جداسازی، تعیین نژاد و مشخصات مولکولی *Fusarium oxysporum* f. sp. *melonis* عامل پژمردگی فوزاریومی خربزه در استان بوشهر

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چکیده

در طی بازدیدی که در اردیبهشت سال ۱۳۹۷ از مزارع خربزه در شهرستان دشتی، منطقه دشت پلنگ استان بوشهر انجام شد، علائم پژمردگی و زردی در گیاهان مشاهده شد. به منظور بررسی عامل بیماری، نمونه‌های آلوده به آزمایشگاه منتقل شدند و پنج پرگنه قارچی از ساقه‌های آلوده جداسازی شد. بر اساس خصوصیات ریخت‌شناختی و توالی‌سنجی سه ناحیه ژنی ITS و *EF-1α* و IGS، قارچ جداسازی شده *Fusarium oxysporum* f. sp. *melonis* شناسایی شد. آزمون بیماری‌زایی و تعیین نژاد با استفاده از ارقام افتراقی Cha-T (فاقد ژن مقاوم)، Cha-*Fom1* (دارای ژن مقاوم *Fom1*) و Cha-*Fom2* (دارای ژن مقاوم *Fom2*) انجام شد. نتایج آزمون تعیین نژاد نشان داد که قارچ *F. oxysporum* f. sp. *melonis* در استان بوشهر متعلق به نژاد ۲،۱ می‌باشد. بر اساس نتایج آنالیزهای فیلوژنی و DnaSp، جدایه‌های جدا شده از استان بوشهر و دیگر جدایه‌های *F. oxysporum* f. sp. *melonis* متعلق به VCG0134 ایران در یک گروه فیلوژنی قرار گرفتند و هیچ تفاوت نوکلئوتیدی بین جدایه‌ها در سه ناحیه ژنی مورد مطالعه در این پژوهش مشاهده نشد. اگرچه *F. oxysporum* f. sp. *melonis* به عنوان بیمارگر قارچی خربزه در نقاط معتدله و سردسیر شناخته شده است، در این مطالعه حضور این بیمارگر در نقاط گرمسیر ایران برای اولین بار گزارش می‌شود.

کلیدواژه: جدایه‌های Fom، خربزه، آنالیزهای فیلوژنی، بیماری‌زایی، منطقه گرمسیری

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Isolation, race determination and molecular characterization of *Fusarium oxysporum* f. sp*. melonis* in Bushehr province

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Abstract

In spring 2018, yellowing and wilting symptoms were observed on melon plants, Janna variety, in Dashti county, Bushehr province, southern Iran. In order to identify the causal agent of disease, infected tissues were transferred to the laboratory and five fungal isolates were isolated. Based on the morphological characteristic and sequence data of three different loci (ITS, *EF-1α*, and IGS), the fungi were identified as *Fusarium oxysporum* f. sp. *melonis* . The disease assay and race determination were performed in greenhouse using three melon differential hosts including Cha-T (no resistance gene), Cha-*Fom1* (contains *Fom1* resistance gene) and Cha-*Fom2* (contains *Fom2* resistance gene). The results of bio-assay experiment reveald that these isolates are belonged to race 1.2 of *F. oxysporum* f. sp. *melonis*. According to the phylogenetic analysis, the isolates from Bushehr and the other Iranian isolates belonging to VCG0134 were clustered in the same linage and there were no nucleotide differences between them based on DnaSp analysis. Although *F. oxysporum* f. sp. *melonis* is known as a fungal pathogen of melon in cold and temperate regions, the presence of this pathogen in the tropical region of Iran is reporting for the first time in this study.

Keywords: Fom isolates, melon, phylogenetic analysis, pathogenicity, tropical region

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Introduction

The Cucurbitacea family involves over 700 species of plants including cucumbers, melons, watermelons, pumpkins, squash, and many others (Blancard *et al.* 1994). Melon, as one of the most important economic plants of this family, yields more than 27 million tons of products worldwide annually (http://faostat3.fao.org, 2018). While the *Cucumis melo* L. (melon) is an eminent crop across tropical and subtropical regions, it is also grown widely in temperate zone countries (Oumouloud *et al.* 2013).

