Egypt. J. Phytopathol., Vol. 38, No. 1-2, pp. 63-75 (2010)

Determination of Genetic Diversity of *Fusarium oxysporum* Isolated from Egyptian Soils by RAPD

H.M. Emara
Botany Dept., Fac. of Sci., Benha Univ., Benha, Egypt.

Fusarium oxysporum is common in soils of Egypt. Wilt is one of the major factors for low productivity of cucumber (*Cucumis sativus* L.). Four Fusarium isolates were isolated from different soils, identified and screened for pathogenicity and genetic variation using RAPD (Random Amplification of Polymorphic DNA) markers. Disease severity and variations in the symptoms were recorded for all isolates. *F. oxysporum* isolates have no typical RAPD profiles and all the four isolates showed a close similarity after the development of a phyllogenetic tree. The RAPD-PCR (RAPD-Polymerase chain reaction) may be valuable in monitoring populations and characterization of different pathotypes of *F. oxysporum*. Clustering analysis based on RAPD fingerprint data revealed two distinct groups within *F. oxysporum*, which often correspond to the origin of the isolate.

Keywords: Cucumber, disease severity, *Fusarium oxysporum*, genetic diversity and RAPD-PCR.

Fusarium oxysporum Schlechtend: Fr. is an anamorphic species with considerable morphological and physiological variations. Most of the interest in this fungus arises from its ability to cause diseases of economically important plant hosts, but its near ubiquity in soils and its ecological activities indicate a much more diverse role in nature. Pathogenic isolates of *F. oxysporum* often display a high degree of host specificity and may be subdivided into formae specials based on the species attacked and into races based on the host cultivars attacked (Brutoon, 1998; Pivonia *et al.*, 1999; Vakalounakis *et al.*, 2004 and Najafinia and Sharma, 2009). Losses caused by vascular wilt showed the necessity to search for an effective plant protection method. However, the identification of isolates is difficult and is based on the assessment of their pathogenicity towards host plants (Rataj-Guranowska and Pieczul, 2002 and Rataj-Guranowska *et al.*, 2007). Currently, molecular diagnostics based on an analysis of subtle differences in DNA sequence offer sensitive means for identification and characterization of fungi (Martin *et al.*, 2000; Taylor *et al.*, 2001; Shokoohi *et al.*, 2004 and Irzykowska, 2006). Application of molecular markers eliminates doubts, which appear during classical research based on morphology evaluation. Molecular studies conducted with different techniques showed that there were inter-specific variations within the genus *Fusarium* and intra-specific differences within *F. oxysporum* (Kistler, 1997). The use of resistant cultivars was one of the most practical and cost-effective strategies for managing Fusarium wilt, but deployment of resistant varieties had not been extensive because of undesirable agronomic characteristics (Jimenez-Gasco *et al.*, 2004). Moreover,

the high pathogenic variability in *F. oxysporum* may limit the effectiveness of resistance (Jimenez-Diaz *et al.*, 1993 and Jimenez-Gasco *et al.*, 2004). The study of structure of pathogen population is pre-requisite for designing a cost effective management strategy for such a devastating disease. Since this disease was primarily managed by the use of resistant cultivars, characterization and identification of pathogenic *F. oxysporum* in a given area has been important for disease resistance breeding and for the effective use of cultivars (Jimenez-Gasco *et al.*, 2001). Therefore, this study was carried out to: (I) identify the different isolates of *F. oxysporum* isolated from different soils, (ii) examine the genetic variability among these isolates, and (iii) determine the geographical distance that could greatly affect the structure in these isolates.

Materials and Methods

Soil analysis:

Four soil samples were collected from different locations (Ismailia, Nobaria, Qualubia, and Fayoum) in Egypt. These soil samples were gathered at 15 cm depth from cultivated vegetable fields for chemical and physical analysis.

Isolation and identification of Fusarium isolates:

For fungal isolation, soil samples were air-dried and passed through a 200-µm sieve. Three samples (10 g per each) were taken. For each sample, four sub-samples (10 mg per each) were spread over Komada's selective medium (Komada, 1975) and were incubated under fluorescent light at room temperature (20-25°C) for 7 days. Fusarium colonies were identified by using cultural characteristics and they were identified as *F. oxysporum*. Then, they were transferred to potato dextrose agar slants (PDA) for further use. Species identification was also confirmed with microscopic observation according to the morphological characteristics described by Nelson *et al.* (1983).

