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Full Length Research Paper

An assessment of wild *Cicer* species accessions for resistance to three pathotypes of *Ascochyta rabiei* (Pass.) Labr. in Algeria

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Twenty-five (25) genotypes of five wild *Cicer* species (*Cicer judaicum, Cicer bijugum, Cicer cuneatum, Cicer echinospermum* and *Cicer reticulatum*) were screened for resistance to ascochyta blight disease caused by *Ascochyta rabiei*, by artificially inoculating the germplasm under glasshouse. Highly significant effect (P<0.01) was observed on their reaction to three pathotypes of *A. rabiei* (Mos02 'pathotype III: highly aggressive', At02 'pathotype II: moderate aggressive', and Sba02 'pathotype I: least aggressive'), there is a difference in genotypes reaction to *A. rabiei* isolates but very important resistance was observed (>50% of accessions collection). All five *C. judaicum* accessions are resistant to *A. rabiei* isolates, two resistant accessions in the wild species *C. echinospermum* (ILWC0 and ILWC246) and three accessions in *C. reticulatum* (ILWC81, ILWC104 and ILWC247), *C. cuneatum* (ILWC37, ILWC40 and ILWC232) and *C. bijugum* (ILWC195, ILWC285 and ILWC286).

Key words: *Ascochyta rabiei*, *Cicer arietinum*, *Cicer* sp., aggressiveness, resistance.

INTRODUCTION

Chickpea is an important food legume crop in the Central, West Asia and North Africaregion (CWANA), accounting for 29% of the total food legume production (Singh, 1990; Zohary and Hopf, 2000; Kerem et al., 2007). 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 (Singh et al., 1992).

In the Mediterranean region, chickpea is traditionally sown in spring and, as a consequence of the low rainfull during the growth period in dry summers, these results in poor biomass development (Kanouni et al., 2011). Work on cold tolerance in chickpea has been intiated since, the advantages of fall-sown crop over traditional spring sown crop were realized (Singh et al., 1997). Winter sowing expands the vegetative growth period and improves the seed yield up to 2 tonnes/ha (Singh et al., 1995; Singh

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Table 1. Wild *Cicer* species accessions origined from ICARDA.

Wild *Cicer* species	Accession
C. judaicum	ILWC4, ILWC43, ILWC148, ILWC168, ILWC256
C. bijugum	ILWC0, ILWC195, ILWC241, ILWC285, ILWC286
C. cuneatum	ILWC37, ILWC40, ILWC185, ILWC187, ILWC232
C. echinospermum	ILWC0, ILWC180, ILWC181, ILWC235, ILWC246.
C. reticulatum	ILWC81, ILWC104, ILWC237, ILWC247,ILWC290

ILWC: International Legume Wild *Cicer*.

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)

and Reddy, 1996), 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 that affects the chickpea fields in Algeria and other Meditrranean countries (Singh and Reddy, 1990). Data of many years of prospections, showed the presence and extension of ascochyta blight with fals of output which can go upto 100% (Bouznad et al., 1996). Mabsoute et al. (1996) annouced that in algeria like other Maghreb countries, the ascochyta blight remains the major constraint of chickpea.

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 (Reddy and Singh, 1983). The use of resistant cultivars appears to be the best management option for this disease (Porta-Puglia et al., 1996). 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). Therefore, breeding of resistant chickpea cultivars against ascochyta blight is efficace to control this disease in chickpea fields. However, limited resistance in existing chickpea germplasm has prompted the search for new sources of resistance to ascochyta blight (Reddy and Singh, 1984). Wild relatives of crops often possess genes that confer resistance to biotic stresses (Malhotra et al., 2000). Sources of resistance to ascochyta blight have been found in a limited number of annual wild *Cicer* species, as reported for *Cicer pinnatifidum* Jaub. & Sp. and *Cicer judaicum* Boiss. (Singh et al., 1981), for *Cicer bijugum* K. H. Rech. (Haware et al., 1992), for *Cicer bijugum*, *Cicer echinospermum* P. H. Davis and *Cicer reticulatum* Ladiz. (Stamigna et al., 1998), and for *Cicer judaicum* and *Cicer pinnatifidum* (Singh and Reddy, 1993). The aim of this study was to evaluate resistance of wild *Cicer* species accessions to three pathotypes of *A. rabiei* from north west region of Algeria.

