Full Length Research Paper

Determination of pathotypes and physiological races in Ascochyta rabiei, the agent of ascochyta blight in chickpea (Cicer arietinum L.) in Algeria

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Pathogenic determination of sixteen *Ascochyta rabies* isolates obtained from seven different provinces of western north of Algeria was the aim of this study. The pathotypes and physiological races were determined using seven differential chickpea lines (ILC1929, F8, ICC1903, ILC247, ILC482, ILC3279 and ICC3996). All isolates were classified into three pathotypes and six physiological races according to their aggressiveness and virulence, respectively. We found only one isolate (6.25%) from pathotype I (the least aggressive), 12 isolates (75%) from pathotype II (moderate aggressive) and three isolates (18.75%) from pathotype III (highly aggressive). Four races of *A. rabiei* were determined in this region (races 1, 4, 5 and 6). Races 1 and 2 were established in pathotype I, race 4 was represented by the pathotype II, and pathotype III included the two races 5 and 6, which were virulent isolates.

Key words: Ascochyta rabiei, Cicer arietinum, pathotypes, physiological races, aggressiveness, virulence.

INTRODUCTION

Chickpea (Cicer arietinum L.) is the third most important grain legume in the world after common bean (Phaseolus vulgaris L.) and pea (Pisum sativum L.) (Pande et al., 2005). It is one of the major protein sources in developing countries such as Algeria and grows even on poor, sandy soil (Sharma and Jodha, 1984). One of the greatest biotic stress reducing potential yields in chickpea is ascochyta blight caused by Ascochyta rabiei Pass. (Labr.) (teleomorph, Didymella rabiei v. Arx. syn. Mycosphaerella rabiei Kovachevski) (Ahmed et al., 2006). The fungus is recognized in many countries of the world including the Mediterranean region, Middle East and Indian subcontinent (Nene and Reddy, 1987). The disease may cause total yield loss if the environmental conditions are favorable (Reddy and Singh, 1990).

In Algeria, data of several years of prospection showed the presence and the extension of ascochyta blight with falls of output which can go up to 100% (Bouznad et al., 1996). Mabsoute et al. (1996) announced that in Algeria like in the other Maghreb countries, the ascochyta blight remains the major constraint of chickpea. The use of resistant chickpea cultivars is the most effective and economical management strategy for ascochyta blight since the application of fungicide is not economical (Gan et al., 2006). 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 physiological races is essential for breeding resistant chickpea cultivars. This determination is based on their reaction on a set of differential chickpea genotypes (Tűrkkan and Dolar, 2009).

The pathogenic variability in *Ascochyta rabiei* was first reported in India in 1969 (Katiyar and Sood, 1985). Subsquently, Vir and Grewal (1974) found 2 races (race 1 and race 2) and 1 biotypes of race 2 in India. Reddy and Kabbabeh (1985) reported 6 physiological races of *A. rabiei* from Syria and Lebanone using 6 differential chickpea lines. Jan and Wiese (1991) identified 11 pathotypes of *A. rabiei* in the Palouse region of the USA.

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Table 1. Differential chickpea lines with their origin.

Chickpea lines	Origin
ILC 1929	ICARDA ¹
F8	ICARDA
ILC 249	ICARDA
ILC 482	ICARDA
ILC 3279	ICARDA
ICC 1903	ICRISAT ²
ICC 3996	ICRISAT

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Table 2. Pathotypes and physiological race groups determined by testing 7 differential chickpea lines separately against each each of 16 *A. rabiei* isolates from western north region of Algeria.

Chickpea lines						_	Dhucial aginal races	Number of isolates		
ILC1929	F8	ICC1903	ILC249	ILC482	ILC3279	ICC3996	Pathotypes	Physiological races	Number of Isolates	
S	R	R	R	R	R	R	l	1	1	
S	S	R	R	R	R	R	I	2	0	
S	S	S	R	R	R	R	I	3	0	
S	S	S	S	S	R	R	П	4	12	
S	S	S	S	S	S	R	111	5	2	
S	S	S	S	S	S	S	III	6	1	

S: Susceptible; R: resistant.

