Indian Journal of Agricultural Sciences **82** (1): 3–7, January 2012

Research Papers

Genetic diversity and mating-type distribution within populations of *Ascochyta rabiei* in Turkey

GÖKSEL ÖZER[1], HARUN BAYRAKTAR[2] and FATMA SARA DOLAR[3]

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

Received: 22 September 2010; Revised accepted: 5 October 2011

ABSTRACT

Genetic diversity and mating-type distribution were studied within five populations of *Ascochyta rabiei* in Turkey. Of 122 isolates tested for mating type, 52.5% were identified as *MAT 1-2* and 47.5% as *MAT 1-1*. Both mating types were observed in all populations, supporting the hypothesis of a randomly mating population. RAPD analysis was performed on 81 isolates of *A. rabiei* representing different pathotypes and geographic origins with 54 primers. Results of RAPD analysis clustered all isolates into nine groups an arbitrary level of 73% similarity. Total gene diversity (H_T=0.221) was mostly attributable to diversity within populations (H_S=0.213). Only 4% of the total variability were attributable to variation among populations. The results revealed low genetic differentiation and high gene flow among populations of *A. rabiei* in Turkey.

Key words: *Ascochyta rabiei*, Chickpea, Genetic diversity, Mating type, RAPD

Ascochyta rabiei [(Pass.) Labr. (teleomorph: *Didymella rabiei* (Kov.) v. Arx.)] is a heterothallic fungus that requires two compatible mating types for sexual reproduction (Wilson and Kaiser 1995). The development of teleomorphic stage plays a major role in the disease epidemiology and pathogenic diversity. Increasing the genetic variability among pathogen populations may result in resistance against fungicide applications and the breakdown of resistance in the cultivated germplasm (Kaiser and Küsmenoğlu 1997). Thus, determining mating types in different growing areas is important for the efficiency of disease management against this pathogen. Many studies have been performed for this purpose in different countries (Kaiser and Küsmenoğlu 1997, Armstrong *et al.* 2001, Rhaiem *et al.* 2007). Barve *et al.* (2003) developed MAT-specific multiplex PCR assay for the characterization of mating types of *A. rabiei*.

Pathogenic variability in populations of *A. rabiei* has been characterized into races or pathotypes based on disease reactions of differential cultivars. However, these classifications varied according to the disease scales and the differential lines used. Chongo *et al.* (2004) detected 14 pathogenic groups among isolates of *A. rabiei* in Canada. Türkan and Dolar (2009) detected three pathotypes and six races among *A. rabiei* isolates representing different regions of Turkey.

To improve the efficiency of breeding schemes and control strategies for Ascochyta blight, knowledge of genetic variability within and among different populations is indispensable. To date, many different molecular methods have been used to reveal genetic polymorphism in populations of *A. rabiei* (Peever *et al.* 2004, Rhaiem *et al.* 2008). RAPD markers have been successfully used in determining pathogenic variability and population structure in *A. rabiei* (Udupa *et al.* 1998, Rhaiem *et al.* 2007). However, little information is available on genetic variability among populations of *A. rabiei* in Turkey (Bayraktar *et al.* 2007a). To our knowledge, RAPD analysis was not used before in examining the intraspecific variations with related to genotypic diversity, pathogenic variability, mating-type distribution, and geographic origins of *A. rabiei* in Turkey.

The objectives of this work were to: (i) reveal the extent and distribution of genetic diversity among populations of *A. rabiei* from different regions of Turkey, and (ii) determine the potential of sexual production and the distribution of mating-type groups in these chickpea growing regions. As a preliminary study, mating-type groups in Central Anatolia region were reported by Bayraktar *et al.* (2007b) according to the original mating-type classification of Trapero-Casas and Kaiser (1992).

