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Molecular Identification and Characterization of Different *Fusarium oxysporum f.sp. lycopersici* Isolates Using RPAD-PCR Marker

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Abstract

Five random primers of RAPD-PCR were done in eight isolates of *Fusarium oxysporum f.sp. lycopersici*, each of them consisted of 10 bases. In accordance with the distinction of the taxa based on RAPD_PCR marker analysis, the results revealed that, the studied isolates of were *F. oxysporum f.sp. lycopersici* clearly distinguished from each other. Genetic similarity coefficients between pair wise varied from (0.040 to 0.429) based on an unweighted paired group method of arithmetic average (UPGMA) cluster analysis of all isolates. The dendrogram obtained from the data showed that, hierarchical clustering separated the isolates into three groups according to their similarity coefficients. Therefore, there were some reliable RAPD markers for the pathogenic isolates, which resulted close relationship at the molecular level under genetic control as it is inherited in a polygenic fashion. Thus, RAPD-PCR technique can be used as an important tool for the genetic identification and characterization of *F. oxysporum f.sp. lycopersici* species.

Keywords:
Fusarium, RAPD-PCR, UPGMA, dendrogram.

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INTRODUCTION

Fusarium species are known to cause a huge range of diseases on an extraordinary range of host plants such as, vascular- wilt, root rot, stem-rot, and fruit and vegetable decay (Booth, 1971; Summeral et al., 2003 and Taylor et al., 2016). The identification and characterization of *fusarium* species is commonly done based on their micro and macroscopic features based on morphological characters, biochemical and allozyme characteristics etc (Kheterpal, 2006). However, these features are mostly reported to be unstable (Nelson et al., 1983, and Szecsi and Dobrovolsky 1985). These methods are time consuming and have proved to be limited and insufficient. Hence, study of distribution and diversity of these species is very important (Aigbe and Fawole, 1999 and Latiffah et al., 2007) based on the nucleotide sequence information from conserved regions using PCR amplification.

PCR based techniques are regularly used for identification, characterization and early diagnosis of microbes and pathogens. Random amplified Polymorphic DNA (RAPD) analysis (Williams et al., 1990; Miller 1996; Gupta et al. 2009 and Ingle et al., 2009) has been used for confirmation of identity among different isolates of fungi (Assigbetse et al., 1994 and Alves-Santos et al., 2002). It has been observed to have a high level of variability among many isolates (Chiocchetti et al., 1999; Edwards et al. 2002; Leslie and Summerell, 2006; Sabir 2006; Lievens et al., 2007; Bayraktar et al., 2008 and Steinkellner et al. 2008). In addition, RAPD is simple and relatively faster as compared with other molecular techniques (Wilson et al., 2004; Guleria et al., 2007 and Niessen 2007). Furthermore, RAPD markers can help to comprehend the mechanisms of pathogenic variation (Albores et al., 2014). So, RAPD markers demonstrated remarkable variation of bacteria, fungi and plants (Skaria et al. 2011 and Singh et al. 2011). There are several reports on characterization of fusarium species using RAPD markers. Gupta et al. (2009) reported the genetic polymorphism of six isolates of *F. solani* causing wilt disease in guava. Ingle and Rai (2011) reported genetic diversity of *F. semitectum* associated with mango malformation. In addition, similarly, a genetic variation in *F. oxysporum f. sp. Fragariae* causing wilt disease in strawberry was identification by Nagarajan et al. (2004).

Therefore, the aim of the present study was to estimate the genetic variation among eight isolates of using R *F. oxysporum f.sp. lycopersici* APD-PCR marker to determine the relationship among them.

MATERIALS AND METHODS

Isolation of fungal cultures

F. oxysporum f.sp. lycopersici isolates were isolated from wilt infested tomato plants. Infested samples were sterilized by dipping in 10% (w/v) sodium hypochlorite solution for (3–5) min and washed thrice with sterile water. Then, the infested samples were cut with a sterile blade and placed on the surface of potato dextrose agar media. PDA was amended with streptomycin antibiotic to minimize chances of any bacterial growth. Plates were incubated at 28 ± 2 ^{o}C and observed periodically. The fungi were identified following sporulation and pure cultures were stored at 4 ^{o}C on PDA slants. All the previous procedures were done in the laboratory of plant Pathology, plant pathology dept., faculty of agric., Sohag University, Sohag, Egypt. The studied *Fusarium* isolates are (Fu-1, Fu-2, Fu-3, Fu-4, Fu-5, Fu-6, Fu-7 and Fu-8). The source of each isolates were infected tomato plants.