As many other crops, melon is susceptible to several foliars and root fungal pathogens that affect melon negatively. Among these, Fusarium wilt is caused by a soil-inhabiting pathogen, *Fusarium oxysporum* Schlechtend: Fr. f. sp. *melonis* (H.N. Hansen) W.C. Snyder & H.N. Hans (Fom) (Oumouloud *et al.* 2013). This fungus causes a sorts of symptoms including yellowing, wilting, and eventually plant death on melon plants. Fom can survive in the soil as chlamydospores and also persist by colonizing the roots of non-susceptible crops cultivated in rotation (Zuniga *et al.* 1997). Evolving locally and transferring from the other places are two general approaches which explane the presence of Fusarium wilt at a particular geographic location (Gordon & Martyn 1997).

The Fom isolates are currently classified according to its pathogenenic activity in melon. In that case, four races (races 0, 1, 2, and 1.2) of Fom has been identified by Risser *et al.* (1976) based on the presence/absence of two resistance genes, *Fom-1 and Fom-2* in muskmelon cultivars. The ability of these races to overcome these resistance genes is currently used to identify races of this pathogen. Race 0 is virulent to muskmelon cultivars without either *Fom-1* or *Fom-2*. Race 1 can infect cultivars carring *Fom-1* and race 2 is able to virulent to cultivars with *Fom-2*. Race 1.2 can infect cultivars possessing both of resistance genes (Risser *et al.* 1976).

According to the surveys, race 2 was the only race known in North America, and races 0 and 1 have been found in Europe and Israel until 1985 (Banihashemi 1968a, Jacobson and Gordon 1991). Races 0, 1, and 1.2 have reported from Lebanon (Abou-Jawdah & Al-Khoury 1996), and races 0, 1, and 2 have reported from South Africa (Schreuder *et al.* 2000). Races 0, 1, 2, and 1.2 have identified in Europe, Israel, Japan, and Turkey (Jocobson & Gordon 1990a, Katan *et al.* 1994, Namiki *et al.* 2000, Kurt *et al.* 2002). The presence of races 1 and 1.2 in Iran has already been reported. Race 1 from Khorasan and Semnan provinces and race 1.2 from Fars, Isfahan, Kermanshah, Yazd, and Markazi provinces have been recovered (Banihashemi 1968b, 1982, 1989, Mirtalebi *et al.* 2013).

A number of fungi are benefited by a vegetative compatibility (it is also known as heterokaryon compatibility) which is an important section for their life cycles. Presently, nine vegetative compatibility groups (VCGs), VCGs 0130-0138, are recognized in Fom isolates worldwide (Jacobson & Gordon 1988, 1990a, Katan *et al.* 1994). In Iran, VCG0134 is the only recognized VCG of Fom (Sarpeleh & Banihashemi 2000). This VCG was also reported in Europe, North America (Jacobson & Gordon 1990a) and South Africa (Schreuder *et al.* 2000). In addition to VCG0134, a new VCG has previously reported from Iran which has been introduced as an unknown VCG becuase of its failure to yield reaction with the available tester isolates (Mirtalebi *et al.* 2013). The correlation between mtDNA haplotypes, the sequence of nuclear ribosomal DNA intergenic spacer (IGS) and Fom VCGs have been indicated previously (Jacobson & Gordon 1990b, Mirtalebi *et al.* 2013).

There is a complex relationship between race and VCG in Fom isolates. One or more physiological races associated to some of the VCGs (Jacobson & Gordon 1988). For example, the VCG0134 has been associated to all four races of Fom isolates (Jacobson & Gordon 1991).

Melon and cantaloupe are the most important vegetable crops in Iran which have about 8081 hectares under cultivation, so they have an important role in national economy (Anonymous 2011). Fusarium wilt is one of the most common diseases on melon plants in Iran mainly in temperate and cold regions which causes great damage to these crops (Hanifei *et al.* 2018). This study was established to determine the race of Fom isolates recovered from melon plants in Bushehr province and also investigate the phylogenetic relationship among Fom strain from Bushehr and the other Iranian isolates.

