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SHORT COMMUNICATION

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Identification of *Fusarium graminearum* and *Fusarium culmorum* Isolates via Conventional and Molecular Methods

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ABSTRACT

Objective: *Fusarium* spp. cause Fusarium head blight (FHB) and crown rot (CR) diseases. They also have harmful effects on animal and human health through their mycotoxins. Within the scope of this study, *F. graminearum* and *F. culmorum* isolates were purified from wheat ears and stalks contaminated with phytopathogens, which had been collected from various regions of Turkey, were identified and characterized by conventional and molecular methods.

Materials and Methods: Sixty-eight *Fusarium* samples were isolated by single spore analysis and classified according to their macroconidia shape and size. Morphologically characterized samples were verified by amplification of SCAR markers. Their mating types (MAT) and chemotypes were also determined through polymerase chain reaction (PCR).

Results: Thirty-eight *F. graminearum* and 30 *F. culmorum* isolates were identified via amplification of UBC85 and OPT18 SCAR markers, respectively. All isolates were determined as trichothecene producers by amplification of the *tri5* gene. All *F. graminarum* isolates carry both *MAT-1* and *MAT-2* loci, whereas 7 of *F. culmorum* isolates were also determined as MAT-1 and 23 of them as MAT-2 mating types. Deoxynivalenol production capacity of all isolates was identified by *tri13* amplification for chemotype determination.

Conclusion: Routine monitoring of phytopathogens and their mycotoxin levels is a requirement since their annual levels may vary depending on environmental factors. This work provides knowledge about the distribution of *Fusarium* spp. leading to FHB and CR in different regions of Turkey between 2010 and 2020. Also, their chemotypes were demonstrated. Our studies will contribute to disease profiling and it is the first step in disease management.

Keywords: *Fusarium* spp., macroconidia, SCAR marker, mating types, trichothecene producers, chemotyping

INTRODUCTION

The soilborne genus *Fusarium* involves a high number of phytopathogenic fungal species that are able to cause diseases by infecting agriculturally important crops including cereals, tobacco, bananas, and carnations, and can also induce health issues in humans and animals through their mycotoxins (1-5). *Fusarium* species are responsible for various dominant diseases on plants including Fusarium head blight (FHB), crown root (CR), or Fusarium wilt via the involvement of different organs like roots, flowers, and spikes in cereal crops. *F. graminearum* and *F. culmorum* are prevalent species causing FHB and CR diseases worldwide as well as in Turkey (6-9). *Fusarium* spp. are widely distributed

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in soil, aerial plant parts, plant debris, and other organic substrates and the causal agents may vary from one agro-ecological region to another (10-12). They are able to produce various mycotoxins at one and the same time. The best known of these endotoxins are trichothecenes, fumonisins, zearalenone, and gibberellic acids. The trichothecenes are divided into four types (A-D) in terms of their additional radical groups onto the heterocyclic core skeleton (13). Deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol, T-2 toxin, and NX-2 are the most studied and well-known trichothecene mycotoxins (14-17). Their toxicity arises from their effects on different cellular mechanisms. Trichothecenes are responsible for oxidative stress-mediated DNA damage, induction of apoptosis through mitochondria-mediated or -independent pathways, and inhibition of protein synthesis by inhibiting the peptidyl transferase activity via binding to the large ribosomal subunit (60S) of eukaryotic cells. Therefore, mycotoxin accumulation as a result of the consumption of contaminated plants and animal products as food leads to potentially severe health problems in humans (18,19). Besides the health concerns based on toxicoses, *Fusarium* spp.-based diseases have both economic effects on agricultural areas and a remarkable number of risks for the vegetation. By considering all these risks, chemotyping, carried out by chemical profile determination of distinct mycotoxins, which are represented by a family or group of related preliminary compounds, is a requirement for the detection of effective methods to cope with the diseases directly caused by pathogens and toxication on animals or humans caused by its natural metabolites. It also has great importance in the characterization of phytopathogenic fungi (20,21).