[1]Scientist (e mail: gokozer@gmail.com), University of Abant Izzet Baysal;

[2,3]Scientist (e mail: bayrakta@agri.ankara.edu.tr, dolars@agri.ankara.edu.tr), University of Ankara

MATERIALS AND METHODS

A total of 122 *A. rabiei* isolates were recovered from chickpea-growing areas in Aegean, Black sea, Central Anatolia, Mediterranean, South Eastern Anatolia regions of Turkey. The experiments were conducted at Department of Plant Protection, Faculty of Agriculture, University of Ankara during 2004–09. Single spore isolates were grown on chickpea seed meal dextrose agar (CSMDA: chickpea meal 40 g, dextrose 20 g, agar 20 g, distilled water 1L) medium at 21 ºC with a 12 hr dark/light cycle. The pathotype groups of 54 isolates were detected in a previous study with a set of three different chickpea cultivars (ILC 1929, ILC 482, and ILC 3279) by Türkan and Dolar (2009).

DNA extraction was performed according to the method of Weising *et al.* (1991). Fungal mycelium was produced in 100 ml of potato dextrose broth medium (Difco Lab., Detroit, USA). Cultures were kept in an incubator shaker at 21°C with a 150 rpm speed for seven days. Mycelia were harvested by filtration through Miracloth, freezed in liquid nitrogen, stored –80 °C, and later used for DNA extraction.

Mating type-specific PCR was carried out in 25 ml reaction containing 10× PCR buffer, 1 mM of $MgCl_2$, 0.4 µM of each primer, 0.2 mM of dNTPs, and 1 unit of *Taq* DNA polymerase (Promega) using primers Com1, SP21 and Tail5 designed by Barve *et al.* (2003). PCR amplifications were performed in a thermal cycler (Whatman-Biometra T1), programmed as follows: one cycle of 96 °C 2 min., 25 cycles of 96 °C 15 sec., 60 °C 30 sec. and 72 °C 45 sec., and then 5 min. at 72 °C (Peever *et al.* 2004). The PCR products were separated electrophoretically on 1.4% agarose gel using 1×TAE buffer. The gels were stained with ethidium bromide and visualized by Gene Tools bio-imaging system.

RAPD–PCR was carried out in 25 µl reaction containing 10x PCR buffer, 1.5 mM of $MgCl_2$, 0.32 µM of primer, 0.125 mM of dNTPs and 0.6 unit of *Taq* DNA polymerase (MBI, Fermantase). PCR amplification was performed as follows: 40 cycles of 94 °C 20 sec., 36 °C 1 min., 72 °C 1 min., and then 8 min. 72 °C. Fifty-four primers selected from the different primer sets were evaluated for their ability to produce polymorphic bands among isolates of *A. rabiei*. The PCR products were separated and visualized as described above.

Chi square values were calculated to test for the hypothesis of random mating with Yates corrections (1 degree of freedom). If the mating-type ratios were not significantly different from 1:1 at $P < 0.01$, the population was concluded to be randomly mating (Milgroom 1996).

Results obtained from RAPD analyses were used to construct binary matrices. The major polymorphic bands were evaluated for statistical analysis. Binary matrices were analyzed by NTSYS–PC software ver. 2.0 using Dice's similarity coefficient (Rohlf 1998). Genetic diversity and population differentiation parameters were calculated using Popgene software ver. 1.32 (Yeh *et al*. 1999) and Tools for Population Genetic Analysis (TFPGA) software ver. 1.3 (Miller 1997).

RESULTS AND DISCUSSION

Mating types

Mating type-specific PCR was amplified a 700 bp fragment from mating-type 1 isolates while a 400 bp PCR product was amplified from mating-type two isolates. Of the 122 isolates tested, 52.5% were identified as *MAT 1-2* and 47.5% as *MAT 1-1* (Table 1). The distribution of mating types occurred at equal frequency over all regions. Chi-squared values for mating-type ratios were not significantly different from 1:1 in all regions. The results indicated that the pathogen has the potential for sexual reproduction in all regions and may be randomly mating in five regions of Turkey. The similarity of mating-types ratios were observed by Kaiser and Küsmenoglu (1997) who reported that *MAT 1-1* was more prevalent relative to *MAT 1-2* in Turkey and the teleomorphic development occurred regularly in disease cycle of pathogen. Testing for mating type of 42 isolates in Saskatchewan, Armstrong *et al.* (2001) observed a similar frequency of mating-type distribution across the region. Rhaeim *et al.* (2007) found both mating types in three of five populations in Tunisia while only *MAT 1-1* was detected in two populations.