Pathogenicity test:

Artificial inoculation was performed under greenhouse conditions. The fungal inocula representing the different samples were used to infest the sterilized soil in pots with 5% (W/W). Fusarium isolates were separately grown on sand wheat-medium in Erlenmeyer flasks (500ml) and collected after two weeks. Sterilized seeds of cucumber var. Beta alpha (with 1% sodium hypochlorite for 2 min.) were sown in the pots (three pots/treatment and 10 seeds per pot) and three non-inoculated pots were served as control. The pots were watered regularly for 12 weeks, and no fertilizers were applied. After 20 days of sowing, the plants were examined and the symptoms were recorded and divided into 5 groups (Table 1).

Table 1. Grouping of symptoms in pathogenicity test

Code of virulence	Symptoms
0	No symptoms
1	Slight chlorsis of cotyledonary leaves
2	Yellowing of the two first leaves
3	Yellowing, wilt and vascular brown discoloration
4	Complete wilt and plant death

DNA extraction:

DNA isolation was performed by using the CTAB method (Doyle and Doyle, 1990). The growth was collected from colony by pipeting 50 µl of triton x 100 up and down several times over the same spot on the plate. The growth/triton x 100 was mixed on 500 µl of CTAB buffer to 1:5 ml tubes by using vortex (Disruptor Genic) for 2 min and incubated at 65°C for 15 min. After that, one volume of phenol/chloroform vortex 1 min was added, and then centrifuged at 12000 g at room temperature for 5 min. To separate phases, we transferred the upper phase (≈ 1ml) to new 1.5-ml tube. Then, added 0.6 volume of isopropyle alcohol and 1/10 volume of 3M NH_4OAc and incubated on ice for 30 min. then centrifuged at 12000 g at room temperature for 15 min to pellet the DNA. The DNA pellet was washed carefully twice with 500µl of cold ethanol (70%), dried at room temperature, and resuspended in 30 µl distilled water. DNA was purified by the incubation of the resuspended sample at 37°C for 30 min with RNase (Borhringer Mannheim). DNA was checked out by using 0.8% agarose gel electrophoresis (Castiglione *et al.*, 1994).

RAPD-PCR test:

DNA amplification was performed in 30 µl react mixture containing 1.0 µl template DNA (25 µg), 0.2 µl taq DNA polymerase (unit), 3.0 µl dNTPs (25 mol of each dATP, dCTP, dGTP, dTTP) 2.0 µl $MgCl_2$ (25 mM), 3.0 µl PCR buffer and 2.0 µl random primers (25 p mol.) and 18.8 µl d-H_2O (Table 2). The mixture was assembled on ice overlaid with a drop of mineral oil. The amplification was carried out in DNA thermal cycler (MWG-BIOTECH Primuse) programmed as follows: One cycle at 94°C for 4 min and then 40 cycles at 94°C for 30 sec., 35°C for 1 min and 72°C for 2 min. One cycle at 72°C for 5 min, then kept at 4°C for long .The products were obtained by 2% agrose gel.

Table 2. Sequences of the primers used in RAPD-PCR test

Code*	Sequence (5'– 3')
OPA_{13}	CAGCACCCAC
OPD_8	TTGGCACGGG
OPE_{20}	AACGGTGACC

* Primer provided by OP technology (USA)

Gel electrophoresis analysis:

All electrophoresis was carried out by using a pharmacia GN-100 submurine gel electrophoresis apparatus 1% agarose gel in TAE buffer was prepared and a total sample volume of 6 µl (4 µl d-H_2O, 1 µl 6x loading dye, and 1µl of PCR product) was loaded in each well. The gel was electrophoresed in 65 V for 1.5 hour and then stained with ethidium bromide solution (10 mg/ml) for around 10-15 min. DNA bands were visualized by UV transilluminator and photographed on UVP Laboratory products (Epichemi 11 Dark room 3 UV transilluminator pharmacia).

Fragment analysis:

Sharp PCR fragments were scored according to their presence or absence and fragments at low intensities were only scored as present when they were reproducible in repeated experiments by using Gel Analyzer 3 (Egygene Co., Egypt) software.

