with nematode infection [root knot- Fusarium wilt complex(McFadden *et al*., 2004)], particularly in acidic, sandy soils. In Egypt, where cotton is grown in alkaline clay soils,there is no evidence for the involvement of nematodes in Fusarium wilt disease.

To date, eight physiologic races of FOV are identified based on their pathogenicity on a differential set of cotton(*Gossypium*) lines and species and up to five non- cotton hosts.these races are geographically isolated because they are found widely separated cotton-growing areas (Watkins,1981).Thus,Races 1 and 2 were mainly described in the United States and Tanzania. The Egyptian race (race 3) of FOV. has long been known in the Nile Valley, where it remains one of the most dangerous pathogen on Egyptian cottons(*G.brbadense* L.)This race also attacks *G.brbadense* in the former Soviet Union(Watkins,1981). And Israel (Netzer *et al* 1985) and was also described in Sudan and China(Davis *et al*., 2006)Race 4 was first described in India but, at present, it was thought to be the most virulent race of FOV in California. Races 5 was initially described in Sudan and then withdrawn because its culture characteristics and virulence were identical to race3. Race6 was described in Brazil and Paraguay. Race 7 and 8 were mainly described in China(Davis *et al*., 2006).

FOV caused heavy losses in the commercial Egyptian. cottons(*G.brbadense* L.) in the late fifties (Barky *et al*.,1958).Since then, an extensive cotton – breeding program was initiated to develop cultivars resistant to the disease. In this program, breeding materials submitted by cotton breeders (Cotton Research Institute)have been screened for Fusarium wilt resistance under greenhouse conditions in soil artificially infested with FOV.This test has been conducted annually for the past 60 years in the greenhouses of Cotton and Fiber Crops Diseases Research Section, Plant Pathology Research Institute. The program has been so successful in developing highly resistant cultivars that the disease no longer occurs in the commercial fields (Aly *et al* 2000).

Fusarium wilt remains a potential threat to cotton production in Egypt because FOV is endemic in the Egyptian soil (Aly *et al*, 2000)Thus, increasing the probability that new races other than race 3 or new biotypes of this race may arise to confound cotton breeders. Therefore, the objective of the present study was to evaluate a collection of cotton germplasm against Fusarium wilt race 3 under greenhouse conditions. The tested germplasm included families of experimental genotypes and commercial cultivars.

MATERIALS AND METHODS

Cotton germplasm

The germplasm evaluated in this test was a piece of the Screening Program of Fusarium Wilt Resistance. This program has been conducted annually in the greenhouses of Cotton and Fiber Crops Diseases Research Section,Plant Pathology Research Institute. The present test included 200 families of ten experimental genotypes (Table 1) supplied by Cotton Breeding Section, Cotton Research Institute. and 933 families of seven commercial cultivars(Table 5) supplied by Cotton Maintenance Section, Cotton Research Institute.

Table 1. Experimental Genotypes supplied by Cotton Breeding Section, Cotton Research institute.

Genotype[a]number	Pedigree
G1	C10229 X Giza86/1
G2	C10229 X Giza86/2
G3	[(Giza89X Karashinky) X Giza86] X Giza94
G4	Giza89XPima (S6)X{(Bahteem105XGiza67)X(Giza72XDelcero)}X(Giza86XGiza89)
G5	Giza85 X Giza89X Giza86
G6	Giza84 X Giza70 X Giza51 XS 62
G7	Giza 93/1
G8	Giza 93/2
G9	Giza84 X Giza70 X Giza51 XS62
G10	C7 X Giza 92 X Pima S1

[a] **Each genotype was represented by 20 families.**

Evaluation of cotton germplasm against Fusarium wilt race 3 under greenhouse conditions.

The fungal inoculum used in the greenhouse test was a mixture of equal parts (w/w) of 50 isolates of FOV race 3. These isolates were obtained from almost all cotton- producing area in Egypt. Autoclaved clay loam soil was infested with the mixture of the isolates at a rate of 10g/kg soil. Substrate for growth of each isolates was prepared according to Aly *et al*.(2000). Infested soil was dispensed in 10-cm diameter clay pots , which were planted with 10 seeds per pot. There were three replications (pots) for each genotype. The greenhouse was equipped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28^{0}c;however,due to the lack of cooling system, the maximum temperature was out of control changeable from 30 to 35 ^{0}c depending on the current temperature during the day (the test was conducted in January and February, 2015).

Assessment of Fusarium wilt incidence

Percentages of infected seedlings were recorded 45 days from planting date. The infected seedlings included the dead seedlings and the surviving seedling, which showed external or internal symptoms. Thus, the seedlings of each genotype were placed in two distinct classes: healthy if they were free of any external or internal symptoms,or infected if the seedlings died or survived showing any external or internal symptoms (Aly *et al*.,2007 and Abd-Elsalam *et* al.,2009).

Statistical analysis of the data.

The design of the greenhouse test was a completely random design with three replications. Data were subjected to analysis of variance. Least significant difference (LSD) was calculated to compare between genotype means. Linear correlation coefficients were calculated to measure the degree of association among wilt symptoms on the genotypes. Commercial cultivars were clustered by the average related technique (unweighted pair- group method) based on the profiles of their reaction classes. Statistical analysis was performed with the software package SPSS6.0.

