Research Article/Araştırma Makalesi

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2017) 30(1): 27-33

Molecular identification of *Fusarium* spp. causing wilt of chickpea and the first report of *Fusarium redolens* in Turkey

Türkiye'de nohutta solgunluğa neden olan *Fusarium* spp.'nin moleküler tanımlaması ve *Fusarium redolens*'in ilk raporu

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ARTICLE INFO

Received 09 August 2016
Received in revised form 14 November 2016
Accepted 14 November 2016

***Keywords*:**

Chickpea
Fusarium oxysporum
Fusarium redolens
Race-specific primers
Species-specific primers

ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important food legume crop and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the most important diseases of chickpea in Turkey. *Fusarium redolens* is known to cause wilt-like disease of chickpea in other countries, but has not been reported from Turkey. Accurate identification of pathogen species and races is important for managing the disease in developing and deploying resistant cultivars. Forty five *Fusarium* spp. isolates caused wilt of chickpea were obtained from 10 provinces in Turkey from 2007 to 2009. Species level identification of these isolates was done using PCR primers specific for *F. oxysporum* f. sp. *ciceris* and *F. redolens* along with sequencing of the *Translation Elongation Factor 1-α* (*Ef-1α*) gene region. Among the isolate collection, eight isolates were identified as *F. redolens*, and eleven isolates as *F. oxysporum* f. sp. *ciceris*, based on PCR with species-specific primers. Seven of the eleven *F. oxysporum* f. sp. *ciceris* isolates were further identified as race 0, based on PCR with race-specific primers. Moreover, sequence data of *Ef-1α* region were used to identify any isolate that did not give amplification with *F. oxysporum* f. sp. *ciceris* race specific primers and *F. redolens* specific primers. This is the first report of *F. redolens* causing chickpea wilt in Turkey.

MAKALE BİLGİSİ

Alınış tarihi 09 Ağustos 2016
Düzeltilme tarihi 14 Kasım 2016
Kabul tarihi 14 Kasım 2016

***Anahtar Kelimeler*:**

Nohut
Fusarium oxysporum
Fusarium redolens
Irk-spesifik primerler
Tür-spesifik primerler

ÖZ

Nohut (*Cicer arietinum* L.) önemli bir yemeklik baklagil türüdür ve *Fusarium oxysporum* f. sp. *ciceris* tarafından neden olunan Fusarium solgunluğu Türkiye'de en önemli nohut hastalıklarından biridir. *Fusarium redolens*'in bazı ülkelerde nohutta solgunluk benzeri hastalığa neden olduğu bilinmektedir, ancak Türkiye'den rapor edilmemiştir. Patojen tür ve ırklarının doğru tanımlanması, hastalık yönetimi için dayanıklı çeşit geliştirme ve kullanımında önemlidir. Nohutta solgunluğa neden olan kırkbeş *Fusarium* spp. izolatı 2007'den 2009'a kadar Türkiye'deki 10 ilden elde edilmiştir. Bu izolatların tür seviyesinde tanımlamaları *F. oxysporum* f. sp. *ciceris* ve *F. redolens* için spesifik PCR primerleri ve *Translation Elongation Factor 1-α* (*Ef-1α*) gen bölgesinin sekanslanması ile yapılmıştır. İzolat koleksiyonunda, tür spesifik primerlerle yapılan PCR'a göre, sekiz izolat *F. redolens* ve onbir izolat *F. oxysporum* f. sp. *ciceris* olarak tanımlanmıştır. Onbir *F. oxysporum* f. sp. *ciceris* izolatının yedisi ırk-spesifik primerlerle yapılan PCR'a göre ırk 0 olarak tanımlanmıştır. Ayrıca, *F. oxysporum* f. sp. *ciceris* ırk-spesifik ve *F. redolens* tür-spesifik primerlerle ürün vermeyen izolatların tanımlamalarında *Ef-1α* bölgesinin sekans verisi kullanılmıştır. Bu, Türkiye'de nohutta solgunluğa neden olan *F. redolens*'in ilk raporudur.