Genetic diversity among isolates

Of 54 primers tested, seven primers (OPG 02, OPJ 10, OPJ 19, OPM 03, OPM 15, OPN 12, and OPB 07) were determined to produce variable banding patterns among 81 isolates, representing different regions and pathogenic groups. UPGMA analysis grouped all isolates into nine major clusters at an arbitrary level of 73% similarity, from which two clusters consisted of one isolate (Fig 1). Group 4 formed the major large group with 40 isolates, divided into three subgroups with 13, 25 and 2 isolates, respectively. In general,

Table 1 Incidences of mating types of *Ascochyta rabiei* in different regions of Turkey

Population	N[a]	Distribution of mating types		χ^{2b}	P[c]
		MAT1-1	*MAT1-2*		
Aegean	18	12	6	1.388	0.239
Black Sea	16	8	8	0.062	0.803
Central Anatolia	45	19	26	0.8	0.371
Mediterranean	8	3	5	0.125	0.724
South Eastern Anatolia	35	16	19	0.114	0.736
All regions	122	58	64	0.205	0.651

[a]N: Number of isolates tested; [b]Chi-squared value for the test of 1:1 ratio with Yates correction; [c]Probability of greater χ^2-value under the null hypothesis of 1:1 ratio (1 degree of freedom).

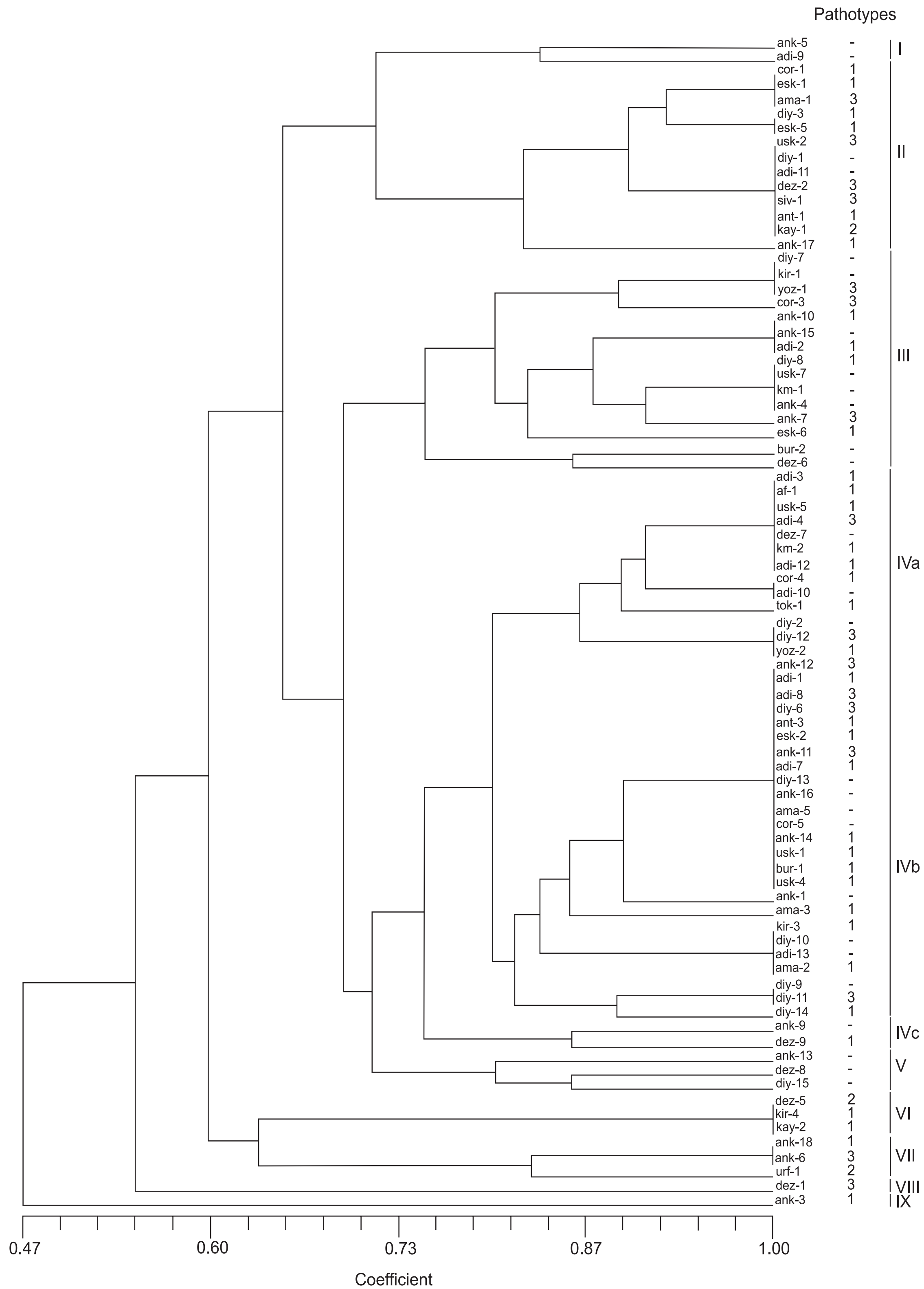

Fig 1 UPGMA dendrogram constructed for 81 isolates of *Ascochyta rabiei* obtained from different regions of Turkey. The isolates were labeled according to their geographical origin. Abbreviations represent dez (Denizli), usk (Usak), af (Afyon), bur (Burdur), ant (Antalya), km (Kahraman Maras), diy (Diyarbakir), adi (Adiyaman), urf (Sanli Urfa), ank (Ankara), esk (Eskisehir), kay (Kayseri), kir (Kirsehir), siv (Sivas), yoz (Yozgat), çor (Çorum), ama (Amasya) and tok (Tokat). The pathotype groups of 54 isolates were described by Türkkan and Dolar (2009).

