Original Article

Morphological and phylogenic analysis of *Fusarium* species associated with vertical system of *Orobanche* spp.

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Abstract: Broomrapes (*Orobanche* spp.) are parasitic weeds and considered as a major limiting factor for the cultivation of various crops in many parts of the world. Due to the special biology of broomrape, including seed production, dispersal and longevity, the control of these species are often extremely difficult. Broomrape poses a serious threat to Iran's agriculture; therefore exploring potential biological agents for these species are necessary. In this study, samples of infected broomrape plants (brown rot on vertical systems) collected from ten provinces of Iran, over the summer period (2014-2015). *Fusarium* isolates were identified according to their cultural and morphological characteristics. For phylogenetic analysis, a part of the *tef1-α* gene was amplified and examined. Based on morphological characters, fourteen species of *Fusarium*, including *F. andiyazi*, *F. equiseti*, *F. flocciferum*, *F. foetens*, *F. hostae*, *F. lacertarum*, *F. oxysporum*, *F. proliferatum*, *F. redolens*, *F. sambucinum*, *F. solani* s. l., *F. thapsinum*, *F. torulosum* and *F. verticillioides*, were identified. *F. solani* s. l., with 25% frequency, was the most common species among species. Eight species namely *F. andiyazi*, *F. hostae*, *F. flocciferum*, *F. foetans*, *F. lacertarum*, *F. redolens*, *F. thapsinum* and *F. torulosum* on broomrape are being reported for the first time on global-scale and *F. lacertarum* is being reported for the first time in Iran.

Key words: *Fusarium lacertarum*, biological agent, new species, *tef1-α* gene.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are one of the most important weeds around the world (Ghotbi et al., 2011). Broomrapes are distributed in more than 80 countries and invaded almost 16 million hectares of agricultural lands around the world. Depending on rate and the amount of infection, broomrape can reduce yield quality and quantity between 30 to 100 percent. Broomrapes only germinate in response to specific chemicals released by the host plants (Perez-de-Luque et al. 2010). After germination, the seedlings attach to the host roots by the production of specialized feeding structures, described as haustoria to develop and accumulate nutrient resources from host plant (Joel et al. 2007). Therefore, broomrape is a damaging and destructive weed to crops and is difficult to control.

Although broomrapes are efficient in mechanisms such as seed production, dispersal and longevity and host roots attachment ability (reaching and entering the vascular tissue and underground development), the control of these species are extremely difficult (Montazeri, 2011; Amsellem et al. 2001b; Mazaheri & Ershad, 1995). Despite the various control practices against broomrapes, such as cultural and mechanical methods, soil fumigation, soil solarization, trap crops and resistant cultivars (Jacobsohn et al. 2001), these available control techniques have not yet proven to be as effective, economical and applicable as expected (Alejandro et al. 2010; Goldwasser & Kleifeld 2004).

Phytopathogenic fungi such as *Fusarium* species (*F. oxysporum* Schelcht, *F. oxysporum* f. sp. *orthoceras*, *F. solani* Mart., *F. arthrosporioides* Sherb., *F. nygamai* Burgess & Trimboli and *F. semitectum* Berk. & Ravenel, especially *F. semitectum* var. *majus*) were reported to be associated with *Orobanche* spp. These *Fusarium* species have shown significant pathogenicity against *Orobanche* spp. when tested under controlled or field conditions (Amsellem et al. 2001b; Bedi & Donchev, 1991; Cohen et al. 2002; Muller-Stover et al. 2002). These fungal pathogens demonstrate their potential ability to be used as bio-herbicides.

TEF is a protein that is translated into an essential part of the encoding high phylogenetic region (Geiser et al. 2004). This gene first was used as a marker for identification and phylogenetic relationship of the species belongs to noctuid moths subfamily Heliothinae in Lepidoptera Order (Cho et al. 1995).

Since, the issue of broomrape has caused serious problems to Iran's agriculture; this study introduce a proper method to overcome the problems associated with *Orobanche* spp. The aim of this study was to identify *Fusarium* species associated with *Orobanche* species that potentially used as biological control agents of broomrape using indigenous antagonistic *Fusarium* species.

MATERIALS AND METHODS

Sampling

Infected broomrape plants (*Orobanche* spp.) with vertically brown rot symptoms were randomly collected from tomato farms in ten provinces of Iran, including Alborz, Tehran, Kermanshah, Kurdistan, Hamadan, Zanjan, East Azerbaijan, Razavi Khorasan, Fars and Markazi provinces, during summertime in 2014-2015. Samples were picked up from their roots using trowel and transferred to the laboratory in paper packets.

Fungal isolation and preservation

Isolation of fungi was carried out according to Nash and Snyder (1962) medium. The roots were separated and rinsed in tap water for 20 minutes to wash away soil particles. The Root part of each samples were then cut into 2 cm pieces and the sterilization steps took place. The pieces were soaked at 1% sodium hypochlorite for 2 minutes and rinsed in sterilized distilled water and air dried on sterile filter paper. The disinfected pieces were cut into 2 cm pieces, placed on Peptone PCNB Agar (PPA) medium and incubated at 25°C in the dark for 3 days. The isolates were sub-cultured into Water Agar (WA) medium and a tip of the hyphae was picked up and transferred to PDA (potato dextrose agar) medium. The purified isolates were then stored on sterile filter papers at -20°C. Fungal isolates were deposited in fungal culture collections of University of Tehran (UTFC).