1. Introduction

Morphology-based identification of *Fusarium* spp. is challenging due to the lack of sufficiently informative morphological features (Leslie et al. 2001). Furthermore, morphological characters are influenced by environmental conditions which make pathogen identification difficult using traditional taxonomic approaches (Leslie et al. 2001). *Fusarium oxysporum* Schlect. Emend. Synd. and Hans. is comprised of a large and highly diverse complex of morphologically

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indistinguishable anamorphic fungi (Baayen et al. 2000; Baayen et al. 2001; Bogale et al. 2006). Within this species complex, some strains are important pathogens of plants causing severe crop losses in a large number of plants (Michelse and Rep 2009). These phytopathogenic fungal strains are classified into formae specialis and pathogenic races based on their pathogenic specificity to host species and cultivars, respectively (Armstrong and Armstrong 1981; Nelson et al. 1983; Edel et al. 1995). Recently, species- and race-specific molecular markers have been developed for *Fusarium* spp. and *F. oxysporum* f. sp. *ciceris*, respectively, for accurate and rapid identification (O'Donnell et al. 1998; Jimenez-Gasco and Jimenez-Diaz 2003; Bogale et al. 2007; Gurjar et al. 2009; O'Donnell et al. 2009; Jimenez Fernandez et al. 2011; Dubey et al. 2014).

Fusarium wilt caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *ciceris* (Padwick) is one of the important diseases limiting chickpea production in Turkey. *F. oxysporum* f. sp. *ciceris* is either soil- or seed-transmitted (Haware and Nene 1982; Kraft and Haware 1988; Chen 2011). The pathogen causes wilting, yellowing, vascular discoloration and eventually death of plants (Haware and Nene 1982; Jimenez-Gasco et al. 2001). Until now, eight pathogenic races of *F. oxysporum* f. sp. *ciceris* have been identified (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) worldwide on the basis of pathogenic reaction of a particular *F. oxysporum* f. sp. *ciceris* isolate to a set of differential chickpea cultivars (Haware and Nene 1982; Jimenez-Gasco and Jimenez-Diaz 2003; Sharma et al. 2005; Sharma and Muehlbauer 2007). *F. oxysporum* f. sp. *ciceris* races have been reported from different countries, such as race 0 from Turkey, Tunisia, Spain, California / USA, Syria, Lebanon, Israel and Iraq; race 1A from India, Spain, California / USA, Israel and Morocco; race 1B/C from Turkey, California / USA, Syria, Tunisia and Iraq; races 2 and 3 from Turkey and India; race 4 from India and Iraq; race 5 from California / USA, Spain, Morocco and Iraq; race 6 from California / USA, Spain, Israel and Morocco (Haware and Nene 1982; Phillips 1988; Rahman et al. 1988; Cabrera et al. 1989; Jimenez-Diaz et al. 1993; Halila and Strange 1996; Dolar 1997; Jimenez-Gasco et al. 2001; Bayraktar and Dolar 2012; Al-Taae et al. 2013). However, identification of *F. oxysporum* f. sp. *ciceris* races based on their pathogenicity on chickpea cultivars is a time-consuming procedure as well as experiments could be affected by environmental factors (Haware and Nene 1982). Therefore, different molecular techniques have been investigated for reliable and rapid identification of races of *F. oxysporum* f. sp. *ciceris*. Randomly amplified polymorphic DNA (RAPD) fingerprinting was able to distinguish races 0, 1B/C, 5 and 6 (Jimenez-Gasco et al. 2001). Sequence characterized amplified regions (SCAR) markers are available for races 0, 1A, 5 and 6 (Jimenez-Gasco and Jimenez-Diaz 2003). Gurjar et al. (2009) identified Indian *F. oxysporum* f. sp. *ciceris* races 1, 2 and 4 by using a combination of DNA-based methods. Even though several studies have been conducted to identify *F. oxysporum* f. sp. *ciceris* races based on classical methods (Dolar 1997; Bayraktar and Dolar 2012) and genetic diversities were investigated among some *Fusarium* pathogens of chickpea using RAPD and ISSR (inter-simple sequence repeats) in Turkey (Bayraktar et al. 2008; Bayraktar and Dolar, 2009), molecular markers have not been applied to describe races and/or species of Turkish *Fusarium* spp. isolates, until now.

