

Genetic characterization of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by using random-amplified polymorphic DNA

E. Yörük¹ and G. Albayrak²

¹Programme of Molecular Biology and Genetics, Institute of Science, Istanbul University, Istanbul, Turkey

²Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey

Corresponding author: G. Albayrak
E-mail: gulruh@istanbul.edu.tr

Genet. Mol. Res. 12 (2): 1360-1372 (2013)

Received August 7, 2012

Accepted January 27, 2013

Published April 25, 2013

DOI <http://dx.doi.org/10.4238/2013.April.25.7>

ABSTRACT. Five *Fusarium graminearum* and 12 *F. culmorum* isolates, primarily pathogenic species of *Fusarium* head blight, were obtained from naturally infected wheat from various agro-ecological regions of Turkey. Genotyping of the isolates was carried out using random-amplified polymorphic DNA (RAPD). Sixty-five 10-mer oligonucleotide primers were used to amplify the RAPD markers. Among them, 50 primers produced strong and reproducible DNA amplicons. The remaining primers generated either insufficient or no amplification patterns. In total, 1200 fragments were scored, 311 of which were determined to be polymorphic and unique to the isolates. The produced RAPD markers ranged from 0.2 to 5 kb. The mean genetic similarity values of the *F. graminearum* and *F. culmorum* isolates were 61.5 and 65%, respectively. The similarity coefficient was 43 to 76.1% among *F. graminearum* isolates and 49 to 81.1% among *F. culmorum* isolates. Genetically, the most similar *F. graminearum* isolates were F6 and F7 (76.1%), which originated from the same agro-ecological region (Sakarya). The most similar *F. culmorum* isolates were F20 and

F21 (81.1%), which were from different geographic regions (Bilecik and Uşak, respectively). Moreover, interspecific variation between the two species was determined to be 86.3 to 93.3%. Cluster analysis generated two branched groups, each containing isolates of one species, except F13 of *F. culmorum*. The sequencing of stable and reproducible monomorphic and polymorphic RAPD markers indicated that the *Fusarium* genome shared high similarity (105-625 bit scores) with the genomes of other organisms as well as with the *F. graminearum* reference genome.

Key words: *Fusarium* head blight; *F. graminearum*; *F. culmorum*; Genotyping; Random-amplified polymorphic DNA

INTRODUCTION

Wheat is one of the most important cereal crops worldwide. In a 2009 ranking of cereal crops in Turkey, wheat was first in terms of production, area harvested, and seed production (FAO, 2012). Wild-type cultivated cereals, especially wheat and barley, are still distributed along the Fertile Crescent, which includes part of south eastern Turkey, where agriculture was first initiated (Harlan and Zohary, 1966). However, cultivated cereals are susceptible to many diseases. Among them *Fusarium* head blight (FHB) is one of the most devastating diseases, and particularly affects wheat. Many *Fusarium* spp cause the disease in Turkey and other countries worldwide (Champail et al., 2004). *Fusarium graminearum* and *F. culmorum* are the most common causal agents of FHB (Miedaner et al., 2001). FHB epidemics cause both severe yield losses and reduction in quality. Mycotoxins produced by the pathogens effectively spread the disease. Moreover, they have long-term harmful effects on both humans and animals entering the food chain. In addition to their carcinogenic effects, they inhibit ribosomal protein synthesis (Gutleb et al., 2002). Research has recently focused on the molecular characterization of causal agents and the production of mycotoxins to improve pathogen recognition (Saharan et al., 2007; Haratian et al., 2008; Pandolfi et al., 2010).

Typing or genotyping, referred to as molecular epidemiology, is as important as the study of global epidemiology of pathogens. Unless the genetic variants collected from various regions of the world can be identified, the long-term struggle with plant pathogens will continue. Nevertheless, our knowledge about the genetic diversity of plant pathogens is limited (Viridi and Sachdeva, 2005). Therefore, understanding the genetic variation of *Fusarium* spp associated with FHB will improve disease-control strategies. Owing to a lack of specific and effective fungicides, the development and selection of resistant cultivars have become an important strategy in disease control and cereal crop breeding programmes (Mesterházy, 1995; Saharan et al., 2004). The detection of *Fusarium* spp among field pathogens, their identification, and the determination of the genetic diversity of their isolates are methodological approaches. The identification of *Fusarium* spp based on morphological characters using traditional techniques is influenced by host age, inoculum quality, and environmental conditions (Champail et al., 2004; Saharan et al., 2004). In addition, traditional techniques are time-consuming and laborious. Therefore, molecular techniques have recently become a promising and versatile tool, especially for the detection of genetic variation (Demir et al., 2010; Abadio et al., 2012; Memati et al., 2012). Because these techniques are reliable, fast, sensitive, and suitable, they can replace traditional methods. Some of them - for example, random-amplified polymorphic DNA (RAPD), microsatellite polymerase chain reaction (PCR),

and inter-simple sequence repeats - are used to determine intra- and interspecific genetic variations in *Fusarium* spp (Carter et al., 2002; Mishra et al., 2003; Heng et al., 2012).

