Turkish Journal of Agriculture - Food Science and Technology, 6(2): 145-149, 2018

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X
www.agrifoodscience.com,
Turkish Science and Technology

Genotyping via Sequence Related Amplified Polymorphism Markers in *Fusarium culmorum*

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ARTICLE INFO

Research Article

Received 15 July 2017
Accepted 11 December 2017

Keywords:
Fusarium culmorum
Genetic diversity
MAT-1
MAT-2
PCR

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ABSTRACT

Fusarium culmorum is predominating causal agent of head blight (HB) and root rot (RR) in cereals worldwide. Since *F. culmorum* has a great level of genetic diversity and the parasexual stage is assumed for this phytopathogen, characterization of isolates from different regions is significant step in food safety and controlling the HB. In this study, it was aimed to characterize totally 37 *F. culmorum* isolates from Turkey via sequence related amplified polymorphism (SRAP) marker based genotyping. *MAT-1/MAT-2* type assay was also used in order to reveal intraspecific variation in *F. culmorum*. *MAT-1 and MAT-2* specific primer pairs for mating assays resulted in 210 and 260 bp bands, respectively. 11 of isolates were belonged to *MAT-1* type whereas 19 samples were of *MAT-2*. Remaining *7* samples yielded both amplicons. Totally 9 SRAP primer sets yielded amplicons from all isolates. Genetic similarity values were ranged from 39 to 94.7%. Total band number was 127 and PCR product sizes were in the range of 0.1-2.5 kb. Amplicon numbers for individuals were ranged from 1 to 16. According to data obtained from current study, SRAP based genotyping is powerful tool for supporting the data obtained from investigations including phenotypic and agro-ecological characteristics. Findings showed that SRAP-based markers could be useful in *F. culmorum* characterization.

DOI: https://doi.org/10.24925/turjaf.v6i2.145-149.1423

Introduction

Head blight (HB) and root rot (RR) are diseases in economically important cereals including wheat and barley worldwide. The diseases are caused by more than ten *Fusarium* species. *Fusarium culmorum*, a single phylogenetic species, is a major causal agent of HB in many agro-ecological regions located in West-Asia, Europe and America (Obanor et al., 2010; Pasquali et al., 2016). Diseases caused by *F. culmorum* resulted in severe economic losses due to decrease in crop quality and quantity (Smiley et al., 2005; Miedaner et al., 2008). Moreover, diseased kernels are contaminated with trichothecenes, zearalenon and fusarins of which are harmful to both humans and animals. Chronic and fatal mycotoxicosis are caused by these mycotoxins and estrogenic effects and protein synthesis inhibition are most famous of those (Desjardins and Proctor, 2007). Thus, struggling with *F. culmorum* is crucial in management of HB and RR diseases.

Several strategies are present in order to struggle with adverse effects caused by *F. culmorum*. Usage of disease resistant cereal cultivars, antagonistic and antimicrobial approaches and gene silencing/genetic manipulations are some of them (Arslan and Baykal, 2002; Wisniewska and Kowalczyk, 2005; Scherm et al., 2011). However, these strategies do not provide an effective and global success in disease management. Reliable, stable and precise strategy which can overcome the *F. culmorum* adverse effects is associated with comprehensive characterization of *F. culmorum* populations worldwide. *F. culmorum* is anamorph species with no known sexual stage. Mating type is controlled via *MAT1-1* or *MAT1-2* alleles (Kerényi et al., 2004). However, parasexual status is suggested by Miedaner et al. (2001, 2008). This suggestion was also supported by mating type analysis by location of two idiomorphic alleles on a single isolates genome (Obanor et al., 2010, Çepni et al., 2013; Albayrak et al., 2016).

Detailed characterization of *F. culmorum* is in need of providing powerful disease management worldwide. Polymerase chain reaction (PCR) based genotyping strategies can provide supportive output associated with morphological and phenotypic diversity characteristics.

PCR based DNA marker methods present reliable, reproducible and fast assays in characterization of heterothallic *F. culmorum* species. Several strategies including random amplified polymorphic DNA, inter simple sequence repeats and simple sequence repeats, have been currently used in *F. culmorum* genotyping (Miedaner et al., 2001; Gurel et al., 2010; Irzykowska et al., 2013; Albayrak et al., 2016). Sequence related amplified polymorphism (SRAP) method uses two primers with nucleotide sequence of conserved coding sites throughout the genomes. The method is powerful tool for genetic mapping and genetic diversity analysis (Li and Quiros, 2001; Irzykowska et al., 2013; Mahmoud, 2016). Moreover reliable and supportive output can be provided for making association between phenotypic characteristics (aggressiveness, linear growth rate etc.) and genotypes. In this study, characterization of *F. culmorum* isolates from different agro-ecological regions of Turkey is carried out. For this purpose, genotypic assays of mating type differentiation and SRAP based characterization were maintained in this study.