F. redolens was considered to be within the *F. oxysporum* complex or *F. solani* for some researchers due to the difficulties of identifying *Fusarium* spp. using traditional approaches (Booth 1971; Nelson et al. 1983). Moreover, those pathogen species cause similar wilting and rotting symptoms on their hosts (Clarkson 1978; Riccioni et al. 2008; Bienapfl et al. 2010) which further complicates their identification. Using DNA-based methods, the taxonomic position of the pathogen became clear and PCR based methods have been developed to identify this pathogen from different crops including chickpea (Bogale et al. 2007; Gambhir et al. 2010; Jimenez-Fernandez et al. 2011; Leisso et al. 2011; Edel-Hermann et al. 2012). However, *F. redolens* has not been previously reported from Turkey as a chickpea pathogen (Asan 2011).

In this study, we performed species and race identification of *Fusarium* spp. and *F. oxysporum* f. sp. *ciceris*, respectively, from chickpea in Turkey using species- and race-specific PCR primers.

2. Materials and Methods

2.1. Fusarium spp. Isolates

Samples of severely wilted plants were collected from 33 fields in 10 provinces in Turkey during July of the years 2007, 2008 and 2009 (Table 1). All collected plant samples showed typical wilt symptoms including discoloration of xylem tissue of stems. Isolations were made from fourth-node stem sections of the plants which showing wilt symptoms (Tekeoglu et al. 2000). These pieces were washed with tap water and surface sterilized with 1 % NaOCl solution for three minutes, rinsed in sterile distilled water and plated on modified potato dextrose agar (PDA) 1/4 strength (9 g PDA Merck, 10 g Bacto agar, 1 L distilled water), amended with streptomycine sulfate (100 mg mL^{-1}) and oxytetracycline (60 mg mL^{-1}). Plates were incubated seven days under cool white fluorescent light at 23 ºC, 15 h photoperiod. Colonies developing on 1/4 strength PDA were sub-cultured and then transferred to synthetic nutrient agar (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose and 20 g agar / L distilled water) at the same condition as described above. Plates were examined under stereo microscope. Colonies on PDA were floccose and range in color from white to pale violet. Both macroconidia and microconidia were formed and chlamydospores were observed in culture for all isolates. *Fusarium* spp. was identified based on descriptions of Booth (1977) and Burgess et al. (1994). After morphologic identification of *Fusarium* spp., single spore isolations were carried out and cultures from single spore colonies were used for further analyses.

2.2. DNA extraction and molecular analyses

DNA was isolated from each of the isolates listed in Table 1. Mycelia from each isolate were grown in potato dextrose broth and harvested for DNA isolation. DNA was isolated using MP Bio DNA isolation kit according to protocol provided by the manufacturer. DNA concentrations were checked on Nanodrop1000 (NanoDrop products, Wilmington, DE, USA) at 260-280 wave length and adjusted to use in PCR reactions. PCR analyses were performed using species-specific primers for *F. redolens* and *F. oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 1B/C, 5 and 6 developed by Bogale et al. (2007) and Jimenez-Gasco and Jimenez Diaz (2003). Primers 'LR3 and CS33' (White et al. 1990; Visser et al. 1995) were used as positive control for successful PCR amplifications from all DNA samples. PCR reactions and cycle conditions were applied as described by Jimenez-Gasco and Jimenez Diaz (2003) and

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Table 1. Isolates of *Fusarium* spp. identified with molecular markers in this study.

