J. Agr. Sci. Tech. (2017) Vol. 19: 1617-1629

Morphological and Molecular Identification and PCR Amplification to Determine the Toxigenic Potential of *Fusarium graminearum* Species Complex (FGSC) Isolated from Wild Grasses in Iran

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ABSTRACT

In order to explore biodiversity of *Fusarium* species associated with the inflorescences of gramineus weeds, heads and inflorescences were collected from wild grasses in west of Iran. Sixty samples, mostly from infected spikes were collected from different sites of western Iran. Nine species from 9 tribes of the Poaceae family were identified based on pollen morphology using light microscopy and scanning electron microscope. Sixty *Fusarium* isolates were obtained from diseased spikes and identified into five species *F. graminearum* (40%), *F. asiaticum* (20%), *F. acuminatum* (20%), *F. equiseti* (10%), and *F. proliferatum* (10%). The identification of the members of *F. Graminearum* Species Complex (FGSC) was confirmed molecularly using Fg16F/Fg16R primers. *F. asiaticum* isolates were distinguished from other FGSC using Fg6CTPSf177/Fg16R primers. The phylogenetic trees based on Translation Elongation Factor-1α (TEF-1α) dataset clearly separated all morphological taxa. PCR-based detection of mycotoxin-synthesis-pathway gene was also used to determine the potential to produce trichothecenes (DON and NIV). Among 60 tested isolates, 16 isolates (27%) belonged to DON chemotype and 10 isolates (17%) were NIV chemotype. These results show that DON was the most common chemotype in western Iran. To our knowledge, this is the first report on molecular identification of *Fusarium* species isolated from poaceous wild grasses in Iran.

Keywords: *Fusarium* spp., Iran, Poaceous wild grasses, *TEF-1α*, Trichothecenes.

INTRODUCTION

Wild grasses (particularly the wild progenitors of cereals) convert solar energy into carbohydrates for their own food and for use by animals. So, wild grasses are the most important annual weeds grown in all continents of the world (Badaeva *et al*., 2002; Inch and Gilbert, 2003). Since the 1950s, grasses have been identified as the major importance in animal nutrition throughout Iran (Parsa, 1950; Yazdanseta *et al*., 2004).

Mycotoxin contamination of agricultural crops, animal feed, and wild grasses has long been a major problem in different regions, which are mostly produced by fungi species (Goswami and Kistler, 2004, 2005; Sanoubar *et al*., 2015; Kononenko *et al*., 2015). Like any other agricultural and non-agricultural plants, wild grasses are also infected by several types of diseases caused by toxigenic fungi (Inch and Gilbert, 2003; Postic *et al.*, 2012; Kononenko *et al*., 2015). Poaceous wild grasses are attacked by various pathogens such as *Fusarium* spp., *Ustilago* spp., and *Aspergillus* spp. (Inch and Gilbert, 2003; Postic *et al.*, 2012). *Fusarium* species are known to be pathogenic to many cereal crops and wild grasses (Boutigny *et al.*, 2011; Postic *et al.*, 2012). One of the most commonly and widely studied poaceous diseases in the world is Fusarium Head Blight (FHB) (Akinsanmi *et*

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[DOR: 20.1001.1.16807073.2017.19.7.16.3]

al., 2003; Boutigny *et al*., 2011; Postic *et al*., 2012). FHB is a disease in the head and kernel of small cereal grains *e.g.*, wheat, barley, rye and triticale and poaceous wild grasses (Boutigny *et al.*, 2011; Postic *et al.*, 2012; Sanoubar *et al*., 2015). The incidence of FHB has increased worldwide over the past decades (Goswami and Kistler, 2004, 2005). At least 18 *Fusarium* species have been found to cause FHB (Bottalico and Perrone, 2002). Members of *F. Graminearum* Species Complex (FGSC) which is one the most important and prevalent species, are able to produce different mycotoxins such as DON and NIV trichothecenes (Boutigny *et al*., 2011). The causal agents of FHB in Europe and Eurasia are *F. graminearum, F. culmorum, F. sporotrichioides, F. tricinctum, F. langsethiae,* and *F. avenaceum* (Waalwijk *et al*., 2003; Yli-Mattila *et al*., 2004; Stepién *et al*., 2008) while *F. asiaticum* has been found at high incidence in oriental Asia (Desjardins and Proctor, 2011; Zhang *et al*., 2012). The Fusarium mycotoxin contamination is a potential health hazard for animals consuming grassy (Desjardins and Proctor, 2011; Postic *et al*., 2012). The mycotoxin-production ability of *Fusarium* species is diverse and particular strains may produce different mycotoxins (Goswami and Kistler, 2005). So, rapid and accurate *Fusarium* spp. identification, as well as detection of their mycotoxin production ability, is therefore vital to reduce the harmful effects of the disease (Eskola *et al*., 2001). The aims of this study were to: (i) Identify the *Fusarium* spp. isolated from grassy weeds in Iran in 2012 and 2014 using morphological and molecular methods; (ii) Determine the genetic potential of *Fusarium* spp. isolates to produce nivalenol and deoxynivalenol.

