International Journal of Agriculture Innovations and Research
Volume 4, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 14/02/2016 | Accepted on : 26/02/2016 | Published : 16/03/2016

Genetic Diversity Among Isolates of *Fusarium* spp Causing Root Rot and Damping off Chick-Pea

Gamal I.A. Mohamed
Genetic department, Faculty of Agriculture, Assiut University, Assiut-Egypt

Gamal Ali Ahmed Mohamed
Plant Pathology department, Faculty of Agriculture, Assiut University, Assiut-Egypt

M.S. Mohamed
Plant Pathology department, Faculty of Agriculture, Assiut University, Assiut-Egypt

Heidi. I.G. Abo-Elnaga
Plant Pathology department, Faculty of Agriculture, Assiut University, Assiut- Egypt

A. Mahran
Plant Pathology department, Faculty of Agriculture, Assiut University, Assiut- Egypt

***Abstract* – Species of *Fusarium* causes root rot and damping-off diseases in chickpea in Egypt. Twenty three *Fusarium* isolates were collected from diseased chick pea plants with root rot and damping off symptoms from different chickpea fields located at Assiut governorate in Egypt and they were tested for their pathogenicity. All tested isolates were infected chickpea plants of Giza 3 cultivar and caused root rot and damping off. The tested fungal isolates were varied in their virulence. *Fusarium oxysporum* isolate Nr 13 caused the highest percentage of post emergence damping off and root rot (40% and 100%, respectively) followed by *F. oxysporum* Nr 6 (30% and 92.5%, respectively). The lowest percentage of pre and post emergence damping off and root rot showed with *Fusarium oxysporum* isolate Nr 17 (30%, 2% and 31.3%, respectively) followed by *F. solani* Nr 19 (28%, 6% and 32.5%, respectively). The isolates were paired in all possible combinations for determined vegetative compatibility and non-compatibility between the isolates of *Fusarium* sp. where some isolates shown compatible and incompatible reactions with all other isolates on CZ medium at 28 °C. While, some isolates shown an exciting result, where it shown self incompatibility reaction. Only nine isolates, belong to *Fusarium oxysporum*, *Fusarium solani* and *Fusarium moniliform* were subjected to molecular investigation. Polymorphism and genetic similarity among the nine *Fusarium* fungi isolates revealed by RAPD markers were obtained using three arbitrary oligonucleotide primers. The number of the amplified fragments per primer varied between one and seventeen giving a total of 192 bands in all samples, with an average 64 bands per primer. These fragments have a size ranged from 4206 to 94 base pairs. Only two bands from a total number of 192 amplified bands were being monomorphic and the other 190 bands were polymorphic with a polymorphism ratio of 98.96 %. The primers used generated unique distinct band which could be used to distinguish the isolates in respect of their species and virulence. Fragments with molecular weight of 2698 bp and 1060 bp could be used as genetic markers (absent and present, respectively) to distinguish *F. oxysporum* Nr 13, which considered the sever pathogenic isolate. Also fragments of 1683, 1336 and 389 bp were present only as a positive marker in *F. solani* Nr 18, which considered the sever pathogenic isolate. The genetic relationships between the nine isolates of *Fusarium* was divided all samples into three main clusters as shown in phylogenetic tree. The results confirming that the evolution direction draws line from *F. moniliform* toward *Fusarium oxysporum*. As well as, the results asserted that RAPD-PCR technique is useful tool for differentiating between species of the genus *Fusarium*.**

***Keywords* – *Fusarium sp*, Fusarium Pathogenicity, Vegetative Compatibility, Fusarium Fingerprinting and RAPD-PCR of Fusarium.**

I. INTRODUCTION

Several species of *Fusarium* survive in fruiting bodies in the soil. These fungi causes root rot and damping-off diseases (Nene and Reddy 1987).

Damping-off and root rot caused by *Fusarium oxysporum, Fusarium solani* and *Fusarium moniliforme* are considered sever diseases in chickpea in Egypt as well as all the world (Noher *et al*., 2009; Sumanti *et al*., 2009; Khetarpal *et al*., 2009; Singh *et al*., 2010; Iqbal *et al*., 2010 and Meki *et al*., 2011). Khan and Abdul Jabbar (2002) tested 20 isolates of *F. oxysporum* from Pakistan for its virulent to chickpea and found the isolates were varied in their virulence (most aggressive, highly virulent, virulent and less virulent). Also, *Fusarium oxysporum* isolates exhibited variation in morphological and cultural characteristics (Iqbal *et al*., 2005). Puhalla, (1985) and Correl *et al.*, (1986) asserted that vegetative compatibility may be a fast and easy way to distinguish pathotypes of *F. oxysporum*. Blok and Bollen (1997) reported that most isolates of *Fusarium oxysporum* f. sp. *asparagi* are not vegetative compatible. While, Di Primo *et al*., (2002) indicated that most of the *Fusarium oxysporum f. sp. gladioli* isolates were vegetative compatibility.

