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FUSARIUM OXYSPORUM F. SP. *LYCOPERSICI* (FOL) RACE 1 AND 3 AS WILT-INCITANTS TO TOMATO PLANTS GROWING AT EL-MINIA GOVERNORATE, EGYPT

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ABSTRACT

Wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, (FOL) is one of the most destructive diseases. This disease causes significant yield losses in the Middle Egypt. Eight *Fusarium* isolates were isolated from wilted tomato plants cv. Super Jackal growing in El-Minia Governorate during 2012 growing season. Isolates were tested for their pathogenic ability on tomato plants cv. Super Jackal under greenhouse conditions. The isolates significantly varied in their ability to cause wilt infection of tomato plants. The most virulent isolates were *F. oxysporum* isolate (F1) and *F. oxysporum* isolate (F2) as they covered the highest area under wilt progress curve (AUWPC). The highest disease severity was obtained by isolate F1 (96% after 45 days) followed by isolate F2 (90% after 45 days).

Polymerase chain reaction (PCR) based markers were used to characterize these two isolates. The PCR analyses confirmed that both isolates were *F. oxysporum* f. sp. *lycopersici* (FOL). Isolate (F1) gave fragments with uni (672 bp) and sp13 (445 bp) specific primer sets for FOL race 1 while, the isolate F2 was observed to amplify fragments with uni(672 bp), sp13 (445 bp), and sp23 (518 bp), indicating FOL race 3. FOL isolates F1 (race 1) and isolate F2 (race 3) were virulent towards all tested cultivars. The highest percentage disease severity was (95 %) for F1 (race 1). Four cultivars gave high resist reaction type to FOL race 1 (F1) and race 3 (F2) namely, cv. Zaman, Super Red cv., Nema Star cv. and Marwa cv. while, hybrid 010-65 showed a resist reaction to both races. However, Basha cv.

was susceptible to race 1 and high susceptible for race 3, hybrid Nema 1400 was highly susceptible to race 1 and susceptible to race 3.
Keywords: Tomato wilt, PCR-based markers, *Fusarium oxysporum* f. sp. *lycopersici*, FOL, race1 and race 3.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) plants are attacked by several soilborne fungal pathogens (Morsy *et al*., 2009). *Fusarium* species are the most important pathogens, which develop in both cultured and non-cultured soils, causing the symptoms of damping off, root rot and wilt diseases to wide range of various plants including tomato (Abu-Taleb *et al*., 2011). In the last decad, *Fusarium* caused severe damage to tomato cultivars in Egypt (Sagitov *et al*., 2008).

Wilt caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp.*lycopersici* (Sacc.) W.C. Snyder and H.N. Hans and *F. oxysporum* f. sp. *radicis-lycopersici* (Jarvis and Shoemaker), respectively, have been the most serious soilborne diseases threatening tomato production (Agrios, 2005). *F. oxysporum* f. sp. *lycopersici* (FOL) infection involves the germination of soilborne spores in the vicinity of growing roots, attachment to the root surface, penetration of the root cortex and proliferation of hyphae within the root vascular system. Eventually, the fungus invades and colonizes the parenchyma of the dying tomato plant and sporulates on the plant surface (Michielse and Rep, 2009). The disease is characterized by yellowing, wilting and browning of the leaves, stunted growth and, eventually, death of the plant. These symptoms result from the obstruction of water and nutrient flow caused by hyphae within the xylem vessels, as well as tyloses, callose, gums and gels produced by the host plant.

