

Research Article

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Determination of pathogenic variability of *Didymella rabiei*, the agent of ascochyta blight of chickpea in Turkey

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Abstract: Pathogenic characterization of 64 *Didymella rabiei* isolates obtained from 5 different regions in Turkey was determined with a set of 7 different chickpea cultivars (ILC 1929, F8, ICC 1903, ILC 249, ILC 482, ILC 3279, and ICC 3996). All the isolates were classified into both 3 pathotypes and 6 physiological races. Of the isolates used in this study, it was determined that 38 (59.4%) belonged to pathotype I, 3 (4.7%) to pathotype II, and 23 (35.9%) to pathotype III. Pathotypes I and III were determined in 5 regions of Turkey including the Mediterranean, Aegean, Southeastern Anatolia, Central Anatolia, and Black Sea regions, but pathotype II was not found in the Mediterranean and Black Sea regions. All 6 races of the *D. rabiei* were determined in Turkey. Race 1, 2, and 3 were established in pathotype I, while race 4 was represented in both pathotypes I and II. Pathotype III included the race 5 and 6, which were aggressive isolates.

Key words: Chickpea, Ascochyta rabiei, pathotype, race

Nohut yanıklık etmeni *Didymella rabiei*'nin Türkiye'deki patojenik değişkenliğinin belirlenmesi

Özet: Türkiye'nin beş farklı bölgesinden elde edilen 64 *Didymella rabiei* izolatının patojenik karakterizasyonu 7 farklı nohut çeşidinden (ILC 1929, F8, ICC 1903, ILC 249, ILC 482, ILC 3279 ve ICC 3996) oluşan bir set ile belirlenmiştir. Tüm izolatlar hem 3 patotip hem de 6 fizyolojik ırk içersinde sınıflandırılmıştır. Çalışmada kullanılan izolatların 38 (59.4%)'i patotip I'e, 3 (4.7%)'ü patotip II'ye ve 23 (35.9%)'ü patotip III'e ait olduğu belirlenmiştir. Patotip I ve III Akdeniz, Ege, Güney Doğu Anadolu, İç Anadolu ve Karadeniz olmak üzere Türkiye'nin 5 bölgesinde de belirlenmiştir. Ancak Patotip II Akdeniz ve Karadeniz bölgelerinde tespit edilememiştir. *D. rabiei*'nin 6 ırkının tümü ülkemizde bulunmuştur. Irk 4 hem patotip I hem de patotip II ile temsil edilmesine karşın, ırk 1, 2 ve 3 patotip I içersinde yer almıştır. Agresif izolatları içeren patotip III ise ırk 5 ve 6'yı içermektedir.

Anahtar sözcükler: Nohut, Ascochyta rabiei, patotip, ırk

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most extensively grown legume crops in Turkey. The total

cultivated area, production, and yield of chickpea in Turkey is 557,800 ha, 551,746 t and 989 kg ha⁻¹, respectively (FAO, 2008). One of the greatest biotic

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stresses reducing potential yield in chickpea is ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. [telemorph: *Didymella rabiei* (Kovachevski) v. Arx] (Singh and Reddy, 1996). The fungus is recognized in many regions of the world including the Middle East, Mediterranean region, and North Africa (Nene, 1982; Nene and Reddy, 1987). The fungus attacks all above ground parts of the plant, causing necrotic lesions, which are circular on leaves and pods, and elongate on petioles and stems. When stems and petioles are girdled, they usually break (Nene and Reddy, 1987). The disease may cause total yield loss if the environmental conditions are favorable (Reddy and Singh, 1990).

