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RESEARCH NOTES

Molecular Identification of Physiological Races of *Fusarium oxysporum* f. sp. *lycopersici* and *radicis lycopersici* Causal Agent of Fusarium Wilt of Tomato in Iran

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ABSTRACT

Recently, vascular wilt disease has caused considerable damages in tomato greenhouses. *Fusarium oxysporum* is a common pathogen of tomato and produces a variety of toxins, pigments, and phytohormones. It has caused huge crop damage in south of Iran. Damage increases in relatively warm climate. *Fusarium oxysporum* were identified from contaminated tissues. Thirty five of 50 isolates from samples collected from tomato greenhouses in southeast of Iran were identified as *F. oxysporom*. Then, *F. oxysporum* f. sp. *lycopersici (Fol)* and *F. oxysporum* f. sp. *radicis lycopersici (Forl)* were differentiated from each other by host range and molecular methods. *Fol* is only pathogenic on tomato and *Forl* is pathogenic on other hosts from Cucurbitaceae family in addition to tomato. Molecular identification of isolates was obtained by Hirano methods. DNAs of each isolates were reproduced by using uni, sp13 and sp23 specific primers. Based on this study, 4 isolates were identified as race1, 4 isolates as race 2, 13 isolates as race 3, and 14 isolates as *Forl*. This is the first report of formae specialis and physiological races of this pathogen in this region. Race 3 is reported for the first time in Iran.

Keywords: Formae speciales, PCR, Physiological races, Specific primer.

INTRODUCTION

Wilt of tomato (*Lycopersicon esculentum* Mill.), caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) WC Snyder and HN Hansen, is an economically important and a destructive disease of tomato crop worldwide (Jones *et al*., 1991). Three different host-specific races of pathogen (races 1, 2, and 3) have been identified (Cai *et al*., 2003). Race 1 was initially observed in 1886 (Booth, 1971) and race 2 was first reported in 1945 in Ohio (Alexander and Tucker, 1945). Race three of *F. o.* f. sp. *lycopersici* was identified in Australia in 1978 (Grattidge and O'Brien, 1982) and was subsequently reported in several US states and Mexico (Davis *et al*., 1988). All commercially cultivated tomatoes (*L. esculentum*) lacking I-genes, are susceptible to *F. o.* f. sp. *lycopersici*. Race 2 of the pathogen was reported in South Korea and in Ohio (Valenzuela, 1996).These races of the pathogen overcome the resistance of cultivars which showed resistance to race 1. The race 3 was observed in Australia and Florida (Grattidge and

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O'Brien, 1982). Races of *F. o.* f.sp. *lycopersici* agents could be distinguished by their differential virulence on tomato cultivars containing different dominant resistance genes (McGrath *et al*., 1987; Mes *et al*., 1999). The pathogenic type (form and race) of *Fusarium oxysporum*, which generates wilt symptoms on tomato, was rapidly identified with a PCR-based technique (Hirano *et al*., 2006).

Fusarium Oxysporum f. sp. *Radicis Lycopersici* (FORL) is a saprophytic fungus occurring in the rhizosphere of many plant species. The pathogen has a broad range of host species but host specialization of isolates is more circumscribed (Kim *et al*. 2001). In tomato, there occur two formae speciales named *Fusarium Oxysporum* f. sp. *Lycopersici* (FOL), and *F. oxysporum* f. sp. *radicis lycopersici* (Armstrong and Armstrong, 1981; Stein-kellner *et al*. 2005). The first reports on *FORL* came from Japan (1969) and California (1971), (Benhamou *et al*. 1989; Fazio *et al*. 1999). Fusarium wilt caused by *F. oxysporum* f. sp. *radicis lycopersici* is one of the most destructive diseases of tomatoes. It is widespread and leads to substantial yield losses in both greenhouse and soil production systems. Katan *et al*. (1991) and Katan and Katan (1999) did not report the physiological races of *FORL*.

The aim of this research was molecular identification of formae speciales*; Fusarium oxysporm* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis lycopersici* and *FOL* physiological races in these isolates.