Three known physiological races (1, 2, and 3) of FOL are distinguished by their specific pathogenicity on differential cultivars carrying dominant race specific resistance genes (Alexander and Tuker 1945; Gerdemann and Finley 1951; Cirulli and Alexander 1966; Grattidge and O'Brien 1982; Masunaga *et al*., 1998). Although the species of *Fusarium* can be identified by their morphological characteristics on selective media (Nelson *et al*., 1983; Burgess *et al*., 1994), the pathogenic types, or formae speciales and races, of *F. oxysporum* cannot be identified morphologically. More recently, Hirano and Arie, 2006 have reported differentiation of *Fusarium oxysporum* f.sp *lycopersici* and f .sp *radicis lycopersici* by a polymerase chain reaction (PCR)-based method using specific primer sets developed from the knowledge of the partial nucleotide sequences of the *endo (pg1*) and *exo* (*pgx4*) polygalacturonases genes of the fungi. Molecular markers are accurate and useful tools to identify formae speciales or races of *F. oxysporum*

would accelerate pathogen identification (Lievens *et al*., 2008).

Tomato cultivars are reacted variously to *Fusarium* infection (Moustafa and Khafagi, 1992). The use of resistant cultivars or resistant rootstocks is the most reliable way to prevent the diseases. To successfully select the most appropriate cultivar for the coming growing season, the form and race of the pathogen that are emerging in the field must be identified (Hirano and Arie, 2006).

Meanwhile, tomato wilt-causing *Fusarium* isolates were obtained from tomato- growing at various locations of the middle region of Egypt mainly at El-Minia. The objectives of this study are mainly to: 1) isolate *Fusarium* associated with wilted tomato plants, 2) carry out the pathogenicity test of the obtained *Fusarium* isolates, 3) identify of *F. oxysporum* formae specials as well as races through PCR and 4) test the reaction of some tomato cultivars to infection by identified races.

MATERIALS AND METHODS

1. Isolation and culture maintenance of tomato wilt-causing *Fusarium*

Samples of tomato plants (cv. Super Jackal) displaying wilt symptoms (Fig.1) were collected from tomato-growing fields of different locations at El-Minia Governorate, Egypt. Morphological and Microscopical examinations were used to select *F. oxysporum* isolates for this study (Booth, 1971; Nelson, 1983).

The isolates of *Fusarium oxysporum* used in this study were recovered from tomato plants showing typical symptoms of wilt. Plant tissues of tomato were washed thoroughly with tap water to remove adhering soil particles. To isolate the fungus, infected plant tissues were first treated with a surface disinfection with 1.0% sodium hypochloride for 2–3 min. Disinfected tissues of about 3–4 mm in length were placed on Petri dishes including 2.0% potato dextrose agar (PDA) and incubated at 25 °C for 5 days.

2. Identification based on morphological features

Identification based on the morphological and microscopical characters of isolates was conducted according to some characteristics of macroconidia, microconidia, phialides, and presence of chlamydospore of *Fusarium oxysporum* (Booth, 1971; Gerlach and Nirenberg, 1982; Nelson *et al*., 1983). The isolates were then stored at 4 °C for further studies.

3. Pathogenicity test

3.1. Inoculum preparation

Inocula of 8 *Fusarium oxysporum* isolates were prepared by culturing each isolate on 50.0 mL Potato Dextrose Broth (PDB) medium in 250 mL Erlenmeyer flasks for 10 days at 25±2°C followed by washing and blending in sterilized water. Colonies forming units (CFU) were adjusted to 10^6 CFU/mL using haemocytometer slide (Elad and Baker, 1985), tween 20 was added to suspension (0.1% v/v) prepared before inculcation.

3.2.Inoculation procedure

Tomato transplants, cv. Super Jackal (35-40 day-old of 3-5 fully compound leaves) were removed from individual cells of the trays using a gentle pulling motion. Care was taken to prevent breaking the stems. After the seedlings were removed from the cell, excess vermiculite above the roots was cleaned by gentle shaking. The plants were submerged into the inoculum to cover the roots and they were allowed to remain in inoculum for 30 min. The inoculated plants were removed and replanted into 5-cm-diameter plastic pots (one plant/each) contained soil (clay/sand) which was used as the growing medium (Ignjatov *et al*., 2012).

