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RAPD-PCR Analysis of Genetic Variation among Isolates of *Fusarium graminearum* and *Fusarium culmorum* from Wheat in Adana Turkey

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Abstract: The aim of the current study was to estimate the genetic diversity of *F. graminearum* and *F. culmorum* isolated from wheat in Adana province of Turkey by investigating by RAPD-PCR polymorphisms. *Fusarium* sp. affecting wheat crops were surveyed for 2 years during growing seasons in Adana, Turkey. Thirty two isolates of *Fusarium* were isolated from seeds and basal stem nodes of wheat displaying disease symptoms. These isolates were identified as *F. aveneacum*, *F. culmorum*, *F. graminearum* and *F. croockwellense* *Fusarium graminearum* was the predominant pathogen isolated, followed by *F. culmorum*, *F. aveneacum* and *F. croockwellense*, respectively. Genetic distances of *F. graminearum* and *F. culmorum* isolates appeared more intense in wheat fields in Adana region were determined by RAPD-PCR (10 primers). One primer (OPU-17) with *F. culmorum* and two primers (OPU-19 and OPU-17) with *F. graminearum* did not react. Amplification products gave 27 polymorphic bands for *F. culmorum* and 9 bands for *F. graminearum*. The RAPD-PCR analysis showed that *F. graminearum* and *F. culmorum* isolates collected from Adana/Turkey were genetically varied.

Key words: *F. culmorum*, *F. graminearum*, *Fusarium* sp., *Triticum* sp., genetic diversity, RAPD-PCR

INTRODUCTION

Fusarium diseases caused by various *Fusarium* species are among of the most economically important wheat disease in humid and semi-humid wheat growing areas worldwide (Parry and Nicholson, 1996; McMullen *et al.*, 1997; Gilbert and Tekauz, 2000). Infection of wheat by the pathogen reduces grain yield and quality, cause kernel contamination with deoxynivalenol (DON) and other mycotoxins, which are known to be harmful to livestock and pose a safety concern in human food (Miller, 1995; Placinta *et al.*, 1999; Bai *et al.*, 2001; Shaner, 2003). Currently, there is no satisfactory cultural or chemical control for *Fusarium* diseases (McMullen *et al.*, 1997; Shaner, 2003; Tekauz *et al.*, 2003).

Turkey is one of the largest wheat producers in the world with 16-21 million tones of production from 9.35 Mha at an average yield of 2 t ha⁻¹ (Braun *et al.*, 2001). In Turkey wheat is grown with natural rainfall alone or with irrigation in cereal fallow rotation. *Fusarium* species contribute to significant yield losses ranging from 24-36% in cultivated winter wheat varieties commonly grown in Central Anatolian Region (Aktaş *et al.*, 1996; Hekimhan *et al.*, 2004). Wheat has been widely grown for many years in Adana province of Turkey. In the last few

or several years *Fusarium* Head Blight (FHB) and Crown Rot (CR) symptoms have been observed in this region. However, to our knowledge no studies have been carried out to determine the *Fusarium* species that are found on wheat in this region.

The aim of the current study was to estimate the genetic diversity of *F. graminearum* and *F. culmorum* isolated from wheat in Adana province of Turkey by using by (RAPD-PCR) polymorphisms and to assess the usefulness of these characters in identifying *F. graminearum* and *F. culmorum* isolates.

MATERIALS AND METHODS

Sampling: Samples of wheat plants infected with *Fusarium* were collected from stem and ears exhibiting symptoms of *Fusarium* sp., in Adana province. The study was undertaken over the period April-May 2002-2003. Symptomatic plants were collected within each field. In order to isolate *Fusarium* sp. from infected plants, both tissue and grains of infected wheat were surface-sterilized. Intact subcrown internodes were excised from the top of the plants and washed thoroughly in tap water. Dead basal leaf-sheaths were removed from the intact subcrown internodes and cut into 3-5 mm length sections. The

tissue sections and the grains were surface sterilized by immersion in 1% sodium hypochlorite for 1 min and, for 10 min, respectively. They were rinsed thoroughly in sterile distilled water, dried on sterile filter paper and placed onto Potato Dextrose Agar (PDA) supplemented with 100 mg L⁻¹ streptomycin sulfate in 9 cm petri dishes. The dishes were incubated for 4-8 d at 25°C under conditions of alternating light/dark photoperiod (16 h light/8 h dark). Uncontaminated mycelium was transferred to fresh PDA medium for identification. Single-spore isolation was performed for each sample. Cultures were identified based on the morphology of macroconidia, microconidia, chlamydoconidia, conidiophores, general colony morphology and taxonomic descriptions as suggested by Nelson *et al.* (1983) and Nicholson *et al.* (1998). Single-spore cultures of selected isolates were transferred to PDA medium in tubes for preservation.

