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RESEARCH ARTICLE

Evaluation of a cotton germplasm collection against Fusarium wilt race 3 isolates from Egypt

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ABSTRACT

Pathogenic variation of 30 isolates of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 was evaluated on seedlings of the highly susceptible cotton cultivar 'Giza 74' in greenhouse assays. FOV isolates were clustered based upon their virulence patterns. The clusters of FOV isolates were not related to their geographic origins. Fifty-five experimental cotton genotypes were evaluated under greenhouse conditions for resistance to Fusarium wilt, in autoclaved clay loam soil infested with a mixture of equal parts (w/w) of 30 isolates of FOV race 3 at a rate of 10 g/kg of soil. Eleven genotypes were rated as highly susceptible (survival frequency from zero to 24.7%) and 21 genotypes were rated as highly resistance (survival frequency of 79.6 to 100%). The remaining genotypes displayed variable levels of partial resistance. Since no wilt nursery has been established in Egypt, greenhouse tests will continue to be the only reliable method for screening cotton breeding materials for FOV resistance. The current absence of Fusarium wilt in commercial cotton fields in Egypt demonstrates the reliability of the adopted screening procedures in discriminating cotton genotypes for resistance to Fusarium wilt.
Key words: *Fusarium oxysporum* f. sp. *vasinfectum*, *Gossypium barbadense*, *Gossypium* spp., germplasm screening.

INTRODUCTION

Fusarium wilt is a destructive disease of cotton (*Gossypium* spp.) in many countries of the world including Australia, USA, Egypt, Tanzania, and China (Feng et al., 2000). The disease is caused by the soil inhabiting fungus *Fusarium oxysporum* f. sp. *vasinfectum* (G.F. Atk.) W.C. Snyder & H.N. Hansen (FOV) (teleomorph: *Neocosmospora vasinfecta*), which causes vascular wilt in susceptible cotton (Watkins, 1981; Chen et al., 1985; Hillocks, 1992; Davis et al., 1996) and okra cultivars (Aguiar et al., 2013). Fusarium wilt of cotton was first observed by Atkinson (1892) in USA. The first report of this disease outside the USA was done in Egypt, where it was rapidly disseminated after the release of the susceptible cultivar 'Sakal' during the 1920s (Fahmy, 1927).

Currently, up to eight races of FOV are recognized worldwide with most of them being geographically isolated (Abd-Elsalam et al., 2004; Abo et al., 2005). The determination of FOV races depends upon the analysis of their virulence profile in a set of differential cotton lines/species and up to five non-cotton hosts (Davis et al., 1996). In Egypt, the occurrence of FOV race 3 has been documented in the Nile Valley, where it remains one of the most damaging pathogens on *G. barbadense* cultivars (Watkins, 1981; Abd-Elsalam et al., 2004). To date, race 3 is the only one found in Egypt. This race was also reported attacking *G. barbadense* in the former Soviet Union (Watkins, 1981).

Techniques for screening cotton germplasm resistance to FOV need to be rigorous because even plants with mild foliar symptoms can be colonized by the pathogen and do exhibit stem discoloration (Davis et al., 2006). The root-cut dip method of inoculation is useful for screening wilt resistance in cotton and has been widely employed (Kim et al., 2005). However, this method may occasionally damage the plants and result in severe wilt even in resistant genotypes (Hillocks, 1992). Other inoculation methods, such as the stem puncture, have been also employed, but they give less discriminating wilt reactions (Ibrahim & Nirenberg, 1993; 2000)

FOV was responsible for serious yield losses in commercial Egyptian cotton (*G. barbadense*) in the late 1950s. Since then, an extensive cotton-breeding program was initiated to develop cultivars highly resistant to the disease. In this program, breeding materials supplied by cotton breeders (Cotton Research Institute) have been screened for Fusarium-wilt resistance under greenhouse conditions using soil infested with FOV isolates. This test has been conducted for the past 50 years at the Cotton and Fiber Crop Diseases Research Section (Plant Pathology Research Institute), Agricultural Research Center, Giza, Egypt. This breeding program has been so successful in developing highly resistant commercial cultivars that the disease no longer occurs at epidemic levels in the major cotton-producing areas of the country. However, Fusarium wilt remains a potential threat to cotton

production because FOV isolates are well established in Egypt as indicated by the severe disease symptoms whenever susceptible cultivars are used (Abd-Elsalam et al., 2009; Aly et al., 2000). In addition, either new races (other than race 3) or new biotypes within the race 3 may arise after continuous growing of resistant cotton cultivars over the years.

