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Screening of 15 Chickpea Germplasm Accessions for Resistance to *Ascochyta rabiei* in North West of Algeria

[1]*Ibrahim Elkhalil Benzohra,* [1]*Boubekeur Seddik Bendahmane,*
[1]*Mokhtar Youcef Benkada and* [2]*Mohamed Labdi*

[1]Laboratory of Plant Protection, Department of Agronomy,
University Abdelhamid Ibn Badis of Mostaganem, Mostaganem, Algeria
[2]INRAA/URO, Algerian National Institute of Agricultural Research /
Western Research Unity, Sidi Bel Abbes, Algeria

Abstract: Ascochyta blight is an economically important disease of chickpea caused by the fungus *Ascochyta rabiei*. The fungus shows considerable variation for pathogenicity in nature. Fifteen chickpea germplasm accessions provided by ICARDA (Aleppo, Syria), their origin from different countries (Table 1), were screened for resistance to Ascochyta blight disease caused by *Ascochyta rabiei*, by artificially inoculating the germplasm under glasshouse at temperature ranged from 20±2°C and humidity was maintained above 80% by sprinkling fresh water. Highly significant effect (P<0.01) was observed on chickpea germplasm reaction to three pathotypes of *Ascochyta rabiei* (Mos02 'pathotype III: highly aggressive', At02 'pathotype II: moderate aggressive' and Sba02 'pathotype I: least aggressive'). We found 5 chickpea germplasm exhibited highly resistant response (ILC72, ILC182, ILC187, ILC200 and ILC202), 2 are susceptible (ILC1929 and Flip1025) and other 8 chickpea germplasm (ILC484, ILC2506, ILC3856, ILC4421, ILC5902, ILC5921, ILC6043 and ILC6090), displayed tolerant reaction.

Key words: *Ascochyta rabiei* · *Cicer arietinum* · Pathotypes · Resistance · Sensitivity

INTRODUCTION

Chickpea is an important food legume crop in the CWANA (Central, West Asia and North Africa) region, accounting for 29% of the total food legume production [1]. It serves as a source of inexpensive high quality production in the diets of many people and provides a rich crop residue for animal feed [2].

In the Mediterranean region, chickpea is traditionnaly sown in spring and, as a consequence of the low rainfull during the growth period in dry summers, these results in poor biomass development [3]. Work on cold tolerance in chickpea has been intiated since, the advantages of fall-sown crop over traditional spring sown crop were realized [4]. Winter sowing expands the vegetative growth period and improves the seed yield up to 2 tonnes/ha [5,6], but is rarely adopted by the farmers because the cool and wet weather, typical for Mediterranean winters, favors the development of fungal diseases. The ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. (Teleomorph, *Didymella rabiei* Kov. v. Arx.), is the major disease affects the chickpea fields in Algeria and other Meditrranean countries [7]. Data of many years of prospections, showed the presence and extension of ascochyta blight with fals of output which can go upto 100% [8]. Mabsoute *et al.* [9] announced that in Algeria like other Maghreb countries, the ascochyta blight remains the major constraint of chickpea [9].

Fungicides such as chlorothalonil are sometimes used to control the disease, but their use is often uneconomical under epiphytotic conditions, because a minimum of four to six sprays can be required [10]. The use of resistant cultivars appears to be the best management option for this disease [11]. The use of resistant chickpea cultivars is the most effective and economical management strategy for ascochyta blight

Corresponding Author: Laboratory of Plant Protection, Department of Agronomy,
University Abdelhamid Ibn Badis of Mostaganem, Mostaganem, Algeria.
Tel: +(213) 791 54 66 99 / +(213) 556 28 00 02, E-mail: ibrahimelkhalil@live.fr.