Cluster analysis:

Data of the similarity matrix were used for cluster analysis by using the unweighed pair-group method with arithmetic averages (UPGMA), [(NTSYS-pc 2.02 software package; Numerical Taxonomy System, Exeter Software) (Rohlf, 2000)]. Bootstrap analysis with 1000 replications was used as Winboot software (Yap and Nelson, 1996).

Results

Physico-chemical properties of soil samples:

The texture of soil collected from Ismailia, Nobaria, Qualubia, and Fayoum were sand, clay, clay, and sandy clay loam with pH values ranged from 6.5 to 8.25, respectively. Soil salinity showed considerable variations among the tested soils where Fayoum soil was the highest (EC= 11. 5 dS/m, in soil paste extract) compared with the other soils. Fayoum soil showed also the highest Na, Ca, and K contents (Table 3).

Table 3. Physico-chemical properties of soils' samples collected from different locations in Egypt

Province	EC	CO_3	Cl^-	Soluble cations (g/kg)			pH	ESP	Mechanical characters				Texture
				Na^+	Ca^{++}	K^+			Sand (%)	Clay (%)	Silt (%)	$CaCO_3$	
Ismailia	0.85	0.50	3.75	3.00	5.50	0.52	6.50	2.25	70.25	21.25	3.50	0.50	Sandy
Nobaria	4.75	0.30	30.00	15.5	14.5	1.20	8.00	12.00	25.80	28.5	2.25	15.50	Clay
Qualubia	5.25	0.25	3.50	2.50	2.50	1.75	7.50	3.50	15.75	27.75	2.50	0.75	Clay
Fayoum	11.50	0.35	70.25	80.25	15.75	3.00	8.25	3.75	26.52	15.25	4.00	25.21	Sandy clay loam

EC: Electrical conductivity (m mhos cm^{-1} 25°C).
pH: The hydrogen ion concenfration (soil water suspension 1:25).
ESP: Exchangeable sodium (%)= Exchangeable sodium meq./Cations exchange capacity (meq./100 g soil).

Characteristics of Fusarium isolates:

Four isolates of Fusarium were obtained from cultivated fields with various vegetable crops including cucumber. They were identified as *F. oxysporum* according to the system described by (Nelson *et al.*, 1983). Under optimal growth conditions, the isolates formed pigmented colonies, showed a loose internal net of hyphae without mucilage which were called "open mesh". These colonies were morphologically and physiologically homogenous. Active growing hyphae were distributed in the colonies, no lysing hyphae, and more conidia were observed. Light

microscopic examinations revealed branched hyphae, shorter phialides in the aerial mycelium, micro conidia usually comma shaped or ellipsoidal, macro conidia of type C, straight and chlamydosdors usually produced singly or in pairs. The morphological characteristics of isolates were very similar to the reference.

Pathogenicity test:

All the tested Fusarium isolates caused the wilt symptoms on cucumber, cv. Beta alpha, but the level of virulence of each isolate differed effectively (Table 4). The highest virulence on the infected plants was observed by isolate F1, which caused complete wilt and plant death, while no symptoms were appeared on control plants. On the other hand, no relationship was observed between the origin of isolates and virulence.

Table 4. Reaction of cucumber plants (Beta alpha) to four isolates of *F. oxysporum* under artificial inoculation

Tested isolate	Pathogenicity test				Virulence*
	No. of diseased plants	Death (%)	No. of healthy plant	Survival (%)	
Ismailia	30	100	0	0	4
Nobaria	27	90	3	10	3
Qualubia	24	80	6	20	3
El-Fayoum	24	80	6	20	3
Control	0	0	30	100	0

* Mean disease severity value, was assessed with 0-4 scale.

DNA amplification and polymorphism of F. oxysporum isolates:

DNA samples prepared before RAPD-PCR amplification were found crucial for fingerprint of four *F. oxysporum* isolates. The purity of DNA genome samples as indicated by A_{280}/A_{260} ratio was 1.8. The quality of genomic DNA extracted (as described here) was a good template for PCR amplification. However, DNA treated with RNase gave sharp and clear amplification products. Ten random screened primers (Operon random primer) were surveyed. For the reproducibility of RAPD primers, two or three independent experiments were performed for each primer. Three primers namely OPA_{13}, OPD_8 and OPE_{20} were more stable and reproducible and gave sufficient polymorphism among the tested Fusarium isolates out of ten primers. Therefore, efforts were focused on these primers. The distribution of the polymorphic bands, (which were generated by using three selected primers among four *F. oxysporum* isolates) is summarized in Tables (5 and 6) and (Fig. 1). The results reveal a total of 100 amplified fragments (34, 33 and 33 genetic markers, by OPA_{13}, OPD_8 and OPE_{20}, respectively). Primer OPD_8 showed four polymorphic amplified fragment (PAF) bands of Fusarium isolate (F_3) with 3054, 2975, 775 and 475bp and 57% polymorphic as well as three monomorphic amplified fragments (MAF) of 4 Fusarium isolates with 1630, 1125, and 850bp. The primer OPL_{20} showed 6 PAF, (2, 3 and 1, for F_1, F_2 and F_3 isolates and with 25, 33, and 12.5% polymorphic respectively) as well as one MAF among Fusarium isolates with 1018bp.