In this context, the objective of the present work was to provide information on the variability of FOV race 3 isolates as well as about the efficiency of the methodology currently used in Egypt to screen cotton germplasm for Fusarium wilt resistance.

MATERIALS AND METHODS

FOV isolates and their geographic origins

A random collection of single-spore *Fusarium* isolates was chosen from the fungal culture collection of the Fiber Crop Diseases Section (Plant Pathology Research Institute). Isolates were originally recovered from symptomatic greenhouse-grown seedlings of the highly susceptible cultivar 'Giza 74' after cultivation in naturally infested soil samples collected from different locations in Egypt. Fusarium isolates were identified at the species level as *F. oxysporum* according to Booth (1971). Pathogenicity of *F. oxysporum* isolates was evaluated on the highly susceptible cultivar 'Giza 74'. The isolates able to induce typical Fusarium wilt symptoms in 'Giza 74' were classified as FOV (Aly et al., 2000).

A random sample of 30 FOV isolates was chosen for further studies. Most of Lower Egypt (LE) isolates were obtained from east Delta governorates (Sharqiya, Daqahliya and Damietta). These represented 41.2% of LE isolates, while south Delta (Qulyubiya) isolates represented the lowest percentage (5.9%). Isolates from north Delta (Kafer El-Sheikh), west Delta (Beheira), and middle Delta (Gharbiya and Minufiya) represented 17.6, 11.8 and 23.5% of the LE isolates, respectively (Table 1). The majority of Upper Egypt (UE) isolates (46.2%) come from south UE (Assiut and Sohag). Isolates of north (Beni suef and Fayoum) and middle (Minya) areas were represented by 38.5 and 15.4%, respectively of the UE isolates (Table 1). Therefore, the two groups of FOV isolates covered most of the cotton-growing regions in Egypt.

Assessment of the pathogenic variation of FOV isolates on 'Giza 74' seedlings

Autoclaved clay loam soil was infested with inoculums of each isolate at a rate of 10 g/kg of soil. Substrate for growth of each isolate was prepared in 500 mL glass bottles. Each bottle contained 50 g of sorghum and 40 mL of tap water. Contents of bottles were autoclaved for 30 minutes. Inoculum (mycelium and conidiospores) was obtained from one-week-old culture on potato dextrose agar (PDA), was aseptically introduced into the bottle and allowed to colonize sorghum for three weeks. Fungus-sorghum mixture was used to infest soil. Infested soil was dispensed in 15 cm diameter clay pots and each pot was planted with 20 seeds of the 'Giza 74' (Aly, 1988). Pots were distributed on greenhouse benches. The greenhouse was equipped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28ºC. However, due to the lack of a cooling system, the maximum temperature was out of control, fluctuating from 30 to 35ºC depending on the prevailing temperature during the day (the test was conducted in January and February). The pathogenicity test was repeated once with essentially the same results. Dead as well as surviving seedlings (with external symptoms) were

TABLE 1 - Geographical origin of monosporic isolates of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 used in the present study.

Lower Egypt (Nile Delta)		Upper Egypt	
Isolate number	**Governorate**	**Isolate number**	**Governorate**
1	Sharqiya	1	Beni Suef
2	Sharqiya	2	Beni Suef
3	Sharqiya	3	Fayoum
4	Daqahliya	4	Fayoum
5	Daqahliya	5	Sohag
6	Daqahliya	6	Sohag
7	Qulyubiya	7	Minya
8	Beheira	8	Minya
9	Beheira	9	Assiut
10	Kafr El-Sheikh	10	Assiut
11	Kafr El-Sheikh	11	Fayoum
12	Kafr El-Sheikh	12	Sohag
13	Damietta	13	Assiut
14	Gharbiya		
15	Gharbiya		
16	Minufiya		
17	Minufiya		

counted daily. In the surviving seedlings, discrete areas of vein discoloration in the cotyledonary leaves usually began at the margin, turned yellow or brown, and eventually the entire leaf wilted. Seedlings that remained apparently healthy six weeks 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, seedlings of 'Giza 74' were placed in four distinct classes: (1) resistant (healthy) if they were free of any external or internal symptoms; (2) slightly susceptible if the surviving seedlings showed discoloration of xylem vessels; (3) susceptible if the surviving seedlings showed vein discoloration in the cotyledonary leaves, and (4) highly susceptible if the seedlings died. Symptoms on 'Giza 74 'seedlings are shown in Figure 1.