DNA extraction: *F. graminearum* and *F. culmorum* isolates appeared more intense in wheat fields in Adana region were cultured at 26°C on Potato Dextrose Agar (PDA). Mycelium was scraped from the surface of 7 days old colonies on PDA, inoculated into 100 mL Erlenmeyer-flasks containing 20 mL liquid media (40 g glucose, 5 g peptone, 3 g yeast extract, 3 g malt extract and water up to 1 L) and incubated at 26°C on rotary shaker (150 rpm) for 5 days. Mycelium of *F. graminearum* and *F. culmorum* were harvested by filtration from liquid cultures and ground to fine powder in liquid nitrogen, DNA was extracted and purified for molecular analysis according to the methods of Peever *et al.* (1999). Mycelium of each isolate was extracted with lysis buffer containing 50 mM EDTA, 100 mM Tris-(TE), pH:8.0 and 3% sodium dodecyl sulfate (SDS) for 30-45 min at 65°C. Mycelium was pelleted by centrifugation at 14,000 rpm for 15 min and the supernatant was precipitated with 8 M potassium acetate at -20°C for 15 min. After spinning the supernatant was subjected to two rounds of phenol:chloroform:isoamyl alcohol (25:24:1) extraction and chloroform:isoamyl alcohol (24:1 v/v) extraction. The final supernatant was precipitated with 2 volumes of 100% ethanol and 0.5 M NaCl. Pellets were resuspended in water, precipitated by 14% polyethylene glycol and 1 M NaCl and finally resuspended in 50 µL of TE buffer (10 mM, 1 mM EDTA). RNA was digested with RNase A (Sigma Chemical Co., St. Lois) at 20 µL and 37°C for 3 h. Extracted DNA samples was stored at -20°C until required.

RAPD-PCR Analysis: The total reaction volume was 25 µL and the conditions were 1.5 mM MgCl₂; 100 µM each dNTP; 200 nM oligonucleotide primers (for RAPD-PCR reactions), 1 u of Taq DNA polymerase,

10-20 ng template DNA, 2.5 µL PCR buffer (10 mM Tris-HCl, pH:8.3, 50 mM KCl). The primers used for RAPD-PCR were from Operon Technologies Inc. (Alameda CA). Ten primers OPT-01, OPT-04, OPT-06, OPT-16, OPT-18, OPU-13, OPU-15, OPU-19, OPU-17 and UBC-85 were selected for a preliminary screen of several kits. Amplifications were performed in a DNA Thermocycler (Techne, Genius). For RAPD-PCR amplifications, the thermocycler was programmed for one cycle at 94°C for 1 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. The final cycle had 5 min extension step at 72°C. The PCR fragments were analyzed through 1.5% agarose gel electrophoresis, stained with ethidium bromide (0.5 g mL⁻¹) and visualized under ultraviolet light.

Data analysis: Data were scored as 1 for the presence and 0 for the absence of a DNA bands for each isolates. The data matrix was entered into the MVSP (MultiVariete Statistical Package) program.

RESULTS

Four species of *Fusarium* were isolated from wheat in Adana province. A total of 35 *Fusarium* isolates were obtained and identified as *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. crookwellense* (Fig. 1a, b-4a, b). From these isolates, 43% were identified as *F. graminearum*, 29% as *F. culmorum*, 17%

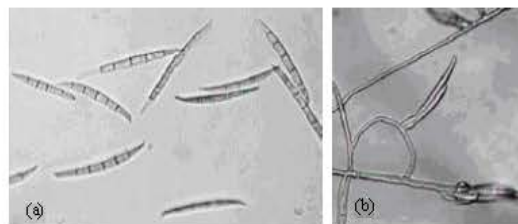


Fig. 1: (a) Macroconidia and (b) conidiophores of *Fusarium avenaceum* (X400)

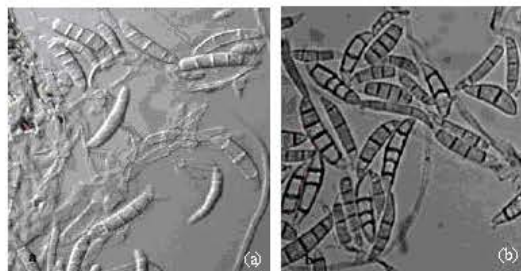


Fig. 2: (a-b) Macroconidia of *Fusarium culmorum* (X400)

