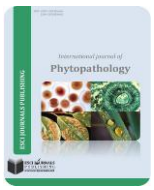

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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS TO DETERMINE THE GENETIC VARIABILITY AMONG VIRULENT AND LESS VIRULENT ISOLATES OF *FUSARIUM MONILIFORME*, *FUSARIUM OXYSPORUM* AND *FUSARIUM SOLANI* ISOLATED FROM INFECTED COTTON SEEDLINGS

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A B S T R A C T

Root-rot of cotton (*Gossypium* spp.) is one of the most important diseases in Upper Egypt. Isolation has been done from diseased cotton roots and seeds which were collected from 11 counties in Assiut province, Egypt. Identification procedures of the isolated fungi confirmed that the isolated fungi were for *Fusarium solani*, *F. moniliforme* and *F. oxysporum* and *Rhizoctonia solani*. Thirty six isolates of *Fusarium* spp. and 10 isolates of *Rhizoctonia solani* were tested for their pathogenicity on both Giza 80 and Giza 83 cotton seedlings to verify their virulence on seedlings. The pathogenicity test results have grouped the *Fusarium* spp. isolates into three groups; highly virulent that caused 91-100% mortalities; moderately virulent that caused 81-90% mortalities and low virulent that caused lower than 81.0% mortalities. Data also shows that, in general Giza 80 cotton cultivar was more susceptible for infection with *Fusarium* spp. when compared with Giza 83 cotton cultivar. In case of *Rhizoctonia solani*, data revealed that the infection percentage was significantly affected by isolates while cotton cultivars had no significant influence on infection. Four 10-mer primers (1:6-d, 2:6-d, 4:6-d and 5:6-d) were used in RAPD-PCR to determine the genetic variability between six isolates, one virulent and one less virulent, of *F. moniliforme*, *F. oxysporum* and *F. solani*. Our results showed that the primer 2:6-d clearly separated *F. moniliforme*, *F. oxysporum* and *F. solani* and proved to be quite powerful in distinguishing the three different species and isolates of *Fusarium* spp.

Keywords: *Fusarium* spp, *Rhizoctonia solani*, Cotton, root rot, Random Amplified Polymorphic DNA.

INTRODUCTION

Root-rot of cotton (*Gossypium* spp.) is one of the most important diseases. Symptoms occur as seedling death before or after emergence and as root damage. Seeds may be infected and contents reduced to a brown gelatinous mass in the seed coat, or the radicle and cotyledons may become brown and soft after emergence (Johnson *et al*., 1978). Root-rot appears suddenly, starting in early summer. It causes rapid wilting and followed by death of the plants within a few days. Usually the leaves of infected plants remain attached but the root of infected plants decay. Scraping the taproot reveals darkened reddish to wine-colored stain (Hashem and Hamada, 2002). Vascular streaking is not present as in wilt disease. Under most conditions spore may appear on the soil surface near diseased plants (Hashem and Hamada, 2002).

F. solani is implicated in cotton root rot in addition to *Rhizoctonia* spp. (Watkins, 1981).

Genetic markers provide more accurate measurement of genetic relationships among the genotypes than other markers (Soller and Beckmann, 1983). The random amplified polymorphic DNA (RAPD) is an attractive complement to conventional DNA finger-printing in ecology (Williams *et al*., 1990). RAPD analysis requires no prior sequence information, simple to perform, inexpensive, and require very little starting DNA template and proved to be useful in detecting intraspecific polymorphisms among organisms (Esselman *et al*., 1999; Assigbetse *et al*., 1994). RAPD technique can generate specific DNA fragments useful

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for identification of isolates and applications in ecology (Hadrys *et al*., 1992). RAPD analysis provided markers to differentiate races of Fusarium solani (Arif *et al*., 2015; Crowhurst *et al*., 1991), Gremmeniella abietina (Hamelin *et al*., 1993), and aggressive and nonaggressive isolates of Phoma lingam (Goodwin and Annis, 1991). RAPD-PCR is a quick and reliable method to determine the variability among and between fungal species and isolates and has been used to study the differences between species, strains and isolates of Fusarium species (Arif *et al*., 2011, 2015; Assigbetse *et al*., 1994; Mattila *et al*., 1996 and 1997; Altomare *et al*., 1997 and Zheng and Ploetz, 2002). Here we report the results of using four random DNA primers RAPD-PCR analysis to determine the genetic variability between three different species of the genus *Fusarium*.