Molecular techniques based on the polymerase chain reaction (PCR) have been used as a tool in genetic mapping, molecular taxonomy, evolutionary studies and diagnosis of several fungal species (Williams *et al*., 1990; Welsh *et al*., 1991; McDonald, 1997; Hyun and Clark, 1998 and Migheli *et al*., 1998). Vakalounakis and Fragkiadakis (1999) concluded that, pathogenicity,

vegetative compatibility group and RAPD-PCR were effective in distinguishing isolates of *F. oxysporum*. Also, Rajendra and Ashok (2011) and Sukumar *et al.*, (2012) reported that RAPD-PCR technique is useful tool for differentiating between species of the genus *Fusarium* either alternatively or complementary to methods based upon morphological and pathological characteristics. On the other hand, Carmer *et al.*, (2003) concluded that RAPD markers had only limited usefulness in correlating pathogenicity among the isolates and races of *Fusarium oxysporum*.

This work aimed to study pathogenicity, vegetative compatibility reaction between isolates of *Fusarium* spp., as well as, to study the genetic diversity using RAPD-PCR technique.

II. Materials and Methods

Isolation and Identification of Pathogenic Isolates

Twenty three *Fusarium* isolates were collected from different chickpea fields located at Assiut governorate (Table 1). Isolates were isolated from infected chick-pea plants showing damping-off and root rot according the methods described by Singh (1994). Isolated fungi were purified using single spore technique and identified on basis of morphological and culture characteristic according to Booth (1997) and Mubasher (1993) and confirmed by Assiut University, Mycological Center (AUMC).

Pathogenicity Test

The Twenty three *Fusarium* isolates were tested for their pathogenicity using Giza3 chick-pea cultivar under greenhouse conditions. Pathogen inoculants were prepared on barley medium as described by Singh et al. (1997). The tested isolates were growing on Barley Medium (150 g barley + 50 g clean sand + 4 g glucose + 0.2 g yeast extract + 200 ml water) in 500 mL flasks. Sterilized barley grains were inoculated with 5 mycelial blocks (0.5-mm in diameter) 5-days old and incubated under room temperature (25±2°C) for three weeks with shaken by hand every alternate day. The autoclaved pots (30 cm in diameter) filled with autoclaved soil and inoculated with fungal inoculant at the rate of 50g/kg of soil. Five pots were used as replicates. Ten seeds of chick-pea Giza3 cultivar were surface sterilized by dipping in 3% sodium hypochlorite solution for 3 min. and washing with sterilized water and seeded in every pot containing infested soil. Pre and post-emergence damping-off were recorded after 15 and 35 days, respectively. Root rot was determined after 60 days from planting using disease index described by Achenbach and Jennifer (1996), using the following equation:

$$\text{Disease index \%} = \frac{0A+1B+2C+3D+4E}{4T}\times 100$$

Where A, B, C, D and E are the number of the plants corresponding to the numerical grade 0, 1, 2, 3, and 4, respectively and (4T) is the total number of plants (T=A+B+C+D+E) multiplied by the maximum disease grade 4. The numerical grade of external root system suggested by Achenbach and Jennifer (1996) were used with some modification:

0 = No infection
1 = 1-25% root tissue exhibiting discoloration.
2 = 26-50% root tissue exhibiting discoloration.
3 = 51-75% root tissue exhibiting discoloration.
4 = More than 75% root tissue exhibiting discoloration or plant died.

Vegetative Compatibility Reaction Between Fusarium sp Isolates in Vitro

Nine isolates of *Fusarium* sp. were grown in 9 cm Petri dishes containing Potato Dextrose agar (PDA) medium for five days at 28°C. Disk (5mm in diameter) from tested isolates were inoculated at 1-1.5 cm from the edge of the new plate and incubated at 28°C for 15 days and three replicates were used. The isolates were paired in all possible combinations on PDA medium according to the procedure of Kohn *et al.*, (1991). The compatible and incompatible isolates were determined. Mycelial reactions were recorded as incompatible when an apparent line of demarcation was observed between the confronting paired isolates.

Molecular Analysis of the Tested Isolates

DNA isolation: 50 ml Czapek's broth media was distributed in 250 ml Erlenmeyer flasks and autoclaved for 15 min. Flasks were inoculated with 5 mm fungal discs from 7 and 15 days old cultures of tested isolates and incubated at 28°C for 15 days. Then, the mycelial growth was filtered using filter paper, washed three times with deionized distilled water and dried in a freeze drier for 30 min. Two grams of the dried mycelia were homogenized with a pestle in liquid nitrogen using upper-chilled mortar. DNA was isolated from 50 mg of homogenized powder using DNA extraction Kit (DNA/RNA extraction purification Kit manufactured by Qiagen Kit). The extracted DNA was dissolved in 100 µl of elution buffer. The concentration and purity of the obtained DNA was determined by using "Gen qunta" system-pharmacia Bio-tech. Samples concentration was adjusted at 60 ng/µl using TE buffer pH 8.0.

Random Amplified Polymorphism DNA Technique (RAPD)

Thirty ng from the extracted DNA were used for amplification reaction. The polymerase chain reaction (PCR) mixture contained PCR beads tablet (manufactured by Amersham Pharmacia Biotech.), which containing all of the necessary reagents except the primer and the DNA template which add to the tablet. The kit, of Amersham Pharmacia Biotech., was also included the primers. Five microliters of the primer (10 mer) were added. The sequences of used primers are as following:

RAPD analysis Primer 4: (5'-AAGAGCCCGT-3')
RAPD analysis Primer 5: (5'-AACGCGCAAC-3')
RAPD analysis Primer 6: (5'-CCCGTCAGCA-3')

The total volume was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as follows using PCR unit (biometra):

a - Denaturation at 95°C for 5 min.
b - 45 cycles each consists of the following steps:
1- Denaturation at 95°C for 1 min.
2- Annealing at 36°C for 1 min.
3- Extension at 72°C for 2 min.
c - Final extension at 72°C for 2 min.
d - Hold at 4°C.