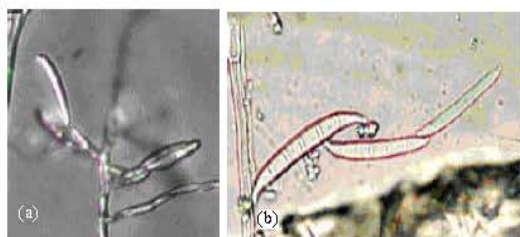


Fig. 3: (a) Conidiophores and (b) macroconidia of *Fusarium graminearum* (X400)

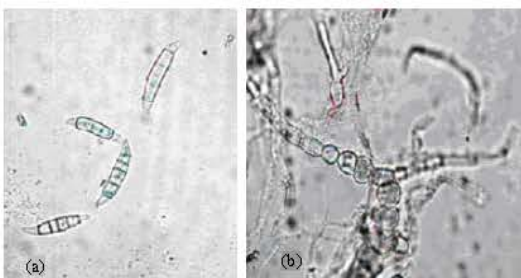


Fig. 4: (a) Macroconidia and (b) chlamydospores of *Fusarium crookwellense* (X400)

as *F. crookwellense* and 11% as *F. avenaceum*. No sexual stage has been identified for *F. culmorum*. The present investigation showed that *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. crookwellense* caused diseases on wheat and *F. graminearum* was the most widely distributed species on winter wheat in Adana province. (data not shown).

RAPD-PCR analysis was performed on 10 isolates of *F. graminearum* and *F. culmorum* appeared more intense in wheat fields in Adana region were determined by RAPD-PCR using 10 different random primers. The genetic variability of Adana province *F. graminearum* and *F. culmorum* isolates was investigated. One primer (OPU-17) with *F. culmorum* and two primers (OPU-19 and OPU-17) with *F. graminearum* did not react. Amplification products gave 27 polymorphic bands for *F. culmorum* and 9 bands for *F. graminearum*. *Fusarium culmorum* isolates were highly varied with most primers, whereas *F. graminearum* was not. *Fusarium graminearum* isolates, all obtained from wheat in Adana province, yielded a 0.4 kb product, whereas *F. culmorum* isolates from wheat yielded a 0.3 kb product (Fig. 5, 6). The data obtained for ten isolates and 10 primers were pooled and a total of 134 bands were scored. Primer OPT.8 amplified a fragment of approximately 0.8 kb from DNA of all isolates of *F.culmorum* and primer OPT-15 amplified a fragment of approximately 0.7 kb from DNA of all isolates of *F. graminearum*.

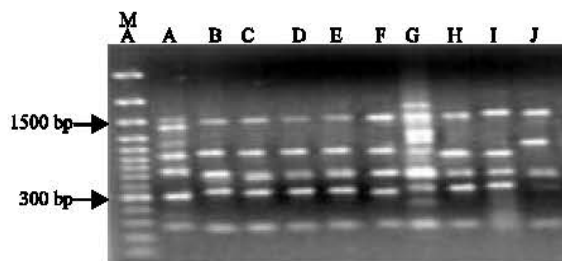


Fig. 5: Agarose gel showing random amplified polymorphic DNA (RAPD-PCR) profiles of *F. culmorum* amplified with arbitrary primer OPT-01 (MA: 100 bp DNA ladder, A: B-1, B: B-2, C: B-3, D: B-4, E: B-5, F: B-8, G: B-9, H: B-10, I: B-11, J: B-12 isolates)



Fig. 6: Agarose gel showing random amplified polymorphic DNA (RAPD-PCR) profiles of *F.graminearum* amplified with arbitrary primer OPT-06 (MA: 100 bp DNA ladder, A: A-15, B: A-8, C: A-6, D: A-2, E: A-3, F: A-4, G: A-11, H: A-13, I: A-16, J: A-9 isolates)

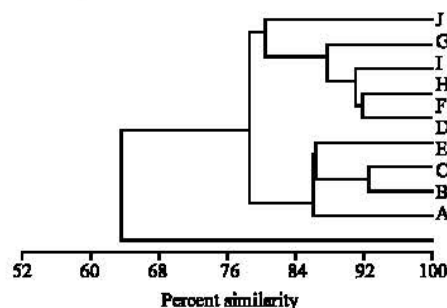


Fig. 7: Dendrogram showing genetic relationships of *Fusarium culmorum* isolates studied. The data matrix was entered into the MVSP (MultiVariate Statistical Package) program

The MVSP analysis by genetic distance failed to identify spatial clustering among the Adana region different geographic regions (Fig. 7). F (B-8), G (B-9), H (B-10), I (B-11), J (B-12) isolates of *F. culmorum* were placed in one group. The isolates of *F. culmorum* could

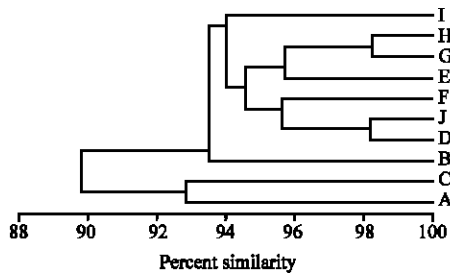


Fig. 8: Dendrogram showing genetic relationships of *F. graminearum* isolates studied. The data matrix was entered into the MVSP (MultiVariate Statistical Package) program

be clustered in 3 groups. The most genetic similarity was observed between isolates F (B-8)/G (B-9). Isolates A (B-1) appeared to be the most genetically distinct.