MATERIALS AND METHODS

Fungal Isolates and Identification of Fusarium Species

Fifty isolates were collected in 2008- 2009 from 175 samples of 10 tomato greenhouses in southeast of Iran. Sections (3-5 cm long) of tomato plant stem showing vascular discoloration were rinsed thoroughly by tap water. After surface-disinfesting in sodium hypochlorite (5%) for 1 min, the plant pieces were rinsed three times in sterile-distilled water, dried on sterile filter paper and plated onto Potato Dextrose Agar (PDA) medium amended with streptomycin sulfate (300 mg L^{-1}) (Nelson *et al.*, 1983). Plates were incubated for two weeks at 24°C. The fungal isolates were cleaned up by sub culturing successively and were selected by single-spore isolation method on dried agar cultures. For identification of Fusarium species, isolates were cultured on Special Nutrient Agar (SNA) media (Nash and Snyder, 1962) and identified by source of Gerlach and Nirenberg (1982). Identification of Fungal isolates was made under the light microscope and fungal structures were placed on slides, stained with methylene blue. Also, identification of fungi was based on colony morphology, conidial characteristics, and phialid type.

Plant Material

The Pathogenicity of isolates was investigated on tomato (Chef cultivar) and cucumber (Negeen cultivar) on seedling stage. Seeds of tomato and cucumber were disinfected with 1% sodium hypochlorite for 5 minutes and rinsed three times in sterile water prior to sowing. Then, the seeds were sown in sand and soil (80:20) in seedling trays (plug size 3.4 by 3.4 by 5 cm, 64 plugs). Trays were maintained in a glasshouse at 23-28°C, 60-70% relative humidity, and 16 hours light, 8 hours darkness. FOL only affects Solanaceous plants; FORL is able to infect a total of some plant species and varieties belonging to Solanaceae and Cucurbitaceae, (Menzies *et al.*, 1990). Therefore, pathogenicity of isolates was investigated on tomato and cucumber on seedling stage.

Inoculum Preparation

The conidia of 10-day-old cultures from PDA were washed with sterile distilled water to obtain suspension of inoculums of the pathogen. Cultures were then filtered through one layer of Mira cloth, centrifuged

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(6,000×g for 15 minutes), washed with sterile water and adjusted to a concentration of 10^6 conidia per mL. Viability of conidia was checked by plating dilutions on PDA media. The spore concentration was measured by using a heamocytometer counting slide.

Pathogenicity Tests

The rate of wilt symptom of each isolate was measured on tomato and cucumber seedling at the four-true-leaf stage. Their roots were dipped into a conidial suspension (10^6 microconidia per mL) of the test isolate for 10 minutes, after which seedlings were transplanted into sterilized soils in pots (10 cm in diameter) and kept in glasshouse for 7 weeks at 20-25°C under supplemental fluorescent light with 14 hours photoperiod at 100-200 hlx.

Disease Severity Index

Disease Severity Index (DSI) was evaluated to assess 35 days after inoculation by using the following scale: 0= No symptoms; 1= < 25% of leaves with symptoms; 2= 26-50% of leaves with symptoms; 3= 51-75% of leaves with symptoms, and 4= 76-100% of leaves with symptoms. Inoculated plants were evaluated as diseased when browning of at least one vascular bundle was visible. (Bora *et al*., 2004; Banerjee, 1990)

Critical Temperature Measurement

Typical classification of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici* is impossible in morphology. However, *F. oxysporum* f. sp. *radicis lycopersici* is classified as psychrophilic fungus since the optimum temperature of disease outbreak of *F. oxysporum* f. sp. *radicis lycopersici* is 18°C, different from the optimum temperature of disease outbreak of *the F. oxysporum* f. sp. *lycopersici* of 27°C (Kim *et al.*, 2005).

In this study, all fungal isolates were cultured on two PDA plates and fungal cultures were incubated for two weeks at 18 and 27°C.

Molecular Identification

Extraction Genomic DNAs from Fungal Mycelium

Genomic DNA of *F. oxysporum* was extracted from fungal mycelia using CTAB method with some modifications (Cullings, 1992). These modifications included adding 2 µL Proteinase K before adding Chloroform Isoamyl alcohol in stage 7 of Cullings protocol to each sample and incubating them for 30 minutes at 37°C.