Isolate	City	Field	Year	*F. oxysporum f. sp. ciceris*[a]	*F. oxysporum* f. sp. *ciceris* and its races[a]					*F. redolens*[a]	GeneBank Accession Number[b]	*Ef1-α* The best match[c]
					Race 0	Race 1B/C	Race 5	Race 6	Race 1A/6			
K29F	Kütahya	Altıntaş 4	2007	-	-	-	-	-	-	-	KT286750	*F. solani* HQ731053.1 (99%) FD_01524 (100%)
K30F	Kütahya	Altıntaş 4	2007	-	-	-	-	-	-	-	KT286751	*F. oxysporum* KF913726.1 (98%) FD_00799 (98%)
K31F	Kütahya	Altıntaş 4	2007	-	-	-	-	-	-	-	KT286752	*F. oxysporum* FJ538243.1 (99%) FD_00786 (99%)
K32F	Kütahya	Altıntaş 4	2007	+	+	-	-	-	-	-		
D55F	Denizli	Tavas 1	2007	-	-	-	-	-	-	-	KT286742	*F. oxysporum* KF574856.1 (99%) FD_00809 (99.7%)
D56F	Denizli	Tavas 2	2007	-	-	-	-	-	-	-	KT286743	*F. oxysporum* KF574856.1 (99%) FD_01216 (99%)
D57F	Denizli	Tavas 3	2007	+	+	-	-	-	-	-		
D58F	Denizli	Tavas 6	2007	-	-	-	-	-	-	+		
D59F	Denizli	Acıpayam 1	2007	-	-	-	-	-	-	+		
D59Fs	Denizli	Acıpayam 1	2007	+	-	-	-	-	-	-	KT286744	*F. oxysporum* KF574859.1 (99%) FD_00799(100%)
D60F	Denizli	Acıpayam 2	2007	-	-	-	-	-	-	+		
D167F	Denizli	Baklan 1	2008	-	-	-	-	-	-	-	KT286745	*F. oxysporum* KF574859.1 (99%) FD_00799 (100%)
B65F	Burdur	Yeşilova 1	2007	-	-	-	-	-	-	-	KT286735	*F. hostae* DQ854862.1 (97%) *F. beomiforme* FD_01329 (88.9%)
B67F	Burdur	Yeşilova 1	2007	-	-	-	-	-	-	-	KT286736	*F. solani* FN689813.1(100%) FD_01390 (100%)
B68F	Burdur	Yeşilova 3	2007	-	-	-	-	-	-	-	KT286737	*F. solani* HQ731048.1 (99%) FD_01054 (99.8%)
I69F	Isparta	Yalvaç 1	2007	-	-	-	-	-	-	-	KT286746	*F. oxysporum* FJ538243.1 (100%) FD_00178 (99.4%)
I70F	Isparta	Yalvaç 1	2007	+	-	-	-	-	-	-	KT286747	*F. oxysporum* KF913726.1 (99%) FD_00799 (100%)
I71F	Isparta	Şarkikaraağaç1	2007	-	-	-	-	-	-	-	KT286748	*F. solani* KF939494.1 (99%) FD_01054 (100%)
I72F	Isparta	Şarkikaraağaç1	2007	-	-	-	-	-	-	+		
I156F	Isparta	Şarkikaraağaç2	2008	+	+	-	-	-	-	-		
I157F	Isparta	Şarkikaraağaç3	2008	+	+	-	-	-	-	-		
I158F	Isparta	Şarkikaraağaç4	2008	-	-	-	-	-	-	-	KT286749	*F. oxysporum* KF574854.1 (99%) FD_00799 (100%)
I159F	Isparta	Şarkikaraağaç4	2008	+	+	-	-	-	-	-		
I160F	Isparta	Yalvaç 2	2008	+	+	-	-	-	-	-		
I161F	Isparta	Yalvaç 2	2008	+	+	-	-	-	-	-		
Ko81F	Konya	Beyşehir 1	2007	+	-	-	-	-	-	-	KT286754	*F. oxysporum* FJ538243.1 (100%) FD_00178 (99%)
Ko82F	Konya	Bozkır 1	2007	-	-	-	-	-	-	-	KT286756	*F. verticillioides* KJ464994.1 (99%) FD_01388 (99.8%)
Ko82Fs	Konya	Bozkır 1	2007	-	-	-	-	-	-	+		

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Table 1 continued. Isolates of *Fusarium* spp. identified with molecular markers in this study.

