TÜBİTAK

Turk J Agric For
33 (2009) 1-10

doi:10.3906/tar-0709-37

Genetic Diversity of Wilt and Root Rot Pathogens of Chickpea, as Assessed by RAPD and ISSR

Harun BAYRAKTAR*, F. Sara DOLAR

Department of Plant Protection, Faculty of Agriculture, University of Ankara, 06110 Ankara - TURKEY

Received: 28.09.2007

Abstract: Intra- and interspecific polymorphisms among fungal pathogens that cause wilt and root rot on chickpea were investigated by using 30 RAPD (random amplified polymorphic DNA) and 20 ISSR (inter-simple sequence repeats) primers. UPGMA (unweighted pair group method with arithmetic average) cluster analysis of RAPD and ISSR datasets using Dice's coefficient differentiated all fungal isolates from each other and revealed considerable genetic variability between the isolates. The isolates that were not easily separable using morphological characteristics were classified again according to their branching in dendrograms. A partial correlation was observed between cluster analysis and geographic origin of *Fusarium solani* isolates. Additionally, high cophenetic correlation values for RAPD and ISSR data, using the MXCOPH algorithm, were observed (0.983 and 0.987, respectively). The results show that both analyses were suitable for the evaluation of genetic polymorphisms among fungal populations.

Key Words: Chickpea, root rot, genetic diversity, RAPD, ISSR

Nohut Solgunluk ve Kök Çürüklüğü Patojenleri Arasındaki Genetik Farklılığın RAPD ve ISSR ile İncelenmesi

Özet: Nohutta solgunluk ve kök çürüklüğüne sebep olan fungal patojenler arasındaki intra ve interspesifik polimorfizimler 30 RAPD (Random amplified polymorphic DNA) ve 20 ISSR (Inter-simple sequence repeats) primeri kullanılarak incelenmiştir. Elde edilen RAPD ve ISSR verilerinin Dice' ın benzerlik katsayısı kullanılarak yapılan UPGMA (Unweighted Pairgroup Method with Arithmetic Average) cluster analizi sonucunda tüm fungus izolatları birbirinden ayrı olarak gruplandırılmış ve aralarında önemli genetik farklılıklar tespit edilmiştir. Morfolojik karakterler kullanılarak ayırt edilemeyen izolatlar dendrogramdaki dağılımlarına göre yeniden sınıflandırılmışlardır. *Fusarium solani* izolatlarının cluster analizi ve coğrafik orijini arasında ise kısmi bir korelasyon bulunmuştur. Ayrıca MXCOPH algoritması kullanılarak belirlenen (RAPD: 0.983, ISSR: 0.987) kofenetik korelasyon değerlerinin oldukça yüksek olduğu tespit edilmiştir. Bu sonuçlar her iki yöntemin fungus populasyonları arasındaki genetik polimorfizimlerin incelenmesi için oldukça faydalı olduğunu göstermiştir.

Anahtar Sözcükler: Nohut, kök çürüklüğü, genetik farklılık, RAPD, ISSR

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume in Turkey, with total acreage and production being 660,000 ha and 620,000 t, respectively (FAO, 2007). Fungal disease is the most destructive factor in chickpea production. To date, more than 50 pathogens have been reported on chickpea from different parts of the world; however, only a few of them cause serious economic losses in chickpea production areas, i.e. *Ascochyta* blight (*Ascochyta rabiei*), *Fusarium* wilt (*F. oxysporum* f. sp. *ciceris*), black root rot (*F. solani*), collar rot (*Sclerotium rolfsii*), wet root rot (*Rhizoctonia solani*),

* Correspondence to: harun.bayraktar@agri.ankara.edu.tr

Phytophthora root rot (*Phytophthora megasperma*), *Pythium* root and seed rot (*Pythium ultimum*), foot rot (*Operculella padwickii*), and stem rot (*Sclerotinia sclerotiorum*) (Nene and Reddy, 1987). Among wilt and root rot pathogens, *Fusarium oxysporum* Schlechtend: Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato is widespread in most chickpea growing areas of the world and results in major economic losses of between 10% and 40% (Nene et al., 1984; Kaiser et al., 1994). Disease control is quite difficult because the pathogens survive in infected soil for years, even in the absence of the host plant (Haware et al., 1996). Other wilt and root rot pathogens have also been reported to cause major crop losses affecting chickpea production in many countries (Trapero-Casas and Jimenez-Diaz, 1985; Pandey and Singh, 1990; Abou-Zeid and Hallila, 2003).

The removal and destruction of chickpea residues, crop rotation, and the use of pathogen-free seed, as well as seed treatment are recommended for the management of wilt and root rot diseases in chickpea. Development of cultivars resistant to or tolerant of soil-borne pathogens is the most practical and effective means of disease control (Nene and Haware, 1980); however, resistant resources are rather restricted by considerable pathogenic variability in fungal populations. Breeders find it necessary to determine the genetic variability within and between pathogen populations in order to develop chickpea cultivars resistant to soil pathogens.

In recent years many molecular techniques have been used to identify and differentiate filamentous fungi at the population, genus, and species level. RAPD analysis has provided many polymorphic DNA markers related to the intra- and interspecific polymorphisms of a wide variety of fungi, including several *Fusarium* species, such as *Fusarium solani* f. sp. *cucurbitae* (Crowhurst et al., 1991), *Fusarium oxysporum* f. sp. *pisi* (Grajal-Martin et al., 1993), and *Fusarium moniliforme* (Huang et al., 1997). ISSR is a molecular technique involving the use of one primer complementary to a target microsatellite region in PCR (Meyer et al., 1993; Zietkiewicz et al., 1994). This fingerprinting technique has been successfully used to determine genetic variability of different plant pathogens, such as *Phytophthora cactorum* (Hantula et al., 2000), *Fusarium culmorum* (Mishra et al., 2003), *Fusarium oxysporum* f. sp. *radicis-lycopersici*, (Balmas et al., 2005), and *Macrophomina phaseolina* (Jana et al., 2005).

