SCREENING A COLLECTION OF COTTON GENOTYPES FOR FUSARIUM WILT RESISTANCE UNDER GREENHOUSE CONDITIONS IN 2017

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Abstract

The genotypes evaluated in this study was a part of the National Screening Program for Fusarium Wilt Resistance. This program is conducted annually in the greenhouses of Cotton and Fiber Crops Diseases Research Dept., PPRI. The present study included 50 of Upper Egypt experimental genotypes and 875 families of six commercial cultivars. The experimental genotypes were supplied by Cotton Breeding Dept., and the six commercial cultivars were provided by Cotton Maintenance Dept., Cotton Research Institute. Healthy survival rate of seedlings of the genotypes was used as a criterion to evaluate the reactions when the genotypes were grown in autoclaved highly infested soil with a mixture of race 3 of *Fusarium oxysporum* f.sp.*vasinfectum* isolates. The majority of the tested genotypes (48%) were very highly resistant while 22% were very highly susceptible. All the families of the commercial cultivars Giza 90, Giza 95, Giza 86, Giza 87, and Giza 88 were very highly resistant while only 99.30% of Giza 92 families showed this level of resistance, which deserves more attention in monitoring its families in the future to Avoid any varietal deterioration. The present work provides new and useful sources of resistance to FOV race 3 isolates.

INTRODUCTION

Fusarium wilt of cotton is a serious fungal disease responsible for significant losses throughout the world.The causal organism *Fusarium oxysporum Schlecht*.f.sp*. vasinfectum* (ATK) Snyd and Hans (FOV), invades the host through the taproots behind the root tip. The combined effect of fungal metabolites and the production of lipodial substances by the host in response to infection may lead to occlusion of the vascular tissue, resulting in wilt of the cotton plant (Hillocks,1984). The pathogen can survive for several decades in soil and cannot be eradicated from infested fields. The pathogen can infect cotton at all stages of growth and produces symptoms, which include seedling death, wilting, vascular discoloration and plant death (Watkins,1981). Apart from, 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 (Aly *et al*., 2000).

Currently, up to eight races of (FOV), most of which are geographically separated, are recognized worldwide. The basis for determining races of FOV depends on their virulence to a differential set of cotton(*Gossypium*) lines and species and up to 5 non- cotton hosts (Watkins,1981).

The Egyptian race (race 3) of FOV has long been known in the Nile Valley, where it remains one of the most damaging pathogen on Egyptian cottons (*Gossypium brbadense* L.). This race also attacks *G.brbadense* in the former Soviet Union(Watkins,1981). and Israel (Netzer *et al*, 1985).

FOV caused serious losses in the commercial Egyptian. Cottons (*G.brbadense* L.) in the late fifties (Barky *et al.*,1958). Since then, extensive cotton breeding programs were conducted to develop cultivars resistant to the disease.

Breeding materials submitted by cotton breeders (Cotton Research Institute) have been screened for resistance under greenhouse conditions in soil highly infested with FOV. This test has been conducted annually for the past 65 years in the greenhouses of Cotton and Fiber Crops Diseases Research Dept, PPRI, ARC. The program has been successful enough in developing highly resistant cultivars. (Aly *et al*, 2000).

Fusarium wilt remains a potential threat to cotton production in Egypt because FOV is still well established in the Egyptain 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.

The objective of the present study was to evaluate a collection of cotton germplasm against Fusarium wilt race 3 under greenhouse conditions by using families of experimental genotypes and commercial cultivars.

MATERIALS AND METHODS

Cotton germplasm

The germplasm used in this study included 50 of Upper Egypt experimental genotypes (Table 1) were supplied by Cotton Breeding Section, as well as 875 families of six commercial cultivars (Table 4) were supplied by Cotton Maintenance Section, Cotton Research Institute.

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

This study was conducted in the greenhouses of Cotton and Fiber Crops Diseases Research Dept, PPRI through 2017.The experimental design used in this study was a completely randomized with three replications (pots) for each genotype.

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 the fungal collection

of Cotton Pathology Dept, PPRI, Giza. Autoclaved clay loam soil was infested with a mixture of the isolates at a rate of 10g/kg soil, prepared in 500-ml glass bottles, each bottle contained 50g of sorghum grains and 40 ml of tap water. The inocula made from one- week old culture on PDA, were allowed to colonize sorghum for 3 weeks. Infested soil was dispensed in 10-cm diameter clay pots, which were planted with 10 seeds per pot..The greenhouse was equipped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28^0c;however,due to the lack of cooling system, the maximum temperature was out of control fluctuating from 30 to 35 ^{0}c depending on the prevailing temperature during the day (the test was conducted in January and February, 2017).