Singh and Reddy (1993), using 3 differential lines, reported that there were 6 races in Syria. Udupa and Weigand (1997) classified the isolates as 3 pathotypes I, II and III according to their aggressiveness in Syria. Navas-Cortes et al. (1998) identified 11 pathotypes in India, Pakistan, Spain and USA. Chongo et al. (2004) reported that there are 14 pathotypes In Canada. Recently, It has been reported that there are 3 pathotypes and 6 physiological races in Turkey according to their aggressiveness and virulence, respectively (Türkkan and Dolar, 2009).

The term 'pathotype' was used recently to describe levels of aggressiveness of isolates with a small set of differential genotypes (Udupa et al., 1998; Jamil et al., 2000; Chen et al., 2004).

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 program. This study was carried to identify the pathotypes and physiological races of *Ascochyta rabiei* using 7 differential chickpea lines in the western north region of Algeria.

MATERIALS AND METHODS

Plant material

A set of 7 differential chickpea lines (ILC 1929, F8, ICC 1903, ILC 249, ILC 482, ILC 3279 and ICC 3996) from ICARDA and ICRISAT

(Table 1). 3 chickpea lines were used to determine the pathotypes of *A. rabiei* and 6 to identify the physiological races according to their aggressiveness and virulence, respectively (Reddy and Kabbabeh, 1985; Udupa and Weigand, 1997) (Table 2).

Fungal material

The isolates of *A. rabiei* used in this study were obtained by isolation from samples of stems, sheets and chickpea pods presenting of the symptoms of ascochyta blight (Table 3). The antagonist was isolated from soil sample in the rhizosphere and its identification was done by optic microscope (x40).

Isolation and purification of cultures

The isolates were conserved in Petri dishes contained CSMDA medium (Chickpea Seed Meal Dextrose Agar) (Jamil et al., 2002). The isolates were maintained on CSMDA medium at 20±2°C (Dolar et al., 1994).

Obtaining the seedlings and inoculum preparation

The seeds of chickpea lines used are sterilized with Sodium hypochlorite (at 2%) for 3 min and washed 3 times with sterile distilled water. They were then sown in pots of 10 cm height and 6 cm in diameter, containing a sterile peatmoss, at rate of 2 seeds per pot and 4 repetitions for each particular treatment. 16 isolates of *A. rabiei* were used in this study (Table 3). The cultures of isolates were flooded with sterile distilled water and spores were scraped

Isolates	Origins	Dates of isolation
At0108	Aïn Temouchent	March 2008
Sba0108	Sidi Bel Abbes	March 2008
Sba0208	Sidi Bel Abbes	March 2008
Msc0108	Mascara	April 2008
Mos0108	Mostaganem	June 2008
Mos0208	Mostaganem	June 2008
Msc0208	Mascara	November 2008
Msc0308	Mascara	November 2008
Msc0408	Mascara	November 2008
At0208	Aïn Temouchent	November 2008
At0308	Aïn Temouchent	November 2008
Rel0109	Relizane	September 2009
Rel0209	Relizane	September 2009
Rel0309	Relizane	September 2009
Chl0110	Chlef	July 2010
Tle 0111	Tlemcen	June 2011

Table 4. ANOVA analysis.

	S.C.E	ddl	C.M.	Test F	Probability	E.T.	C.V.
Variance global	3739.964	447	8.367				
Variance Factor 1	598.035	15	39.869	31.336	0		
Variance Factor 2	2293.245	6	382.208	300.402	0		
Var. Inter F1*2	421.184	90	4.68	3.678	0		
Var. Résiduelle 1	427.5	336	1.272			1.128	19.65%

S.C.E., Sum of square differencies; ddl: free degre; C.M.: mean square, E.T.: error type, C.V.: coefficient of variation.

with sterile glass spatula. The concentrated spores' suspensions were filtered through filter paper to remove mycelia fragments. Spores suspensions were adjusted to 5×10^5 spores ml⁻¹ using a hemacytometer (Labdi, 1995). All isolates used in this study origined from single conidia.