The present study was undertaken to assess wilt and root rot diseases on chickpea in Turkey, and to assess the relationship between the geographic origin and genetic diversity of these pathogens using RAPD, ISSR, and pathogenicity testing. The results provided information useful to future population studies of wilt and root rot complex on chickpea, as well as data that can help breeding programs in Turkey.

Materials and Methods

Fungal Isolates

Fungal isolates were obtained from diseased chickpea samples in different vegetative stages from some important chickpea growing provinces in Turkey (Ankara, Eskişehir, Kırşehir, Kayseri, Konya, Burdur, Denizli, Kütahya, Uşak, Tokat, Antalya, Kahramanmaraş, and Diyarbakır). Single spore isolations of the cultures were made on potato dextrose agar (Merck, Darmstadt, Germany) medium and preserved on Microbank® tubes (Pro-Lab Diagnostics, UK) at –80 °C. The study included 11 isolates belonging to different fungal species, which were provided by Prof. Dr. F. Sara Dolar (University of Ankara, Turkey), Prof. Dr. Gülay Turhan (Ege University, Turkey), and Dr. Steiner Stenzel (University of Bonn, Germany). Geographical origin and pathogenicity of the fungal species used in this study are shown in Table 1.

Pathogenicity Tests

Pathogenicity tests were performed using a susceptible chickpea cultivar (ILC-482) according to the pot-culture inoculation method of Nene and Haware (1980). Inoculum was grown in mixtures of sand and chickpea meal (45 g of sand and 5 g of chickpea meal) in 250-ml flasks at 23 ± 1 °C for 10 days. After incubation the inoculum was mixed with 0.6 kg of autoclaved soil in 15-cm pots. Four days later 5 seeds were sown in each pot and grown for 30-40 days at 25 ± 1 °C, with a relative humidity of 30%-50% and a 14-h photoperiod (light intensity, 297 $\mu mol \cdot s^{-1} \cdot m^{-2}$). Three pots were used for each isolate. Control plants were grown in a comparable mixture of non-infested sand, chickpea meal, and autoclaved soil. Disease development on inoculated plants was evaluated on a 0-4 scale, as described by Trapero-Casas and Jimenez-Diaz (1985).

Table 1. Distribution, morphological identification, and virulence of the fungal isolates studied.

Provinces	Morphological identification	Accession no	Years	Virulence[a]
Ankara	*F. oxysporum*	Ank-3	2002	L
	F. oxysporum	Ank-4	2002	H
	F. oxysporum	Ank-5	2002	M
	F. oxysporum	Ank-9	2002	H
	F. oxysporum	Ank-10	2002	M
	F. oxysporum	Ank-11	2002	M
	F. oxysporum	Ank-12	2002	M
	F. oxysporum	Ank-13	2002	H
	F. oxysporum	Ank-17	2002	M
	F. oxysporum	Ank-21	2002	M
	F. oxysporum	Ank-32	2002	M
	F. solani	Ank-25	2002	M
	F. solani	Ank-26	2002	M
	F. solani	Ank-27	2002	M
	F. solani	Ank-28	2002	M
	F. semitectum	Ank-29	2002	M
	F. equiseti	Ank-30	2002	M
	F. equiseti	Ank-31	2002	H
	R. solani	Ank-33	2002	H
	R. solani	Ank-34	2002	M
	M. phaseolina	Ank-36	2002	M
	F. oxysporum[1]	Ka2	1995	-
	F. solani[1]	Ka4	1995	-
	F. solani[1]	Ka8	1995	-
	F. solani[1]	Hay6	1995	-
	F. equiseti[1]	Ank-35	1995	-
	F. moniliforme[1]	Ank-37	1995	-
	F. acuminatum[1]	Ank-38	1995	-
	C. tonkinense[1]	Ank-39	1995	-
Eskişehir	*F. oxysporum*	Esk-2	2002	M
	F. oxysporum	Esk-4	2002	M
	F. oxysporum	Esk-5	2002	L
	F. oxysporum	Esk-6	2002	M
	F. oxysporum	Esk-8	2002	H
	F. equiseti	Esk-12	2002	L
Kayseri	*M. phaseolina*	Kay-1	2002	H
Kırşehir	*F. oxysporum*	Kır-1	2002	L
	F. solani	Kır-3	2002	H
Konya	*F. oxysporum*	Kon-1	2002	M
	F. oxysporum	Kon-3	2002	H
Denizli	*F. oxysporum*	Dez-5	2002	M
	F. oxysporum	Dez-6	2002	M
	F. oxysporum	Dez-8	2002	M
	F. oxysporum	Dez-11	2002	M
	F. oxysporum	Dez-12	2002	M
	F. oxysporum	Dez-14	2002	H
	F. solani	Dez-16	2002	M
	F. solani	Dez-18	2002	M
	F. solani	Dez-19	2002	M
	F. equiseti	Dez-20	2002	M
	F. semitectum	Dez-21	2002	M
Denizli	*F. semitectum*	Dez-22	2002	M
	M. phaseolina	Dez-23	2002	M
	M. phaseolina	Dez-24	2002	H
Uşak	*F. oxysporum*	Uşk-3	2002	H
	F. oxysporum	Uşk-6	2002	M
	F. solani	Uşk-7	2002	M
	F. solani	Uşk-8	2002	M
	F. solani	Uşk-9	2002	M
	F. acuminatum	Uşk-11	2002	M
Kütahya	*F. oxysporum*	Küt-1	2002	M
	F. oxysporum	Küt-2	2002	M
Diyarbakır	*F. oxysporum*	Diyar-6	2001	M
	F. oxysporum	Diyar-13	2001	L
	F. oxysporum	Diyar-16	2001	M
	F. solani	Diyar-19	2001	M
	F. solani	Diyar-20	2001	M
	F. solani	Diyar-21	2001	M
	M. phaseolina	Diyar-22	2001	H
Tokat	*F. oxysporum*	Tok-1	2002	M
	F. oxysporum	Tok-3	2001	M
	F. solani	Tok-4	2001	M
	F. solani	Tok-5	2001	M
Burdur	*F. oxysporum*	Bur-5	2002	M
	F. oxysporum	Bur-6	2002	M
	F. solani	Bur-8	2002	M
	F. solani	Bur-9	2002	L
	F. semitectum	Bur-10	2002	H
	F. semitectum	Bur-11	2002	H
Antalya	*F. solani*	Ant-1	2001	H
K.Maraş	*F. oxysporum*	Km-2	2001	M
	F. oxysporum	Km-4	2001	M
	F. oxysporum	Km-6	2001	M
	F. oxysporum	Km-7	2001	M
	F. oxysporum	Km-9	2001	M
	F. oxysporum	Km-11	2001	L
	F. oxysporum	Km-13	2001	M
	F. oxysporum	Km-14	2001	M
	F. oxysporum	Km-19	2001	H
	F. oxysporum	Km-27	2001	M
	F. solani	Km-21	2001	M
	F. solani	Km-23	2001	M
	F. solani	Km-25	2001	M
	M. phaseolina	Km-28	2001	M
Not known	*F. oxysporum*	Ova-1	2002	H
	F. oxysporum	Gergen	2002	M
	F. moniliforme[2]	Ref-3	-	-
	F. equiseti[2]	Ref-5	-	-
	F. solani[3]	Ref-1	-	-