7 µl of X buffer (as manufactured instructions) were added to 25 µl of the amplification product.

Amplification Product Analysis

The amplified DNA for all samples (15 µl) were electrophoresed using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD) on 1% agarose gel containing ethedium bromide (0.5 µg/ml). The run was carried out at

75 constant volts, and the result was determined with UV transilluminator.

Gel Results Analysis

The gel results were scanned using gel documentation system (AAB, Advanced American Biotechnology). The different molecular weights (MW) of bands were determined against PCR marker (1kb DNA Ladder RTU, promega), then the banding profiles were analyzed by unweighted pair-group method based on arithmetic mean (UPGMA) to estimate similarity, genetic distances and reconstruct the dendogram.

Statistical Analysis

All data were subjected to statistical analysis and means were compared using L.S.D. test (Gomez and Gomez, 1984).

III. Results

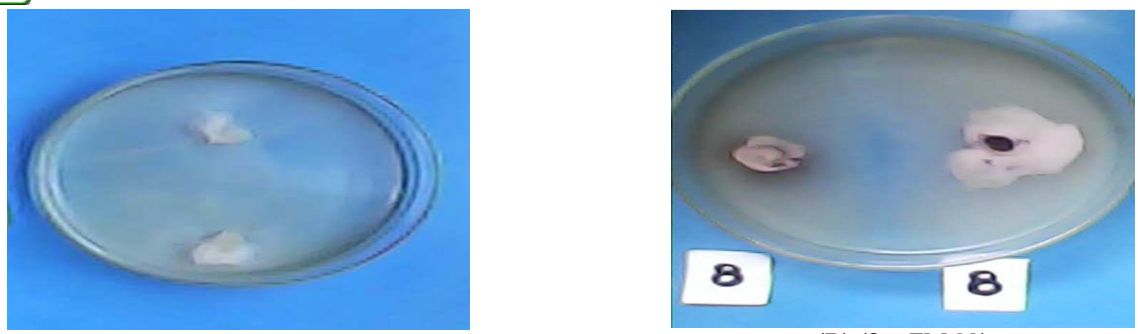
Twenty three *Fusarium* isolates were collected from different chick-pea fields located at Assiut governorate (Table 1). The *Fusarium* isolates were tested for their pathogenicity and shown different disease index (Table 1).

Data in table (1) indicated that all tested isolates were able to infect chick-pea plants of Giza-3 cultivar and caused root rot and damping-off diseases. The tested fungal isolates were varied in their virulence, where *F. oxysporum* isolate Nr. 13 caused the highest percentage of post-emergence damping-off and root rot (40% and 100%, respectively) followed by *F. oxysporum* isolate Nr. 6 (30% and 92.5%). The lowest percentage of pre and post-emergence damping-off and root rot caused by *F. oxysporum* isolate Nr. 17 (30%, 2% and 31.3%, respectively) followed by *F. solani* isolate Nr. 19 (28%, 6% and 32.5%, respectively).

Also, Vegetative compatibility reactions between *Fusarium* sp. isolates were carried out (Figure 1 and Table 2). Data presented in Table (2) indicated that vegetative compatibility and non-compatibility were existed between the isolates of *Fusarium* sp. The isolate No. 23 shows incompatible reaction with all other isolates. Also, isolate Nr. 13 shows incompatible reaction with all other isolates except with isolate Nr. 18. On the other hand, isolate Nr. 21 shows compatible reaction with all other isolates except isolate Nr. 13. Also, isolate Nr. 18 shows compatible reaction with all other isolates except isolate Nr. 20 and 23. Figure (1-B) had shown an exciting result, where some isolates shown self-incompatibility reaction as shown with *F. moniliform* Nr 22.

Table 1. Source and pathogenicity of Fusarium spp. isolates on Giza (3) chick-pea cultivar under greenhouse conditions.

No. of isolates	Isolates	Source of fungal	Damping-off%		Root rot %
			pre	post	
1	*Fusarium* ***oxysporum***	El-Ghanayim	**56**	**12**	**65**
2	***F. oxysporum***	El-Kussiah	**52**	**12**	**62.5**
3	***F. oxysporum***	El-Kussiah	**28**	**10**	**35**
4	***F. oxysporum***	Sahil-Selim	**32**	**8**	**40**
5	***F. oxysporum***	Sahil-Selim	**56**	**18**	**72.5**
6	***F. oxysporum***	Al-badary	**64**	**30**	**92.5**
7	***F. oxysporum***	Sahil-Selim	**24**	**22**	**43.8**
8	***F. oxysporum***	Abnoub	**58**	**2**	**60**
9	***F. oxysporum***	Assiut	**58**	**8**	**63.8**
10	***F. oxysporum***	Assiut	**50**	**12**	**61.3**
11	***F. oxysporum***	Sahil-Selim	**52**	**8**	**60**
12	***F. oxysporum***	Abnoub	**38**	**4**	**41.5**
13	***F. oxysporum***	Abo-Teg	**60**	**40**	**100**
14	***F. oxysporum***	Assiut	**60**	**20**	**80**
15	***F. oxysporum***	Assiut	**50**	**4**	**52.5**
16	***F. oxysporum***	Al-Fath	**32**	**12**	**42.5**
17	***F. oxysporum***	Al-Fath	**30**	**2**	**31.3**
18	***F. solani***	El-Kussiah	**70**	**20**	**90**
19	***F.solani***	El-Kussiah	**28**	**6**	**32.5**
20	***F. solani***	Abo-Teg	**56**	**14**	**70**
21	***F. moniliforme***	Sahil-Selim	**70**	**10**	**80**
22	***F. moniliforme***	Abo-Teg	**52**	**6**	**55**
23	***F. moniliforme***	Abo-Teg	**66**	**20**	**83.8**
	control		**0**	**0**	**0**
L.S.D. 0.05%			**5.58**	**4.57**	**8.574**