MATERIALS AND METHODS

Wild *Cicer* species accessions

Accessions of wild *Cicer* species including *C. judaicum, C. bijugum*, *C. echinospermum*, *C. reticulatum* and *C. cuneatum* (Table 1) were obtained from International Center for Agricultural Research in the Dry Areas (ICARDA). A total of 25 wild *Cicer* accessions was screened for resistance to three pathotypes of *A. rabiei* in glasshouse trials.

Fungal isolates

The 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 2).

Obtaining seedlings and inoculum preparation

The seeds of chickpea and its wild relatives used are sterilized with Sodium hypochlorite (at 0.2%) for 10 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 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 (Iqbal et al., 2003). All isolates used in this study originate from single conidia.

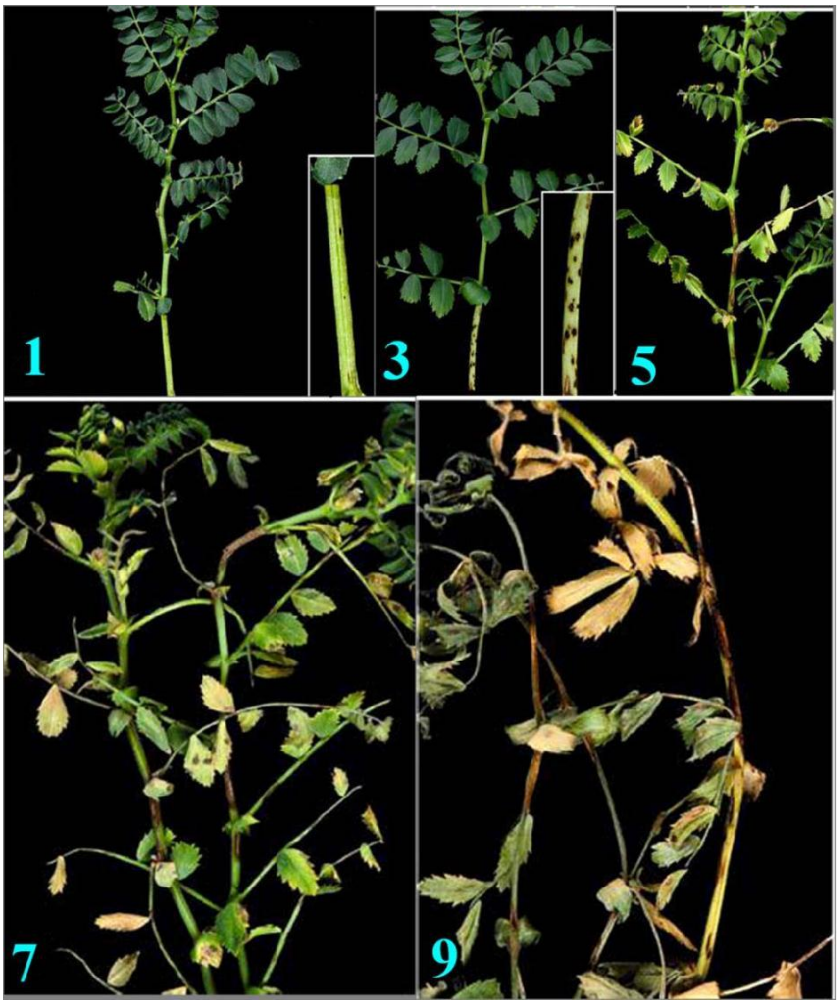

Figure 1. Rating scale of ascochyta blight disease's severity.

Ino culation of see dlings

Tw weeks old s eedlings of ea ch line were in oculated with the isol ates of *A. rabi ei* using 4 pots of 2 plants p er isolate. In each experiment, as co ntrol, inoculated set of plants were sprayed with sterile distilled w ater by press re sprayer in growth chamber (Pie ters and Tahiri 1986). After s raying, plants were inoculated by spore suspension. In order to maintain humidity, seedlings were spr yed with steril e distilled water 2 times a da y with a humidifier (Setti et al., 2009).