Morphological characterization

Isolates of *Fusarium* were identified according to their cultural and morphological characteristics as described by (Gerlach & Nirenberg, 1982; O'Donnell et al., 2004; Leslie & Summerell, 2006; Saremi, 2005). The isolates were grown on PDA medium to determine their growth rate and colony pigmentation, so the cultures were incubated at 26°C and 30°C for 7–10 days in the dark. Colony diameter was measured and Colony color recorded with naked eyes. Isolates were also placed on CLA and SNA, then incubated for 14 days under fluorescent and near-ultraviolet lights conditions to investigate the presence and shape of the macroconidia, microconidia and chlamydospores.

Phylogenetic analysis

DNA extraction: Liquid cultures were initiated by adding 2 pieces of 5 days old fungal cultures to 250-mL Erlenmeyer flasks containing 100 mL PDB medium (potato dextrose broth plus 2 g yeast extract per liter). Flasks were incubated at room temperature approximately 25°C on a rotary shaker for 6–8 days. Mycelium was collected by filtration through the sterile filter paper with a vacuum funnel. Mycelia were harvested, frozen and stored at −20°C. DNA was extracted using a modified hexadecyl trimethyl-ammonium bromide (CTAB) procedure (Doyle and Doyle 1987). The DNA was visualized on a 1% agarose gel (wt/v) (Boehringer Mannheim) stained with ethidium bromide and viewed under ultra-violet light. DNA concentrations were estimated by comparing the intensity of ethidium bromide fluorescence of the DNA sample to a known concentration of lambda DNA marker (marker III, Roche Diagnostics). Extracted DNA (50–90 ng) was used as the template for the PCR reaction.

Molecular characterization

A part of the *tef1-α* gene was amplified by PCR using the primers Ef1 F (5'-ATGGGTAAGGAGGA CAAGAC-3') and Ef2 R(5'GGAAGTACCAGTGAT CATGTT-3') (O'Donnell et al. 1998) in a final volume of 25 μL containing 50-60 ng of DNA, 0.1 μM of each primer, 150 μM dNTP, 3 U Taq DNA polymerase and PCR reaction buffer. Amplifications were conducted in a Master-cycler (Eppendorf) with an initial denaturation of 5 min at 95°C followed by 35 cycles of 60 s denaturation at 95°C, 75 s annealing at 56°C, 60 s extension at 72°C and a final extension of 7 min at 72°C. The presence of PCR products was confirmed by gel electrophoresis. The *tef1-α* amplicons were sequenced by Macrogene Co. (South Korea) using the two PCR primers as sequencing primers. Sequence identities were determined using Blast analysis from NCBI available online and most identic sequences from each species were recorded together with their information to use in phylogenetic analysis (table 1).

Sequence analysis

Sequences were aligned and compared by Kimura's two parameters distance model and the neighbor-joining (NJ) and Maximum Likelihood (ML) methods with Tamura-Nei distance model using the program MEGA ver. 6.0 software (Gouy et al. 2010). The topology of the resulting tree was tested by bootstrapping with 1000 re-samplings of the data.

RESULTS AND DISCUSSION

A total of 203 isolates from 385 collected samples were identified as the genus of *Fusarium*. Based on morphological characters, only fourteen *Fusarium* species, including *Fusarium*, including *F. andiyazi*, *F. equiseti*, *F. flocciferum*, *F. foetens*, *F. hostae*, *F. lacertarum*, *F. oxysporum*, *F. proliferatum*, *F. redolens*, *F. sambucinum*, *F. solani* s. l., *F. thapsinum*, *F. torulosum* and *F. verticillioides*, have been identified. *Fusarium solani*, *F. oxysporum* and *F. redolense* with 25%, 20% and 15% frequency are common among all the species, respectively (table 2).

Table 1. Information of refer sequences from NCBI gene bank used in phylogenic analysis.