The fundamental step in the taxonomy of any fungal species is morphological characterization. The classification of closely related *Fusarium* species is carried out according to the distinction of asexual spore types; macroconidia, microconidia and chlamydospores. Remarkable expertise in morphological analyses in *Fusarium* taxonomy is required. Some *Fusarium* species consist of very closely related members. Because differences among them are unclear, they are mentioned as members of the species complex (22). Therefore, the classification of a species complex based on morphological analyses is insufficient for taxonomic characterization. Hence, molecular approaches along with morphological characterization are effectively used in this field, as they provide rapid access to delicate and accurate findings for the identification of species. The amplification of a targeted definite genomic region also known as genotyping, nucleic acid sequencing both any specific regions or the whole genome and chemotyping carried out by using DNA amplification methods or chromatographic-based methods are efficiently used for classification and characterization of *Fusarium* species (2,21,23).

In the current study, a total of 68 isolates belonging to *Fusarium* species collected from infected cereal plants were identified and characterized based on conventional and molecular approaches. After the classification of the fungal isolates was carried out according to their macroconidia types, genus and species identifications were performed by using molecular assays. Also, their mating types (MAT) and chemotypes were determined through amplification methods.

MATERIALS AND METHODS

Single Spore Isolation and Growth Conditions

Wheat ears and stalks with FHB and CR symptoms were collected from different agricultural regions of Turkey (Çanakkale, Balıkesir, Tekirdağ, Amasya) between 2010 and 2020 (Figure 1). Thirty-eight *F. graminearum* (Table 1) and 30 *F. culmorum* (Table 2) samples included for analysis were isolated by the single spore isolation technique (24). After plant samples were treated with 15% NaOCl for 3 minutes, surface sterilization was completed by washing with sterile distilled water three times. Explants, which were dehumidified on blotting paper, were transferred to potato dextrose agar (PDA) medium (4 g/L potato extract, 20 g/L dextrose, 15 g/L agar) and incubated for 3 days at 25±2°C with 60% humidity. After incubation, 0.25 cm^2 diameter plates taken from *in vitro* fungal cultures were suspended

Figure 1. The isolates used in the study were obtained from where marked on the map.

Table 1. The list of *Fusarium graminearum* isolates originated from infected wheat ears and stalks. Their agroecological distributions, mating types and chemotypes.

Species	Isolate	Region/ Year	Mating Type		DON Chemotype
			MAT-1	*MAT-2*	
F. graminearum	Fg19W1	Amasya (2019)	+	+	+
	Fg19W2	Amasya (2019)	+	+	+
	Fg19W3	Amasya (2019)	+	+	+
	Fg19W4	Amasya (2019)	+	+	+
	Fg19W5	Amasya (2019)	+	+	+
	Fg19W6	Amasya (2019)	+	+	+
	Fg19W7	Amasya (2019)	+	+	+
	Fg19W8	Amasya (2019)	+	+	+
	Fg19W9	Amasya (2019)	+	+	+
	Fg19W10	Amasya (2019)	+	+	+
	Fg19W11	Amasya (2019)	+	+	+
	Fg19W12	Amasya (2019)	+	+	+
	Fg19W13	Amasya (2019)	+	+	+
	Fg19W14	Amasya (2019)	+	+	+
	Fg19W15	Amasya (2019)	+	+	+
	Fg19W16	Amasya (2019)	+	+	+
	Fg19W17	Amasya (2019)	+	+	+
	Fg19W18	Amasya (2019)	+	+	+
	Fg19W19	Amasya (2019)	+	+	+
	Fg19W20	Amasya (2019)	+	+	+
	Fg19W21	Amasya (2019)	+	+	+
	Fg19W22	Amasya (2019)	+	+	+
	Fg19W23	Amasya (2019)	+	+	+
	Fg19W24	Amasya (2019)	+	+	+
	Fg19W25	Amasya (2019)	+	+	+
	Fg19W26	Amasya (2019)	+	+	+
	Fg19W27	Amasya (2019)	+	+	+
	Fg20W28	Amasya (2020)	+	+	+
	Fg20W29	Amasya (2020)	+	+	+
	Fg20W30	Amasya (2020)	+	+	+
	Fg20W31	Amasya (2020)	+	+	+
	Fg20W32	Amasya (2020)	+	+	+
	Fg20W33	Amasya (2020)	+	+	+
	Fg20W34	Amasya (2020)	+	+	+
	Fg20W35	Amasya (2020)	+	+	+
	Fg20W36	Amasya (2020)	+	+	+
	Fg20W37	Amasya (2020)	+	+	+
	Fg20W38	Amasya (2020)	+	+	+