MATERIALS AND METHODS

Sample Collection and Identification of Poaceus Weeds

A survey was carried out during the 2012–2014 growing seasons in five agroecological zones in western Iran including Sarpole-Zahab, Mahidasht, Eslam Abad, Bisetoon, and Kermanshah districts. In each agroecological zone, 12 farms were randomly selected, giving a total of 60 farms. In each farm infected heads and inflorescences of wild grasses were collected. All native grasses of Poaceae family were identified based on pollen morphology using Light Microscopy (LM) and Scanning Electron Microscope (SEM). To identify members of Poaceae, plants were transferred to the herbarium at Razi University. For Light Microscopy (LM) observations, pollens were acetolysed following the technique of Erdtman (1960) and mounted in glycerine jelly. Size measurements were taken based on 25 pollen grains per sample; the values of *P* (Polar axis length) and *E* (Equatorial diameter) were measured and the *P/E* ratios were calculated. Measurements were recorded using both a 40X objective, and a crossed micrometer eyepiece graticule. For SEM studies unacetolysed pollen grains were examined.

Isolation and Identification of *Fusarium* Isolates

Infected heads and inflorescences were randomly hand-collected and plated onto water-agar amended with Peptone-pentaChloronitro Benzene (PCNB) plates (Nash and Snyder 1962). The Petri dishes were incubated at 25°C for 4 days. The resulting single-spored Fusarium colonies were transferred onto Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA) plates to study the morphological characteristics. The species were identified on the basis of macroscopic and microscopic characteristics such as pigmentations and growth rates on PDA plates, size of macroconidia, presence of microconidia and chlamydospore, and the type of conidiogenous cells (monophialidic and polyplialidic conidiophores). Identification of *Fusarium* species was based on species description of Leslie and Summerell (2006).

[DOR: 20.1001.1.16807073.2017.19.7.16.3]

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification and DNA Sequencing Alignment

Potato Dextrose Broth (PDB, Sigma) medium was used to grow the *Fusarium* strains to produce mycelium for DNA extraction. Selected strains of all species were grown on PDB with shaking at 150 rpm at 25±2°C for 5 days. Mycelia were harvested by filtration through Whatman paper 1 and freeze-dried for 20 hours and DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's protocol. Amplification of the Translation Elongation Factor-1*α* (TEF-1α) gene was conducted using the primer pairs of ef1 and ef2 (O'Donnell *et al*., 1998). Amplification reactions were performed in a total volume of 50 µl, by mixing 0.4 µl of template DNA with 16.35 µl ddH_2O, 1 µl of deoxyNucleotide TriPhosphate (dNTP) (Promega); 8 µl of each primer; 0.25 µl of *Taq* DNA polymerase (Promega); 8 µl of $MgCl_2$ (Promega) and 8 µl of PCR 5X reaction buffer (Promega, Madison, Wl, USA) (Chehri *et al*., 2014). The PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) according to the manufacturers' instructions. The purified PCR products were sent to Centre for Chemical Biology Laboratories in Malaysia. The sequences of *TEF-1α* gene received were aligned and edited using BioEdit version 7.0.5. The edited alignments were used as query to search for similarities using BLAST network services at the FUSARIUM-ID database (http://fusarium,cbio.psu.edu). Maximum-parsimony analyses were performed on the aligned DNA sequences of the individual and combined datasets using MEGA4.0 version (Tamura *et al*., 2007).

Molecular Identification of *F. graminearum* Species Complex Using Species-Specific PCR

Studied isolates belonging to *F. graminearum* species complex were identified with species-specific PCR assay using previously published primer pairs for FGSC and *F. asiaticum* (Table 1). Amplification reactions were performed in a total volume of 25 µl, by mixing 1 µl of template DNA with 17.8 µl ddH_2O, 1 µl of deoxyNucleotide TriPhosphate (dNTP) (Promega); 1 µl of each primer; 0.2 µl of *Taq* DNA polymerase (Promega); 0.5 µl of $MgCl_2$ (Promega) and 2.5 µl of PCR 5X reaction buffer (Promega, Madison, Wl, USA). PCR amplification was carried out in the Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) with the following programs: An initial denaturation step at 94°C for 5 minutes, 35 cycles of 94 (1 minute)/56 (1 minute)/72°C (3 minutes), and a final extension step at 72°C for 10 minutes. The PCR products were visualized by 1X TBE electrophoresis in ethidium-bromide-stained, 1% agarose gel.