MATERIALS AND METHODS

Isolation and identification of the causal pathogens

From diseased cotton roots: Eleven diseased cotton roots samples were collected from 11 counties in Assiut province, Egypt. Each sample approximately included 10 seedlings have shown the symptoms of root rots. Infected cotton roots were washed thoroughly under running tap water to remove any adhering soil particles, cut into small pieces (1cm), then surface sterilized by soaking in 1% sodium hypochlorite solution for two minutes and then washed thoroughly with sterile distilled water for two times. Surface sterilized pieces were put on sterilized filter papers to dry and transferred to petri plates (9cm diam) containing 25ml potato dextrose agar (PDA) medium. Four pieces were used per plate. Plates were incubated at 28°C for 5-7 days.

From cotton seeds: Ten seed samples of Giza83 cultivar were collected from different counties in Assiut Province during season 2001. Cotton seeds were washed in tap water then soaked in 1% sodium hypochlorite for 10 minutes followed by complete washing in sterile distilled water. Surface sterilized seeds were put on sterilized filter papers to dry and transferred to petri plates (9cm diam) filled with 25ml potato dextrose agar (PDA) medium. In all isolation procedures, the antibiotic procaine penicillin was added to PDA medium after autoclaving (1200 antibiotic units/20 ml medium) in order to prevent bacterial contamination. Five seeds were used per plate. Plates were incubated at 28°C for 5-7 days.

The obtained isolates were divided into three categories of *Fusarium* spp. and two categories of *Rhizoctonia* spp., according to their external features and growing characters. Purification of the obtained fungal isolates was carried out by using the hyphal tip and single spore isolation technique according to (Toussoun and Nelson, 1976). Thirty six isolates of *Fusarium* spp. and 10 isolates of *Rhizoctonia* spp. were selected to be tested for their virulence on both Giza83 and Giza80 cotton cultivars in order to verify their virulence on seedlings.

Isolated fungi were grown for 6 days on PDA medium in agar slants at 28±2°C then kept as stock cultures for further studies. Identification procedures for the isolated fungi was carried out on 3 weeks old cultures using the cultural characteristics and their microscopic features according to Ainsworth (1971) and Booth (1977); confirmed by Assiut University Mycological Center (AUMC).

Pathogenicity tests: Thirty six isolates of *Fusarium* spp. and 10 isolates of *Rhizoctonia solani* were tested for their pathogenicity on both Giza 80 and Giza 83 cotton seedlings to verify their virulence on seedlings. Inocula of the isolated fungi were prepared by inoculating sterilized bottle 500 ml, containing barley medium (75 g barley + 25 g clean sand + 2 g sucrose + 0.1 g yeast + 100 ml water) (Abd-El-Moneem, 1996). Fungal inoculum was taken from 5-7 days old cultures on PDA, was aseptically introduced into the bottle and allowed to grow for 3 weeks. Pathogenicity tests were carried out by using autoclaved clay loam soil. Batches of autoclaved soil were infested separately with inoculum of each isolate at the rate of 50 g/kg of soil in case of *Fusarium* spp. while 10, 20 g/kg of soil were used in case of *Rhizoctonia solani*, dispersed in 25 cm diameter clay pots, then pots were planted with 5 seeds per pot of Giza 83 and Giza 80 cultivars. Five replicates of each treatment were maintained for both Giza 83 and Giza 80 cotton cultivars. In the control treatment sterilized barley medium was mixed thoroughly with soil at the rate of 50 g/kg of soil. Pots were randomly distributed inside greenhouse. The prevailing temperatures during pathogenicity tests was 24.5°C (minimum) and 28.5°C (maximum). Percentage of infection was recorded 45 days after planting. Based on the percentage of infection the isolates were categorized into 3 groups as highly, moderately and low virulent.

DNA extraction: Pure cultures of each isolates were maintained on PDA slants and incubated at 28±2 °C for 6 days under controlled temperature and mycelia were aseptically transferred to flasks of potato dextrose broth and incubated for 5 days at 28±2 °C without shaking.