[a] Low virulence (L), moderate virulence (M), and high virulence (H)

These isolates were provided by [1]Professor Dr. F.S. Dolar, [2]Professor Dr. Gülay Turhan, and [3]Dr. Steiner Stenzel.

Purification of Nucleic Acids

Isolates were grown in 100 ml of potato dextrose broth (Difco, Detroit, MI, USA) at 26 °C on an orbital shaker (150 rpm) for 7 days. Mycelium was harvested by filtration through sterile Miracloth, frozen with liquid nitrogen, and stored at –80 °C until use. Fungal genomic DNA was extracted according to a modified method of Reader and Broda (1985). Ground mycelium in liquid nitrogen was suspended in 500 µl of 200 mM Tris-HCl (pH: 8.5), 25 mM of NaCl, 25 mM of EDTA, and 0.5% SDS. Samples were incubated for 30 min at 65 °C. After adding an equal volume of phenol-chloroform, the mixture was centrifuged at 13,000 ×g for 15 min. Then, 25 µl of RNAse-A was added and incubated for 30 min at 37 °C. The suspension was extracted once with chloroform-isoamyl alcohol and precipitated with 1 volume of isopropanol. The pellet was then rinsed with ethanol, suspended in TE buffer (pH 7.4) and stored at –20 °C.

Amplification Conditions

RAPD-PCR was performed in a total volume of 25 µl of reaction containing 10 mM of Tris-HCl (pH 8.8), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.32 µM of primer, 0.125 mM of dNTPs, and 0.6 U of Taq polymerase (MBI, Fermantase). Thirty oligonucleotides were randomly selected from the OPK and OPA primer series (Operon Technologies Inc. Alameda, CA, USA). Amplification was carried out in a thermal cycler (Biometra) programmed as follows: 40 cycles of 20 s at 94 °C, 1 min at 36 °C, 1 min at 72 °C, and a final cycle of 8 min at 72 °C.

ISSR-PCR was performed in a total volume of 25 µl of reaction containing 10 mM of Tris-HCl (pH 8.8), 50 mM of KCl, 2.5 mM of $MgCl_2$, 0.24 µM of primer, 0.2 mM of dNTPs, and 1 U of Taq polymerase (MBI, Fermantase). Twenty primers with di- or tri- nucleotide repeats in ISSR analysis were used and annealing temperatures were adjusted depending on the G+C contents (Table 2). Amplification was performed as follows: 35 cycles of 30 s at 94 °C, 30 s at annealing temperature, 2 min at 72 °C, and a final cycle of 10 min at 72 °C.

The PCR products were separated electrophoretically in 1.4% agarose gels using 1 × TAE buffer and were stained with ethidium bromide (Sambrook et al., 1989). Gel images were scanned into a computer. All PCR amplifications were performed at least twice for each isolate.

Table 2. Sequences, annealing temperatures (Ta) and G+C content (%) of the primers employed in ISSR analysis.

Primer sequence	(Ta)	G+C Content (%)
$(AG)_8G$	52 °C	52.9
$(GA)_8T$	50 °C	47.1
$(GA)_8C$	50 °C	52.9
$(AC)_8T$	50 °C	47.1
$(AG)_8YT$	52 °C	47.2
$(AG)_8YC$	55 °C	52.8
$(GA)_8YT$	53 °C	47.2
$(GA)_8YC$	54 °C	52.8
$(CA)_8RT$	52 °C	47.2
$(AC)_8YT$	52 °C	47.2
$(AC)_8YA$	53 °C	47.2
$(ATG)_6$	50 °C	33.3
$BHB(GA)_7$	53 °C	51
$(GC)_9YR$	55 °C	95
$(AC)_9RY$	54 °C	50
$(GA)_9RY$	54 °C	50
$(AT)_9YR$	36 °C	5
$(TG)_8RT$	53 °C	47.2
$(AG)_8T$	50 °C	47.1
$(CT)_8RG$	54 °C	52.8

Y = Pyrimidine; R = Purine; B = C, G, or T; H = A, C, or T.