Polymerase Chain Reaction (PCR) to Identify *FORL* and Races of *FOL*

Amplification was performed using an Eppendorf Thermal Cycler, Mastercycler™(Gradient). The PCR reaction mixture (25 µL) consisted of 0.4 mM dNTPs, 0.4 mM of primer, 2 mM MgSO4, 1X PCR buffer, 1 unit of Taq polymerase with template DNA 40 ng. The thermal conditions were set as follows: initial denaturation at 94°C for 5 minutes; 40 cycles of denaturation at 94°C for 60 seconds, annealing at an appropriate temperature for 60 seconds, and elongation at 72°C for 2 minutes; final extension at 72°C for 7 minutes. Amplification products were resolved using 1.2% agarose gel under 3 V/cm potential and 90 mA currency in TBE buffer for 2 hours. Gels were stained with ethidium bromide for 30 minutes and visualized under UV light using UVP™ (Baysal *et al.*, 2009).

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According to Hirano *et al*.. (2006), amplified of Endo and Exopolygalacturonase fragments differentiate of physiological races and species of *F. oxysporum* (Table 2).

The Uni Primers (Unif and Unir) differentiate FOL and FORL from other formae specialis of *F. oxysporum*, Uni primers amplified a 670-672 bp fragment. Primer sp23 differentiate races 2 and 3 of *FOL* from race1 of *FOL* and *FORL*, primer sp23 amplified a 518 bp fragment.

Primer sp13 amplified a 445 bp fragment in *FOL* races 1 and 3, but not any fragment in *FORL* race 2 and *FORL* (Hirano *et al.*, 2006). The nucleotide sequence for these primers is shown in Table 1.

Table 2. Identification of isolates according to Hirano *et al*. (2006).

	Primer set		
	Uni	Sp13	Sp23
F. oxysporum f. sp. *Lycopersici* race1	+	+	-
F. oxysporum f. sp. *Lycopersici* race2	+	-	+
F. oxysporum f. sp. *Lycopersici* race3	+	+	+
F. oxysporum f. sp. *radicis lycopersici*	+	-	-

RESULTS

Fungal Isolates

Fifty isolates of the fungi were collected from tomato greenhouses in southeast of Iran (Table 3).

Identification of Fusarium Species

Identification of *F. o.* f. sp. *lycopersici* was based on morphological criteria according to Gerlach and Nirenberg (1982). 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, 4-12×2.1-3.5 µm. Macroconidia, spores were born abundantly on branched conidiophores or on the surface of sporodochia and were thin walled, three to five septate, fusoid-subulate and pointed at both ends, and had 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. They are generally solitary, but occasionally form in pairs or chains. No perfect stage is known. Thirty five isolates were identified as *F. oxysporum.* These isolates were: F1, F4, F5, F6, F7, F8, F9, F10, F11, F13, F16, F21, F22, F24, F26, F27, F28, F29, F30, F31, F32, F33, F34, F35, F36, F39, F40, F41, F42, F43, F44, F45, F46, F47, and F50.

Table 1. Nucleotides sequences of different used primers.

Primers	Melting Point (°c)	Sequence	Ampelicons size (bp)
Unif	55.9	*ó ATCATCTTGTGCCAACTTCAG 3ó5*	670-672
Unir	56.5	*ó GTTTGTGATCTTTGAGTTGCCA 3ó5*	
Sp 13f F	59.4	*ó GTCAGTCCATTGGCTCTCTC 3ó5*	445
Sp 13r R	57.3	*ó TCCTTGACACCATCACAGAG 3ó5*	
Sp 23f F	57.9	*ó5 CCTCTTGTCTTTGTCTCACGA 3ó*	518
Sp 23r R	59.4	*ó GCAACAGGTCGTGGGGAAAA 3ó5*	

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Table 3. Geographicreign and host tissue and morphological diagnosis of collected isolates.