The mycelia were filtered from liquid medium through four cheesecloth layers. Total DNA was isolated from 50 mg mycelium of each isolate according to the CTAB protocol of Murray and Thompson (1980). DNA concentration and purity of the obtained DNA was determined by using "Gen qunta" system (Pharmacia Biotech, MN, USA). The concentration was adjusted to 6 ng/µl for all samples.

Random Amplified Polymorphism DNA (RAPD): RAPD-PCR analysis was used to determine the genetic variability among a virulent and less virulent isolates of Fusarium moniliforme (Fm), F. oxysporum (Fo) and F. solani (Fs). The codes used for the virulent isolates were Fm-13, Fo-15 and Fs-7 and the less virulent isolates were Fm-20, Fo-28 and Fs-19.

PCR reactions were conducted using four arbitrary 10-mer primers (Amersham Pharmacia Biotech). The code numbers and sequences of these four primers were:

1:6-d (5`-GGTGCGGGAA-3`), 2:6-d (5`-GTTTCGCTCC-3`), 4:6-d (5`-AAGAGCCCGT-3`) and 5:6-d (5`-AACGCGCAAC-3`). The PCR was performed in a total volume of 25 µl, containing 30 ng of template DNA, 10 picomole of random primer, 0.2 mM of dNTPs, 2 mM of MgCl2, 1.5 U of Hot start Taq DNA polymerase (Fermentas, USA). The amplification protocol was carried out as follow using PCR (unit II Biometra, Göttingen, Germany); a) Initial denaturation at 95ºC for 5 min; b) 45 cycles each consists of the following steps: denaturation at 95ºC for 1 min, annealing at 37ºC for 1 min and extension at 72ºC for 2 min; c) final extension at 72ºC for 5 min and d) Hold at 4ºC.

The amplified DNA for all samples (15µl) were resolved by electrophoresis (unit of WIDE mini-sub-cell GT Bio-RAD, CA, USA) on 1% agarose containing ethidium bromide (0.5 µg/ml) at 75 constant voltage, and determined with UV-transilluminator (UVP, Cambridge, UK).

Gel analysis: The gels were scanned using gel documentation system (AAB Advanced American Biotechnology, Fullerton, CA, USA). Hierarchical cluster analysis to produce a dendrogram was performed using unweighted pair group method based on arithmetic mean (UPGMA) according to Sneath and Sokal (1973).

RESULTS

Pathogenicity tests: Data in table 1 indicated that the mean percentage of fungal recovery from diseased samples showed that *Fusarium* spp. (33.4%) were the most dominant fungi present followed by *R. solani* (23.4%), while *Aspergillus* spp. (13.8%), *Penicillium* spp. (9.4%), and *Alternaria* spp. showed the lowest percentage of recovery (7.7%).

The identification process showed that the isolated *Fusarium* spp. were *F. moniliforme*, *F. oxysporum* and *F. solani*. Data presented in table 2 indicates that *Fusarium* spp. isolates (No. 5, 7, 11, 13, 15, 24) were highly virulent that caused 91-100% mortalities. Isolates (No. 8, 9, 10) were moderately virulent (81-90%) while isolate (No.28) showed the lowest virulence on the two cotton cultivars and caused 60.0% infection. Data also shows that, in general Giza 80 cotton cultivar was more susceptible for infection with *Fusarium* spp. when compared with Giza 83 cotton cultivar.

Table 1. Frequency of isolated fungi isolated from diseased cotton roots collected from different counties of Assiut Province during 2001 growing season.