Table 5. Polymorphism and polymorphic percentage of four Fusarium isolates

Tested Fusarium isolate	Total bands	OPD_8				OPE_{20}				OPA_{13}			
		TAF	MAF	PAF	Polymorphic (%)	TAF	MAF	PAF	Polymorphic (%)	TAF	MAF	PAF	Polymorphic (%)
F_1	25	9	9	-	0	8	6	2	25	8	7	1	12.5
F_2	26	9	9	-	0	9	6	3	33	8	7	1	12.5
F_3	24	7	3	4	57	8	7	1	12.5	9	8	1	11.1
F_4	25	8	8	-	0	8	8	-	0	9	8	1	11.1
Polymorphism (%)	100	33	29	4	12.12	33	27	6	18.18	34	30	4	11.76

TAF : Total amplified fragments.
MAF: Monomorphic amplified fragments or common amplified fragments.
PAF : Polymorphic amplified fragments or specific amplified fragments.

Table 6. Genetic markers of four *F. oxysporum* isolates by RAPD analysis using PCR technique

bp	OPD_8				OPE_{20}				OPA_{13}				
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	Polymorphism
4525	-	-	-	-	-	-	-	-	+	-	-	-	Unique
3054	-	-	+	-	-	-	+	+	-	+	+	+	Polymorphic
2975	-	-	+	-	-	-	-	-	-	-	-	-	Unique
2036	+	+	-	+	-	+	+	+	-	+	+	+	Polymorphic
1985	+	+	-	+	+	+	-	-	-	-	-	-	Polymorphic
1850	-	-	-	-	-	-	-	-	-	+	-	-	Unique
1630	+	+	+	+	+	+	-	-	-	-	-	-	Polymorphic
1595	-	-	-	-	-	-	+	+	+	+	+	+	Polymorphic
1350	-	-	-	-	-	-	-	+	+	-	+	+	Polymorphic
1250	+	+	-	+	+	+	-	-	-	-	-	-	Polymorphic
1125	+	+	+	+	+	+	-	-	-	-	-	-	Polymorphic
1018	+	+	-	+	+	+	+	+	+	+	+	+	Monomorphic
952	-	-	-	-	-	-	-	-	+	-	-	+	Polymorphic
920	-	-	-	-	-	-	+	+	-	+	+	+	Polymorphic
875			-			-	+	+	+	+	+	+	Polymorphic
850	+	+	+	+	-	+	-	-	-	-	-	-	Polymorphic
775	-	-	+	-	+	-	-	-	-	-	-	-	Polymorphic
725	-	-	-	-	-	-	+	+	+	+	-	-	Polymorphic
550	-	-	-	-	-	-	-	-	-	-	+	-	Unique
506	+	+	-	+	-	-	-	-	-	-	-	-	Polymorphic
475	-	-	+	-	-	-	-	-	-	-	-	+	Polymorphic
450	+	+	-	-	-	+	-	-	-	-	-	-	Polymorphic
420	-	-	-	-	+	-	-	+	+	-	+	-	Polymorphic
396	-	-	-	-	-	-	+	-	-	-	-	-	Unique
315	-	-	-	-	+	-	-	-	-	-	-	-	Unique
No. of markers	9	9	7	8	8	8	8	9	8	8	9	9	