Although FHB can be diagnosed on all small-grain crops, it has been reported most frequently on wheat in Turkey (Mamluk et al., 1997). Tunali et al. (2006a,b) obtained 116 single-spore isolates belonging to *Fusarium* - including *F. graminearum* and *F. culmorum* - from wheat spikes in the north central parts of Anatolia and Marmara region between 2000 and 2005. Three of these species (*F. culmorum*, *F. graminearum*, and *F. pseudograminearum*) were observed as the most aggressive pathogens, and they reduced vitality under greenhouse conditions. The authors also reported that *F. graminearum* was the primary pathogen responsible for FHB and that the disease incidence was positively correlated with the content of deoxynivalenol that accumulated during that period of time. Gürel et al. (2010) determined the genetic variation of 15 Turkish *F. culmorum* isolates from nine agro-ecological regions using repetitive DNA-based fingerprinting methods. This report was the first to describe the molecular characterization of these isolates and their relationship with the wide geographic distribution of that species. Arici and Koç (2010) also investigated the genetic variation of 10 isolates belonging to *F. graminearum* and *F. culmorum* from the Adana region using RAPD markers. Moreover, chemotyping of 11 *F. graminearum* and 21 *F. culmorum* isolates from various agro-ecological regions of Turkey has been carried out using PCR analysis (Yörük and Albayrak, 2012).

The identification of *Fusarium* spp and their produced mycotoxins are important in treatment strategies for FHB and the development of new species-specific diagnostics in Turkey. In this study, we aimed to 1) analyze the genetic variation of *F. graminearum* and *F. culmorum* isolates collected from various regions of Turkey, 2) detect nucleotide sequences that are sequence characterized amplified regions (SCAR) markers in the genomes of these pathogens, and 3) investigate the homologies of these sequences with the genomes of other organisms as well as the reference genome of *F. graminearum*.

MATERIAL AND METHODS

Fungal material and genomic DNA extraction

Seventeen isolates of *Fusarium*, including 5 *F. graminearum* and 12 *F. culmorum* produced from single spores (Bentley et al., 2006) were provided by Dr. Berna Tunali (Department of Plant Protection, Agricultural Faculty, Samsun Ondokuz Mayıs University; Table 1). All isolates were grown on potato dextrose agar plates at 25°C for 6 days. Genomic DNA was extracted using a genomic DNA isolation kit (Macherey-Nagel, A026218, Germany) based on the established cetyltrimethylammonium bromide procedure.

Identification of trichothecene-producing groups and species

Primer set Tox5-1/Tox5-2 (5'-GCTGCTCATCACTTTGCTCAG/CTGATCTGGTCACG CTCATC-3') was used to amplify the trichodiene synthetase (*tri5*) gene (Table 2) to identify trichothecene-producing *Fusarium* spp (Hue et al., 1999). PCR was carried out in a volume of 25 µL containing 50 ng genomic DNA, 1.5 mM MgCl₂, 1X PCR buffer, 10 pmol of each primer, 0.1 mM of each deoxyribonucleoside triphosphate, and 1 U *Taq* DNA polymerase (9PIM829; Promega, USA). PCR amplification was performed by predenaturation at 94°C for 5 min, followed by 35

Table 1. Hosts and geographic origins of *Fusarium culmorum* and *F. graminearum* isolates used in this study.

Isolate	Species	Host	Geographic origin
F2	<i>F. culmorum</i>	Wheat	Sakarya
F3	<i>F. culmorum</i>	Wheat	Konya Merkez
F4	<i>F. culmorum</i>	Wheat	Sakarya
F5	<i>F. graminearum</i>	Wheat	Sakarya
F6	<i>F. graminearum</i>	Wheat	Sakarya
F7	<i>F. graminearum</i>	Wheat	Sakarya
F8	<i>F. graminearum</i>	Wheat	Sakarya
F9	<i>F. graminearum</i>	Wheat	Balikesir
F10	<i>F. culmorum</i>	Wheat	Bilecik
F13	<i>F. culmorum</i>	Wheat	Osmaneli/Bilecik
F14	<i>F. culmorum</i>	Wheat	Bilecik
F15	<i>F. culmorum</i>	Wheat	Boyabat/Sinop
F17	<i>F. culmorum</i>	Wheat	Konya Merkez
F19	<i>F. culmorum</i>	Wheat	Sarayönü/Konya
F20	<i>F. culmorum</i>	Wheat	Bilecik
F21	<i>F. culmorum</i>	Wheat	Uşak
F24	<i>F. culmorum</i>	Wheat	Cumra/Konya

cycles of amplification at 94°C for 1 min, 68°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 10 min. *F. graminearum* isolates were confirmed via Fg16F/Fg16R (5'-CTCCGGATA TGTTGCGTCAA-3'/5'-GGTAGGTATCCGACATGGCAA-3') and UBC85F/UBC85R (5'-GCA GGGTTTGAATCCGAGAC-3'/5'-AGAATGGAGCTACCAACGGC-3') primer sets designed by Nicholson et al. (1998) and Schilling et al. (1996), respectively. Similarly, *F. culmorum* isolates were verified via Fc01F/Fc01R (5'-ATGGTGAACCTCGTCGTGGC-3'/5'-CCCTTCTTACGCCA ATCTCG-3') and OPT18F/OPT18R (5'-GATGCCAGACCAAGACGAAG-3'/5'-GATGCCAGA CGACTAAGAT-3') primers, also designed by Nicholson et al. (1998) and Schilling et al. (1996), respectively. PCR mixing and thermal cycling were carried out as previously described, with the exception of the annealing temperature, which was adjusted to 61°C. Amplification products were separated with electrophoresis on 1.5% agarose gel and visualized under ultraviolet light after staining with ethidium bromide.

