J. Agric. Sci. Mansoura Univ., 34 (7): 8335 - 8351, 2009

PATHOLOGICAL AND MOLECULAR VARIATION AMONG SOME ISOLATES OF *Fusarium oxysporum* F.SP. *lycopersici* FROM TOMATO ROOTS

Ahmed, M. A. M.*; M. A. Kararah*; S. M. Abdel-Momen and Omnia M. Elharrany****

*** Plant Pathology Dept., Fac. Agric., Cairo Univ.**

**** Plant Pathology Res. Inst., Agric. Res. Center**

ABSTRACT

Fusarium wilt disease is among the serious diseases that attack different tomato cultivars causing loss of stand yield. Pathological and Molecular variations among certain isolates of *Fusarium oxysporum lycopersici*, collected from diseased tomato plants grown in different governorates of Egypt, were studied in this investigation. The pathogenicity tests were performed for the isolates *F. oxysporum* f.sp. *lycopersici* which isolated from samples of tomato roots cv. Castle Rock. These isolates were collected from different locations in Egypt (El-Gharbiya, El-Qalubia, Beni-Sueif, El-Nubaria, South Tahrir, Kafr El-Sheikh, El-Salhiya, Giza, Al-Arish, El-Ismailia, Sohag and El-Fayoum). The results showed that all isolates were pathogenic, with different degrees of pathogenicity. The electrophoretic study of *F. oxysporum* f.sp*. lycopersici* had high degrees of similarity and no relation between the protein profiles and the geographic source. On the other hand, the fingerprinting technique with DNA through using RAPD-PCR showed that there was no clear relationship between genetic and geographic origin.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop of *Solanaceae*. It is considered as an important human nutritional source containing minerals and vitamins. It is a short duration crop and can grow all over the year under the Egyptian conditions giving a high yield.

Tomato is subjected to some destructive diseases. Fusarium wilt disease is among the serious diseases that attack different tomato cultivars causing loss of stand yield. The spread of this disease was recorded in some tomato fields in some governorates of Egypt. The most important pathogens infecting roots are different species of Fusarium fungus (Sneh *et al.,* 1989).

Fusarium oxysporum f.sp. *lycopersici* causes tomato Fusarium wilt. The fungus invades susceptible plants through wounds and natural openings created by newly emerging roots or other mechanical injuries. Early symptoms in tomato seedlings include stunting, yellowing, and premature loss of cotyledons and lower leaves. A pronounced brown lesion that girdles the hypocotyl (root/shoot junction), root rot, wilting, and death are advanced symptoms.

The identification and taxonomy of fungal species and isolates are mainly based on their morphological, physiological characters and the hosts they infect. Recently, molecular techniques have been used to identify species and races of fungi and study their taxonomical relationships (Mazzola *et al*., 1996). Randomly amplified polymorphic DNA RAPD, assay a modified

polymerase chain reaction (PCR) technique has been increasingly used to study genetic variability of plants and several microorganisms (Turner *et al*., 1999). These techniques have accurate and quick results and simplify but dose require prior sequence information to generate primers.

The objectives of the present work were to investigate (i) Cultural and pathological variation among certain isolates of *Fusarium oxysporum lycopersici* collected from diseased tomato plants grown in different governorates of Egypt. (ii) Electrophoretic studies includes protein profiles and molecular differentiating, RAPD-PCR among the same isolates using six primers.

MATERIALS AND METHODS

1. Source of samples, isolation and identification

Samples of diseased tomato plants (cv. Castle Rock) were collected from different tomato fields in different governorates of Egypt: El-Gharbiya, El-Qalubia, Beni-Sueif, El-Nubaria, South Tahrir, Kafr El-Sheikh, El-Salhiya, Giza, Al-Arish, El-Ismailia, Sohag and El-Fayoum. Symptomatic roots were collected and washed with distilled water then cut into 5-10-cm-long sections with a sterilized sharp blade. The root pieces were surface disinfected for two minutes in 1% sodium hypochlorite, rinsed in several changes of distilled water and dried before plating on potato dextrose agar (PDA) medium containing 0.2% streptomycin and incubated at 25°C ± 2 for 3-5 days. Fungi which developed from the root pieces were purified using single spore technique (Brown, 1924 and Hawker, 1960). Identification of the isolated fungi was carried out at Mycology Res. and Disease Survey Dept., Plant Pathology Research Institute, A.R.C., Giza, Egypt, according to description given by Chidambaram *et al*. (1973) and Domsch *et al*. (1980). The obtained isolates of *Fusarium oxysporum*, the most frequent fungi, were identified to the forma specials depending on their infection to tomato roots and subcultured on PDA slants and kept in refrigerator at 5^{o}C for subsequent use.