Table 2. The list of *Fusarium culmorum* isolates originated from infected wheat ears and stalks. Their agroecological distributions, mating types and chemotypes.

Species	Isolate	Region/ Year	Mating Type		DON Chemotype
			MAT-1	*MAT-2*	
F. culmorum	CM61	Çanakkale (2014)	+	-	+
	BB7	Balıkesir (2014)	-	+	+
	BB18	Balıkesir (2014)	-	+	+
	BG14	Balıkesir (2014)	-	+	+
	BH149	Çanakkale (2010)	-	+	+
	BH192	Tekirdağ (2010)	+	-	+
	BBR15	Balıkesir (2014)	-	+	+
	BBR16	Balıkesir (2014)	+	-	+
	BBR7	Balıkesir (2014)	-	+	+
	BBR8	Balıkesir (2014)	-	+	+
	D823	Çanakkale (2010)	-	+	+
	C606	Çanakkale (2010)	+	-	+
	L901	Balıkesir (2010)	-	+	+
	CE291	Çanakkale (2014)	-	+	+
	CE293	Çanakkale (2014)	-	+	+
	CB205	Çanakkale (2014)	+	-	+
	M211	Balıkesir (2010)	-	+	+
	M213	Balıkesir (2010)	-	+	+
	M214	Balıkesir (2010)	-	+	+
	TK309	Tekirdağ (2014)	-	+	+
	N201b	Balıkesir (2010)	-	+	+
	TC13	Tekirdağ (2014)	-	+	+
	BH226	Çanakkale (2010)	-	+	+
	TY13	Tekirdağ (2014)	-	+	+
	TY21	Tekirdağ (2014)	-	+	+
	TY82	Tekirdağ (2014)	-	+	+
	CA202	Çanakkale (2014)	-	+	+
	D815	Çanakkale (2010)	+	-	+
	CB206	Çanakkale (2014)	+	-	+
	TC14	Tekirdağ (2014)	-	+	+

in 1 mL of phosphate buffered saline. Forty microliters of the suspension was transferred to water-agar (WA) for 16 hours under the same conditions, explained above. Single spores were selected and transferred to PDA medium for a 7-day incubation with the same environmental conditions above and pure cultures were obtained.

Species Description Based on Morphological Characters

Species identification of isolates was carried out *in vitro* cultures grown on PDA medium from a single spore according to their macroconidia structures under the light microscope by following the characteristics explained below (16). Macroconidia of *F. graminearum* are relatively rare *in vitro* cultures while sporodochia are abundant. The macroconidium is medium length and thick-walled. Its ventral surface is moderately curved to straight while the dorsal side is neatly arched. Macroconidia of *F. culmorum* are uniform in shape and size. Their sporodochia are abundant. The macroconidium is relatively short, and thick-walled. Its midpoint is wide. The dorsal side is somewhat curved, but the ventral is nearly straight. It is quite wide in comparison to its size.

Genomic DNA (gDNA) Isolation and Analysis

In this study, gDNAs were extracted from 40 mg mycelium for each sample by using Nucleospin Tissue, Mini Kit for DNA from Cells and Tissue (Macherey-Nagel™, Germany) with the manufacturer's instructions. The quality and quantity of gDNAs were spectrophotometrically determined by using NanoDrop™ 2000 (Thermo Fisher Scientific, USA). Also, their integrity was controlled by agarose gel electrophoresis conducted at 65V for 60 min. Amplicons were visualized under a UV transilluminator (Avegene X-Lite 200).