Assessment of Fusarium wilt incidence

Percentages of infected seedlings were recorded 45 days from planting date. The infected seedlings included the dead and the surviving seedling, which showed external or internal symptoms. The external symptoms usually began at the margin of cotyledons as yellowing along the veins (vein clearing), eventually, the entire cotyledonds turned yellow and dropped from the seedlings. Seedlings that remained apparently healthy 45 days after planting were cut diagonally across the root and stem to examine the internal symptoms. If discoloration of xylem vessels was observed, they were considered infected. If seedlings were free of such a discoloration, they were considered healthy. 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).

RESULTS

Seedlings within each of the tested genotypes showed variable symptoms expression (Table 1) due to the fact that many of these genotypes were not pure lines.

Healthy survival rate was used as a criterion to evaluate the reactions of the tested genotypes to Fusarium wilt (Table 2). The majority of the tested genotypes (48 %) were VHR while 22 % were VHS. The other reaction classes ranged from 2 to 16 % of the tested genotypes (Table 3).

All the families of the commercial cultivars Giza 90, Giza 95, Giza 86, Giza 87, and Giza 88 were VHR while only 99.30 % of Giza 92 families showed this high leve of resistance (Table 4).

Table 1. Symptoms of Fusarium wilt disease of a random collection of Upper Egypt Cotton genotypes when they tested under greenhouse conditions in 2017.

	Symptoms[a] (%)		
Gnoetype[b] no.	**VD**	**CY**	**DS**
1	4.54	0.00	95.46
8	17.39	0.00	73.91
18	9.52	0.00	90.47
29	4.17	0.00	95.80
30	0.00	0.00	100.00
42	0.00	0.00	100.00
50	0.00	11.53	57.69
58	10.53	0.00	89.47
59	0.00	0.00	100.00
66	0.00	0.00	100.00
77	0.00	0.00	0.00
86	0.00	0.00	0.00
88	0.00	0.00	0.00
97	0.00	3.84	0.00
105	0.00	0.00	10.00
114	0.00	0.00	0.00
117	0.00	0.00	0.00
129	0.00	0.00	0.00
136	0.00	0.00	0.00
142	0.00	0.00	0.00
151	0.00	0.00	0.00
163	0.00	0.00	0.00
170	0.00	4.00	0.00
176	0.00	0.00	0.00
179	0.00	0.00	0.00
187	0.00	0.00	0.00
196	0.00	0.00	0.00
206	0.00	0.00	0.00
211	7.69	0.00	0.00
219	0.00	0.00	0.00
229	0.00	0.00	0.00
239	0.00	0.00	0.00
240	0.00	0.00	0.00
247	0.00	0.00	0.00
252	0.00	0.00	0.00
260	0.00	20.00	30.00
270	0.00	52.94	11.76
271	0.00	57.89	31.50
281	0.00	27.27	9.09
285	10.52	42.10	15.78

Table 1. cont.

Gnoetype[b] no.	Symptoms[a] (%)		
	VD	CY	DS
289	0.00	18.18	31.81
298	0.00	0.00	53.33
300	3.84	0.00	42.30
309	0.00	60.00	0.00
313	0.00	0.00	100.00
321	4.76	0.00	80.90
328	0.00	0.00	100.00
329	0.00	0.00	83.35
334	0.00	15.78	42.1
341	5.88	0.00	76.47

Symptoms[a] were Vascular discoloration (VD), Cotyledonary yellowing (CY) and Dead seedlings (DS).

[b]Each genotype was represented by 20 to 25 seedlings.

Table 2. Reaction classes of a random collection of Upper Egypt Cotton genotypes to Fusarium wilt disease based on the percentage of healthy survival(HS)

Gnoetype no.	HS (%)	Reaction class[a]
1	0.00	VHS
8	8.69	VHS
18	0.00	VHS
29	0.00	VHS
30	0.00	VHS
42	0.00	VHS
50	30.77	S
58	0.00	VHS
59	0.00	VHS
66	0.00	VHS
77	100.00	VHR
86	100.00	VHR
88	100.00	VHR
97	96.16	VHR
105	90.00	HR
114	100.00	VHR
117	100.00	VHR
129	100.00	VHR
136	100.00	VHR
142	100.00	VHR
151	100.00	VHR
163	100.00	VHR
170	96.00	VHR
176	100.00	VHR
179	100.00	VHR
187	100.00	VHR
196	100.00	VHR
206	100.00	VHR
211	92.31	VHR
219	100.00	VHR
229	100.00	VHR
239	100.00	VHR
240	100.00	VHR
247	100.00	VHR
252	100.00	VHR
260	50.00	S
270	35.29	S
271	10.60	HS
281	63.63	R
285	31.57	S

Table 2. cont.

Gnoetype no.	HS (%)	Reaction class[a]
289	50.00	S
298	46.66	S
300	53.84	R
309	40.00	S
313	0.00	VHS
321	14.34	HS
328	0.00	VHS
329	17.65	HS
334	42.11	S
341	17.64	HS

[a]Reaction class was determined based on the percentage of healthy survival (HS) according to the following scale:-
Very highly susceptible (VHS) = 0-10
highly susceptible (HS)= 11-30
Susceptible (S)= 31- 50
Resistant (R) = 51-70
Highly resistant (HR) = 71- 90
Very Highly resistant (V H R) = 91 – 100

Table 3. Distribution of a random collection of Upper Egypt cotton genotypes based on their reaction classes to Fusarium wilt disease.