2. Pathogenicity tests

a. Pot experiment

The pathogenicity of *F.o.* f.sp. *lycopersici* and was tested under the greenhouse conditions at the Agriculture Research Center, Giza. The used tomato cultivar was Castle Rock. Twenty-cm-diameter plastic pots were surface sterilized by formalin (5%) for 15 minutes and left for 15 days to dry and get rid of any toxic residues.. Inocula of *F.o.* f.sp. *lycopersici* was prepared by growing each isolate in 500-ml Erlenmeyer flasks containing 100 g of sorghum grains, 90 ml of distilled water and 50 g sand. The flasks were autoclaved at 121°C for 20 min and inoculated with mycelia disks that were cut from the edges of 3-day-old isolates of *F.o.* f.sp. *lycopersici*. The inoculated flasks were incubated at 25°C ± 2 for 10 days.. Each pot was filled with 2 kg of soil and infested with the tested fungus colonized sorghum grains (5% w/w).. Two apparently healthy tomato seedlings (30-day-old), cv. Castle Rock were planted in each pot. Ten isolates of *F. o*. f.sp*. lycopersici* were used in the pathogenicity tests.

Disease incidence and severity were determined 15, 30 and 45 days after inoculation. Also, plant height and number of leaves and dry and fresh weight for each plant were assessed.

b. Agar experiment

The isolates of *F.o.* f.sp*. lycopersici* were grown separately on water agar medium for three to five days at 20°C. Seeds of tomato cv. Castle Rock were allowed to germinate, under moistened and aseptic conditions at 25°C for 10 days. Four uniform seedlings were placed on the cultures of the respective fungus for five days after which colonization of seedling particularly roots were carefully exrooted and photographed.

3. Electrophoretic studies

a. Detection of protein in the mycelia

Sodium dodecyle sulphate, polyacrylamide gel electrophoresis (SDS-PAGE) technique was performed for separating proteins by the method described by Laemmili (1970), and modified according to Hames (1995). The modification, was reduced TEMED from 30 µl to 25 µl and also APS was reduced from 1.5 ml to 1.3 ml.

The mycelial growth from any of the tested isolate of *F. o.* f.sp*. lycopersici* was extracted by grinding 1g freeze-dried mycelium with pestle and mortar in liquid nitrogen and 200 µl extraction buffer (50 m M tris-HCl buffer, pH 6.8, glycerol 10 % w/v, ascorbic acid 0.1%, cycteine hydrochloride 0.1 w/v). The mixture was centrifuged for 30 min at 18.000 rpm and the supernatant collected.

The protein content in supernatant was estimated according to the method of Bradford (1976) with bovine serum albumin as standard protein. Protein content was adjusted to 2 mg/ml per sample.

1. Preparation of gels

Preparation of gels was made as described by Laemmili (1970).

a. Resolving (separating) gradient gel

Gel mixture for polyacrylamide gel electrophoresis 11% (SDS-PAGE) was prepared as follows:

30% Acrylamide	10 ml
1.5 M Tris-HCL pH 8.8	7.5 ml
10% SDS	0.3 ml
1.5% APS	1.5 ml
TEMED	15 ml
Deionized water	11 ml

b. Staking gel

30% Acrylamide	3.5 ml
0.5 M Tris-HCL pH 6.8	7.5 ml
10% SDS	0.3 ml
1.5% APS	1.5 ml
TEMED	30 ml
Deionized water	17.8 ml

The resolving gel was prepared according to the previously mentioned recipe. It is useful to add all the reagents except the TEMED to the gel (once

the TEMED and APS have been added to the polyacrylamide solution will polymerize in few minutes).

2. Preparation of samples

Protein samples were diluted by sodium dodecyl sulphate (SDS), added to the sample at a rate of 4 mg SDS/1 mg protein and 50 µl 2-mercaptoethanol were applied to each 950 ml of the sample, then the mixture was heated at 100ºC (boiled) in water bath for 3-5 min.