Screening of cotton genotypes for Fusarium wilt resistance under greenhouse conditions

The genotypes evaluated in this assay were part of the Cotton Screening Program for Fusarium wilt resistance. The test, which included 55 experimental breeding materials (Table 2), was conducted in Cotton and Fiber Crop Diseases Research section, Plant Pathology Research Institute, Agric. Res. Cent., Egypt. The inoculum used in the test was a mixture of equal parts (w/w) of 30 isolates of FOV race 3. Autoclaved clay loam soil was infested with the mixture of isolates isolate at rate of 10 g/kg of soil. The infested soil was dispensed in 15 cm diameter clay pots and 20 seeds per each pot were planted (three pots for each genotype). The test was repeated once with almost the same results. Seedlings of each genotype were evaluated based on the previously mentioned four grade scale (resistant, slightly susceptible, susceptible, and highly susceptible).

Statistical analyses

FIGURE 1 - Symptoms of Fusarium wilt of cotton. **A.** Wilted cotton seedlings (cultivar Giza 74) grown in naturally infested field soil. **B.** Vein discoloration at the margin of cotyledonary leaf. **C.** Isolation of FOV from surface-sterilized hypocotyls vascular tissue from selected plants was used to confirm that the symptoms observed were due to Fusarium wilt. **D.** Severely affected seedlings after planting in artificially infested soil in a greenhouse test. **E.** General view of cotton genotype test for Fusarium-wilt resistance in a greenhouse **F.** Longitudinal section of healthy hypocotyl tissue compared with **G.** Discoloration of xylem vessels in infected hypocotyl.

TABLE 2 - Cotton genotypes evaluated for resistance to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates.

Genotype number (#)	Pedigree
01	Line 488/2002
02	Line 490/2002
03	Line 491/2002
04	Line 494/2002
05	Line 496/2002
06	Line 499/2002
07	Line 501/2002
08	Line 507/2002
09	Line 508/2002
10	Line 509/2002
11	Line 511/2002
12	Line 514/2002
13	(Giza 83 *x* Tamcot) *x* Austalian1 / 88/2002
14	(Giza 83 *x* Tamcot) *x* Austalian1 / 89/2002
15	(Giza 83 *x* Tamcot) *x* Austalian1 / 90/2002
16	(Giza 83 *x* Tamcot) *x* Austalian1 / 91/2002
17	(Giza 83 *x* Tamcot) *x* Austalian1 / 95/2002
18	(Giza 83 *x* Tamcot) *x* Austalian1 / 96/2002
19	Giza 90
20	Australian 2
21	(Giza 90 *x* Australian 2) / 98
22	(Giza 90 *x* Australian 2) /100
23	(Giza 90 *x* Australian 2) /103
24	(Giza 90 *x* Australian 2) /107
25	Australian 3
26	(Australian 3 *x* Giza83R) /138
27	(Australian 3 *x* Giza83R) /139
28	(Australian 3 *x* Giza83R) /142
29	(Australian 3 *x* Giza83R) /143
30	Giza 83 R
31	Karashinky
32	(Giza 83 R *x* Karashinky) /147
33	(Giza 83 R *x* Karashinky) /148
34	(Giza 83 R *x* Karashinky) /149
35	(Giza 83 R *x* Karashinky) /150
36	(Giza 83 R *x* Karashinky) /154
37	(Giza 83 R *x* Karashinky) /157
38	Australian 4
39	Giza 72
40	(Australian 4 *x* Giza 72) /158
41	(Australian 4 *x* Giza 72) /161
42	(Australian 4 *x* Giza 72) /163
43	(Australian 4 *x* Giza 72) /164
44	Giza 80
45	Giza 83
46	Australian 5
47	(Giza 80 *x* Australian 5) *x* Giza83 /178
48	(Giza 80 *x* Australian 5) *x* Giza83 /179
49	(Giza 80 *x* Australian 5) *x* Giza83 /182
50	(Giza 80 *x* Australian 5) *x* Giza83 /184
51	(Giza 80 *x* Australian 5) *x* Giza83 /185
52	(Giza 80 *x* Australian 5) *x* Giza83 /187
53	(Giza 80 *x* Australian 5) *x* Giza83 /189
54	Dandera
55	Australian 6

