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Physiological Characterisation of *Ascochyta rabiei* (Pass.) Lab. Isolated from Diseased Chickpea Fields in Six Regions of Northwestern Algeria

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Abstract: The ascochyta blight fungus *Ascochyta rabiei* (Pass.) Lab. is considered the most damaging pathogen of chickpea (*Cicer arietinum* L.). Sixteen isolates of the pathogen were collected from six regions in north-western Algeria and were studied for cultural diversity, morphology, pathogenicity and mating type. These isolates showed variation in colony colour as mouse gray, light green, olive green, brown and dark brown, when grown on chickpea seed meal dextrose agar. The produced Conidia had a length ranging from 5.2 to 17.2 µm and a width between 2.4 and 6.7µm. Their number varied from 0.13 x 10^7/ml to 2.52 x 10^7/ml. The aggressiveness of isolates was evaluated on 11 chickpea lines. Disease rating on a 1-9 scale indicated a large variability among them and revealed occurrence of the highly aggressive pathotype III in two regions. Both mating types 1-1 and 1-2 were detected. Results from this study show that local strains may overcome resistance in commonly used chickpea cultivars. Some of the described isolates will be useful for breeding Ascochyta blight-resistant chickpea cultivars adapted to geographical regions and for developing necessary control measures.

Key words: Ascochyta Rabiei · *Cicer arietinum* L. · Disease Resistance · Pathotype · Plant Breeding · Mating Type

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important food legume in the world after dry bean (*Phaseolis vulgaris* L.) and field pea (*Pisum sativum* L.). It is a major source of high-quality protein in human diets and also provides high quality crop residues for animal feed. Legume plants such as chickpea maintain soil fertility through biological nitrogen fixation.

Unfortunately, several factors are responsible of low crop yields in the world. Drought, salinity and cold [2,3,4,5] are a serious problem. The crop also is subject to infection by several fungal affecting all the plant parts (seed, leaves, crown and roots).

Foliar diseases that affecting leaves and stems of the plants constitute one of the most serious groups causing great damage to the crop.

One of the main diseases which reduce potential yield in chickpea is ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. [6]. Frequently, it devastates chickpea crop areas and causes heavy losses (up to 100 %) in severely affected fields [7].

Ascochyta rabiei has been reported in 29 countries world-wide [8]. Symptoms of the disease include wilting leaf tips, leaf lesions, stem lesions causing stem breakage and lesions on pods resulting in seed infection. The two most damaging symptoms are stem breakage and pod infection .

The occurrence of differences in cultural characteristics and pathogenicity among isolates of this pathogen is well known. The genetic variability in *A. rabiei* is enhanced by the presence of its teleomorph (*Didymella rabiei* (Kav.) under field conditions [9]. Pathogenic variability among isolates of *A. rabiei* has

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been reported from India [9], Syria and Lebanon [10,11], USA [12], Italy [13], Pakistan [9,14], Spain [9], Australia [15], Tunisia [16] and in Canada [17]. These studies have used between 3 and 15 differential host genotypes and classified between 11 and 130 isolates of *A. rabiei* into 3 to 14 pathotypes. Pathotypes can be characterised by differences in the amount of disease (differential virulence) caused on a set of differential host genotypes in artificial inoculation experiments employing single-spore isolates of the pathogen.

Udupa *et al*. [18] suggested that as few as three differentials from each class (susceptible, moderately resistant and highly resistant) of the host plant are sufficient for classifying *A. rabiei* isolates into 3 pathotypes based on increasing level of aggressiveness. The standard set of chickpea differentials used at ICARDA consists of 'ILC 3279' as a resistant genotype, 'ILC 482' as tolerant and 'ILC 1929' as a susceptible genotype [14]. Similarly, the United States Department of Agriculture (USDA) uses cultivars 'Dwelley' and 'Spanish White' for pathotyping isolates into Pathotype I (low pathogenicity) and Pathotype II (high pathogenicity) respectively [19].

Development of resistant or tolerant chickpea cultivars is the most practical way to control Ascochyta blight [20]. However, cultivating resistant cultivars will eventually result in the selection of new pathotypes or races able to overcome the resistance gene(s) introduced by breeding [21]. Hence the design of proper breeding strategies requires biological and genetic characterisation of the pathogen and the comprehension of disease mechanisms.

The aim of the present study was to characterise *A. rabiei* isolates obtained from different chickpea growing areas of North-western Algeria using cultural and pathological characteristics as well a molecular marker for mating type.

MATERIALS AND METHODS

Sampling, Isolation and Maintenance of Fungal Isolates: During chickpea growing season of 2009, chickpea plants with brown or black lesions on stem, leaves and pods were collected from farmer's fields in various areas of North western Algeria (Figure 1; Table 1).

Sampling sites were at least 100 km away from each other and belong to regions with different altitudes and climate. Regions near the sea (Mostaganem, Ain Temouchent) have a temperate climate with high air humidity during the whole year, whereas regions in the interior plains (Relisane, Mascara, Sidi Belabes) have harsher winters and a dry summer season between May and October and the interior mountains of region Ain Defla region have the highest rainfall and even snow in winter.

