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GENETIC DIVERSITY AMONG *FUSARIUM GRAMINEARUM* AND *F. CULMORUM* ISOLATES BASED ON ISSR MARKERS

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Abstract: To characterize the isolates of *F. graminearum* and *F. culmorum* fungi from Turkey and Iran, we performed ISSR analysis with 18 non-anchored and 23 anchored (including ten novel) primers. Amplification product sizes were 0.2-3.5 kb. In total, 405 bands were scored, 24 of which (5.92%) were polymorphic. The similarities among *F. graminearum* isolates were calculated as 62.3-99%, and among *F. culmorum* as 65.7-94.3%. Moderate genetic variation at intra- and inter-specific levels was determined, and the average intraspecific genetic diversity values were 80.65% for *F. graminearum*, and 80% for *F. culmorum*. Cluster analysis separated the isolates into two main clades. Group I consisted of *F. culmorum* isolates from Turkey that produced DON mycotoxin. Group II contained all *F. graminearum* isolates that were deoxynivalenol (DON) and nivalenol (NIV) chemotypes from Turkey and Iran. Both groups I and II were divided into two subgroups including their divisions. Phenons in group II included isolates distributed in the same geographic region. ISSR markers clustered isolates within a definite order according to their species. Isolates from the same agro-ecological locations were also kept together in subdivisions. The novel ISSR markers developed for the first time in this study contribute to differentiating between *Fusarium* isolates according to their species and geographic regions.

Key words: *Fusarium graminearum*; *Fusarium culmorum*; chemotype; inter simple sequence repeats (ISSRs); mating type

INTRODUCTION

Fusarium genus has more than ten phytopathogenic species, including *F. graminearum* and *F. culmorum*, and infect several plant species. *Fusarium graminearum* and *F. culmorum* cause diseases such as Fusarium head blight (FHB) and root rot in cereals, resulting in severe yield loses, reduction in crop quality and quantity [1,2]. *Fusarium graminearum* is a predominating species all over the world [3-5]. According to molecular studies, the pathogen is a species complex consisting of at least nine phylogenetically distinct and biogeographically structured species [6-10]. The predominant *F. graminearum* complex in Turkey, Europe and temperate regions of Asia is *F. graminearum sensu stricto* (lineage 7) [11]. However, *F. graminearum sensu lato* (lineage 7) has been reported as the dominant FHB species in northern and northwestern provinces of Iran [12]. Outbreaks caused by *F. culmorum* have increased worldwide [3,4]. Contamination of plants with these two species results in the accumulation of mycotoxins, including deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA), which threaten human health [13, 14].

Fusarium species possess high levels of phenotypic and genotypic diversity [15-20]. Information about the genetic structure of *F. graminearum* and *F. culmorum* populations, obtained from different agro-ecological regions, is still limited [21]. However, a high level of genetic variation in these phytopathogenic species has been reported by different researchers [22-24]. Miedaner et al. [21,22] suggest that the high level of variation in *F. culmorum* is at the parasexual stage. The

mating types of these species were identified by the amplification of specific genes (*MAT-1* and *MAT*-2). Besides, transcription of both of the genes was investigated in *F. culmorum* [25], but detailed information is still lacking. Furthermore, sexual reproduction is not reported in *F. culmorum* as yet [26]. Therefore, reliable, reproducible and fast approaches are needed to determine the genetic structure and diversity of these species.

In Turkey, more than ten different *Fusarium* species were reported to cause FHB and root rot on cereals including wheat, barley and maize, and *F. graminearum* and *F. culmorum* were the most commonly isolated *Fusarium* species [27], whereas *F. graminearum* species caused FHB in Iran [28]. Studies of *F. graminearum* and *F. culmorum* isolates from Turkey and Iran, including chemotype and genotype analyses, have increased [29-31].

Molecular marker techniques based on polymerase chain reaction (PCR) are currently used in determining the genetic variability of *Fusarium* species. Some of these techniques, such as universally primed PCR (UP-PCR) [32], random amplified polymorphic DNA (RAPD) [33], microsatellite PCR [34], restriction fragment length polymorphism (RFLP) [35] and amplified fragment length polymorphism (AFLP) [36], detect genetic variation at the genomic level. In addition, the internal transcribed (ITS) region [37], restriction polymorphism of the intergenic spacer (IGS) region [38], rRNA coding regions of nuclear DNA [39] and mitochondrial DNA [40] were also analyzed in the genotyping of *Fusarium* species.