(A) (B) (8 = FM 22)

(A) Compatible reaction of *Fusarium* isolates (B) Non-compatible reaction (self-incompatibility)

Fig. 1.

Table 2. Vegetative compatibility reaction between Fusarium isolates
(FO = *F. oxysporum*, FS = *F. solani* and FM = *F. moniliform*)
(C = Compatible reaction, N = Non-compatible reaction)

***Fusarium* isolates**	**FO (6)**	**FO (13)**	**FS (18)**	**FS (19)**	**FS (20)**	**FM (21)**	**FM (22)**	**FM (23)**
FO (5)	N	N	C	C	C	C	N	N
FO (6)		N	C	N	C	C	N	N
FO (13)			C	N	N	N	N	N
FS (18)				C	N	C	C	N
FS (19)					N	C	N	N
FS (20)						C	C	N
FM (21)							C	N
FM (22)								N

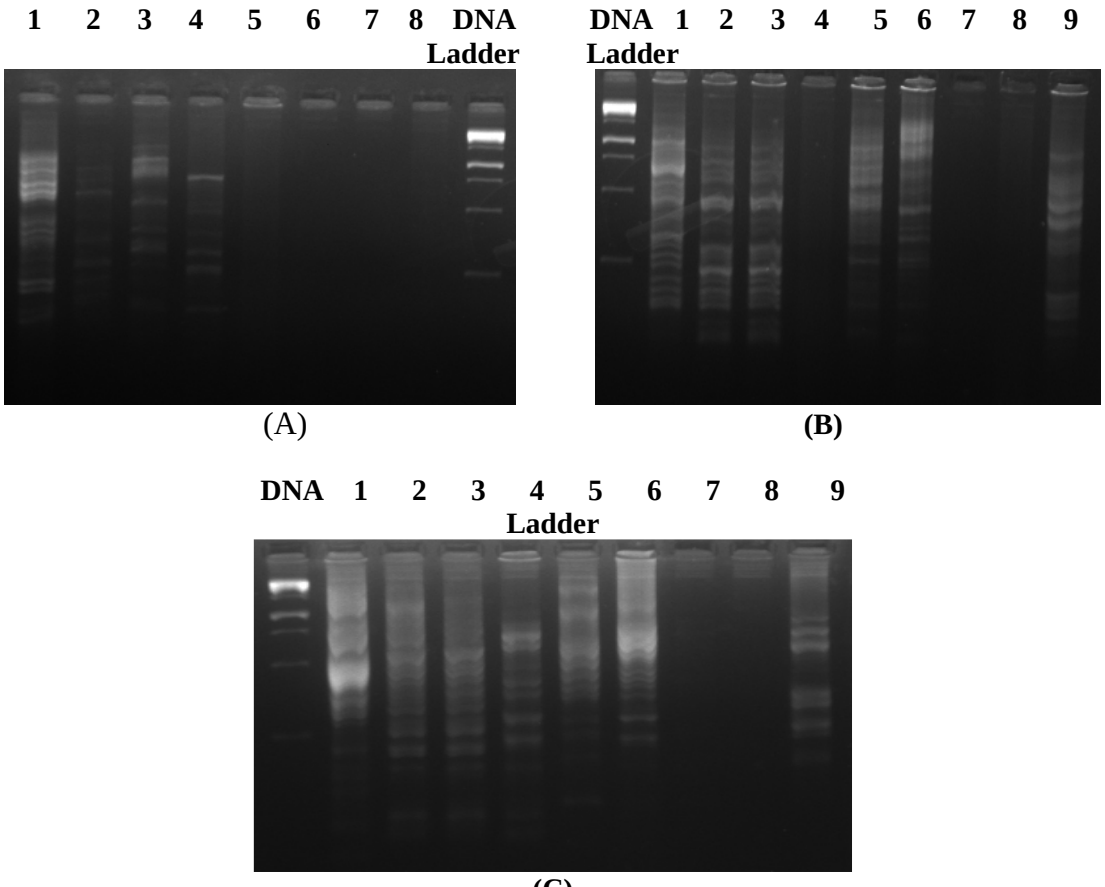


Figure (2): Agarose-gel electrophoresis of RAPD products generated by primers P4 (A), P5 (B) and P6 (C) in the isolated samples of *Fusarium.*

Random Amplified Polymorphic DNA (RAPD) Technique

Only nine of twenty three isolates were subjected to molecular investigation. These isolates belong to *Fusarium oxysporum* (FO, isolates Nr. 5, 6 and 13), *Fusarium solani* (FS, isolates Nr. 18, 19 and 20) and *Fusarium moniliform* (FM, isolates Nr. 21, 22 and 23).