Rating scale

1: N o lesion is visib le on the whole of the plants.
3: V isible lesions on less than 10 % of the plants, the stems are not rea ched.
5: L esions on 25 of the plants, with damage on approximately 10% of the stems.
7: L esions on all the plants, approximately 50% of the stems are rea ched, which results in the death of certain plants because of serious damage.
9: esions diffused on all the plants, the stem s are reached in pro portions higher than 50% wit h the death of the majority of he plants.

The chickpea lines rated 1.0 to 4. were considered resistant and those rated 5.0 to 9.0 were considered susceptible (Türkkan and Dolar, 2009) (Figure 1).

Statistical analysis

The ariances (σ^2), averages an d standard deviation (SD) o f various repetitions w e re calculated a nd analyzed b the software o f statistics (STAT BOX 6.0.4. GRIMMERSOFT) and the device used are the global bifact rial randomization (two studi d factors, F1 is aggressiveness and F2 is chickp a germplasm and wild *Cice r* specie s accessions reactions) by t he test of New man and Keuls ($P_{0.05}$ and $P_{0.01}$). Isolates were cla ssified in three groups by their aggressiveness on t hree chickpea lines, and chickpea lines were classif ied according to their reaction to ascochyta blight disease . Mean disease score s for control accessions were subjected to analys is of varianc e (ANOVA) in order to detect differences between separate trials. For ea ch separate trial, differences between mean disease scores of ILC1929, the sus ceptible control, and m ean disease s cores of individual accessions were calculated using *t*-tests.

Table 3. Aggressiveness of three pathotypes of *A. rabiei* on chickpea germplasm and wild *Cicer* accessions.

Organism	Aggressiveness (Mean ± SD)			F value	C.V.
	Sba 02	At 02	Mos 02		
Wild *Cicer* accessions	4.01^{c} ± 0.5	4.69^{b} ± 0.66	5.01^{a} ± 1.01	95.11**	20.7%

**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 25 wild *Cicer* accessions to pathotype I (Sba02) of *A. rabiei*.

Species	Genotypes	Mean ± SD
Cicer arietinum	ILC1929	6.5^{a} ± 2.51
	ILWC4	3.5^{c} ± 1
	ILWC43	3^{c}
C. judaicum	ILWC148	4^{bc} ± 1.15
	ILWC168	3^{c}
	ILWC256	4^{bc}
	ILWC0	4.5^{abc} ± 1
	ILWC195	3^{c}
C. bijugum	ILWC241	4.5^{abc} ± 1
	ILWC285	3.5^{c} ± 1
	ILWC286	3^{c}
	ILWC37	3.5^{c} ± 1
	ILWC40	3.5^{c} ± 1
C. cuneatum	ILWC185	4^{bc} ± 1.15
	ILWC187	5^{abc}
	ILWC232	3.5^{c} ± 1
	ILWC0	4^{bc} ± 1.15
	ILWC180	5^{abc}
C. echinospermum	ILWC181	6^{ab} ± 1.15
	ILWC235	3.5^{c} ± 1
	ILWC246	3.5^{c} ± 1
	ILWC81	3^{c}
	ILWC104	3^{c}
C. reticulatum	ILWC237	5^{abc}
	ILWC247	3.5^{c} ± 1
	ILWC290	5^{abc}
F value		4.11**
C.V.		23.63%

**Highly significant effect (*P* <0.01, Test of Newmann-Keuls at 1%) ; SD: sandard deviation ; C.V.: Coefficient of variation.

Table 5. Reaction of 25 wild *Cicer* accessions to pathotype II (At02) of *A. rabiei*.