Materials and methods

Fungal isolates

In spring 2018, melon plants showing Fusarium wilt symptoms were collected randomly from melon fields in Bushehr province in southern Iran. Stem segments (3-4mm) showing Fusarium wilt symptoms with adjacent healthy tissue were cut off and then surface disinfected with 0.5% sodium hypocholorite for 5 min, and then washed thoroughly with sterile distilled water. The plant tissues were spread on the filter paper to dry, placed onto acidified PDA (potato dextrose agar, pH4.2) (Banihashemi & de Zeeuw 1969) and incubated at 25°C for 5 days. Single spore methods was used to purify of the isolates (Sinclair & Dhingra 1995). For microscopic observation, the pure isolates were transferred to carnation leaf-specie agar (CLA) (Fisher *et al.* 1982), potassium chloride agar (KCL) (Fisher *et al.* 1983), and PDA. Morphological identification was performed by standard keys (Leslie & Summerell 2006).

Pathogenicity test and race determination

The following muskmelon cultivars were used: Cha-T (no resistant genes), Cha-*Fom1* (resistant to races 0 and 2), and Cha-*Fom2* (resistant to races 0 and 1) (kindly supplied by ENZA Zaden) (Shmidt *et al.* 2016), as well as two local hosts Shahde-Shiraz (resistant to races 0 and 1) and Kharboze-Mashhad (resistant to races 0 and 2) (Banihashemi 2010).

The following Fom isolates were used for race determination: Fom-Bushehr-3s and Fom-Buhehr-5s. Fom-I1-1 (race 0), Fom-I-17 (race 1), Fom-NYFom3 (race 2), and Fom-P-13 (race 1,2) isolates from the fungal collection of the Plant Protection Department (Shiraz University) were also include as positive controls.

For bioassay experiments, conidia were scraped from five-day-old cultures in NO3-medium (0.17% yeast nitrogen base, 3% sucrose, and 100mM KNO_3) by filtering through miracloth (Merk; pore size of 22-25 µm). spores were centrifuged, resuspended in sterile distilled water, counted with a haemocytometer and 10 ml of a 10^7 conidia/ml suspension was used for melon plants inoculation by the root dip method (Wellman 1939). 10-day-old melon seedlings were uprooted, rinsed with water,inoculated, individually potted and incubated at 25 °C in the greenhouse. For each strain, five seedlings of each cultivar were inoculated. Control plants were inoculated with sterile, distilled water. Two weeks after inoculation, disease was scored using a disease index ranging from 0 to 4 including 0: no symptoms; 1: slight root rot symptoms only at tip of main root; 2: brown lesion just above roots, thinner stem or only brown root tip and much smaller; 3: large lesion visible and clear root rot syptoms; 4: plant is dead or almost dead. Disease severity was also scored by determining the plant fresh weight (FW), two weeks after inoculation (van Dam *et al.* 2016).

DNA extraction and PCR amplification

For DNA isolation, mycelium of the representative isolates (Fom-Bushehr-3s and Fom-Bushehr-5s) were grown on potato dextrose agar (PDA). Genomic DNA was extracted from 7-to 10-day-old mycelium according to the protocol described by Van Dam *et al.* (2018).

Sequences of internal transcribed spacer (ITS), translation elongation factor 1-alpha genes (*Ef-1α*), and nuclear ribosomal DNA intergenic spacer (IGS) were amplified using primer pairs including ITS1/ITS4 (White *et al.* 1990), EF1-728F/EF2 (O'Donnell *et al.* 1998, Carbone & Kohn 1999), and CNL12/CNS2 (Anderson & Stasovski 1992, Mirtalebi *et al.* 2013), respectively.

PCR was performed using Sphaero-Q Super Taq (Gorinchem) in a total volume of 20 µl. the PCR mixture contained 1× Sphaero-Q Super Taq buffer, 0.25 U of Sphaero-Q Super Taq (Gorinchem), 3 pmol of each primer, dNTPs (0.2 mM each), 0.1× ROX reference dye (Thermo Fisher Scientific), 1 µl of template DNA, and sterile Milli-Q (to bring the volume to 20 µl). Sterile Milli-Q was used substituted for the DNA template as a negative control sample.