3.3. Postinoculation care

Inoculated plants were watered daily for 3 days after transplanting with sufficient water to keep the soil wet, but not as wet as to cause leaching. After the initial 3 days, seedlings were watered as needed. The experiment was done on Randomized Complete Block design with 4 replications (each 20 plants) and the experiment was repeated 3 times.

3.4. Disease assessments

Wilt severity was estimated at 15, 25, 35 and 45 days after replanting using a rating scale of (0 – 5) based on leaf yellowing grading, viz., 0 = healthy, 1= one leaf yellowing 2= more than one leaf yellowing, 3= one wilted leaf, 4= more than one leaf wilted, and 5= completely dead plants (Rep, 2005). Disease severity index (DSI) described by Liu *et al*. (1995) was adapted and calculated as follows:

$$DSI = \Sigma d/(d\ max \times n) \times 100$$

Where: d is the disease rating of each plant, d max the maximum disease rating and n the total number of plants/samples examined in each replicate.

The mean of area under wilt progress curve (AUWPC) for each replicate was calculated as suggested by Pandy *et al*. (1989).

$$AUWPC = D\ [1/2\ (Y1+Yk) + (Y2+Y3+\ldots\ldots..+Yk-1)]$$

Where D= Time interval; Y1= First disease severity; Yk= Last disease severity;

Y2, Y3,……Yk-1= Intermediate disease severity.

Re-isolation of *F. oxysporum* from artificially inculcated tomato plants was conducted to achieve Koch`s postulates.

4. Identification based on molecular biology technique

In this experiment, selected two *Fusarium oxysporum* (isolate 1 and isolate 2) isolates, which were identified according to the morphological, microscopical and pathological characteristics, were used.

4.1. Molecular biology study

In the present study, the identification of *F. oxysporum* isolates originating from tomato growing in different locations at El-Minia Governorate using morphological, microscopical and PCR methods. Identification of FOL races based on PCR was done at the Lab of Plant Breeding, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan.

4.1.1. DNA extraction

A CTAB DNA extraction protocol was used to acquire DNA from the fungal isolates. Fungal isolates were grown in 20 ml of liquid medium (4 g l -1 of malt extract broth (Merck) or ¼ MYEDP medium (4.75 g malt extract, 0.5 g yeast extract, 0.75 g dextrose, 0.45 g peptone, 500 mM thiamine in a final volume of 1 l, pH 5.6). Five to six 0.5 cm^2 agar discs of each isolate were used to inoculate liquid medium. The cultures were incubated for four to five days at 28 °C. The mycelia were centrifuged and washed once in sterile water and twice in 500 mM $NaCl_2$ and 50 mM EDTA pH 8.0. The mycelia were blotted dry and macerated in liquid nitrogen with 2800 µl of DNA extraction buffer [1.0% CTAB, 0.5 M $NaCl_2$, 69 mM EDTA pH 8.0, 34 mM Tris pH 8.0, 0.05% N-lauryl sarcosine, 1% SDS, and 0.009% β- mercaptoethanol]. Twenty µl of proteinase K (20 mg l-1) was added and the solution was incubated at 55 °C for 2 h. After incubation, the solution was centrifuged for 10 min at 12000xg and phenol/chloroform extracted. DNA was precipitated in 0.8 volumes of isopropanol, centrifuged, and washed with 70% ethanol, dried, resuspended in 100 µl of sterile water and stored at 4 °C (Ausubel *et al*., 1994).

4.1.2. Polymerase Chain Reaction (PCR) detection

To determine the formae speciales of *Fusarium oxysporum* as *F. oxysporum* f. sp. *lycopersici* (FOL race 1, 2, or 3) or *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), PCR was performed with the specific primer sets uni, sp13, sp23, and sprl as described by Hirano and Arie (2006). Primer sequences as well as the amplified DNA fragments detected in the present experiment are shown in Table 1. Fifty cycles of PCR was carried out for 1 min/94°C, 1 min/62°C and 1 min/72°C using PCR Thermal Cycler (Takara, Japan). The PCR products were fractionated in a 4% NuSieve 3:1 Agarose (Lonza, Rockland, ME, USA) by using gel electrophoresis apparatus (EIDD, Japan). After electrophoresis, the gel was stained with ethidium bromide and viewed with a UV illuminator.