Table 1. Species-specific primers used for the identification of *F. graminearum* species complex and *F. asiaticum* isolated from wild grasses in Iran.

Species	Primer name	Sequence 5′-3′	Product size (bp)	Source
F. graminearum	Fg16F	*CTCCGGATATGTTGCGTCAA*	400-500	Nicholson *et al.* (1998)
	Fg16R	*GGTAGGTATCCGACATGGCAA*		
F. asiaticum	Fg6CTPSf177	*GTCTCACTTCAAGCCA*	162	Yang *et al.*(2008)
	FgCTPSrR306	*CCTTGGTCATCCATAGAG*		

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Molecular Analyses of the Toxigenic Potential of *Fusarium* spp. Isolates

The potential of *Fusarium* spp. isolates to produce trichothecenes was determined by the PCR-based molecular analyses using the Tri13F and Tri13DONR, and Tri13NIVF and Tri13R specific primers pairs (Table 2) which target the mycotoxin-synthesis pathway genes were Tri13DON (DeOxyNivalenol), and Tri13NIV (Nivalenol) respectively. A total volume of 25 µl amplification reactions for each isolate was prepared containing the mixture of 4 µl 10X buffer (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 0.2 mM deoxyNucleotide TriPhosphate (dNTP) (Promega), 0.4 µM each primer, 0.75 units of Taq DNA polymerase (Promega), and 50 ng of template DNA. PCR amplification was carried out in the Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) according to temperature profiles described by Lenc *et al.* (2008) and Lenart *et al.* (2013). The PCR products were visualized by 1X TBE electrophoresis in ethidium-bromide-stained, 1% agarose gel.

RESULTS

Sixty samples, mostly from infected spikes were collected from different sites of western Iran. Nine species from 9 tribes of Poaceae family were identified based on pollen morphology using light microscopy and scanning electron microscope (Table 4). A total of 60 *Fusarium* isolates were recovered from diseased inflorescences of wild grasses. All isolates were identified into five species i.e. *F. graminearum, F. asiaticum, F. acuminatum, F. proliferatum,* and *F. equiseti* (Table 3). Members of FGSC were the most prevalent species in all samples. Based on morphological characters, 36 isolates belonged to FGSC, and 18 isolates were classified into two known species among *F. Incarnatum-F. Equiseti* Species Complexes (FIESC), namely *F. acuminatum* (12) and *F. equiseti* (6), and 6 isolates were *F. proliferatum* in the *Gibberella Fujikuroi* Species Complex (GFSC). Macroscopic and microscopic characteristics including means and ranges of spore dimensions of individual isolates of FSSC are summarized in Table 3.

Members of FGSC were also distinguished molecularly using Fg16F/Fg16R primers, and *F. asiaticum* isolates were identified using specific primers Fg6CTPSf177/FgCTPSrR306. The primers Fg16F/Fg16R produced fragments of 450 bp in 36 isolates and hence they were identified as FGSC along with morphological features (Figure 1) and the specific primers Fg6CTPSf177/FgCTPSrR306 produced fragments of 162 bp only in 12 isolates, which belonged to *F. asiaticum*. Therefore, molecular studies were useful in differentiating closely related species especially among members of FGSC (Figure 2).

Two PCR assays (nivalenol, deoxynivalenol) were used for the identification of the chemotypes of all *Fusarium* isolates. The expected product size for DON and NIV (282 and 312 bp)

Table 2. Primers used for detection of the *Fusarium* spp. potential to produce trichothecenes.

Primer name	Sequence 5′-3′	Product size (bp)	Target sequence	Source
Tri13F	*CATCATGAGACTTGTKCRAGTTTGGG*	282	Tri13DON gene	Chandler *et al.* (2003)
Tri13DONR	*GCTAGATCGATTGTTGCATTGAG*			
Tri13NIVF	*CCAAATCCGAAAACCGCA*	312	Tri13NIV gene	Chandler *et al.* (2003)
Tri13R	*TTGAAAGCTCCAATGTCGTG*			

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Table 3. GenBank accession numbers, cultural and morphological characteristics of strains of *Fusarium* spp. associated with inflorescences of wild grasses in Iran.