Materials and Methods

Fungal Material and DNA extraction

37 *F. culmorum* isolates obtained from diseased wheat and barley kernels of different regions in Turkey were used in this study (Table 1). Each isolates were identified at species level via morhological characteristics and species-specific SCAR markers formerly by Yörük et al. (2016). Fungal isolates were grown at 25°C on Czapek Dox Agar (CDA). 7-day-old cultures were used in extraction of genomic DNA. That was isolated by using slightly modified version of the sodium dodecyl sulphate-based protocol of Niu et al. (2008). Quantitative and qualitative analyzes of genomic DNAs were examined by 1% agarose gel electrophoresis and spectrophotometer (Shimadzu, Japan). DNAs were photographed via gel visualization system under UV light (Maestrogen, USA).

Mating Type Analysis

Primer pairs, targeting *MAT1-1* specific α box and *MAT2* specific HMG box, developed by Kerényi et al. (2004) were used in mating type characterization of *F. culmorum* isolates (Table 2). PCR mixture was conducted on 25 µL including 25 ng genomic DNA, 1 X PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP's, 0.25 µM for each primer, 1 U *Taq* DNA polymerase (Solis Bio Dyne, Estonia). The cycling conditions were as the same as reported by Kerényi et al. (2004). PCR bands were analyzed on 1.5% agarose gels as given before.

SRAP Assays

Totally 9 primer set combinations were formed using 3 forward and 3 reverse primers (see Table 2). PCR mixture was conducted on 25 µL. The mixture was containing components as follows; 50 ng genomic DNA, 1 X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP's, 0.4 µM for each primer, 1 U *Taq* DNA polymerase (Solis Bio Dyne, Estonia). The cycling conditions were as the same protocol provided by Zhang et al. (2014). PCR bands were visualized on agarose electrophoresis as described before.

Statistical Analysis

SRAP bands were scored according to their presence and absence as ''1'' and ''0'', respectively. A similarity matrix was formed using Nei-Li's coefficient (Nei and Li, 1979). A dendrogram was generated via the unweighted pair group method with arithmetic average algorithm (UPGMA) in MVSP 3.1 software (Kovach, United Kingdom).

Results and Discussion

The *Fusarium culmorum* isolates used in this study were characterized by their morphological characteristics. Fungal isolates were successfully grown on CDA medium. Genomic DNAs with high quality ($A_{260/280}$= ~1.8) were isolated from 7-day-old cultures in the amount of 0.5-3µg/µL.

After DNA extraction, 37 *F.culmorum* isolates were subjected to mating type analysis. According to the mating type analysis, 11 isolates yielded 210 bp amplicon corresponding to *MAT-1* (data not shown), 19 isolates were belonged to *MAT-2* type since their mating type analysis resulted in 260 bp (Figure 1). The remaining seven samples corresponded to both *MAT-1* and *MAT-2* alleles.

Genetic variation was investigated among 37 *F. culmorum* isolates using Sequence Related Amplified Polymorphism (SRAP) technique. For this purpose, 9 SRAP primer combinations (ME1-EM1, ME1-EM3, ME1-EM6, ME3-EM1, ME3-EM3, ME3-EM6, ME5-EM1, ME5-EM3 and ME5-EM6) were used. In SRAP analysis, each primer sets yielded amplicons in all isolates (Figure 2). PCR product sizes were in the range of 0.1-2.5 kb. Totally 114 markers were obtained from 9 primer sets. Minimum and maximum band numbers for individuals were as 1 to 16, respectively. Even if majority of isolates from related and close agro-ecological regions were co-clustered in the same sub-clusters. Some isolates were located with isolates from different regions at same sub-cluster meaning that heterogeneity was present Turkish *Fusarium* isolates.

The dendrogram constructed using the SRAP data shows that the isolates to be divided into two divisions (Figure 3). Each cluster also included two sub-divisions. In SRAP analysis, the similarity matrix among 37 isolates of *F. culmorum* were determined by Nei and Li (1979) coefficient (data not shown). The genetic similarity rate was ranged from a minimum value of 39 % to a maximum value of 94.7%. The highest similarity rate (94.7%) was observed between F4 isolate and F12 isolate. Both isolates were originating from Marmara Region. The lowest similarity rate was observed between *F. culmorum* 14-1 isolate and 19F. While *F. culmorum* 14-1 isolate was obtained from diseased barley kernels in Sivas city, *F. culmorum* 19 isolate of wheat was originated from Eskisehir.