Table 1. Specific primer sets used to identify *Fusarium oxysporum* f. sp. *lycopersici* or *radicis-lycopersici* as well as races of *F. oxysporum* f. sp. *lycopersici*

primer name	sequence 5'-3'	mer	Product (bp)
uni-f	ATCATCTTGTGCCAACTTCAG	21	672
uni-r	GTTTGTGATCTTTGAGTTGCCA	21	
sp13f	GTCAGTCCATTGGCTCTCTC	20	445
sp13r	TCCTTGACACCATCACAGAG	20	
sp23f	CCTCTTGTCTTTGTCTCACGA	21	518
sp23r	GCAACAGGTCGTGGGGAAAA	20	
sprlf	GATGGTGGAACGGTATGACC	20	947
sprlr	CCATCACACAAGAACACAGGA	21	

(Hirano and Arie, 2006)

5. Reaction of certain tomato cultivars and hybrids to infection by FOL races 1 and 3

Seven cultivars of tomato plants e.g. Super Jackal, Basha, Marwa, Nema Star, Zaman, Super Red, and Rawan plus three hybrids e.g. 777, Nema 1400 and 010-65 were subjected to infection by Race 1 and Race 3 of *Fusarium oxysporum* f. sp. *Lycopersici* (FOl). Inoculum preparation, inoculation and disease severity were essentially done as above mentioned. The experiment was repeated 3 times (4 replicates, 20 plants /each replicate).

6. Statistical analysis

All recorded data were subjected to the analysis of variance procedures and treatment means were compared using t Standard Deviation (SD) as described by Gomez and Gomez (1984).

RESULTS

Isolation, purification and identification of the *Fusarium* associated with wilted tomato plants

Eight *Fusarium* isolates were isolated from wilted tomato plants collected from different locations in the Middle Egypt mainly at El-Minia Governorate. Hyphal tip cultures of grown fungi were maintained on PDA medium. All fungi were purified using single spore technique cultures, and then they were identified. Results indicated that all fungal isolates which identified are belonging to *Fusarium oxysporum* as described below.

The mycelia of pathogen were white cottony to pink, often with purple tinge or reddish coloration of the medium. Microconidia were born on simple phialides arising laterally and were abundant, oval-ellipsoid, straight to curved, 4-12x2.1-3.5 µm. Macroconidia, spares to abundant, were borne on branched conidiophores or on the surface of sporodochia and were thin walled, three to five septate, fusoid-subulate and pointed at both ends, have pedicellate base. Three septate spores were more common. Chlamydospores, both smooth and rough walled, which are abundant and form terminally or on an intercalary basis Gerlach and Nirenberg (1982).

Pathogenicity test

The *Fusarium* isolates obtained from naturally infected plants (Fig. 1A), were tested for their pathogenic ability on tomato plants cv. Super Jackal under greenhouse conditions (Fig. 1B). The tested fungal isolates significantly varied in their ability to cause wilt infection of tomato plants (Table 2). Area under wilt progress curve (AUWPC) and disease severity (DS) after 15, 25, 35 and 45 days was calculated. The most aggressive isolates were *F. oxysporum* isolate F1 followed by isolate F2 as they exhibited 2640 and 2300 AUWPC, respectively. On the other hand, *F. oxysporum* isolate F5 and isolate F8 caused least potentiality of infection in tomato plants, 755 and 735 AUWPC, respectively. The highest disease severity was caused by isolate F1 (96% after 45 days) followed by isolate F2 (90% after 45 days) while, isolate F5 gave the lowest disease severity (26%).