Counties	Frequency of isolated fungi %					
	Fusarium sp.	*Rhizoctonia* sp.	*Alternaria* sp.	*Aspergillus* sp.	*Penicillium* sp.	Others
Sedfa	25.80	29.00	5.00	14.5	11.2	14.5
Alghanaim	27.40	24.20	7.5	19.6	9.2	12.1
Albadary	34.50	29.50	3.4	14.7	8.1	9.8
Sahel Seleem	26.90	25.30	9.5	17.5	11.3	9.5
Alqusia	42.40	25.70	7.5	9.3	4.5	10.6
Manfalout	30.50	22.70	9.0	10.6	12.1	15.1
Abnoob	32.80	20.80	8.9	14.9	10.7	11.9
Abuteeg	35.20	19.50	10.2	16.1	10.2	8.8
Diroot	38.20	19.30	8.8	14.7	10.2	8.8
Elfath	35.50	16.90	7.8	13.8	10.7	15.3
Assiut	39.00	25.20	7.8	6.2	6.2	15.6
Means	33.47	23.48	7.76	13.80	9.49	12.00

Table 2. Pathogenicity tests, number and sources of 36 isolates of *Fusarium* spp. from different localities of Assiut Governorate on both Giza 80 and Giza 83 cotton cultivars.

Isolates		Root-rot %		Means
Source	No.	Giza 80	Giza 83	
Seeds	6	95.00*	85.00	90.00
	10	86.00	86.00	86.00
	13	97.00	95.00	96.00
	14	100.00	89.00	94.50
	16	91.00	81.00	86.00
	31	93.00	44.00	68.50
	20	90.00	40.00	65.00
Abnob	1	97.00	63.00	80.00
	12	92.00	87.00	89.50
	32	76.00	78.00	77.00
	21	79.00	81.00	80.00
	29	90.00	51.00	70.50
	36	86.00	74.00	80.00
Alqusia	2	91.00	86.00	88.50
	19	76.00	78.00	77.00
	22	92.00	95.00	93.50
	25	81.00	88.00	84.50
	26	78.00	48.00	63.00
	30	83.00	79.00	81.00
Assiut	11	96.00	91.00	93.50
	17	87.00	75.00	81.00
	23	91.00	84.00	87.50
	24	91.00	91.00	91.00
	27	86.00	94.00	90.00
	33	86.00	49.00	67.50
Sedfa	5	95.00	93.00	94.00
	9	83.00	89.00	86.00
	15	96.00	95.00	95.50
	34	86.00	52.00	69.00
	35	91.00	54.00	72.50
Alghanaium	3	97.00	85.00	91.00
	4	98.00	81.00	89.50
Sahel seleem	8	89.00	82.00	85.50
	7	94.00	97.00	95.50
Albadary	18	74.00	81.00	77.50
	28	77.00	43.00	60.00
Control	C	10.00	11.00	10.50
Mean		86.48	74.81	80.72

LSD(0.05) for:
Isolates 9.38
Cultivars x Isolates 13.28
* These values represent infection percentage.

In case of *R. solani*, data revealed that percentage of infection was significantly affected by isolates table 3. The highest values (100%) were obtained from isolates (No. 1, 2, 4, 6, 8 and 9) while the lowest infection percentage (73.5%) caused by isolate (No. 5) table 3. Data also showed that cotton cultivars had no significant influence on infection. However, the percentage of infection was 94.10% and 93.55% for Giza 80 and Giza 83 cultivars, respectively. Data also showed that weight of inoculum exerted a significant influence on the infection percentage. The percentages of infection were 92.9% and 94.7% when 10 and 20g were used, respectively. The second order interaction was not significantly affected the percent of infection by all interactions. In general, the lowest infection percentage was obtained from isolate (No. 5) and Giza 80 cultivar when inoculum weight was 10 g.

Random Amplified Polymorphism DNA (RAPD): Four 10-mer primers (1:6-d, 2:6-d, 4:6-d and 5:6-d) were used in RAPD-PCR to determine the genetic variability between two isolates (one virulent and one less virulent) of each Fusarium spp.: F. moniliforme (isolates Fm 13 and 20), F. oxysporum (isolates Fo 15 and 28) and F. solani (isolates Fs 7 and 19).

1- Primer 1:6-d

The DNA fragments generated by the primer 1:6-d from *F. moniliforme* (isolates Fm 13 and 20), *F.oxysporum* (Fo 15 and 28) and *F.solani* (Fs 7 and 19) were separated using agarose gel electrophoresis and illustrated in Figure 1. The banding patterns of these DNA fragments were analyzed by AAB computer software program and clustered by unweighted pair group method based on arithmetic mean (UPGMA) as shown in Figure 2.

