African Journal of Agriculture Vol. 1 (4), pp. 066-069, November, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Genetic affinities of *Fusarim* spp. and their correlation with origin and pathogenicity

Mohmed S. Khalil[1], Mohmed A. Abdel-Sattar[2], Ibrahim N. Aly[2], Kamel A. Abd-Elsalam[1,3*] and Joseph A. Verreet[3]

[1]Agricultural Research Center, Plant Pathology Research Institute, Giza, Egypt. [2]Suez Canal University, Faculty of Agriculture, Ismailia, Egypt.
[3]Christian Albrechts Universität zu Kiel, Institut für Phytopathologie, Kiel, Germany.

Accepted 14 September, 2013

Random amplified polymorphic DNA (RAPD) analyses was used in combination with pathogenicity assays to study the taxonomic kinships among five *Fusarium* species. A total of 46 isolates of *Fusarium* spp. obtained from diseased cotton seedlings showing typical root rot and dampping-off symptoms were characterized. Of 10 primers tested, four primers produced polymorphic amplification patterns with taxon-specific bands, in addition to individual- specific bands. Genetic analysis indicated into 2 main clusters, with the minor cluster included all *F. moniliforme* and *F. solani* at the genetic similarity of GS=57.82%. The major cluster consisted of all *F. oxysporum, F. avenaceum* and *F. chlamydosporum* clustered at 71% similarity. There was no clear-cut relationship between clustering in the RAPD dendrogram, pathogenicity test and geographic origin of tested isolates. The results suggest that RAPD-PCR is a useful method for analysing genetic variation within and between *Fusarium* spp.

Key words: DNA-fingerprinting, *Fusarium chlamydosporum,* genetic homology, RAPD-PCR.

INTRODUCTION

Since *Fusarium* species are among the most common fungi associated with seedling roots of cotton, it is important to determine their effects on seedling growth. These fungal pathogens are frequently isolated from diseased roots of cotton seedlings (Johnson et al., 1978; Roy and Bourland, 1982; Colyer, 1988; Zhang et al., 1995; Aly et al., 1996; El-Samawaty 1999). Pathogenicity tests are cumbersome, time-consuming (50–60 days), require extensive facilities, and are influenced by variability inherent in the experimental system. Furthermore, pathogenicity data alone provide no information about genetic diversity within, or relatedness among, races of the pathogen. Knowledge of genetic diversity is needed for resistance deployment to be effective and to identify shifts in race or population structure that might occur (McDonald, 1997).

In recent years numerous DNA-based fingerprinting methods that reveal the genetic diversity of similar organisms have arisen. Random amplified polymorphic DNA (RAPD) analysis is a fast, PCR-based method of genetic typing based on genomic polymorphisms. A technique widely used to assess inter- and intra-specific genetic variation on a nuclear level is based on the use of random amplified polymorphic DNA (RAPD) markers, produced by the polymerase chain reaction (PCR) (Williams et al., 1990; Welsh and McClelland, 1990). RAPD analysis has been applied widely in the detection and genetic characterization of phytopathogenic fungi (Lanfranco et al., 1995; Miller, 1996; Brown, 1998), including race differentiation in several formae speciales of *F. oxysporu*m, such as *cubense* (Bentley et al., 1994), *dianthi* (Manulis et al., 1994; Migheli et al., 1998), *pisi* (Grajal-Martin et al., 1993), and *vasinfectum* (Assigbetse et al., 1994). Abd-Elsalam et al., (2003) used RAPD markers to study inter- and intra-specific variation of twelve *Fusarium* species isolated from cotton-growing areas in Egypt. In this study, we examined the possibility of identifying and differentiating *Fusarium* spp. isolates using the RAPD method and their association with origin and pathogenicity.

*Corresponding author; phone: (49 431) 880 2993, fax: (49 431) 880 1583, e-mail: kaabdelsalam@msn.com

Abbreviations: RAPD, random amplified polymorphic DNA; GS, genetic similarity; UPGMA, unweighted pair group method using arithmetic.