Figure 1. Wilt symptoms on tomato plant cv. Super Jackal growing under natural (A) showing yellowed, browned and dangled leaves (A-1), cortex discoloration (A-2) plus vascular discoloration (A-3) and artificial inoculation (B) by *Fusarium oxysporum* f. sp. *Lycopersici* (FOL) race 1 (B-1) and race 3 (B-2 and B-3). Bar=2cm.

Identification of *F. oxysporum* f. sp. *lycopersici* races based on molecular biology

The uni primer set amplifies 670–672 bp fragments from all isolates (FOL and FORL). The sp13 primer set amplifies 445 bp fragments for FOL races 1 and 3, while the sp23 primer set amplifies 518 bp fragments for FOL races 2 and 3. With the sprl primer set, a fragment of 947 bp was amplified specifically from FORL but not from the FOL isolates (Table 3).

The PCR analyses of the formae speciales and races of two *Fusarium oxysporum* isolates (F1 and F2) obtained from tomato plants were found to be effective method as described by Hirano and Arie (2006). The isolates were analyzed with the uni primer set, confirming the presence of *F. oxysporum*, and the sprl primer set confirming the presence of FORL. Isolates for which specific fragments did not amplify with the sprl primer were amplified by sp13 (FOL races 1 and 3) and sp23 (FOL races 2 and 3) primer sets.

The electrophoresis of PCR products obtained for isolates belonging to Samalot (F1 and F2) where intensive tomato growing is practiced are given in Figure 2. The PCR analyses of the F1 and F2 isolates indicated that the F1 gave fragments with uni (672 bp) and sp13 (445 bp) specific primer sets for FOL race 1. The tomato isolate F2 was observed to amplify fragments with uni(672 bp), sp13 (445 bp), and sp23 (518 bp), indicating FOL race 3 (Fig. 2A and B).

Table 2. AUWPC of the *Fusarium oxysporum* isolated from Super Jackal cv. in some locations of El-Minia Governorate, Egypt.

Fusarium oxysporum isolates	Locations	Disease severity (%)				AUWPC*
		After 15 days	After 25 days	After 35 days	After 45 days	
F1	Samalot	26±0.95	42±0.95	65±0.91	96±1.06	2640±1.16
F2	Samalot	24±1.16	32±1.05	51±1.00	90±1.05	2300±0.58
F3	Bani Mazar	5±1.01	18±1.04	25±0.75	36±1.05	995±0.12
F4	Matai	6±0.94	12±1.00	20±1.10	31±1.05	815±0.40
F5	Samalot	7±1.01	16±0.40	17±0.95	26±1.00	755±0.15
F6	Minia	3±1.05	13±0.96	18±0.91	37±1.00	880±0.76
F7	Minia	5±1.07	7±1.07	20±0.99	42±1.03	925±0.06
F8	Minia	6±0.91	15±0.62	15±0.80	27±0.90	735±0.44

Data are means of 3 experiments (4 replicates, 20 plants/each)±SD.
*AUWPC= Area Under Wilt Progress Curve was calculated as described in Materials and Methods

Table 3. Identification of *F. oxysporum* (*F. oxysporum* f. sp. *radicis-lycopersici* (FORL) or *F. oxysporum* f. sp. *lycopersici* (FOL) races) based on the results of PCR with specific primer sets as described by Hirano and Arie, 2006.

Isolate	FOL/FORL	uni	spr1	sp13	sp23
Fusarium oxysporum isolate 1 (F1)	FOL race 1	+	-	+	-
	FOL race 2	+	-	-	-
	FOL race 3	+	-	-	-
	FORL	+	-	-	-
Fusarium oxysporum isolate 2 (F2)	FOL race 1	+	-	-	-
	FOL race 2	+	-	-	-
	FOL race 3	+	-	+	+
	FORL	+	-	-	-