The UPGMA clustering analysis showed that all tested isolates were clustered together in sub clusters with a branched-off at genetic similarity of 68.39%. The isolates of F. moniliforme (Fm 13 and 20) were clustered together and showed very high similarity of 98.0%. The next cluster consisted of F. oxysporum (Fo 15 and 28) at the genetic similarity of 93.67. While the two isolate of F. solani were extremely clustered together with a branched-off at genetic similarity of 68.39%. These results suggested that the primer 1:6-d can clearly separate the isolates of Fusarium spp.

2- Primer 2:6-d

RAPD-PCR patterns generated by the primer 2:6-d from the isolate of *F.moniliforme* (Fm 13 and 20), *F.oxysporum* (Fo 15 and 28) and *F.solani* (Fs 7 and 19) are shown in Figure 1. The Primer 2:6-d was reacted with all six isolate.

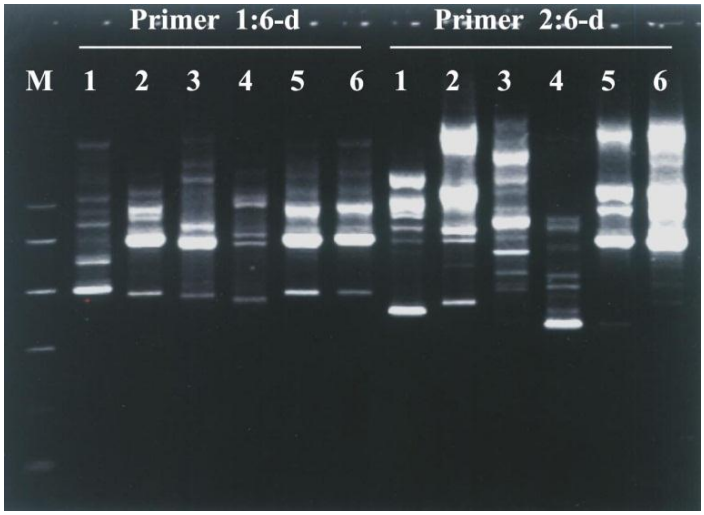
Table 3. Pathogenicity test of 10 isolates of Rhizoctonia solani selected from 250 isolates taken from different counties of Assiut Province.

Isolate no.	County	Infection %				*Means*
		Giza 80		Giza 83		
		10 g	20 g	10 g	20 g	
1	Sedfa	100	100	100	100	100
2	Sedfa	100	100	100	100	100
3	Alghanaim	83	84	80	85	83
4	Alghanaim	100	100	100	100	100
5	Abnoob	69	79	71	75	73.5
6	Albadary	100	100	100	100	100
7	Alghanaim	100	100	90	100	97.5
8	Albadary	100	100	100	100	100
9	Assiut	100	100	100	100	100
10	Abnoob	80	87	85	85	84
Means		93.2	95.0	92.6	94.5	
		94.10		93.55		

Control for Giza 80= 11% Control for Giza 83= 10%

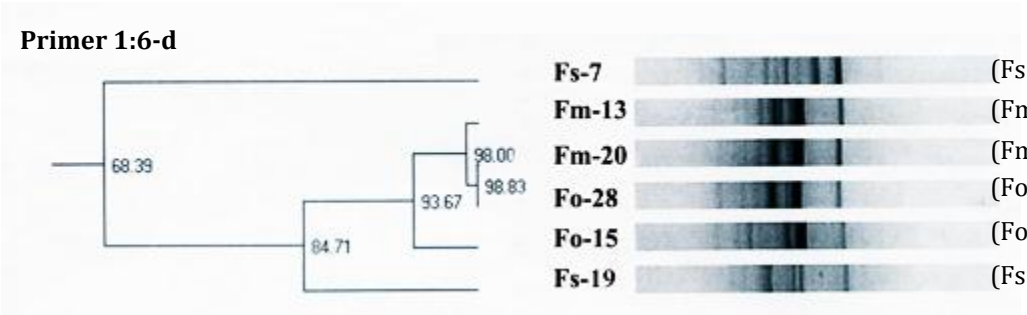
LSD (0.05) for:

Isolates (I) = 3.15, Weight (W): ** ,I x W: NS, Cultivar (C): NS, I x C: NS, C x W: NS, I x V x W: NS



(M): RAPD marker
(1): Fusarium solani (Fs7).
(2): Fusarium moniliforme (Fm13).
(3): Fusarium oxysporum (Fo15).
(4): Fusarium solani (Fs19).
(5): Fusarium moniliforme (Fm20)
(6): Fusarium oxysporum (Fo28).