Isolate	City	Field	Year	*F. oxysporum f. sp. ciceris*[a]	*F. oxysporum* f. sp. *ciceris* and its races[a]					*F. redolens*[a]	GeneBank Accession Number[b]	*Ef1-α* The best match[c]
					Race 0	Race 1B/C	Race 5	Race 6	Race 1A/6			
Ko83F	Konya	Kadınhanı 1	2007	-	-	-	-	-	-	-	KT286757	*F. oxysporum* KF574856.1 (99%) FD_00809 (100%)
Ko83Fs	Konya	Kadınhanı 1	2007	-	-	-	-	-	-	+		
Ko143F	Konya	Kadınhanı 2	2008	-	-	-	-	-	-	-	KT286753	*F. oxysporum* KF913726.1 (99%) FD_00799 (100%)
Ko148F	Konya	Derbent 1	2008	+	-	-	-	-	-	-	KT286758	*F. oxysporum* FJ538243.1 (100%) FD_00178 (99.5%)
Ko151F	Konya	Hüyük 2	2008	-	-	-	-	-	-	+		
S99F	Sivas	Yıldızeli 1	2007	-	-	-	-	-	-	-	KT286760	*F. oxysporum* KF574856.1 (99%) FD_00809 (100%)
S100F	Sivas	Yıldızeli 2	2007	-	-	-	-	-	-	-	KT286759	*F. oxysporum* KF574856.1 (99%) FD_00809 (100)
Y118F	Yozgat	Yozgat 1	2007	-	-	-	-	-	-	-	KT286762	*F. oxysporum* KF913726.1 (99%) FD_00799(100%)
Y119F	Yozgat	Yozgat 1	2007	-	-	-	-	-	-	-	KT286763	*F. verticilloides* KF562131.1 (99%) FD_01388 (99%)
Ç124F	Çorum	Alaca 1	2007	-	-	-	-	-	-	-	KT286738	*F. oxysporum* KF913726.1 (99%) FD_00799 (99.8%)
Ç196F	Çorum	Külah	2008	-	-	-	-	-	-	-	KT286739	*F. proliferatum* JF747030.1 (100%) FD_01379 (99.7%)
Ç198F	Çorum	Mecitözü 1	2008	-	-	-	-	-	-	-	KT286740	*F. oxysporum* KF913726.1(100%) FD_00799(100%)
Ç202F	Çorum	Çorum	2009	-	-	-	-	-	-	-	KT286741	*F. oxysporum* KF913726.1 (99%) FD_00799 (99.9%)
Ant131F	Antalya	Korkuteli 4	2008	-	-	-	-	-	-	+		
Ant132F	Antalya	Korkuteli 5	2008	-	-	-	-	-	-	-	KT286733	*F. proliferatum* JF747030.1 (100%) FD_01379 (99.7%)
Ant133F	Antalya	Elmalı 1	2008	-	-	-	-	-	-	-	KT286734	*F. oxysporum* KF574859.1 (99%) FD_00799 (100%)
Sa209F	Samsun	Havza	2009	-	-	-	-	-	-	-	KT286761	*F. equiseti* JQ412101.1(100%) FD_01695(99.8%)

[a]: PCR analyses were performed using species-specific primers for *F. redolens* and *F. oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 1B/C, 5 and 6 developed by Bogale *et al.* (2007) and Jimenez-Gasco & Jimenez Diaz (2003). Primer information was shown in Table 2. [b]: GenBank accession number of *Translation Elongation Factor-1α* of the *Fusarium* spp. isolates used in this study.
[c]: Sequence similarity with one of the most related sequences from NCBI-GeneBank and from FUSARIUM-ID v.1.0 databases, respectively, based on the *Translation Elongation Factor-1α* sequences.