RAPD analysis

In this study, 65 random-10-mer oligonucleotide primers from Operon Technologies (USA) were examined through RAPD analysis (see Table 2). Amplification reactions were carried out in volumes of 25-µL containing 50 ng genomic DNA, 1X PCR buffer, 2.5 mM MgCl₂, 0.4 mM of each deoxyribonucleoside triphosphate, 10 pmol primer and 1 U *Taq* DNA polymerase (Promega). The thermal cycling conditions for all primers consisted of DNA denaturation at 94°C for 5 min; 4 cycles of 1.5 min at 94°C, 1.5 min at 37°C, and 3 min at 72°C; followed by 40 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C, and a final extension at 72°C for 10 min. RAPD products were electrophoresed on 1.7% agarose gel. RAPD assays for each of the primers were carried out three times to verify reproducible amplicons.

Statistical analysis

RAPD markers were visually scored as the presence (1) or absence (0) of a band. A similarity matrix was constructed from these data using Nei-Li's coefficient (Nei and Li, 1979). A dendrogram was generated according to the cluster analysis of RAPD markers using the unweighted pair group method with arithmetic average algorithm (UPGAM) in MVSP 3.1.

Table 2. RAPD primers used in this study.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPA02	TGCCGAGCTG	OPC19	GTTGCCAGCC	OPF06	GGGAATTCGG
OPA06	GGTCCCTGAC	OPD01	ACCGCGAAGG	OPG02	GGCACTGAGG
OPA09	GGGTAACGCC	OPD02	GGACCCAACC	OPG03	GAGCCTCCA
OPA13	CAGCACCCAC	OPD03	GTCGCCGTCA	OPG07	GAACCTGCGG
OPA18	AGGTGACCGT	OPD04	TCTGGTGAGG	OPG10	AGGGCCGTCT
OPB03	CATCCCCCTG	OPD07	TTGGCACGGG	OPG11	TGCCCGTCTG
OPB05	TGCGCCCTTC	OPD08	GTGTGCCCA	OPG13	CTCTCCGCA
OPB06	TGCTTGCC	OPD13	GGGGTGACGA	OPG16	AGGCTCCTCC
OPB07	GGTGACGCAG	OPD14	CTTCCCAAG	OPG17	ACGACCGACA
OPB09	TGGGGGACTC	OPD17	TTCCACGG	OPZ02	CCTACGGGGA
OPB10	CTGCTGGGAC	OPD20	ACCCGGTCAC	OPZ03	CAGCACCGCA
OPB13	TTCCCCCGCT	OPE01	CCCAAGGTCC	OPZ04	AGGCTGTGCT
OPB14	TCCGCTCTGG	OPE02	GGTGCGGGAA	OPZ07	CCAGGAGGAC
OPB19	ACCCCGAAG	OPE03	CCAGATGCAC	OPZ08	GGGTGGGTAA
OPC02	GTGAGGCGTC	OPE06	AAGACCCTC	OPZ09	CACCCAGTAC
OPC04	CCGCATCTAC	OPE13	CCCATTCCG	OPZ10	CCGACAAACC
OPC05	GATGACCGCC	OPE14	TGCGGCTGAG	OPZ12	TCACGGGAC
OPC07	GTCCCGACGA	OPF01	ACGGATCCTG	OPZ15	CAGGGCTTTC
OPC08	TGGACCGGTG	OPF02	GAGGATCCCT	OPZ16	TCCCATCAC
OPC09	CTCACCGTCC	OPF03	CCTGATCACC	OPZ19	GTGGCAGCAA
OPC14	TGCGTGCTTG	OPF04	GGTGATCAGG	OPZ20	ACTTTGGCGG
OPC17	TTCCCCCAG	OPF05	CCGAATTC		

Screening of stable and reproducible polymorphic and monomorphic RAPD markers

Distinctive stable and reproducible polymorphic and monomorphic RAPD markers with the potential to differentiate the two species were detected. These markers were then purified from agarose gels using a High Pure PCR Product Purification Kit (Roche, 11732668001, Switzerland). The amplicons were cloned into plasmids using a p-GEM Easy Vector System (Promega, TM042) and then sequenced with universal M13 primers. The sequencing process was carried out based on a chain termination method. Nucleotide sequences were then monitored as chromatograms using ChromasPro and subjected to homology analysis. Sequence similarity (e value <0.05; bit score >50) was searched with nucleotide Basic Local Alignment Search Tool (BLASTN) through the National Center for Biotechnology Information (NCBI) network (Altschul et al., 1997). Some criteria in particular, such as nucleotide collection and highly similar sequences, were considered within BLASTN.