MATERIALS AND METHODS

Fungal isolates

Forty-six monosporic isolates of *Fusarium* species, reported to be associated with seedlings disease of cotton, were obtained from different geographic locations in Egypt (Table 2).

Pathogenicity test of *Fusarium* spp. on Giza 89

The growth medium consists of 100 g of sorghum grains and 80 ml of tap water in 500 ml glass bottles which were autoclaved for 30

minutes. Isolated inoculum, taken from one- weak-old culture on potato dextrose agar (PDA), was aseptically introduced into the bottle and allowed to colonize the sorghum for 3 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isolate at the rate of 50 g/kg of soil. Infested soil was dispensed in 10- cm-diameter clay pots and these were planted with 10 seeds per pot of cotton cultivar Giza 89. In the control treatment, sterilized sorghum grains were mixed thoroughly with soil at the rate of 50 g/kg of soil. Pots were randomly distributed on greenhouse bench under temperature regime ranging from 25 to 35 C. Preemergence damping-off was recorded 15 days after planting. Postemeergence damping damping-off, survival, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

Genomic DNA extraction

DNA was isolated from mycelial mats of each single- spore isolate grown for 6 days at 28°C in 5ml of potato dextrose broth (PDB) (Difco) in 15-ml Falcon tubes. The mycelial mats were collected by filtration, mats were ground with a mortar and pestle in liquid nitrogen and stored immediately at -20°C. DNA was extracted from these preparations according to the method of Liu et al. (2000).

RAPD using arbitrary primers

For the development of the RAPD method for *Fusarium* isolates, the 10 different 10- mer primers (Kit A, Roth, Germany) (Table 1) were screened for the amplification of template DNA from five isolates of *Fusarium* spp. four primers were selected for final RAPD typing. Since PCR in the RAPD method is affected by the experimental parameters (Tyler et al., 1997). All of the screening steps in this study were performed under the conditions described below. DNA amplifications for the screening of primers or final typing were carried out in buffer (50 mM KCl, 1.5 mM MgCl2 , 10 mM Tris HCl, pH 8.8) containing 200 mM (each) dATP, dCTP, dGTP, and dTTP; 0.50 mM primer; 2.0 U of *Taq* DNA polymerase (Promega); and 50 ng of template DNA. The temperature cycling program used with a thermocycler (Primus 69 HPL, MWG Biotech, Germany) was as follows: 2 initial cycles consisting of 94°C for 4 min, 35°C for 2 min, and 72°C for 2 min, followed by 35 cycles consisting of 94°C for 30 s, 35°C for 1 min, and 72°C for 2 min and a final extension step consisting of 72°C for 5 min. All manipulations were carried out with dedicated DNA-free pipettes in a sterile hood to minimize the risk of contamination.

Table 1. Code and nucleotide sequence of primers used in the random amplified polymorphic DNA (RAPD) reactions, G+C content, and number of polymorphic bands produced in the *Fusarium* spp. isolates studied.

Primer	5'-Sequence-3'	G+C (%)	Polymorphic bands/isolates
01	CAGGCCTTCA	60	3
02	**TGCCGAGCTG**	**70**	**4**
03	**AGTCAGCCAC**	**60**	**4**
04	AATCGGGCTG	60	3
05	AGGGGTCTTG	60	2
06	GGTCCCTGAC	70	3
07	**GAAACGGGTG**	**60**	**4**
08	GTGACGTAGG	60	3
09	**GGGTAACGCC**	**70**	**4**
10	GTGATCGCAG	60	2

Gel electrophoresis

The amplified products were electrophoresed at 75 V in a horizontal 10- by 15-cm 1.5% agarose gel in Tris-borate buffer for about 4.5 h. The amplified DNA bands were visualized after ethidium bromide staining and photographed under UV light. A 100-bp ladder (Promega, Germany) was used as a marker in determining the sizes of the amplification products.