3. Pouring the separating and staking gel

The resolving gel was poured between glass sandwich using scientific instruments (San Francisco CA, USA, Model XPO77 Hoefer) and gently covered with 1 cm of water. Polymerization started within 25-30 min. after pouring. The stacking gel was then poured and allowed for polymerization after about 30 min.

4. Loading of the samples

Twenty micro-liters of the crude protein solution were applied to the wells of the stacking gel. The samples were covered with electrode buffer. Few drops of bromophenol blue (4 mg/100 ml deionized water) were added to the electrode (tracking dye).

5. Gel running

Electrophoresis was performed in a vertical slab mold (Hoefer Scientific Instruments, San Francisco, CA, USA, model LKB 2001, measuring 16 x (18 x 0.15 cm). Electrophoresis was carried out at 30 milliamper (m.A.) at 10º C for 3 hours.

6. Staining the gel with silver nitrate

The silver staining method for protein described by Sammons *et al.* (1981) was used. This method of staining is sensitive and detects as little as 2 ng of protein in a single band, staining procedure is shown herein:

b. PCR analysis:

The PCR for RAPD analysis was performed in a 25 ul volume that contained 2ul of genomic DNA (30 ng), 2.5 ul of 10 × reaction buffer, 2.5 ul of 25 mM MgCl2, 0.75ul of (2.5 mM) dNTPs, 1ul primer, and 0.5ul (0.5 unit) Taq DNA polymerase (Promega). The total volume was completed to 25 ul using sterile distilled water. The six used random primers are indicated in Table (1).

The DNA amplification was performed in a PCR unit II-Biometra. The amplification program started with an initial step of 95°C for 5 min. and then a repeated 45 cycles of the following: denaturation at 95°C for 1 min., annealing at 36°C for 1 min. and extension at 72°C for 2 min. At the end of the amplification program samples were soaked at 4°C.

Table (1): List of primers used in RAPD analysis.

Primer no.	Sequence 5' _____ 3'	Annealing temperature
1	GGTGCGGGAA	36ºC
2	GTTTCGCTCC	36ºC
3	GTAGACCCGT	36ºC
4	AAGAGCCCGT	36ºC
5	AACGCGCAAC	36ºC
6	CCCGTCAGCA	36ºC

c. Electrophoresis

The PCR products were electrophoresed using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD) on 1% agarose made of 1x TBE buffer and containing 0.5 mg/ml ethidium bromide, at 75 constant volt and photographed on a UV transilluminator.

d. Gel analysis

The gel was scanned for band Rf using gel documentation system called Advanced American Biotechnology system (AAB) (1166 E. Valencia Dr. Unit 6 C, Fullerton CA 92631). The different M.W. of bands was determined against PCR marker Promega G 317A.

RESULTS

According to morphological and cultural characteristics, the fungus *F. oxysporum* was the most frequent on the isolating substrate. The identification by specialists was confirmed with the results of the pathogenicity tests shown later. Ten isolates of *F.o.* f.sp. *lycopersici*, were identified

Linear growth of *F.o.* f.sp. *lycopersici* ranged from 70 mm to 90 mm for all isolates. The lowest growth was for isolates 2 and 6 which isolated from El-Ismailia and El-Salhiya, respectively. The highest growth was observed for the isolates 5, 9 and 1 originated from El-Qalubia, Al-Arish and El-Nubaria, respectively (table 2).

The sporulation of *F.o.* f.sp. *lycopersici* was greatly varied among the isolates, and ranged from 10-95 x 10^5 per ml. Lowest sporulation was observed in case of isolates 9, 6, 4 and 3 being 10, 20, 25 and 25 x 10^5 for the four isolates, respectively. Sporulation of isolates 1, 5, 8 and 10 was mostly the same and ranged from 50-65 x 10^5 per ml. In particular isolate 2 exhibited ample amount of spores on PDA, being 95 x 10^5 spores per ml (as shown in table 2).

Table 2. Cultural characteristics of *Fusarium oxysporum* f.sp. *lycopersici* grown on PDA medium after 10 days of incubation at 25 ºC.