RESULTS

Families within each of the tested genotypes showed variable symptom expressions due to the fact that many of these genotypes were not pure lines. However, the majority of the families of each genotype were completely free of any internal or external symptoms (healthy survival) (Table2).

Table 2. Symptoms used in evaluating the reactions of cotton genotypes to Fusarium wilt disease .

Genotype numbe	Symptoms(%)			
	DS[a]	CY	VD	HS
G1	0.00[b]	1.39	0.00	98.61
G2	0.00	2.05	0.00	97.95
G3	2.03	1.01	0.50	96.12
G4	2.71	1.53	0.67	95.08
G5	2.07	1.87	0.85	94.47
G6	0.00	2.53	0.00	97.47
G7	0.00	0.52	0.00	99.48
G8	0.00	2.58	0.00	97.42
G9	0.33	0.33	0.33	99.00
G10	2.03	0.67	0.51	96.79

[a]DS= Deed seedlings, CY = Cotyledonary yellowing, VD = Vascular discoloration, and HS =Healthy survival.[b]Mean of 20 replicates(families).

The highest correlations among symptom expressions were observed between vascular discoloration and each of dead seedlings (r=o.961, P=0.000) and healthy survival (r=0.793,p=0.000)(Table 3).

Table3.Correlation coefficients between symtoms of fusarium wilt disease .

Symptoms	Symptoms		
	X1	X2	X3
Deed seedlings(X1)			
Cotyledonary yellowing(X2)	-0.338[a](0.339)[b]		
Vascular discoloration(X3)	0.961(0.000)	-0.218(0.545)	
Healthy survival(X4)	-0.643(0.045)	-0.285(0.475)	0.793(0.006)

[a]Linear correlation coefficient. [b] Prpbabilit level and n=10

Healthy survival rate was used as a criterion to evaluate the reactions of the tested genotypes to Fusarium wilt. The tested genotypes showed a very narrow range of reactions to Fusarium wilt with healthy survival rates ranging from 94.73 to 99.48%. Thus, all the tested genotypes were classified as very highly resistant (VHR). However, within this narrow range, significant differences were abserved among some of the tested genotypes (Table4).

Table4.Reactions of some experimental cotton genotypes to fusarium wilt disease

Genotypes no.	Healthy Seedling [a]	Reaction class [a]
G1	98.61[c]	VHR
G2	97.95	VHR
G3	96.12	VHR
G4	95.08	VHR
G5	94.73	VHR
G6	97.47	VHR
G7	99.48	VHR
G8	97.42	VHR
G9	99.00	VHR
G10	96.79	VHR
LSD ($P \leq 0.05$)	2.32	

[a] **Seedlings completely free from any external or internal symptoms.**
[b] **Reaction class based on the percentage of healthy seedlings according to the following scale:**

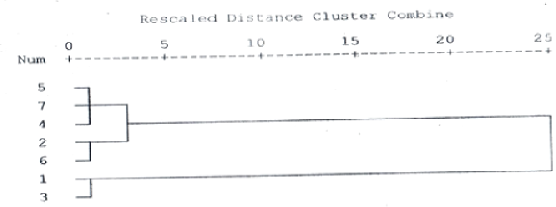
Most of the Families of the tested commercial cultivars were classified as VHR. The percentage of families in this reaction class ranged from 57.82 (Families of Giza87) to 100%(Families of Giza88)(Table5).

Table 5. frequencies (%) Commercial cotton cultivars supplied by Cotton were tested against Fusarium wilt disease .

Cultivar	Total No. of		Reaction	Class(%)[a]			
	tested families	VHR	HR	R	S	HS	VHS
Giza 90	161	62.12	37.88	0.00	0.00	0.00	0.00
Giza 80	160	85.63	14.37	0.00	0.00	0.00	0.00
Giza 87	147	57.82	42.18	0.00	0.00	0.00	0.00
Giza 92	118	93.23	6.77	0.00	0.00	0.00	0.00
Giza 86	156	96.79	3.21	0.00	0.00	0.00	0.00
Giza 45	73	80.82	19.18	0.00	0.00	0.00	0.00
Giza 88	118	100.00	0.00	0.00	0.00	0.00	0.00

[a] **Reaction class was determined based on the percentage of healthy seedlings according to the following scale:**
Very highly susceptible (VHS) = 0-10, Highly susceptible (HS)= 11 – 31,Susceptible (S) = 31- 50
Resistant (R) = 51- 70, Highly resistant (HR)=71- 90,Very highly resistant (VHR)= 91 – 100.