Determination of inter simple sequence repeats (ISSR) is widely used in genotyping *Fusarium* species as well as other fungal genera [17,18,41,42]. ISSR analysis is a PCR-based technique. ISSR primers are generated from simple sequence repeat (SSR) loci. They can be anchored, consisting of repeated motifs fallowed by one or a few additional nucleotides, or non-anchored composed of repeated nucleotides. ISSR primers produce dominant molecular markers. ISSR fingerprinting shows higher levels of polymorphisms than some of the other PCR-based techniques. The method does not require previous knowledge of the nucleotide sequences in a target region. Since ISSR fingerprinting is reliable and reproducible, it is preferred over other dominant molecular marker techniques [32,43,44].

Knowledge from the genetic characterization of these phytopathogenic species provides valuable resources for the development of disease control strategies. Therefore, in this study, our focus was on genetic characterization, and intra- and interspecific genetic variation among *F. graminearum* and *F. culmorum* isolates from different hosts in different agro-ecological locations in Turkey and Iran. The determination of genetic diversity among *Fusarium* isolates based on ISSR analysis will contribute to integrated pest management (IPM) programs against head blight disease and may also help to develop new control strategies of the disease.

MATERIALS AND METHODS

Fungal isolates and DNA extraction

Twenty *F. culmorum* and 43 *F. graminearum* single-spore isolates from scabby kernels were kindly provided by Prof. Dr. Berna Tunali, Department of Plant Protection, Agricultural Faculty, Samsun Ondokuz Mayis University and Prof. Dr. Bahram Sharifnabi, Department of Plant Protection, College of Agriculture, Isfahan University of Technology. Collection places, hosts and codes of fungal isolates used in this study are listed in Table 1. *Fusarium* isolates were grown on potato dextrose agar (PDA) plates for six days at 25°C. Genomic DNA was isolated from 50 mg of fresh mycelia grown on PDA using a genomic DNA isolation kit (Macherey-Nagel, Germany), which was developed on the basis of the established cetyltrimethylammonium bromide (CTAB) method.

Sequence characterized amplified region (SCAR) assay

Fungal isolates that were identified by morphological characteristics were confirmed at the species level using SCAR markers developed by Nicholson et al.

Table 1. *Fusarium* spp. isolated from different hosts in different agro-ecological locations, their chemotypes and mating types.