The use of resistant cultivars is the most effective and economical management strategy for ascochyta blight since the application of fungicide is not economical (Singh et al., 1981). However, breeding of resistant chickpea cultivars against ascochyta blight is more difficult because of the variation in pathogenicity of A. rabiei (Singh, 1990). Thus, determination of pathotypes or races is essential for breeding resistant chickpea cultivars. A. rabiei isolates have been classified into physiological races or pathotypes based on their reaction on a set of differential chickpea genotypes. Pathogenic variability in A. rabiei was first reported in India in 1969 (Katiyar and Sood, 1985). Subsequently, Vir and Grewal (1974) and Grewal (1981) found 2 races (races 1 and 2) and 1 biotype of race 2 in India. Reddy and Kabbabeh (1985) reported 6 physiological races of A. rabiei from Syria and Lebanon using 6 chickpea differentials. Jan and Wiese (1991) identified 11 pathotypes of A. rabiei in the Palouse region of the USA. Dolar and Gürcan (1992) determined 3 races of A. rabiei in Turkey. Singh and Reddy (1993), using 3 differentials, reported that there were 6 races of A. rabiei in Syria. Thereafter, 3 pathotypes of A. rabiei, which were classified as pathotypes I, II, and III, were identified by Udupa and Weigand (1997) in Syria. Navas-Cortés et al. (1998) identified 11 pathotypes in India, Pakistan, Spain, and the United States. Recently, it has been reported that there are 14 and 17 pathotypes in Canada and Turkey, respectively (Chongo et al., 2004; Maden et al., 2004). The number of pathogenic group or race of Didymella rabiei has changed according to country because of different disease scoring methods

and different set of differentials. *D. rabiei* is a heterothallic ascomycete with 2 mating types, and both mating types are present in most chickpea production areas (Trapero-Casas and Kaiser, 1992; Kaiser and Küsmenoğlu, 1997; Bayraktar et al., 2007). Pseudothecia of *D. rabiei* usually forms on chickpea debris during winter months under cold and moist conditions, contributing to the long-term survival of the pathogen (Navas-Cortés et al., 1998). Pathogenic variability of *A. rabiei* is enhanced by the presence of the teleomorphic stage (Kaiser, 1997). There is a need to understand the pathogenic variation in the pathogen population in the production area in order to maintain an efficient resistance breeding programme.

This study was carried out to determine the distribution of the races and pathotypes of *A. rabiei* in different chickpea growing regions of Turkey and to explore a possible relationship between pathotype and race categorization of *A. rabiei*.

Materials and methods

Plant materials

Pathogenic characterization of A. rabiei isolates was determined with a set of 7 different chickpea cultivars (ILC 1929, F8, ICC 1903, ILC 249, ILC 482, ILC 3279, and ICC 3996), which were obtained from the germplasm collection of the ICARDA (International Centre for Agricultural Research in the Dry Areas, Syria) and ICRISAT (International Crops Research Institute for the Semi-Arid, India). A set of 3 different chickpea cultivars (ILC 1929, ILC 482, and ILC 3279) was used to determine the pathotype groups (Udupa and Weigand, 1997) (Table 1). Furthermore, a set of 6 different chickpea cultivars (ILC 1929, F8, ICC 1903, ILC 249, ILC 3279, and ICC 3996), which was suggested by Reddy and Kabbabeh (1985), were used to characterize isolates for physiological race groups (Table 1). The seeds were surface sterilized with sodium hypochlorite (1%) for 3 min and washed 3 times with sterile distilled water (SDW). Ten seeds were sown in 14 cm pots containing sterilized mixtures of soil, sand, and fertilizer (1:1:0.5, v/v/v) and, after germination, they were thinned to 7 seedlings per pot. Plants were grown at 22 ± 1 °C with a 14 h light-photoperiod

Table 1. Pathotype and physiological race groups characterized by testing 7 differential chickpea lines separately against each of 64 *Ascochyta rabiei* isolates from Turkey.

	Differential chickpea lines						Race	Pathotype	Number of isolates
ILC	F8	ICC	ILC	ILC	ILC	ICC			
1929		1903	249	482	3279	3996			
S	R	R	R	R	R	R	1	I	2
S	S	R	R	R	R	R	2	I	11
S	S	S	R	R	R	R	3	I	9
S	S	S	S	R	R	R	4	I	16
S	S	S	S	S	R	R	4	II	3
S	S	S	S	S	S	R	5	III	14
S	S	S	S	S	S	S	6	III	9

S = Susceptible

R = Resistant

(light intensity, 260 μmoles sec⁻¹ m⁻²) for 14 days. Plants were watered every 2-3 days. The experiment consisted of 3 replicates and was performed twice.