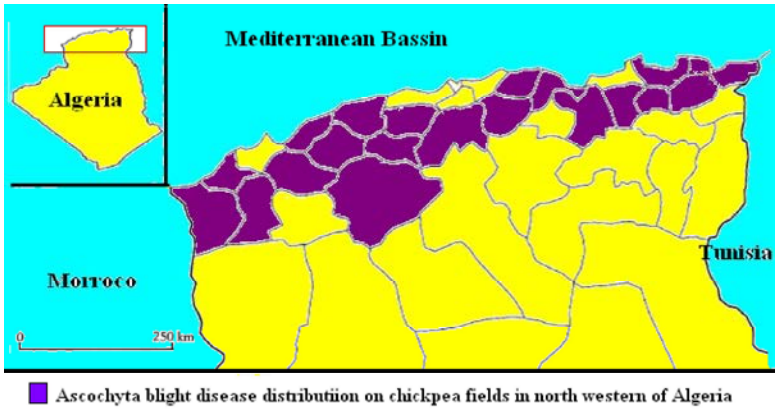

Fig. 1: Ascochyta blight disease if chickpea distribution in North West of Algeria.

since the application of fungicide is not economical [12]. Therefore, breeding of resistant chickpea cultivars against Ascochyta blight could be efficace to control this disease in chickpea fields.

This study has the objective to screening of chickpea germplasm for resistance to Ascochyta blight disease in the Western North region of Algeria (Figure 1).

MATERIALS AND METHODS

Chickpea germplasm: A set of fifteen differential chickpea lines from ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria), (Table 1), were screened for resistance to 3 pathotypes of *Ascochyta rabiei*.

Fungal Materials: The isolates of *Ascochyta rabiei* used in this study were obtained by isolation from samples of stems, shoots and chickpea pods showing of the tipical symptoms of Ascochyta blight (Table 2). The method to determine this pathotyping in *Ascochyta rabiei* isolates, was recently published by Udupa *et al*. [10], who reported that there are 3 pathotypes and 6 physiological races in Syria according to their aggressiveness and virulence, respectively.

Isolation and Purification of Cultures: The isolates were conserved in Petri dishes contained CSMDA medium (Chickpea Seed Meal Dextrose Agar) [13]. The isolates were maintained on CSMDA medium in temperature at 20±2°C [14] and relative humidity above 70% [15].

Obtaining Chickpea Seedlings and Fungal Suspension Preparation: The seeds of chickpea lines used are 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 sterile peat-moss, at rate of 2 seeds per pot and 4 repetitions for each particular treatment.

Three isolates of *A. rabiei* were used in this study (Table 2), each one of them represents one pathotype. 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 [16]. All isolates used in this study originated from single conidia.

Table 1: Differential chickpea lines with their origin.

Chickpea germplasm	Origin	Type of grains	Institutes
ILC 1929	Syria	Kabuli	ICARDAa
Flip 1025	Syria	Desi	ICARDA
ILC72	Russia	Kabuli	ICARDA
ILC 182	Russia	Kabuli	ICARDA
ILC 187	Russia	Kabuli	ICARDA
ILC 200	Russia	Kabuli	ICARDA
ILC 202	Russia	Kabuli	ICARDA
ILC 484	Syria	Kabuli	ICARDA
ILC 2506	Russia	Kabuli	ICARDA
ILC 3856	Russia	Kabuli	ICARDA
ILC 4421	Russia	Kabuli	ICARDA
ILC 5902	Bulgaria	Kabuli	ICARDA
ILC 5921	Bulgaria	Kabuli	ICARDA
ILC 6043	Russia	Kabuli	ICARDA
ILC 6090	Russia	Kabuli	ICARDA

[a] International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.

Table 2: *Ascochyta rabiei* isolates with their origin, date of isolation and pathotype groups.

Isolates	Origin	Dates of isolation	Pathotypes
Sba01	Sidi Bel abbes	March 2008	I (least aggressive)
At02	Ain Temouchent	November 2008	II (moderately aggressive)
Mos02	Mostaganem	June 2009	III (Highly aggressive)

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 [17]. 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 [18].

Rating Scale: The severity of the disease is noted from 1 to 9, according to the scale of Reddy and Singh (1984) [19] based on the intensity of the symptoms, 21 days after inoculation presents itself as follows:

- No lesion is visible on the whole of the plants.
- Visible lesions on less than 10% of the plants, the stems are not reached.
- Lesions on 25% of the plants, with damage on approximately 10% of the stems.
- Lesions on all the plants, approximately 50% of the stems are reached, which results in the death of certain plants because of serious damage.
- 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 [20].