Species	Genotypes	Mean ± SD
Cicer arietinum	ILC1929	8.5^{a} ± 1
	ILWC4	4.5b ± 1
	ILWC43	4^{b} ± 1.15
C. judaicum	ILWC148	5^{b}
	ILWC168	4.5^{b} ± 1
	ILWC256	4.5^{b} ± 1
	ILWC0	5^{b}
	ILWC195	4.5^{b} ± 1
C. bijugum	ILWC241	5^{b}
	ILWC285	4.5^{b} ± 1
	ILWC286	4^{b} ± 1.15
	ILWC37	4^{b} ± 1.15
	ILWC40	4.5^{b} ± 1
C. cuneatum	ILWC185	4^{b} ± 1.15
	ILWC187	5.5^{b} ± 1
	ILWC232	4^{b} ± 1.15
	ILWC0	4.5^{b} ± 1
	ILWC180	6^{b} ± 1.15
C. echinospermum	ILWC181	6.5^{b} ± 1
	ILWC235	5.5^{b} ± 1
	ILWC246	4.5^{b} ± 1
	ILWC81	5^{b}
	ILWC104	4^{b} ± 1.15
C. reticulatum	ILWC237	5.5^{b} ± 1
	ILWC247	4^{b} ± 1.15
	ILWC290	5.5^{b} ± 1.91
F value		3.82**
C.V.		20.99%

** Highly significant effect (*P*<0.01, Test of Newmann-Keuls at 1%); SD : sandard deviation ; C.V.: Coefficient of variation.

RESULTS

Higly significant effect (*P*<0.01) was observed on chickpea germplasm and wild *Cicer* accessions reaction to *A. rabiei* isolates (Tables 3, 4, 5 and 6). The mean diseases scores and their standard deviations (SD) for all chickpea germplasm and wild relatives accessions tested in four separate trials are mentionned in the Tables 3, 4,

Table 6. Reaction of 25 wild *Cicer* accessions to pathotype III (Mos02) of *A. rabiei*.

Species	Genotypes	Mean ± SD
Cicer arietinum	ILC1929	9^{a}
	ILWC4	$4.5^{cd} \pm 1$
	ILWC43	$4^{cd} \pm 1.15$
C. judaicum	ILWC148	$4.5^{cd} \pm 1$
	ILWC168	$4^{cd} \pm 1.15$
	ILWC256	$5.5^{cd} \pm 1$
	ILWC0	5^{cd}
	ILWC195	$3.5^{d} \pm 1$
C. bijugum	ILWC241	$5.5^{cd} \pm 1$
	ILWC285	$4.5^{cd} \pm 1.91$
	ILWC286	$4^{cd} \pm 1.15$
	ILWC37	$4^{cd} \pm 1.15$
	ILWC40	$4^{cd} \pm 1.15$
C. cuneatum	ILWC185	5^{cd}
	ILWC187	6^{bcd}
	ILWC232	$4.5^{cd} \pm 1$
	ILWC0	$5.5^{cd} \pm 1$
	ILWC180	$6^{bcd} \pm 1.15$
C. echinospermum	ILWC181	$8^{ab} \pm 1.15$
	ILWC235	$6^{bcd} \pm 1.15$
	ILWC246	$4^{cd} \pm 1.15$
	ILWC81	$3.5^{d} \pm 1$
	ILWC104	$3.5^{d} \pm 1$
C. reticulatum	ILWC237	$6.5^{bc} \pm 1$
	ILWC247	$4^{cd} \pm 1.15$
	ILWC290	$6^{bcd} \pm 1.15$
F value		6.55**
C.V.		21 28%

** Highly significant effect (P<0.01, Test of Newmann-Keuls at 1%) ; SD: sandard deviation ; C.V.: Coefficient of variation.

5 and 6. The mean disease score that were significantly different (P<0.01) from the susceptible line ILC1929, are also cited in the Tables 4, 5 and 6.

The wild *Cicer* species accessions showed important and interesting source of resistance to *A. rabiei* isolates (>50% of accessions collection (Table 7); but unfortunately, only two species (*Cicer reticulatum* and *C. echinospermum*) are fertile and can be used as a source of resistance (Collard et al., 2003). The evaluation of the resistance in wild *Cicer* species reaction showed broad but important, which will be used in the future in the program of creation of new hybrids of chickpea cultivars resistant to ascochyta blight disease. There was very important resistance was observed in the accessions of wild species *Cicer judaicum*, *C. cuneatum* and *C. reticulatum*, compared to others. For the 25 accessions of five species (Figure 2), 13 accessions were resistant to Sba02, 15 to At02 and 14 accessions to Mos02.