Genomic Extraction

From each isolate, DNA concentration was estimated used electrophoresis unit, then stored at 20 ^{o}C until further use.

RAPD amplification

Total genomic DNA isolated from each isolates *F. oxysporum f.sp. lycopersici* were subjected to RAPD-PCR analysis used random set of primers (OPAB-4, OPA-3, OPA-2, OPA-15 and OPY-5) in the molecular biology lab, central laboratories of faculty of agriculture, Assiut university, which showed different amplification pattern with the studied *Fusarium* isolates. The nucleotide sequences of the primers are presented in (Table NO.1).

Table (1): Nucleotide sequences of the used RAPD-PCR primers.

OPAB-4	GGCACGCGTT
OPA-3	AGTCAGCCAC
OPA-2	TGCCGAGCTG
OPA-15	AGATGCAGCC
OPY-5	GGCTGCGACA

Preparations of the reactions buffers

A master mix was prepared in a 1.5 ml microcentrifuge tube, according to the performed number of PCR reactions included an extra reaction to be compensate for the loss part of the solution due to frequent pipetting. Each reaction contained as showed in Table (NO.2).

Table (2): PCR reaction content in each tube (sample).

Master Mix	12.5µ 2x master mix
Primer F	2µ 20 mmole
Primer R	----------
Deionized H_2O	9.5µ
DNA	1µ (10ng – 30 ng)
Reaction	25µ

PCR program and temperature profile

Amplification of the DNA was performed by placing the tubes containing the reaction buffers in the thermal cycler programmed to fulfill 36 cycles. The temperature profile in the different cycles was observed in Table (N0.3).

Table (3): PCR reaction temperature, time and cycles.

	Temperature	Time	Cycles
Initial Denaturation	95^0C	5 min	1
Denaturation	94^0C	30 sec	35
Annealing	36^oC	1 min	
Extension	72^oC	2 min	
Final extension	72^0C	10 min	

Electrophoresis of PCR products

When the PCR program was terminated the mineral oil was removed from the surface of the reactions and the amplified PCR products were analyzed by electrophoretic separation in a 1% agarose gel. An amount of 8µl of each PCR product was mixed with 1µl loading buffer and loaded into the wells of the gel. Gels were run at 90 volts for about 70 minutes. The gel stained with ethidium-bromide to easy view under the UV light.

Binary Data

After electrophoresis, the RAPD patterns were visualized with an UV transilluminator. Then, RAPD markers were scored from the gels as DNA fragments present (1) or absent (0) in all the lanes. The DNA bands were determined against a ladder of DNA bands marker consisted of (3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bP).

The application of the Jaccard's coefficient to the binary matrix served to calculate the pairwise distance among the isolates, which were clustered by UPGMA (Unweighted Pair Group method using arithmetic means).

Genetic diversity for each primer was calculated as previously described (Owen et al., 1998) using the following formula ($H = 1–\Sigma xi^2$), where H is the expected heterozygosity and xi is the frequency of allele i, including "nulls" as a separate band. It works on the assumption that the rates of evolution in all lineages are same and gives output clustering in increasing order of distance (Sahoo et al. 2010).

RESULTS

Variation between infected and uninfected isolates of *F. 0xysporum f.sp. lycopersici* by the random amplified polymorphic DNA (RAPD)

RAPD markers were carried out to study the variation among the infected and uninfected isolates of *F. oxysporum f.sp. lycopersici* at molecular level. In this investigation, five random ten-mer primers OPAB-4, OPA-3, OPA-2, OPA-15 and OPY-5 were used for studying the polymorphism and genetic similarity among the tested isolates.

Polymorphism

The characterization of the fragments generated by the array of the five RAPD primers used, the description of each primer and the bands produced was summarized as follows:

OPAB-4 Primer

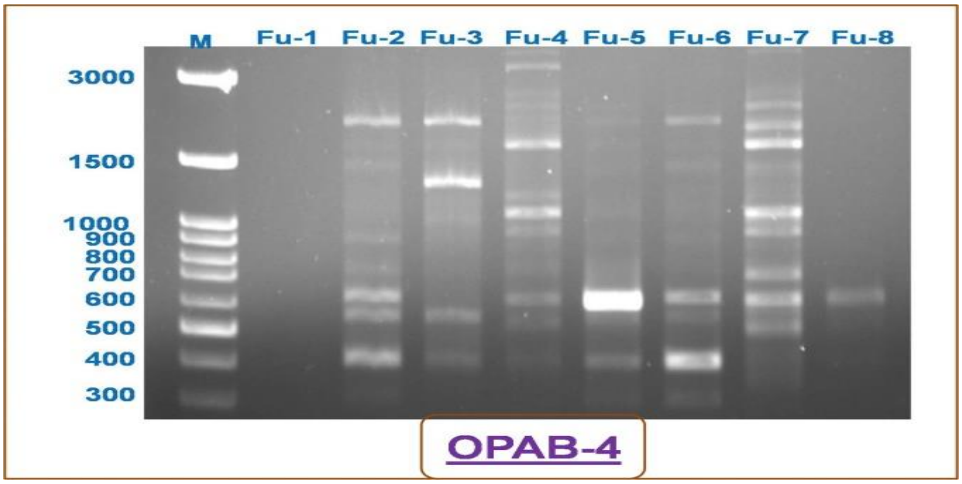

Fig. (1) Agarose gel electrophoresis of RAPD-PCR products by OPAB-4 primer of eight isolates of *F. oxysporum f.sp. lycopersici.*

Table 4 : Presence of RAPD-PCR bands in *F. oxysporum f.sp. lycopersici* isolates after using OPAB-4 Primer

	Bands No.	M	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
OPAB-4	1	2000	-	+	+	-	-	+	-	-
	2	1800	-	-	-	+	-	-	+	-
	3	1350	-	-	+	-	-	-	-	-
	4	1000	-	-	-	+	-	-	+	-
	5	900	-	-	-	-	-	-	+	-
	6	700	-	-	-	-	-	-	+	-
	7	600	-	+	-	+	+	+	+	+
	8	500	-	+	+	-	-	-	+	-
	9	400	-	+	-	-	+	+	-	-

The primer (OPAB-4) was used in the present experiment to scan the isolates, for RAPD-PCR markers. Each lane showed the amplification products of each isolate. The number of bands were found to be variable among isolates and shown in Fig(1) .The detailed information about the bands found in each isolate was given in Table(4). Band 1 with (2000 bp) was noted in the isolates Fu-2, Fu-3 and Fu-6. Whereas band 2 with (1800 bp) was founded in the isolates Fu-4 and Fu-7.While, band 3 are uniquely exhibited in (1350 bp) for the isolate Fu-3. On the other hand, band 4 was contributed only in the isolates Fu-4 and Fu-7 with (1000 bp). However, bands (5 and 6) were presented uniquely in the isolate Fu-7 with (900 and 700 bp), respectively. Whereas, band 7 with 600 bp was shared in the isolates Fu-2, Fu-4, Fu-5, Fu-6, Fu-7 and Fu-8. In addition, band 8 with was showed in the isolates Fu-2, Fu-3 and Fu-7. Furthermore, band 9 with 400 bp was revealed in the isolates Fu-2, Fu-5 and Fu-6.

Regarding the pathogenic isolates, the results viewed that bands (1, 7, 8, and 9) with (2000, 600, 500 and 400 bp), respectively, could be a reliable marker for the pathogenic isolates included in this study, in spite of, there are no bands contributed for the isolate Fu-1 toward primer OPAB-4.

OPA-3 primer

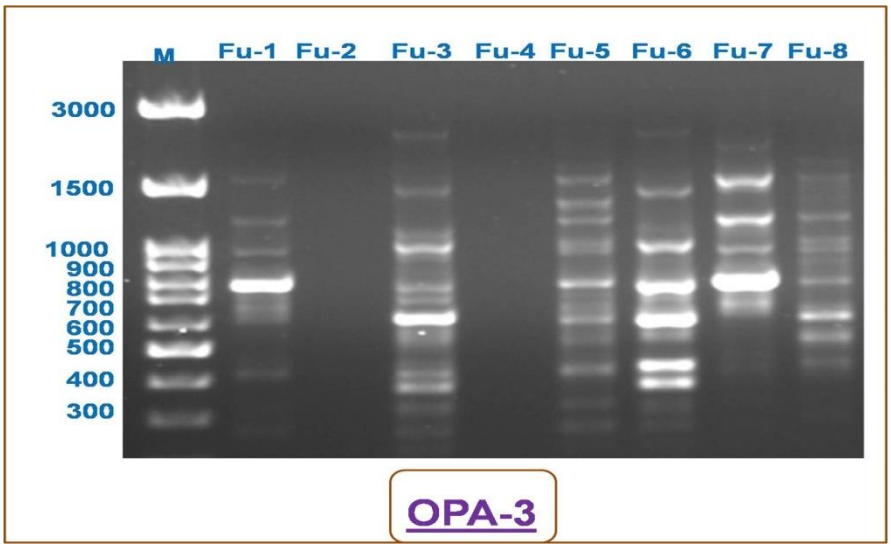

Fig.2: Agarose gel electrophoresis of RAPD-PCR products by OPA-3 primer of eight isolates of *F. oxysporum f.sp. lycopersici.*

Table. 5: Presence of RAPD-PCR bands in isolates after using OPA-3 Primer.