Culture no.	Name of species	Chlamydospores	Pigmentation on PDA	Microconidia		Types of conidigenious cells		General morphology		Length×width of macroconidia (µm) [a]	*tef1* [b]
				Number of septa		Poly [c]	Mono [d]	Apical cell	Basal cell		
FGSCHaz34weed	*F. graminearum*	+	Red	5-6	- [e]	-	+[f]	Tapered	fs [g]	34-58 × 4.0-6.5	KU664648
FGSCHaz134weed	*F. graminearum*	+	Red	5-6	-	-	+	Tapered	fs	35-60 × 4.0-6.5	KU664649
FGSCHaz33weed	*F. asiaticum*	+	Red	5-6	-	-	+	Tapered	fs	33-53 × 4.0-6.3	KU664646
FGSCHaz033weed	*F. asiaticum*	+	Red	5-6	-	-	+	Tapered	fs	34-55 × 4.0-6.3	KU664647
FIESCK261weed	*F. equiseti*	+	Brown	5-7	-	-	+	Tapered , elongate	fs	45-80 × 3.5-5.6	KU664650
FIESCK0261weed	*F. equiseti*	+	Brown	5-7	-	-	+	Tapered , elongate	fs	48-81 × 3.8-5.6	KU664651
FIESCK266weed	*F. acuminatum*	+	Red	3-5	-	-	+	Tapered and elongate	fs	42-70 × 3.9-6.0	KU664652
FIESCK272weed	*F. acuminatum*	+	Red	3-5	-	-	+	Tapered and elongate	fs	48-72 × 4.1-6.0	KU664653
FFSCK281weed	*F. proliferatum*	-	Violet	3-5	+	+	+	Curved	pdfs	28-51 × 3.0-5.0	KU664654
FFSCK285weed	*F. proliferatum*	-	violet	3-5	+	+	+	curved	pdfs	32-55 × 3.0-5.0	KU664655

[a] Mean values of 50 random conidia±standard deviation. [b] GenBank numbers for translation elongation factor 1-alpha (*tef1*) gene partial sequences; [c] Polyphialidic; [d] Monophialidic; [e] Absence; [f] Presence; [g] Foot shape. [h] Poorly developed foot shape.

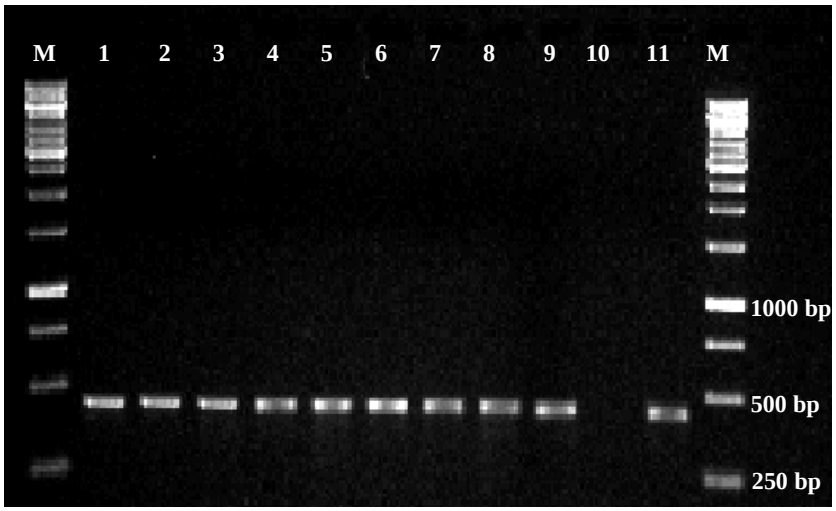

Figure 1. PCR products obtained with specific primer pairs Fg16F / Fg16R (band, 450 bp) from 10 isolates of *F. graminearum*. (Lane M) GeneRuler 1 kb DNA Ladder; (1)= FGSC Haz33 weed; (2) FGSC Haz34 weed; (3) FGSC Haz134 weed; (4) FGSC Haz033 weed; (5) FGSC Haz35 weed; (6)= FGSC Haz36 weed; (7)= FGSC Haz37 weed; (8)= FGSC Haz38 weed; (9) FGSC Haz39weed; (10) *F. solani*, and (11) FGSC Haz40 weed isolates in wells (1-11) amplified with primers Fg16F and Fg16R.