The PCR cycles began with an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s, extension at 72°C for 40 s, and final extansion at 72°C for 5 min. the annealing temperatures differed for each gene: ITS (54°C), EF-1α (54°C), and IGS (65°C).

DNA sequencing and phylogenetic analysis

The PCR products were sent to a service provider to be sequenced via Sanger sequencing

Table 1. *Fusarium oxysporum* isolates used as comparison in phylogenetic analysis, with the geographic origion, original designation, vegetative compatibility group, and their accession numbers in the GenBank database.

Isolates	Species, formae speciales	Original designation	Geographic origin	VCG	GenBank Accession number
Fom-I-17	*F. oxysporum* f. sp. *melonis*	I-17 [a]	Iran,Khorasan	0134	JAANSG000000000
Fom-Yazd2	*F. oxysporum* f. sp. *melonis*	Yazd2 [a]	Iran,Yazd	0134	WUBN00000000
Fom-KT2a	*F. oxysporum* f. sp. *melonis*	KT2a [a]	Iran,Kashmar-Khorasan	0134	JAANSF000000000
Fom-Seif3a	*F. oxysporum* f. sp. *melonis*	Seif3a[a]	Iran,Seifabad-Fars	0134	JAAIIX000000000
Fom-P13	*F. oxysporum* f. sp. *melonis*	P13[a]	Iran,Poshtpar-Fars	0134	JAANSE000000000
Fom-660A-17	*F. oxysporum* f. sp. *melonis*	660A/17[b]	France	0134	JAAIJG000000000
Fom-NYFom62	*F. oxysporum* f. sp. *melonis*	NYFom62[b]	USA	0134	JAAIIZ000000000
Fom-Khaf1	*F. oxysporum* f. sp. *melonis*	Khaf1[a]	Iran,Khaf-Khorasan	GNA	JAAIJC000000000
Fom-Kavar-22	*F. oxysporum* f. sp. *melonis*	Kavar 22[c]	Iran,Kavar-Fars		WUBP00000000
Fom-18L	*F. oxysporum* f. sp. *melonis*	18L[b]	USA	0130	WUBQ00000000
Fom-T61-1	*F. oxysporum* f. sp. *melonis*	T61/1[b]	Japan	0132	JAAIIW000000000
Fom-R12-13	*F. oxysporum* f. sp. *melonis*	R12/13[b]	France	0133	JAAIIY000000000
Fom-NYFom3	*F. oxysporum* f. sp. *melonis*	NYFom3[b]	USA	0131	WUBO00000000
Fom-I1-1	*F. oxysporum* f. sp. *melonis*	I1/1[b]	Israel	0135	JAAIJD000000000
Fonon-2Ma4-5	*F. oxysporum- nonpathogen*	2Ma4-5[a]	Iran,Khorasan		JAANSJ000000000
Fonon-TO1	*F. oxysporum-nonpathogen*	TO1[a]	Iran,Khorasan		JAANSI000000000
F.nonpath- Barmshour	*Fusarium* sp.	Barmshour [a]	Iran,Barmshour-Fars		JAAIJJ000000000

a: Mirtalebi *et al.* 2013, b: Gordon, T.R., Risser, G. and Zitter, T. Z., c: unpublished; GNA: group not assigned

technology. Newly obtained sequences were blasted against databases available at BLAST (https://blast.ncbi.nlm.nih.gov/) on NCBI-GenBank database. Sequences of the targeted genes (ITS, *EF-1α*, and IGS) from additional isolates were retrieved from whole genome sequences to be used in the phylogenetic analysis (Table 1). The sequences were aligned using the Clustal-W in Molecular Evolutionary Genetic Analysis version 6.06 (MEGA 6.06) (http://www.megasoftware.net/).

Phylogenetic analysis was conducted in MEGA 6.06 using the maximum likelihood method (Tamura *et al.* 2013). The sequence of F.nonpath-Barmshour (Table 1) was used as an outgroup with bootstrap value of 1,000 replications.

Genetic diversity DnaSP 5.10 software was used to estimate the number of nucleotide diversity among the Iranian Fom isolates (Rozas *et al.* 2009).