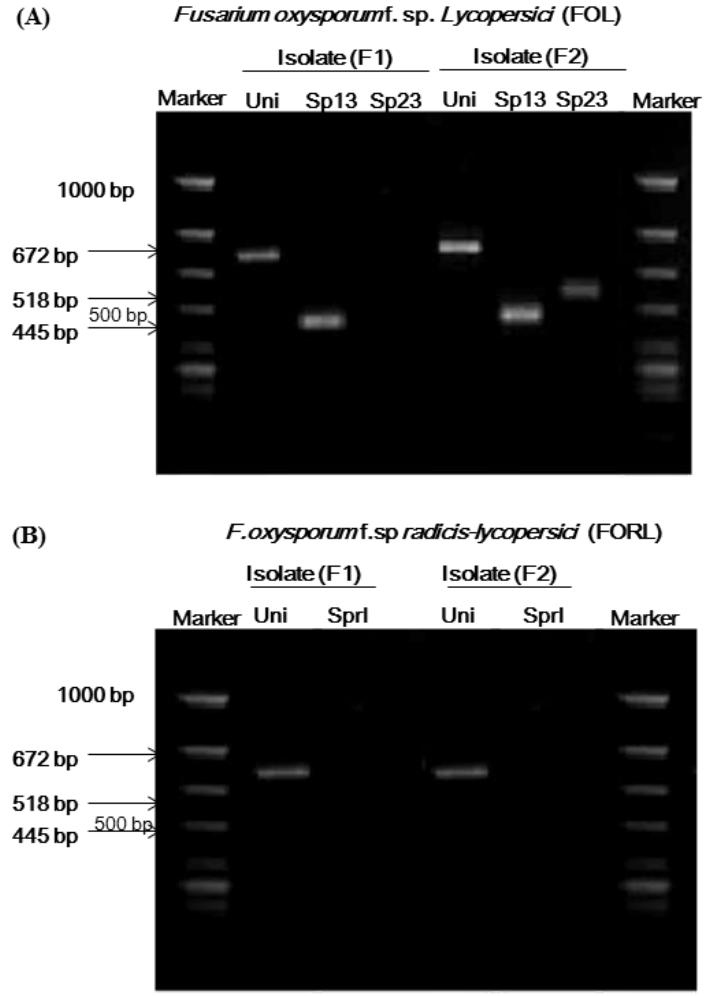

Figure 2. Agarose gel electrophoresis PCR products using 4 specific primer sets to detect *F. oxysporum isolates*.; The PCR analyses of the F1 and F2 isolates indicated that the F1 gave fragments with uni (672 bp) and sp13 (445 bp) specific primer sets for FOL race 1. The tomato isolate F2 was observed to amplify fragments with uni(672 bp), sp13 (445 bp), and sp23 (518 bp), indicating FOL race 3 (A). The isolates were analyzed with sprl primer set and did not gave any amplification confirming the absence of FORL (B). All PCR reaction products were electrophoresed in a 4% agarose gel, stained with ethidium bromide, and visualized under UV light.

Reaction of different tomato cultivars and hybrids to infection by *F. oxysporum* f. sp. *lycopersici* (FOL) races 1 and 3

Data presented in Table (4) showed that there was a significant variation in response of tomato cultivars and hybrids to wilt-causing *Fusarium oxysporum* f. sp. *Lycopersici* (FOL). Four cultivars gave high resist reaction type to FOL race 1 (F1) and race 2 (F2) namely, Zaman cv., Super Red cv., Nema Star cv. and Marwa cv. having the lowest disease severity percentage 5, 6, 8 and 12 DS%, respectively. Hybrid 010-65 showed a resist reaction to both races, DS% recorded 16% and 28%, respectively. While, Basha cv. was susceptible for race 1 and high susceptible for race 3 with 56 and 86 DS%, respectively, hybrid Nema 1400 was high susceptible for race 1 and susceptible for race 3 with 80 and 61 DS%, respectively. The highest percentage DS in case of both tested races (95 %) for race 1. Meanwhile, FOL isolates F1 (race 1) and isolate F2 (race 3) were virulent towards all tested cultivars.

Table 4. Reaction of ten tomato cultivars which are grown the most at El-Minia. Data expressed as disease severity (DS) percentage and reaction type of the selected isolates of *Fusarium oxysporum* f. sp. *Lycopersici* race 1 and 3.