Data Analysis

Comparison of each primer's profile was made on the basis of the presence or absence of PCR fragments at positions. Only bands repeatable in at least 2 experiments with the same primer at different times were evaluated. Using the NTSYS-pc v.2.0 numerical taxonomy package program (Rohlf, 1998), a genetic similarity matrix was created with Dice's coefficient of similarity. The genetic similarity matrix was subjected to cluster analysis with an unweighted pair-grouped method with arithmetic average (UPGMA) to generate a dendrogram. In addition to comparison of the dendrograms formed using these marker systems, cophenetic value matrices were calculated, which were later compared by the Mantel test.

Results

Pathogenicity Test

The virulence spectrum of the fungal pathogens obtained from diseased chickpea plants from 13 Turkish provinces was determined on a susceptible chickpea cultivar (ILC-482). They included 48 isolates of *Fusarium oxysporum*, 22 isolates of *F. solani*, 6 isolates of

Macrophomina phaseolina, 5 isolates of *F. semitectum*, 4 isolates of *F. equiseti*, 2 isolates of *Rhizoctonia solani*, and 1 isolate of *F. acuminatum* (Table 1). The virulence groups of some *F. oxysporum* isolates were described previously (Bayraktar et al., 2008). One or 1.5 months after inoculation all isolates were grouped into 3 categories based on their effect: low virulent isolates (0-1.0 scale value), moderately virulent isolates (1.1-3.0), and highly virulent isolates (3.1-4.0). Most of the *Fusarium oxysporum* isolates, the most common in chickpea growing areas in Turkey, caused vascular wilt and were moderately virulent. A few isolates caused typical yellowing symptoms on affected seedlings. *F. solani*, the second most important species of the *Fusarium* complex on chickpea caused black root rot symptoms. The other *Fusarium* species caused different levels of wilt and root rot disease. The isolates of dry root rot, *M. phaseolina*, and wet root rot, *R. solani*, were more virulent than *F. oxysporum* and *F. solani*, and induced severe pre-emergence damping-off in chickpea seedlings.

RAPD-PCR Analysis

To assess genetic variability within the wilt and root rot complex on chickpea, 99 isolates, including 88 isolates obtained from diseased chickpea and 11 isolates provided by different researchers, were analyzed (Table 1). RAPD-PCR generated very distinct amplification products with considerable variability between the isolates belonging to different fungal species. In total, 392 fragments were generated from 30 RAPD primers, none of which were monomorphic. The number of RAPD fragments produced per primer varied between 1 and 8, and ranged in size from 0.2 to 3.6 kb. Isolates belonging to the same fungal species formed a similar amplification profile within each group. The most useful primers were OPK-7, OPK-19, and OPA-18, which yielded polymorphic bands within and between fungal species.

UPGMA cluster analysis of RAPD data separated the isolates of each particular species into unique groups based on high genetic similarity, except *F. oxysporum* and *F. solani* isolates (Figure 1). *F. semitectum* isolates were clustered into the first main cluster and shared a genetic similarity of about 84%. The isolates of *F. oxysporum* were classified into 3 major groups. As isolate Kon-3 does not sporulate it could not be accurately identified based on morphological characteristics and was included in group 1 with 76% genetic similarity. Similarly, isolate Ank-32 does not sporulate and was included in group 2 with 75% genetic similarity, while isolates KA2, Km-11, and Tok-3 were included in group 3. Six isolates of *F. equiseti* clustered together with genetic similarity of about 67%. Isolate Ank-39 (*C. tonkinense*) clustered separately from other isolates with genetic similarity of only 17.8% in the dendrogram. *F. solani* isolates were grouped into 2 major clusters. Partial geographical structuring was observed in populations from southeast and northern Turkey. Group 1 contained 11 isolates from Ankara, Uşak, Denizli, and Antalya provinces with 70% genetic similarity. Group 2 was composed of isolates (with 81.4% genetic similarity) obtained from Burdur, Tokat, Kırşehir, Diyarbakır, and K. Maraş provinces, as well as the provinces above. Isolates Ank-37 and Ref-3 (*F. moniliforme*) clustered with 80% genetic similarity. Isolates of *F. acuminatum*, *M. phaseolina*, and *R. solani* showed a high genetic similarity within the group, with similarity coefficients over 85%, 75%, and 79% in dendrogram analysis, respectively.

ISSR Analysis

Twenty ISSR primers were tested with the fungal pathogens that were used in RAPD analysis to determine which primers generated reproducible banding patterns. Additionally, PCR conditions for DNA amplification were standardized for all primers. From these, primers $(GC)_9YR$, $(AT)_9YR$, $(CT)_8RG$, $(CA)_8RT$, and $(GA)_8YT$ did not produce clear scorable bands in these isolates or amplify any bands. In total, 346 distinct bands, which were consistent in repeated amplifications, were amplified using the DNA from all isolates as templates. The number of ISSR fragments produced per primer varied between 1 and 9, and ranged in size from 4.6 to 0.18 kb. According to ISSR analysis, a higher similarity coefficient was observed both within and between groups than with RAPD analysis. Primers $(GA)_8T$, $(AG)_8G$, and $(AC)_8T$ produced amplification profiles that differentiated each fungal species.