Computer comparisons of RAPD patterns

Digitized RAPD fingerprints were analyzed by using one-dimensional software (Advanced American Biotechnology and Imaging, Fullerton CA 92831, USA). After normalization and background subtraction with mathematical algorithms, the levels of genetic similarity between RAPD patterns were calculated with the Pearson product-moment correlation coefficient (r) (Pot et al., 1994). For cluster analysis of RAPD banding patterns, the unweighted pair group method using average linkages (UPGMA) was used (Vauterin and Vauterin, 1992)

RESULTS

Pathogenicity test

Of the 46 isolates of *Fusarium* spp. tested under greenhouse conditions 38 isolates (82.4%) were pathogenic to seedlings of Giza 89. They included twenty three isolates of *F. oxysporum,* seven isolates of *F. moniliforme,* and five isolates of *F. solani.* Also, two isolates of *F. avenaceum* and one isolate of *F. chlamydosporum* were pathogenic (Table 2) . Only one isolates of *F. moniliforme* was non- pathogenic during pre-emergence stage. Isolate no. 24 of *F. oxysporum* showed peculiar behavior because it significantly reduced pre-emergence damping-off, that is, it significantly improved germination. Thirty-seven isolates were induced post-emergence damping-off cotton seedlings. They included twenty one isolates of *F. oxysporum*, four isolates of *F. solani*, two isolates of *F. avenaceum* and seven *F. moniliforme* isolates. More than 75% *F. oxysporum*, *F. moniliforme* and *F. solani* isolates were pathogenic.

Identification of informative primers

To identify primers that generate informative arrays of PCR products, five *Fusarium* isolates were selected from the entire panel of isolates. They had been isolated from different geographic sites and belonged to different species. Ten oligonucleotides, each 10 nucleotides long, with a G/C content of 60 to 70%, and containing no palindromic sequences, were tested (Table 1). The choice of selected primers was based on the number of bands generated (with as few low-intensity bands as possible) as well as the quantity of different and reproducible patterns yielded. Four primers (2, 3, 7, and 9) were selected because they satisfied the characteristics described above. A set of reproducible bands produced for a particular primer was defined as a "pattern."

Table 2. List of *Fusarium* isolates indicating location and effect of *Fusarium* spp. isolates on Giza 89 cotton seedlings growing in artificially infested soil under greenhouse conditions (1998).