Code	Species	Host	Location	Chemotype		Mating type	
				DON	NIV	MAT-1	MAT2
F1	*F. culmorum*	Wheat	Marmara/Turkey	+	-	+	-
F2	*F. culmorum*	Wheat	Marmara/Turkey	+	-	-	+
F3	*F. culmorum*	Wheat	Konya/Turkey	+	-	-	+
F4	*F. culmorum*	Wheat	Marmara/Turkey	+	-	-	+
F5	*F. graminearum*	Wheat	Sakarya/Turkey	+	-	+	+
F6	*F. graminearum*	Wheat	Sakarya/Turkey	+	-	+	+
F7	*F. graminearum*	Wheat	Sakarya/Turkey	+	-	+	+
F8	*F. graminearum*	Wheat	Sakarya/Turkey	+	-	+	+
F9	*F. graminearum*	Wheat	Balikesir/Turkey	+	-	+	+
F10	*F. culmorum*	Wheat	Bilecik/Turkey	+	-	+	-
F12	*F. culmorum*	Wheat	Balikesir/Turkey	+	-	-	+
F14	*F. culmorum*	Wheat	Bilecik/Turkey	+	-	-	+
F15	*F. culmorum*	Wheat	Sinop/Turkey	+	-	+	-
F16	*F. culmorum*	Wheat	Konya/Turkey	+	-	+	+
F17	*F. culmorum*	Wheat	Konya/Turkey	+	-	+	-
F19	*F. culmorum*	Wheat	Konya/Turkey	+	-	+	-
F20	*F. culmorum*	Wheat	Bilecik/Turkey	+	-	-	+
F21	*F. culmorum*	Wheat	Usak/Turkey	+	-	+	-
F24	*F. culmorum*	Wheat	Konya/Turkey	+	-	-	+
1F	*F. graminearum*	Wheat	Bolu/Turkey	-	+	+	+
2F	*F. graminearum*	Wheat	Cankırı/Turkey	+	-	+	+
3F	*F. graminearum*	Maize	Samsun/Turkey	+	-	+	+
4F	*F. graminearum*	Barley	Bolu/Turkey	+	-	+	+
5F	*F. graminearum*	Maize	Samsun/Turkey	+	-	+	+
6F	*F. graminearum*	Maize	Samsun/Turkey	+	-	+	+
7F	*F. graminearum*	Maize	Samsun/Turkey	+	-	+	+
8F	*F. culmorum*	Wheat	Ankara/Turkey	+	-	-	+
9F	*F. culmorum*	Wheat	Isparta/Turkey	+	-	+	+
10F	*F. culmorum*	Wheat	Samsun/Turkey	+	-	-	+
11F	*F. culmorum*	Wheat	Corum/Turkey	+	-	-	+
12F	*F. culmorum*	Wheat	Amasya/Turkey	+	-	-	+
13F	*F. culmorum*	Wheat	Konya/Turkey	+	-	+	+
M10	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T7	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T11	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T16	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T2	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T10	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T9	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
56	*F. graminearum*	Wheat	Gorgan/Iran	-	+	+	+
T12	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
M6	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
M1	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
165	*F. graminearum*	Wheat	Kordkooy/Iran	-	+	+	+
170	*F. graminearum*	Wheat	Gorgan/Iran	-	+	+	+

Table 1. continued:

Code	Species	Host	Location	Chemotype		Mating type	
				DON	NIV	MAT-1	MAT2
18	*F. graminearum*	Wheat	Moghon/Iran	-	+	+	+
sh4	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
sh14	*F. graminearum*	Wheat	Unknown/Iran	+	-	+	+
sh15	*F. graminearum*	Wheat	Unknown/Iran	+	-	+	+
sh7	*F. graminearum*	Wheat	Mazandaran/Iran	+	-	+	+
sh1	*F. graminearum*	Wheat	Mazandaran/Iran	+	-	+	+
sh13	*F. graminearum*	Wheat	Unknown/Iran	-	+	+	+
M5	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
sh5	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
F49	*F. graminearum*	Wheat	Moghon/Iran	+	-	+	+
Fg4	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
T3	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
M3	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
M9	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
174	*F. graminearum*	Wheat	Gorgan/Iran	-	+	+	+
Fg5	*F. graminearum*	Wheat	Sari/Iran	-	+	+	+
sh10	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+
M7	*F. graminearum*	Wheat	Mazandaran/Iran	-	+	+	+

Table 2. ISSR primers used in this study

Primer	Sequence	Results	P/M	Primer	Sequence	Results	P/M
C3	$(AT)_8C$	-	0/0	UBC872	$(GATA)_4$	+	2/29
C4	$(GT)_8C$	-	0/0	UBC873	$(GACA)_4$	+	2/7
UBC813	$(CT)_8T$	±	0/0	UBC874	$(CCCT)_4$	+	1/10
UBC825	$(AC)_8T$	+	3/26	UBC877	$(TGCA)_4$	-	0/0
UBC828	$(TG)_8A$	+	4/19	UBC878	$(GGAT)_4$	+	0/11
UBC831	$(AT)_8YA$	-	0/0	B5	$(GATA)_5$	-	0/0
UBC835	$(AG)_8YC$	±	0/0	B6	$(GACA)_5$	-	0/0
UBC845	$(CT)_8RG$	-	0/0	B7	$(CTAG)_4$	-	0/0
UBC850	$(GT)_8YC$	+	1/29	AYS1	$(TAAT)_4AA$	-	0/0
UBC855	$(AC)_8YT$	+	3/7	AYS2	$(ATTA)_4CT$	-	0/0
UBC859	$(TG)_8RC$	+	0/13	AYS3	$(ACCA)_4GC$	+	2/24
C6	$(TC)_8RG$	-	0/0	AYS4	$(GTTG)_4TA$	+	1/9
UBC862	$(AGC)_6$	+	0/9	AYS5	$(GTCT)_3GT$	+	0/5
UBC864	$(ATG)_6$	+	0/18	AYS6	$(GAGG)_3GG$	+	0/11
UBC865	$(CCG)_6$	+	0/11	AYS7	$(AGGG)_3AG$	±	0/0
UBC866	$(CTC)_6$	-	0/0	AYS8	$(GTTC)_3GT$	+	0/13
UBC867	$(GGC)_6$	+	0/14	AYS9	$(CCTG)_3CC$	+	0/10
UBC868/B1	$(GAA)_6$	+	0/8	AYS10	$(CGTG)_3CG$	+	0/14
UBC869	$(GTT)_6$	+	0/15	UBC840	$(GAGA)_4YT$	+	1/29
UBC871	$(TAT)_6$	-	0/0	UBC876	$(GATA)_2(GACA)_2$	+	4/27
(CAC)5	$(CAC)_5$	+	0/13				