Classification of Isolates by Using Polymerase Chain Reaction (PCR)

Classification of all isolates at species level, identification of their mating types and chemotypes were determined by using PCR. The Tox 5-1/2 primer set (Table 3) was used for the classification of the *Fusarium* genus. Species-level determination was performed with two different sets. The UBC85F/R and OPT18F/R primer pairs (Table 3) were used for the identification of *F. graminearum* and *F. culmorum* isolates, respectively. Amplification reactions were carried out in a volume of 25 µL containing; 50 ng gDNA, 1×PCR buffer, 2.5 mM $MgCl_2$, 10 pmol for each primer, 0.4 mM for dNTP mix and 1 U *Taq* DNA polymerase (Thermo Fisher Scientific, Germany) under conditions at 94°C for 5 min as predenaturation and followed by 30 cycles as denaturation, annealing and extension steps at 94°C for 45 sec, 55°C for 45 sec and 72°C for 1.5 min, respectively. Finally, 72°C for 5 min was applied as the final extension step. Amplification products were observed on agarose gel as described in section "Genomic DNA (gDNA) Isolation and Analysis".

The fusαF/R and fusHMGF/R primer pairs targeting the *MAT-1* and *MAT-2* loci, respectively, were used for the determination of the mating types of the isolates (Table 3). The reaction volume was adjusted as 25 µL including 50 ng gDNA, 1×PCR buffer, 1.5 mM $MgCl_2$, 5 pmol for each primer, 0.5 mM for dNTP mix and 1 U *Taq* DNA polymerase. The mixtures were incubated at 94°C for 2 min as predenaturation and followed by 30 cycles as denaturation, annealing and extension steps at 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, respectively. The final extension was obtained at 72°C for 5 min. Amplification products and Gene Ruler 100 bp DNA ladder (Thermo Fisher Scientific, Germany) were run on agarose gel electrophoresis and visualized as previously mentioned in "Genomic DNA (gDNA) Isolation and Analysis".

Chemotype determination was performed by amplifying the *tri13* gene region of isolates. For that purpose, the primer pairs of Tri13NIVF/Tri13R and Tri13F/Tri13DONR were chosen for the determination of NIV and DON chemotypes, respectively (Table 3). PCR mixtures were prepared for 25 µL containing 50 ng gDNA, 1×PCR buffer, 2 mM $MgCl_2$, 5 pmol for each primer, 0.4 mM for dNTP mix and 1 U *Taq* DNA polymerase. PCR conditions were set at 94°C for 2 min as predenaturation, 30 cycles as denaturation, annealing and extension steps at 94°C for 30 sec, 53°C for 45 sec and 72°C for 1.5 min, respectively, and 72°C for 5 min as a final extension. Amplification products were controlled as described above in "Genomic DNA (gDNA) Isolation and Analysis".

RESULTS AND DISCUSSION

F. graminearum and *F. culmorum* are the predominant species responsible for FHB and CR infections in Turkey. Accurate identification of the causal agents is the fundamental step in the diagno-

Table 3. Primers, their target regions on gDNA, their nucleotide sequences and product sizes used for PCR analysis (*gene region, **marker, ***locus)

Primer	Target	Sequence 5' - 3'	Band Size (bp)	References
Tox 5-1 **Tox 5-2**	*tri5**	GCTGCTCATCACTTTGCTCAG CTGATCTGGTCACGCTCATC	658	Niessen and Vogel (1998) (25)
UBC85F **UBC85R**	SCAR**	GCAGGGTTTGAATCCGAGAC AGAATGGAGCTACCAACGGC	332	Schilling et al. (1996) (26)
OPT18F **OPT18R**	SCAR	GATGCCAGACCAAGACGAAG GATGCCAGACGCACTAAGAT	472	
fusαF **fusαR**	*MAT-1****	CGCCCTCTKAAYGSCTTCATG GGARTARACYTTAGCAATYAGGGC	210	Kerényi et al. (2004) (27)
fusHMGF **fusHMGR**	*MAT-2****	CGACCTCCCAAYGCYTACAT TGGGCGGTACTGGTARTCRGG	260	
Tri13NIVF **Tri13R**	*tri13**	CCAAATCCGAAAACCGCAG TTGAAAGCTCCAATGTCGTG	312	Chandler et al. (2003) (28)
Tri13F **Tri13DONR**	*tri13**	CATCATGAGACTTGTKCRAGTTTGGG GCTAGATCGATTGTTGCATTGAG	282	