Isolates No.	Locations	*Fusarium* spp.	Damping-off			Plant vigors	
			Pre-emergence (%)[a]	Post-emergence (%)	Survival (%)	Plant height (cm)	Dry weight (g)
1	Dumyat	*F. oxysporum* (**Fo**)	18.00 c-h *[b]	18.00 j-n	64.00 b-g*	24.88 f-h*	257.0 j-s*
2	Gharbiya	*F .oxysporum*	22.00 c-h*	30.00 f-l*	48.00 f-m*	25.45 fg	245.4 l-s*
3	Minufiya	*F. oxysporum*	22.00 c-h*	32.00 g-m*	46.00 g-n*	28.99 b-e	304.2 c-n
4	Gharbiya	*F. oxysporum*	22.00 c-h*	18.00 j-n	60.00 c-i*	27.66 c-f	241.2 o-s
5	Sharqiya	*F. oxysporum*	10.00 g-k	46.00 b-g*	44.00 h-n*	30.14 b-d	274.0 g-r
6	Daqahliya	*F. oxysporum*	12.00 g-k	34 00 d-j *	54.00 f-l*	26.25 e-g	548.0 a
7	Minufiya	*F. oxysporum*	8.00 g-l	44.00 b-h*	48 00 f-m*	36.66 a	500.6 a
8	Gharbiya	*F. oxysporum*	6.00 i-l	34.00 d-g*	60.00 c-i*	31.93 b	328.6 d-h
9	Minufiya	*F. oxysporum*	10.00 g-l	48.00 b-g*	42.00 i-o*	27.08 d-g	375.6 cd
10	Daqahliya	*F. oxysporum*	16.00 e-i*	34 00 d-j *	50.00 f-m*	27.27 d-f	261.4 j-s
11	Fayium	*F. oxysporum*	14.00 f-j*	42.00 c-i*	44.00 h-n*	26.61 e-g	244.2 m-s
12	Beheira	*F. oxysporum*	14.00 f-j*	30.00 f-l*	56:00 e-k*	30.99 b	293.2 f-o
13	Beheira	*F. oxysporum*	6.00 i-l	44.00 b-h*	50.00 f-m*	25.81 e-g	436.6 b
14	Beheira	*F. oxysporum*	14.00 f-j	62.00 a-c*	28.00 n-p*	30.94 b	411.6 bc
15	Beheira	*F. oxysporum*	0.00 l	56.00 a-d*	44.00 h-n*	30.46 bc	267.2 i-r
16	Beheira	*F. oxysporum*	14.00 f-j*	52.00 b-f*	34.00 m-o*	25.19 fg	317.2 e-j
17	Beheira	*F. oxysporum*	6.00 i-l	52.00 b-f*	42.00 i-o*	26.58 e-g	697.6 f-c
18	Beheira	*F. oxysporum*	8.00 g-l	54.00 b-e*	38.00 k-o*	29.95 b-d	333.0 d-g*
19	Beheira	*F. oxysporum*	28.00 b-f	28.00 g-m*	44.00 h-n*	13.13 j	152.6 t*
20	Beheira	*F. oxysporum*	8.00 g-l	26.00 g-m*	66.00 b-f*	23.91 gh	290.0 f-d*
21	Beheira	*F. oxysporum*	10.00 g-l	74.00 a*	16.00 p*	26.80 e-g*	305.2 e-m*
22	Minufiya	*F. oxysporum*	20.00 c-h*	66.00 ab*	14.00 p*	26.36 e-g*	343.6 d-f*
23	Daqahliya	*F. oxysporum*	2.00 kl	44.00 b-h*	54.00 f-l*	27.27 d-f	310.8 e-k