Results

Occurrence and pathogenicity

A total of five isolates of *Fusarium oxysporum* were successfully isolated from the melon plants with Fusarium wilt symptoms in Bushehr province (Table 2). All isolates were deposited in the fungal collection of the Plant Protection Department, College of Agriculture, Shiraz University. All isolated strains produced a pale to dark violet pigment on PDA. The macroconidia were falcate to almost straight, thin walled and usually 3 septate. The apical cell was short and was slightly hoocked. The basal cell was notched or foot-shaped. Microconidia were formed abundantly in false-heads on short monophialides on hyphae. The microconidia were usually non-septate and are oval, elliptical or reniform. chlamydospores were formed in hyphae

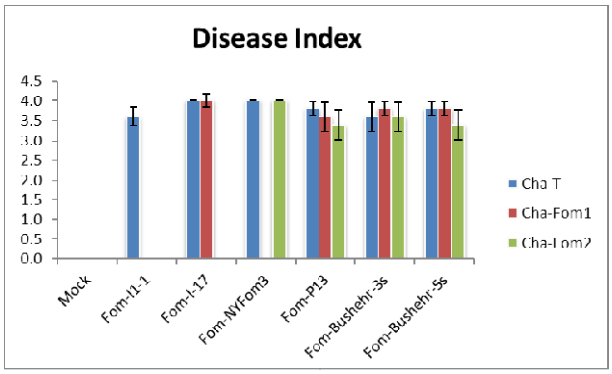
Table 2. *Fusarium oxysporum* f. sp. *melonis* strains were isolated from melon plants and GenBank accession number of their sequence data.

Isolates	Location	Race	ITS accession number	*Ef-1α* accession number	IGS accession number
Fom-Bushehr-1s	Dashti country, Bushehr				
Fom-Bushehr-2s	Dashti country, Bushehr				
Fom-Bushehr-3s	Dashti country, Bushehr	1.2	MT573333	MT672305	MT890562
Fom-Bushehr-4s	Dashti country, Bushehr				
Fom-Bushehr-5s	Dashti country, Bushehr	1.2	MT573334	MT672306	MT890563

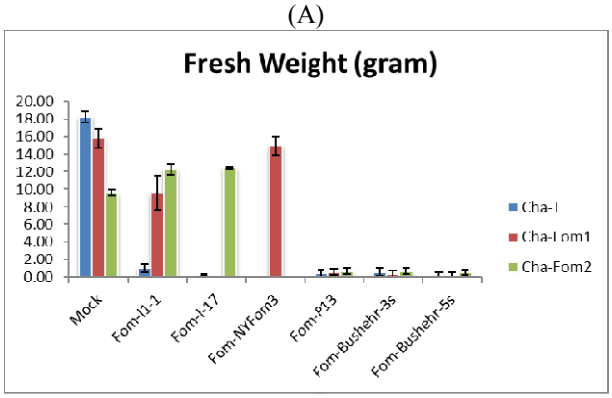
Table 3. The plant frash weight and disease index score of melon differential cultivars inoculated with *Fusarium oxysporum* f. sp. *melonis* isolates.