Cluster analysis of ISSR data clearly separated all the isolates of each fungal species into distinct branches (Figure 2). Five isolates of *F. semitectum* clustered together with genetic similarity of about 93% in the dendrogram. Isolates of *F. oxysporum* were clustered similarly into 3 distinct groups based on RAPD analysis. The non-sporulated isolates Kon-3, Ank-32, KA2, Km-11, and Tok-3 were grouped with *F. oxysporum* isolates. Isolates of *F. moniliforme* clustered together with genetic similarity of about 70%. Isolates of *F. equiseti* showed

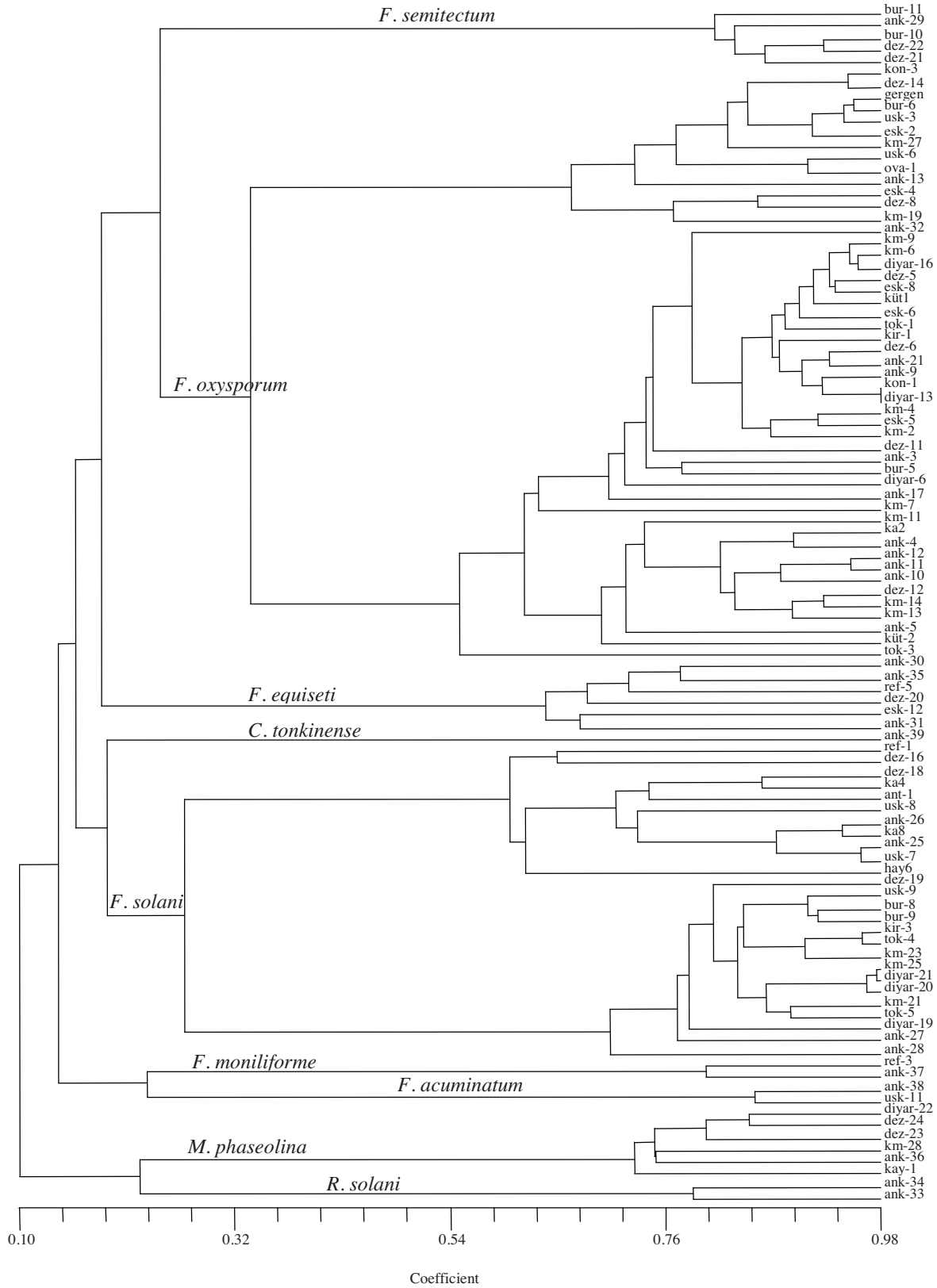

Figure 1. UPGMA cluster analysis of the data from DNA amplification of chickpea root rot isolates with RAPD analysis.