Tomato plants	*Fusarium oxysporum* f. sp. *Lycopersici* races			
	Race 1		Race 3	
	DS%	Reaction Type*	DS%	Reaction Type
Cultivars				
Super Jackal	95±0.74	HS	89±1.05	HS
Basha	56±0.95	S	86±1.00	HS
Marwa	12±1.00	HR	10±1.09	HR
Nema Star	8±1.05	HR	10±1.00	HR
Zaman	5±1.06	HR	9±1.100	HR
Super Red	6±1.00	HR	2±0.00	HR
Rawan	74±1.05	HS	78±0.06	HS
Hybrids				
777	88±1.05	HS	77±1.00	HS
Nema 1400	80±0.88	HS	61±0.52	S
010-65	16±1.15	R	28±1.16	R

Data are means of 3 experiments (4 replicates, 20 plant/each)±SD.
*Reaction Type; High Susceptible (HS) 76~100%, Moderate Susceptible (MS) 61~75%, Susceptible (S) 46~60%, Moderate resistant (MR) 31~45%, Resistant (R) 16~30% and High Resistant (HR) 1~19%. Disease severity (DS) was monitored 45 days after inoculation and replanting time.

DISCUSSION

In 1991, MSc. student (Moustafa, 1999) under supervision of the last author isolated *Fusarium oxysporum* f. sp. *Lycopersici* from tomato plants grown from seeds labelled that

resistant to Fusarial wilt. Isolates varied in their ability to cause wilt symptoms under artificial inoculation. At that time, the team work could not make further identification studies. Thus, identification of FOL races became the main target of this study before further research to make an appropriate method for better Fusarial wilt management and to help tomato breeder in Egypt to produce new resistant tomato hybrids.

Results obtained indicated that all isolates were varied in their pathogenicity on tomato plants. Of the 8 *F. oxysporum* isolates obtained, the most aggressive isolates were *F. oxysporum* isolate (F1) and *F. oxysporum* isolate (F2). These two isolates were selected molecular identification. As a result of the current study, FOL race 1 and 3 were, for the first time, identified through a molecular method of PCR based-markers to be one of the most common pathogens in tomato fields at El-Minia Governorate, Egypt. These 2 races are difficult to differentiate morphologically. Furthermore, since these races may cause disease symptoms on the same host tomato plant at the same time, they are sometimes confused with each other. The previously conducted race and vegetative conformity group (VCG) identification has not been sufficient in determining these *F. oxysporum* forms. Particularly in VCG identification, while some formae speciales conform to only one VCG, others may belong to many VCGs. For this reason, although VCG identification may be beneficial, it cannot be utilized as a universal method in identifying formae specials or isolates that are not pathogenic (Attitalla *et al*., 2004). However, due to the greater accuracy and reliability of PCR for identification purposes, putting into practice the diagnosis of the disease as a result of this method will be quicker and easier (Hirano and Arie, 2006).

Wilt-resistant cultivars have been generated by the introgression of genes for *Fol* resistance, designated *Immunity* genes, into the cultivated tomato *Solanum lycopersicum*, from wild relatives. The *I* gene was identified in the wild tomato *Solanum pimpinellifolium* (accession PI79532) and confers resistance against *Fol* race 1 (Bohn and Tucker, 1939). However, the rapid emergence of race 2, virulent on tomato cultivars carrying the *I* gene, soon necessitated the search for a new source of resistance. The *I-2* gene was identified in an *S. lycopersicum* × *S. pimpinellifolium* hybrid (accession PI126915; Stall and Walter, 1965) and confers resistance to *Fol* race 2. *I-2* encodes a coiledcoil, nucleotide-binding, leucine-rich repeat (CC-NB-LRR) resistance protein (Simons *et al*., 1998). The eventual emergence of race 3, virulent on tomato cultivars carrying the *I-2* gene, necessitated another search for a new source of resistance. Two genes for resistance to *Fol* race 3 were identified in *Solanum pennellii*, one from accession LA716 (Scott and Jones, 1989) and the other from accession PI414773 (McGrath *et al*., 1987). Originally, both genes were designated *I-3*, but gene-mapping

work by Lim *et al*. (2006) revealed that the two genes were not the same. The *I-3* gene from *S. pennellii* LA716 encodes an S-receptor-like kinase (SRLK) protein (Catanzariti *et al*., 2015; Lim *et al*., 2008).