Table 2 Estimates of genetic diversity within five populations of *Ascochyta rabiei*

Population	N	Polymorphic loci (%)	h	I
Aegean	13	81.82	0.212±0.146	0.343±0.209
Black sea	9	81.82	0.217±0.127	0.354±0.194
Central Anatolia	28	100	0.261±0.109	0.422±0.140
Mediterranean	6	45.45	0.157±0.189	0.238±0.282
South-eastern Anatolia	25	72.73	0.216±0.155	0.342±0.234
All populations	81		0.236±0.110	0.390±0.141

[a]N: Number of isolates h: Gene diversity I: Shannon's Information index

Table 3 Genetic distance (below diagonal) and genetic identity (above diagonal) between pairwise combinations of *Ascochyta rabiei* populations

Population	Central Anatolia	Aegean	South-eastern Anatolia	Black Sea	Medite-rranean
Central Anatolia		0.9766	0.9828	0.9865	0.9841
Aegean	0.0237		0.9891	0.9766	0.9842
South Eastern Anatolia	0.0173	0.0109		0.9883	0.9919
Black Sea	0.0135	0.0237	0.0118		0.9838
Mediterranean	0.0160	0.0159	0.0081	0.0164	

isolates from the same regions were placed in different groups in the cluster analysis, indicating that the variation was less among different regions. For example, with an approximate 750 km distance from each other, isolates ank 15 and adi 2 shared the same genotype. However, no correlation was detected between pathotype groups and mating types of the isolates and their clustering in dendrogram. Similar results were obtained in previous studies performed by different researchers (Santra *et al.* 2001, Bayraktar *et al.* 2007a). However, Udupa *et al.* (1998) grouped all the genotypes into 22 different clusters with related to three pathotypes of *A. rabiei* in Syria. Rhaiem *et al.* (2007) detected that all *MAT 1-2* isolates had unique RAPD patterns among the 40 isolates from Tunisia.