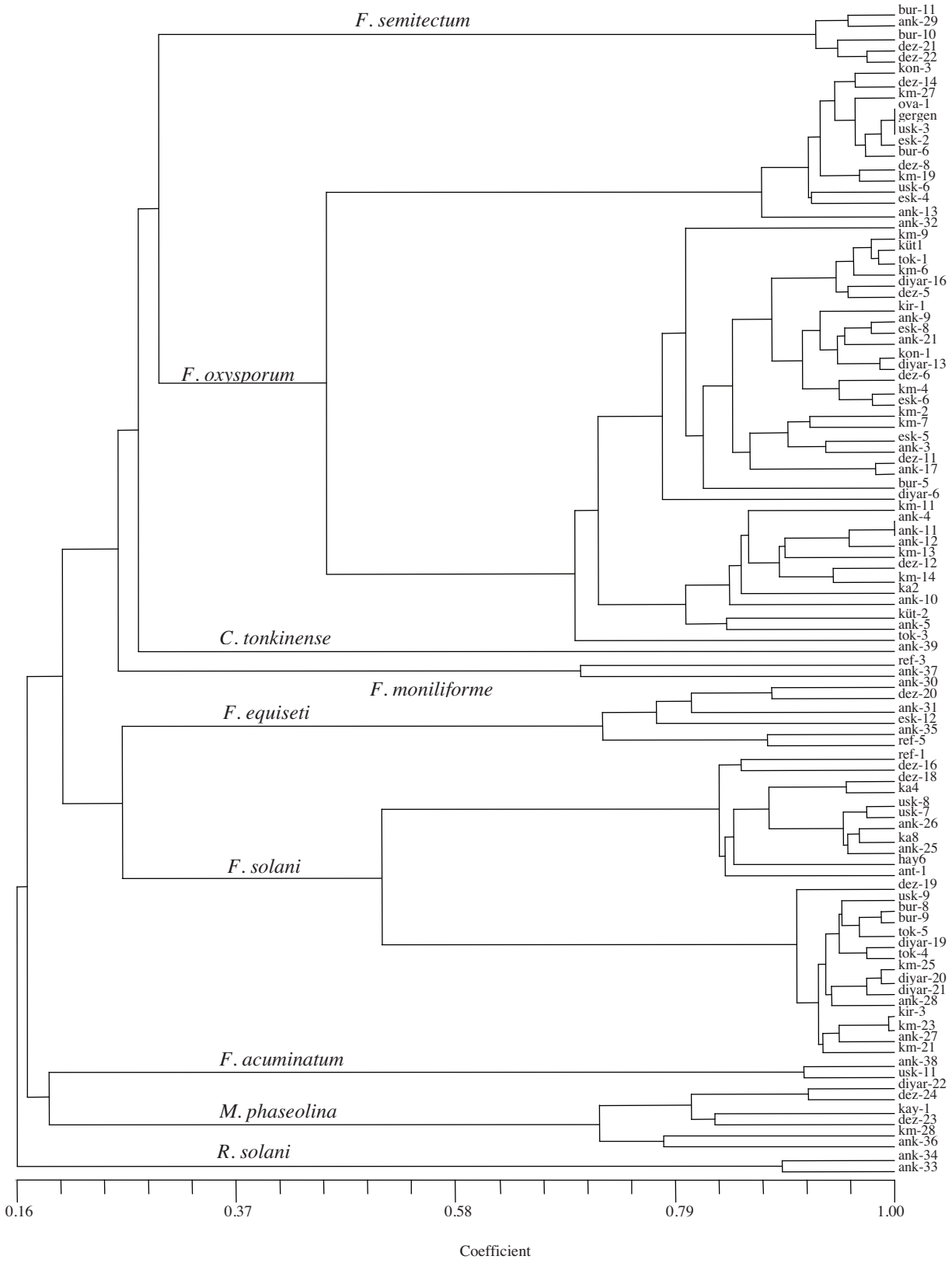

Figure 2. UPGMA cluster analysis of the data from DNA amplification of chickpea root rot isolates with ISSR analysis.

76% genetic similarity. Isolates of *F. solani* were clustered into 2 distinct groups. Group 1 consisted of *F. solani* isolates from 4 different provinces with genetic similarity of about 87%, while group 2 (with genetic similarity of 93.2%) was composed of isolates from the same provinces as mentioned in the RAPD analysis section. Isolates of *F. acuminatum*, *M. phaseolina*, and *R. solani* showed 91%, 76%, and 89% genetic similarity within groups, respectively. Isolates of *C. tonkinense* clearly separated from other fungal isolates with 24% genetic similarity in the dendrogram.

To test the fit of the cluster analyses, the original similarity matrix was compared to the cophenetic value matrix using the matrix comparison model (MXCOPH algorithm) in NTSYS-pc. The cophenetic correlation values for RAPD and ISSR were 0.983 and 0.987, respectively. Based on the high cophenetic correlation values we concluded that both molecular markers were powerful tools for analyzing the intra- and interspecific variability within and between wilt and root rot pathogens of chickpea. The correlation value between cophenetic matrix values obtained from these analyses was r = 0.59, suggesting a very poor correlation between RAPD and ISSR dendrograms.

Discussion

We observed considerable genetic diversity within wilt and root rot complexes on chickpea, based on pathogenicity, and RAPD and ISSR markers. Isolates were classified into 3 major groups based on their effects on the susceptible chickpea variety. Generally, aggressiveness was observed among the isolates with moderate virulence. *M. phaseolina* and *R. solani* isolates were not frequently observed in the chickpea growing areas. The isolates of these 2 species were more virulent than the other root pathogens and resulted in the death of chickpea seedlings in the pre-emergence stage due to rot. Similarly, in a study of wilt and root rot pathogens of the chickpea cv. Aziziye-94, Demirci et al. (1999) reported that *Fusarium solani* f. sp. *pisi* and *Fusarium oxysporum* f. sp. *ciceris* were more prevalent than other chickpea pathogens. Moreover, *Fusarium solani* f. sp. *pisi* and *Rhizoctonia solani* were shown to be the most virulent of the fungal species detected on this cultivar. *Fusarium oxysporum*, *Fusarium solani*, *F. acuminatum*, *F. equiseti*, *M. phaseolina*, and *R. solani* were also reported to be the most common agents of wilt and root rot complex on chickpea in Turkey (Yücel and Güncü, 1991; Dolar, 1996).

Many molecular studies have been performed to identify genetic polymorphisms between races of *Fusarium oxysporum* f. sp. *ciceris*, and to differentiate other wilt and root rot pathogens (Barve et al., 2001, Chakrabarti et al., 2001; Jimenez-Gasco et al., 2001; Sivaramakrishnan et al., 2002). *Fusarium oxysporum* f. sp. *ciceris* is monophyletic; however, it exhibits considerable variation in symptom types and pathogenicity on chickpea according to geographical location (Jimenez-Gasco et al., 2002). With RAPD analysis, Kelly et al. (1994) separated isolates of *F. oxysporum* f. sp. *ciceris* into 2 groups that correlated with the pathotypes that cause yellowing and wilt disease syndrome in chickpea. They also differentiated *F. oxysporum* f. sp. *ciceris* from other formae speciales and *Fusarium* species, and observed 11%-18% genetic similarity between *F. oxysporum* and *F. solani*. Yet, Khalil et al. (2003) observed genetic similarity of 57% between these fungal species on cotton. Zamani et al. (2004) reported that RAPD analysis revealed considerable genetic variation among *F. oxysporum* obtained from chickpea and that these isolates belonged to 3 different groups based on vegetative compatibility and virulence assay. Conversely, they did not find a relationship between RAPD analysis, and vegetative compatibility and virulence assay. In another study, Bayraktar et al. (2008) detected a high degree of genetic variability among *Fusarium oxysporum* isolates and separated them into 3 distinct groups. There was no relationship between their clustering in dendrograms, and geographic origin and virulence of the isolates.