Polymorphism and genetic similarity among nine *Fusarium* fungi isolates revealed by RAPD markers were obtained using three arbitrary oligonucleotide primers (P4, P5, and P6), which used to generate RAPD profiles from nine isolates of *Fusarium*. All primers worked successfully on the genomic DNA from taken isolates yielding distinct RAPD patterns (Figure 2) except primer P5 doesn't work with samples 4 (FS 18), 7 (FM 21) and 8 (FM 22). Unfortunately, for techniqual work error, only eight samples were subjected to primer P4. The number of the amplified fragments per primer varied between one and seventeen for total bands of 192, with an average 64 bands per primer. These fragments have a size ranged from 4206 to 94 base pairs (bp). A total number of 192 bands were amplified with only two bands being monomorphic (shared by all examined samples) and the other 190 bands were polymorphic with a polymorphism ratio of 98.96 %. Primer P5 generated the largest number of fragments (72), while primer P4 generated the lowest number of fragments (52).

The first Primer P4: The results of RAPD analysis of the eight isolate samples of *Fusarium* as obtained by primer P4 are illustrated in Figure (2). Primer P4 produced 52 bands in the eight samples. It reacted with the eight samples generating 21 fragments ranged in size from 4206 to 121 bp. The number of fragments generated by this primer varied among samples where the lowest number was two observed in samples Nr. 5 (FS 19), 6 (FS 20), 7 (FM 21) and 8 (FM 22), while the highest number was 15 in sample Nr. 1 (FO 5).

The second primer P5: The results of RAPD analysis of the nine isolate samples of *Fusarium*, as obtained by primer P5, are illustrated in Figure (2). Primer P5 did not work with samples 4 (FS 18), 7 (FM 21) and 8 (FM 22), it produced 72 bands in the six samples. It reacted with the six samples generating 22 fragments having sizes of 850 to 94 bp. The number of fragments generated by this primer varied among samples from eight in sample Nr. 5 (FS 19) to 17 in sample Nr. 2 (FO 6).

The Third Primer P6: The results of RAPD analysis of the nine isolate samples of *Fusarium* as obtained by primer P6 are illustrated in Figure (2). Primer P6 produced 68 bands in the nine samples. It reacted with the nine samples generating 18 fragments having sizes from 1045 to 184 bp. The number of fragments generated by this primer varied among samples where the lowest number was one observed in sample Nr. 7 (FM 21) and 8 (FM 22), while the highest number was eleven in samples Nr. 2 (FO 6), 5 (FS 19) and 6 (FS 20).

The summary of all fragments generated by the three primers are illustrated in (Table 3).

Genetic Similarity and Cluster Analysis Based on Rapd Results

The genetic relationships between the nine isolates of *Fusarium* was illustrating in the dendrogram generated by cluster analysis (UPGMA) with the RAPD data (Figure 3). All samples divided into three main clusters. The first cluster contains three clades first of them, which represent the main root of dendrogram, contains two isolates [Nr. 7 (FM 21) and 8 (FM 22)] with genetic similarity of 1.00 %, both isolates belong of *Fusarium moniliforme*. The second clade contains isolate Nr. 4 (*Fusarium solani* Nr. 18) with genetic distance 0.323. The third clade contains isolate of *Fusarium moniliforme* Nr. 23 (Nr. 9) which represents the direct clade for the rest of samples with genetic distance of 0.374. The second cluster composed two isolates Nr. 5 (FS 19) and 6 (FS 20) which belongs to *Fusarium solani* with genetic similarity of 0.667. The third cluster composed three isolates belong to *Fusarium oxysporum*, where isolate Nr. 1 (FO 5) represent the ancestor clade for the other two isolates of *Fusarium oxysporum* [Nr. 2 (FO 6) and 3 (FO 13)] with genetic distance of 0.608.

IV. Discussion

Damping-off and root rot of chickpea in Egypt caused by *Fusarium* spp (*F. oxysporum*, *F. solani* and *F. moniliforme*) caused several loses in chickpea (Khalil, 2007; Noher et al., 2009 and Montaser, 2011).

The objectives of this study were to investigate the pathogenicity, vegetative compatibility and molecular differences of twenty three *Fusarium* isolates, collected from different chickpea fields located at Assiut governorate. The isolates of *Fusarium* shown different disease index (Table 1) and all tested isolates were able to infect chickpea plants of Giza 3 cultiver. These results are in agreement with those reported by Khan *et al*., (2002) and Iqbal *et al*., (2005). The highest and lowest percentage of pre and post-emergence damping-off and root rot shown by *F. oxysporum* (Nr. 13) and (Nr. 17), respectively. This results agree with Nene and Reddy (1987) and Leisso et al. (2011). The vegetative compatibility reaction showing compatible and non-compatible reaction between *Fusarium* isolates. The isolate Nr. 21 (*F. moniliforme*) shown compatible reaction with all other isolates except with isolates Nr. 13 (*F. oxysporum***)** and 23 (*F. moniliforme*). The isolate Nr. 23 shown non-compatible reaction with all other isolates, also isolate Nr. 13 shown the same result except with isolate Nr. 18 (*F. solani*). Moreover, isolate Nr. 22 (*F. moniliforme*) shown incompatible reaction with all other isolates, except isolates Nr. 18, 20 (*F. solani*) and 21 (*F. moniliforme*). These results are in harmony with those reported by Blok and Bollen (1997); Di Primo *et al*., (2002) and Ana et al., (2009). An exciting result was found with vegetative compatibility reaction using the same kind of isolate in Petri dish (self-incompatibility). The isolate Nr. 8 shown line of demarcation between the confronting fungi (Figure 1, B). These result may explained by the feedback inhibition, where the mycelium of fungi make secretion production that cause growth