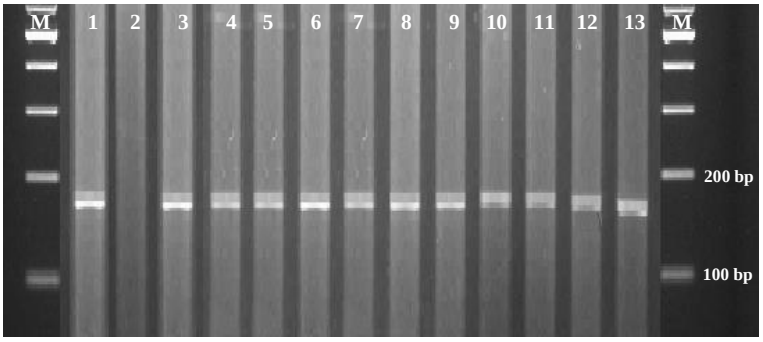

Figure 2. PCR products obtained with specific primer pairs Fg6CTPSf177/FgCTPSrR306 (band, 162 bp) from 10 isolates of *F. asiaticum*. Lane M: GeneRuler DNA Ladder Mix, 100–10,000 bp Ladder; (1) FGSC Haz33 weed, 2. *F. graminearum* (FGSC Haz34weed), FGSC Haz033 weed; (4) FGSC Haz25 weed; (5) FGSC Haz025 weed; (6)= FGSC Haz35 weed; (7) FGSC Haz36 weed; (8) FGSC Haz27 weed; (9) FGSC Haz28weed; (10) FGSC Haz29 weed; (11) Haz30 weed; (12) FGSC Haz40 weed, and (13) FGSC Haz41 weed amplified with primers Fg6CTPSf177 and FgCTPSrR306 and one *F. graminearum* (FGSC Haz34 weed) (well 2).

were amplified in PCR reaction (Figures 3 and 4). From among 60 tested isolates, detection of the deoxynivalenol, gave positive results for 16 *Fusarium* isolates that belonged to *F. graminearum* (12) and *F. asiaticum* (4) (Figure 3). Also the detection of potential nivalenol producers was positive for 10 *Fusarium* isolates which belonged to *F. asiaticum* (Figure 4). The highest proportion of DON producing isolates was observed in Sarpole-Zahab site (10%) followed by Kamyaran (5%). while NIV producing isolates were dominant in Ravansar (5%) followed by Sahneh (3%). Frequencies and percentage of potential mycotoxin producing strains are presented in Table 4.

Ten strains were selected for DNA sequence analysis using the *TEF-1α* gene. A single band of DNA fragments 700-bp was

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Table 4. Place of sample collection, frequencies and percentage of potentially toxigenic (DON and NIV) isolates isolated from inflorescences of wild grasses in Iran.

Place of sample collection	Isolation sources	No of spike samples infected with *Fusarium* spp. (Percentage)	*Fusarium* spp. Identified [a]	No. of potentially toxigenic (DON) isolates (Percentage)	DON producer strain	No. of potentially toxigenic (NIV) isolates (Percentage)	NIV producer strain
Sarpol Zohab	*Avena wiestii, Bromus sericeus, Dactylis glumerata*	6 (100%)	*F. gr, F. as, F. pr*	6 (10%)	*F. gr* (5), *F. as* (1)	-	-
Kamyaran	*Lolium perenne, Melica Jacquemontii, Aegilops cylindrical*	6 (100%)	*F. gr, F. as, F. pr, F. ac*	3 (5%)	*F. gr* (2), *F. as* (1)	-	-
Ravansar	*Aegilops cylindrical, Stipa barbata, Agropyron repens*	6 (100%)	*F. gr, F. as, F. pr, F. ac*	-	-	3 (5%)	*F. as* (3)
Sahneh	*Avena wiestii, Bromus sericeus, Dactylis glumerata*	6 (100%)	*F. gr, F. as, F. pr, F. ac*	-	-	2 (3/3%)	*F. as* (2)
Gilan Gharb	*Avena wiestii, Bromus sericeus*	6 (100%)	*F. gr, F. as, F. ac*	1 (1/6%)	*F. gr* (1)	1 (1/6%)	*F. as* (1)
Kermanshah	*Lolium perenne, Melica Jacquemontii, Stipa barbata*	6 (100%)	*F.gr, F. eq, F. as, F. ac*	2 (3/3%)	*F. gr* (1)	1 (1/6%)	*F. as* (1)
Bisotun	*Dactylis glumerata Melica Jacquemontii, Agropyron repens*	6 (100%)	*F. gr, F. as, F. pr, F. ac*	2 (3/3%)	*F. gr* (1), *F. as* (1)	1 (1/6%)	*F. as* (1)
Eilam	*Ermopoa persica, Aegilops cylindrical, Stipa barbata*	6 (100%)	*F.gr, F. as, F .ac*	1 (1/6%)	*F. gr* (1), *F. as* (1)	1 (1/6%)	*F. as* (1)
Gorveh	*Ermopoa persica, Lolium perenne*	6 (100%)	*F.eq, F. as, F. ac*	-	-	1 (1/6%)	*F. as* (1)
Asad Abad	*Avena wiestii, Bromus sericeus*	6 (100%)	*F. gr, F. ac*	1 (1/6%)	*F. gr* (1)	-	-

[a] *F. eq=F. equiseti; F. gr=F. graminearum; F. as=F. asiaticum; F. ac=F. acuminatum. F. pr=F. proliferatum.*