There are few reports on the genetic diversity of other wilt and root rot pathogens on chickpea. *F. solani* is the second most frequently isolated fungus among the *Fusarium* complex on diseased chickpea plants in Turkey. All isolates can be divided into 2 distinct groups with all the molecular markers analyzed. A partial correlation was detected between the geographical origin and distribution of southeastern and northern isolates of *F. solani* in both dendrograms. The results showed low-level genetic diversity within the other root rot pathogens, except *F. oxysporum* and *F. solani*. Isolates belonging to the same species formed distinct branches of the dendrograms in RAPD and ISSR analysis. Amplification profiles of all the

isolates within each group were very similar to the same primers. Jana et al. (2003) classified isolates of *M. phaseolina* obtained from chickpea into 3 distinct groups. Primer OPA-13 was able to distinguish *M. phaseolina* from *Fusarium* species, including *F. oxysporum* f. sp. *ciceris*. Abd-Elsalam et al. (2003) measured the genetic diversity among 171 isolates belonging to 12 *Fusarium* spp. on cotton using AFLP markers. They found that genetic similarity ranged from 28% to 34% for inter-specific and from 34% to 98% for intra-specific comparisons.

The goal of the present study was to assess the distribution of genetic variation among chickpea pathogens in Turkey. High levels of interspecific genetic diversity among the tested pathogens were observed with all the molecular markers analyzed, and they were divided into distinct groups. The clusters formed from UPGMA analysis of both RAPD and ISSR corresponded broadly to the morphological species classification. The 2 methods provided similar resolution, although there were differences in the distribution of isolates and the ratio of genetic similarity in the dendrograms. The cophenetic value matrices supported that the branching isolates belonged to different species in the dendrogram. Lu et al. (2004) studied the diversity and host specificity of *Colletotrichum* strains and stated that ISSR and RAPD analysis produced considerably similar dendrograms, and that both methods are useful for population analysis of this species. ISSR analysis grouped *Colletotrichum* isolates into distinct clusters with similarities ranging from 44% to 90%, while this ratio was about 18%-85% in RAPD analysis. In this study, more similarity coefficients were detected within and between groups with ISSR analysis than with RAPD analysis.

The development of chickpea cultivars resistant to wilt and root rot disease is very important. Cultivars resistant to a single disease have become susceptible to other diseases over time. Thus, chickpea breeders need different strategies for developing multi-disease-resistant cultivars. Nonetheless, treating seeds with fungicides does not provide sufficient control of multiple diseases (Jimenez-Diaz and Trapero-Casas, 1985). The present study highlights the genetic basis of these potential diagnostic markers and provides additional insights into the molecular evolution of chickpea pathogens. Both RAPD and ISSR analyses were very useful in assessing the intra- and interspecific diversity of these pathogens. In addition to contributing to the understanding of the diseases caused by these pathogens and improving crop productivity, these results will be useful for developing integrated strategies for disease management and breeding programs.

Acknowledgements

This research was supported in part by Ankara University (Scientific Research Project). We are thankful to Professor Dr. Gülay Turhan (Ege University, Turkey) and Dr. Steiner Stenzel, (University of Bonn, Germany) for providing the fungal isolates. We would also like to thank Professor Dr. Salih Maden (University of Ankara, Turkey) for fungal identification.

References

Abd-Elsalam, K.A., F. Schnieder, M.S. Khalil, A. Asran-Amal and J.A. Verreet. 2003. Use of AFLP fingerprinting to analyze genetic variation within and between populations of *Fusarium* spp. Derived from Egyptian cotton cultivars. J. Plant Pathol. 85: 99-103.

Abou-Zeid, N.M. and H. Hallila. 2003. Current status of chickpea diseases in Egypt. In: International Chickpea Conference, Chickpea Research for Millennium Raipur, Chhattisgarh, January, pp. 156-166, India.

Balmas, V., B. Scherm, P.D. Primo, D. Rau, A. Marcello and Q. Migheli. 2005. Molecular characterisation of vegetative compatibility groups in *Fusarium oxysporum* f. sp. *radicis-lycopersici* and f. sp. *lycopersici* by random amplification of polymorphic DNA and microsatellite-primed PCR. Eur. J. Plant Pathol. 111: 1-8.

Barve, M.P., M.P. Haware, M.N. Sainani, P.K. Ranjekar and V.S. Gupta. 2001. Potential of microsatellites to distinguish four races of *Fusarium oxysporum* f. sp. *ciceri* prevalent in India. Theor. Appl. Genet. 102: 138-147.

Bayraktar, H., F.S. Dolar and S. Maden. 2008. Use of RAPD and ISSR markers in detection of genetic variation and population structure among *Fusarium oxysporum* f. sp. *ciceris* isolates on chickpea in Turkey. J. of Phytopathol. 156: 146-154.

Chakrabarti, A., P.K. Mukherjee, P.D. Sherkhane, A.S. Bhagwat and N.B.K. Murthy. 2001. A simple and rapid molecular method for distinguishing between races of *Fusarium oxysporum* f. sp. *ciceris* from India. Curr. Sci. 80: 571-575.

Crowhurst, R.N., B.T. Hawthrorne, E.H.A. Rikkerink and M.D. Templeton. 1991. Differentiation of *Fusarium solani* f. sp. *cucurbitae* races 1 and 2 by random amplification of polymorphic DNA. Curr. Genet. 20: 391-396.

Demirci, E., C. Eken and F. Kantar. 1999. Pathogenicity of wilt and root rot pathogens of chickpea cv. Aziziye-94. J. Turk. Phytopath. 28: 25-32.