RESULTS

Identification of trichothecene-producing groups and species

In PCR analysis, the Tox5-1/Tox5-2 primer produced a 658-bp fragment in all isolates (Figure 1A). Therefore, we determined that 17 isolates had trichothecene-producing potentials.

Fg16F/Fg16R generated a common band of 450 bp (Figure 1B), whereas UBC85F/UBC85R amplified a band of 332 bp (data not shown) in only 5 isolates (F5-F9). Thus, we confirmed 5 isolates as *F. graminearum*. Similarly, the Fc01F/Fc01R and OPT18F/OPT18R primer pairs yielded product sizes of 570 bp (Figure 1B) and 472 bp (data not shown), respectively. These PCR results indicated that 11 isolates (not including F13) belonged to *F. culmorum*. Thus, we identified all isolates at the species and group levels.

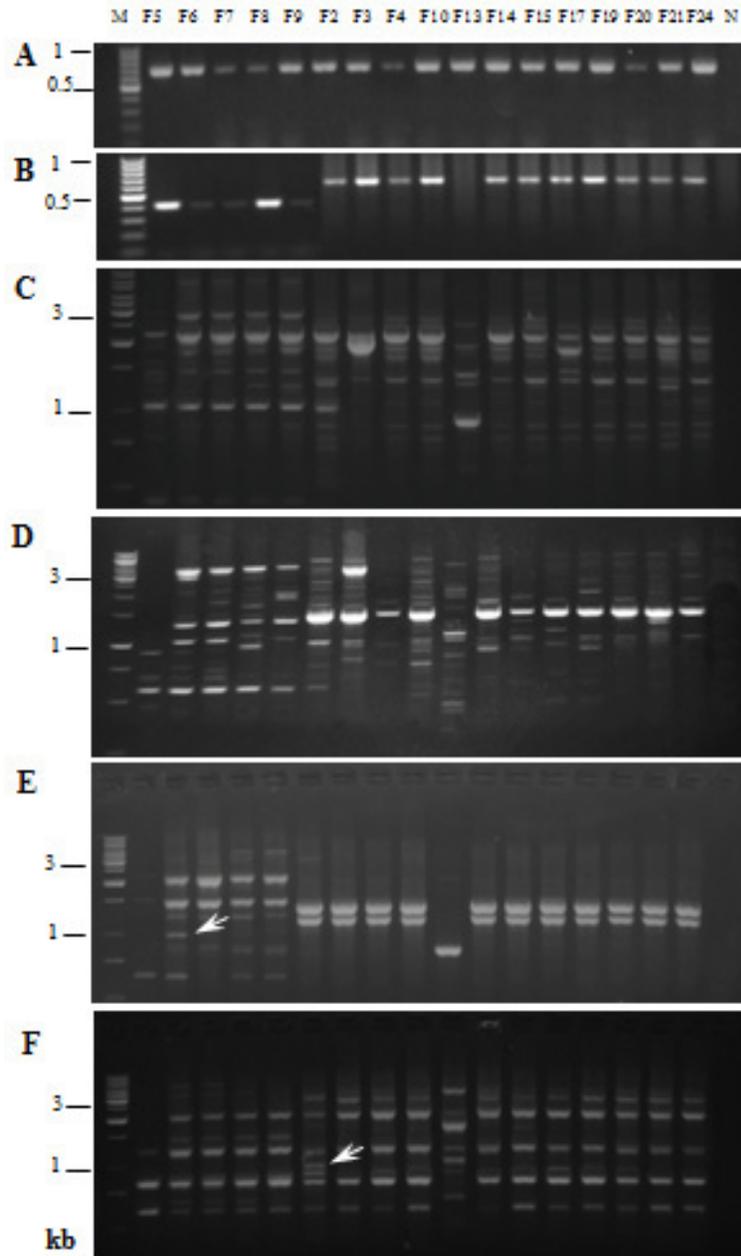


Figure 1. DNA amplification products (A) of a 658-bp fragment with Tox5-1/Tox5-2 primer in all isolates, (B) of 450 bp with Fg16F/Fg16R primer specific to *Fusarium graminearum* isolates (F5, F6, F7, F8, F9) and of 570 bp with Fc01F/Fc01R primer specific to *F. culmorum* isolates (F2, F3, F4, F10, F13, F14, F15, F17, F19, F20, F21, and F24). RAPD profiles of 5 *F. graminearum* and 12 *F. culmorum* (F2, F3, F4, F10, F13, F14, F15, F17, F19, F20, F21, and F24) isolates amplified with (C) OPC07, (D) OPC17, (E) OPG11, and (F) OPG13 primers. White arrows indicate polymorphic markers. Lane M = 100-bp (A and B) and 1-kb ladder (C, D, E, F) molecular weight standards; lane N = negative control.