24	Minufiya	*F. oxysporum*	6.00 i-l	16.00 j-n	78.00 ab	24.88 f-h*	300.4 f-o*
25	Minufiya	*F. oxysporum*	22.00 c-h*	18.00 j-n	60.00 c-i*	21.89 h	216.8 rs*
26	Minufiya	*F. oxysporum*	30.00 b-f*	12.00 l-n	58.00 d-j*	24.52 f-h	271.0 h-r*
27	Fayium	*F. oxysporum*	10.00 g-l	10.00 m-n	80.00 ab	23.96 gh*	227.6 q-s*
28	Fayium	*F. oxysporum*	10.00 g-l	14.00 j-n	76.00 a-c	17.77 i*	205.8 s*
29	Sharqiya	*F. moniliforme* (**Fm**)	38.00 a-d*	14.00 j-n	48.00 f-m*	18.95 i*	323.2 d-i*
30	Beheira	*F. moniliforme*	40.00 a-c*	16.00 j-n	44.00 h-n*	13.09 j*	264.8 i-s*
31	Minufiya	*F. moniliforme*	62.00 a	20.00 i-m*	18.00 p*	12.22 j*	283.2 fe
32	Sharqiya	*F. moniliforme*	48.00 ab*	32.00 e-k*	20.00 p*	11..93 j*	307.4 e-k*
33	Gharbiya	*F. moniliforme*	34.00 b-e*	20.00 i-m*	46.00 g-n*	16.97 i*	253.8 k-s*
34	Gharbiya	*F. moniliforme*	40.00 a-c*	22.00 i-m*	38.00 k-o*	18.20 i*	323.0 o-i*
35	Minufiya	*F. moniliforme*	40.00 a-c*	12.00 l-n	48.00 f-m*	17.01i*	244.2 m-s*
36	Minufiya	*F. moniliforme*	50.00 ab*	28.00 g-m*	22.00 p*	6.42 k*	116.6 t*
37	Gharbiya	*F. moniliforme*	36.00 b-e*	24.00 g-m*	40.00 j-o*	16.14 i*	306.8 e-l*
38	Daqahliya	*F. solani* (**Fs**)	4.00 j-l	22.00 i-m*	74.00 a-d*	27.27 d-f	292.4 f-o*
39	Daqahliya	*F .solani*	14.00 f-j*	12.00 l-n	74.00 a-d*	31.93 b	266.2 l-s*
40	Dumyat	*F .solani*	16.00 e-i*	48.00 b-g*	36.00 l-o*	24.52 f-h*	261.8 i-s*
41	Dumyat	*F. solani*	8.00 g-l	28.00 g-m*	64.00 b-h*	31.93 b	271.8 h-r*
42	Dumyat	*F. solani*	16.00 e-i*	34 00 d-j *	50.00 f-m*	30.49 bc	264.8 i-s*
43	Beheira	*F. solani*	10.00 g-l	46.00 b-g*	44.00 h-n*	31.22 b	242.8 n-s*
44	Sharqiya	*F. avenaceum* (**Fa**)	20.00 c-h*	54.00 b-e*	26.00 op*	27.08 d-g	264.8 i-s*
45	Fayium	*F. avenaceum*	8.00 g-l	42.00 c-i*	50.00 f-m*	26.56 e-g*	226.8 q-s*
46	Beheira	*F. chlamydosporum* (**Fc**)	16.00 e-i*	48.00 b-g*	36.00 l-o*	27.25 d-f	230.0 p-s*
47	-------	Control	8.00 g-l	6.00 n	86 a	30.08 b-d	363.2 c-e*