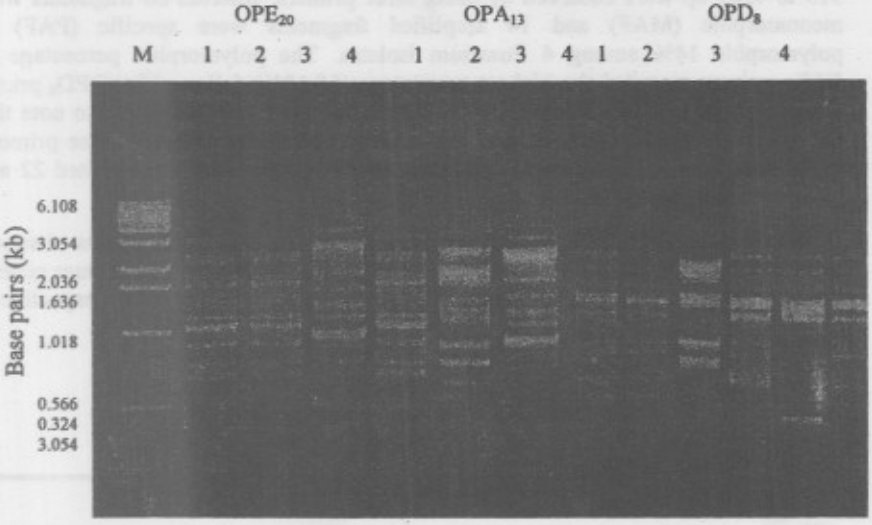

Fig. 1. The bands that have been created by using three primers RAPD-PCR test.

The primer OPA_{13} showed 4 PAF one marker for each of Fusarium isolates with 4525, 1850, 550 and 725 bp for F_1, F_2, F_3 and F_4, respectively, as well as 3 MAF among four Fusarium isolates with 1545, 1918 and 875 bp. Meanwhile, 14 PAF were detected in four Fusarium isolates with polymorphism 14% and 86 MAF with 86% polymorphism (Table 7).

Table 7. Genetic markers, polymorphism and polymorphic of four Fusarium isolates using RAPD-PCR

Primer	Polymorphism *				Genetic marker of Fusarium isolates			
	TAF	MAF	PAF	M.W (bp)	F_1	F_2	F_3	F_4
OPD_8	33	29	4	3054			+	
				2975			+	
				775			+	
				475		+	+	
OPE_{20}	33	27	6	850		+		
				775	+			
				506		+		
				450		+		
				396			+	
				215	+			
OPA_{13}	34	30	4	4525	+			
				1850		+		
				550			+	
				475				+
Total	100	86	14	-	3	4	6	1
Polymorphism (%)	-	86	14	-	3	4	6	1

* TAF, MAF and PAF: as described in footnote of Table (5).

A total of 100 revealed scorable amplified DNA fragments ranging in size from 315 to 4525 bp were observed by using three primers, whereas 86 fragments were monomorphic (MAF) and 14 amplified fragments were specific (PAF) or polymorphic 14% among 4 Fusarium isolates. The polymorphic percentage of OPE_{20} primer recorded the highest percentage (18.18%) followed by OPD_8 primer with percentage of 12.12% and OPA_{13} with 11.76%. It was interesting to note that the four *F. oxysporum* isolates differed in their characters using the three primers. Whereas isolate F_4 exhibited 23 markers, other Fusarium isolates exhibited 22 and 18 for F_1 and F_3, respectively.

In cluster analysis based on genetic distance, the shortest distance was observed in F1 and F3 from Ismailia and Qualubia provinces. However the maximum genetic distance was observed in F2 and F4 from Nobaria and Fayoum, respectively, (Fig. 2).

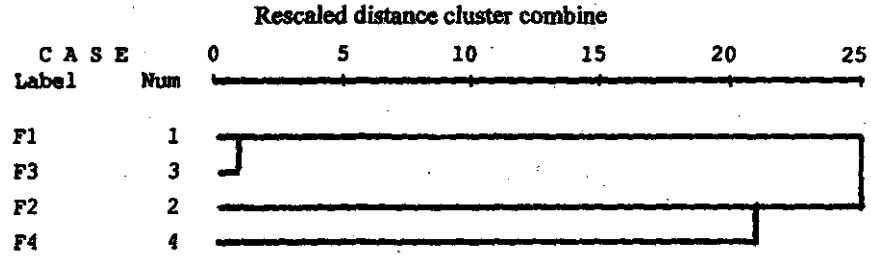

Fig. 2. Dendrogram of compinated data of primers showing relationships among four *F. oxysporum* isolates. 1- *F. oxysporum* F_1 isolate, 2- *F. oxysporum* F_2 isolate, 3- *F. oxysporum* F_3 isolate and 4- *F. oxysporum* F_4 isolate.