B (A-8), D (A-2), E (A-3), F (A-4), J (A-9) and I (A-16) isolates of *F. graminearum* were placed in one group. Isolates of I (A-16) and J (A-9) were the most genetically similar. The isolates of *F. graminearum* could be clustered in 2 groups. Isolates A (A-15) and C (A-6) constituted a different group and were the most genetically distinct (Fig. 8). As a result, the RAPD-PCR analysis revealed that *F. graminearum* and *F. culmorum* isolates collection from Adana/Turkey were genetically varied.

DISCUSSION

In this study the fungal isolates were identified a combination of morphological and molecular marker techniques. *Fusarium avenaceum*, *F. culmorum*, *F. graminearum* and *F. crookwellense* have been reported to cause foot rot and head blight on winter wheat in Europe (Parry and Nicholson, 1996; Ahmad Khan *et al.*, 2005). The present investigation showed that *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. crookwellense* caused diseases on wheat and *F. graminearum* was the most widely distributed species on Winter wheat in Adana province. The results clearly support the results of other European surveys indicating the increasing importance of *F. graminearum* as a major wheat pathogen. *F. graminearum*, *F. moniliforme*, *F. culmorum*, *F. sporodotricoides*, *F. tritricana*, *F. heterosporium* and *F. avenaceum* had previously been reported on wheat plants in Turkey (Aktaş *et al.*, 1996, 1999). The present study is the report of *Fusarium* species of wheat in Adana, Turkey.

The objective of this study was to estimate the extent of genetic diversity of *F. graminearum* and *F. culmorum*

appeared more intense in wheat fields in Adana region, Turkey by means of RAPD-PCR markers. It was successfully applied for differentiation of *F. graminearum* and *F. culmorum*. One primer (OPU-17) with *F. culmorum* and two primers (OPU-19 and OPU-17) with *F. graminearum* did not react. Amplification products have given 27 polymorphic bands for *F. culmorum* and 9 bands for *F. graminearum*. RAPD-PCR analysis revealed a high level of genetic variability in populations of *F. graminearum* and *F. culmorum*. The RAPD-PCR analysis suggests that the Adana/Turkey *F. graminearum* and *F. culmorum* isolate collection was genetically varied. Some researchers have reported high levels of genotypic variation using RFLP analysis of *F. culmorum* and *F. avenaceum* strains in England (Mieadaneer and Schilling, 1996). On the other hand, little variation was observed among isolates of *F. culmorum* based on RAPD-PCR analysis (De Nijs *et al.*, 1997). The fungal isolates identified using a combination of morphological and DNA marker techniques. Each isolate had to show the correct morphology on PDA and yield 0.8 kb from DNA of all isolates of *F. culmorum* and a fragment of approximately 0.3 kb from DNA of all isolates of *F. graminearum* in the RAPD-PCR analysis. RAPD-PCR assays are more convenient, because there is no need for radioactive probes. Results obtained so far indicate that it will be continued to generate molecular markers necessary to DNA fingerprint *F. graminearum* and *F. culmorum*. Isolates of *F. graminearum* and *F. culmorum* from wheat kernels and stem were analyzed. RAPD-PCR analysis revealed a high level of genetic variability in populations of *F. culmorum*. Other researchers have reported high levels of genetic diversity in local populations of *F. culmorum*. Schilling *et al.* (1996) found a high degree of genetic variability for aggressiveness within single field populations of *F. culmorum* and *F. graminearum*. De Nijs *et al.* (1997) reported that RAPD-PCR analysis of 17 *F. culmorum* isolates revealed that most of the genetic diversity was within population while genetic diversity between populations represented a small proportion of the total.

In conclusion *F. graminearum* and *F. culmorum* isolates collection from Adana/Turkey was genetically varied. The ability of RAPD-PCR analysis for isolates of *F. graminearum* and *F. culmorum* provided a tool for further investigations into these pathogens. Knowledge of *Fusarium* population diversity in Turkey is essential for developing effective disease management strategies. This information will be useful for ecological and epidemiological studies, especially in the development of resistant cultivars through improved screening procedures and will also optimize chemical and biological controls.

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