Population differentiations of A. rabiei

Gene diversity within all populations of *A. rabiei* was the averaged 0.236±0.11 (Table 2). The highest gene diversity became in Central Anatolia region (0.261) with 100% polymorphic loci while the lowest gene diversity occurred in Mediterranean region (0.157) with 45.4% polymorphic loci. The Shannon's index (I) was the averaged 0.39±0.14, showing the same trend within populations.

Estimation of genetic distances provided to a better understanding of genetic relationships among populations. Genetic distance among populations ranged from 0.0237 (Central Anatolia to Aegean, Black sea to Aegean) to 0.0081 (South Eastern Anatolia to Mediterranean) while genetic identity varied from 0.9919 to 0.9766 (Table 3). UPGMA analysis of genetic distances among five populations revealed two clusters with 100% bootstrap support values (Fig 2). The populations geographically more similar to each other grouped into the same cluster in dendrogram. Although, the genetic distance between Central Anatolia and Black Sea regions was higher than that between Black Sea and South Eastern Anatolia regions, they included in the same cluster. This situation may result from that the levels of gene diversity or the numbers of sample are different in populations. The geographical proximity of Central Anatolia and Black Sea regions may affect genetic levels among populations.

The analysis of population structure revealed that genetic diversity within regions accounted for most of the total genetic diversity. The total gene diversity (H_T) was 0.221 ±0.014 while the gene diversity within populations (H_S) was 0.213±0.013, accounting for 96.3% of the total gene diversity. The coefficient of genetic differentiation relative to the total population (G_{ST}) was 0.045, revealed a low level of genetic differentiation. These low values indicated that most of the genetic diversity of *A. rabiei* occurred within populations and there was little differentiation among populations. The level of gene flow (N_M) was 10.48 among all populations, demonstrating very high levels of gene flow. Similarly, detecting the low level of gene diversity (H_T= 0.02) among Australian populations of *A. rabiei* based on STMS markers, Phan *et al.* (2003) observed a high level of diversity (H_T= 0.45) among the overseas populations (H= 0.38 in Canada, H= 0.36 in USA, and H= 0.32 in Syria). Rhaiem *et al.* (2007) detected that genetic differentiation among populations of *A. rabiei* in Tunisia was a very high level, 33% (G_{ST}) of genetic variation occurred among populations.

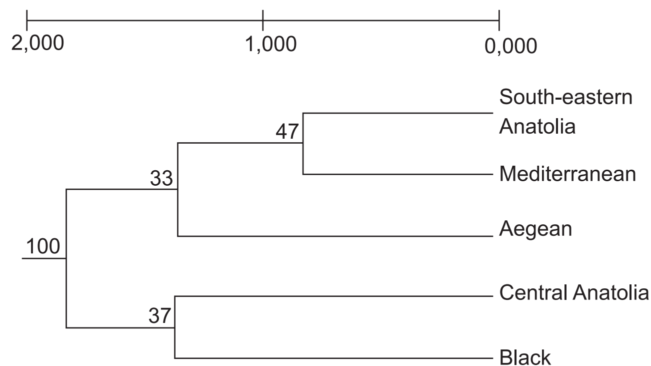

Fig 2 UPGMA dendrogram based on Nei's genetic distances among five populations of *Ascochyta rabiei*. Bootstrap support values (based on 1000 permutations) are indicated above their respective branches

The high genetic similarities and the clustering among populations supported that gene flow occurred across long distances in Turkey. Air-borne ascospores lead to the increasing of genetic variation and disease spread in different region of Turkey (Kaiser and Küsmenoğlu 1997). The transportation of infected seeds was considered to be a reasonable source for the observed low level of genetic differentiation and high level of gene flow among populations.

Our results revealed high genetic diversity within five populations of *A. rabiei*, but low genetic differentiation and high gene flow among populations. Regular gene flow and random mating between isolates from different populations appeared to have a considerably influence on population structure of *A. rabiei* in Turkey. Thus, the integrated approach should be taken into account to disease control such as using pathogen-free seeds, proper debris management, crop rotation and fungicide treatment.

ACKNOWLEDGEMENTS

This work was financially supported by a grant from State Planning Organization (DPT-YUUP, Project no: DP2004K120657 GENPRO) and Ankara University (Scientific Research Project) as a doctoral thesis project.