Table 1 Isolates used in this study

Isolate	Year	Host	Region	Chemotype	Mating type
F1	2006	Wheat	Marmara	DON + 3-AcDON	MAT1-1
F2	2006	Wheat	Marmara	DON + 3-AcDON	MAT1-2
F3	2006	Wheat	Konya	DON + 3-AcDON	MAT1-2
F4	2006	Wheat	Marmara	DON + 3-AcDON	MAT1-2
F10	2006	Wheat	Bilecik	DON + 3-AcDON	MAT1-1
F12	2006	Wheat	Balıkesir	DON + 3-AcDON	MAT1-2
F14	2006	Wheat	Bilecik	DON + 3-AcDON	MAT1-2
F15	2006	Wheat	Sinop	DON + 3-AcDON	MAT1-1
F16	2006	Wheat	Konya	DON + 3-AcDON	MAT1-1
F17	2006	Wheat	Konya	DON + 3-AcDON	MAT1-1
F19	2006	Wheat	Konya	DON + 3-AcDON	MAT1-1
09-1TR	2009	Wheat	Kastamonu	DON + 3-ADON	MAT1-1/2
15-3TR	2015	Barley	Tekirdağ	DON + 3-ADON	MAT1-2
F20	2006	Wheat	Bilecik	DON + 3-AcDON	MAT1-2
F21	2006	Wheat	Uşak	DON + 3-AcDON	MAT1-1
F24	2006	Wheat	Konya	DON + 3-AcDON	MAT1-2
15-4TR	2015	Wheat	Edirne	DON + 3-ADON	MAT1-2
17F	2009	Wheat	Ankara	DON + 3-AcDON	MAT1-1/2
18F	2010	Wheat	Eskişehir	DON + 3-AcDON	MAT1-1
19F	2010	Wheat	Eskişehir	DON + 3-AcDON	MAT1-1/2
20F	2011	Barley	Afyon	DON + 3-AcDON	MAT1-1/2
14-1TR	2014	Barley	Sivas	DON + 3-ADON	MAT1-1/2
14-2TR	2014	Wheat	Samsun	DON + 3-ADON	MAT1-1/2
15-1TR	2015	Wheat	Tekirdağ	DON + 3-ADON	MAT1-1/2
14-3TR	2014	Wheat	Yozgat	DON + 3-ADON	MAT1-1
15-2TR	2015	Wheat	Edirne	DON + 3-ADON	MAT1-2
8F	2009	Wheat	Ankara	DON + 3-AcDON	MAT1-2
9F	2008	Wheat	Isparta	DON + 3-AcDON	MAT1-1
10F	2007	Wheat	Samsun	DON + 3-AcDON	MAT1-2
11F	2009	Wheat	Çorum	DON + 3-AcDON	MAT1-2
13F	2008	Wheat	Konya	DON + 3-AcDON	MAT1-1
12-1TR	2012	Wheat	Konya	DON + 3-ADON	MAT1-2
09-2TR	2009	Wheat	Ankara	DON + 3-ADON	MAT1-2
14-9TR	2014	Barley	Sivas	DON + 3-ADON	MAT1-2
12F	2009	Wheat	Amasya	DON + 3-AcDON	MAT1-2
14-8TR	2014	Wheat	Amasya	DON + 3-ADON	MAT1-2
15-5TR	2015	Wheat	Edirne	DON + 3-ADON	MAT1-2

Table 2 Primers used this study.

Primer	5'-3' sequence	Target Region	Band size in kb
ME1	TGAGTCCAAACCGGATA	Conserved exon	0.2-2.5
EM3	GACTGCGTACGAATTGAC		
EM6	GACTGCGTACGAATTGCA		
ME3	TGAGTCCAAACCGGAAT		
EM1	GACTGCGTACGAATTAAT		
ME5	TGAGTCCAAACCGGAAG		
FusALPHAfor	CGCCCTCTKAAYGSCTTCATG	MAT-1	0.21
FusALPHArev	GGARTARACYTTAGCAATYAGGGC		
FusHMGfor	CGACCTCCCAAYGCYTACAT	MAT-2	0.26
FusHMGrev	TGGGCGGTACTGGTARTCRGG		