+ – amplification in all isolates; **±** – amplification in some isolates; **-** – no amplification products; **P** – the number of polymorphic markers; **M** – monomorphic markers.

[45] and adjusted as described previously [30]. Fg16F/Fg16R (5′-CTCCGGATATGTTGCGTCAA-3′/5′-GGTAGGTATCCGACATGGCAA-3′) and Fc01F/Fc01R (5′-ATGGTGAACTCGTCGTGGC-3′/5′-CCCTTCTTACGCCAATCTCG-3′) primer sets were used in *F. graminearum*- and *F. culmorum*-specific PCR assays.

Anchored primer design and ISSR assays

Forty-one ISSR primers were used in this study (Table 2), of which 31 belonged to the Universal ISSR primer set (NAPS-UBC, Canada) and the remaining primers were newly designed according to the nucleotide sequence information about *F. graminearum* [46]. SSR motifs in the *Fusarium* genus that had not been studied before were selected via "blasting" of four chromosomes of the *F. graminearum* PH-1 strain. All contigs and supercontigs were separately screened for microsatellite regions by the online microsatellite repeats finder. After selection of SSR regions, all primers were designed with 2-mer anchors.

Amplification of ISSR loci by PCR was conducted in a volume of 25 μL reaction mixture containing 1 x buffer, 25 ng genomic DNA, 0.5 μM primer, 0.2 mM of dNTPs, 2.5 mM $MgCl_2$ and 1 U of *Taq* polymerase. PCR was performed under the following program: 35 cycles at 94°C for 45 s, 48-55°C for 45 s and 72°C for 2 min. The first cycle had an extra 5 min at 94°C and the final extension an extra 10 min at 94°C. Amplicons were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide. Images were photographed with a gel visualization system (Avegene, Taiwan). Each experiment set was replicated at least three times.

Mating type assays

MAT-1 and *MAT-2* specific primer pairs fusALPHA-for/rev (5'-CGCCCTCTKAAYGSCTTCATG-3'/5'-GGARTARACYTTAGCAATYAGGGC-3') and fusHMGfor/rev (5'-CGACCTCCCAAYGCYTACAT-3'/5'-TGGGCGGTACTGGTARTCRGG-3') were used in mating type determination according to Kerényi et al. [25]. PCRs were carried out in a reaction volume of 25 μl containing 1 × buffer, 50 ng of genomic DNA, 0.5 μM of primer, 0.2 mM of dNTPs, 2.5 mM $MgCl_2$ and 1 U of *Taq* polymerase. PCRs were performed under the following program: 35 cycles at 94°C for 1 min, 53°C for 1 min and 72°C for 1 min. The first cycle had an extra 3 min at 94°C and the final extension had an extra 3 min at 94°C. Amplicons were analyzed as mentioned before. *MAT-1* and *MAT-2* experiment sets were replicated three times.

Statistical analysis

ISSR markers were visually scored as presence (1) or absence (0) of a band. A similarity matrix was generated from the data using Nei-Li's coefficient [47]. The dendrogram was created according to cluster analysis of ISSR markers using UPGMA (unweighted pair-group method with arithmetic average) algorithm via MVSP 3.1 software.