Isolate No. and source		Linear growth (mm)	Growth description	Sporulation Per ml x 10^5
1	El-Nubaria	83	Light violet, mostly surface and aerial	65
2	El-Ismailia	70	White, soft surface and aerial	95
3	South-Tahrir (1)	85	White, mostly surface and oppressed	25
4	South-Tahrir (2)	75	White, mostly surface and oppressed	25
5	Toukh (Qalubia)	90	Light violet, soft surface and aerial	50
6	El-Salhiya	70	Light violet, soft surface and aerial	20
7	Sohag	79	Violet, soft surface and aerial	40
8	El-Fayoum	73	Dark violet, soft surface and aerial	50
9	Al-Arish	85	Violet, surface and aerial	10
10	Giza	90	Dark violet, mostly surface and aerial	50

a. Pot experiment

1. Inoculation with the different isolates:

All isolates used were able to infect tomato transplants. The percentages of transplants showed symptoms were varied greatly according to the tested isolates (Table 3). The growth and number of leaves on plants of the different treatments assumed not to be varied from treatment to other until the end of the experiment. Thus, the number of leaves in the control ranged from 9-13 and from 9-12 leaves in the inoculated treatment through the periods of the experiment. However, there was normal growth of the control plants and the plant height showing 36.0, 47.0 and 52.0 cm after 15, 30 and 45 days after inoculation, respectively. The analogous values in the diseased plants due to different isolates were ranged from 28 – 30.5, 29 – 39 and 30 – 43 cm, respectively, (Table 3).

The percentages of plants that exhibited disease symptoms were zero in the control treatment, for the all periods, and even in soil which was inoculated with isolates 2, 3, 7, 8, 9 until 30 days after inoculations.

Isolates 4 and 6 induced 83 and 50% wilt, respectively, 15 days after inoculation. The analogous values after 30 days from inoculation were 66% and 50%; the least percentages of wilt 30 days after inoculation were caused by isolates 10 and 1 which induced 16 and 33% wilt, respectively, (Table 3). After 45 days from inoculation the plants showed wilt symptoms in all treatments, and the percentages of wilt ranged from 16.0 for isolate 10 to 83.0 for isolate 1 (Table 3). After 15 days from inoculation, the percentages of dead plants were zero in soil inoculated with any of the isolates 1, 2, 4, 5, 7, 9 and 10. On the other hand, isolates 3, 6 and 8 gave an equal percentage of dead plants (16 %). After 30 days from inoculation, there were no dead plants raised in soil inoculated with any of the isolates 2, 4, 9 and 10, while in the other isolates, 1, 3, 5, 6, 7 and 8 the percentages dead plants was 16%. After 45 days from inoculation, isolate 10 showed no dead plants, while the other isolates were able to cause dead plants ranged from 16 (by isolate 1) to 66% (by isolate 3) (Table 3).

On the basis of the aforementioned results the isolates of *F. o.* f.sp. *lycopersici* could be classified into three groups in respect to cause wilt symptoms 45 days after inoculation, group 1: includes isolates 3, 7 and 10 which caused 16-33% wilt; group 2: included isolates 2, 4, 5, 6, 8 and 9, caused 50-66% wilt; group 3: includes isolate 1, caused 83% wilt, grouping the isolates in respect to their ability to cause death of the plants assumed to be not correlated with their ability to cause wilt symptoms. Though the isolates could be classified as follows: group 1: included isolate scored 0.0% for plant death (isolate 10) group 2 caused relatively low % death, 16-33%, (isolates 1, 2, 4, 6, 8 and 9) and group 3 which caused 50-66% plant death (isolates 3, 5 and 7).

The total dry weights of the plants were affected at various degrees according to the inoculated isolate. The least dry weights, 0.91 and 0.95 g/plant, were recorded for plants inoculated with isolates 1 and 9, respectively. The other isolates gave plant dry weight values ranged from 1.25 to 3.9 g according to isolate as compared with 5.3 g in the control (Table 3).

t3

b. Agar experiment

All isolates of *F. o.* f.sp. *lycopersici* were able to colonize seedling of tomato in particular their roots. There were some variations among the isolates in respect to severity of colonizing and rot symptoms caused by the end of inoculating periods.