Species	Isolate	GB Accession no.	host	Authors
Fusarium andiyazi	2193	EU620627.1	sorghum grain	Petrovic et al., 2008
Fusarium andiyazi	M051749S2_	KM462947.1	sorghum grain	Funnell-Harris et al., 2014
Fusarium andiyazi	M051946S-3	KM462919.1	sorghum grain	Funnell-Harris, et al., 2015
Fusarium equiseti	XJ-CJ-F11-11	KT224315.1	sugar beet	Wang & Wu et al. , 2015
Fusarium equiseti	UBOCC-A-	KF225018.1	---------	Lecellier, et al., 2013
Fusarium equiseti	MOS879	KP008978.1	Soil	Oskiera, et al., 2014
Fusarium equiseti	ITEM 3190	JF966238.1	Soil	Stepien, et al., 2012
Fusarium flocciferum	VI01420	AJ543572.1	*Hordeum*	Kristensen, et al., 2005
Fusarium flocciferum	GS-WW-4-1	KT224194.1	Potato	Wang and Wu, 2015
Fusarium flocciferum	GS-WW-1-3	KT224192.1	Potato	Wang and Wu, 2015
Fusarium foetens	10-137b	JX298790.1	*Begonia elatior*	Saurat, et al., 2013
Fusarium foetens	NRRL 52749	JF740825.1	---------	O'Donnell, et al., 2012
Fusarium foetens	NRRL 31852	HM057337.1	Tomato	Huang et al., 2010
Fusarium hostae	NRRL 29889	HM057340.1	---------	Huang, et al., 2010
Fusarium hostae	O-2081	AF331819.1	---------	Geiser, et al., 2001
Fusarium hostae	NRRL29642	AF324322.1	---------	O'Donnell and Geiser, 2000
Fusarium lacertarum	NRRL 52753	JF740828.1	---------	O'Donnell, et al., 2012
Fusarium lacertarum	NRRL 20423	GQ505593.1	cucumber	O'Donnell, et al., 2009
Fusarium oxysporum	ATCC 16612	KT323866.1	cucumber	Ortiz et al., 2017
Fusarium oxysporum	CBS 127.73	KF913725.1	*Pisum sativum*	Bani, et al., 2014
Fusarium proliferatum	G3-1	KX215078.1	strawberry	Pastrana, et al., 2016
Fusarium proliferatum	M05-1749S-1	KM462938.1	sorghum grain	Funnell-Harris, et al., 2015
Fusarium proliferatum	FV4	KF715258.1	Barley	Molnar, O. 2014
Fusarium redolens	MOS681	KP008977.1	Tomato	Oskiera et al., 2014
Fusarium redolens	NRRL 25123	JF740748.1	Tomato	O'Donnell et al., 2012
Fusarium sambucinum	IM-WL-SD-	KT224139.1	Potato	Wang and Wu, 2015
Fusarium sambucinum	YN-KM-DC	KT224160.1	Potato	Wang and Wu, 2015
Fusarium sambucinum	XJ-YN-1	KT224159.1	Potato	Wang and Wu, 2015
Fusarium solani	NRRL 52778	JF740846.1	---------	O'Donnell, et al., 2012
Fusarium solani	NRRL 25083	JF740714.1	---------	O'Donnell, et al., 2012
Fusarium solani	MOS615	KP008979.1	---------	Oskiera, et al., 2014
Fusarium thapsinum	FT-2	KM589049.1	Tomato	Kandan, et al., 2014
Fusarium thapsinum	M05-1711S-	KM462956.1	sorghum grain	Funnell-Harris et al., 2015
Fusarium thapsinum	M05-1874S-	KM463006.1	sorghum grain	Funnell-Harris, et al., 2015
Fusarium torulosum	NRRL 52772	JF740840.1	---------	O'Donnell, et al., 2012
Fusarium torulosum	F110	JX534443.1	---------	Chen, et al., 2014
Fusarium verticillioides	A71	KY173009.1	---------	Tupaki- et al., 2016
Fusarium verticillioides	638ES	KR905555.1	Maize	Velarde Felix et al., 2015
Fusarium verticillioides	F36	KM598766.1	Maize	Madrigal, et al., 2014
Fusarium scirpi	NRRL 36478	GQ505654.1	---------	O'Donnell et al., 2009
Fusarium scirpi	NRRL 29134	GQ505605.1	---------	O'Donnell et al., 2009
Fusarium scirpi	NRRL 26922	GQ505601.1	---------	O'Donnell et al., 2009
Fusarium sp.	NRRL 52720	JF740802.1	---------	O'Donnell et al., 2012
Fusarium sp.	NRRL 25085	JF740716.1	---------	O'Donnell, et al., 2012
Fusarium sp.	45997	GQ505672.1	---------	O'Donnell, et al., 2009
Fusarium sp.	ITEM 13005	LN901570.1	Wheat	Villani, et al., 2015
Microdochium majus	10149	JX280543.1	*Triticum* sp.	Jewell, & Hsiang, 2012

Fungi associated with *Orobanch* spp. including *Alternaria* spp*., Bipolaris austransis, F. equiseti, F. oxysporum, F. semitectum, F. solani, Rhizoctonia solani, Ulocladium atrum* and *Verticillium allbo-atrum*, were isolated from *O. aegyptiaca* (Mohammadi et al. 2014). *Fusarium* species are well distributed across many geographical regions and substrates, and also widely distributed in different soils, plants and air (Booth 1971; Burgess et al. 1994; Nelson et al. 1994; Summerell et al. 2003). So, in this research different species of genus *Fusarium* associated with *Orobanch* spp. from different geographical regions of Iran were identified.

The morphological identification of the *Fusarium* species was confirmed by the sequencing of *tef1-α*

gene. So, the Standard Nucleotide BLAST search for similarities showed the similarity percentage of the strains ranged from 98 to 99 percent. The *tef*1-α sequence of *Fusarium* strains were searched for homology in GeneBank database. Then, the result of *tef*1-α gene sequencing demonstrates that all tested isolates belong to the genus *Fusarium*. Also, the similarities of *tef*1-α sequence between our isolates and the reference sequences from the GeneBank, were supported by bootstrap values of more than 50 percent. All the analyzed sequences data were deposited in the GeneBank database. To our knowledge, this is the first report of eight species including *F. redolens, F. torulosum, F. hostae, F. foetans, F. andiyazi, F. flocciferum, F. lacertarum* and *F. thapsinum* on

Table 2. Information of fifteen species of Fusarium isolated from infected broomrapes.