[a] Percentage data were transformed into arsine angles before carrying analysis of virulence.
[b] Values of a column followed by the same letter(s) are not significantly (P 0.05) according to Duncan´s multiple range test.
* an asterisk denotes a significant difference from the control.

Interpretation of RAPD fingerprints performed with selected primers

To investigate the degree of genetic diversity among and within collections of *Fusarium* species, a total 46 isolates comprised of one isolate of *F. chlamydosporum*, two isolates of *F. avenaceum*, six isolates of *F. solani*, nine isolates of *F. moniliforme* and twenty-eight isolates of *F. oxysporum* were analyzed. RAPD patterns amplified with primers 2, 3, 7, and 9 (Figure 1) were clearly differentiated within and between species. The PCR bands were more polymorphic between the species and less variable within the species. Genetic similarity (GS) values obtained among isolates of *F. oxysporum, F. moniliforme* and *F. solani* revealed remarkable differences for inter- and intraspecific comparisons. A

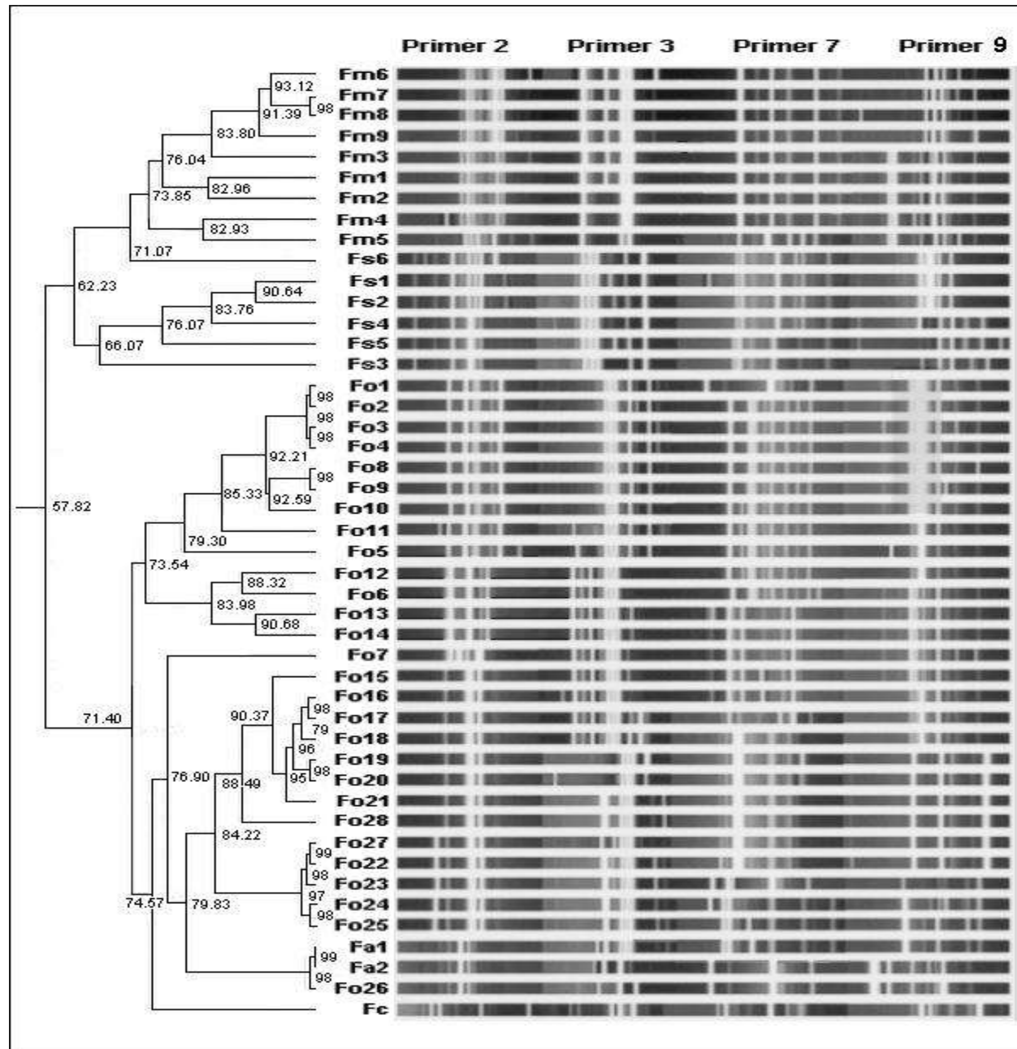

Figure 1. Dendrogram obtained by genomic DNA fingerprinting with the random amplification of polymorphic DNA (RAPD) method. The percentage of genetic homology between banding patterns is indicated. Patterns of *Fusarium* spp. were combined with one-dimensional analysis software (Advanced American Biotechnology and Imaging, Fullerton CA 92831, USA) and grouped with the unweighted pair group algorithm with arithmetic averages (UPGMA).

UPGMA dendrogram is shown in Figure 1. The tree obtained indicated that genetic similarity between these isolates was high (57%). Nine *F. moniliforme* isolates were clustered into the first main cluster, the genetic similarity values between populations of this cluster ranged from 62 to 98%. Isolates Fm7 and Fm8 showed high genetic relatedness (98.91%). Isolates Fs1, Fs2, Fs3, Fs4 and Fs5 were grouped into a separate subcluster in the first main cluster. The second major cluster consist of all *F. oxysporum* isolates and *F. avenaceum* isolates at the genetic similarity of 71.40%, Isolates Fo1, Fo2, Fo3 and Fo4 showed very high genetic similarity of 98.50. Between *Fusarium* spp., similarities ranged from 57 to 71% for inter-specific comparisons. The genetic similarities ranged from 71 to 99% for intra-specific comparisons. High intra-specific variability was observed in *F. oxysporum, F. solani, F. moniliforme* and *F. avenaceum*. No tendency was detected between geographical origin and pathogenicity of isolates and genetic diversity.