	Bands No.	M (bp)	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
OPA-3	1	1500	+	-	+	-	+	+	+	+
	2	1250	-	-	-	-	+	-	+	+
	3	1000	+	-	+	-	+	+	+	+
	4	800	+	-	+	-	+	+	+	+
	5	700	+	-	+	-	-	-	+	-
	6	600	-	-	+	-	+	+	-	+
	7	500	-	-	-	-	-	-	-	+
	8	430	-	-	+	-	-	+	-	-
	9	380	-	-	+	-	-	+	-	-

The primer (OPA-3) was used in this study to screen the isolates, for RAPD-PCR markers. Each lane showed the amplification products of each isolate. The number of bands was found to be variable among isolates. The RAPD-PCR bands after using primer OPA-3 were presented in Fig. (2). The detailed information about the bands found in each isolate was noticed in Table (5). Band 1 with (1500 bp) was founded in the isolates Fu-1, Fu-3, Fu-5, Fu-6, Fu-7 and Fu-8. While, band 2 was only shared in the isolates Fu-5, Fu-7 and Fu-8 with (1250 bp). On the other side, bands (3 and 4) with (1000 and 800 bp, respectively) were shared in the isolates Fu-1, Fu-3, Fu-5, Fu-6, Fu-7 and Fu-8. Meanwhile, band 5 with (700 bp) was noted in the isolates Fu-1, Fu-3 and Fu-7. Whereas, band 6 with (600 bp) was exhibited in the isolates Fu-3, Fu-5, Fu-6 and Fu-8. However, band 7 with (500 bp) was uniquely noticed in the isolate Fu-8. In spite of, bands (8 and 9) were distinguished in the isolates Fu-3 and Fu-6 with (430 and 380 bp, respectively). Although, there are no bands shared in the primer OPA-3 for the isolates Fu-2 and Fu-4. So, the results showed that, the bands (1, 3, 4 and 5) with (1500, 1000, 800 and 700 bp, respectively) should be a reliable markers for the pathogenic isolates involved in this study.

OPA-2 Primer

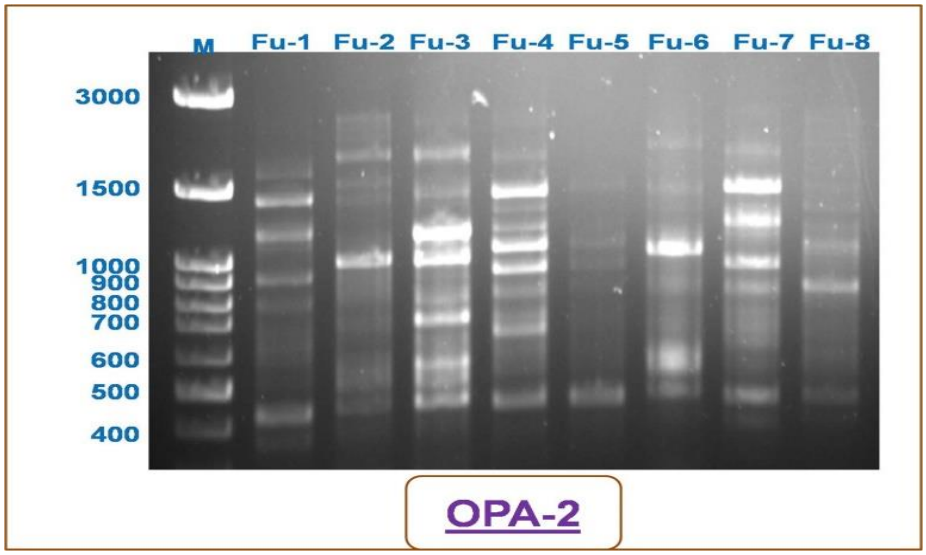

Fig. 3: Agarose gel electrophoresis of RAPD-PCR products by OPA-2 primer of eight isolates of *F. oxysporum f.sp. lycopersici*