Isolate	Species	Sampling region	GB Accession no.	Collection Accession no.
AH1	*F. flocciferum*	Alborz province	MF588955	ABRII 10265
FSE3-1	*F. foetens*	Fars province	MF588956	ABRII 10257
HT1-2	*F. solani*	Alborz province	MF588957	ABRII 10258
KG1	*F. torolosum*	Kermanshah province	MF611744	ABRII 10259
KG7-1	*F. hostae*	Kermanshah province	MF611745	ABRII 10260
KK23-2	*F. verticillioides*	Kurdistan province	MF611746	ABRII 10261
KK27-2	*F. andiyazi*	Kurdistan province	MF611747	ABRII 10262
KK32-1	*F. sambucinum*	Kurdistan province	MF611748	ABRII 10263
KK32-2	*F. verticillioides*	Kurdistan province	MF611749	ABRII 10264
KM4	*F. lacertarum*	Kermanshah province	MF611751	ABRII 10266
NA2-1	*F. equiseti*	Hamedan province	MF611752	ABRII 10267
NA9	*F. redolans*	Hamedan province	MF611753	ABRII 10268
ZCH2-2	*F. oxysporum*	Zanjan province	MF611755	ABRII 10269
ZCH6	*F. proliferatum*	Zanjan province	MF611754	ABRII 10270

Orobance spp. in global scale. Idetification of *F. lacertarum* is from Orobanch spp. is recorded for the first time for the mycobiota of Iran.

Fusarium lacertarum Subrahm., Mykosen 26 (9): 478. 1983.

Colonies on PDA were reached to 38-44 mm average growth after three days at 26°C. Mycelia were white and the bottom of the colony showed brown-orange pigmentation after seven days. On CLA, the aerial mycelium produced solitary chlamydospores with 7.7-12.3µm diameters, and no microconidia was formed. Macroconidia originated from abundant sporodochia with strong orange color, in short monophialids, usually with not more elongated apical cells, rarely curved and the basal cell of macroconidia presented a foot form. Apicali cell form in hook form, smoothly curved, 5 septa, 42-58×3.5–4.5 µm (Fig. 1). Morphological characters of *F. lacertarum* approved by sequencing results of *tef1-α* part gene.

Specimens examined. Iran, Kermanshah Province, Mahidasht, isolated from crown and root of *Orobanche aegyptiaca*, 28 July 2014, *A. Rostami*, (IRAN UTFC-FO11, isolate KM4).

In this study, based on molecular data and morphological characterization, *F. lacertarum* is reported for the first time in Iran and also on broomrape across the world. Morphology of examined specimens agrees with the description provided by Poletto et al. (2015).
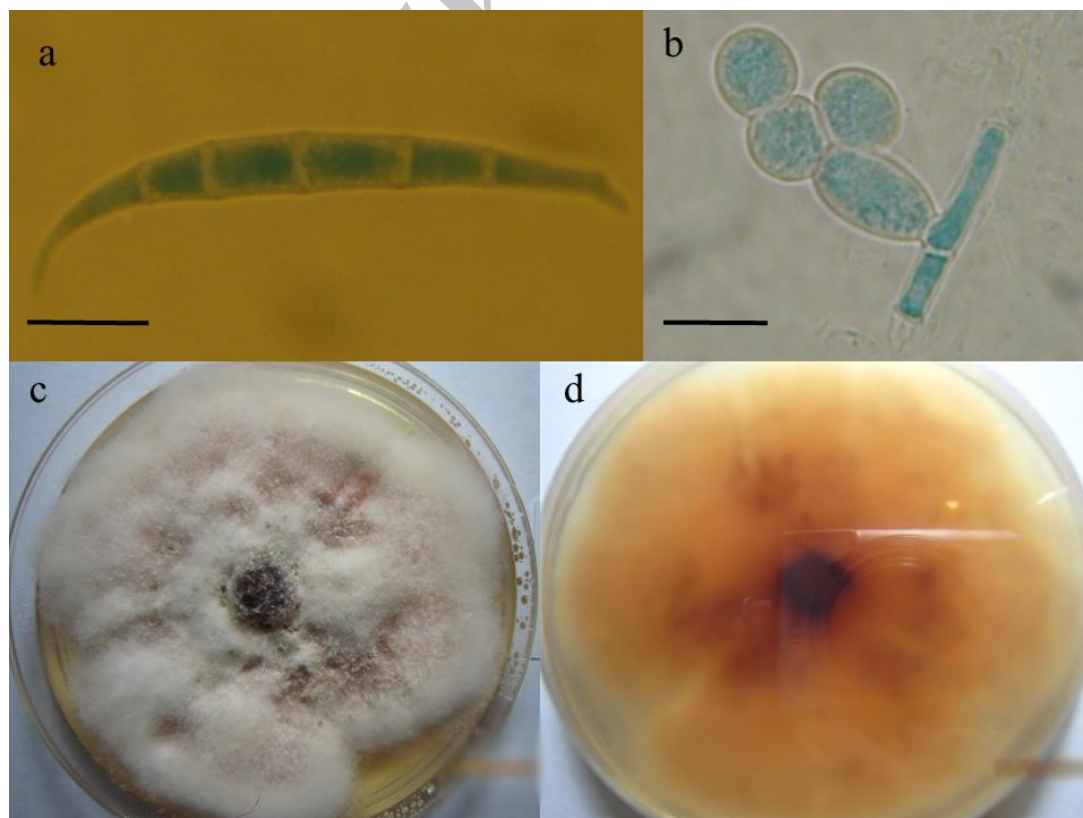

Fig. 1. Morphological characters of *F. lacertarum*. a. A single spore of Macroconidia. b. A clump of clamydospores. c. Front and d. back views of PDA cultured colony. Scale bar: 10 µm.