	Genotypes	
Reaction class[a]	No	%
VHS	11	22
HS	4	8
S	8	16
R	2	4
HR	1	2
VHR	24	48
Total	50	100

[a]Reaction classes were determined based on the based on the percentage of healthy survival according to the scale shown in Table2.

Table 4. Distribution of commercial cotton cultivars supplied by Cotton Maintenace Section , Cotton Research Institute based on their reaction classes to Fusarium wilt disease.

Cultivar	Total no. of tested families	Reaction class[a] (%)					
		VHS	HS	S	R	HR	VHR
Giza 90	155	0.00	0.00	0.00	0.00	0.00	100.00
Giza 92	138	0.00	0.00	0.00	0.00	0.70	99.30
Giza 95	151	0.00	0.00	0.00	0.00	0.00	100.00
Giza 86	156	0.00	0.00	0.00	0.00	0.00	100.00
Giza 87	123	0.00	0.00	0.00	0.00	0.00	100.00
Giza 88	152	0.00	0.00	0.00	0.00	0.00	100.00

[a]Reaction classes were determined based on the percentage of healthy survival according to the scale showen in Table 2.

DISCUSSION

In the present study, genotypes were screened against 50 FOV isolates collected 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. However, there is no consensus regarding the diagnostic importance of this vascular discoloration for evaluation of the host germplasm reaction to Fusarium wilt. For example, Armstrong and Armstrong (1978) stated that vascular discoloration is a questionable standard for judging susceptibility to wilt in seedling tests. Zink *et al*. (1983) found no clear relationship between the severity of external symptoms in surviving muskmelon seedlings and the extent and degree of internal vascular discoloration. On the other hand, salgado *et al*. (1994) used vascular discoloration as a criterion for judging susceptibility of tepary bean (*phaseoulus acutifolius* Gray) seedlings to Fusarium wilt. Osman (1996) found a highly significant correlation ($r=0.98, p \leq 0.01$) between external wilt symptoms and vascular discoloration of cotton seedlings (cultivar Giza74). 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 inoculum 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. 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 testing 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. However, one should keep in mind that Giza 92 families deserve more any varietal detewrioration.

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تقييم مجموعة من الأصول الوراثية للقطن من حيث المقاومة لمرض ذبول الفيوزاريوم تحت ظروف الصوبة خلال عام ٢٠١٧

أمل عبد المنجي عسران ، محمود توفيق محمود منصور، محمد سيد خليل ، عبد الودود زكي عبد الله عاشور شوقي محمد المتولي زايد، ماريان منير حبيب و علي عبد الهادي علي

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التراكيب الوراثية التي قيمت في الدراسة الحالية هي جزء من البرنامج القومي لتقييم الأصول الوراثية للقطن من حيث المقاومة لمرض الفيوزاريوم.هذا البرنامج ينفذ سنويا في صوب قسم بحوث أمراض القطن،معهد بحوث أمراض النباتات ، إشتملت الدراسة الحالية علي نوعين من الأصول الوراثية هي ٥٠ تركيب وراثي تجريبي مخصصة للزراعة في الوجة القبلي ٨٧٥ عائلة تتبع ستة اصناف تجارية. التراكيب الوراثية التجريبية أمكن الحصول عليها من قسم بحوث تربية القطن، معهد بحوث القطن،أما عائلات الاصناف التجارية فقد أمكن الحصول عليها من قسم بحوث المحافظة علي أصناف القطن، معهد بحوث القطن. إستعملت النسب المئوية للبادرات السليمة الباقية علي قيد الحياة كمعيار لتقييم الأصول الوراثية من حيث المقاومة أو القابلية للاصابة بالمرض،وذلك عند زراعتها في تربة معقمة ملوثة بتركيز مرتفع لخليط عزلات فطر الذبول التابعة للسلالة ٣.أغلب التراكيب الوراثية (٤٨%) أظهرت درجة عالية جدا من المقاومة للمرض، في حين أظهرت ٢٢% من التراكيب الوراثية درجة عالية جدا من القابلية للاصابة بالمرض. جميع عائلات الاصناف التجارية جيزة ٩٠و جيزة٩٥ و جيزة٨٦ و جيزة٨٧ و جيزة٨٨ أظهرت درجة عالية جدا من المقاومة للمرض، في حين أظهرت ٣٠و٩٩ % فقط من عائلات جيزة ٩٢ هذا المستوي المرتفع من المقاومة. مما يستلزم المتابعه الدقيقة لعائلات هذا الصنف . تتيح الدراسة الحالية مجموعة من التراكيب الوراثية المفيدة في مقاومة مرض ذبول الفيوزاريوم الناجم عن الاصابة بعزلات السلالة رقم ٣ من فطر الذبول.