3. Electrophoretic studies

a. Protein profile in *Fuzarium oxysporum* f.sp. *lycopersici* isolates

Protein patterns of the ten isolates of *F. o.* f.sp. *lycopersici* are shown in (Figure 1). The dendrogram in Figure (2) was constructed based on similarity levels generated from cluster analysis of electrophoretic banding patterns of SDS-dissociated proteins shown in the same figure. A cluster analysis was performed by using the UPG-MA algorithm. This programme calculated the similarities and differences among protein profiles. Based on protein profiles markers, similarities were calculated among ten isolates of *F. o. lycopersici* from El-Fayoum, El-Ismailia, Toukh, El-Salhiya, Sohag, Al-Arish, El-Nubaria and South Tahrir. Cluster analysis of the protein profiles placed the *F. o.* f.sp. *lycopersici* isolates into two main groups (Figure2). The similarity between *F. o.* f.sp. *lycopersici* isolates ranged from 79.44 to 98.63%. The first main cluster included isolates 8 (El-Fayoum), 2 (El-Ismailia), 5 (Toukh) and 6 (El-Salhiya). The second main cluster included 7 (Sohag), 9 (Al-Arish), 10 (Giza), 3 & 4 (South Tahrir) and 1 (El-Nubaria). Two isolates 3 & 4 (South Tahrir) at similarity of 95.89%, and two isolates from El-Ismailia and 5 From Toukh showed the highest similarity, ranged from 95.89% to 98.63%, thus the obtained protein profiles (Figure2) could not distinguish the *F. o.* f.sp. *lycopersici* isolates based on the geographic source.

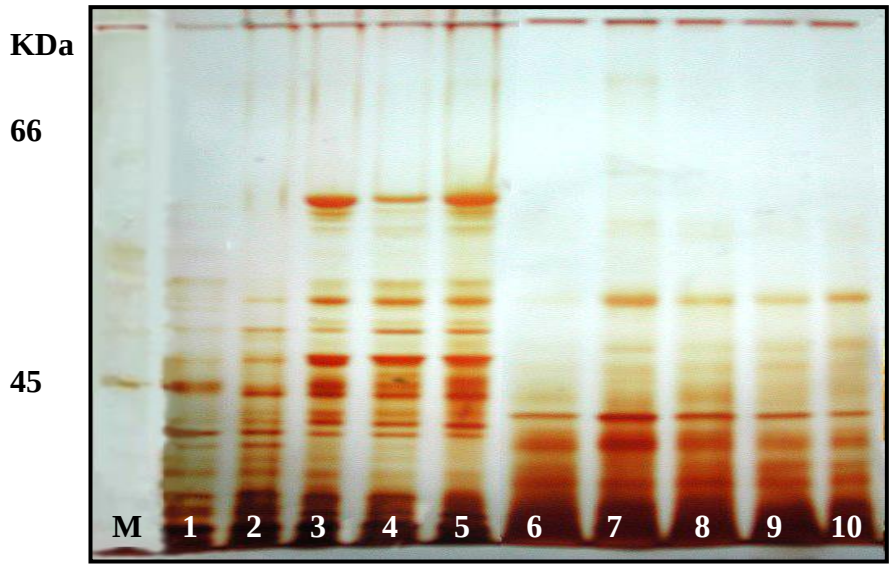

Fig.1. Protein profile analysis of ten *Fusarium oxysporum* f.sp. *lycopersici* isolates using SDS-polyacrylamide gel electrophoresis stained with silver nitrate. (M = marker).

c. RAPD-PCR analysis in *Fusarium oxysporum* f.sp. *lycopersici*

The dendrogram was constructed based on the similarity levels from cluster analysis of electrophoretic banding patterns of DNA of ten isolates of *F. o.* F.sp. *lycopersici* (Figures 3-11). Primer 1 classified the ten isolates into two main groups with similarity 87.53 (Figure3). The individual isolates were invariably subclustered according to their sources, with similarity levels ranging from 92.22 to 97.21% (Figure 4).

When primer 2 was used, El-Nubaria (isolate No. 1) was placed in one separate cluster and the other tested isolates placed in separate cluster (Figure5), with similarity level reached 83.62%. The highest percentage of similarity (95.1%) was observed between El-Fayoum and El-Ismailia isolates, (No. 8 and 2), (Figure 6).