Isolates	Cha-T		Cha-*Fom1*		Cha-*Fom2*	
	DI	FW	DI	FW	DI	FW
Mock	0	20.8	0	13.55	0	10.46
Mock	0	18.5	0	13.80	0	8.35
Mock	0	16.77	0	15.39	0	9.56
Mock	0	17.5	0	16.06	0	9.87
Mock	0	17.66	0	19.89	0	9.74
Fom-I1-1	4	0.3	0	3.2	0	13.2
Fom-I1-1	4	0.5	0	6.2	0	9.8
Fom-I1-1	3	2.41	0	11.9	0	12.8
Fom-I1-1	4	0	0	11.5	0	13.6
Fom-I1-1	3	2.04	0	15	0	11.7
Fom-I-17	4	0.5	4	0	0	11.68
Fom-I-17	4	0.4	4	0	0	12.40
Fom-I-17	4	0.6	4	0	0	12.55
Fom-I-17	4	0	4	0.2	0	13.08
Fom-I-17	4	0	4	0	0	12.33
Fom-NYFom3	4	0	0	13.80	4	0
Fom-NYFom3	4	0	0	13.61	4	0
Fom-NYFom3	4	0	0	13.41	4	0
Fom-NYFom3	4	0	0	19.51	4	0
Fom-NYFom3	4	0	0	14.35	4	0
Fom-P-13	4	0	4	0	3	0.94
Fom-P-13	4	0.3	2	1.97	4	0
Fom-P-13	3	2.2	4	0.66	2	1.90
Fom-P-13	4	0	4	0	4	0.67
Fom-P-13	4	0	4	0.37	4	0
Fom-Bushehr-3s	4	0	4	0	2	1.93
Fom-Bushehr-3s	4	0	4	0	4	0.89
Fom-Bushehr-3s	2	2.5	4	0	4	0
Fom-Bushehr-3s	4	0.5	4	0.11	4	0.68
Fom-Bushehr-3s	4	0	3	1.89	4	0
Fom-Bushehr-5s	3	1.3	3	1.30	3	1.15
Fom-Bushehr-5s	4	0	4	0	4	0
Fom-Bushehr-5s	4	0	4	0.15	4	0
Fom-Bushehr-5s	4	0	4	0	2	1.46
Fom-Bushehr-5s	4	0.2	4	0.05	4	0.39

DI: Disease Index
FW:Fresh Weight



(A)



(B)

Fig 1. Pathogenicity and race determination of strains Fom-Bushehr-3s and Fom-Bushehr-5s using three melon diferential hosts: Cha-T (without resistance gene), Cha-*Fom1* (*Fom1* resistance gene), and Cha-*Fom2* (*Fom2* resistance gene). Mock inoculated seedlings were used as a negative control. Fom-I1-1, Fom-I-17, Fom-NYFom3, and Fom-P13 inoculated plants were used as positive controls for raes 0, 1, 2, and 1.2, respectively. A) the disease index score and B) the plant fresh weight were determined two weeks after inoculation.

on the surface of the agar of the CLA plate. Morphological characteristics of all isolates were consistent with Burgess *et al.* (1994) and Leslie and Summerell (2006) description.

Based on our pathogenicity testing results, Fom strain which was able to infect Cha-T and Cha-*Fom1* melon cultivars was classified as race 1. The strain that was pathogenic on Cha-T and Cha-*Fom2* cultivars was classified as race 2. Fom strain that was able to infect on Cha-T was classified as race 0 and the strain that was pathogenic on all of melon cultivars was classified as race 1.2. in that case, Fom-Bushehr-3s and Fom-Bushehr-5s were belonged to race 1.2 (Table 3; Fig. 1).

Sequence and phylogenetic analysis

On the basis of BLAST searches against NCBI-GenBank database, the morphologically identified *Fusarium oxysporum* was confirmed on the basis of three different loci (ITS, *Ef-1α*, and IGS) sequences. Similarity of 99-100% was showed that isolates belong to *Fusarium oxysporum* f. sp. *melonis*.

The results of phylogenetic tree which constructed with concatenated sequences of the ITS, *EF-1α*, and IGS showed that the Fom isolates were divided into six lineages. Each lineage of