Inoculation of plants

Two weeks old plants of each line were inoculated with the isolates of A. rabiei using 4 pots of 2 plants per isolate. In each experiment, as control, inoculated set of plants were sprayed with sterile distilled water by pressure sprayer in growth chamber. After spraying, plants were inoculated by spore suspension. In order to maintain humidity, plants were sprayed with sterile distilled water 2 times a day with a humidifier (Tűrkkan and Dolar, 2009).

Rating scale

The severity of the disease is noted from 1 to 9, according to the scale of Reddy and Singh (1984) which is based on the intensity of the symptoms, 21 days after inoculation presents itself as follows:

1 : No lesion is visible on the whole of the plants.

3 : Visible lesions on less than 10% of the plants, the stems are not reached.

5: Lesions on 25% of the plants, with damage on approximately 10% of the stems.

7: Lesions on all the plants, approximately 50% of the stems are reached, which results in the death of certain plants because of serious damage.

9: Lesions diffused on all the plants, the stems are reached in proportions higher than 50% with the death of the majority of the plants.

The chickpea lines rated 1.0 to 4.9 were considered resistant and those rated 5.0 to 9.0 were considered susceptible (Türkkan and Dolar, 2009).

Statistical analysis

The variances (σ^2), averages and standard deviation (SD) of various repetitions were calculated and analyzed by the software of statistics (STAT BOX 6.0.4. GRIMMERSOFT) and the device used are the unifactorielle total randomization (one studied factor) by the test of Newman and Keuls (P_{0.05} and P_{0.01}).

RESULTS

Sixteen Algerian isolates of *A. rabiei* used in this study were classified into 3 pathotypes based on disease reaction on a set of 3 chickpea genotypes, and 6 physiological races based on a set of 6 chickpea genotypes. Highly significant effect (P < 0.01) was

Isolates	Aggressiveness (Mean ± SD)	Test F	C.V.
At0108	5.32 ^c ± 1.16		
Sba0108	$5.32^{\circ} \pm 0.83$		
Sba0208	$2.46^{\circ} \pm 0.76$		
Msc0108	$5.25^{\circ} \pm 1.29$		
Mos0108	$5.75^{\circ} \pm 0.95$		
Mos0208	$8.32^{a} \pm 0.60$		
Msc0208	$7.03^{b} \pm 0.88$		
Msc0308	$6.96^{b} \pm 0.81$	31.33**	19.65%
Msc0408	$5.60^{\circ} \pm 0.66$		
At0208	5.75 ^c ± 1.18		
At0308	5.75 ^c ± 1.28		
Rel0109	$5.92^{\circ} \pm 0.73$		
Rel0209	$5.20^{\circ} \pm 0.83$		
Rel0309	5.71 [°] ± 1.14		
ChI0110	$5.39^{c} \pm 0.83$		
Tle 0111	$5.67^{\circ} \pm 1.43$		

Table 5. Comparison of severity degrees between Ascochyta rabiei isolates.

Highly significant effect P<0.01 (Newmann-Keuls test at 1%). SD: Standard deviation, C.V.: coefficient of variation.

Table 6. Reaction of chickpea lines to the agressiveness and virulence of A. rabiei isolates.

Lines	ILC1929	F8	ICC1903	ILC249	ILC482	ILC3279	ICC3996	Test F
Mean±SD	8.43 ^a ±0.75	1.34 ^b ±0.75	6.92 ^c ±0.83	6.46 ^d ±1.04	6.28 ^d ±1.0	3.07 ^e ±1.6	1.65 ^e ±0.55	300.4**



Provinces

Figure 1. Distribution of *A. rabiei* pathotypes in a western north region of Algeria according to their aggressiveness.

observed on a comportment of *A. rabiei* isolates (Tables 4, 5 and 6).

All 3 pathotypes were obtained in a western north region of Algeria although distribution of each pathotype

was different (Figure 1).

Pathotype II (moderately aggressive) was found in all the provinces of this region. Just 1 isolate (6.25%) was represented in pathotype I (least aggressive), 3 isolates (18.75%) were in pathotype III (Highly aggressive), and all another 12 isolates (75%) were represented in pathotype II and found in all provinces of this region.