Figure (7) shows the cluster analysis for RAPD products of *F. o.* f.sp. *lycopersici* isolates using primer 3. The ten isolates placed into two main groups with similarity level reached 61.89%. It is worth mentioned to explore that the similarity level between the two isolates 8 and 6, collected from Fayoum and Salhiyia, respectively was 100%. The same was for isolates 2 and 1 from El-Ismailia and El-Nubaria. The whole similarity levels for the ten isolates ranged from 61.89 to 100%. The similarity levels among the three subclusters which included isolates 6, 8, 9; 1, 2, 5, and 4 and 10 were alike, being 90.91% (Figure 8).

Primer 4 placed the different isolates in two main clusters (Figures 6 and 7). The similarity between these two groups was relatively low (45.18%). Isolates of El-Ismailia (No. 3), Al-Arish (No. 9) and South Tahrir-1 (No. 3) came in one group with 87.12% similarity, while the other isolates placed in another group with 60.55% similarity level (Figure8).

Primer 5 placed the isolates into different and distinct main groups (Figures 9 and10). Similarities ranged from 52.01% to 94.74%. Two main clusters were detected, the first included isolates 4 and 9, with similarity 61.54%. The second main cluster included the other tested isolates, similarity levels between them ranged from 58.48 to 94.74%. The similarity between the two main clusters was 52.0% (Figure10).

The cluster analysis of RAPD of the tested isolates in case of using primer 6 Figure (11) showed that two main groups were observed in the dendrogram. Similarities ranged from 34.86 to 100%. The first main cluster included one isolate (8) from Al-Fayoum, while the second main cluster included the other tested isolates. The similarity between the two main clustered groups was 34.86%. The similarity among isolates of the second group ranged from 77.36 to 100% (Figure11). Three subcluster groups (*i.e.* isolates 2 and 6, 5 and 9 and 7 and 10) scored the same similarity level, being 100%.

2-3

4-5

6-7

8-9

10-11

DISCUSSION

Results revealed that certain isolates of *Fusarium oxysporum* f.sp. *lycopersici*, grown in different governorates ,were isolated from tomato plants, exhibited typical wilt symptoms,. Pathogenicity tests proved that all isolates were pathogenic to tomato plants cv Castle Rock but they were greatly varied in this respect.

Fusarium isolates showed some morphological differences which could be due to some physiological differences (Lamprecht *et al*., 1988).

All the isolates of *F. oxysporum* f.sp. *lycopersici* caused different degrees of disease incidence to tomato indicating variations in their pathogenicity to the tested tomato cultivar. The variation in pathogenic ability of the tested isolates could be due to some genetic variability that make some isolates able to produce degrading enzymes, toxins or acids such as oxalic acid that increase the aggressiveness of the pathogens. The results were most obvious among the tested isolates as it caused different degrees of wilt 15 or 45 days after inoculation. Similar results has already been observed by Ramalingam (1984).

Protein profiles of ten *F. oxysporum* f.sp. *lycopersici* isolates indicated different degrees of similarity that reached 98.63% in some cases, and 79.44% in some other cases. This finding gave an indication of the genetic variability among the tested isolates. The obtained results are in agreement with those obtained by Ibrahim *et al.* (2003) who revealed that variation could be considered as a reflection of the genetic variability in the tested isolates.These results are consistent with those by (Mohammadi *et al.*, 2003).

The geographical source of *F. oxysporum* f.sp. *lycopersici* did not affect on the variability among isolates indicating that the variability is not due to the environmental conditions but may be due to some degrees of genetic variability.

When six random primers were used to detect the genetic variability among ten isolates of *F. oxysporum* f.sp. *lycopersici*, considerable degrees of genetic variability were observed. The genetic variability differed in its appearance with different primers used. This could be attributed to differences in the amplification loci of the used primers. Some of the used primers placed a single isolate in a separate clustering group giving an indication of the high genetic variability between this isolate and the others. These results are in agreement with those obtained by Vakalounaskis and Fragkiadakis (2000) who stated that isolates of *F. oxysporum* from cucumber were classified into three groups by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). They recorded, also, that all isolates of *F. oxysporum* f.sp. *radicis-cucumerinum* fell into one group and they noticed that isolates of races 1 and 2 of *F. oxysporum* f.sp. *cucumerinum* fell into a second group related to isolates of *F. oxysporum* f.sp. *melonis* and *niveum*. Additionally, isolates of race 3 were placed into a third group, related to *F. oxysporum* f.sp. *momordicae*

On the other hand, some isolates showed 100% genetic similarity with each other with some of the used primers could be attributed to have identical amplification locus of that certain primer. The genetic variability among The ten isolates of *F. oxysporum* f.sp. *lycopersici* was mostly not correlated with geographic sources of the tested isolates. This is an indication of the genetic variability was not due to the ambient conditions (Gherbawy, 1999) since the genetic diversity was detected in the tested isolates and was not related to the geographic sources.