RAPD analysis

Sixty-five 10-mer oligonucleotide primers were examined for amplification of RAPD markers of 16 isolates. Genomic DNA of isolate F13 displayed different amplification patterns with all primers. Moreover, neither Fc01F/Fc01R nor OPT18F/OPT18R generated any DNA fragments in species-specific PCR of this isolate. Hence, F13 was excluded from the evaluation of RAPD findings and generation of relationships. Fifty primers produced strong and reproducible DNA amplicons. The highest number of amplification patterns was observed using OPC17 (Figure 1C). The remaining primers generated either similar amplicons or no amplification products. In total, 1200 fragments were scored for multivariate analysis, 311 of which were determined to be polymorphic and unique to the isolates (Figure 1D-F). The RAPD amplicon sizes ranged from 0.3 to 4 kb among 5 *F. graminearum* isolates and from 0.2 to 5 kb among 11 *F. culmorum* isolates. According to Nei-Li's coefficient, the mean value of genetic similarity was 61.5% for *F. graminearum* isolates and 65% for *F. culmorum* isolates (Table 3). The similarity coefficient among *F. graminearum* and *F. culmorum* isolates ranged from 43 to 76.1% and 49 to 81.1%, respectively. Isolates F6 and F7 (*F. graminearum*) were the most genetically related isolates and originated in the same geographic region (Sakarya). Similarly, F20 and F21 (*F. culmorum* isolates), which belonged to geographically different regions (Bilecik and Uşak, respectively) were determined to be highly similar (81.1%). Moreover, interspecific variation between two species ranged from 86.3 to 93.3%. Although F7 (*F. graminearum*) and F2 (*F. culmorum*) were genetically the most similar (13.7%) isolates belonging to the same geographic region (Sakarya), F5 (*F. graminearum*) and F19 (*F. culmorum*) were dissimilar (6.7% similarity) and originated from different regions (Sakarya and Sarayönü/Konya, respectively). F5 and F13 were the most distant isolates, and F13 was excluded from data analysis.

Table 3. Similarity matrix belong to 16 isolates of *Fusarium culmorum* and *F. graminearum*.

	F5	F6	F7	F8	F9	F2	F3	F4	F10	F14	F15	F17	F19	F20	F21	F24
F5	1.000															
F6	0.446	1.000														
F7	0.444	0.761	1.000													
F8	0.430	0.673	0.691	1.000												
F9	0.437	0.665	0.727	0.750	1.000											
F2	0.096	0.132	0.137	0.127	0.135	1.000										
F3	0.090	0.117	0.134	0.115	0.129	0.684	1.000									
F4	0.075	0.118	0.117	0.129	0.127	0.708	0.683	1.000								
F10	0.082	0.122	0.121	0.120	0.125	0.724	0.721	0.784	1.000							
F14	0.075	0.118	0.111	0.107	0.108	0.680	0.709	0.732	0.794	1.000						
F15	0.074	0.120	0.118	0.115	0.122	0.613	0.622	0.691	0.725	0.734	1.000					
F17	0.071	0.080	0.095	0.087	0.107	0.491	0.545	0.545	0.569	0.589	0.607	1.000				
F19	0.067	0.103	0.124	0.111	0.112	0.602	0.633	0.649	0.690	0.710	0.705	0.602	1.000			
F20	0.070	0.109	0.104	0.110	0.114	0.630	0.622	0.697	0.704	0.733	0.808	0.610	0.741	1.000		
F21	0.071	0.116	0.108	0.114	0.115	0.613	0.622	0.675	0.685	0.724	0.794	0.590	0.690	0.811	1.000	
F24	0.088	0.126	0.131	0.124	0.132	0.598	0.640	0.659	0.682	0.719	0.688	0.611	0.717	0.742	0.739	1.000

Cluster analysis generated two groups, each containing isolates of one species with the exception of F13, which was of *F. culmorum* (Figure 2). As shown in the dendrogram, group I was larger cluster than group II because it consisted of 11 isolates, whereas group II contained only 5 isolates. Moreover, both groups were subdivided further into two subgroups. Isolates F5 (*F. graminearum*) and F17 (*F. culmorum*) were absent from these subgroups. They were related to their main groups.

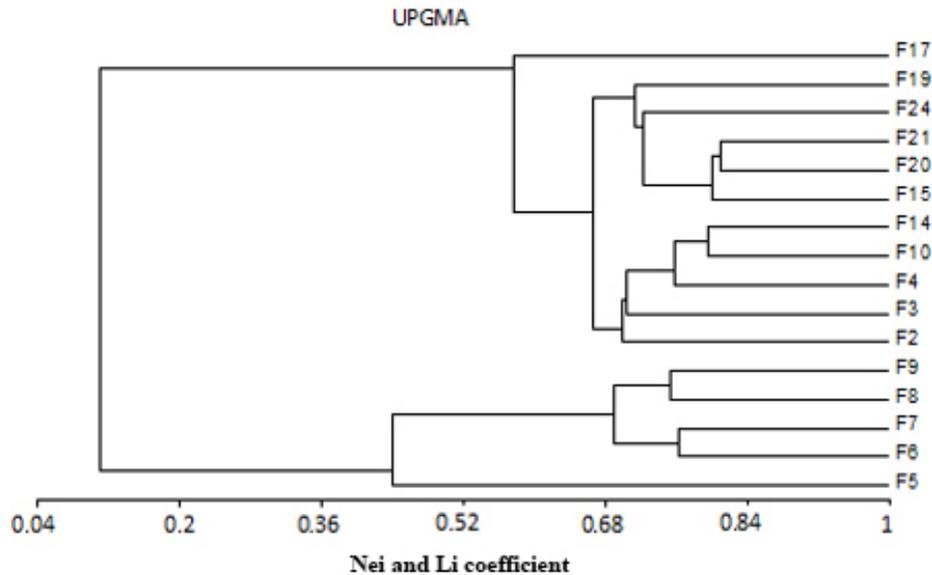


Figure 2. Cluster analysis of genetic similarity obtained from 16 isolates of *Fusarium culmorum* and *F. graminearum*.