Concerning the physiological race groups of *A. rabiei*, were found 4 races (race 1, 4, 5 and 6). Distribution among isolates obtained from 7 provinces was quite different. Race 1 (avirulent) was represented by just one isolate from the province 'Sidi Bel Abbes'. Race 4 (Moderately virulent) was found from all provinces. However, race 5 (highly virulent) was observed in just one province (Mascara) and race 6 (highly virulent) was found in Mostaganem by one isolate from Mostaganem province.

DISCUSSION

Pathogenic variability among *A. rabiei* was reported from many countries including India (Vir and Grewal, 1974; Singh, 1990; Singh and Pal, 1993; Ambarder and Singh, 1996), Syria and Lebanon (Reddy and Kabbabeh 1985; Udupa and Weigand, 1997; Udupa et al., 1998), the Palouse region of USA (Jan and Wiese, 1991; Navas-Cortes et al., 1998; Chen et al., 2004), Italy (Porta-Puglia et al., 1996), Pakistan (Jamil et al., 2000; Iqbal et al., 2004), Spain (Navas-Cortes et al., 1998), Australia (Khan et al., 1999), Tunisia (Hamza et al., 2000), Canada (Chongo et al., 2004; Vail and Banniza, 2008) and recently Turkey (Türkkan and Dolar, 2009). These studies were based on 3 to 15 differential chickpea genotypes tested with 11-130 isolates of *A. rabiei*, classified into 3 to 14 differential pathotypes or races.

Pathogenic variation of *A. rabiei* has been been expressed by various terms such as pathogenic group, biotype, pathovar, pathotype and race (Navas-Cortes et al., 1998). Udupa and Weigand (1997) suggested that standard set of 3 differential chickpea genotypes 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. Reddy and Kabbabeh (1985) proposed a set of 6 differential genotypes (ILC1929, F8, ICC1903, ILC249, ILC3279 and ICC 3996) to determine 6 physiological races.

The pathotypes of *A. rabiei* were obtained using 130 and 64 isolates from Pakistan and Turkey, respectively (Jamil et al., 2000; Türkkan and Dolar, 2009). We showed that 16 algerian isolates of *A. rabiei* could be classified into 3 pathotypes and 4 physiological races. The results revealed that aggressiveness of the isolates was generally moderate. Pathotype II was predominant in almost all provinces, pathotype III was existed in two provinces (Mascara and Mostaganem) and we found just 1 isolate from pathotype I. In contrast, Udupa et al. (1998) found just 5 (9.5%) isolates from pathotype II in Svria.

All 6 physiological races of *A. rabiei* were found by Reddy and Kabbabeh (1985) using 64 isolates from Syria

and Lebanon. By using the same set, Dolar and Gürcan (1992) reported races of *A. rabiei* 1, 4 and 6 in Turkey. In 2009, Türkkan and Dolar reported all 6 races in Turkey. Thus, in our study, we found races 1, 4, 5 and 6 (race 2 and 3 not found) using the same differential chickpea genotypes. In this region, it was found that race 4 was the largest and most widely distributed race.

Chen et al. (2004) reported that the 5 races of *A. rabiei* 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 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. Algerian isolates of *A. rabiei* showed a high level pathogenic variability and all the pathotypes were found in Algeria.

Conclusion

In the present study, A. rabiei isolates showed difference in their aggressiveness and virulence. We found that the pathotype II was predominant in all provinces of western north region of Algeria, the pathotype I in the province Sidi Bel Abbes and pathotype III, we found it in 2 provinces (Mascara and Mostaganem). By using a set of 6 differential chickpea genotypes, 4 races were determined in this region (races 1, 4, 5 and 6), the race 4 (moderate virulent) is a predominant (75%). Thus, pathotype I was designed to races 1, 2 and 3, pathotype II to race 4 and pathotype III is a both races 5 and 6. However, now almost studies in the world use the term race for identify the virulence of their isolates. It is difficult to study the pathogenic variability of this pathogen and compare it with other researches, because they used different methods and chickpea genotypes.

These data can be used in chickpea breeding program for resistance to ascochyta blight. It is necessary to determine in future, the reactions of local chickpea cultivars to *A. rabiei* for their recommendation for Algerian breeders.

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