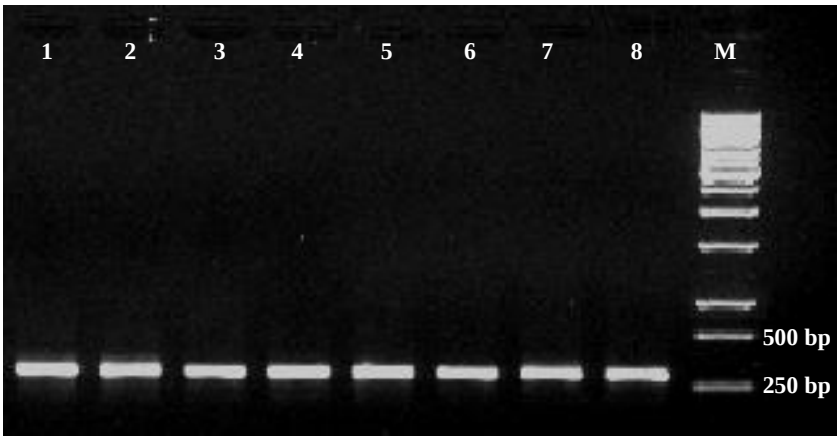

Figure 3. PCR detection of DON production potential indicated by the presence of Tri13DON marker (Tri13F/Tri13DONR). (Lane M) GeneRuler 1 kb DNA Ladder; (Lanes 1-4) *F. asiaticum* (1= FGSC Haz33 weed; 2= FGSC Haz033 weed; 3= FGSC Haz25 weed, 4= FGSC Haz025 weed), and (Lanes 5-8) *F. graminearum* (5= FGSC Haz34 weed; 6= FGSC Haz134 weed; FGSC Haz37 weed, 8= FGSC Haz38 weed) positively identified presence of Tri13- DON marker.

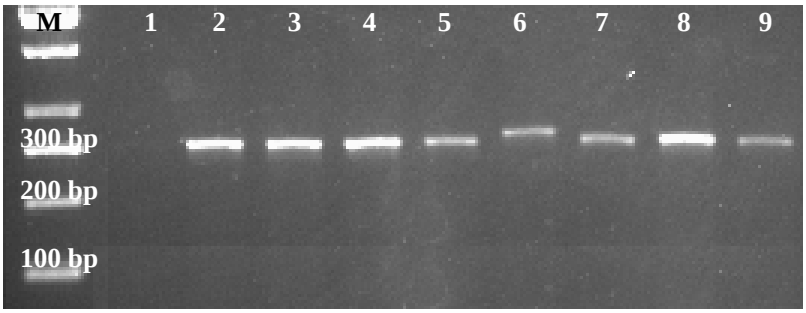

Figure 4. PCR detection of Tri13NIV production potential indicated by the presence of Tri13NIV marker (Tri13NIVF/Tri13R). Lane M: GeneRuler DNA Ladder Mix, 100–10,000 bp Ladder; (Lanes 1) *F. graminearum* (FGSC Haz34 weed); (Lanes 2-9) *F. asiaticum* (2= FGSC Haz033 weed; 3= FGSC Haz25 weed; 4= FGSC Haz025 weed; 5= FGSC Haz35 weed; 6= FGSC Haz36 weed; 7= FGSC Haz27 weed; 8= FGSC Haz28 weed, 9= FGSC Haz29 weed) positively identified presence of Tri13NIV marker.

amplified for the *TEF-1α* gene from all *Fusarium* spp. isolates. Obtained sequences were aligned and edited using BioEdit version 7.0.5 and compared with FUSARIUM-ID database. From similarities searched at FUSARIUM-ID database, identification of all *Fusarium* spp. was confirmed with statistical significance. GenBank accession numbers used in this study are shown in Table 3. Also, this was confirmed by a phylogenetic analysis of the combined dataset of *TEF-1α* gene data (Figure 5). The phylogenetic tree generated from the combined dataset of *TEF-1α* gene revealed a monophyly among 2 isolates (FFSC K281 and FFSC K285 weeds) in this study, and *F. proliferatum* (NRRL 31071) obtained from GenBank (87% MP). The tree also showed 2 isolates (FIESC K266 and FIESC K272 weeds) with 99% bootstrap support being placed in distinct lineage of *F. acuminatum*. The tree showed a well supported relationship (99% MP bootstrap) between *F. equiseti* (NRRL 46916 and NRRL 46628) obtained from GenBank and 2 isolates in this study based on morphological features were identified as *F. equiseti* (FIESC K0261 and FIESC K261 weeds). Phylogenetic tree showed a monophyly between *F. graminearum* (CBS