Table.6: Presence of RAPD-PCR bands in *F. oxysporum f.sp. lycopersici* isolates after using OPA-2 Primer

	Bands No.	M (bp)	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
OPA-2	1	1800	-	+	+	-	-	-	-	-
	2	1500	+	-	-	+	-	-	+	-
	3	1350	-	-	+	-	-	-	+	-
	4	1100	+	-	-	+	-	+	-	-
	5	1000	+	+	+	+	-	+	+	+
	6	850	+	-	-	-	-	-	+	+
	7	700	-	-	+	+	-	-	-	-
	8	600	-	-	+	-	-	+	-	-
	9	480	-	-	+	+	+	+	+	-
	10	420	+	-	-	-	-	-	-	-

The primer (OPA-2) was used in the present study to screen the isolates for RAPD-PCR markers. Each lane showed the amplification products of each isolate. The number of bands was found to be variable among isolates. The RAPD-PCR bands after using primer OPA-2 were shown in Fig.(3). The detailed information about the bands found in each isolate was given in Table(6). Band 1 with (1800 bp) was exhibited in the isolates Fu-2 and Fu-3. Band 2 with (1500 bp) was founded in the isolates Fu-1, Fu-3 and Fu-7. In addition, band 3 was shared in the isolates Fu-3 and Fu-7 with (1350 bp). Moreover, band 4 with (1100 bp) was noted in the isolates Fu-1, Fu-4 and Fu-6. Furthermore, band 5 with (1000 bp) was revealed in the isolates Fu-1, Fu-2, Fu-3, Fu-4, Fu-6, Fu-7 and Fu-8. Besides, band 6 with (850 bp) was noticed in the isolates Fu-1, Fu-7 and Fu-8. On the other hand, band 7 with (700 bp) was screened in the isolates Fu-3 and Fu-4. In spite of, band 8 with (600 bp) was presented in the isolates Fu-3 and Fu-6. Whereas, band 9 was distinguished in the isolates Fu-3, Fu-4, Fu-5, Fu-6 and Fu-7 with (480 bp). While, band 10 with (420 bp) was uniquely found in the isolate Fu-1.

Considering the pathogenic isolates, the results exhibited that band 5 with (1000 bp) and band 9 with (480 bp) may be a reliable marker for the studied pathogenic isolates.

OPA-15 Primer

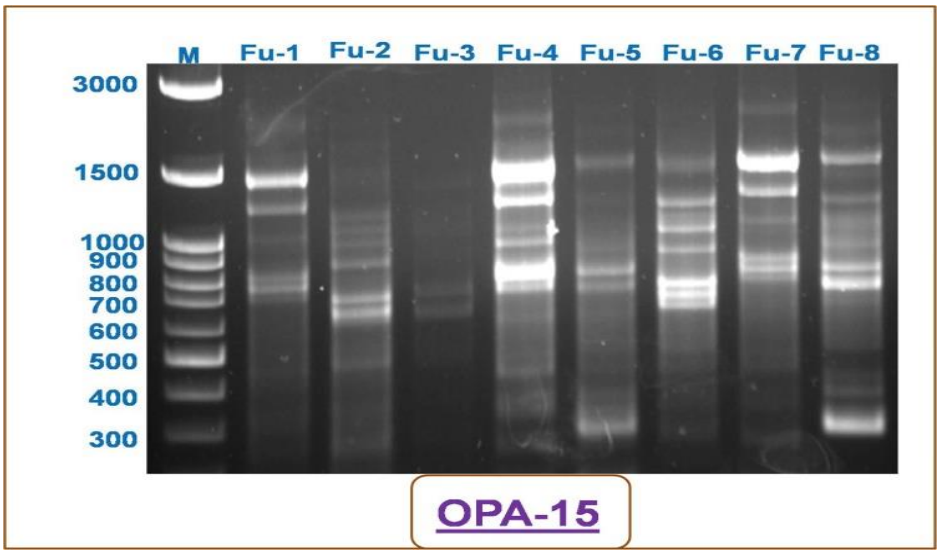

Fig. 4: Agarose gel electrophoresis of RAPD-PCR products by OPA-15 primer of eight isolates of *F. oxysporum f.sp. lycopersici.*

Table .7: Presence of RAPD-PCR bands in *F. oxysporum f.sp. lycopersici* isolates after using OPA-15 Primer.