Bogale et al. (2007). 25 µL volume of PCR contained 5X reaction buffer, 4 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 1 U of Taq polymerase (Promega, Madison, WI) and 30-50 ng of DNA. All PCR reactions were carried out in a thermal cycler GeneAmp PCR Systems 9700 (Applied Biosystems, Foster City, CA, USA) using the programs described by Jiménez-Gasco and Jiménez-Díaz (2003), Bogale et al. (2007), Jimenez-Fernandez et al. (2011). PCR products were detected on 1 % agarose gels, stained with ethidium bromide and visualized under UV light on a gel documentation system ChemiDOC$_{TM}$XRS (BIO-RAD, USA). Sequence data of *Translation Elongation Factor 1-α* (*Ef-1α*) region were used to identify any isolate that did not give amplification with *F. oxysporum* f. sp. *ciceris* race specific primers and *F. redolens*-specific primers for species level identification at molecular level. The primer pair of EF1 and EF2 (O'Donnel et al. 1998) was used. A PCR reaction contained 10-15 ng of template DNA, 1X PCR buffer (Applied Biological Materials Inc., Canada), 4mM $MgCl_2$ (Applied Biological Materials Inc., Canada) 200 µM dNTPs, and 1 µM of each primer. The PCR conditions were 94 °C for 2 min, 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min followed by a final step at 72 °C for 10 min. Reactions were carried out with a Bio-Rad T100 thermalcycler (Bio-Rad, USA). PCR products were detected on 1.5 % agarose gels, stained with 5 µL 100 mL^{-1} of SafeView (Applied Biological Materials Inc., Canada) dye and visualized under UV light on a gel documentation system Vilber Lourmat Quantum ST4 1100 (Vilber Lourmat, France). After successful

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amplifications, PCR products were sequenced in ABI 3500xL Genetic Analyzer (Applied Biosystems). DNA sequences were edited with Bioedit v7.0.53 for Windows software (Hall 1999) and aligned using clustalW implemented in BioEdit software. Each sequence data used as a query to search similarities using BLASTn of the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990) and the FUSARIUM-ID v.1.0 database (http://fusarium.cbio.psu.edu) (Geiser et al. 2004).

3. Results

Forty-five isolates were obtained, each from a single diseased plant. Exceptionally, isolates D59F and D59Fs were isolated from the same plant, as it was the case with isolates Ko82F and Ko82Fs, isolates Ko83F and Ko83Fs. PCR amplification with primer pair LR3/CS33 as a positive control produced a 900 bp amplicon successfully for all 45 isolates, indicating that the DNA samples were suitable for PCR amplification. Within sample collection, 11 isolates produced a positive amplicon of expected size in PCR with primer pair Foc0-12f and Foc0-12r specific for *F. oxysporum* f. sp. *ciceris*. Seven of the 11 isolates of *F. oxysporum* f. sp. *ciceris* produced a positive PCR product of 900 bp size in amplification with primers FocR0-M15 f/r specific for race 0 (Jimenez-Gasco and Jimenez Diaz 2003) (Table 1). The seven race 0 isolates were detected in Denizli, Isparta and Kütahya provinces. The rest four of the 11 isolates did not give successful amplification in PCR using race specific primer pairs to detect race 1A, 1B/C, 5 and 6 even though these isolates were confirmed as *F. oxysporum* f. sp. *ciceris* based on positive amplicon of Foc0-12f and Foc0-12r primer pairs.

Among the collection of 45 isolates, eight isolates produced an amplicon of expected 386 bp size in PCR with *F. redolens*-specific primers (Redolens F/R) (Bogale et al. 2007) (Table 1) and they were found in Antalya, Denizli, Isparta, and Konya provinces.

Ef-1α region of *Fusarium* spp. samples that did not give amplification with neither *F. oxysporum* f. sp. *ciceris* race specific primers nor *F. redolens*-specific primers were sequenced for species level identification. Each isolate resulted ~750 bp amplicon and control reactions without template DNA gave no product. All of the sequenced isolates were belong to *Fusarium* genus based on the partial sequencing of *Ef-1α* region and the sequences were deposited in GenBank (accession numbers KT286733-KT286763, Table 1). Results of identification based on both the best matches of partial sequencing of *Ef-1α* region deposited in NCBI genebank and the most related sequences FUSARIUM-ID v.1.0 database at http://fusarium.cbio.psu.edu (Geiser et al. 2004) presented in Table 1.

4. Discussion

Accurate and rapid identification of *F. oxysporum* f. sp. *ciceris* races and other *Fusarium* species related in chickpea diseases such as *F. redolens* is important for the appropriate and efficient management of Fusarium wilt. Since the most practical management of the disease is using resistant cultivars, identification of new species and races would help breeders to select appropriate germplasm for cultivar development. Identification of disease causing *Fusarium* spp. also may help cultural practices for management, e.g., crop rotation and sowing time. This study demonstrated species and race level identifications and distributions of *Fusarium* spp. related with wilt disease of chickpea in Turkey by using PCR techniques and sequencing. Using species and race specific PCR-primers for identification of pathogens is more rapid and less costly technique when compared to traditional taxonomic or RFLP based approaches and we successfully applied some species and race specific PCR primers for *Fusarium* spp. and *F. oxysporum* f. sp. *ciceris*, respectively, to our samples.