RESULTS

Isolates used in this study were completely identified at species level by their morphological characteristics [27,29]. In addition, verification of fungal isolates at species level was carried out via amplification of SCAR markers. A UBC85F/UBC85R primer set yielded 332 bp of PCR product in 43 *F. graminearum* isolates. The primer pair OPT18F/OPT18R produced a common amplicon of 472 bp in all *F. culmorum* isolates (data not shown). All isolates characterized at species level were incorporated into further ISSR fingerprinting analysis.

In this study, a total of 10 novel ISSR primers were designed according to the SSR regions located in the *F. graminearum* strain PH-1's genome. Twenty-five of the 42 ISSR primers produced amplicons in all isolates and 7 of these were novel primers (Fig. 1a, b, c, d). Fourteen of the remaining primers did not yield any fragment in any of the isolates, while three primers produced amplicons in only some isolates. A total of 405 bands were scored and 24 of them (5.92%) were polymorphic. PCR product sizes ranged from

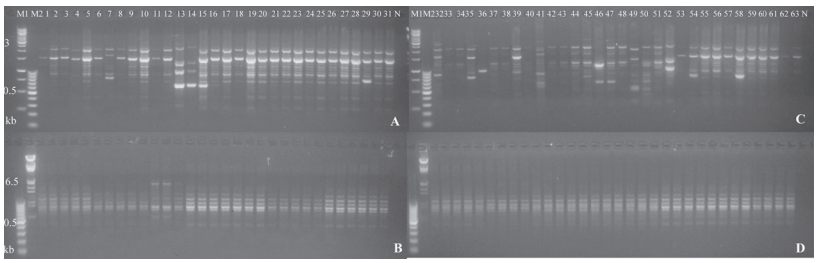

Fig. 1. ISSR profiles obtained from *F. graminearum* (A, B) and *F. culmorum* isolates (C, D) with primers AYS8 (A, C) and AYS6 (B, D) primers. M1: 1 kb DNA ladder (Thermo, SM0313), M2: λ/HindIII DNA marker (Thermo, SM0101), 1-31 and 52-63: *F. graminearum* isolates, 32-51: *F. culmorum* isolates listed in Table 1.

Table 3. Similarity matrix of *F. culmorum* isolates used in this study. Decimal places were accepted as "2".

	F1	F2	F3	F4	F10	F12	F14	F15	F16	F17	F19	F20	F21	F24	8F	9F	10F	11F	12F	13F
F1	1																			
F2	0.86	1																		
F3	0.87	0.9	1																	
F4	0.81	0.87	0.91	1																
F10	0.8	0.83	0.85	0.86	1															
F12	0.88	0.84	0.88	0.86	0.87	1														
F14	0.86	0.85	0.92	0.89	0.87	0.91	1													
F15	0.81	0.77	0.83	0.82	0.81	0.87	0.87	1												
F16	0.82	0.81	0.86	0.81	0.81	0.84	0.87	0.86	1											
F17	0.84	0.83	0.85	0.83	0.83	0.87	0.84	0.83	0.88	1										
F19	0.81	0.79	0.81	0.79	0.78	0.85	0.83	0.81	0.85	0.88	1									
F20	0.85	0.81	0.85	0.8	0.81	0.86	0.86	0.84	0.88	0.9	0.92	1								
F21	0.8	0.78	0.83	0.82	0.78	0.82	0.85	0.78	0.79	0.81	0.86	0.86	1							
F24	0.78	0.75	0.8	0.77	0.8	0.81	0.82	0.79	0.81	0.84	0.82	0.88	0.85	1						
8F	0.71	0.68	0.73	0.68	0.69	0.72	0.75	0.71	0.77	0.71	0.75	0.78	0.78	0.79	1					
9F	0.73	0.7	0.74	0.7	0.7	0.74	0.76	0.75	0.77	0.74	0.76	0.8	0.77	0.81	0.91	1				
10F	0.75	0.72	0.77	0.73	0.68	0.76	0.77	0.75	0.78	0.75	0.78	0.82	0.8	0.78	0.86	0.85	1			
11F	0.73	0.69	0.74	0.71	0.66	0.73	0.75	0.71	0.74	0.72	0.73	0.76	0.75	0.73	0.84	0.8	0.91	1		
12F	0.75	0.71	0.76	0.72	0.67	0.75	0.76	0.75	0.78	0.72	0.76	0.79	0.77	0.75	0.87	0.86	0.94	0.92	1	
13F	0.73	0.74	0.78	0.74	0.7	0.75	0.79	0.73	0.75	0.72	0.75	0.78	0.8	0.75	0.84	0.83	0.94	0.9	0.94	1