REFERENCES

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72:248–254.

Brown, N. (1924). Two mycological methods. II. A method of isolated single strain fungi by cutting hyphal tip. Ann. Bot., 38: 402-406.

Chidambaram, P.; Mathur, S.B. and Neergaard, P. (1973). Identification of seed-borne *Drechslera* species. Freesia, 10:165-207.

Domsch, K.H.; Games, W. and Anderson, T. (1980). Compendium of Soil Fungi. Acad. Press., London, New York, Toronto, Sydney and San Francisco, 859 pp.

Gherbawy, Y.A.M.H. (1999). RAPD profile analysis of isolates belonging to different *formae speciales* of *Fusarium oxysporum*. Cytologia, 64 (3): 269-276.

Hames, B.D. (1995). One-dimensional polyacrylamide gel electrophoresis. In: "Gel Electrophoresis of Proteins. A Practical Approach" (Eds. Hames, B.D. and Rickwood, D.). Oxford Univ. Press, New York, p. 1-147.

Hawker, L.E. (1960). Physiology of Fungi. Univ. of London Press, LTD Warwish Square, London. 360pp.

Ibrahim, N.; Aly, M.A.; Abdel-Sattar, M.A.; Abd-Elsalam, K.A.; Khalil, M.S. and Joseph, A. (2003). Comparison of multi-locus enzyme and protein gel electrophoresis in the discrimination of five *Fusarium* species isolated from Egyptian cottons. African J. Biotechnol., 2(7): 206–210.

Laemmili, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

Lamprecht, S.C.; Marasas, W.F.O.; Van Wyk, P.S. and Davies, P.S.K. (1988). *Fusarium tricinctum* (family: Hyphomycetes) in South Africa - morphology and pathogenicity. Bothalia, 18(2): 189-194.

Mazzola, M.; Wong, O.I. and Cook, R.J. (1996). Virulence of *Rhizoctonia oryzae* and *R. solani* AG-8 on wheat and detection of *R. oryzae* in plant tissues by PCR. Phytopathology, 86(4):354-360.

Mohammadi, M.; Banihashemi, M.; Hedjaroude, G.A. and Rahimian, H. (2003). Genetic diversity among Iranian isolates of *Rhizoctonia solani* Kuhn anastomosis group 1 subgroups based on isozyme analysis and total soluble protein pattern. J. Phytopathol., 151(3):162-170.

Ramalingam, P. (1984). Production of cellulolytic enzyme by *Rhizoctonia solani* Kuhn. Current Sci. India, 53(6): 327-328.

Sammons, D.W.; Adams, L.D. and Nishizawa, E.E. (1981). Ultra-sensitive silver based color staining of polypeptides in polyacrylamide gels. Electrophoresis, 2:135.

Sneh, B.; Auster, I. and Shomer, M. (1989). Comparative anatomy of colonization of cotton hypocotyls and roots by virulent and hypovirulent isolates of *Rhizoctonia solani*. Can. J. Bot., 67(7):2142-2149.

Turner, A.S.; O'hara, R.B.; Rezamoor; H.N.; Nutall, M.; Smith, J.N. and Nicholsop, P. (1999). Visual disease and PCR assessment of stem base diseases in winter wheat. Plant Pathol., 48(6):742-748.

Vakalounaskis, D.J. and Fragkiadakis, G.A. (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility and RAPD finger printing. Phytopathology, 89(2):161-168.