Isolates and inoculum preparation

A total of 67 isolates of *A. rabiei* were used in this study, 64 of which were obtained from the culture collection of the Department of Plant Protection,

Faculty of Agriculture, University of Ankara (Table 2). Pathotype I (least aggressive), pathotype II (moderately aggressive), and pathotype III (most aggressive) were provided from ICARDA. The isolates maintained on Microbank beads were grown on CSMDA (Chikpea Seed Meal Dextrose Agar: chickpea meal 40 g, dextrose 20 g, agar 20 g, distilled water 1L) at 22 ± 1 °C with a 12 h light-photoperiod

Table 2. Distribution of Ascochyta rabiei isolates used in this study according to geographic regions.

Regions	Provinces	Names of isolates	Number of isolates	
Aegean	Afyon	Afy	1	
	Denizli	Dez	7	
	Uşak	Uşk	5	
Black Sea	Amasya	Ams	4	
	Çorum	Çor	4	
	Tokat	Tok	1	
Central Anatolia	Ankara	Ank	9	
	Eskişehir	Esk	4	
	Kayseri	Kay	2	
	Kırşehir	Kır	2	
	Sivas	Siv	1	
	Yozgat	Yoz	3	
Mediterranean	Antalya	Ant	3	
	Burdur	Bur	1	
Southeastern Anatolia	Adıyaman	Ady	8	
	Diyarbakır	Diy	7	
	Kahramanmaraş	Kmar	1	
	Şanlıurfa	Urf	1	
Total			64	

for 14 days. The cultures were flooded with SDW and spores were scraped with sterile glass spatula. The concentrated spore suspensions were filtered through filter paper to remove mycelial fragments. Spore suspensions were adjusted to 5×10^5 spores ml⁻¹ in SDW using a hemacytometer. All isolates utilized in this study originated from single conidia.

Inoculations of plants and disease assessment

Two-week-old plants of each cultivar were inoculated with the isolates of A. rabiei using 3 pots of 7 plants per isolates. Spore suspensions (5×10^5 spores ml $^{-1}$) were applied to the plants until run off using a pressure sprayer. In each experiment, as control, inoculated set of plants were sprayed with SDW. After spraying, plants were immediately covered with transparent polyethylene bags to maintain leaf wetness and incubated for 21 days in a growth chamber adjusted to 20 ± 1 °C and a 12 h photoperiod. The polyethylene bags were opened at the bottom after day 3 and removed on day 4 after inoculation. In order to maintain humidity, plants were sprayed with SDW 4 times a day with a humidifier.

Twenty-one days after inoculation, cultivars were assessed using 1-9 rating scale as described by Singh et al. (1981) with 1 = no visible lesions on any plants; 3 = lesions visible on less than 10% of the plants, no stem girdling; 5 = lesions visible on up to 25% of the plants, stem girdling on less than 10% of the plants but little damage; 7 = lesions on most plants, stem girdling on less than 50% of the plants resulting in the death of a few plants; 9 = lesions profuse on all plants, stem girdling on more than 50% of the plants and death of most plants. Cultivars rated 1.0 to 5.0 were considered resistant and those rated 5.1 to 9.0 were considered susceptible.

Results

Sixty-four Turkish isolates of *A. rabiei* used in this study were classified into both 3 pathotypes and 6 physiological race groups based on disease reaction on a set of 7 differential chickpea genotypes (Table 1).

All 3 pathotypes were determined in Turkey although distribution of each pathotype was different (Figure 1). Pathotype I was found in all the provinces of the Aegean, Black Sea, Central Anatolia,

Mediterranean, and Southeastern Anatolia regions, except in Burdur, Sivas, and Şanlıurfa provinces (Figure 1). Thirty-eight isolates (59.4%) were represented in pathotype I. However, pathotype II included only 3 isolates (4.7%), which were found in Denizli (Aegean), Kayseri (Central Anatolia), and Şanlıurfa (Southeastern Anatolia). Pathotype III was found in all the regions including 23 isolates (35.9%). Pathotype I was the highest and most widely distributed pathotype in Turkey, whereas pathotype II was the lowest. Pathotype III was the second largest group in Turkey, but it was not as widespread as pathotype I (Figure 1).