Statistical Analysis: The variances ($ó^2$), 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 bifactorial randomization (two studied factors, F1 is aggressiveness and F2 is chickpea germplasm reaction) by the test of Newman and Keuls ($P_{0.05}$ and $P_{0.01}$). Isolates were classified in three groups by their aggressiveness on three chickpea lines and chickpea lines were classified according to their reaction to ascochyta blight disease. Pearson's linear correlation coefficients were calculated between components across chickpea germplasm. The principal component analysis (PCA) was performed to detect the main components that defined significant structures within the data set. PCA was followed by an ascending hierarchical classification (AHC). Chickpea germplasm were then classified in groups by their reaction to three pathotypes of *A. rabiei*. To as certain how the components were associated with each other, Pearson's linear correlation coefficients were calculated between components across cultivars. All statistics were determined using the R Statistics software (Version 8.0.1).

RESULTS AND DISCUSSION

Highly significant effect ($P < 0.01$) was observed on a chickpea germplasm reaction to *A. rabiei* isolates (Tables 3, 4). The screening of 15 chickpea germplasm against 2 pathotypes (I and II) revealed, 2 chickpea lines (ILC 1929 and Flip 1025) were susceptible, 5 (ILC72, ILC182, ILC187, ILC200 and ILC202) resistant and other 8 lines were tolerant (ILC484, ILC2506, ILC3856, ILC4421, ILC5902, ILC5921, ILC6043 and ILC6090) (Fig. 2; Table 5). While, all 15 chickpea germplasm were susceptible to the highly aggressive isolate of Mostaganem region 'Mos 02' (pathotype III) (Table 5; Fig. 2). No seedling without any lesions was found. The best resistance reaction was observed in three chickpeas germplasm ILC187, ILC200 and ILC 202. The chickpea line ILC72 is generally resistant to pathotypes I and II and tolerant to pathotype III. Thus, ILC 1929 is susceptible to all three pathotypes and ILC202 is resistant to all three pathotypes of *A. rabiei*.

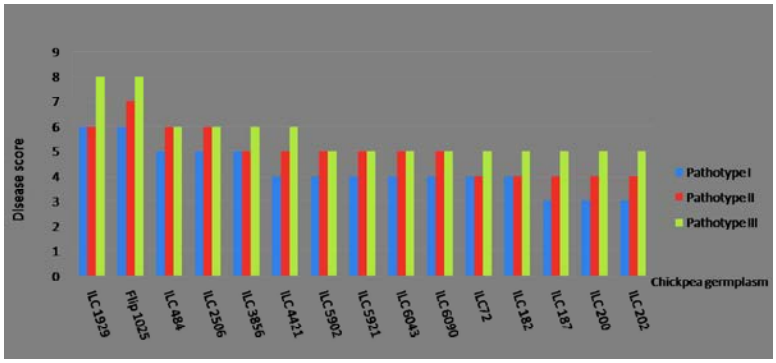

Fig. 2: Chickpea germplasm reaction to three isolates of *A. rabiei.*

Table 3: Aggressiveness of three pathotypes of *A. rabiei* on chickpea germplasm

	Isolates				
	Sba 02	At 02	Mos 02	F value	C.V.
Aggressiveness (Mean ± SD)	7.76^{c}±0.59	4.84^{b}±0.68	6.80^{a}±1.43	98.22**	21.8%

** Highly significant effect at P<0.01, SD : Standard deviation, C.V. : Coefficient of variation, a, b and c : homogenate groups.

Table 4: Reaction of 13 chickpea germplasm to 3 pathotypes of *A. rabiei*

Chickpea germplasm	Ascochyta blight score (x ±SD)	t-test
ILC 1929	6.83a±0.52	9.45**
Flip 1025	7a±0.85	
ILC 484	5.83ab±0.52	
ILC 2506	5.83ab±1.16	
ILC 3856	5.33bc±0.6	
ILC 4421	5bcd±0.85	
ILC 5902	4.83bcd±1.16	
ILC 5921	4.83bcd±1.31	
ILC 6043	4.83bcd±1.78	
ILC 6090	4.83bcd±0.85	
ILC72	4.33cd±0.52	
ILC 182	4.33cd±0.54	
ILC 187	4.16d±1	
ILC 200	4.16d±1.16	
ILC 202	4.16d±1	

** Highly significant effect at *P*<0.01, SD : standard deviation, *x* :Mean of disease score, C.V. : Coefficient of variation, a, b, c and d : homogenate groups.