Table 1: Geographical origin and designation of *Ascochyta rabiei* isolates from North-western Algeria

Province (Wilaya)	Site	Designation of isolate
Mascara	Rachidia	C1
	El- Houaire	C2
	Ain Fares	C3
Ain Témouchent	Ain Témouchent	A1
	Hammam Bouhadjar	A2
	Ain Tolba	A3
Sidi Belabbes	Attouche	B1
	Tessala	B2
Mostaganem	Hessainia	M1, M2
Relizane	Relizane	R1, R2, R3
Ain Defla	Oued Zeboudj	Z1, Z2, Z3

Diseased samples were surfaces sterilized in 0.1% mercuric chloride for 1 minute and plated on chickpea seed dextrose agar (CSMDA; 40 g chickpea seed meal; 20 g dextrose; 20 g agar per liter of water)[22]. Each isolate was purified by single spore culture [23] and incubated at 22 ± 2°C for 2 weeks. The pycnidiospores were stored in 25% sterile glycerol at -80°C until use.

Sixteen isolates were obtained from chickpea presenting typical ascochyta blight symptoms, from fields in 6 regions of north western Algeria with different agro climatic conditions. They were maintained as monosporic cultures and characterised.

Koch's postulate was checked to assess the responsibility of each isolate in the disease. Each isolate was inoculated (10^6 conidia/ml) on two plants of chickpea line ILC 263 (susceptible). All plants showed typical symptoms of *A. rabiei* ten days after inoculation and the fungus could be re-isolated from diseased plants.

***A. rabiei* Cultural and Morphological Parameter Assessment:** Discs of 5 mm diameter from actively growing cultures of *A. rabiei* were placed in the center of 90 mm diameter Petri dishes containing CSMDA. Inoculated plates were incubated at 20 ± 2°C and observed for colour and diameter (mm) of colony, amount and size of conidia.

For quantification of conidia, the Petri dish was flooded with 10 ml of sterile distilled water. After 20 minutes of incubation, the colony was gently scraped with a glass rod to release pycnidiospores. The spore suspension containing spores was collected in a beaker and spore concentration was determined with a Malassez haematocytometer. The experiments were conducted with six replicates.

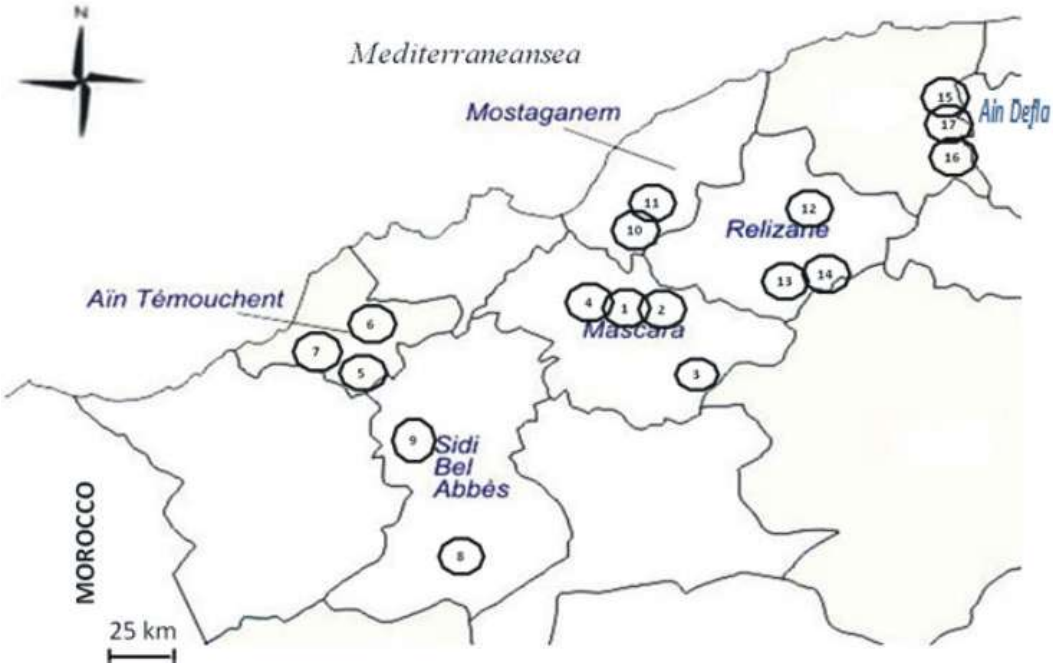

Fig. 1: Collecting sites of *Ascochyta rabiei* in regions of north-western Algeria
The isolates were collected from fields in the following villages: 1-Rachidia, 2-El Houaire, 3-Ain fares, 4- Zakor 5- Ain Temouchent, 6- Hammam Bouhdjar, 7- Ain Tolba, 8-Attouche, 9- Tessala, 10-Hessainia, 11-Hessainia, 12-Relizane, 13-Relizane, 14- Relizane, 15-Oued Zeboudj, 16- OuedZeboudj, 17-Oued Zeboudj.