F [a]	Geographic reign	Host tissue	Species
F1	Zahedan	Crown	*F. oxysporum*
F2	Zahedan	Root	*F. solani*
F3	Zahedan (Suffi)	Crown	*F. solani*
F4	Zahedan (Suffi)	Crown	*F. oxysporum*
F5	Zahedan (Gavdaran)	Crown	*F. oxysporum*
F6	Nehbandan	Crown	*F. oxysporum*
F7	Nehbandan	Root	*F. oxysporum*
F8	Zabol	Crown	*F. oxysporum*
F9	Zabol(Shibab)	Crown	*F. oxysporum*
F10	Khash	Root	*F. oxysporum*
F11	Khash (Nukabad)	Root	*F. oxysporum*
F12	Khash (Irandegan)	Crown	*F. oxysporum*
F13	Nehbandan (30 Km to center)	Crown	*F. oxysporum*
F14	Nehbandan (30 Km to center)	Root	*F. solani*
F15	Taftan	Root	*F. solani*
F16	Saravan	Crown	*F. oxysporum*
F17	Saravan (Jalgh)	Crown	*F. solani*
F18	Saravan (Center)	Root	*F. solani*
F19	Saravan (Bamposht)	Root	*F. solani*
F20	Saravan	Crown	*F. solani*
F21	Saravan (Bamposht)	Crown	*F. oxysporum*
F22	Saravan (Jalgh)	Root	*F. oxysporum*
F23	Ladiz	Root	*F. solani*
F24	Mirjave (Ladiz)	Root	*F. oxysporum*
F25	Mirjave (Ladiz)	Crown	*F. solani*
F26	Mirjave	Crown	*F. oxysporum*
F27	Mirjave (Tamin)	Root	*F. oxysporum*
F28	Mirjave	Root	*F. oxysporum*
F29	Zahedan (Razaghzade)	Crown	*F. oxysporum*
F30	Iranshahr	Crown	*F. oxysporum*
F31	Iranshahr (Bazman)	Root	*F. oxysporum*
F32	Iranshahr (Bampur)	Crown	*F. oxysporum*
F33	Iranshahr	Root	*F. oxysporum*
F34	Iranshahr (Bazman)	Crown	*F. oxysporum*
F35	Iranshahr (Bampur)	Root	*F. oxysporum*
F36	Zahedan (Gavdaran)	Crown	*F. oxysporum*
F37	Dashtyari	Root	*F. solani*
F38	Zarabad	Crown	*F. solani*
F39	Chabahar	Crown	*F. oxysporum*
F40	Chabahar (dashtyari)	Root	*F. oxysporum*
F41	Chabahar (yalan)	Crown	*F. oxysporum*
F42	Chabahar (Zarabad)	Crown	*F. oxysporum*
F43	Nikshahr (Fanuj)	Crown	*F. oxysporum*
F44	Nikshahr (Ghasreghand)	Root	*F. oxysporum*
F45	Chabahar	Root	*F. oxysporum*
F46	Zahedan	Crown	*F. oxysporum*
F47	Zabol	Root	*F. oxysporum*
F48	Zahedan	Root	*F. solani*
F49	Shibab	Root	*F. solani*
F50	Zabol	Crown	*F. oxysporum*

[a] Isolates

Critical Temperature Measurement

In this section, observation of PDA plates and fungal cultures after 2 weeks showed that the growth of F1, F4, F5, F10, F11, F33, F28, F50, F16, F36, F46, F47, F40, F41 isolates was different from other isolates. After 14 days of fungal cultures incubation at temperature of 18 and 27°C, the colony diameter measurements showed that these isolates had the highest growth at 27°C. This test was repeated five times for each isolate and the average of colony diameter is given in Figure 1.

Pathogenicity Testsn

The pathogenicity test of all isolates was confirmed on tomato and cucumber seedling at the four-true-leaf stage. Symptoms in plants infected appeared two weeks after inoculation. Fourteen isolates caused typical symptoms of Fusarium wilt on the tomato and cucumber seedlings and 21 isolates caused typical symptoms of Fusarium wilt on only tomato host (Table 4). Disease Severity Index (DSI) was evaluated on tomato plants (Table 5).

Molecular Identification

Primer set unif and unir differentiated *FOL* and *FORL* from other formae speciales of *F. oxysporum*. This uni primer set amplified a 670-672 bp fragment from isolates of *FOL* and *FORL*. In this study, F1, F4, F5, F6, F7, F8, F9, F10, F11, F13, F16, F21, F22, F24, F26, F27, F28, F29, F30, F31, F32, F33, F34, F35, F36, F39, F40, F41, F42, F43, F44, F45, F46, F47, and F50 isolates with uni primer set, made a 672 bp band (Figure 3).