Figure 1. Agarose gel electrophoresis of RAPD profile generated by the primers 4:6-d and 5:6-d from Fusarium isolates.



(Fs7): Fusarium solani.
(Fm13): Fusarium moniliforme.
(Fm20): Fusarium moniliforme.
(Fo28): Fusarium oxysporum.
(Fo15): Fusarium oxysporum.
(Fs19): Fusarium solani.

Figure 2. Dendrogram demonstrating the relationships among Fusarium isolates based on the polymorphism of RAPD primer 1:6-d.

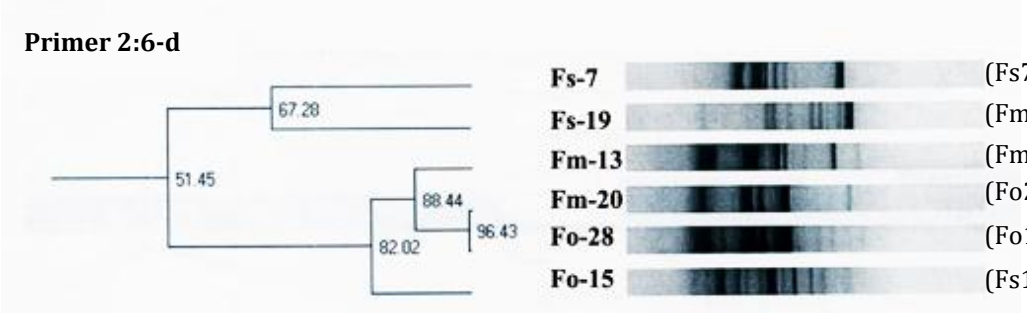


Figure 3. Dendrogram demonstrating the relationships among Fusarium isolate based on the polymorphism of RAPD primer 2:6-d.

Figure 3 showed the dendogram based on average linkage cluster analysis of RAPD marker 2:6-d obtained from the six isolates of Fusarium spp. The primer 2:6-d proved to be quite powerful in distinguishing the different species and isolates of F. moniliforme, F. oxysporum and F. solani. Therefore, the dendrogram tree was divided into two main clusters with a branched-off at genetic similarity of 51.45%. Within this cluster, F. solani (Fs 7 and 19) have a genetic similarity of 67.28%. The second main cluster included the isolates of F. moniliforme and F. oxysporum in two sub clusters. The isolates (Fm 13 and 20) were clustered together in the first subcluster and displayed high genetic similarity of 88.44%. However, F. oxysporum showed high genetic similarity of 82.02% and formed the second subcluster. The phenogram showed that the isolates of F. monliforme exhibited higher similarity (88.44%) than those of F. solani (67.28%) and F. oxysporum (82.02%). These results suggested that DNA profiles generated by the primer 2:6-d clearly separated Fusarium spp and isolates.

3- Primer 4:6-d

The DNA fragments generated by the primer 4:6-d from the isolates of *F.moniliforme* (Fm 13 and 20), *F.solani* (Fs 7 and 19) and *F.oxysporum* (Fo 15 and 28) were separated using agarose gel electrophoresis and illustrated in Figure 4. The banding patterns of these DNA fragments were analyzed by AAB computer software program and clustered by unweighted pair group method based on arithmetic mean (UPGMA) as shown in Figure 5.