Phylogenetic analysis

A phylogenetic tree with 3 clades including clades A, B and C was drown during phylogenic analysis of tested taxa based on NJ method (Fig. 2). Clade A was containing *Gibberella fujikuroi* species complex and divided into four subclades. Sublade A1 showed strain of *F. andiyazi* that were supported by bootstrap value of 99%, subclade A2 consisted of isolates *F. verticilliodes* and subclad A3 including strains of *F. foetans*, *F. oxysporum* and *F. thapsinum.* An isolate which was morphologically identified as *F. verticilliodes* (kk 23-2), according to no observation of globose microconidia, was placed within the *F. thapsinum* isolates from NCBI and subclade A4 contained strains of *F. proliferatum* species.

Clad C is a group with members of Gibbosum complex of *Fusarium* species and divided into four subclades names C1, C2, C3 and C4. Subclade C1 was supported by the strong bootstrap value of 94% and included *F. laceratum* strains, Subclade C2 included strains of *F. scirpi* and was located as sister group of

subclade C3 including, strains of *F. equiseti* and some *Fusarium* sp. isolates.

Clade B includes other species and divided into four subclades (B1, B2, B3 and B4). Subclade B1 included the strain HT 1-2 and demonstrated high similarities of *tef1-α* gene sequence to the referred isolates of *F. solani*, subclade B2 contained two species of *F. hostae* and *F. redolens* by strong bootstrap value of 98%, subclade B3 consists of *F. torulosum* and *F. flociferatum* strains, subclade B4 consisted of strain KK32-1 which gave high similarity of *tef*1-α gene sequence to the referred *F. sambucinum* from NCBI and was supported by the strongest bootstrap value of 100%.

Maximum likelihood (ML) analysis of the *tef1-α* gene sequence alignment recovered a tree with significant similarities to the phylogenetic tree in NJ method (Fig. 3). However, there are some differences between the two trees. For example, subclade B1 in NJ analysis which includes *F. solani* strains is located as a separate clade in maximum likelihood method that
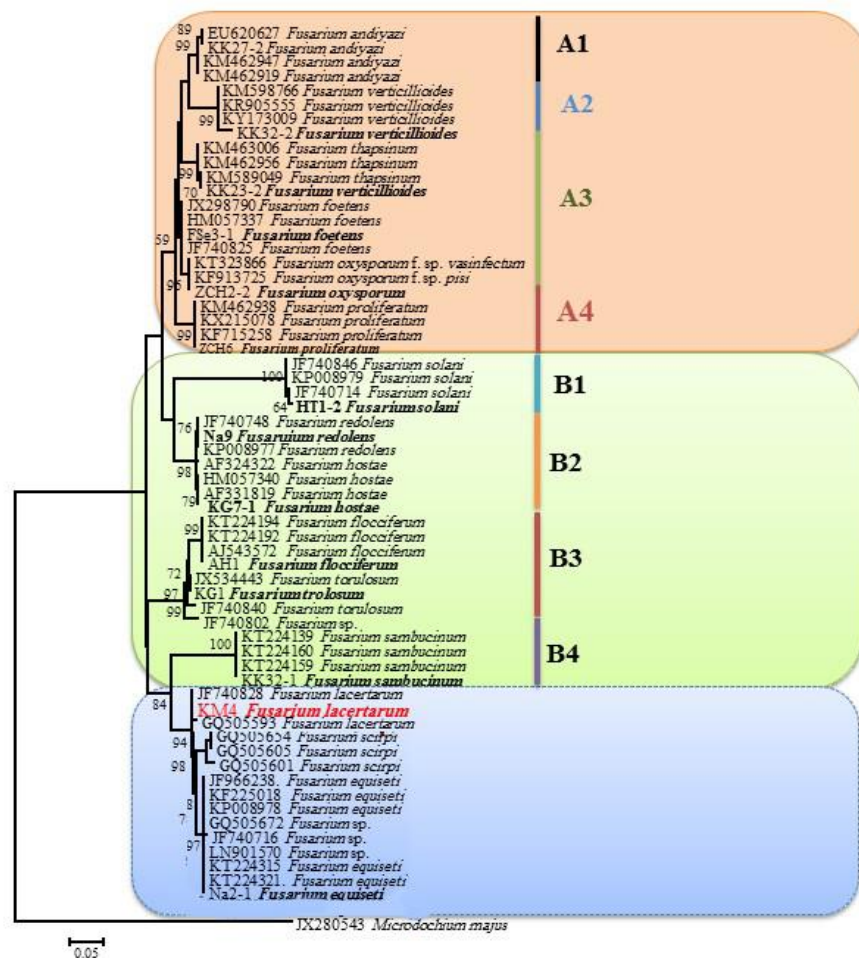

Fig. 2. Phylogenetic tree showing the relationship of 34 *Fusarium* species strains based on *tef1-α* gene sequence using the neighbor-joining (NJ) method. The percentage values of replicate trees in which the linked taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The bootstraps values > 50% were shown next to the branches. *Microdochium majus* was used as an out-group.

was named as clade D. Subclade B4 consisted of strain KK32-1 and strains of *F. sambucinum* was placed in clade C in maximum likelihood analysis by the strongest bootstrap value of 100%. The location of isolate KM4 (*F. lacertarum*) in ML tree is the same as NJ tree.