	Bands No.	M (bp)	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
OPA-15	1	1500	+	-	-	+	-	-	+	+
	2	1350	+	-	-	+	-	+	+	-
	3	1000	-	-	-	+	-	+	-	-
	4	900	-	-	-	+	-	+	+	-
	5	800	-	+	-	+	-	+	+	+
	6	750	-	+	-	+	-	+	-	+
	7	700	-	-	-	+	-	+	-	+
	8	600	-	+	-	-	-	-	-	-
	9	315	-	-	-	-	+	-	-	+

The primer (OPA-15) was used in the present study to screen the isolates for RAPD-PCR markers. Each lane showed the amplification products of each isolate. The number of bands was found to be variable among isolates. The RAPD-PCR bands after using primer OPA-15 were shown in Fig.(4). The detailed information about the bands found in each isolate was given in Table(7). Band 1 with (1500 bp) was showed in the isolates Fu-1, Fu-4, Fu-7 and Fu-8. Moreover, band was found in the isolates Fu-1, Fu-4, Fu-6 and Fu-7 with (1350 bp). Furthermore, band 3 with (1000 bp) was noted in the isolates Fu-4 and Fu-6. In addition, band 4 with (900 bp) was shared in the isolates Fu-4, Fu-6 and Fu-7. Meanwhile, band 5 with (800 bp) was exhibited in the isolates Fu-2, Fu-4, Fu-6, Fu-7 and Fu-8. Whereas, band 6 with (750 bp) was founded in the isolates Fu-2, Fu-4, Fu-6 and Fu-8. However, band 7 with (700 bp) was noticed in the isolates Fu-4, Fu-6 and Fu-8. In spite of, band with (600 bp) and band 9 with (315 bp) were presented in the isolates Fu-2, and Fu-5 and Fu-8, respectively. Although, there are no bands founded in the isolates Fu-3.

Thus, the results exhibited that, the bands (1, 2, 5 and 6) with (1500, 1350, 800 and 750 bp), could be a reliable markers for the pathogenic isolates.

OPY-5 primer

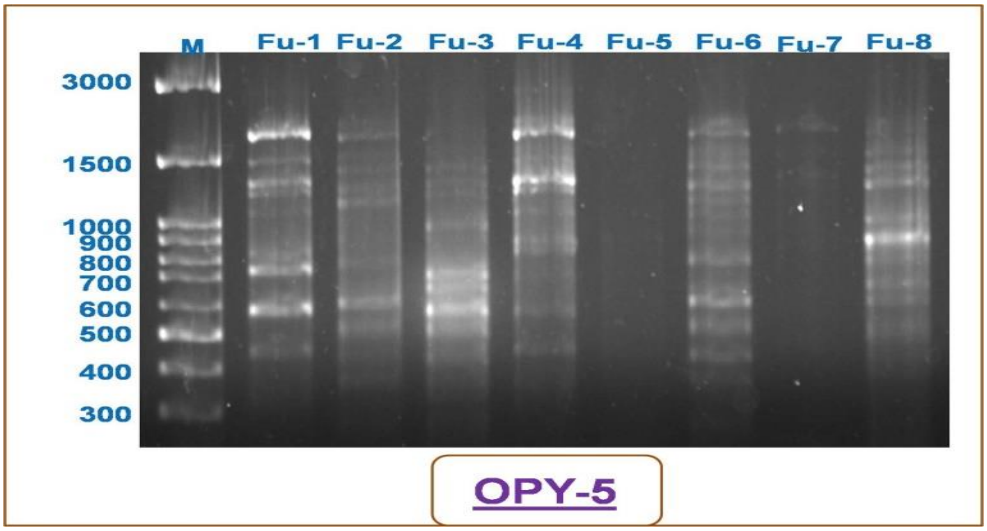

Fig.5 -: Agarose gel electrophoresis of RAPD-PCR products by OPY-5 primer of eight isolates of *F. oxysporum f.sp. lycopersici.*

Table .8 : Presence of RAPD-PCR bands in *F. oxysporum f.sp. lycopersici* isolates after using OPY-5 Primer.