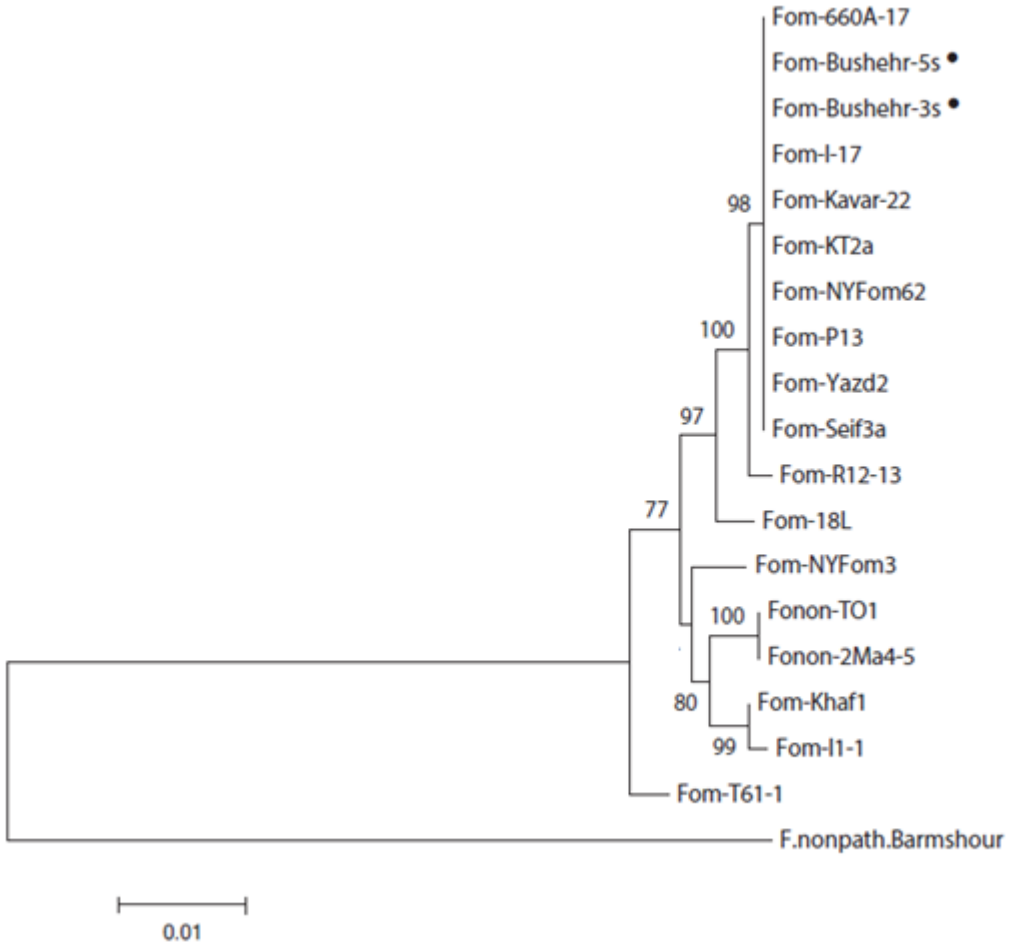


Fig. 2. Phylogeny of *Fusarium oxysporum* f. sp. melonis strains of Bushehr province based on combined ITS, *EF-1α*, and IGS sequences in Maximum likelihood phylogenetic tree. Bootstrap values (> 75%) are shown as percentages of 1,000 replicates at the branch point. F.nonpath-Barmshour is the outgroup. Black circle indicates strains isolated in this study.

Fom isolates corresponds to a single VCG, VCG0130-0135. Two non-pathogenic isolates grouped into separate clade.

Based on phylogenetic analysis, two race 1.2 Fom isolates recovered from Bushehr province grouped together with the other Iranian Fom isolates races 1 and 1.2 belonging to VCG0134 which isolated from Khorasan, Fars, and Yazd provinces and also foreign isolates belonging to VCG0134 (Fig. 2).

Based on the results of DnaSP analysis, there was no nucleotides differences between two Fom isolates from Bushehr province and the other Iranian isolates belonging VCG0134 in the ITS, *EF-1α*, and IGS sequences.

Fom-Bushehr-3s and Fom-Bushehr-5s were seprated from Fom-Khaf1 strain from Khorasan province with six nucleotides difference in *EF-1α* sequences (site: 26, 104, 175, 383, 440, and 462) and also 40 nucleotides difference in IGS sequences (see Fig. 3 for detailed information).

Discussion

Melon Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis*, is one of the most destructive disease of melon (Oumouloud *et al.* 2013). Four races of Fom, races 0, 1, 2, and 1.2, have been distinguished based on their ability to overcome *Fom1* and *Fom2* resisitance genes (Risset *et al.* 1976).