In this study, in addition to the morphological identification, the sequence data analysis of the *tef1-α* region was employed. The molecular data and phylogenetic relationships allow reliable differentiation between the major *Fusarium* species. For this purpose, sequence of *tef1-α* gene was used to assess 15 identified species of genus *Fusarium* isolated from broomrape samples. Use of translation elongation factor 1α (*tef1-α*) and β-tubulin genes can lead to more clear fungal identification (Vitale *et al*. 2011; Wang *et al*. 2011). Overall, Molecular analysis had harmony with morphological grouping, except for isolate kk23-2 in clade A and subclade A3, where *F. verticilliodes* isolate (kk23-2) was located between the *F. thapsinum* isolates. Some strains of *F. thapsinum* that do not produce diagnostic yellow pigment, is

morphologically identical to *F. verticillioides* therefore in similar situations molecular characterization can be helpful in identifying these isolates (Leslie & Summerell, 2006). This sort of discrepancy between morphological and molecular data in fungal studies has been seen frequently (Darvishnia 2013; Watanabe 2013).

Although the morphological species concept does not completely reflect the phylogenetic tree of the genus *Fusarium* (O'Donnell et al., 2000), this does not imply that morphological characteristics are not useful for identification and taxonomy. To identify unknown species, morphological characteristics can be widely applied to any species, not only the genus *Fusarium* but also to other fungi (Taylor et al. 2000). *Fusarium* isolates can be initially classified on the basis of morphological similarity, with the awareness that sections are in fact a means of artificial grouping, but the morphological approach fails to detect many biological factors but phylogenetic approach can be useful in detection of this factors (Liddell, 2003).
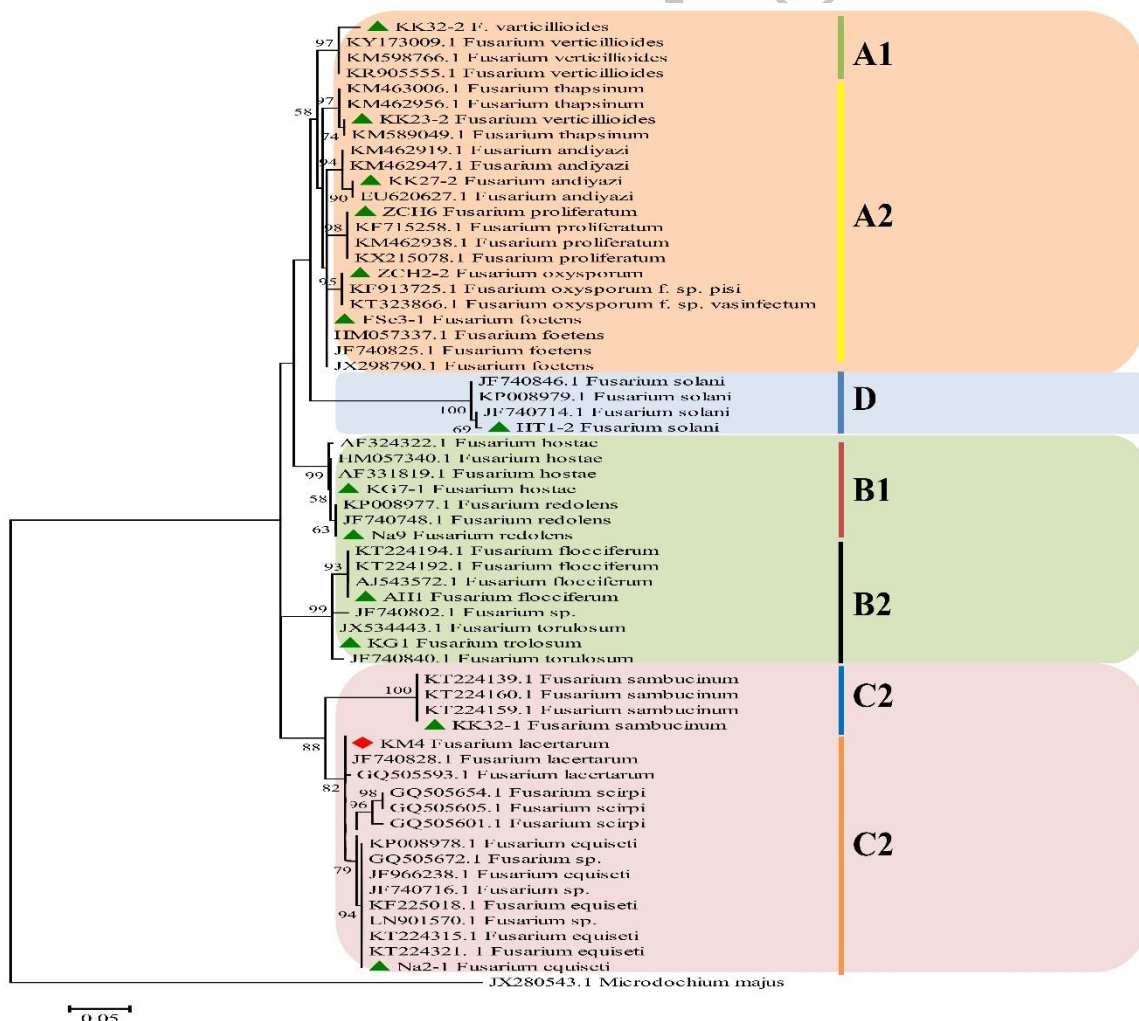