0.2 to 3.5 kb. Maximum and minimum number of ISSR bands were obtained from primers UBC872 and UBC876 (31 amplicons) and IF5 (five amplicons). The calculated similarity coefficient among *F. graminearum* isolates ranged from 62.3 to 99% (data not shown). The mean value of similarity was calculated as 0.809±0.002 (SE). Genetically, the most similar (99%) isolates were sh10 and M7. However, the maximum variation level was 62.3% between M3 and sh7. These four isolates belonged to the same geographic location (Neka). The similarity coefficients among *F. culmorum* isolates were 65.7-94.3% (Table 3). The mean value of similarity was 0.798±0.004 (SE). Genetically, the closest (94.3%) isolates were 10F and F12. Also, the most dissimilar (65.7%) were F10 and 11F from Bilecik and Corum, respectively (Table 1). Interspecific similarity between two species ranged from 42.5 to 59.2%. The mean value of similarity belonging to two species was 0.525±0.001 (SE). The most closely related isolate of *F. culmorum* F10 from Bilecik was sh1 of *F. graminearum* from Mazandaran. Similarly, the most distinct isolates were *F. culmorum* 12F from Amasya and *F. graminearum* M9 from Mazandaran.

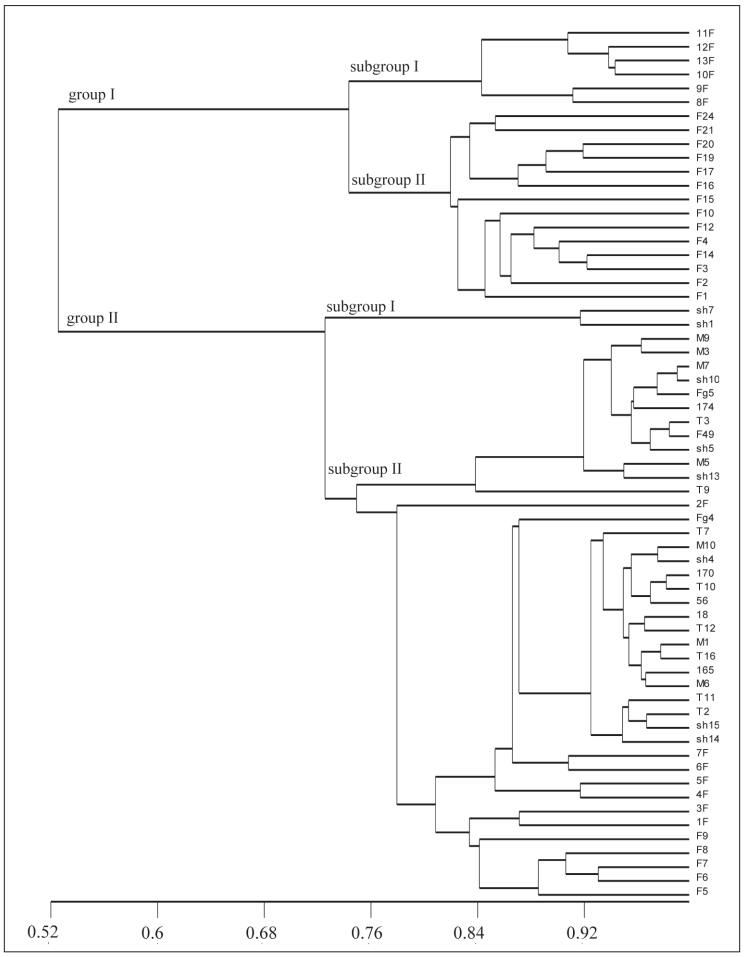

Fig. 2. UPGMA dendrogram generated via ISSR markers amplified in *Fusarium* sp. genome.

