Pathotyping study of Algerian *Ascochyta rabiei* isolates using screening test of ascochyta blight severity assessment

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Abstract

Determination of twenty *Ascochyta rabies* isolates obtained from different provinces of western north of Algeria, to study their pathotyping. The pathotypes were determined using four chickpea germplasm accessions ILC1929, ILC482, ILC3279 and ICC12004, were inoculated to detect the four pathotypes I, II, II and IV. All isolates were classified into four pathotypes according to their aggressiveness on these accessions. We found three isolates represents the pathotype I (least aggressive), 13 isolates were the pathotype II (moderate aggressive), two isolates for pathotype III (more aggressive), and two isolates represent the pathotype IV (highly aggressive). We noticed that these results confirm the ascochyta blight of chickpea in these regions is moderately to highly aggressive when the season conditions will be favorable to devepment this disease.

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

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. Recently, Imtiyaz et al. (2011) reported the presence the highly aggressive pathotype named pathotype IV in Syria.

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 necessary to know the pathogenic variation in this pathogen population in the field in order to maintain an efficient resistance breeding program. This study was carried to identify these four pathotypes of *Ascochyta rabiei* using 4 differential chickpea genotypes.

Material and Methods

Plant material

A set of 4 differential chickpea genotypes (ILC 1929, ILC 482, ILC 3279 and ICC 12004) from ICARDA and ICRISAT (Table 1). These chickpea genotypes were used to determine four pathotypes of *A. rabiei* according to their aggressiveness (Udupa and Weigand, 1997 ; Türkkan and Dolar, 2009 ; Imtiyaz et al., 2011).

Fungal material

The isolates of *A. rabiei* used in this study were obtained by isolation from samples of chickpea stems, sheets and pods presenting of the typical symptoms of ascochyta blight (Table 2).

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 inoculums 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. Twenty 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 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 conidia ml-1 using a hemacytometer (Labdi, 1995). All isolates used in this study originated from monosporal culture.

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 (Türkkan 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 global unifactorial randomization (one studied factor) by Newman and Keuls test ($P_{0.05}$ and $P_{0.01}$).

Results

Twenty Algerian isolates of *A. rabiei* used in this study were classified into four pathotypes based on disease reaction on a set of four chickpea genotypes (Table 3). Highly significant effect ($P < 0.01$) was observed on a inoculation of these genotypes by *A. rabiei* isolates (Table 4). All four pathotypes were obtained in a western north region of Algeria although their distribution and pourcentage of each pathotype were different (Figure 1). Pathotype II (moderately aggressive) was found in all the provinces of the north-western region with 13 isolates. Just 2 isolates were represented the pathotype I (least aggressive), 3 isolates were in pathotype III (Highly aggressive), and 2 isolates represented pathotype IV, highly aggressive from Mascara region.

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; Peever et al., 2012), 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), Turkey (Türkkan and Dolar, 2009), and also recently in Syria (Imtiyaz et al., 2011). 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 the twenty algerian isolates of *A. rabiei* could be classified into 4 pathotypes.

The results revealed that aggressiveness of the isolates was generally moderate (pathotype II represents 65%), and was predominant in almost all provinces, pathotype III was existed in two provinces (Mascara and Sidi Bel Abbes) and we found 3 isolates from pathotype I. The highly aggressive pathotype (IV) represented in Mascara region.

In contrast, Udupa et al. (1998) found just 5 (9.5%) isolates from pathotype II in Syria. 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. 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. Results of our study are more or less in agreement with those of Chen et al. (2004).

The term physiologic race was mostly replaced by the term pathotype. Imtiyaz et al. (2011) reported a new highly aggressive pathotype named IV in Syria. We are also observed this aggressive pathotype in *A. rabiei* Algerian isolates of Mascara region, which showed a high level pathogenic variability with all the pathotypes were found in this region.

Conclusion

In this study, we determinate the pathotyping of *A. rabiei* isolates using a set of four chickpea genotypes for aggressiveness study. We observed the presence of pathotype II when it was predominant in all provinces of western north region of Algeria, the pathotype I in the province Sidi Bel Abbes, pathotype III, in 2 provinces (Mascara and Ain Temouchent), and pathotype IV in Mascara. However, now almost studies in the world use the term pathotype than 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. It is necessary to transfer thses informations to breeders and farmers about regions where the aggressive pathotypes are presents like Mascara, when the ascochyta blight disease is destructive.

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Table 01: Differential chickpea genotypes with their origin.

Chickpea genotypes	Origin
ILC 1929	ICARDA
ILC 482	ICARDA
ILC 3279	ICARDA
ICC 12004	ICRISAT

ICARDA: International Center of Agricultural Research on Dry Areas, Aleppo, Syria. ICRISAT: International Crops Research Institute for the Semi Arid Tropics, Patanchero, India.

Table 02: *Ascochyta rabiei* isolates with their origin and seasons of sampling.

Isolates	Origin	Seasons of sampling
Ar1	Mascara	2011
Ar2	Mascara	2011
Ar3	Mascara	2011
Ar4	Mascara	2011
Ar5	Mascara	2011
Ar6	Mascara	2011
Ar7	Ain Temouchent	2011
Ar8	Ain Temouchent	2011
Ar9	Ain Temouchent	2011
Ar10	Ain Temouchent	2011
Ar11	Ain Temouchent	2011
Ar12	Ain Temouchent	2011
Ar13	Ain Temouchent	2011
Ar14	Tlemcen	2012
Ar15	Tlemcen	2012
Ar16	Tlemcen	2012
Ar17	Tlemcen	2012
Ar18	Tlemcen	2012
Ar19	Sidi Bel Abbes	2012
Ar20	Sidi Bel Abbes	2012

Ar: *Ascochyta rabiei*.

Table 3: Pathotyping groups of *Ascochyta rabiei* determinated by using four differential chickpea genotypes.

Chickpea genotypes				Pathotypes	Number of isolates	Pathotyping pourcentage
ILC 1929	ILC 482	ILC 3279	ICC 12004			
S	S	S	S	I	3	15
S	S	R	R	II	13	65
S	S	S	R	III	2	10
S	S	S	S	IV	2	10

R: Resistant; S: Susceptible.

Table 5: ANOVA of statistical analysis of results.

t-test at $P_{0.01}$	Error type	C.V
39,8**	1.01	19.01
Probability at 1% was 0.0003		

C.V.: Coefficient of variation

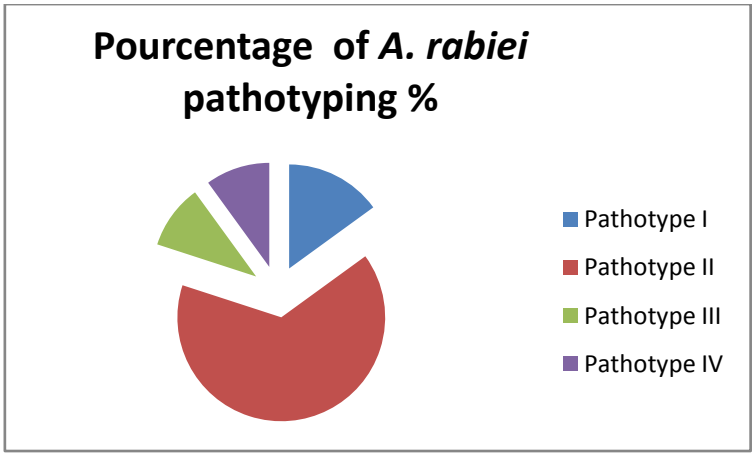

Figure 1. Pourcentage distribution of *A. rabiei* pathotypes according to their aggressiveness.