DISCUSSION

The advent of DNA-based molecular methods has provided useful tools with which to study the phylogeny of *Fusarium* and to differentiate species, formae speciales, races, and strains. RAPD markers have been previously used to study inter- and intra-specific variation of twelve *Fusarium* species isolated from cotton-growing areas in Egypt (Abd-Elsalam et al., 2003). Donaldson et al. (1995) examined the usefulness of primer sets designed to amplify introns within conserved genes in filamentous ascomycetes to differentiate 35 isolates representing six different species of *Fusarium* commonly found in association with conifer seedlings.

The genetic similarity values of 57% between *F. solani* and *F. oxysporum* isolates in this study are in contrast with that of 20% between *F. solani* and *F. oxysporum* found by Szecsi and Dobrovolsky (1985). We can draw no conclusions for *F. chlamydosporum* because of the limited number of isolates tested. The polymorphisms observed for RAPD markers revealed a high degree of genetic diversity in *Fusarium* spp at the inter-specific level. Donaldson et al. (1995) found that the degree of inter-specific polymorphism observed in the PCR products from the six *Fusarium* species allowed differentiation by a limited number of amplifications and restriction endonuclease digestions. Our findings indicate no clear relationship between the RAPD profiles and pathogenicity tests/geographic origin for *Fusarium* spp., and the conclusions are in accord with these obtained by Abdel-Sattar et al. (2003) and Kiprop et al. (2002). Analyzing the association of specific RAPD markers and aggressiveness did not reveal a clear relation in a world-wide collection of 41 *F. culmorum* isolates (Miedaner et al., 1997). There were also no relationships among pathogenicity and RAPD profiles of *F. avenaceum* (Satyaprasad et al., 2000). Yli-Mattila et al. (1996) also found no correlation between isozymes, RAPD-PCR and UP-PCR patterns on the one hand, and either geographic origin or particular host plants on the other. Nevertheless, in a RAPD analysis of *F. oxysporum*, Assigbetse et al. (1994) reported a correlation between genetic similarity and geographic origin. RAPD analysis is extremely powerful and can separate individuals having intra- and inter-specific variability. It gives more comprehensive information regarding the genetic variability among the pathogen populations as it is based on the entire genome of an organism (Achenback et al., 1997). Our study showed that, RAPD technique is influential tool for discriminating different *Fusarium* species as well as *Fusarium* isolates within species.

REFERENCES

Abd-Elsalam KA, Schnieder F, Khalil MS, Aly AA, Verreet JA (2003). Genetic variation at the intra- and interspecific level in *Fusarium* spp. associated from Egyptian cottons. J. Plant Dis. Prot. 110: 46-53.

Abdel-Sattar MA, Khalil MS, Mohmed IN, Abd-Elsalam KA, Verreet JA (2003). Molecular phylogeny of *Fusarium* species by AFLP fingerprint. Afr. J. Biotechnol. 2: 51-56.

Achenback LA, Patrick JA, Gray LE (1997).Genetic homogeneity among isolates of *Fusarium solani* that cause soybean sudden death syndrome. Theor. Appl. Genet. 95:474-478.

Aly AA, Hussein EM, Mostafa MA, Ismail AI (1996). Distribution, identification, and pathogenicity of *Fusarium* spp. isolated from some Egyptian cottons. Menofiya J. Agric. Res. 4: 819-836.

Assigbetse KB, Fernandez D, Doubios MP, Geiger JP (1994). Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84: 622-626.

Bentley S, Pegg KG, Dale JL (1994). Optimization of RAPD-fingerprinting to analyze genetic variation within populations of *Fusarium oxysporum* f. sp. *cubens*e. J. Phytopathol. 142: 64-78.

Brown JKM (1998). Surveys of variation in pathogen populations and their application to disease control. In: Jones DG (ed) The Epidemiology of Plant Diseases (pp 73-102) Kluwer Publishers, Dordrecht.

Colyer PD (1988). Frequency and pathogenicity of *Fusarium* spp. associated with seeding diseases of cotton in Louisiana. Plant. Dis. 72: 400-402.