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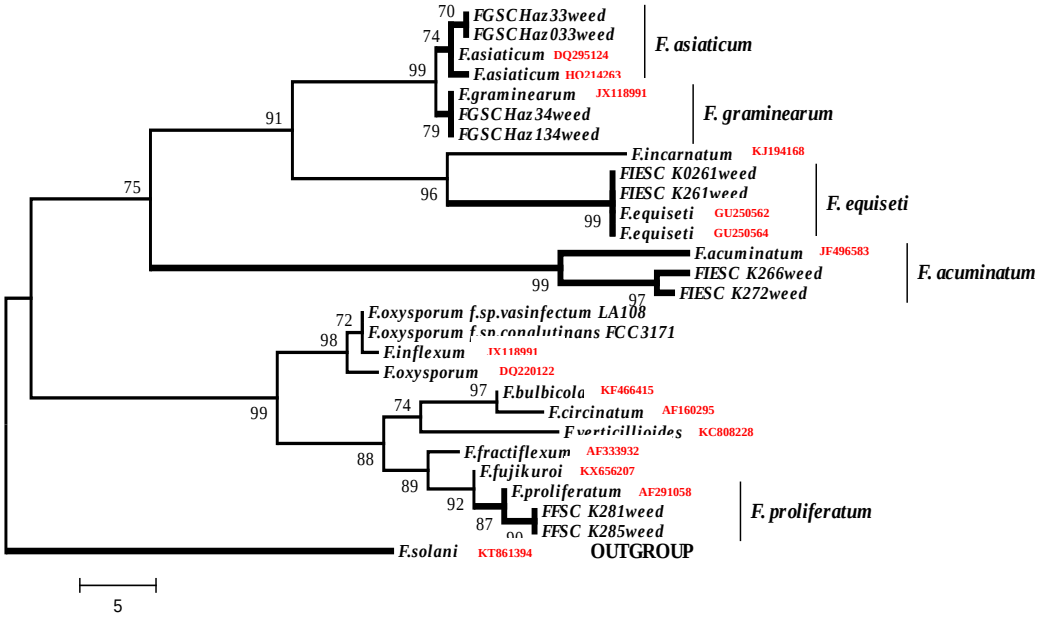

Figure 5. A maximum parsimony phylogeny for 28 taxa of the *Fusarium* spp. inferred from combined *tef1* gene sequence. Bootstrap tests were performed with 1,000 replications. *Fusarium solani* (NRRL 22586) obtained from GenBank was treated as the outgroup.

131776) and isolates FGSC Haz34 and FGSC Haz134 weeds (79% MP), and based on morphological features in which all strains were identified as *F. graminearum*. Also, the tree demonstrated that isolates FGSC Haz33 and FGSC Haz033 weeds, based on morphological characters which were identified as FGSC, are monophyletic with *F. asiaticum* (FO 442 and Nep 350) (74% MP).

DISCUSSION

Fusarium head blight is a destructive disease on poaceous plants caused by different complexes of *Fusarium* species (Goswami and Kistler, 2004). The most prevalent species involved in Fusarium head blight are members of *F. graminearum*, *F. incarnatum*- *F. equiseti*, *F. fujikuroi*, and *F. avenaceum* species complexes (Yli-Mattila *et al*., 2002; Goswami and Kistler, 2004, 2005). Although *F. avenaceum* species complex is considered less pathogenic than other species complexes, that infect cereals and grasses (Turner *et al*., 1998). In this study the *Fusarium* spp. were isolated and identified from grassy weeds in Iran during the 2012–2014 growing seasons using morphological and molecular methods and *F. graminearum* (40%) was the dominant *Fusarium* species in inflorescences of gramineus weeds which was in agreement with previous studies (Boutigny *et al*., 2011; Postic *et al*., 2012; Yli-Mattila *et al*., 2015). The high frequency of these species in the current study provides further evidence of their potential importance in *Fusarium* diseases of gramineus weeds as the most important animal feeds in Iran. Wild grasses may also act as the source of *Fusarium* species for infection of cultivated species (Boutigny *et al*., 2011; Postic *et al*., 2012; Skladanka *et al*., 2013). The widespread nature of FGSC in all grassland sites in western Iran is further evidence of its ability to interactions with plant hosts and mycotoxin production.