Cluster analysis showed that all isolates were divided into two main clades: group I and group II (Fig. 2). As illustrated in the dendrogram generated by UPGMA, group I included 20 isolates belonging to *F. culmorum* species and was smaller than group II, which consisted of 43 *F. graminearum* isolates. Group I consisted of *F. culmorum* isolates from Turkey that produced DON mycotoxin. Group II had only *F. graminearum* isolates from both Turkey and Iran. Their chemotypes showed heterogeneity, and both have DON and NIV chemotypes. Phenons in group II included isolates distributed in same geographic region.

According to mating type analysis, all *F. graminearum* and three *F. culmorum* isolates (13F, 9F, F16) yielded specific amplicons of both *MAT-1* and *MAT-2* genes of 210 and 260 bp, respectively. The 210 bp of DNA fragment corresponding to *MAT-1* was amplified from all *F. graminearum* and nine *F. culmorum* isolates (Fig. 3). Similarly, all *F. graminearum* and 14 *F. culmorum* isolates produced a common band of 260 bp belonging to *MAT-2*. Findings obtained from this analysis showed that all *F. graminearum* isolates have sexual reproduction, while three of *F. culmorum* (13F, 9F, F16) isolates have potential of a sexual stage.

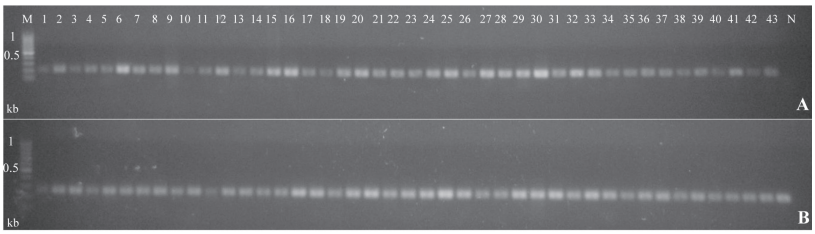

Fig. 3. PCR bands of 210 (A) and 260 (B) bp corresponding to MAT-1 and MAT-2 genes amplified from 43 *F. graminearum* isolates. M: 100 bp DNA ladder (Thermo, SM0242), N: Negative control.

DISCUSSION

More than ten *Fusarium* species are responsible for FHB and root rot diseases worldwide. Among them, *F. graminearum* and *F. culmorum* are the main causal agents of the diseases. However, predominating species in one location can vary according to climatic conditions, their hosts and other environmental factors [1,14,48,49]. *Fusarium* species possess a high level of phenotypic and genotypic diversity. Genotyping of *F. graminearum* and *F. culmorum* has a great importance to the genetic characterization of these phytopathogenic species; however, comprehensive and detailed studies are required.

Mating type analysis provides valuable knowledge about the reproduction of fungal pathogens. Therefore, the identification of mating type is important for the characterization of a fungus. In this study, all isolates belonging to *F. graminearum* yielded both mating type genes, i.e. a sexual stage is present in the species. In *F. culmorum* isolates, no sexual stage was determined in 17 isolates according to either *MAT-1* or *MAT-2* amplification profiles. The presence both of two mating type genes in three *F. culmorum* isolates (13F, 9F and F16) is an interesting finding of asexual reproduction. Çepni et al. [50] used the same *F. culmorum* isolates from Turkey in their study including genetic diversity and mating type analysis. They reported that two *F. culmorum* isolates (F4 and F15) contained both *MAT-1* and *MAT-2* loci. Also, they stated that no amplification product was obtained from the F21 isolate. In this study, mating type analysis of *F. culmorum* and *F. graminearum* isolates was performed. In contrast to these authors' findings, we observed that the F21 isolate possesses only the *MAT-1* gene. In addition, while F4 carries only *MAT-2*, F15 carries only *MAT-1*. There are incompatible findings between two studies. Nevertheless, the presence of both mating types in *F. culmorum* could indicate a parasexual recombination as previously reported [22]. In the wild, hyphal anastomosis can arise when genetically different hyphae of the same species come into contact. After the fusion of two cells, different types of nuclei come together in the heterokaryon, giving a somatic diploid nucleus. During the division of such nuclei, mitotic crossing-over may occur, resulting in parasexual recombination. This is the reason why both *MAT-1* and *MAT-2* idiomorphs can be amplified in the 13F, 9F and F16 isolates of *F. culmorum* [51].