The experimental design of greenhouse tests of FOV isolates and cotton genotypes was randomized complete block with three replications. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used to compare isolate and genotype means. ANOVA was performed with MSTAT-C statistical package. Isolates were clustered by the average linked technique (unweighted pair-group method) and the results were expressed as a phenogram. Cluster analysis was performed with the software package SPSS 6.0.

RESULTS

Pathogenic variation of FOV isolates

A distinctive characteristic of LE isolates was their inability to induce vascular discoloration when compared with UE isolates (Tables 3 and 4). On the other hand, the percentage of LE isolates which induced cotyledonary yellowing and seedling death was greater than those of UE. There were no significant differences between FOV isolates from LE and UE regarding rate of plant survival, vascular discoloration, and dead seedlings. However, LE isolates caused significantly more cotyledonary yellowing than UE isolates (Tables 4 and 5). Symptoms used for evaluating pathogenicity of FOV isolates were better correlated in the case of LE isolates (Table 6). Isolates of FOV were clustered based on their virulence patterns on seedlings of 'Giza 74' (Figure 2). The observed clustering of the isolates was not related to their geographical origins.

Screening of cotton genotypes for Fusarium wilt resistance

Plant survival rate was used as a criterion to evaluate the reaction of the tested genotypes to Fusarium wilt. The tested genotypes showed a wide range of reactions to Fusarium wilt with survival rates ranging from 0 to 100% (Table 7). Genotypes #07, #13, #16, #17, #25, #39, #41, #43, #46, and #55 were classified as highly susceptible with survival rates ranging from 0 to 24.7%. Seedlings within each of these genotypes showed variable symptom expression due to the fact that many of these genotypes were not pure lines. On the other hand, genotypes #05, #19, #21, #24, #29, #30, #31, #32, #33, #34, #35, #36, #37, #38, #40, #45, #50, #51, #52, #53, and #54 were classified as highly resistant with plant survival rate ranging from 79.6 to 100%. The grade scale including the slightly susceptible, susceptible and highly susceptible categories, enabled us to differentiate within the susceptible group the genotypes that were statistically indistinguishable in terms of plant survival rate. For example, 5.56% of the tested seedlings of the genotype #5 were placed in the susceptible category (dead seedlings), while 5.56% of the tested seedlings of the genotype #19 were placed in the slightly susceptible category (displaying only vascular discoloration). This comparison implies that genotype #19 has superior levels of resistance to Fusarium wilt when compared to genotype #5 because

TABLE 3 - Variation in pathogenicity among *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates on cotton 'Giza 74' seedlings under greenhouse conditions.