The sp13 primer set composed of primers sp13r and sp13f amplified a 445 bp fragment from *FOL* races 1 and 3, but did not amplify any fragment from race 2 and *FORL*. In this study, F6, F7, F8, F9, F13, F21, F22, F24, F26, F27, F29, F30, F31, F32, F34, F35, and F39 isolates with **sp13** primer set, made a

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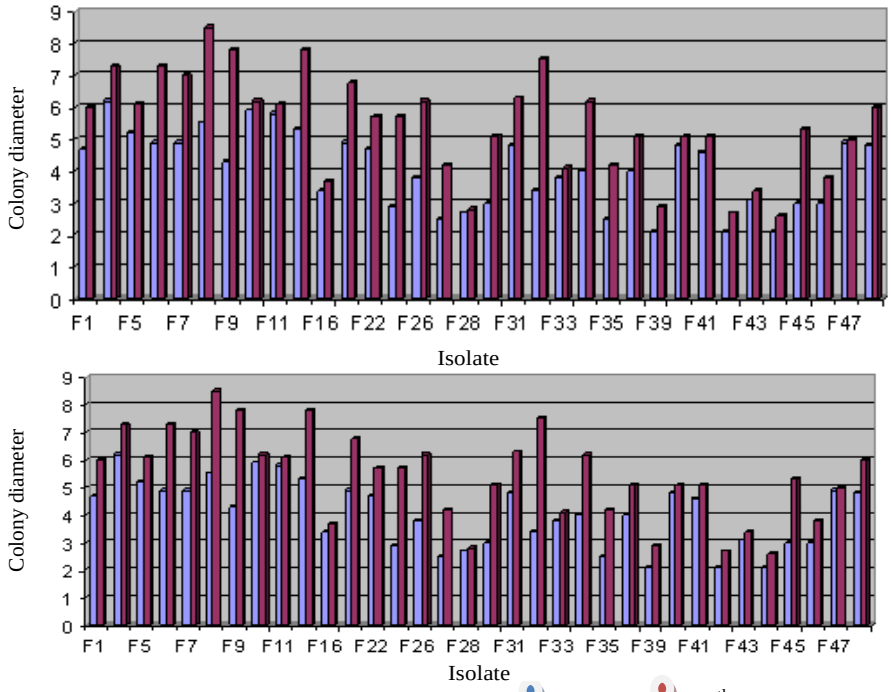

Figure 1. Growth of isolates in 18 and 27°C at 7th day.

(a) (b) (c)

Figure 2. (a) Pathogenicity tests; (b) Symptoms of Fusarium wilt on tomato by F16 (DSI= 4), and (c) Pathogenicity test on cucumber with root dip method.

445 bp band (Figure 4). Primer sp23 differentiated *FOL* races 2 and 3 from race1 and *FORL*. This sp23 primer amplified a 518 bp fragment from *FOL* races 2 and 3, but did not amplify any fragment from *FOL* race 1 and *FORL*. In this study, F6, F7, F8, F9, F13, F22, F24, F26, F27, F29, F30, F32, F39, F42, F43, F44, and F45 isolates with **sp23** primer set, made a 518 bp band (ture 5).

The final results are summarized in Table 5. Based on this, 11.43% of isolates were identified as race1, 11.43% as race 2, 37.14% as race 3, and 40% as *Forl* (Table 6). It was the first report of formae speciales and physiological races of this pathogen in southeast of Iran. Race 3 was reported for the first time in Iran.

DISCUSSION

Tomato is one of the most widely grown vegetable crops in the world (Barone *et al.*,

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Table 4. Symptoms on tomato and cucumber.