A wide range of molecular marker techniques based on PCR was employed in genotyping *Fusarium* isolates. Many of them involve the amplification of genomic DNA. RAPD [24], AFLP [36] and microsatellite-PCR [52] are just some of them and they target chromosomal DNA. Moreover, mitochondrial DNA analysis is also used for genotyping [40]. The ISSR technique is one of the most widely used and versatile tools in genetic characterization in fungi. ISSR markers obtained from PCR amplification represent differentiation between SSR regions [42]. In ISSR analysis, no prior genomic information is required. Both anchored and non-anchored primers are effectively used in PCR amplification. Although non-anchored primers in particular are a good choice for DNA fingerprinting, stable and reproducible fragments are also obtained from anchored ones [43]. As a result, only a limited number of polymorphic markers (5.92%) could be produced in the current study because (i)

anchored primers were used for amplification and (ii) more than half of the primers contained four nucleotide repeats. Nevertheless, a moderate genetic variation at intra- and interspecific levels was determined in *Fusarium* isolates. Mishra et al. [18,19] reported that there are high levels of genetic diversity using ISSR marker amplification in *F. graminearum* and *F. culmorum* isolates from different countries. They found 81% of ISSR amplicons to be polymorphic. Similarly, from ISSR analysis among *F. poae* isolates from England and Argentina, Dinolfo et al. [42] showed that there was a high level of genetic variation. They reported that 89% of the produced ISSR bands were polymorphic. Moreover, Li et al. [53] and Mishra et al. [54] reported a high level of polymorphic bands (82.3% and 98.3%, respectively) obtained from in their ISSR analysis. Both Mishra et al. [18] and Dinolfo et al. [42] used 4 and 25 oligonucleotide primers, respectively, most of which were non-anchored. However, in the current study, 41 primers were examined for ISSR fingerprinting, of which 23 were anchored and 18 non-anchored. Among them, 10 were novel and were developed and used for the first time in this study. Of the 24 primers (60%), 7 were novel amplified scorable ISSR markers. As a result, variations at the intra- and interspecific level were determined via these primers. Anchored primers have the potential to produce more monomorphic markers. A high number of anchored primers could result with a stable and reproducible but low number of polymorphic bands. The mechanisms accounting for variations in ISSR markers are replication slippage, structural rearrangements of chromosomes and insertion/deletion SNPs occurring in amplified regions [18]. ISSR analysis is not time consuming and laborious, and it also provides reproducible and reliable results in fungal genetics [19,42-44].

Many genotyping studies of *Fusarium* species resulted in no clear correlation between genetic variability and the host and/or geographic origin of isolates [18,19,32]. Isolates either from a single population and/or a few populations, or from limited agro-ecological regions have been used in ISSR studies to date. In this study, the 63 isolates used in this study belonged to two different species, from 3 host plants in 13 different agro-ecological regions of two neighboring countries. Wheat is the prevalent host and therefore, there was no correlation between genetic diversity and host plant. However, isolates were found in subdivisions, especially phenons in group II clustered together at the same agro-ecological locations. *F. graminearum* and *F. culmorum* isolates were definitely separated into clades. Group I consisted of only *F. culmorum* isolates producing DON mycotoxin from Turkey and group II involved only *F. graminearum* isolates from both Turkey and Iran. Their chemotypes and mating types showed heterogeneity, with both having DON and NIV chemotypes and *MAT-1* and *MAT-2* mating types.

The fingerprinting results obtained from this study showed that ISSR fingerprinting is a powerful tool for the discrimination of phytopathogenic species at the species level. Moreover, the technique provides reliable and reproducible results for the correlation between genotype and geographic origins. The novel ISSR markers developed for the first time in this study, should contribute to the differentiating of *Fusarium* isolates according to their species and geographic region. Genotyping via molecular marker techniques is currently a promising and reliable tool for fungal genetic characterization.

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