In previous studies which have been conducted in Iran, race 1 from Khorasan and Semnan provinces and race 1,2 from Fars, Isfahan, Kermanshah, Khorasan, Markazi, and Yazd provinces were reported (Banihashemi 1968b,

	EF-1α					IGS															
	26	104	175	383	440	462	641	646	737	807	812	848	847	898	916	968	987	1041			
Fom-Khaf1	C	T	T	C	C	C	C	A	A	T	G	A	G	T	T	T	A	G			
Fom-Bushehr-3s	T	C	C	A	G	T	T	G	T	G	A	G	A	C	A	C	T	C			
Fom-Bushehr-5s	T	C	C	A	G	T	T	G	T	G	A	G	A	C	A	C	T	C			
	IGS																				
	1055	1092	1116	1121	1124	1252	1273	1294	1324	1381	1388	1452	1515	1646	1655	1678	1775				
Fom-Khaf1	A	A	A	C	T	C	A	T	C	C	C	A	T	T	T	A	A				
Fom-Bushehr-3s	G	T	G	T	C	T	T	G	G	T	T	T	C	C	C	G	G				
Fom-Bushehr-5s	G	T	G	T	C	T	T	G	G	T	T	T	C	C	C	G	G				
	IGS																				
	1802	1810	1834	1881	1892	1958	1964	2030	2044	2110	2250										
Fom-Khaf1	T	A	A	G	T	A	G	C	A	C	C										
Fom-Bushehr-3s	C	C	C	A	C	G	A	T	G	T	T										
Fom-Bushehr-5s	C	C	C	A	C	G	A	T	G	T	T										

Fig. 3. Nucleotide differences observed between Fom-Bushehr-2s, Fom-Bushehr-5s, and Fom-Khaf1 strains. Base positions include spaces caused by alignment gaps and refer to the position in the alignment deposited in DnaSP 5.10.

1982, 1989, Sarpeleh & Banihashemi, 2000, Mirtalebi *et al.* 2013). In the present study, five isolates of Fom were isolated from plants infected melons in Bushehr province. Two isolates, Fom-Bushehr-3s and Fom-Bushehr-5s were investigated by determining the race in the greenhouse. The results showed that these isolates are belonged to race 1.2.

Fusarium wilt of melon is prevalent in cold and temperate regions where it causes a worldwide problem (Banihashemi 1968a). Trough this study we found that Fom is also presented in tropical and subtropical regions of Iran (Bushehr) and has caused Fusarium wilt in melon fields. Bushehr has a hot semi-arid climate (https://en.wikipedia.org). In some parts of the world, which has a climate similar to Bushehr, the presence and survival of this pathogen has been reported. For example, Suarez-Estrella *et al.* (2003, 2004) indicated that Fom which is an important plant pathogen fungus in the province of Almeria, southeast Spain, can persist in plant wastes for more than 12 months.

In the present study multi-gene esquences of three loci (ITS, *EF-1α*, and IGS), were used for phylogenetic analysis of Fom isolates from Bushehr. Sequence analyses of the IGS and *EF-1α* regions of Iranian Fom isolates was performed previously. According to Mirtalebi *et al.* (2013) IGS lineages were correlated with the seven VCG (VCG0130-0136), while VCG0130 and 0131 could not be differentiated with *EF-1α* sequences (Mirtalebi & Banihashemi, 2014). In the current study, the phylogenetic analysis based on combined three loci sequences showed that six VCG (0130-0135) are grouped to seprate clades. In the phylogenetic tree, a close genetic relationship between the Fom isolates from Bushehr and the other Iranian isolates with VCG0134 were observed. No differences were found between the two Fom isolates from Bushehr and other Iranian isolates with VCG0134 in the tree loci sequences we evaluated in this study. According to the results, it can be spectulated that the VCG of Fom-Busher-3s and Fom-Bushehr-5s may be VCG0134, but we did not examine this in the laboratory. Also, Fom isolates which recovered from melon plants of tropical region in this study are not genetically different in three loci we evaluated from other Fom isolates with VCG0134 isolated from the other parts of Iran.

According to phylogenetic tree, Fom strains from Bushehr and Fom-Khaf1 strain from Khorasan province were clustered into seprate linages. Based on DnaSP analysis six nucleotides difference in *EF-1α* sequences and 40 nucleotides difference in IGS sequences were observed between these linages, but there was no nucleotide difference in ITS region between them.

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