sis of plant diseases. At the same time, it also aids in establishing efficient methods to understand and control them. Macroconidia of fungi play an important role in the dissemination of fungal diseases (29). Their shape and size are the main primary morphological features used for the differentiation of *Fusarium* species as a conventional classification approach (16,30).

Since traditional morphological techniques are time-consuming and inaccurate, preferring new techniques for the diagnosis of disease and characterization of pathogens has become obligatory. Within this context, nucleic acid amplification-based methods provide accurate and reliable diagnosis and classification of species, identified by conventional approaches, in a short time. In the current study, at first, 38 *F. graminearum* and 30 *F. culmorum* samples isolated from wheat ears and stalks with FHB and CR symptoms collected from different agricultural regions of Turkey were identified at species level based on their macroconidia shape and size (Figure 2). PCR-based molecular methods, targeting the amplification of different genomic regions, were used for the classification of all isolates at species level and the determination of their mating types and chemotypes. Within this context, after the gDNA isolation of 68 *Fusarium* isolates, their amount (70-120 ng/µl) and purity (1.7-1.9) were controlled and found to be convenient for use in PCR amplification. Since, SCAR markers were generated from cloned RAPD or AFLP fragments, linked to a trait of interest or not, those species-specific DNA fragments are amplified for use in species determination (26). Schilling et al. (1996) developed species-specific oligonucleotide primers, that are capable of differentiating two *Fusarium* species (*F. graminearum* and *F. culmorum*) by the amplification of species-specific SCAR markers derived from RAPD fragments (26). Since amplicons with 332 bp specific to *F. graminearum* were amplified in this study from 38 isolates by using a UBC85 primer pair, they were classified as *F. graminearum* (Figure 3). Similarly, 30 isolates were diagnosed as *F. culmorum* by amplifying the 472 bp SCAR marker region specific to *F. culmorum* by using OPT18 primers (Figure 4). In this manner, the identification of both species, already

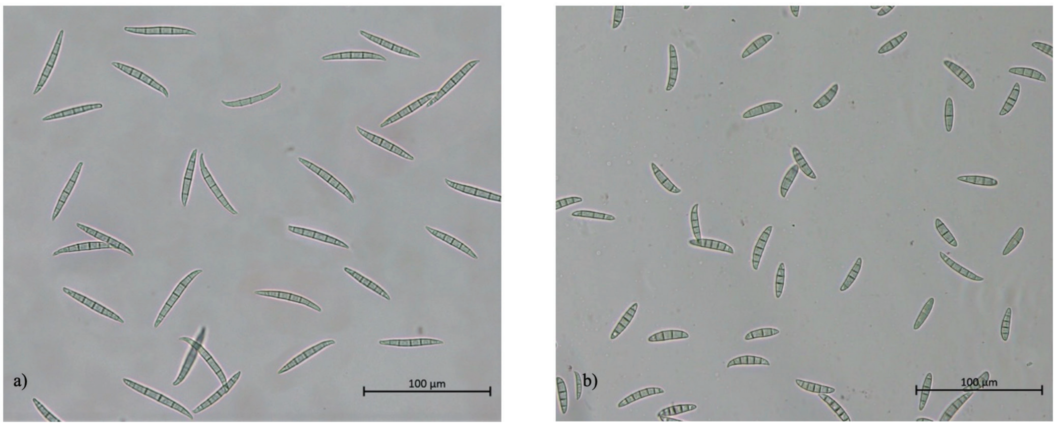

Figure 2. Macroconidia structures of a) *Fusarium graminearum* b) *Fusarium culmorum* under light microscope with total 40X magnification (Eclipse E100, Nikon Instruments, Japan).