inhibition in the line area between the two growing mycelium by the accumulative of the secretion production. To determine the genetic diversity of *Fusarium* isolates, nine isolates representing the three species of *Fusarium* (*F. oxysporum*, *F. solani* and *F. moniliform*) were subjected to the molecular analysis of RAPD-PCR using three arbitrary primers. The primers used showed clear differences among the nine isolates of *Fusarium*. The primers used generated unique distinct band which could be used to distinguish the isolates in respect of their species (*oxysporum*, *solani* and *moniliform*) and virulence. These results are in agreement with those reported by Migheli *et al*., (1998); Rajendra and Ashok (2011) and Sukumar *et al.*, (2012). The first primer P4 generated many fragments which could be used as genetic marker to distinguish *F. oxysporum* by generating fragments with molecular weight (M.W) of 715, 361 and 287 bp which are present only in the isolates of *F. oxysporum* and absent in all other samples. Also, this primer generated fragments with M.W. of 1683, 1336 and 389 bp which are present only in the isolate of *F. solani*. Moreover, primer P4 generated fragment (genetic marker) of 1060 bp with *F. oxysporum* Nr. 13, the highest virulence isolates and fragments of 203, 167 and 121 bp with *F. oxysporum* Nr. 5. The second primer P5 also could be used as a genetic marker to distinguish isolates of *F. oxysporum* by generating fragments with special size (M.W). As example, fragments with size of 181, 162 and 147 bp are present only in isolates of *F. oxysporum*. Moreover, the third primer P6 could be used as a genetic marker to distinguish isolates of *F. oxysporum* by generating fragment with special size of 256 bp which present only with *F. oxysporum* isolates and absent in all other species samples. In addition, this primer (P6) generated fragments with M.W. of 381 and 243 bp, that fragments present only with isolates of *F. solani* and absent in all other species samples, which could be used as a genetic marker to distinguish isolates of *F. solani*. The dendogram is shown the isolates Nr. 7 and 8 represent the ancestor of the other isolates, both isolates are *F. moniliform*. Also, the isolates Nr. 1, 2 and 3 represent the most recent isolates in the dendogram with about 73 % genetic similarity, these isolates are *F. oxysporum*. These results confirming that the evolution direction draws line from *F. moniliforme* toward *F. oxysporum*. As well as, these results asserted that RAPD-PCR technique is useful tool for differentiating between species of the genus *Fusarium*.

Table 3. Summary of all fragments generated by the assay of the three primers, and their molecular weight in all nine isolate samples of *Fusarium* where (1) means presence and (0) means absence.

Primer code	M.W (bp)	Samples								
		1 (FO5)	2 (FO6)	3 (FO13)	4 (FS18)	5 (FS19)	6 (FS20)	7 (FM21)	8 (FM22)	9 (FM23)
P4	4206	1	1	1	1	1	1	1	1	0
P4	2698	1	1	0	1	1	1	1	1	0
P4	1683	0	0	0	1	0	0	0	0	0
P4	1336	0	0	0	1	0	0	0	0	0
P4	1060	0	0	1	0	0	0	0	0	0
P6	1045	1	1	1	1	1	1	1	1	1
P6	967	0	1	0	0	1	1	0	0	0
P4	857	1	0	1	0	0	0	0	0	0
P5	850	1	0	0	0	0	1	0	0	0
P6	845	1	1	0	0	0	1	0	0	0
P6	765	1	0	0	0	1	0	0	0	1
P4	715	1	1	1	0	0	0	0	0	0
P5	685	1	1	1	0	1	0	0	0	0
P6	645	1	1	0	1	0	0	0	0	1
P4	598	1	1	0	1	0	0	0	0	0
P5	597	0	0	0	0	1	1	0	0	1
P5	545	0	1	1	0	1	0	0	0	0
P6	538	0	1	0	1	0	0	0	0	0
P4	507	1	1	0	0	0	0	0	0	0
P6	492	1	0	0	1	1	1	0	0	1
P5	488	1	1	1	0	0	1	0	0	0
P5	448	0	1	1	0	1	0	0	0	0
P4	439	0	0	1	1	0	0	0	0	0
P6	432	0	1	1	0	1	1	0	0	0
P5	393	1	0	0	0	0	1	0	0	1
P4	389	0	0	0	1	0	0	0	0	0
P6	381	0	0	0	1	1	1	0	0	0

Table 3. Continue the summary of all fragments generated by the assay of the three primers, in all nine isolate samples of Fusarium where (1) means presence and (0) means absence.