Table 5: Chickpea germplasm chowing their resistance or sentivity to 3 pathotypes of *A. rabiei.*

	Reactiona		
Chickpea germplasm	Pathotype I	Pathotype II	Pathotype III
ILC 1929	S	S	S
Flip 1025	S	S	S
ILC 484	R	S	S
ILC 2506	R	S	S
ILC 3856	R	S	S
ILC 4421	S	S	S
ILC 5902	R	S	S
ILC 5921	R	S	S
ILC 6043	R	R	S
ILC 6090	R	R	S
ILC72	R	R	S
ILC 182	R	R	S
ILC 187	R	R	S
ILC 200	R	R	S
ILC 202	R	R	S

[a]chickpea germplasm reaction was rated 1.0 to 4.9 for resistant (R) seedlings and those rated 5.0 to 9.0 for susceptible (S) (Türkkan and Dolar, 2009).

The primary objective of this research was the screening of chickpea germplasm collection for resistance to *A. rabiei*. Many reports on the screening of chickpea for resistance to ascochyta blight have appeared in the literature and a long list would be required to mention all the chickpea genotypes that have been reported to be resistant. The screening of chickpea germplasm was reported from many countries including India [7, 10, 21, 22], the Palouse region of USA [24, 25], Italy [11], Pakistan [18, 25-29], Syria, Lebanon [30-32], Spain [33], Australia [15, 34], Tunisia [35], Canada [36, 37], Turkey [14, 20] and Algeria [38].

It was [39] suggested that the possibility to determine the resistance and sensitivity of chickpea germplasm according to their reaction to the three pathotypes of *A. rabiei*, consisting of pathotype I to determine the susceptible chickpea lines, pathotype II for tolerant and pathotype III for resistant chickpea lines.

In Pakistan, the sensitivity of chickpea germplasm ILC1929 was reported by Iqbal *et al*. [27] and Reddy & Kabbabeh [31]. The chickpea cultivars ILC72 and ILC200 which were recorded as resistant to Ascochyta blight for many years of world chickpea production [15, 22, 40], became susceptible in these last years [30]. Thus, our results confirm this sensitivity reaction. Despite the importance of use the resistant cultivars to control this disease, it's difficult to obtain a stable resistance [16]. The causes of this rapid breakdown varietal resistance are due to pathogenic variability of pathogen agent and the presence of the teleomorph *Didymella rabiei* (Kov. v. Arx.) under fields conditions [41,42]. Ascospores of *D. rabiei* (perfect stage of *A. rabiei*) are a major source of primary inoculum which play an important role in the pathogenicity and epidemiology of *A. rabiei* [15].

The tolerant chickpea germplasm ILC 484, which is become susceptible to pathotypes II and III of *A. rabiei*. Similarly, the sensitivity behavior of these two chickpea germplasm were reported by other authors like Singh & Reddy [7].

CONCLUSION

In the present study, the screening of chickpea germplasm showed a different behavior to three pathotypes of *A. rabiei*. We found 2 chickpea lines (ILC 1929 and Flip1025) were susceptible, 5 lines (ILC72, ILC182, ILC187, ILC200 and ILC202) resistant and other 8 lines were tolerant (ILC484, ILC2506, ILC3856, ILC4421, ILC5902, ILC5921, ILC6043 and ILC6090). Such results could be useful for choosing representative pathotypes that may be used to identify specific resistant groups for utilization in breeding program. It's necessary to apply this test on commercial chickpea cultivars for reduce crop damage caused by this disease. The knowledge generated

on *A. rabiei* resistance in chickpea germplasm indicated that can be exploited for disease control by building disease resistance pyramids due to complex nature of Ascochyta blight disease.

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