Figure 3. PCR products with 332 bp length obtained by using UBC85 F/R primers for species diagnosis of *Fusarium graminearum*. PH-1 was used as positive control. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

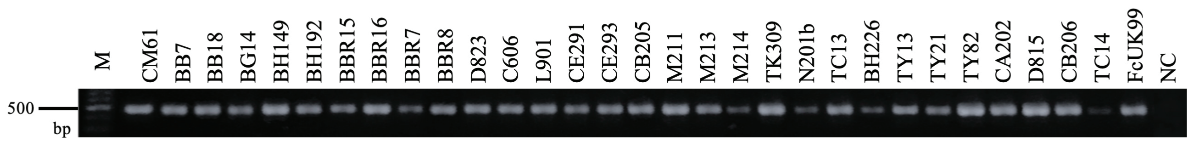

Figure 4. Fragments with 472 bp amplified with OPT18 F/R primers for *Fusarium culmorum* species diagnosis. Electrophoresis was conducted at 65V for 60 min. Fragments were visualized under UV transilluminator (Avegene X-Lite 200). FcUK99 was used as positive control. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

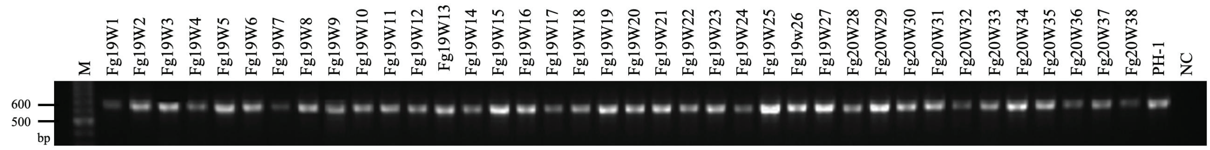

Figure 5. PCR products of 658 bp, amplified with Tox5-1/2 primers for genus diagnosis purpose. Electrophoresis was conducted at 65V for 60 min. Fragments were visualized under UV transilluminator (Avegene X-Lite 200). PH-1 was used as positive control. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

carried out based on morphological characters, were verified by using molecular methods. In the same way, Yörük and Albayrak (2012) described 12 fungal isolates as *F. graminearum* with a UBC85 primer pair, while 20 were identified as *F. culmorum* with OPT18 primers (20). In another study, Yörük et al. (2014) described eight *F. graminearum* and four *F. culmorum* isolates among 17 fungal samples by targeting the same SCAR regions (21). Moreover, Abedi-Tizaki and Sabbagh (2012) identified 51 *F. culmorum* isolates among 344 studied specimens by using the OPT18 primer pair for amplification of the SCAR marker (31).

Trichothecenes are toxic secondary metabolites produced by various fungal species belonging to *Fusarium*, *Myrothecium*, *Stachybotrys* and *Trichoderma* genera. The *tri5* gene, encoding the trichodiene synthase, is located at the beginning of the trichothecene biosynthetic gene cluster and has highly conserved nucleotide sequences. Those sequences enable the detection of trichothecene producers based on amplification (25, 32-34). Therefore, in the current study, the amplification of the *tri5* gene was carried out for the characterization of trichothecene producing *F. graminearum* and *F. culmorum* isolates. A 658 bp fragment belonging to the *tri5* gene was amplified from all isolates with the Tox 5-1/2 primer pair designed by Niessen and Vogel (1998) (25) (Figure 5). Hence, it was shown that isolates of both *Fusarium* species were trichothecene producers. Similarly, trichothecene producers among different *Fusarium* species, isolated from infected crops and fast food, were confirmed by using Tox5 primers (35,36). As can be understood from these studies, *tri5* amplification with Tox5 primer pair is a suitable approach for characterization in terms of the mycotoxin production patterns of phytopathogenic *Fusarium* species obtained directly from crops or processed products.