Screening of stable and reproducible polymorphic and monomorphic RAPD markers

Owing to their reproducibility and stability, 22 polymorphic and 4 monomorphic RAPD markers were selected as SCAR markers to generate detailed sequence information. After screening the sequence data of markers using ChromasPro, we evaluated high-quality signal peaks in chromatograms. Seven of the polymorphic and all monomorphic RAPD markers showed significant nucleotide sequence homologies to the genomes of various organisms as well as to the *F. graminearum* genome registered in NCBI (Table 4). All similarity indexes had acceptable *e* values (<0.05) and bit scores (>50). Among polymorphic RAPD markers, the insert from 2.17.D07 and 7.08.A09 clones had identical nucleotide sequences with a polymerase protein and coat protein in hepatitis B virus. In addition, the insert from the 6.13.D08 clone shared identical nucleotide sequences with the marenostriin protein in *Homo sapiens*. Another insert from 22.06.E14 showed 20% nucleotide similarity with a transfer RNA (tRNA)-Lys and maturase K (matK) in *Nymphaea macrosperma*. Moreover, the BLASTN analysis indicated that three inserts from 20.24.D02, 14.04.C17, and 16.17.C17 clone sequences were similar (53, 81, and 91%, respectively) to β -lactamase TEM-116 and blaCTX-M-15 gene sequences. In addition to polymorphic markers, all monomorphic RAPD markers had significant sequence homologies with the genomes of other organisms (see Table 4). The insert from the D3G clone shared sequence similarity (25%) with PH-1 hypothetical mRNA in *Gibberella zeae* (asexual *F. graminearum*). The D3C clone insert displayed nucleotide sequence similarity (17%) with a predicted protein-coding region in *Nectria haematococca* (asexual name *F. solani*). Finally, the inserts from the G11.1C and G11.2C clones revealed 10 and 15% nucleotide homologies with genomic fragments of *Lepilemur dorsalis* and 18S ribosomal DNA (rDNA) of uncultured *Trebouxia photobiont*, respectively.

Table 4. Nucleotide sequence homologies between RAPD markers amplified in this study and genomic fragments of different organisms.

Marker	Associated organism	Accession No.	Product	e value
2.17.D07	Hepatitis B virus	FJ621799.1	Polymerase (P) protein	0.0
7.08.A09	Hepatitis B virus	AB263407.1	Coat protein	0.0
6.13.D08	<i>Homo sapiens</i>	NG_007871	Marenostrin protein	0.0
22.06.E14	<i>Nymphaea macrosperma</i>	DQ185540.1	tRNA-Lys and maturase K	5e-46
20.24.D02	<i>Aeromonas jandaei</i>	FJ767909.1	β -lactamase TEM-116	2e-94
14.04.C17	<i>Klebsiella pneumoniae</i>	GQ845086	β -lactamase blaCTX-M-15	2e-177
16.17.C17	<i>Enterobacter</i> sp	EU531513.1	β -lactamase blaCTX-M-28	5e-111
D3G	<i>Gibberella zeae</i>	XM_389641.1	Hypothetical protein	5e-61
D3C	<i>Nectria haematococca</i>	XM_003046704.1	Predicted protein	4e-37
G11.1C	<i>Lepilemur dorsalis</i>	AJ244005.1	Unknown	1e-33
G11.2C	Uncultured <i>Trebouxia photobiont</i>	AJ969582	18S ribosomal RNA	1e-36

DISCUSSION

The occurrence of a disease is closely related not only to plant-fungal pathogen interactions but also to environmental conditions (Gutleb et al., 2002). More recent global climatic changes can contribute to the spread of a disease from geographical area in which it was not previously observed to regions throughout the world (Saharan et al., 2004). Therefore, strategies for combating disease must be revised. Controlling the disease and the genotyping of causal agents depend on accurate diagnosis (Ward et al., 2004). Conventional characterization methods for *Fusarium* spp are time-consuming, inconclusive, and laborious. Conversely, nucleic acid-based molecular techniques are reliable, fast, and suitable for diagnosis, identification of pathogens or their specific markers, and genotyping. In this study, 5 *F. graminearum* and 11 *F. culmorum* isolates identified using conventional methods were confirmed through PCR analysis of SCAR markers. SCAR analysis is a powerful tool for definitive diagnosis at the species level. *F. culmorum* F13 was excluded from the comparison of all isolates because its genomic DNA was not amplified using species-specific primers.