Geographic origin									
	Lower Egypt (LE)					Upper Egypt (UE)			
Isolate number	HS% [a]	VD%	CY%	DS%	Isolate number	HS%	VD%	CY%	DS%
01	38.53 B-D[b]*	11.2 A*	26.87 A*	23.50 A-C*	01	77.83 B*	2.57 AB*	15.97 AB*	3.6 DE
02	31.27 B-D*	6.87 AB	36.6 A*	25.33 A-C*	02	20. 00 EF*	0.0 C	15.00 BC	65.0 A*
03	36.03 B-D*	8.47 AB	30.9 A*	24.63 A-C	03	50.47 B-E*	0.0 C	16.20 A-C	33.33 A-D*
04	42.03 B-D*	6.07 AB	19.3 A*	32.53 A*	04	19.43 F*	12.03 BC	11.10 BC	57.4 A*
05	30.0 CD	0.0 B	40.47 A*	29.53 AB*	05	34.87 D- F*	0.0 C	56.07 A	9.1 C-E
06	33.70 B-D*	8.3 AB	24.77 A*	33.23 A*	06	60.93 B-D*	3.03 AB*	19.07 AB*	17.0 B-E
07	57.76 B-D*	0.0 B	24.07 A*	18.13 A-D	07	36.47 C-F*	3.7 AB*	16.23 AB*	43.6 AB*
08	21.63 D*	1.97 AB	32.40 A*	44.03 A*	08	51.70 B- E*	6.67 A*	20.80 AB*	20.8 A-E
09	36.63 B-D*	0.0 B	35.00 A*	28.33 A-C*	09	41.53 B-F*	0.0 C	16.90 AB*	41.6 AB*
10	63.63 B-D*	6.07 AB	27.30 A*	3.03 CD	10	68.77 BC*	3.03 BC	9.70 BC	18.5 B-E
11	46.22 BC*	6.10 AB	21.09 A*	5.57 CD	11	78.07 B*	5.8 A*	8.60 BC	7.53 B-E
12	72.50 B*	0.0 B	19.17 A*	8.33 B-D	12	53.90 B-E*	8.77 A*	21.40 AB*	15.97 B-E
13	27.50 D*	4.17 AB	34.53 A*	33.93 AB*	13	57.17 B-E*	2.57 AB*	22.57 AB*	17.70 B-E
14	57.17 B-D*	2.57 AB	22.57 A*	17.7 A-D					
15	53.90 B-D*	8.77 AB	21.37 A*	15.97 A-D					
16	27.37 D*	4.17 AB	34.53 A*	33.93 AB*					
17	72.50 B*	0.0 B	19.17 A*	8.33 B-D					
Control	100 A	0.0 B	0.0 B	0.0 D	Control	100 A	0.0 C	0.00 C	0.0 E

[a]HS=Healthy survival rate, VD=Vascular discoloration, CY=Cotyledonary yellowing, DS=Dead seedlings.
[b]Values followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
*Significant difference from the control.

TABLE 4 - Distribution of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates based on their pathological effects on cotton seedlings (cultivar 'Giza74') under greenhouse conditions.

Geographic origin	Number of tested isolates	Percentage of isolates which significantly affected [a]			
		HS[b]	VD	CY	DS
Lower Egypt	17	100	5.9	100	58.8
Upper Egypt	13	100	53.8	61.5	38.5

[a]The tested isolates significantly decreased HS, while they significantly increased the other disease symptoms
[b]HS=Healthy survival rate, VD=Vascular discoloration, CY=Cotyledonary yellowing ,and DS=Dead seedlings.

TABLE 5 - Relative pathogenicity (%) of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates from Lower and Upper Egypt on cotton seedlings (cultivar 'Giza 74').

Symptom (%)	Origin of the Isolates				
	Lower Egypt	Upper Egypt	Difference	F. value	P >F
Healthy survival	43.96[a]	50.09[b]	6.13	0.905	
Vascular discoloration	4.40	3.71	0.69	0.256	
Cotyledonary yellowing	27.83	19.20	8.64	6.258	0.02
Dead seedlings	22.71	27.01	4.30	0.565	

[a]Average of 18 isolates including the control.
[b]Average of 14 isolates including the control.

TABLE 6 - Correlation among symptoms used for evaluating pathogenicity of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates from distinct geographic regions of Egypt.

Geographic origin of isolates	Symptoms	Symptoms[a]		
		X1	X2	X3
Lower Egypt	Healthy survival (X1)			
	Vascular discoloration (X2)	-0.860**[a]		
	Cotyledonary yellowing (X3)	-0.731**	0.571*	
	Dead seedlings (X4)	-0.259	0.43	-0.126
Upper Egypt	Healthy survival (X1)[a]			
	Vascular discoloration (X2)	-0.831**		
	Cotyledonary yellowing (X3)	-0240	-0.312	
	Dead seedlings (X4)	-0.039	0.041	-0.317

[a]Pearson's correlation coefficient is significant at P<0.01 (**) or P<0.05 (*)