The occurrence of mycotoxins produced by members of the *F. graminearum*, *F. fujikuroi*, and *F. avenaceum* species

[DOR: 20.1001.1.16807073.2017.19.7.16.3]

complexes in family *Gramineae* is of great concern worldwide, because their presence in processed feeds seems unavoidable (IARC, 1993; Bottalico and Perrone, 2002; CAST, 2003; Boutigny *et al*., 2011; Postic *et al*., 2012; Skladanka *et al*., 2013; Duan *et al*., 2016). Lenart *et al.* (2013) applied the positive-negative PCR assay based on the *Tri13* gene to identify the genetic potential of DON production of *Fusarium* strains isolated from maize ears in southern Poland and indicated 11 *Fusarium* strains were found to be potential DON producing strains. The presence of DON and NIV genes was found in 24 strains of the species *F. graminearum*, which can produce significant amounts of deoxynivalenol and nivalenol in wheat ears in Poland (Wolny-Koładka et *al*., 2015). In this survey, as the potent producers of mycotoxin, *F. graminearum* and *F. asiaticum* comprised the highest frequencies in gramineus weeds and our results are in agreement with previous studies in the world (Tóth *et al*., 2004; Jurado *et al*., 2005; Pasquali *et al*., 2010). From 60 tested isolates, 16 isolates (27%) being DON chemotype and 10 isolates (17%) were NIV chemotype. These results show that DON was the most common chemotype in western Iran. Haratian *et al*. (2008) investigated the genetic potential of DON and NIV production of *F. graminearum* strains isolated from grain cereals in Iran and demonstrated 11 *Fusarium* strains were found to be potential DON and NIV producing strains. They revealed that both DON and NIV *Fusarium* spp. chemotypes existed in Iran, but NIV producers were more frequently reported. The results obtained in this study confirmed and completed the previous data about genetic potential of DON and NIV production of *F. graminearum* strains in Iran and revealed in addition of grain cereals, wild grasses can also be one of the great concerns in Iran.

Several molecular systematic studies have successfully utilised *TEF-1α* sequence variation to investigate phylogenetic relationships of *Fusarium* spp. (O'Donnell *et al.*, 2004). Our study demonstrated that *TEF-1α* sequence was an excellent phylogenetic marker for resolution of *Fusarium* species. It is very difficult to distinguish *F. graminearum* and *F. asiaticum* by their morphological characteristics. The close relationship between these species is supported by the *TEF-1α* sequence (O'Donnell *et al*., 2004). Based on our result, we can conclude that FGSC are the causal agents for mycotoxin contamination in poaceous wild grasses in Iran and application of molecular biology techniques provides new information on the DON and NIV producers in Iran.

ACKNOWLEDGEMENTS

Khosrow Chehri acknowledges Razi University, Kermanshah, Iran for providing necessary facilities to carry out this research.

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[DOR: 20.1001.1.16807073.2017.19.7.16.3]

شناسایی ریخت شناسی و مولکولی و بررسی پتانسیل توکسین زایی جمعیت‌های گونه مرکب *Fusarium graminearum* جدا شده از غلات وحشی در ایران مبتنی بر PCR

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چکیده

به منظور بررسی تنوع زیستی گونه های فوزاریوم مرتبط با گندمیان وحشی، ۶۰ نمونه سنبله و گل آذین متعلق به گندمیان وحشی بیمار از غرب ایران جمع آوری شد. بر اساس ریخت شناسی دانه گرده با استفاده از میکروسکوپ نوری و میکروسکوپ الکترونی، نه جنس متعلق به تیره گندمیان شناسایی شد. ۶۰ جدایه فوزاریوم از سنبله های بیمار جداسازی شد که همه جدایه ها متعلق به ۵ گونه شامل *F. graminearum*(۴۰٪)، *F. asiaticum* (۲۰٪)، *F. acuminatum* (۲۰٪)، *F. proliferatum*(۱۰٪) و *F. equiseti* (۱۰٪) بودند. شناسایی جمعیت‌های گونه مرکب *F. graminearum* با استفاده از پرایمرهای Fg16F/Fg16Rتایید شد. جدایه های*F. asiaticum* با استفاده از پرایمرهای Fg6CTPSf177/Fg16Rاز سایر گونه های موجود در گونه مرکب *F. graminearum* تشخیص داده شد. درخت فیلوژنی بر اساس تعیین توالی قسمتی از ژن Translation Elongation Factor- 1α (TEF- 1α)، گونه هایی که بر اساس مطالعات ریخت شناسی شناسایی شدند را در گروه های متفاوت قرار داد. پتانسیل توکسین زایی جدایه های تولید کننده تریکوتسین (DON و NIV) با استفاده از روش مبتنی بر PCR ژن های سنتز کننده مایکوتوکسین بررسی شد. از بین ۶۰ جدایه، ۱۶ جدایه (۲۷٪) تولید کننده DONو ۱۰ جدایه (۱۷٪) تولید کننده NIVبودند. همچنین نتایج این تحقیق نشان داد که DON شایع ترین کموتیپ در غرب ایران بود. بر اساس تحقیقات ما این اولین گزارش از شناسایی گونه های فوزاریوم مرتبط با گندمیان وحشی در ایران است.

[DOR: 20.1001.1.16807073.2017.19.7.16.3]