Primer code	M.W (bp)	Samples								
		1 (FO5)	2 (FO6)	3 (FO13)	4 (FS18)	5 (FS19)	6 (FS20)	7 (FM21)	8 (FM22)	9 (FM23)
P4	361	1	1	1	0	0	0	0	0	0
P5	359	1	1	1	0	1	0	0	0	0
P6	353	1	1	1	0	1	1	0	0	0
P4	351	1	0	1	1	0	0	0	0	0
P5	332	0	1	1	0	0	1	0	0	1
P6	327	0	1	1	1	1	1	0	0	0
P6	298	0	0	1	1	1	1	0	0	1
P5	293	1	1	1	0	1	1	0	0	1
P4	287	1	1	1	0	0	0	0	0	0
P6	279	1	0	0	0	0	0	0	0	1
P5	269	0	1	1	0	0	1	0	0	0
P4	257	0	0	1	1	0	0	0	0	0
P6	256	1	1	1	0	0	0	0	0	0
P5	248	1	0	0	0	0	1	0	0	0
P6	243	0	0	0	1	1	1	0	0	0
P5	236	0	0	0	0	1	0	0	0	1
P4	225	1	1	0	1	0	0	0	0	0
P5	221	1	1	1	0	0	0	0	0	1
P6	219	0	1	1	0	0	0	0	0	1
P6	204	0	0	0	1	1	1	0	0	1
P4	203	1	0	0	0	0	0	0	0	0
P5	201	1	1	0	0	1	1	0	0	0
P6	184	1	1	0	0	0	0	0	0	0
P4	183	1	1	0	0	0	0	0	0	0
P5	181	1	1	1	0	0	0	0	0	0
P4	167	1	0	0	0	0	0	0	0	0
P5	162	1	1	1	0	0	0	0	0	0
P5	147	1	1	1	0	0	0	0	0	0
P5	137	0	1	0	0	0	0	0	0	1
P4	135	1	0	0	1	0	0	0	0	0
P5	126	1	1	1	0	0	0	0	0	1
P4	121	1	0	0	0	0	0	0	0	0
P5	108	0	1	1	0	0	0	0	0	1
P5	94	0	1	1	0	0	0	0	0	1
Total	**61**	**37**	**37**	**31**	**20**	**21**	**22**	**3**	**3**	**18**

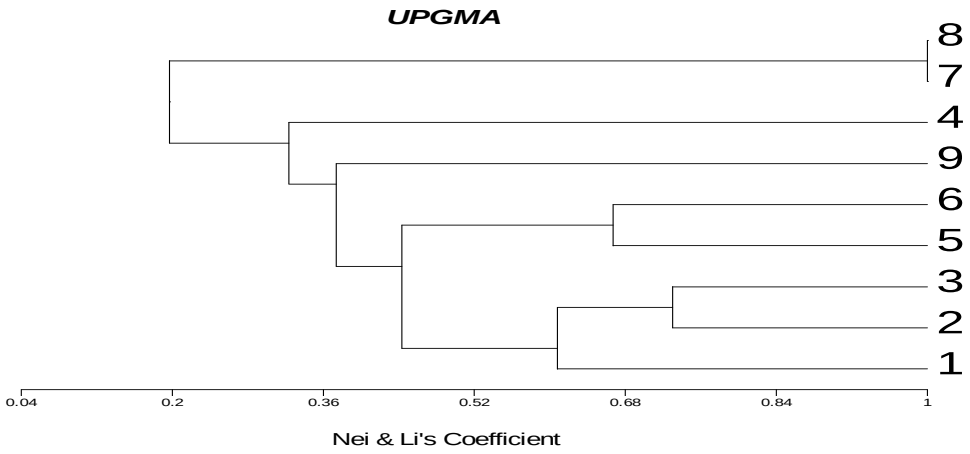


Fig. 3. UPGMA dendrogram of the nine isolates of *Fusarium* based on values of genetic distances calculated from data of all three primers in RAPD analysis

REFERENCE

[1] Achenbach, L.A. and Jennifer, P. (1996). Use of RAPD markers as a diagnostic tool for the identification of *Fusarium solani* isolates that cause soybean sudden death syndrome. Plant Disease, 80: 1228-1232.

[2] Ana, M.N.M.; Diaz, R.M.J. and Artes, E.P. (2009). Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *ciceri.* And *F. oxysporum* non-pathogenic to chickpea. J. Phytopathol., 157: 729-735.

[3] Blok, W.J. and Bollen, G.J. (1997). Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum* f. sp. *asparagi*. Canadian Journal of Botany, 75: 383-393.

[4] Booth, C. (1997). *Fusarium* laboratory guide to identification of the major species. Commonwealth Mycological Institute Kew, Surrey, England, pp. 1-158.

[5] Carmer, R.A.; Byrne, P.F.; Brick, M.A.; Panella, L.; Wickliffe, E. and Schwartz, H.F. (2003). Characterization of *Fusarium oxysporum* isolates from common bean and sugar bet using pathogenicity assays and random amplified polymorphic DNA markers. J. Phytopathologische Zeitschrift. 151: 352-360.

[6] Correll, J.C.; Puhalla, J.E. and Schneider, R.W. (1986). Identification of *Fusarium oxysporum* f. sp. *ciceri*. on the basis of colony size, virulence and vegetative compatibility. Phytopathology, 76: 396-400.

[7] Di Primo, P.; Cappelli, C. and Katan, T. (2002). Vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *gladioli* from saffron. European Journal of Plant Pathology, 108: 869-875.

[8] Gomez, K.A. and Gomez, A.A. (1984). Statically procedures for Agricultural research, 2 nd Edn., John Wily and Sons Inc., New York, USA. 680.

[9] Hyun, J.W. and Clark, C.A. (1998). Analysis of *Fusarium lateritium* using RAPD and rDNA-RFLP techniques. Mycol Res., 102: 1259-1264.