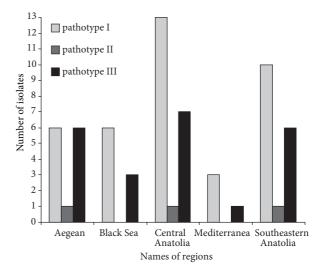


Figure 1. Distribution of pathotypes of *Ascochyta rabiei* according to geographic regions.

Pathogenic variability was evaluated to determine the physiological races of *A. rabiei* using 6 chickpea lines. The results of our study showed that all 6 races of *A. rabiei* existed in Turkey. Distribution of races among isolates obtained from 5 different regions were quite different. Race 1 was represented by 2 isolates collected from only Ankara and Yozgat provinces of the Central Anatolia region. Races 2, 4, and 6 were found in all the provinces of the Aegean, Black Sea, Central Anatolia, Mediterranean, and Southeastern Anatolia regions. However, races 3 and 5 were not observed in the Black Sea and Mediterranean regions. Race 4 included 19 isolates (29.7%) out of 64 so it was the largest and most widely distributed race, whereas

race 1 was the lowest. Race 5 was found to be the second widespread race (21.9%). Races 3 and 6 exhibited the same distribution (14.1%).

The result of our study indicated that the isolates representing pathotype I have a high variability in the pathogenicity spectrum (Table 1). Pathotype I isolates were designated as races 1, 2, 3, and 4. On the other hand, it was detected that race 4 was represented into pathotype I and pathotype II. Pathotype III included races 5 and 6, which were aggressive isolates (Table 1, Figure 2).

Discussion

Pathogenic variability among A. rabiei isolates has been reported from many countries including India, USA, Syria, Pakistan, Turkey, and Canada. The isolates used in these studies have been classified into 3 to 17 pathotypes based on their reaction on 3 to 16 host genotypes (Vir and Grewal, 1974; Jan and Wiese, 1991; Udupa and Weigand, 1997; Jamil et al., 2000; Maden et al., 2004; Chongo et al., 2004). It is difficult to compare the results of these studies as they used different methods and chickpea genotypes (Navas-Cortés et al., 1998; Chen et al., 2004). Therefore, Udupa and Weigand (1997) suggested that a standard set of 3 different chickpea cultivars consisting of 'ILC 1929' as susceptible, 'ILC 482' as tolerant, and 'ILC 3279' as resistant genotype is sufficient for pathotyping A. rabiei isolates into 3 pathotypes based on increasing level of aggressiveness. In Pakistan, 130 isolates of A. rabiei were classified into 3 pathotypes with the standard set used at ICARDA (Jamil et al., 2000). We showed that 64 Turkish isolates of A. rabiei could be classified into 3 pathotypes. The results from our study revealed that aggressiveness of the isolates was generally low. Pathotype I was predominant in almost all provinces. It could not be determined in Burdur, Sivas, and Şanlıurfa provinces, probably because of the limited samples. Similar results were observed by Maden et al. (2004), who classified 134 isolates into 17 pathotypes with 5 local chickpea genotypes (Canıtez 87, Er 99, Gökçe, ILC 195/2, Uzunlu 99). They reported that 69 (51.5%) of 134 A. rabiei isolates belonged to pathotype 1. In contrast, Jamil et al. (2000) reported that almost all isolates of A. rabiei obtained from Pakistan belonged to pathotypes II and III. In the present study, pathotype II appeared in a very limited number of provinces including Denizli, Kayseri, and Şanlıurfa. Only 3 (4.7%) of all isolates were represented by pathotype II. Udupa et al. (1998) reported that 5 (9.5%) of 53 Syrian isolates of *A. rabiei* belonged to pathotype II. Moreover, they determined that pathotype diversity existed in the northeast provinces in which chickpea is cultivated extensively. In our study we found that all 3 pathotypes were present in the Southeastern Anatolia region including Şanlıurfa, Adıyaman, K. Maraş, and Diyarbakır provinces, which have a border with or are close to Syria. Kaiser (1997) determined that pseudothecia and ascospore developed on