REFERENCES

Armstrong C L, Chongo G, Gossen B D and Duczek L J. 2001. Mating type distribution and incidence of the teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. *Canadian Journal of Plant Pathology* **23**: 110–3.

Barve M P, Arie T, Salimath S S, Muehlbauer F J and Peever T L. 2003. Cloning and characterization of the mating type (*MAT*) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a *MAT* phylogeny of legumes-associated *Ascochyta* spp. *Fungal Genetics and Biology* **39**: 151–67.

Bayraktar H, Dolar F S and Tör M. 2007a. Determination of genetic diversity within *Ascochyta rabiei* (Pass.) Labr., the pathogen of ascochyta blight of chickpea in Turkey. *Journal of Plant Pathology* **89**: 341–7.

Bayraktar H, Dolar F S and Maden S. 2007b. Mating type groups of *Ascochyta rabiei* (teleomorph: *Didymella rabiei*), the causal agent of chickpea blight in Central Anatolia. *Turkish Journal of Agriculture and Forestry* **31**: 41–6.

Chongo G, Gossen B D, Buchwaldt L, Adhikari T and Rimmer S R. 2004. Genetic diversity of *Ascochyta rabiei* in Canada. *Plant Disease* **88**: 4–10.

Kaiser W J and Küsmenoğlu I. 1997. Distribution of mating types and the teleomorph of *Ascochyta rabiei* on chickpea in Turkey. *Plant Disease* **81**: 1284–7.

Milgroom M G. 1996. Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology* **34**: 457–77.

Miller M P. 1997. *Tools for Population Genetic Analysis.* Version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

Peever T L, Salimath S S, Su G, Kaiser W J and Muehlbauer F J. 2004. Historical and contemporary multilocus population structure of *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) in the Pacific Northwest of the United States. *Molecular Ecology* **13**: 291–309.

Phan H T T, Ford R and Taylor P W J. 2003. Population structure of *Ascochyta rabiei* in Australia based on STMS fingerprints. *Fungal Diversity* **13**: 111–29.

Rhaiem A, Cherif M, Dyer P S and Peever T L. 2007. Distribution of mating types and genetic diversity of *Ascochyta rabiei* populations in Tunisia revealed by mating-type specific PCR and random amplified polymorphic DNA (RAPD) markers. *Journal of Phytopathology* **155**: 596–605.

Rhaiem A, Cherif M, Peever T L and Dyer P S. 2008. Population structure and mating system of *Ascochyta rabiei* in Tunisia: evidence for the recent introduction of mating type 2. *Plant Pathology* **57**: 540–51.

Rohlf F I. 1998. NTSYS-pc. *Numerical Taxonomy and Multivariate Analysis System*. Version 2.0. Applied Biostatistics, New York, USA.

Santra D K, Singh G, Kaiser W J, Gupta V S, Ranjekar P K and Muehlbauer F J. 2001. Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of Ascochyta blight in chickpea. *Theoretical and Applied Genetics* **102**: 676–82.

Trapero-Casas A and Kaiser W J. 1992. Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathology* **82**: 1261–6.

Türkan M and Dolar F S. 2009. Determination of pathogenic variability of *Didymella rabiei*, the agent of ascochyta blight of chickpea in Turkey. *Turkish Journal of Agriculture and Forestry* **33**: 585–91.

Udupa S, Weigand F, Saxena M and Kahl G. 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. *Theoretical and Applied Genetics* **97**: 299–307.

Weising K, Kaemmer D, Epplen J T, Weigand F, Saxena M and Kahl G. 1991. DNA fingerprinting of *Ascochyta rabiei* with synthetic oligodeoxynucleotides. *Current Genetics* **19**: 483–9.

Wilson A D and Kaiser W J. 1995. Cytology and genetics of sexual incompatibility in *Didymella rabiei*. *Mycologia* **87**: 795–804.

Yeh F C, Yang R C, Boyle T J, Ye Z H and Mao J X. 1999. Popgene, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.