Discussion

Soils play an important role in transmission of pathogens, carrying pathogens to cultivated areas and disease distribution. Isolates with some morphological and microscopical characteristics had been placed in different groups. Therefore, morphological characters were not assured factors for distinguishing the isolates. The pathogenicity test was performed for the four *F. oxysporum* isolates and the obtained results indicated that these isolates differed according to their aggressiveness, infection percentage on cucumber and subsequently the harmful effect on the survival of healthy plants. The current findings are similar to those reported by Vakalounakis *et al.* (2004), Shafagh *et al.* (2008) and Najafinia and Sharma (2009). The pathogenicity test also revealed that *F. oxysporum* isolate (F_1) was the most virulent isolate in increasing the infection percentage and subsequently decreasing the healthy survival of cucumber plants.

Little is known about the genetic complexity of *F. oxysporum* populations in Egypt or about the origin and spread of the pathogen, *i.e.* whether it occurs as a single lineage in one location or occurs independently in several locations. Random amplified polymorphic DNA (RAPD) analysis has many advantages as a means of characterizing genetic variability such as speed, low cost, minimal requirement for DNA and non-use of radioactivity (Williams *et al.*, 1990). Genetic diversity among genotypes could occur due to the variation in genes nature that might be affected by environmental stresses (Nei, 1972; Prasad and Singh, 1986). Three random primers gave reproducible and very stable results in an unexpected way compared to the same species found in other locations. The other primers did not always give the exact fingerprints for the tested Fusarium isolates. Accordingly, it might be suggested to use bulked DNA samples to different species to eliminate intraspecific variations. The dendrogram of the four Fusarium isolates by RAPD showed two cluster groups. Clusters of the first group (F_1 and F_3) were isolated from Ismailia and Qualubia, respectively .Those for the second group (F_2 and F_4) were isolated from Nobaria and Fayoum, respectively. This show that each of F_1 and F_3 as well as those of F_2 and F_4 strains are monophyletic, but the two groups of Fusarium are polyphyletic to each other. Genetic similarity between each of the isolates calculated by using cluster analysis was used to generate a dendrogram by showing relationship between them. The isolates could be grouped into two subpopulations based on RAPD analysis. The results indicate little genetic variability among subpopulations of *F. oxysporum* as identified by RAPD markers and that there is a slight relationship with geographical origin or aggressiveness of isolates. The high amount of genetic diversity resulted virtually in two clusters of clearly identified regions of pathogenicity of isolates. Therefore, a region could not be used for determining genetic diversity as suitable measurement.

Using the RAPD technique, *F. oxysporum* produced clear reproducible and polymorphic bands that allowed the characterization of isolates to be examined in this study. In cluster analysis based on genetic distance, the shortest distance was observed in F_1 and F_3 from Ismailia and Qualubia provinces, whereas the maximum genetic distance was observed in F_2 and F_4 from Nobaria and Fayoum, respectively. Although isolates F_1, F_2, F_3, and F_4 were derived from different regions, all of them were settled in two genotypic groups. However, cluster analysis of the RAPD banding pattern, revealed a substantial amount of genetic diversity among all isolates examined. Analysis of cluster showed two genotypic groups taking into consideration the fact that all pathogenic isolates belong to *F. oxysporum*. According to the results, all *F. oxysporum* isolates obtained in this study showed similar RAPD profiles. Other researchers (Migheli *et al.*, 1997 and Khalil *et al.*, 2003) observed a close relationship between RAPD pattern and geographic location. Major polymorphisms in RAPD pattern indicated to genetic distinctness that could be used to distinguish unrelated groups. Minor polymorphisms may indicate a genetic distinctness within groups because of experimental variability that might have occurred. RAPD analysis had been used effectively to distinguish between species of *Fusarium* (Voigt *et al.*, 1995 and Yli-Mattila *et al.*, 1996).The unique band might need further work for sequencing of gene. Sequencing would provide more information about the regions from which the differences observed in

RAPD-PCR. These bands would also show whether the sequences have similarities to any known genes (Paran and Michelmore, 1993; Vakalounakis *et al.* 2004 and Shafagh *et al.* 2008). The current results suggest that molecular genetics techniques could be used to separate these four phenotypes. Furthermore, these techniques could be used to compare the phylogenetic relationships of virulent *Fusarium* spp. isolates from Egypt. These techniques and tools could also determine these markers variation in Fusarium for their precise classification, which may aid in disease management. The present study could generate significant information in terms of pathogenic and genetic variability of *F. oxysporum*, which could be used further for development of area-specific resistant varieties of cucumber. The study also highlighted the facts that both pathogenic virulence analysis and RAPD markers could be useful tools for analyzing the structure of the pathogen population, but further studies are needed to make them complementary to each other.