The obtained results revealed that some tomato cultivars and hybrids are highly resistant while, others are highly susceptible or susceptible. Dealing with the wilt disease caused by FOL could be possible through developing resistant varieties of tomato and improving disinfection practices of the soil. Moreover, if irrigation, fertilization, and similar cultivation practices are improved, the disease could be kept within tolerable limits (Takahashi *et al*., 2005; Colak and Bicici, 2013). However, as mentioned in the present study, since the FOL race 1 and race 3 are identified in the region, growing the varieties of tomato that are resistant to these races will particularly decrease the rate of infection.

Meanwhile, the development of resistant tomato cultivars to specific form and race identified will be more appropriate to plant in this area. Nowadays, breeding programs are mostly focused on developing resistant varieties, since this method is the most effective in dealing with the diseases that cause huge economic losses (Colak and Bicici, 2013). Consequently, the identification of formae speciales and races of *F. oxysporum* will promote the breeding of resistant tomato varieties to tomato wilt disease caused by FOL races 1 and 3 especially under El-Minia environmental conditions.

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الملخص العربي

سلالتي فيوزاريوم أوكسيسبورم *Fusarium oxysporum* شبه النوع *lycopersici* (1 , 3) كمسببات لذبول الطماطم في محافظة المنيا

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من الأمراض الأكثر خطورة علي نباتات الطماطم مرض الذبول الناتج عن الاصابة بالفطر الفيوزاريوم أوكسيسبورم *Fusarium oxysporum* شبه النوع *lycopersici*، حيث انه يسبب خسائر كبيرة للمحصول في منطقة مصر الوسطي. واختلفت القدرة المرضية للعزلات معنويا. وأظهرت العزلات F1 ، F2 اعلي قدرة مرضية والتي تمثلت في تسجيل اعلي نسبة مئوية لشدة المرض حيث اعطت العزلة F1 (96٪ بعد 45 يوما)، واعطت العزلة F2 (90٪ بعد 45 يوما).

باستخدام المعلمات الوراثية (DNA markers) عن طريق تفاعل البلمرة المتسلسل (PCR) لتمييز هذه العزلات وراثيا. أكدت تحليلات PCR أن كلا العزلتين الفيوزاريوم أوكسيسبورم *Fusarium oxysporum* شبه النوع *lycopersici* والتي يرمز لها (FOL). العزله (F1) أعطت حجم (672 bp) لل uni و (445 bp) لل sp13 والذي يؤكد ان هذه العزله هي السلاله 1 (race 1) بينما العزله (F2) أعطت حجم (672 bp) لل uni و (445 bp) لل sp13 و(518 bp) لل sp23 الذي يؤكد ان هذه العزله هي السلاله 3 (race 3).

أظهرت الدراسة أن السلالتين race 1 ، race 2 للفطر لهما القدرة علي احداث الذبول لجميع الأصناف وهجن الطماطم المختبرة حيث اظهرت اربعة أصناف من الطماطم (Nemastar–Super Red–Zaman–Marwa) اعلي مقاومة بينما الهجين (65–010) اعطي مقاومة لكلا السلاتين في حين أن الصنف (Basha) ذات قابلية عالية للاصابة بالسلالة (1) واكثر قابلية للاصابة بالسلالة (3) وبينت الدراسة ان الهجين (نيما 1400) اكثر قابلية للاصابة بالسلالة (1) وقابل للاصابة بالسلالة (3).