Biological species concepts can not be used for many species including *Fusarium* as the sexual stage has not been observed under laboratory conditions. Therefore, the determination of MAT alleles via PCR amplification is required to provide information about the sexual stage and compatibility between haploid individuals in heterothallic ascomycetes (37,38). Kerényi et al. (2004) accomplished the determination of mating types in several *Fusarium* species including *F. graminearum* and *F. culmorum* by developing degenerated and semi degenerated primer pairs targeting the conserved α-box and the high-mobility-group (HMG) domains of *MAT-1* and *MAT-2* alleles, respectively. They reported that *F. graminearum*, which is a homothallic species, carries both *MAT-1* and *MAT-2* idiomorphs together whereas heterothallic *F. culmorum* carries only one of the MAT alleles (27). With the aim of isolates' MAT determination, two primer sets for targeting the alleles which were two idiomorphs found in a single locus were used for PCR analysis in the present study. Likewise, it was determined that all *F. graminearum* isolates carried both the *MAT-1* and *MAT-2* loci (Table 1). The 210 bp long amplification products obtained from seven *F. culmorum* isolates with the fusαF/R primer pair revealed these isolates contained only the *MAT-1* allele (Figure 6). The 260 bp long fragments, amplified with fusHMGF/R primers, demonstrated that the remaining 23 *F. culmorum* samples bear only the *MAT-2* locus (Figure 7). These findings obtained from the study were exactly compatible with

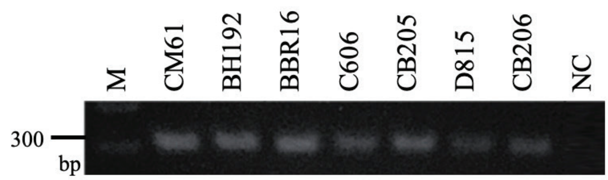

Figure 6. Agarose gel electrophoresis image of PCR products displayed amplicons (210 bp) were obtained from reaction with fusαF/R primers for mating type diagnosis purpose. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

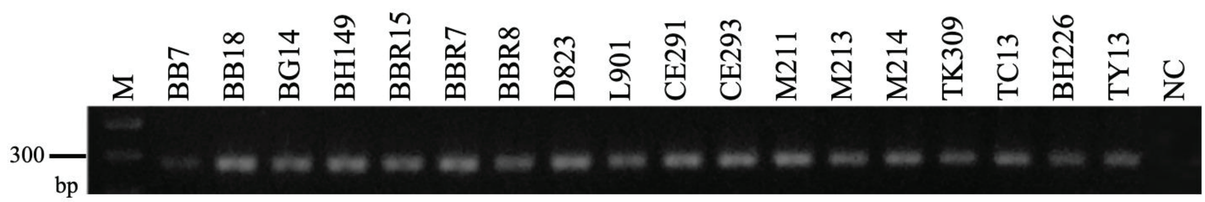

Figure 7. Agarose gel electrophoresis image of PCR products displayed amplicons (260 bp) were obtained from reaction with fusHMG-F/R primers for mating type diagnosis purpose. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

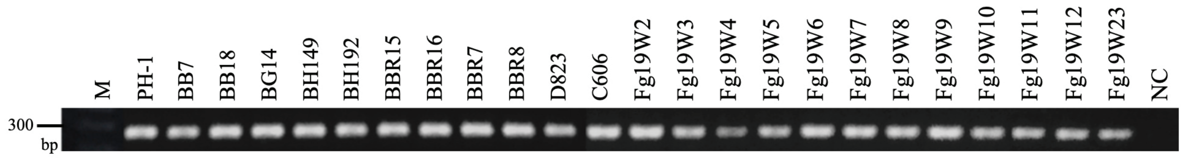

Figure 8. Agarose gel electrophoresis image of PCR products displayed amplicons (282 bp) were obtained from reaction with Tri13F/ Tri13DONR primers for chemotype diagnosis purpose. M: 100 bp (Thermo Scientific, ABD), NC: Negative control.

that of Kerényi et al. (2004) (27). Likewise, it was reported in the previous studies that both *MAT-1* and *MAT-2* alleles were carried together in *F. graminearum* whereas either the *MAT-1* or *MAT-2* idiomorph was detected in *F. culmorum* (39,40).