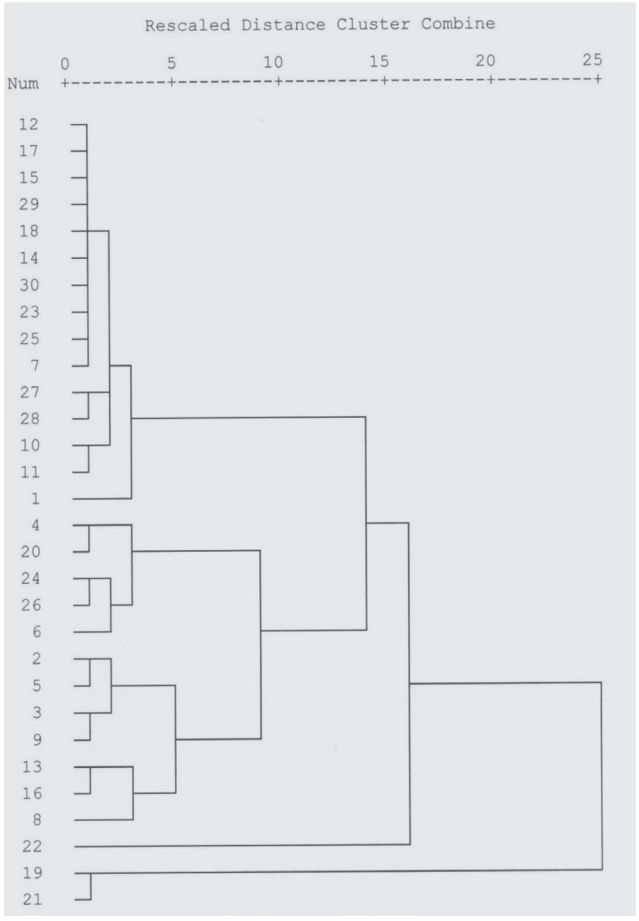

FIGURE 2 - Phenogram based on average linkage cluster analysis of virulence of 30 isolates of FOV on cultivar 'Giza 74'. Isolates 1 to 17 are from Lower Egypt governorates while isolates 18 to 30 are from Upper Egypt governorates.

TABLE 7 - Frequency (%) in each reaction class of cotton genotypes to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates under greenhouse conditions.

Genotype number	HS[a]	VD	CY	DS
01	67.78 F-N	10.74 A-D	17.78 B-I	3.67 IJ
02	76.40 I-N	3.67 DE	12.86 D-J	7.04 G-J
03	45.24 D-K	0.00 E	27.38 A-H	27.38 D-J
04	47.22 D-K	20.37 A	25.00 B-I	7.41 F-J
05	94.44 MNO	0.00 E	0.00 J	5.56 IJ
06	75.93 I-N	3.67 DE	12.04 C-J	8.33 H-J
07	8.93 A-D	4.76 DE	22.62 B-I	63.69 AB
08	73.75 H-N	0.00 E	11.43 D-J	15.0 F-J
09	40.0 D-I	0.00 E	19.44 B-J	40.56 B-F
10	40.99 D-J	3.03 DE	27.42 A-H	28.56 C-I
11	65.28 F-M	3.67 DE	13.89 C-J	17.13 E-J
12	65.08 F-M	13.29 A-D	15.87 C-J	4.76 IJ
13	22.56 A-E	15.07 ABC	36.87 A-D	25.50 D-I
14	47.78 D-K	0.00 E	30.0 E	22.22 E-J
15	39.78 C-I	0.00 E	37.31 A-D	22.78 D-I
16	20.37 A-E	14.07 A-D	37.79 A-D	27.78 D-I
17	9.52 A-C	6.67 A-E	47.02 AB	36.79 B-G
18	26.67 B-G	10.0 A-E	39.17 A-D	24.17 D-I
19	94.44 MNO	5.56 CDE	0.00 J	0.00 J
20	29.64 B-F	16.67 AB	25.22 A-H	27-78 D-I
21	79.63 J-O	3.67 DE	16.67 C-J	0.00 J
22	43.86 D-K	10.37 A-E	37.3 A-D	8.47 F-J
23	70.37 G-N	0.00 E	18.52 B-I	22.22 D-I
24	100.0 O	0.00 E	0.00 J	0.00 J
25	3.33 AB	0.00 E	56.55 A	40.12 B-F
26	46.31 D-K	7.50 A-E	32.02 A-F	14.17 F-J
27	39.09 C-I	0.00 E	35.15 A-E	25.76 D-I
28	48.98 E-L	4.17 D-E	25.74 A-H	21.11 E-J
29	100.0 O	0.00 E	0.00 J	0.00 J
30	100.0 O	0.00 E	0.00 J	0.00 J
31	94.44 MNO	0.00 E	0.00 J	5.56 IJ
32	100.0 O	0.00 E	0.00 J	0.00 J
33	100.0 O	0.00 E	0.00 J	0.00 J
34	100.0 O	0.00 E	0.00 J	0.00 J
35	95.1 MNO	0.00 E	4.76 IJ	0.00 J
36	96.33 NO	0.00 E	3.67 IJ	0.00 J
37	96.33 NO	0.00 E	0.00 J	3.67 IJ
38	91.53 MNO	0.00 E	8.47 E-J	0.00 J
39	7.41 K-O	0.00 E	37.03 A-D	55.56 A-E
40	96.33 NO	0.00 E	3.67 IJ	0.00 J
41	18.15 A-E	0.00 E	42.59 A-C	39.26 B-G
42	28.70 B-H	0.00 E	33.98 A-F	37.31 B-G
43	0.00 A	7.41 B-E	31.02 A-F	61.57 A-D
44	35.56 B-H	0.00 E	24.44 B-I	40.0 B-F
45	91.67 MNO	0.00 E	8.33 HIJ	0.00 J
46	0.00 A	0.00 E	25.56 B-I	74.44 A
47	25.55 B-G	3.67 DE	41.85 A-C	25.18 D-I
48	44.81 D-K	3.33 DE	27.78 A-H	24.07 D-I
49	24.68 A-E	8.59 A-E	32.25 A-F	34.49 B-H
50	95.83 NO	0.00 E	4.17 IJ	0.00 J
51	88.43 MNO	3.67 DE	7.87 F-J	0.00 J
52	91.67 MNO	0.00 E	8.33 E-J	0.00 J
53	85.83 L-O	4.17 DE	10.0 G-J	0.00 J
54	90.00 MNO	0.00 E	10.0 G-J	0.00 J
55	16.2 A-E	0.00 E	16.20 C-J	