Dolar, F.S. 1996. Determination of chickpea diseases in Ankara, Turkey. Int. Chickpea Newsl. 3: 33-34.

Food and Agriculture Organization of the United Nations (FAO). 2007. http//:www.fao.org/, accessed date: 10.06.2007.

Grajal-Martin, M.J., C.J. Simon and F.J. Muehlbauer. 1993. Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f. sp. *pisi*. Phytopathol. 83: 612-614.

Hantula, J., A. Lilja, H. Nuorteva, P. Parikka and S. Werres. 2000. Pathogenicity, morphology and genetic variation of *Phytophthora cactorum* from strawberry, apple, rhododendron and silver birch. Mycol. Res. 104: 1062-1068.

Haware MP, Y.L. Nene and M. Natarajan. 1996. Survival of *Fusarium oxysporum* f. sp. *ciceri* in soil absence of chickpea. Phytopathol. Mediterr. 35: 9-12.

Huang, R., M. Galperin, Y. Levy and R. Perl-Treves. 1997. Genetic diversity of *Fusarium moniliforme* detected by vegetative compatibility groups and random amplified polymorphic DNA markers. Plant Pathol. 46: 871-881.

Jana, T., T.R. Sharma, R.D. Prasad and D.K. Arora. 2003. Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. Microbiol. Res. 158: 249-257.

Jana, T., T.R. Sharma and N.K. Singh. 2005. SSR-based detection of genetic variability in the charcoal root rot pathogen *Macrophomina phaseolina*. Mycol. Res. 109: 81-86.

Jimenez-Diaz, R.M. and A. Trapero-Casas. 1985. Use of fungicide treatments and host resistance to control the wilt and root rot complex of chickpeas. Plant Dis. 69: 591-595.

Jimenez-Gasco, M., E. Perez-Artes and R.M. Jimenez-Diaz. 2001. Identification of pathogenic races 0, 1 B/C, 5, and 6 of *Fusarium oxysporum* f. sp. *ciceris* with random amplified polymorphic DNA (RAPD). Eur. J. Plant Pathol. 107: 237-248.

Jimenez-Gasco, M., M.G. Milgroom and R.M. Jimenez-Diaz. 2002. Gene genealogies support *Fusarium oxysporum* f. sp. *ciceris* as a monophyletic group. Plant Pathol. 51: 72-77.

Kaiser, W.J., A.R. Alcala-Jimenez, A. Hervas-Vargas, J.L. Trapero-Casas and R.M. Jimenez-Diaz. 1994. Screening of wild Cicer species for resistance to race 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. Plant Dis. 78: 962-967.

Kelly, A., A.R. Alcala-Jimenez, B.W. Bainbridge, J.B. Heale, E. Perez-Artes and R.M. Jimenez-Diaz. 1994. Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea. Phytopathol. 84: 1293-1298.

Khalil M.S., M.A. Abdel-Sattar, I.N. Aly, K.A. Abd-Elsalam and J.A. Verreet. 2003. Genetic affinities of Fusarium spp. and their correlation with origin and pathogenicity. Afr. J. Biotech. 2: 109-113.

Lu, G., P.F. Cannon, A. Reid and C.M. Simmons. 2004. Diversity and molecular relationships of endophytic *Colletotrichum* isolates from the Iwokrama Forest Reserve, Guyana. Mycol. Res. 108: 53-63.

Meyer, W., T.G. Mitchell, E.Z. Freedman and R. Vilgays. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J. Clin. Microbiol. 31: 2274-2280.

Mishra, K.P., R.T.V. Fox and A. Culham. 2003. Inter-simple sequence repeat and aggressiveness analyses revealed high genetic diversity, recombination and long-range dispersal in *Fusarium culmorum*. Ann. Appl. Biol. 143: 291-301.

Nene, Y.L. and M.P. Haware. 1980. Screening chickpea for resistance to wilt. Plant Dis. 66: 379-380.

Nene, Y.L., V.K. Sheila and S.B. Sharma. 1984. A world list of chickpea (*Cicer arietinum* L.) and pigeon pea (*Cajanus cajan* (L.) Millsp.) pathogens. ICRISAT Pulse Pathology Progress Report 32. pp.19.

Nene, Y.L. and M.V. Reddy. 1987. Chickpea diseases and their control. In: Saxena, M.C. and Singh K.B. (eds.), The Chickpea, C.A.B. Wallingford, Oxon, UK. pp. 233-270.

Pandey, G. and R.B. Singh. 1990. Survey of root diseases of chickpea in Allahabad region. Curr. Nematol. 1: 77-78.

Reader, U. and P. Broda. 1985. Rapid preparation of DNA from filamentous fungi. Lett. Appl. Microbiol. 1: 17-20.

Rohlf, F.I. 1998. NTSYS-pc. Numerical taxonomy and multivariate analysis system, Version 2.0. Applied Biostatistics, New York, USA.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular cloning: A Laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Sivaramakrishnan, S., S. Kannan and S.D. Singh. 2002. Genetic variability of *Fusarium* wilt pathogen isolates of chickpea (*Cicer arietinum* L.) assessed by molecular markers. Mycopathol. 155: 171-178.

Trapero-Casas, A. and R.M. Jimenez-Diaz. 1985. Fungal wilt and root rot diseases of chickpea in Southern Spain. Phytopathol. 75: 1146-1151.

Yücel, S. and M. Güncü. 1991. Fungal diseases determined on food legumes in Mediterranean region. Plant Protection Bulletin, 3: 19-30.

Zamani, M.R., M. Motallebi and A. Rostamian. 2004. Characterization of Iranian isolates of *Fusarium oxysporum* on the basis of RAPD analysis, virulence and vegetative compatibility. J. Phytopathol. 152: 449-453.

Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genome 20: 176-183.