Fig. 3. Phylogenetic tree showing the relationship of 34 *Fusarium* species strains based on *tef1-α* gene sequence using the Maximum likelihood (ML) method. The percentage values of replicate trees in which the linked taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The bootstraps values > 50% were shown next to the branches. *Microdochium majus* was used as an out-group.

Due to the deficiencies and problems within morphological identification knowledge and also due to the large number of fungal species and inadequate available morphological information, the use of molecular information can be helpful (Davari et al. 2013). So, we need to construct more reliable taxonomic system in combination with the morphological, phylogenetic, toxicological, biological, and other recognition methods. The use of another gene in molecular identification can be useful in better identifying and better phylogenetic analyses.

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REFERENCES

Amsellem Z, Barghouthi S, Cohen B,Goldwasser Y,Gressel J. 2001a. Recent advances in the biocontrol of Orobanche (Broomrape) species. Biocontrol 46: 211-228.

Amsellem Z, Kleifeld Y, Kerenyi Z, Hornok L, Goldwasser Y,Gressel J. 2001b. Isolation, identification and activity of mycoherbicidal pathogens from juvenile broomrape plants. Biological Control 21: 274–284.

Bedi JS, Donchev N. 1991. Results on mycoherbicide control of sunflower broomrape (Orobanchc cernua wall.) under glasshouse and field conditions. Proceeding of the 5th International Symposium on Parasitic Weeds, Jun. 24-30, Nairobi, Kenya, Cimmyt. Pp. 76-82.

Booth C. 1971. The genus Fusarium. Commonwealth Mycological Institue, Kew, UK.

Burgess LW, Summerell Ba, Bullock S, GottKp, Bakhous D. 1994. Laboratory manual for Fusarium research. Fusarium research laboratory. Department of Crop Science, University of Sydney and Royal Botanic Gardens, Australia.

Cho SW, Mitchell A, RegierJc, Mitter C, Poole RW, Frienlander TP, Zhao SW. 1995. A highly conserved nuclear gene for low-level phylogenetics-elongation factor-1-alpha recovers morphology-based tree for Heliothine moths. Molecular Biology and Evolution 12: 650- 656.

Cohen B, Amsellem Z, Lev-Yadun S, Gressel J. 2002. Infection of tubercles of the parasitic weed Orobanche aegyptiaca by mycoherbicidal Fusarium species. Annals of Botany 90:567–578.

Darvishnia M. 2013. Morphological and phylogenetic studies of Fusarium species in Iran. Journal of Novel Applied Sciences 2:1134-1142.

Davari M, Babai-Ahari A, Arzanlou M, Zare R, Van Diepeningen AD, De Hoog GS. 2013. Morphological and molecular characterization of three new Fusarium species associated with

inflorescence of wild grasses for Iran. Rostaniha 14: 124-134.

Geiser DM, Jimenez-Gasco MM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, KuldauGA, O'Donnell K. 2004. Fusarium-ID v.1.0: a DNA sequence database for identifying Fusarium. European Journal of Plant Pathology 110: 473-479.

Ghotbi M, Amini Dehghan M, Ghotbi M. 2011. Effect of nutritional regime on expression of pathogenicity of Fusarium oxysporum against Orobanche aegyptiaca Pers. International Journal of AgriScience 1(4): 012-031.

Gouy M, Guindon S, Gascuel O. 2010. Seaview version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27: 221-224.

Joel DM, Hershenhorn J, Eizenberg H, Aly R, Ejeta G, Rich PJ, Ransom JK, Sauerborn J, Rubiales D. 2007. Biology and management of weedy root parasites. Horticultural Reviews 33: 267-349

Leslie JF, and Summerell BA. 2006. The Fusarium laboratory manual. Blackwell Publishing Professional. Ames, IA, USA.

Liddell CM. 2003. Systematics of Fusarium species and allies associated with fusarium head blight. The American Phytopathological Society, St. Paul, MN, USA, 2003; pp. 35–43.

Maiko W. 2013. Molecular phylogeny and identification of Fusarium species based on nucleotide sequences. Mycotoxins 63: 133-142.

Mazaheri A, Ershad J. 1995. Reactions of different crops to Fusarium solani fungal pathogen of broomrape. Paper presented at the 12th Iranian Plant Protection Congress, Karadj, Iran.

Mohammadi A. 2014. Biological control of Orobanche Ramosa by Fusarium solani. International Journal of Advanced Biological and Biomedical Research 2:2751-2755.