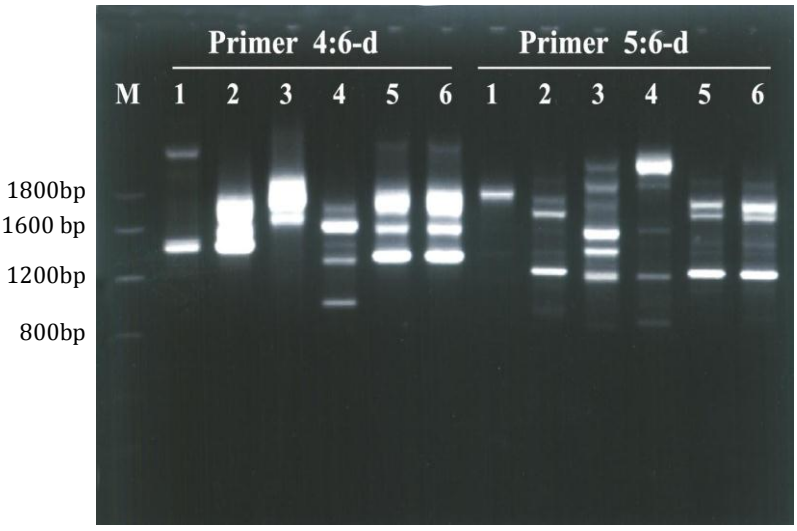


Figure 4. Agarose gel electrophoresis of RAPD profile generated by the primers 4:6-d and 5:6-d from Fusarium isolates.

The UPGMA clustering analysis showed that all tested isolates were clustered together in subclusters with a branched-off at genetic similarity of 66.01%. F. moniliforme isolates were clustered together and showed high similarity of 82.85%. The next cluster consists of F. oxysporum isolates at the genetic similarity of 80.96. These isolate were also clustered with F. solani (Fs 19) within similarity of 75.44%. While F. solani isolates (Fs 7 and 19) were extremely clustered together with a branched-off at genetic similarity of 66.01%. These results suggested that the primer 4:6-d can clearly separate the isolates of Fusarium spp.

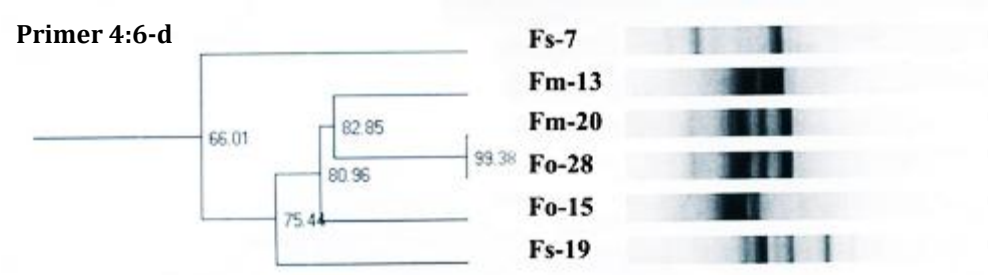


Figure 5. Dendrogram demonstrating the relationships among Fusarium isolates based on the polymorphism of RAPD primer 4:6-d.

4- Primer 5:6-d

The DNA fragments generated by the primer 5:6-d from the isolate of F.moniliforme (Fm 13 and 20), F.solani (Fs 7 and 19) and F.oxysporum (Fo 15 and 28) were separated using agarose-gel electrophoresis and illustrated in Figure 4. The banding patterns of these DNA fragments were analyzed by AAB computer software program and clustered by unweighted pair group method based on arithmetic mean (UPGMA) as shown in Figure 6. The UPGMA clustering analysis showed that all tested isolate were clustered together in subclusters with a branched-off at genetic similarity of 49.68%. F. moniliforme (Fm 13 and 20) were clustered together and showed very high similarity of 96.59%. Then these isolates were clustered with F. oxysporum (Fo 15 and 28), within genetic similarity of 75.68%. These isolates were also clustered with F. solani (Fs7) within similarity of 68.60% and then with (Fs 19) with a branched-off at genetic similarity of 49.68%. These results suggested that the primer 5:6-d could be used to differentiate between the three Fusarium spp.