DISCUSSION

The primary objective of this research was the screening of wild *Cicer* species accessions for resistance to *A. rabiei*. Many reports on the screening of Wild *Cicer* species for resistance to ascochyta blight have appeared in the literature and a long list would be required to mention all wild *Cicer* accessions that have been reported to be resistant.

The screening of chickpea germplasm was reported from many countries including India (Reddy and Singh, 1984; Singh et al., 1984; Singh and Reddy, 1990; Haware et al. 1995), Syria, Lebanon (Reddy and Kabbabeh, 1985; Udupa et al., 1998; ICARDA, 2003), the Palouse region of USA (Jan and Wiese, 1991; Chen et al., 2004), Italy (Porta-Puglia et al., 1996), Pakistan (Jamil et al., 2002; Iqbal et al., 2003; Iqbal et al., 2004; Malik et al., 2005; Ilyas et al., 2007; Ghazanfar et al., 2010), Spain (Navas-Cortes et al., 1998), Australia (Khan et al., 1999; Nasir et al., 2000), Tunisia (Hamza et al., 2000), Canada (Chongo et al., 2004; Vail and Banniza, 2008), Turkey (Dolar et al., 1994; Türkkan and Dolar, 2009) and Algeria (Zikara-Zine and Bouznad, 2007).

Udupa and Weigand (1997) suggested that is possible 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 ILC 263 was reported by Iqbal et al. (2004), and ILC 1929 by Reddy and Kabbabeh (1985). The chickpea cultivars ILC 3279 and ICC 3996 which were recorded as resistant to ascochyta blight for many years of world chickpea production (Singh et al., 1984; Labdi, 1995; Nasir et al., 2000), became susceptible in these last years (ICARDA, 2003). 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 (Iqbal et al., 2003). 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 (Navas -Cortes et al., 1990; Trapero-Casas and Kaiser, 1992). Ascospores of *D. rabiei* (Perfect stage of *A. rabiei*) are a major source of primary inoculums which play an important role in the pathogenicity and

Table 7. A wild *Cicer* species accessions showing their Sensibility or resistance to three pathotypes of *A. rabiei*.

Species	Genotype	Reaction[a]		
		Pathotype I	Pathotype II	Pathotype III
Cicer arietinum	ILC1929	S	S	S
C. judaicum	ILWC4	R	R	R
	ILWC43	R	R	R
	ILWC148	R	S	R
	ILWC168	R	R	R
	ILWC256	S	R	S
C. bijugum	ILWC0	S	S	S
	ILWC195	R	R	R
	ILWC241	S	S	S
	ILWC285	R	R	R
	ILWC286	R	R	R
C. cuneatum	ILWC37	R	R	R
	ILWC40	R	R	R
	ILWC185	S	R	S
	ILWC187	S	S	S
	ILWC232	R	R	R
C. echinospermum	ILWC0	S	R	S
	ILWC180	S	S	S
	ILWC181	S	S	S
	ILWC235	S	S	S
	ILWC246	R	R	R
C. reticulatum	ILWC81	R	S	R
	ILWC104	R	R	R
	ILWC237	S	S	S
	ILWC247	R	R	R
	ILWC290	S	S	S

[a]Wild *Cicer* accessions 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).