These findings can be explained in that F13 contains genomic alterations such as transition/transversion or rearrangements such as insertion/deletion in the primer annealing sites. Carter et al. (2000) excluded WL6 and MK4 isolates from their *Fusarium* analysis according to SCAR results. They have shown that both of the isolates are classified as *F. napiforme* and *F. fujikuroi* using internal transcribed spacer sequence analysis. These outcomes cause uncertainty about the reliability of conventional methods for the diagnosis or identification of pathogenic fungi. As has already been stated in the literature, findings obtained with conventional methods must be confirmed using molecular techniques. Therefore, combining conventional techniques with molecular methods provides accurate diagnosis of the disease and proper identification of fungal organisms.

The RAPD technique has been effective for investigating genetic diversity in *Fusarium* spp. Walker et al. (2001) and Miedaner et al. (2001) reported high levels of genetic variation between *F. culmorum* and *F. graminearum* isolates. On the contrary, various studies (Quellet and Seifert, 1993; Carter et al., 2000; Saharan et al., 2007) have indicated a relatively low amount of genetic diversity among *F. graminearum* strains. In this study, intra- and interspecific genetic variations of Turkish *F. graminearum* and *F. culmorum* isolates were investigated using an RAPD method. Interspecific similarity among the isolates ranged from 6.7 to 13.7% according to the similarity matrix. Moderate and low levels of intraspecific

variation were detected among *F. culmorum* (18.9 to 51%) and *F. graminearum* (23.9 to 57%) isolates. Cluster analysis of RAPD data showed that the isolates were divided into two main clusters (groups I and II), each containing isolates of one species, with the exception of F13 *F. culmorum*. Although four of *F. graminearum* isolates were collected from the same region (Sakarya), they showed moderate variation (23.9 to 57%). With the exception of F5, they were separated into two subgroups. Whereas F6 and F7 isolates from Sakarya were divided into one subgroup, F8 joined F9 from Balıkesir in the other. Although F5 was obtained from Sakarya, it was excluded from both subgroups. Genetically, the most similar *F. graminearum* isolates were F6 and F7, and they originated from the same geographic region.

According to Nei-Li's coefficient, similarity among *F. culmorum* isolates was 13.7 to 81.1%, including F13. Isolates F20 and F21 were the most similar, and they originated from Bilecik and Uşak, respectively. The dendrogram indicated that all Marmara region isolates except F20 clustered in the same subgroup. The other subgroup showed heterogeneity in terms of geographic origin. This subgroup also contained the isolates with the highest similarity value - for example, 81.1% (between F20 and F21) and 80.9% (between F15 and F20). The RAPD dendrogram illustrated that *F. culmorum* isolates, except F17, were divided into two subgroups. *F. graminearum* isolates had a higher polymorphism potential than those of *F. culmorum*. We detected no correlation between genotypes and geographic origin that was influenced by *F. graminearum* because the sampling number was very low. The number of *F. culmorum* isolates was sufficient to evaluate this correlation, and we found that isolates present in group I showed geographic heterogeneity.

Many studies have reported that some isolates belonging to the same geographic region are found in different clusters, and others from different regions are grouped in the same cluster (Carter et al., 2002; Saharan et al., 2007). Determining the correlation between polymorphism/similarity and geographic origin of *Fusarium* isolates is extremely difficult. Considerably high intraspecific variation (60 to 100%) exists among the isolates of worldwide collections even within populations from individual fields (Miedaner et al., 2008). However, compared to this high diversity, a moderate level of interspecific variation (18.9 to 57%) was presented among Turkish *Fusarium* isolates obtained from nine regions. These findings suggest that the genetic composition of the Turkish *Fusarium* population has stability. Saharan et al. (2007) concluded that genetic diversity is also associated with variation in climatic conditions, farm practices, and crop rotations. Moreover, sexual or parasexual recombination is responsible for intraspecific variation within *F. graminearum* isolates, whereas asexual or parasexual recombination accounts for variability within *F. culmorum* isolates (Miedaner et al., 2001). Gürel et al. (2010) reported relatively high genetic variability (11.3 to 84.2%) within *F. culmorum* isolates from Turkey by using repetitive DNA-based fingerprinting methods. Some *F. culmorum* isolates examined in the present study were also used by Gürel et al. (2010). A comparison of repetitive element palindromic and RAPD dendrograms has shown that F2, F3, F4, F10, and F14 cluster in the same subgroup, whereas F15, F19, F20, and F21 cluster in the other subgroup. These compatible data indicate that selected molecular marker techniques are reliable for genotyping.

Genotyping of closely related *Fusarium* isolates can contribute to diagnosis, disease control strategies, and selection of suitable plant breeding programmes related to FHB. The development of SCAR markers derived from RAPD is an important advancement for species-specific diagnosis. SCAR markers have been used to identify pathogenic fungi and dis-

tinguish wild-type and mutant organisms. These markers have also provided knowledge about nucleotide sequence homology and orthologous genes among genomes (Nicholson et al., 1998; Adinolfi et al., 2007; Saharan et al., 2007). They are frequently used in the molecular characterization of *Fusarium* at the genus and species levels (Schilling et al., 1996; Haratian et al., 2008).