References

Brutoon, P.D. 1998. Soil borne disease in cucurbitaceae; pathogen virulence and host resistance. Pp. 143-166. In: *Cucurbitace*. J. McCrirgt (ed.), Amer. Soc. of Hort. Sci., Alex., VA, USA.

Castiglione, S.; Wang, W.; Bao, P.; Li, W.; Giordoni, E.; De Stanchina, E.; Domiania, G.; Bandi, C.; Bldoffi, S. and Sala, F. 1994. RAPDs for the assessment of DNA plasticity and genetic diversity. *Current Topics in Molecular Genetics*, **2**: 219-243.

Doyle, J.J. and Doyle, J.L. 1990. Isolation of DNA from fresh tissues. *Focus*, **12**: 13-15.

Irzykowska, L. 2006. Markery molekularne w diagnostyce chorób podstawy źdźbła i korzeni zbóż. *Post. Nauk Roln.*, **6**: 31-40.

Jimenez-Diaz, R.M.; Alcala-Jimenez, A.R.; Hervar, A. and Trapero-Casas, J.L. 1993. Pathogenic variability and host resistance in the *Fusarium oxysporum* f.sp. *ciceris/Cicer arietinum* pathosystem. 3rd Proc. Europ. Seminar: Fusarium-mycotoxins Taxonomy, Pathogenicity and Host Resistance, pp. 57–94.

Jiménez-Gasco, M.M.; Pérez-Artés, E. and Jiménez-Díaz, R.M. 2001. Identification of pathogenic races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f.sp. *ciceris* with random amplified polymorphic DNA (RAPD). *Europ. J. Plant Pathol.*, **107**: 237-248

Jimenez-Gasco, M.M.; Navas-Cortes, J.A. and Jimenez-Diaz, R.M. 2004. The *Fusarium oxysporum* f.sp. *ciceris/Cicer arietinum* pathosystem:a case study of the evolution of plant-pathogenic fungi into races and pathotypes. *Int. Microbiol.*, 7: 95-104.

Khalil, M.S.; Abdel-Sattar, M.A.; Aly I.N.; Abd-Elsalam, K.A. and Verreet, J.A. 2003. Genetic affinities of *Fusarium* spp. and their correlation with origin and pathogenicity. *African J. Biotechnol.* **2**: 109-113.

Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology*, **87**: 474-479.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Protec. Res.*, **8**: 114-124.

Martin, R.R.; Delano, J. and Lévesque, C.A. 2000. Impact of molecular diagnostic technologies on plant disease management. *Annu. Rev. Phytopathol.*, **38**: 207–239.

Migheli, Q.; Briatore, E.; Andrina, M. and Garibaldi, A. 1997. Physiological race determination in *Fusarium oxysporum* f.sp. *dianthi* by RAPD-PCR. Pp. 203–206. In: *Diagnosis and Identification of Plant Pathogens*. H.W. Dehne (ed.). Kluwer, Dordrecht.

Najafinia, M. and Sharma P. 2009. Development of specific PCR-based marker for identification of pathogenic groups of *Fusarium oxysporum* f.sp. *cucumerinum* in India. *J. Phytopathol.*, **157** (11-12): 776.

Nei, M. 1972. Genetic distance between populations. *The Amer. Naturalist*, **106**: 283-292.

Nelson, P.E.; Toussoun, T.A.; Marasas, W.F.O. 1983. *Fusarium species. An Illustrated Manual for Identification*, Pennsylvania State Univ., Univ. Park, PA., USA.

Paran, I. and Michelmore, R.W. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical Applied Genetics*, **85**: 985-993.

Pivonia, S.; Cohin, R.; Katon, G. and Levita, R. 1999. Effect of soil temperature on the development of sudden wilt of melons. *Phytoparasitica*, **27**: 42-43.