A phenogram based on dissimilarity distance (DD)generated from cluster analysis of the profiles of reaction classes of the tested cultivars is presented in Fig.1. The smaller the DD,the more closely the cultivars were related in the profiles of their reaction classes. Three groups of cotton cultivars were identified by cluster analysis. The first group included cultivars Giza 86, Giza 88,and Giza 92. The second group included cultivars Giza 80 and Giza 45. The third group, which was unrelated to the first two groups included Giza 90 and Giza 87. Within each group, the cultivars were almost identical in the patterns of the profiles of their reaction classes



Fige1. Phenogram based on average linkage cluster analysis of reaction classes profilesof seven commercial cotton cultivars when they were inoculated with isolates of FOV races3.The tested cultivars were Giza90(1), Giza80(2), Giza87(3), Giza92(4), Giza86(5), Giza45(6) and Giza86(7)

DISCUSSION

In the present study, genotypes were screened against 50 FOV isolates from almost all cotton- growing areas in Egypt. The use of such a large number of isolates is a strategy to maximize the probability that resistant genotypes identified under greenhouse conditions will maintain their resistance levels under field conditions in distinct geographic locations. On the contrary, if genotypes were screened against a limited number of isolates, they may not perform as expected due to potential presence of isolates differing in their virulence profile from those used in the greenhouse test.

A distinctive characteristic of Fusarium wilt is the olive brown discoloration of the root and stem xylem. Some cotton pathologists believe that vascular discoloration is a questionable standard for judging susceptibility to wilt in seedling tests (Armstrong and Armstrong,1978 and Zink *et al*., 1983) On the other hand, some other workers used vascular discoloration as a criterion for judging susceptibility of seedlings to Fusarium wilt(Salgado *et al*., 1994 and Osman,1996). In the present study, we used more rigorous criteria for disease rating. According to these criteria, the seedlings were considered slightly susceptible if they showed internal discoloration even though they were free of external symptoms. Thus, the seedlings were considered resistant only if they were completely free of any internal and external symptoms. In our study, cotton genotypes were screened under very favorable conditions for FOV development. The soil was sterile, temperature was optimal most of the time, and the inoculums density was relatively high. Under these conditions, it is unlikely that any susceptible genotypes would have escaped from infection. However, one should keep in mind that evaluation in the greenhouse precludes identifying genotypes that may possess useful levels of field resistance to wilt. The soil infestation method, which we used for seedling inoculation, had several advantages. Assays were simple, did not damage the seedlings and provided discriminating and reproducible disease reactions. In many cotton-producing countries, cotton genotypes are screened for Fusarium wilt resistance in experimental field plots heavily infested with highly pathogenic (hot) isolates of FOV.These plots are called wilt nurseries. Since no cotton wilt nurseries have been established in Egypt, greenhouse tests will continue to be the only reliable method for screening cotton breeding materials for Fusarium wilt resistance. The current absence of Fusarium wilt in commercial cotton field using cultivars derived from our breeding program demonstrates the reliability of these screening procedures, which we have adopted in testing cotton genotypes for Fusarium -wilt resistance. The most successful strategy to manage Fusarium wilt is the use of resistant cultivars(Doan and Davis,2014). Therefore the present work provides new and useful sources of resistance that might be employed in breeding programs aiming to develop cotton cultivars with resistance to FOV race 3 isolates.

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مقاومة مرض ذبول الفيوزاريوم في بعض التراكيب الوراثية التجريبية وأصناف القطن التجارية

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يهدف الاختبار الحالي إلي تقييم مجموعة من الأصول الوراثية للقطن من حيث المقاومة أو القابلية للاصابة بمرض ذبول الفيوزاريوم . الاختبار الحالي هو جزء من أختبار أكبر يجري سنويا في صوب قسم بحوث أمراض القطن , معهد بحوث أمراض النباتات. إشتمل الاختبار الحالي علي نوعين من الأصول الوراثية تمثلت في ٢٠٠ عائلة تتبع عشر تراكيب وراثية تجريبية و ٩٣٣ عائلة تتبع سيعة أصناف تجارية . إستعمات النسب المئوية للعائلات السليمة الباقية علي قيد الحياة كمعيار للمقارنة بين الاصول الوراثية من حيث المقاومة أو القابلية للاصابة بالمرض . أظهرت التراكيب الوراثية نسب مرتفعة من العائلات السليمة تراوحت من ٩٤.٧٣ إلي ٩٩.٤٨% وعلي ذلك فقد صنفت هذة التراكيب الوراثية علي أنها ذات مقاومة عالية جدا للمرض. بالرغم من هذا المدي الضيق للتباين في نسب العائلات السليمة, إلا ان ذلك لم يمنع من إمكانية ملاحظة فروق معنوية بين بعض التراكيب الوراثية, أغلب عائلات الاصناف التجارية أظهرت أيضا درجة عالية جدا من المقاومة للمرض.هذا وقد تراوحت نسب هذة العائلات المقاومة جدا للمرض من ٥٧.٨٢% في صنف جيزة ٨٧ إلي ١٠٠% في صنف جيزة ٨٨. أمكن - باستعمال التحليل العنقودي - تقسيم الأصناف التجارية إلي ثلاث مجموعات محددة, بناء علي ما بينها من تباين في أنماط المقاومة أو القابلية للاصابة بالمرض. أظهرت الاصناف داخل كل مجموعة , درجة عالية من التماثل, الذي كاد أن يكون تاما, من حيث أنماط المقاومة أو القابلية للإ صابة بالمرض.