Montazeri M. 2005. Achievements in weed science (with special attention to biological control). Agricultural Research and Education Organization Press, Tehran. Iran. (In Persian).

Muller-Stover D, Kroschel J, Thomas H, Sauerborn J. 2002. Chlamydospores of Fusarium oxysporum schlecht f. sp. orthoceras (Appel & Wollenw) as inoculum for wheat flour-kaolin granules to be used for the biological control of Orobanche cumana wallr. European Journal of Plant Pathology 108: 221–228.

Nash SM, Snyder WC.1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology 52: 567–572.

Nelson PE, Dignani MC, Anaissie EJ. 1994. Taxonomy, biology and clinical aspect of Fusarium species. Clinical Microbiology Review 7:479–504.

O'Donnell K. 1998. Molecular genetic approaches for measuring microbial diversity: examples from

filamentous fungi. National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan.

O'Donnell K. 2000. Molecular phylogeny of the Necteria haematococca-Fusarium solani species complex. Mycologia 92: 919- 938.

O'Donnell K, Sutton DA, RInaldi MG, Magnon KC, Cox PA, van burik JH, Padhye A, Aniassie EJ, Francesconi A, Walsh TJ, Robinson JS. 2004. Genetic diversity of human pathogenic members of the Fusarium oxysporum complex inferred from multilocus DNA sequence data and amplified fragment lenth polymorphism analyses. Journal of Clinical Microbiology 42: 5109-5120.

Parry DW, Jenkinson P, Mcleod PL. 1995. Fusarium ear blight (scab) in small grain cereals a review. Plant Pathology 44: 207–238.

Perez-de-Luque A, Eizenberg H, Grenz JH, Sillero JC, Avila C, Sauerborn J, Rubiales D. 2010. Broomrape management in faba bean..Field Crops Research. 115: 319–328.

Poletto T, Gonzatto MC, Muniz MFB, Blume E, Poletto I, Harakava R,Bagiotto C. 2015. First report of Fusarium lacertarum causing damping-off in Casuarina equisetifolia in Brazil. Plant Disease 99: 1503.

Summerell BA, Salleh B, Leslie JF. 2003. A utilitarian approach to Fusarium identification. Plant Disease 87: 117-128

Saremi H. 2005. Fusarium: biology, ecology and taxonomy. Jihad Daneshgahi Press, University of Mashhad, Iran. (In Persian).

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21-32.

Vitale S, SantoriA, Wajnberg E, Castagnone-Sereno P, Luongo L, Belisario A. 2011. Morphological and molecular analysis of Fusarium lateritium, the cause of gray necrosis of hazelnut fruit in Italy. Phytopathology 101: 679–686.

Wang H, Xiao M, Kong F, Chen S, Dou HT, Sorrell T. 2011. Accurate and practical identification of 20 Fusarium species by Seven-Locus sequence analysis and reverse line blot hybridization, and an in vitro antifungal susceptibility study. Journal of Clinical Microbiology 49: 1890–1898.

شناسایی ریخت شناختی و فیلوژنیکی گونه های *Fusarium* مرتبط با سیستم آوندی علف هرز گل جالیز

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چکیده: گل جالیز (.Orobanche spp) از علف های هرز انگلی به عنوان یکی از عوامل مهم محدود کننده در کشـت محصـولات مختلف در بسـیاری از نقاط جهان مورد توجه می‌باشـد. با توجه به مکانیزم‌های موثر این علف هرز از جمله: تولید بذر، پراکندگی و طول عمر زیاد، کنترل آن اغلب بسـیار دشوار است. گل جالیز یک مشکل جدی در کشاورزی ایران می باشد، لذا شناسایی عوامل زنده با قابلیت کنترل این علف هرز حائز اهمیت می با شد. در این مطالعه، در طول تاب ستان سال های ۹۳ و ۹۴ از نمونه‌های بیمار این علف هرز از مزارع گوجه فرنگی در ده ا ستان کشور نمونه برداری صورت گرفت. جدایه های *Fusarium* با توجه به ویژگی های ریخت شناختی شناسایی شدند. بخشی از ژن *tef1-α* برای تکثیر و تجزیه و تحلیل فیلوژنتیک مورد بررسی قرار گرفت. بر اساس ویژگی های ریخت شناختی، چهارده گونه از جنس *Fusarium*، شامل *F. andiyazi؛ F. equiseti، F. flocciferum، F. foetens، F. hostae، F. lacertarum، F. oxysporum، F. proliferatum، F. redolens، F. sambucinum، F. solani* s. l.، *F.thapsinum، F. torulosum* و *F. verticillioides* شنـاسـایی شـدند که *F. solani* s. l. با فراوانی ۲۵٪، شـایعترین گونه در میان آنها بود. بر اساس اطلاعات ما هشت گونه شامل *F. andiyazi، F. foetans، F. flocciferum، F. hostae، F. lacertarum، F. redolens، F. thapsinum* و *F. torolosum* برای اولین بار در مقیاس جهانی روی گل جالیز و گونه *F. lacertarum* برای اولین بار برای فلور قارچی ایران گزارش می شوند.

کلمات کلیدی: *Fusarium lacertarum*، عوامل بیولوژیک، گونه جدید، ژن *tef1-α*