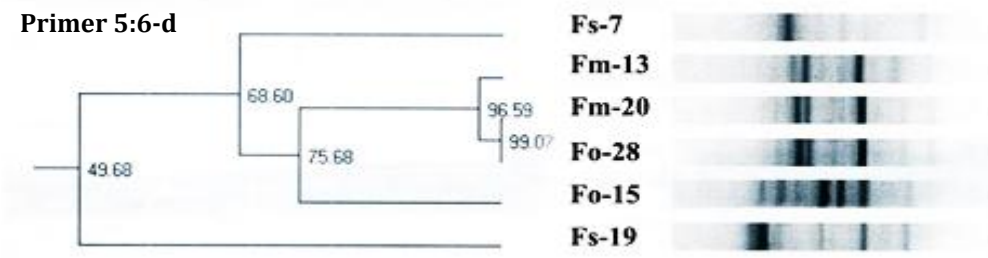


Figure 6. Dendrogram demonstrating the relationships among *Fusarium* isolate based on the polymorphism of RAPD primer 5:6-d.

DISCUSSION

Isolates of F. moniliforme, F. oxysporum and F. solani and R. solani varied in their virulence on both Giza 80 and Giza 83 cotton varieties. The two varieties also varied in their susceptibility. Variation in virulence of the isolates and in susceptibility of the two cotton varieties may be due to the presence of genetic differences among isolates and cotton varieties. In previous studies, F. solani showed high variation in pathogenicity among the isolates (Arif *et al*., 2013, 2015). Our research proved that F. moniliforme, F. oxysporum and F. solani can spread by cotton seeds which is in agreement with (Soleymani *et al*., 1993; Wahid *et al*., 1994). These findings indicate that cotton seeds may play a significant role in the disease transmission. It should be recommended that cotton seeds must be tested for the presence of these three species of Fusarium spp. before distributing cotton seeds to farmers to prevent the spread of the disease to new areas that are free of this disease.

Random amplified polymorphic DNA (RAPD) markers are based on the amplification of unknown DNA sequences using single, short, and random oligonucleotide primers (Williams *et al*., 1990). The ability of RAPD technique to produce multiple bands using a single primer means that a relatively small number of primers can be used to generate a very large number of fragments. These fragments are usually generated from different regions of the genome and hence multiple loci may be examined (Edwards 1998 and Ovesna *et al*., 2002). In the present study, four random primers (1:6-d, 2:6-d, 4:6-d and 5:6-d) were used to determine the genetic variability between six isolates of Fusarium moniliforme (Fm 13 and Fm 20), F. oxysporum (Fo15 and Fo28) and F. solani (Fs7 and

Fs19). The dendrograms obtained by the primers 1:6-d, 4:6-d and 5:6-d were nearly identical except the differences were only recorded for similarity level according to the primer. In these dendrograms the isolates arranged as F. solani (Fs 7) followed by F. moniliforme (Fm 13 and 20), F. oxysporum (Fo 28 and 15) and F. solani (Fs 19). The UPGMA clustering analysis showed that the primer 2:6-d clearly separated F. moniliforme, F. oxysporum and F. solani and proved to be quite powerful in distinguishing the three different species and isolates of Fusarium spp. However, the other three primers also were valuable in distinguishing between F. moniliforme and F. oxysporum where the isolates of each species were clustered together in the phenogram. These results suggested that RAPD-PCR analysis appeared to be a useful tool for identification and characterization of the genetic differences among Fusarium species and isolates. RAPD-PCR analysis has also been used successfully to study the differences between species, strains and isolates of different fungal genera such as Fusarium spp. (Arif *et al*., 2011), F. solani (Arif *et al*., 2015), F. oxysporum (Assigbetse *et al*., 1994 and Mattila *et al*., 1997), F. avenaceum (Mattila *et al*., 1996), F. acuminatum, F. sporotrichioides and F. tricinctum (Altomare *et al*., 1997), F. subglutinans (Zheng and Ploetz 2002), Beauveria bassiana (Castrillo and Brooks 1998), Pythium ultimum var. ultimum (Tojo *et al*., 1998), Macrophomina phaseolina (Su *et al*., 2001), Verticillium dahliae and V. albo-atrum (Ramsay *et al*., 1996 and Perez-Artes *et al*., 2000), Assigbetse *et al*. (1994) used pathogenicity and random amplified polymorphic DNA (RAPD) markers to assess genetic diversity among 46 isolates of F. oxysporum f. sp. vasinfectum of worldwide origin. These authors suggested that RAPD markers are a quick and reliable method for differentiating isolates of F. oxysporum f. sp. vasinfectum into their respective pathogenicity group.

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