	Bands No.	M (bp)	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
OPY-5	1	1850	+	-	-	+	-	+	-	-
	2	1500	+	-	-	-	-	-	-	-
	3	1430	+	-	-	+	-	-	-	+
	4	900	-	-	-	-	-	-	-	+
	5	750	+	-	+	-	-	+	-	-
	6	600	+	-	+	-	-	+	-	-
	7	450	+	-	-	-	-	-	-	-

The primer (OPY-5) was used in the existing study to scan the isolates for RAPD-PCR markers. Each lane showed the amplification products of each isolate. The number of bands was found to be variable among isolates. The RAPD-PCR bands after using primer OPY-5 were shown in Fig.(5). The detailed information about the bands found in each isolate was given in Table (8). Band 1 with (1850 b) was noticed in the isolates Fu-1, Fu-4 and Fu-6. While, band 2, band 7 and band 4 were uniquely founded in the isolates Fu-1 and Fu-8 with (1500 bp) for band 2, (450 bp) for band 7 and (900 bp) for band 4. Whereas, band 3 with (1430 bp) was presented in the isolates Fu-1, Fu-4 and Fu-8. On the other hand, bands (5 and 6) were contributed in the isolates Fu-1, Fu-3 and Fu-6.

Therefore, the results viewed that, bands (1, 3, 5 and 6) with (1850, 1430, 750 and 600 bp) may be a trusted marker for the studied pathogenic isolates.

Genetic similarity

Jaccard Coefficient similarity index was performed to estimate the level of similarity and dissimilarity among the eight studied *F. oxysporum f.sp. lycopersici* isolates against pathogenic properties according to (Jaccard, 1901). The correlation matrix between the different isolates Table (6) were done according to their amplification pattern. It could be concluded that, there was high similarity between Fu-3 and Fu-6 (0.429) with high pathogenic prosperities. This trend might be due to the molecular affinity. While, the lowest similarity was between the isolates Fu-1 and Fu-2 (0.040).

The dendrogram tree (UPGMA) among the studied eight isolates of which developed fro *F.*

oxysporum f.sp. lycopersici m five random primers of RAPD-PCR was showed in the Figure(3) and the results revealed that, the studied eight isolates of *F. oxysporum f.sp. lycopersici* were clustered to three groups. The first group illustrated isolates Fu-1, Fu-3, Fu-4, Fu-6 and Fu-7. Whereas, the second group showed the isolates Fu-5 and Fu-8. On the other hand, the third group exhibited the isolate Fu-2 uniquely. These clusters had a stable performance in pathoenicity, which presented variable response in performance during the time of the study. Therefore, similarity index (Jaccard Coefficient) was used to identify superior pathogenic isolates for accuracy and trusted index (Shamim et al., 2014).

Table.9: Values of genetic similarity calculated from the DNA fragments amplified from eight different isolates of *F. oxysporum f.sp. lycopersici* used five random primers.

	Fu-1	Fu-2	Fu-3	Fu-4	Fu-5	Fu-6	Fu-7	Fu-8
Fu-1	**1.000**							
Fu-2	**0.040**	**1.000**						
Fu-3	**0.250**	**0.174**	**1.000**					
Fu-4	**0.259**	**0.182**	**0.094**	**1.000**				
Fu-5	**0.130**	**0.125**	**0.227**	**0.083**	**1.000**			
Fu-6	**0.300**	**0.240**	**0.429**	**0.393**	**0.292**	**1.000**		
Fu-7	**0.321**	**0.160**	**0.267**	**0.370**	**0.261**	**0.273**	**1.000**	
Fu-8	**0.269**	**0.190**	**0.172**	**0.269**	**0.389**	**0.310**	**0.333**	**1.000**

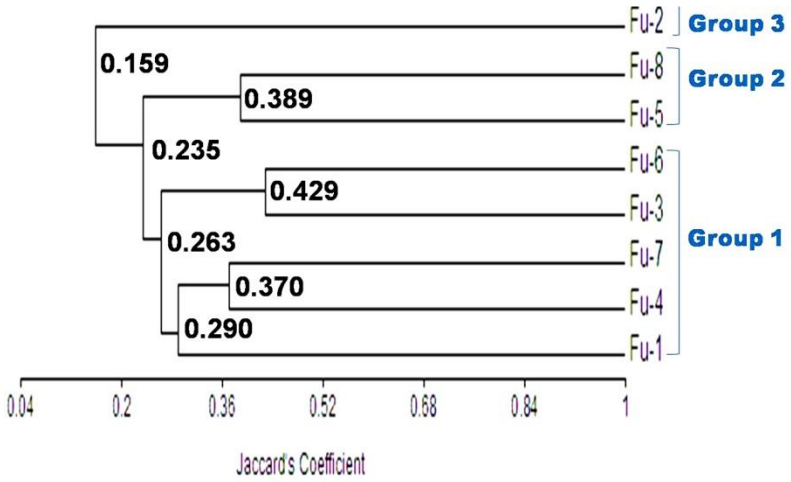

Fig.6: Dendrogram of eight isolates of *F. oxysporum f.sp. lycopersici* shows the clustered groups of PCR-RAPD developed from five primers by UPGMA analysis. The scale used is based on the Jaccard coefficient of similarity.