Within the sample collection, eleven isolates gave amplification with *F. oxysporum* f. sp. *ciceris* specific primers. Race specific primers for *F. oxysporum* f. sp. *ciceris* detected seven race-0 isolates and none of the other primer pairs for other races (1A, 1B/C, 5 and 6) were successful to give product for the remaining isolates which are possibly *F. oxysporum* f. sp. *ciceris*. It might be caused that those *F. oxsyporum* f. sp. *ciceris* race specific primer pairs may not universal for all *F. oxsyporum* f. sp. *ciceris* populations or maybe the other isolates are belong to race 2 or 3 or 4 for which race-specific primers were not available. Moreover, races 2 and 3 were detected in Turkey by using traditional methods (Dolar 1997; Bayraktar and Dolar 2012). Race detection by conventional methods was not intended in this study, but it would be informative for those isolates to detect race groups by using specific host varieties.

Our study has shown that molecular markers could solve misidentification problems for morphologically and pathogenically similar pathogens. Some of the isolate pairs (D59F and D59Fs, Ko82F and Ko82Fs, K83 and Ko83Fs) were isolated from the same plant sample. Morphologically, one of these isolate pairs (coded Fs) seemed to be *F. solani* while the others (coded F) identified as *F. oxysporum*. But, identification based on sequence data of *Ef-1α* prevented misidentification (Table 1). Sequencing of *Ef-1α* region was useful to solve misidentifications of *Fusarium* spp. in other researches, as well (Gurjar et al. 2009; Jimenez-Fernandez et al. 2011). Moreover, some of the isolates in the collection causing wilt symptoms of chickpea were identified as different *Fusarium* species rather than *F. oxysporum* (Table 1) which indicates pathogenicity characteristics also may cause misinterpretation. Thus, in addition to morphologic and pathogenic features, consistent and strong molecular approaches should be applied for accurate specification.

In this study, *F. redolens* isolates, for the first time, were identified and reported from chickpea in Turkey. Jimenez-Fernandez et al. (2011) also stated that those *F. redolens* isolates had been misdiagnosed as *F. oxysporum* f. sp. *ciceris* previously. Even though *F. oxysporum* f. sp. *ciceris* and *F. redolens* are morphologically indistinguishable and cause similar symptoms, molecular techniques are very useful to differentiate these species as shown in this study and previous studies (Bogale et al. 2007; Jimenez-Fernandez et al. 2011). Similar situation was observed for *F. redolens* from tomato (Edel-Herman et al. 2012), as well. Three isolates were distinguished as *F. redolens* among *Fusarium* spp. isolates sampled from diseased tomato in Algeria by using molecular markers (Edel-Herman et al. 2012). Recently, the association of *F. redolens* with wilting-like symptoms in chickpea in Lebanon, Morocco, Pakistan and Spain was reported (Jimenez-Fernandez et al. 2011). It is needed to understand etiology and epidemiology of the chickpea disease caused by *F. oxysporum* f. sp. *ciceris* and *F. redolens*. Additionally, *F. redolens* has been referred as a pathogen on different legumes (Clarkson 1978; Riccioni et al. 2008; Bienapfl et al. 2010; Gambhir et al. 2010). In addition to that, it would be useful to test pathogenicity of

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these isolates on other different plants to understand whether those isolates show host specificity or not. Besides, *F. redolens* isolates responsible for chickpea disease may lead to grouping into a new *formae specialis* as previously applied to *F. oxysporum* f. sp. *dianthi* and *F. redolens* f. sp. *dianthi* isolates causing carnation diseases (Baayen et al. 1997). It is important to detect these species distribution both on chickpea and other crops to know better what the problem source is and how to manage it.

Acknowledgment

Turkish Higher Education Board (YÖK) was supported part of this research by a grant awarded to Mücella Tekeoğlu.

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