Genetic homology is evaluated on the basis of the detection of both amino acid and nucleic acid sequence similarity by using whole-genome scanning methods. The fact that two organisms contain homologous or highly similar sequences indicates that they originated from a common ancestor. In particular, the determination of sequence homology provides data not only for the construction of evolutionary trees but also for the development of new model organisms that can be used in basic research in the future. In this study, we examined the utility of RAPD marker sequences as SCAR markers. We also searched for similarities between these sequences and those of the genomes of other organisms and the *F. graminearum* genome. In this study, stable, reproducible, polymorphic RAPD markers amplified in *F. graminearum* or *F. culmorum* genomes showed significant nucleotide sequence similarities or homologies with the genomes of other organisms, especially of unrelated species. The high level of homology demonstrated that these sequences were evolutionary conserved from viruses to humans. In particular, the similarity between RAPD markers obtained from *F. culmorum* and antibiotic resistant gene sequences in bacteria is crucial with regard to natural selection.

The most remarkable finding in the BLASTN analysis was the high nucleotide sequence similarity between marker 6.13.D08 of isolate F13 and the Mediterranean fever gene on chromosome 16 *H. sapiens*. The marenstrin protein is the final product of this gene, and it is related to familial Mediterranean fever. Although we excluded isolate F13 from diversity analysis, we found that polymorphic marker 6.13.D08 had 93% coverage and 98% maximum identity values to this gene. For this reason, F13 can be used as model organism in molecular studies related to Mediterranean fever. Another important similarity (coverage: 91%, maximum identity: 90%) was detected between the 16.17.C17 marker amplified in F17 and β -lactamase bla CTX-M-28 type of *Enterobacter* spp. In addition, the β -lactamase bla CTX-M-15 type of *Klebsiella pneumoniae* shared homology with the 14.04.C17 marker in F4 of the same fungal pathogen. Both the 2.17.D07 and 7.08.A09 markers of *F. culmorum* and *F. graminearum* showed high similarity (coverage: 81%, maximum identity: 95%; coverage: 75%, maximum identity: 97%, respectively) to the polymerase protein and coat protein of hepatitis B virus, respectively. The similarity of the 22.06.E14 marker sequence of *F. graminearum* to the tRNA-Lys and matK gene sequences of *N. macrosperma* was also a remarkable finding in terms of calculated coverage and maximum identity values (81 and 95%, respectively).

In addition to these BLASTN results, similarities between stable, strong monomorphic RAPD marker sequences, which separated two *Fusarium* spp, and genomes of other organisms were observed. Despite their relatively low coverage value, these marker sequences shared significant maximum identity, even identical nucleotide sequences, with part of genome sequences of other organisms. Homology values of monomorphic marker sequences were lower than those of polymorphic ones. The monomorphic D3G marker amplified in *F. graminearum* resembled the PH-1 hypothetical partial mRNA of *G. zae* (anamorph *F. graminearum*) registered in the database (NCBI, 2012). Despite having a low coverage value (25%), the maximum identity was 100%. This result indicates that the generated D3G marker contains common sequences with *F. graminearum* genes published in the NCBI database. The D3C marker of *F. culmorum* was similar (coverage: 17%, maximum identity: 87%) to the mpVI 77-

13-4 predicted protein coding genomic region of *N. haematococca* (asexual name of *F. solani*). This finding is also valuable in terms of determining conserved sequences among *Fusarium* spp. Moreover, a short genomic region associated with unrelated species was detected in this study. For example, G11.2C marker sequences showed similarity with the available uncultured *T. photobiont* 18S rDNA region (coverage: 15%; maximum identity: 100%). The *F. culmorum* genome had sequences closely related to those within algal genomes. Knowledge from both polymorphic and monomorphic RAPD markers produced within isolates belonging to two *Fusarium* spp may be useful for evaluating evolutionary relationships.

In the present study, we identified 17 isolates of *Fusarium*, including 5 *F. graminearum* and 12 of *F. culmorum* at the species level by using PCR-based techniques. We realized that the number of *F. graminearum* isolates was very low; we decided that identifying them was important. We reliably confirmed all isolates in a short time at the species level and determined the relationship among them using RAPD analysis. We found a moderate level of intraspecific genetic variation. Simultaneously, we investigated the potential of the produced RAPD markers to be used as SCAR markers. However, 11 RAPD markers were not allele specific. Hence, they cannot be used as species-specific SCAR markers. Nevertheless, marker sequence information is useful for molecular biologists. Therefore, the sequence of the D3G RAPD marker, generated from the *F. graminearum* F7 isolate was registered in the NCBI database (GenBank) under accession No. JN638063. The remaining sequence data will also be deposited in the database. We believe that the findings of this research will contribute to genome studies of *Fusarium* spp, especially *F. culmorum*.

ACKNOWLEDGMENTS

Research supported by the Research Fund of the Istanbul University (#T1494/04092007 and #5765). The authors thank Dr. Berna Tunali from the Department of Plant Protection, Agricultural Faculty, Samsun Ondokuz Mayıs University, for providing fungal material.

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