DISCUSSION

The non reproducible primers interpreted according to the primers themselves such as no detectable product or low yield of the desired product, the presence of nonspecific background bands due to mispriming or misextension of the primers; the formation of primer (Innis et al.,1988). This also could be due to the experimental conditions such as $MgCl_2$ concentration (Wolf et al., 1993), presence of glycerol in the reaction buffer (Hai Lu and Negre.1993), Taq polymerase quantity and quality (Schierwater and Ender, 1993) and the type of DNA thermal cycler (Wolf et al., 1993).

RAPD-PCR technique is a suitable method for identification and characterization of Fusarium species (Pujo et al. 1997; El-Fadly et al. 2008 and Leon et al. 2011). Our findings are in agreement with those obtained by Vakalounaskis and Fragkiadakis (1999) who stated that isolates of *F. oxysporum* from cucumber which classified into

three groups by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Moreover, Leslie and Summerell, 2006, observed inter- and intra specific genetic variation in different Fusarium species. So, RAPD-PCR technique was used to rapid identification and characterization of *Fusarium* species (El-Fadly et al. 2008). Moreover, RAPD marker used to estimate genetic variation among 12 isolates of the *F. solani* isolates causing dry root rot of sweet orange (Citrus sinensis osbeck) (Sankar et al., 2014). Edel et al. (2001) observed the isolates of *F. oxysporum* isolated from soil samples in France and showed genetic diversity. Furthermore, Gupta et al. (2009) reported the genetic polymorphism and diversity in isolates of *F. solani* isolated from wilt disease of Guava in India. In addition, Costano et al., 2014 studied genetic diversity of *F. oxysporum f. sp. dianthi* in Southern Spain in 132 isolates collected from carnation wilted plants. Besides, Assigbetse et al. (1994) differentiated races of *F. oxysporum f. sp. vasinfectum* on cotton by using RAPD as molecular tool and Bonde et al. (2013) studied genetic variation of *F. equiseti* isolated from fruits and vegetables.

UPGMA is a simple agglomerative or hierarchical clustering method used in bioinformatics for the phylogenetic analysis. The results obtained in the present study are noteworthy and showed the similarity with the observations of Abd-Elsalam et al. (2003) Ingle and Rai (2011), Bonde et al. (2013) and Gupta et al. (2009).

CONCLUSION

There were some reliable RAPD markers for the pathogenic isolates, which resulted close correlation at the molecular level under genetic control as it is inherited in a polygenic fashion. There were also some RAPD markers refer to the high pathogenic isolates. Therefore, RAPD markers offered a promising alternative to morphological marker and mostly display co dominant inheritance, which be used in many biological applications.

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التعريف الجزيئي والتوصيف لعزلات مختلفة من *Fusarium oxysporum f.sp. lycopersici* باستخدام محدد RPAD-PCR

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تم عمل خمس بادئات عشوائية من RAPD-PCR في ثماني عزلات من *Fusarium oxysporum f.sp. lycopersici*، كل منها يتكون من 10 قواعد. وفقًا لتمييز الأصناف بناءً على تحليل العلامات RAPD_PCR ، أوضحت النتائج أن العزلات المدروسة من *F. oxysporum f.sp. lycopersici* تتميز بوضوح عن بعضها البعض. تفاوتت معاملات التشابه الوراثي بين الزوجين من (0.040 إلى 0.429) بناءً على طريقة المجموعة الزوجية غير الموزونة لتحليل كتلة المتوسط الحسابي (UPGMA) لجميع العزلات. أظهر مخطط الشجرة الذي تم الحصول عليه من البيانات أن التجميع الهرمي فصل العزلات إلى ثلاث مجموعات وفقًا لمعاملات التشابه بينهما. لذلك ، كانت هناك بعض علامات RAPD الموثوقة للعزلات المسببة للأمراض ، والتي نتج عنها علاقة وثيقة على المستوى الجزيئي تحت السيطرة الوراثية حيث أنها موروثة بطريقة متعددة الجينات. وبالتالي ، يمكن استخدام تقنية RAPD-PCR كأداة مهمة للتعرف الجيني وتوصيف *F. oxysporum f.sp. lycopersici* الأنواع.