التفرقة المرضية والجزيئية فيما بين بعض عزلات *Fusarium oxysporum* f.sp. *lycopersici* التى تصيب جذور الطماطم

محمد أحمد محمد احمد *، محمد عبد الجليل كرارة* ، صلاح محمد عبد المؤمن و أمنية محمد الحرانى****

*** كلية الزراعة جامعة القاهرة**

**** معهد بحوث امراض النباتات- مركز البحوث الزراعية**

تعتبر الطماطم من أهم محاصيل الخضر فى مصر ولكن من سوء الحظ أنها موضع مهاجمة من العديد من فطريات التربة التى تؤثر بالتبعية على نموها وإنتاجها. وقد أجريت هذه الدراسة بهدف دراسة الإختلافات المرضية والجزيئية بين بعض عزلات فطر الفيوزاريوم أوكسيسبورم ليكوبرسيسى والتى تصيب جذور نباتات الطماطم. وتم إجراء عدوى صناعية بالفطريات المعزولة وهى الفيوزاريوم أوكسيسبورم ليكوبرسيسى من جذور نباتات طماطم مصابة وقد أدى ذلك إلى ظهور الأعراض المرضية مع إختلافات فى القدرة المرضية للعزلات المختبرة. وعند استخدام الفصل الكهربى لتحديد الطرز البروتينة للعزلات المختلفة لفطر *Fusarium oxysporum* f.sp. *lycopersici* وجد أن هناك تشابهاً كبير بين العزلات وبعضها أى أنه لا يعتمد فى التفرقة على أساس المنطقة الجغرافية المأخوذ منها العزلات. وكذلك استخدمت البصمة الوراثية لمعرفة وجود إختلافات من عدمها لعزلات فطر الفيوزاريوم أوكسيسبورم ليكوبرسيسى عن طريق التضاعف العشوائى للـ DNA فأظهرت النتائج أن هناك تشابه بين عزلات الفطر المأخوذة من مناطق مختلفة ما يدل على عدم وجود إرتباط وثيق بين مصدر العزلة وتركيبها الوراثى.

Table 3. Pathogenicity tests of *Fusarium oxysporum* f.sp. *lycopersici* isolates to tomato transplants (cv. Castle Rock) 15, 30 and 45 days after inoculation.

* Isolate and source	15 days				30 days				45 days				Dry weight/plant (g)**		
	Plant height (cm)	No. of leaves	% wilted plants	% dead plants	Plant height	No. of leaves	% wilted plants	% dead plants	Plant height	No. of leaves	% wilted plants	% dead plants	Shoots	Roots	Total
1. El-Nubaria	29.0	10.0	33.0	0.0	31.8	12.0	33.0	16.0	31.0	12.0	83.0	16.0	0.77	0.14	0.91
2. El-Ismailia	30.0	10.0	0.0	0.0	33.7	11.0	0.0	0.0	34.0	11.0	66.0	33.0	1.8	0.2	2.0
3. South Tahrir (1)	28.0	9.0	0.0	16.0	29.0	12.0	0.0	16.0	30.0	12.0	33.0	66.0	2.8	0.16	2.96
4. South Tahrir (2)	26.0	10.0	83.0	0.0	30.0	11.0	66.0	0.0	31.0	11.0	66.0	33.0	1.4	0.29	1.69
5. Toukh	30.5	10.0	16.0	0.0	33.0	12.0	16.0	0.0	33.0	12.0	50.0	50.0	2.2	0.38	2.58
6. El-Salhiya	29.0	10.0	50.0	16.0	31.5	12.0	50.0	16.0	32.0	12.0	66.0	16.0	1.0	0.25	1.25
7. Sohag	30.0	9.0	0.0	0.0	32.0	10.0	0.0	16.0	32.9	10.0	33.0	50.0	2.5	0.14	2.64
8. El-Fayoum	29.0	11.0	0.0	16.0	32.0	12.0	0.0	16.0	32.0	12.0	50.0	33.0	2.2	0.39	2.59
9. Al-Arish	29.0	9.0	0.0	0.0	33.0	10.0	0.0	0.0	33.0	10.0	50.0	33.0	0.7	0.25	0.95
10. Giza	31.0	9.0	0.0	0.0	39.0	11.0	16.0	0.0	43.0	11.0	16.0	0.0	2.8	1.1	3.9
Control (not-inoculated)	36.0	9.0	0.0	0.0	47.0	13.0	0.0	0.0	52.0	13.0	0.0	0.0	4.6	0.7	5.3
L.S.D. (0.05)											3.8	2.5	0.21	0.053	

*** , re-isolation procedures produced the same isolates originally inoculated while control samples did not produce any fungi.**

**** ,Assessed 45 days after inoculation.**