[10] Iqbal, S.M.; Rauf, C.A.; Ahmad Bakhsh and Umer Iqbal (2005). Variability in *Fusarium oxysporum* f. sp. *ciceri*. Mycopath, 3: 47-51.

[11] Iqbal, S.M.; Ghafoor, A.; Bakhsh, A.; Ahmad, I. and Sher, A. (2010). Identification of resistant sources for multiple disease resistance in chickpea. Pakistan Journal of Phytopathology, 22(2): 89-94.

[12] Khalil, M.S.M. (2007). Studies on some chickpea (*Cicer arietinum* L.) fungal diseases M.Sc. Thesis, Fac. Agric. Minia Univ. Egypt. Pp. 144.

[13] Khan, I.A. and Abdul Jabbar, A.S. (2002). Genetic variation among *Fusarium oxysporum* f. sp. *ciceri* isolates in Pakistan as determined by biological path typing and vegetative compatibility. Pakistan Journal of Botany, 34(4): 433-440.

[14] Khetarpal, R.K.; Khetarpal, S.; Kumar, P.; Pal, M.; Chand, D. and Lata, S. (2009). Effect of association of *Fusarium Solani* and *F. moniliforme* on photosynthetic characteristics in chickpea (*Cicer arietinum* L.). Indian Phytopathology, 62(4): 484-487.

[15] Kohn, L.M.; Stasovski, E.; Carbone, I.; Royer, J. and Anderson, J.B. (1991). Mycelial incompatibility and molecular marker in identify genetic variability in field population of S. sclerotiorum. Phytopathology, 81: 480-485.

[16] Leisso, R.; Miller, Z.; Jacobsen, B. and Burrows, M. (2011). Pathogenicity of *Fusarium* spp. To chickpea seed seedlings (*Cicer arietinum* L.). Canadian Journal of Plant Patholog., 33: 400- 409.

[17] Mc Donald, B.A. (1997). The population genetic of fungi: tools and techniques. Phytopathology, 87: 448-453.

[18] Meki, S.; Ahmed, S. and Sakhuja, P.K. (2011). Control of chickpea wilt *Fusarium oxysporum* f. sp. *ciceri* using *Trichoderma* spp. in Ethiopia. Archives of Phytopathology and Plant Protection, 44(5): 432-440.

[19] Migheli, Q.; Briatore, E. and 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. PI Pathol., 104: 49-57.

[20] Montaser, F.A. (2011). Integrated Management of damping-off, root and/or Stem rot diseases of chickpea and efficacy of the suggested formula. Plant Pathology Research, Agriculture Research Center, Giza, Egypt. 3(3): 80-88.

[21] Mubasher, A.H. (1993). Soil fungi in Qatar and other Arab countries. The Scientific and Applies Research center, University of Qatar, pp. 566.

[22] Nene, Y. and Reddy, M.V. (1987). Chickpea diseases and their control. Pp233-270. In: Saxena, MC, Singh, K.B. (Eds.). The chickpea, Commonwealth Agricultural Bureaux International, Oxon, UK, 320.

[23] Noher, A.M.; Abdalla, M.Y.; Abou Attia, M.A. and Abou Zeid, N.M. (2009). Pathological and genetic variability among isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea in Egypt. Association Francaise de Protection des Planets, 9eme conference international sure les maladies des planets, Tours, France, 9: 143-151.

[24] Puhalla, J.E. (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Canadian Journal of Botany, 63: 179-183.

[25] Rajendra, B.K. and Ashok, M.C. (2011). Molecular characterization of *Fusarium* spp. isolates by using RAPD technique. Journal of Ecobiotechnology, 3(11): 1-4.

[26] Singh, R.S. (1994). Plant pathogens: the fungi 3rd Ed. Oxford and IBH publishing Co. LTD, New Delhi, India, 443p.

[27] Singh, R.S.; Singh, D.; Singh, H.V. and Singh, D. (1997). Effect of fungal antagonists on the growth of chickpea plants and wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Plant Disease Research, 12: 103-107.

[28] Singh, R.K.; Abul Hasan and Chaudhary, R.G. (2010). Variability in *Fusarium oxysporum* f. sp. *ciceri* causing vascular wilt in chickpea. Archives of Phytopathology and Plant Protection, 43(10): 987-995.

[29] Sukumar Mesapogu, Achala Bakshil, Bandamravuri Kishore Babu, S.S. Reddy, Sangeeta Saxena and Dilip K. Arora (2012). Genetic diversity and pathogenic variability among Indian isolates of *Fusarium udum* infecting pigeonpea (*Cajanus cajan* L. millsp). International Research Journal of Agricultural Science and Soil Science, 2(1): 51-57.

[30] Sumanti, G.; Chakraborti, D.; Rangi, R.K.; Basu, D. and Das, S. (2009). A molecular insight into the early events of chickpea (*Ccer arietinum* L) and *Fusarium oxysporum* f. sp. *ciceri* (race 1) interaction through cDNA-AFLP analysis. Phytopathology, 99(11): 1245-1257.

[31] Vakalounakis, D.J. and Fragkiadakis, S. (1999). Genetic variation among *Fusarium oxysporum* isolates from Cucumber. Bull. Plant Pathol., 30(2): 175-177.

[32] Welsh, J.; Petersen, C. and Me Clelland, M. (1991). Polymorphisms generated by arbitrary primed PCR in mouse: application to strain Identification and genomic mapping. Nucleic Acids Res., 19: 303-306.

[33] Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalsk, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.