According to the findings of the dendrogram, the Turkish *F. culmorum* isolates used in the study were clustered according to the geographical regions. In general, isolates from the same agro-ecological regions were co-clustered in the same sub-division. Moreover sub-division II included both wheat and barley phytopathogenic isolates, whereas sub-division I contained just wheat pathogens. Similarly, isolates with collection year between 2006-2009 included in sub-division I, while 2009-2015 isolates were belonged to sub-division II. In mating type analysis, it was also clear that isolates carrying two alleles are replaced in sub-division II. These findings showed that SRAP-based markers could be useful in detailed genotyping of *F. culmorum* isolates with different characteristics such as geographic region, host type and mating type.

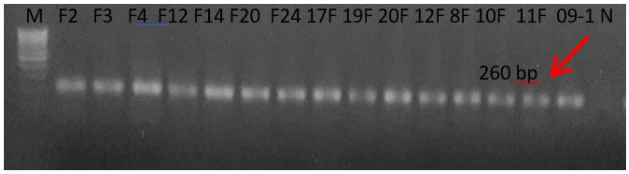

Figure 1 *Mat1-2* profile of *F. culmorum* isolates. M: 100 bp DNA size marker (Thermo, USA), N: no template control

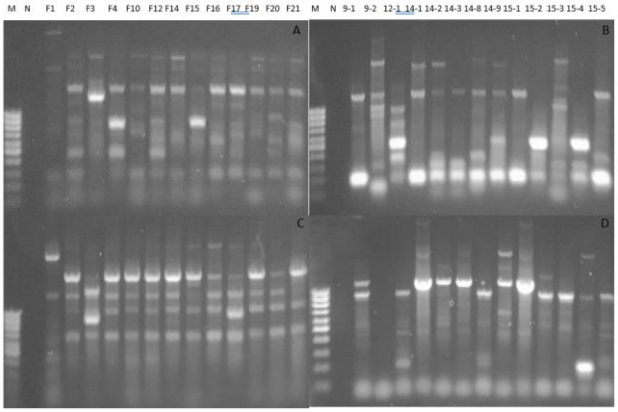

Figure 2 SRAP profiles of isolates by ME5/EM6 (A), ME5/EM1(B), ME1/EM3 (C), ME1/EM6 (D) M: 100 bp DNA markers, N: negative control

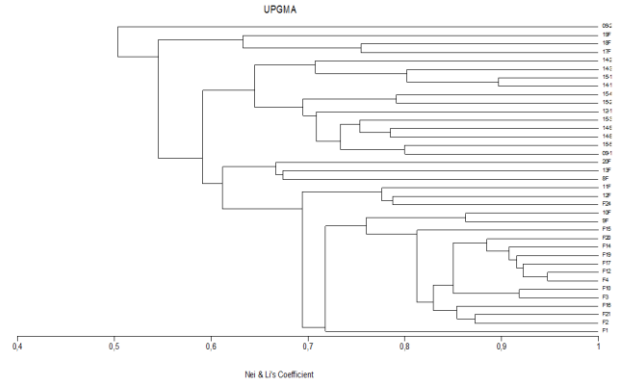

Figure 3 Dendogram showing genetic relatedness among *F. culmorum* isolates based on SRAP analysis

Conclusion

In this study, *F. culmorum* isolates, collected from diseased wheat and barley samples in different agro-ecological regions of Turkey, were subjected to genotyping analysis via mating type and SRAP markers. Findings obtained from both assays showed that isolates with similar and/or same characteristics investigated in this study were co-clustered in UPGMA-dendogram. Isolates with similar profiles could be evaluated in terms of practicing the similar disease control strategies. In this regard, SRAP based genotyping provide fast, reliable and reproducible results for detailed characterization of phytopathogenic isolates. 12 band per primer set was obtained from SRAP analysis which could be accepted as high level of polymorphism profile for a single primer set. Since genetic characterization of the quarantined phytopathogenic species *F. culmorum* is crucial in fight with HB and RR diseases, powerful tools to determine the genotypic variation among *F. culmorum* isolates are needed. SRAP results distinguished isolates according to their several important traits in this study. However further studies, including higher number of isolates and characteristics such as region and host type, should be carried out to get more knowledge about comprehensive genetic characteristics of this phytopathogenic species.

Acknowledgement

This study was supported by the Board of Regents of Istanbul Yeni Yüzyil University and TÜBİTAK 109O476 and 111O835 numbered projects.

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