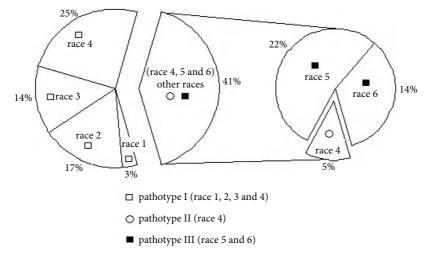


Figure 2. Pathotype and race percentages of Ascochyta rabiei in Turkey.

infested chickpea samples collected from Syrian province of Hassakeh. It was reported that infested chickpea samples play an important role in natural spread of the pathogen. Moreover, it was observed that sexual recombination (teleomorph) led to genetic diversity and novel pathotypes of the pathogen (Kaiser, 1997). It is known that 2 mating types (MATs) of A. rabiei are present in most chickpea production regions (Trapero-Casas and Kaiser 1992; Barve et al. 2003; Bayraktar et al., 2007). Of the 145 isolates collected from 23 provinces of Turkey, Kaiser and Küsmenoglu (1997) reported that 85 (59%) belonged to MAT 1-1, 60 (41%) to MAT 1-2, and both mating types were present in the Southeastern Anatolia region, which explains why all the 3 pathotypes existed in the Southeastern Anatolia region.

We observed that pathotype diversity existed in 5 different regions of Turkey including the Aegean, Black Sea, Central Anatolia, Mediterranean, and Southeastern Anatolia regions. All the 3 pathotypes were determined in 3 (Agean, Southeastern Anatolia, and Central Anatolia) regions where chickpea is grown extensively. However, Pathotype II was not found in the Mediterranean and Black Sea regions. We thought that it may be associated with the limited isolates collected from these regions.

Pathogenic variation of *D. rabiei* isolates has been expressed by various terms such as pathogenic group, pathotype, and race (Navas-Cortés et al., 1998). In this study, all 64 isolates were classified as races of *D. rabiei*. Six races of *A. rabiei* were originally determined on a set of 6 different chickpea cultivars (ILC 1929, F8, ICC 1903, ILC 249, ILC 3279, and ICC 3996) (Reddy and Kabbabeh, 1985). By using the same set, Dolar and Gürcan (1992) reported races of *A. rabiei* 1, 4, and 6 in Turkey. It was found that races 4 and 6 were more widespread than race 1. We determined that race 4 was the largest and most widely distributed race. The race spectrum determined in our study is different from that of previous studies in Turkey, where also races 2 and 5

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are widespread together with races 4. Thus, we detected that the race spectrum of *A. rabiei* has fairly varied in Turkey in the last 16 years.

The results of our study showed that the 3pathotype system of Udupa and Weigand (1997) could represent the 6 race system of Reddy and Kabbabeh (1985). The chickpea cultivars (ILC 1929, F8, ICC 1903, and ILC 249) are susceptible to all 3 pathotypes. The chickpea cultivar (ILC 482) is susceptible to both pathotypes II and III, but resistant to pathotype I. The chickpea cultivar (ILC 3279) is susceptible to both pathotype III and race 5, but resistant to pathotypes I and II. Thus, in our study, Pathotype I was designated as races 1, 2, 3, and 4. On the other hand, we detected that race 4 was represented in pathotype II. Chen et al. (2004) reported that the 5 races of *A. rabie* without race 6 are pathotype I. The chickpea cultivars (ILC 3279 and ICC 3996) were identified to be susceptible to race 6. Thus, pathotype III was designated to both race 5 and race 6 (Table 1 and Figure 2). Results of our study are more or less in agreement with those of Chen et al. (2004). However, they reported that race 6 is pathotype II and the other 5 races are pathotype I. The term physiologic race was mostly replaced by the term pathotype. Turkish isolates of *A. rabiei* showed a high level pathogenic variability and all the pathotypes and races were found in Turkey. These data from the present study can be used in chickpea breeding programmes. In other words, reactions of local chickpea genotypes to all 3 pathotypes of A. rabiei should be determined and after the best chickpea genotypes are selected, they should be recommended for Turkish breeders.

Acknowledgements

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