Since the mycotoxin type and quantity have a considerable effect on epidemics, the designation of mycotoxin production pattern has an influence on the development of control strategies against pathogenic organisms (20). Trichothecenes are the largest group of mycotoxins known to date. In this context, chemotyping of *Fusarium* species is carried out by targeting conserved functional genes located in the *Tri5* gene cluster via PCR-based molecular methods (41). Therefore, for the discrimination of mycotoxins, researchers developed various PCR methods based on revealing the sequence differences of genes that are responsible for the encoding of mycotoxin production enzymes.

Discrimination between DON and NIV-producing isolates was mainly determined from *tri7* and *tri13* genes in the *Tri5* gene cluster in *Fusarium* species. NIV-producing isolates carry functional copies of both genes (28,42), whereas DON producers have deleted regions on both of them (42-44). However, it was also reported that there are certain DON-producing isolates with full-length *tri7* while carrying nonfunctional *tri13* (44). Therefore, as functional *tri7* is a rare event for DON producers, targeting *tri13* instead of *tri7* became a more consistent strategy in chemotyping for isolates in terms of detection of DON or NIV producing capacity (28,45-47). Chandler et al. (2003) performed chemotyping of *Fusarium* isolates by amplifying the *tri13* gene region encoding P450 monooxygenase (28). In the current study, all *Fusarium* isolates were identified according to the amplification of two different regions of the *tri13* gene. For that purpose, two different primer sets designed by Chandler et al. (2003) were chosen (28). The potential of NIV production was not determined because the expected 312 bp amplicons could not be obtained from any of the isolates by PCR using Tri13NIVF/ Tri13R primers. However, all isolates were determined as having potential DON production via obtaining 282 bp amplicons by PCR assay which was performed with the Tri13F/Tri13DONR primer pair (Figure 8). Similarly, Tóth et al. (2004) identified the DON and NIV producer *F. graminearum* and *F. culmorum* isolates based on *tri13* amplification (40). Although it is known that the DON chemotype is common in Turkey (48,49), after the *F. graminearum* isolate with NIV production capacity was reported by Yörük and Albayrak (2012) for the first time (20), this chemotype was also detected by different researchers (50,51).

Species identification of phytopathogenic fungal agents and their chemotyping via PCR assay ensure a reliable diagnosis, and also provide developing influential struggle strategies against pathogens (20). For that purpose, genotypic and chemotypic identification of 68 *Fusarium* isolates, purified by single spore analysis, through different amplification strategies was performed in this study. Since studied isolates originated from four different populations of Turkey (Tekirdağ, Çanakkale, Balıkesir and Amasya) and their geographical discrimination inferred two different regions (Marmara and Black Sea), data became supportive for revealing the agro-ecological distribution of *Fusarium* spp. chemotypes.

Since the annual levels of phytopathogenic fungi spread and their produced mycotoxin levels may vary depending on environmental factors, continuous monitoring of phytopathogenic profile and levels of mycotoxins is essential for controlling diseases and ensuring risk assessment arising from contaminated food products. According to these findings from the present study, it could be said that PCR becomes a significant tool for screening plant infecting mycotoxigenic fungi. In addition, determination of mycotoxin distribution via chemotyping in agro-ecological regions is an effective approach for understanding the severity and spread of pathogens. Lastly, further research from other regions of Turkey with more isolates will improve the identification of the regional distribution of both *F. graminearum* and *F. culmorum* and their produced mycotoxins. Through this research, a new wild-type culture collection including *F. graminearum* and *F. culmorum* isolates was established from different agricultural areas with the aim of being utilized in future projects.

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