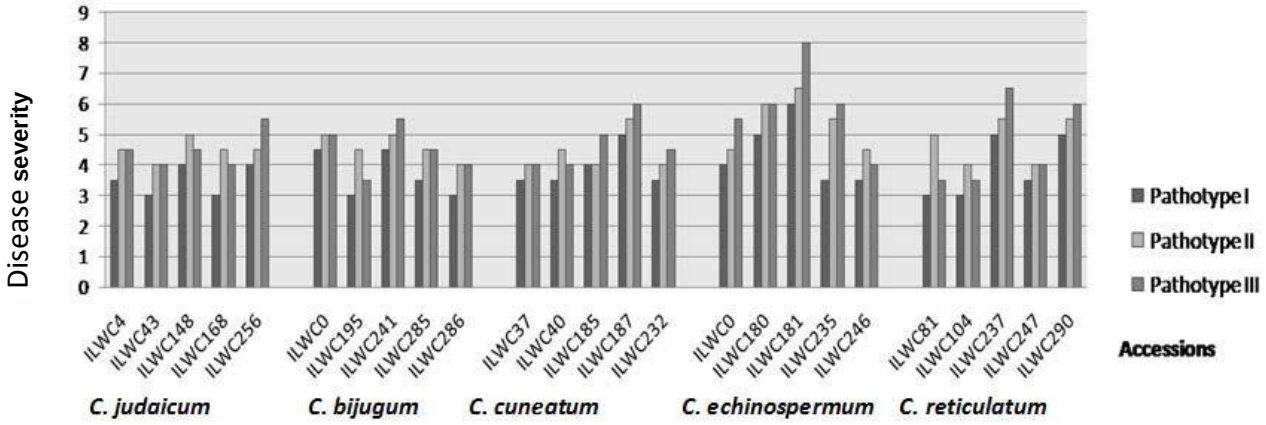

Figure 2. Aggressiveness of three pathotypes of *A. rabiei* against 25 wild *Cicer* species accessions.

epidemiology of *A. rabiei* (Nasir et al., 2000).

The tolerant chickpea germplasm ILC 482 and ILC 483, which are become susceptible to pathotypes II and III of *A. rabiei*. Similarly, the sensitivity behavior of these two chickpea germplasm was reported by other authors like Singh and Reddy (1990).

Many authors around the world have reported the importance of wild *Cicer* species in resistance to different stresses that affect the culture of chickpea (Nene and Haware, 1980; Haware et al., 1992; Singh and Reddy, 1993; Singh and Weigand, 1994 ; Singh et al., 1994; Singh et al., 1998; Shah et al., 2005; Pande et al., 2006; Aryanmanesh 2007; Trapero-Casas and Kaiser, 2009; Saeed et al., 2010).

In the pathological aspect, there is a wide spectrum of variability among isolates of *A. rabiei* (Navas-Cortes et al., 1998; Chongo et al., 2004 ; Banniza and Vail, 2008; Türkkan and Dolar, 2009). We must therefore use the screening test 2 or 3 aggressiveness classes of the pathogen to facilitate the interpretation of results (Udupa et al., 1998). In our test, we used three isolates representing the three pathotypes of *A. rabiei* according to their degree of aggressiveness (Table 2).

Similarly, in Australia, Collard et al. (2001) used one isolate for screening test accessions and reported the existence of significant resistance among these wild species to this isolate. The *C. Judaicum* accessions have a greater resistance than other species. These results have also been reported by Singh et al. (1991) in Syria, Lebanon and Turkey. We note that the majority of accessions tested in our test, have not been studied elsewhere, except seven lines showed a similarity in their reaction against *A. rabiei*. The accession ILWC 81 (*Cicer reticulatum*) seems resistant in our test, but sensitive in the results of Stamigna et al. 1998 and Collard et al. (2001).

The lack of results completely similar with other research, may be linked to the methods chosen (number of isolates, nature and concentration of the inoculum, seedlings inoculated with isolates separately or mixed etc.).

Conclusion

The screening of wild *Cicer* species accessions showed a different behavior to three pathotypes of *A. rabiei*. The evaluation of wild *Cicer* species accessions for resistance to *A. rabiei* showed the presence of significant resistance compared to known cultivars of chickpea (ILC 3279, and ILC72 ICC3996) in different countries (Labdi, 1995; Aryamanesh, 2007).

The wild species *C. Judaicum*, *C. bijugum* and *C. reticulatum* gave a very high level of resistance to ascochyta blight disease. But just *C. reticulatum* can be used in the future to transfer its resistance traits important chickpea cultivars by hybridization or other appropriate methods. Due to the fact that more resistant chickpea cultivars selected and cultivated in the world for several years later became susceptible when they were cultivated on a large scale.

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.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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