Donaldson GC, Ball L.A, Axlrood PE, Glass NL (1995). Primer sets developed to amplify conserved genes from filamentous ascomycetes Are useful in differentiating *Fusarium* species associated with conifers. Theor. Appl. Genet. 61: 1331-1340.

El-Samawaty MA (1999). Studies on cotton root rot disease. MS.c. Thesis. Assiut Univ., Egypt.

Grajal-Martin MJ, Simon CJ, Muehlbauer FJ (1993). Use or Random Amplified Polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f. sp. *pis*i. Phytopathology 83: 612-614.

Johnson LF, Baird DD, Chambers AY, Shamiyeh NB (1978). Fungi associated with postemergence seedling disease of cotton in the soils. Phytopathology 68: 917-920.

Kiprop EK, Baudoin JP, Mwang'ombe AW, Kimani PM, Mergeai G, Maquet A (2002). Characterization of Kenyan isolates of *Fusarium udum* from Pigeonpea [*Cajanus cajan* (L.) Millsp.] by cultural characteristics, aggressiveness and AFLP analysis J. Phytopathol. 150: 517-527.

Lanfranco L, Wyss P, Marzachi C, Bontante P (1995). Generation of RAPD-PCR primers for the identification of isolates of *Glomus mosseae*, an arbuscular mycorrhiza fungus. Mol Ecol. 4: 61-68.

Liu D, Coloe S. Baird R, Pedersen J (2000). Rapid mini-preparation of fungal DNA for PCR. J. Clinic. Microbiol. 38: 471.

Manulis S, Kogan N, Reuven M, Ben-Yephet Y (1994). Use of RAPD technique for identification of *Fusarium oxysporum* f. sp. *dianthi* from carnation. Phytopathology 84: 98-101

McDonald BA (1997). The populations genetics of fungi: tools and techniques. Phytopathology 87: 448-453.

Miedaner T, Gang G, Schilling AG, Geiger HH (1997). Aggressiveness and mycotoxin production of populations of *Fusarium culmorum* and *Fusarium graminearum* in winter rye. Cer. Res. Commun. 25: 471-475.

Migheli Q, Briatore E, Garibaldi A (1998). Use of random amplified polymorphic DNA (RAPD) to identify races 1, 2, 3 and 8 of *Fusarium oxysporum* f. sp. *dianthi* in Italy. Eur. J. Plant Pathol. 104: 49-57.

Miller SA (1996) Detecting propagules of plant pathogenic fungi. Ad. Bot. Res. 23: 73-102.

Pot B, Vandamme P, Kersters K (1994). Analysis of electrophoretic whole-organism protein fingerprints, p 493-521. In Goodfellow M, O'Donnell AG (ed.), Chemical methods in prokaryotic systematics. J. Wiley and Sons, Chichester, United Kingdom.

Roy KW, Bourland FM (1982). Epidemiological and relationships in cotton seedling disease in Mississippi. Phytopathology 72: 868-872.

Satyaprasad K, Batemen GL, Ward E (2000). Comparisons of isolates of *Fusarium avenaceum* from white lupin and other crops by pathogenicity tests, DNA analyses and vegetative compatibility tests. J. Phytopathol. 4: 211-219.

Szecsi A, Dobrovolsky A (1985).Genetic distance in fungus genus *Fusarium* measured by comparative computer analysis of DNA thermal denatueration profiles. Mycopathologia 89: 95-100.

Tyler KD, Wang G, Tyler SD, Johnson WM (1997). Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol. 35: 339-346.

Vauterin LA, Vauterin P (1992). Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Eur. Microbiol. 1: 37-41.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers.Nucleic Acids Res. 18: 6531-6535.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Yli-Mattila T, Paavanen S, Hannukkala A, Parikka P, Tahvonen R, Karjalainen R (1996). Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathol. 45: 126-134.

Zhang J, Howell CR, Starr JL, Weheeler MH (1995). Frequency of isolation and pathogenicity of *Fusarium* species associated with roots of healthy cotton seedlings. Mycol. Res. 100: 747-752.