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Fungal Inoculum Potential on Severity of Ascochyta Blight of Chickpea (*Cicer arietinum* L.)

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Abstract: The effect of inoculum concentration on severity of ascochyta blight in chickpea was studied using two chickpea lines, one resistant (ICC 3996) another susceptible line (ILC 1929). These two chickpea germplasm were inoculated with two *Ascochyta rabiei* isolates (Mos02, virulent; Sba02, avirulent) each of them at four different concentrations (10^4, 10^5, 5×10^5 and10^6 conidia/ml). The results obtained showed that the disease severity increased with increasing of inoculum concentration from 10^5 conidia/ml for the resistant line, while the susceptible one was affected with all the tested fungal conidial concentrations.

Key words: *Ascochyta rabiei* · *Cicer arietinum* · Inoculum concentration · Resistance · Sensitivity

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important grain legume in the world space after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) [1-3]. It is one of the major protein sources in developing countries such as Algeria and grows even on poor, sandy soil [4,5].

Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. is major constraint in chickpea production in Algeria and elswhere [6,7]. *A. rabiei* can cause 100% crop loss when conditions are favorable for the development of the disease [8,9]. The most effective ways to control this disease are through the use of resistant cultivars and certificated seeds.

Spore concentration is important factor affecting resistance reaction of the chickpea lines [10]. Previous reports suggest that the expression of resistance may vary with the inoculum concentration and the race of pathogen. This study has the objective to evaluate the effect of inoculum concentration on seveirity of ascochyta blight disease in two chickpea lines.

MATERIALS AND METHODS

Plant Material: A set of 2 chickpea lines, ILC 1929 from ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria) and ICC 3996 from ICRISAT (International Crops Research Institute for the Semi Arid Tropics, Patanchero, India). ILC 1929 is susceptible chickpea line to Ascochyta blight, but ICC 3996 is resistant.

Fungal Material: Two isolates of *Ascochyta 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 1).

Isolation and Purification of Cultures: The isolates were conserved in Petri dishes contained CSMDA medium (Chickpea Seed Meal Dextrose Agar) [11,12]. The isolates were maintained on CSMDA medium at 20±2°C [13].

Obtaining the Seedlings and Inoculum Preparation: The seeds of chickpea lines used are surface sterilized with Sodium hypochlorite (at 2%) for 3 minutes 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 autoclaved peatmoss, at rate of 2 seeds per pot and 4 repetitions for each particular treatment.

Sixteen 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

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Table 1: Ascochyta rabiei isolates with their origin, dates of isolation and aggressiviness degrees.

Isolates	Origin	Date of isolation	Pathotype
Sba 02	Mostaganem	March 2008	I (Least aggressive)
Mos 02	Sidi Bel Abbes	June 2008	III (Highly aggressive)

were filtered through paper of Watman to remove mycelia fragments. Spores suspensions were adjusted using a hemacytometer [14]. All isolates used in this study originated 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 [15].

Rating Scale: The severity of the disease is noted from 1 to 9, according to the scale of Reddy and Singh (1984) [16] 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 2.9 were considered resistant and those rated 3.0 to 9.0 were considered susceptible [11].

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

A significant effect ($P<0.05$) was observed on reaction of both chickpea lines (except the concentration 10^4 conidia/ml in the isolate Sba 02) (Tables 2 and 3).

Disease symptoms appeared on plants with inoculum concentrations (10^5, 5×10^5 and 10^6 conidia/ml), but not on the concentration 10^4 conidia/ml except on the susceptible line ILC 1929. If we compare the reaction of two chickpea lines to 2 isolates, we observe that the line ILC 1929 is sensitive to all concentrations of the isolate Mos 02 and ICC 3996 is resistant to the concentration 10^4 conidia/ml.

Table 2: Ascochyta blight severity on two chickpea lines inoculated with 4 different inoculums concentration (conidia/ml).

		Inoculum concentrations (conidia/ml)				
Lines	Isolates	10^4	10^5	5×10^5	10^6	F value
ILC1929	Sba02	R	S	S	S	5.12*
	Mos02	S	S	S	S	
ICC3996	Sba 02	R	S	S	S	
	Mos02	R	S	S	S	

*Significant effect at 5% ($P<0.05$); R, Resistant; S, Susceptible.

Table 3: ANOVA analysis.

	S.C.E	ddl	C.M.	Test F	PROBA	E.T.	C.V.
Var globale	125,938	15	8,396			1,436	22,31%
Var factor 1	76,563	1	76,563	37,121	0,00007		
Var factor 2	14,063	1	14,063	6,818	0,02191		
Variance Interaction F1*2	10,562	1	10,562	5,121	0,0413		
Var iance Residual 1	24,75	12	2,063				

S.C.E. Sum of square differences, ddl, Free degree, C.M. Mean square, E.T. Error type, C.V. Coefficient of variation. var. Variance, Factor1, Isolates, Factor2, Lines.

DISCUSSION

The inoculum concentration of pathogens on many host plants were reported by a lot of authors [10,17-19]. In our study, we observed that the disease severity of ascochyta blight caused by *A. rabiei* on chickpea increased when the inoculum concentration increase. These results were reported by others researchs [10, 20, 21].

Dolar (1997) [21] reported that the inoculum concentration 5×10^5 conidia/ml, is very important of symptoms appearance and it used in screening program for resistance diseases by another researchers in the world. Trapero-Casas and Kaiser (1992) [10] suggested that increases in disease severity with increasing inoculum concentration depended on the chickpea cultivars. Stewart (1990) [19] reported that spore concentration had an important role on the resistance of potato to *Phytophthora infestans* and the incidence of immune reactions decreased with increasing inoculum concentrations is a significant factor influencing disease severity and its effect depends on susceptibility of the chickpea cultivar. The results on the effect of inoculum concentrations are of great importance in screening chickpea germplasm for Ascochyta blight resistance.

This study showed that lower and higher doses of inoculum were not appropriate for screening for disease resistance. We observed that the severity of ascochyta blight disease vary according to type of race or pathotype and chickpea lines [22].

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