Prasad, S.K. and Singh T.P. 1986. Heterosis in relation to genetic divergence in maize (*Zea mays* L.). *Euophitica*, **35**: 919-924.

Rataj-Guranowska, M. and Pieczul, K. 2002. Charakteryzowanie form specjalnych *Fusarium oxysporum*. *Post. Nauk Roln.*, **1**: 33-44.

Rataj-Guranowska, M.; Pieczul, K.; Wach, I. and Saniewska, A. 2007. Limited cross-infectivity and vegetative incompatibility between *Fusarium oxysporum* f.sp. *callistephi*, *F. oxysporum* f.sp. *dianthi*, *F. oxysporum* f.sp. *narcissi* and *F. oxysporum* f.sp. *tulipae*. *Phytopathol. Pol.*, **43**: 77-92.

Rohlf, F.G. 2000. NTSYSpc: Numerical Taxonomy and Multivariate Analysis System, version 2.02. Setauket (New York), Exeter Publishing.

Shafagh, N.; Rastegar, M.F. and Tafarpour, B. 2008. Physiological race and genetic diversity determination of *Fusarium oxysporum* f.sp. *melonis* by differential hosts and molecular marker RAPD in Northern and Razavi Khorasan provinces. *Res. J. Biol. Sci.*, **3**: 790-793.

Shokoohi, F.F.; Bagheri, A. and Rastegar, M. 2004. Genetic diversity determination of causal agent of pea blight (*Ascochyta rabiei* (Pass) Lab.) in Iran with molecular marker RAPD. *Sci., Agric. and Hort.*, 7: 193-203.

Taylor, E.; Bates, J., Kenyon, D.; Maccaferri, M. and Thomas J. 2001. Modern molecular methods for characterization and diagnosis of seed-borne fungal pathogens. *J. Plant Pathol.*, **83**: 75-81.

Vakalounakis, D.J.; Wang, Z.; Fragkiadakis, G.A.; Skaracis, G.N. and Li, D.B. 2004. Characterization of *Fusarium oxysporum* isolates obtained from cucumber in China by pathogenicity, VCG and RAPD. *Plant Dis.*, **88**: 645-649.

Voigt, K.; Schleier, S. and Brückner, B. 1995. Genetic variability in *Gibberella fujikuroi* and some related species of the genus *Fusarium* based on random amplification of polymorphic DNA (RAPD). *Curr. Genet.*, **27**: 528-535.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers as useful genetic markers. *Nucleic Acids Res.*, **18**: 6531-6535.

Yap, I.V. and Nelson, R.J. 1996. Winboot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion Paper Series No. 14, Internat. Rice Res. Inst., Manila, Philippines.

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

(Received 06/03/2010; in revised form 12/05/2010)

تقدير التنوع الوراثي لعزلات الفطر فيوزاريوم اكسيسبورم المعزولة من الأراضي المصرية باستخدام تفاعل البلمرة المتسلسل ببوادئ عشوائية

حسن محمود عمارة
قسم النبات - كلية العلوم - جامعة بنها.

الفطر فيوزاريوم اكسيسبورم واسع الانتشار في الأراضي المصرية وعادة ما يعتبر من الفطريات القاطنة في التربة المصرية، كما يعتبر الذبول الذي يسببه احد الأمراض الهامة التي يعاني منها الخيار. ونظرا لأهمية هذا الفطر فقد تم جمع عدد من عزلاته من أراضي تمثل أربع محافظات هي الإسماعيلية والنوبارية والفيوم والقليوبية وهي ذات نوعية مختلفة من الأراضي. وقد تم تعريف العزلات واختبار قدرتها الامراضية على نباتات الخيار حيث أظهرت اختلافا واضحا في ذلك. أيضا تم تسجيل شدة المرض والاختلافات في الأعراض المرضية لكل العزلات وأيضا تم تقدير الاختلافات الوراثية بين العزلات. وقد أوضح الاختبار باستخدام تكنيك RAPD أن العزلات ليست متماثلة وأن الاختلاف يضعهم في مجموعتين وراثيتين يتوافقان مع العزلة الأسلية التي نشأ منها وذلك بعد دراسة شجرة التشعب. وهكذا فإن استخدام الـ RAPD-PCR يمكن أن يكون ذو أهمية في تحديد الصفات المرضية لعزلات الفيوزاريوم أوكسيسبورم.