Isolates	Symptoms on tomato	Symptoms on cucumber
F1	+	+
F4	+	+
F5	+	+
F6	+	-
F7	+	-
F8	+	-
F9	+	-
F10	+	+
F11	+	+
F13	+	-
F16	+	+
F21	+	-
F22	+	-
F24	+	-
F26	+	-
F27	+	-
F28	+	+
F29	+	-
F30	+	-
F31	+	-
F32	+	-
F33	+	+
F34	+	-
F35	+	-
F36	+	+
F39	+	-
F40	+	+
F41	+	+
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F45	+	-
F46	+	+
F47	+	+
F50	+	+

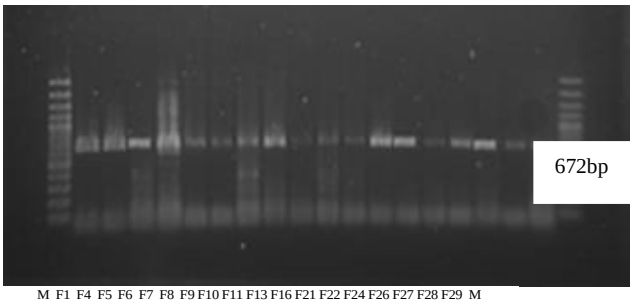

Figure 3. PCR product with uni primer in some isolates. The Uni Primers (Unif and Unir) differentiate *FOL* and *FORL* from other formae specials of *F. oxysporum*. Uni primers amplified a 670-672 bp fragment.

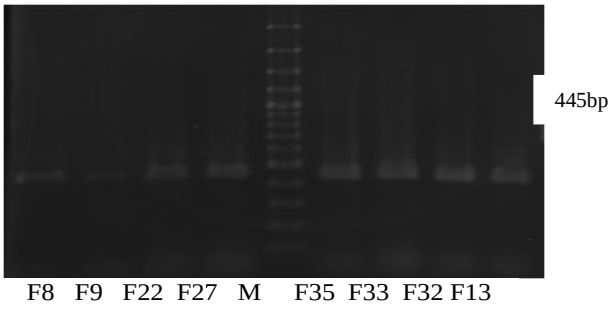

Figure 4. PCR product with sp13 primer in some isolates. Primer sp13 amplified a 445 bp fragment in *FOL* races 1 and 3, but not any fragment in *FORL* race 2 and *FORL*.

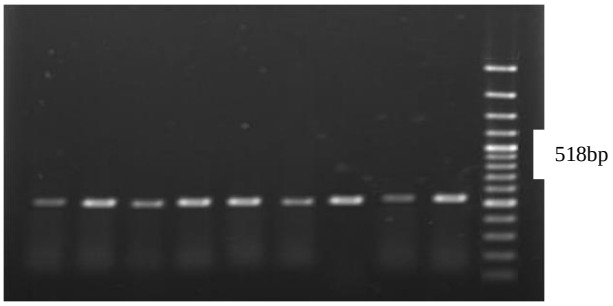

Figure 5. PCR product with sp23 primer in isolates. Primer sp23 differentiate races 2 and 3 of *FOL* from race 1 of *FOL* and *FORL*. Primer sp23 amplified a 518 bp fragment.

2007). One of the most important diseases of tomato is Fusarium wilt caused by three races of *F. oxysporum* f. sp. *lycopersici.* Races 1 and 2 are distributed worldwide, whereas race 3 has a more limited geographic distribution. The most susceptible plants inoculated by root-dipping developed typical symptoms of wilt, slight vein clearing on outer leaflets, stunting, dark brown vascular discoloration and death. *F. o.* f.sp. *lycopersici* was recovered from all symptomatic plants, whereas non inoculated tomato seedlings showed no symptoms. In Iran, race 1and 2 of .*F. o*. f. sp. *lycopersici* was reported from Kordestan province (Amini, 2009), but molecular identification

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Table 5. Disease Severity Index of some isolates.

Isolates repeats	F1	F5	F8	F11	F13	F16	F28	F33	F45	Control
1	3	2	2	2	2	3	3	2	2	0
2	4	0	1	4	1	4	4	0	4	0
3	4	1	1	4	1	4	1	0	3	0
4	4	0	4	3	0	4	4	4	4	0
5	2	4	4	4	2	4	4	4	4	0
DSI	3.6	1.4	2.4	3.4	1.2	3.8	3.2	1.8	3.4	0

Table 6. Identification of formae specialis; *Forl* and *Fol* and their physiological races by uni, sp13, sp23 primers.