[a]HS=Healthy survival rate, VD=Vascular discoloration, CY=Cotyledonary yellowing, and DS=Dead seedlings.

it did not include any dead seedlings. Another example is the comparison between genotypes #36 and #37, which indicates the superiority of the first. The other genotypes showed variable levels of resistance/susceptibility between the two extremes of highly susceptible and highly resistant genotypes.

Among the significant correlations, those which included vascular discoloration showed the lowest r values. The correlation between vascular discoloration and dead seedlings was non significant (Table 8).

DISCUSSION

Advances in breeding cotton for resistance to FOV have been difficult because of the complex interaction of the host, pathogen, and soil environment. Host resistance offers the best opportunity to protect the cotton industry from virulent populations of FOV that may be introduced and from new virulent strains of FOV that may arise within cotton production areas.

Pathogenic variation within FOV isolates, as we have demonstrated herein, is well documented in the literature (Mohamed, 1958; Fahim et al., 1973; Osman, 1996; Abd-Elsalam et al., 2009). In the present study, genotypes were screened against 30 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 tests.

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 & 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 discolorations. On the other hand, Salgado et al. (1994) used vascular discolorations as a criterion for judging susceptibility of tepary bean (*Phaseolus acutifolius* Gray) seedlings to Fusarium wilt. Osman (1996) found a highly significant correlation ($r= 0.98$, $p<0.01$) between external wilt symptoms and vascular discoloration of cotton seedlings (cultivar 'Giza 74').

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 discolorations 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 are 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 fields 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. In addition, 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.

TABLE 8 - Correlation coefficients among symptoms used in evaluating the reactions of cotton genotypes to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 3 isolates from Egypt.

Symptoms	Symptoms[a]		
	X1	X2	X3
Healthy survival (X1)			
Vascular discoloration (X2)	0.336*[a]		
Cotyledonary yellowing (X3)	0.870**	0.308*	
Dead seedlings (X4)	0.853**	0.044	0.650**

[a]Pearson's correlation coefficient is significant at P<0.01 (**) or P<0.05 (*).

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