Isolates	Uni	Sp13	Sp23		Isolates	Uni	Sp13	Sp23	
F1	+	-	-	Forl	F30	+	+	+	Race3
F4	+	-	-	Forl	F31	+	+	-	Race1
F5	+	-	-	Forl	F32	+	+	+	Race3
F6	+	+	+	Race3	F33	+	-	+	Forl
F7	+	+	+	Race3	F34	+	+	-	Race1
F8	+	+	+	Race3	F35	+	+	+	Race3
F9	+	+	+	Race3	F36	+	-	-	Forl
F10	+	-	-	Forl	F39	+	+	+	Race3
F11	+	-	-	Forl	F40	+	-	-	Forl
F13	+	+	+	Race3	F41	+	-	-	Forl
F16	+	-	-	Forl	F42	+	-	+	Race2
F21	+	+	-	Race1	F43	+	-	+	Race2
F22	+	+	+	Race3	F44	+	-	+	Race2
F24	+	+	+	Race3	F45	+	-	+	Race2
F26	+	+	+	Race3	F46	+	-	-	Forl
F27	+	+	+	Race3	F47	+	-	-	Forl
F28	+	-	-	Forl	F50	+	-	-	Forl
F29	+	+	+	Race3					

has not been reported from Iran. In this research, race 3 was reported from Iran for the first time. The presence of this formae speciales and race 3 in Southeast Iran could be associated with high temperatures in the area. *F. oxysporum* f. sp. *radicis lycopersici* causes Fusarium crown and root rot of tomato often referred to as 'crown rot' which also inflicts substantial damage to crops (Fazio *et al.*, 1999). Conventionally, genetic diversity is estimated on the basis of morphological and phenotypic characters. Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features (Noori *et al.*, 2010). According to the research results, molecular methods confirmed morphological keys to identify the species of Fusarium with high ability and separated the races of .*F. o.* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici.*

It is proposed to track and analyze the role of polygalacturonase gene on pathogenicity of *F. oxysporum* using specific primers and identify races and forms of specific resistant varieties for each region according to its pathogenic form of the dominant race and formae speciales in each region.

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تشخیص مولکولی نژادهای فیزیولوژیک *Fusarium oxysporm* f. sp. *Lycopersici* و *Fusarium oxysporm* f. sp. *radicis lycopersici* عامل پژمردگی فوزاریومی گوجه فرنگی درایران

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چکیده

درسالهای اخیر بیماری پژمردگی آوندی خسارات بسیاری به گلخانه‌های گوجه‌فرنگی وارد کرده است. قارچ *Fusarium oxysporum* به عنوان یک پاتوژن عمومی شناخته شده و توانایی بالایی در تولید توکسین ها، پیگمان‌ها و هورمون‌های مختلف دارد. خسارات بسیاری از این پاتوژن در نواحی جنوب ایران گزارش شده است و این خسارات در آب و هوای نسبتا گرم بیشتر است. در این تحقیق ۵۰ جدایه از قارچ فوزاریوم از گلخانه گوجه فرنگی جنوب شرقی ایران جمع آوری شدند. ۳۵ جدایه *Fusarium oxysporum* بودند که فرم‌های اختصاصی *F.oxysporum* f. sp. *lycopersici (Fol)* و *F. oxysporum* f.sp.. *radicis lycopersici (Forl)* و نژادهای فیزیولوزیک آن با روش‌های مورفولوژیک، دامنه میزبانی و مولکولی از هم تفکیک شدند. فرم Fol فقط روی گوجه فرنگی و فرم Forl روی میزبانهای دیگری از خانواده Cucurbitaceae نیز بیماریزاست. پس از استخراج DNA از کلیه جدایه ها با متود هیرانو و با استفاده از آغازگرهای uni، sp13 و sp23 مشخص شد. از این جدایه ها، ۴ تا نژاد ۱، ۴ تا نژاد۲، ۱۳ جدایه نژاد ۳ و ۱۴ جدایه فرم اختصاصی *Forl* بودند. این اولین گزارش از این قارچ در نواحی جنوب شرق ایران و اولین گزارش از نژاد ۳ پاتوژن از ایران است.

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