Plant Culture: Eleven chickpea genotypes (Flip 9393 C, ILC 263, ILC 8068, ILC 7795, ILC 249, ILC 483, ILC 3996, ILC 484, ILC 1929, ILC 482 and ILC 3279) were obtained from Algiers's ITGC (Institut Technique des Grandes Cultures. This collection belongs to ICARDA (International Center for Agricultural Research in the Dry areas, Syria) and ICRISAT (International Crops Research Institute for the semi-arid, India). The seeds were surface sterilized with 1% sodium hypochlorite for 3 min and washed 3 times with sterile distilled water [24], then sown (1 seed per pot) in 14 cm pots containing sterilized mixtures of soil and sand (1:1, v/v). Plants were grown in the green house at 22 ± 2°C for 14 days.

Plant Inoculation: A spore suspension of each isolate was prepared from 15-day-old cultures as described above. The suspension was filtered through two layers of muslin cloth and spore concentration was determined with a haematocytometer and adjusted to 5 x 10^5 spores per ml with sterile distilled water. Tween 20 (0.05%) was added to the spore suspension as a surfactant agent for sticking the spore to leaves of chickpea plants. The plants were inoculated by spraying the spore suspension until run-off and kept under a humid chamber for 48 hours in a glasshouse [25]. Relative humidity was maintained at 95-100 %. Each treatment consisted of three replicates and each replication consisted of 4 plants.

Disease Symptom Scoring: The disease reactions of individual plants were scored 14 days after inoculation on a scoring scale from 1 to 9 [26] as follows:

1 = no infection;
2 = highly resistant (lesions on 1–5% of the plant);
3 = resistant (lesions on 6–10%);
4 = moderately resistant (lesions on 11–15%);
5 = intermediate (lesions on 16–40%);
6 = moderately susceptible (lesions on 41–50%);
7 = susceptible (lesions on 51–75%);
8 = highly susceptible (lesions on 76–100%);
9 = highly susceptible, plant killed.

According to Reddy & Singh [26], resistance for an individual plant was defined as a disease score less than or equal to five. Accessions possessing mean disease scores of less than five were described as resistant.

Mean disease scores were subjected to analysis of variance (*ANOVA*) in order to detect differences between different accessions.

For Determination of *A. rabiei* pathotypes, a set of three chickpea cultivars (ILC 1929, ILC 482, ILC 3279) was used [11]. Two independent experiments were conducted. Each experiment consisted of three replications with 20 plants in each replication. The conditions of inoculation and culture of plants are the same as described above. Fourteen days after inoculation, cultivars were assessed using the 1-9 scale described by Singh et al. [25] as follows:

1= no visible lesions on any plants (highly resistant);
3= lesions visible on less than 10% of the plants, no stem girdling (resistant);
5= lesions visible on up to 25% of the plants, stem girdling on less than 10% of the plants but little damage (tolerant);
7= lesions on most plants, stem girdling on less than 50 % of the plants resulting in the death of a few plants (susceptible);
9= lesions profuse on all plants, stem girdling on more than 50 % of the plants and death of most plants (highly susceptible).
Cultivars rated less than5.0 were considered resistant.

Molecular Characterization of *A. rabiei* Isolates: Discs from the growing margin of single spore colonies on CSMDA were used to inoculate 200 ml potato dextrose broth (PDB, Difco) in 250 ml flasks. After incubation at 20°C for 7 days, mycelia mats were harvested by filtering through Whatman paper n°1. Genomic DNA was extracted with 2% hexadecyltrimethyl-ammonium bromide (CTAB) extraction buffer (1.4 M NaCl, 0.1 M Tris-HCl pH: 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol) using the method of Weising *et al*. [27]. DNA concentration was measured in a spectrophotometer at 260 nm.

PCR amplifications were carried out in a 25 µl reaction volume containing 20mM Tris-HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 unit *Taq* DNA polymerase. Fungal DNA concentration was 2 µg/ml for ITS regions and 4 µg/ml for mating type locus. Primer concentrations were 0.2 µM for ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [28] and 0,4 µM for mating type primers Tail5 (CGCTATTTTATCCAAGACACACC), Com 1 (GCATGCCATATCGCCAGT) and SP21 (ACAGTGAGCCTGCACAGTTC) as described by Barve *et al*. [29]. Amplifications were performed in a thermal cycler with an initial denaturation step for 1 min at 94°C, followed by 38 cycles for ITS primers and 45 cycles for mating type primers of 30 sec at 94°C, 1 min at annealing temperature, 1 min at 72°C, with a final extension for 2.5 min at 72°C. The annealing temperature was 50°C for ITS and 58°C for mating type primers. *A. rabiei* DNA from isolates of known mating types was obtained from Dr. Sabine Banniza/ (University of Saskatchewan, Canada) and used as a control.

The PCR products were separated electrophoretically in a 1.4% agarose gel in 40 mM Tris-acetate, 1mM EDTA pH 8.0 (TAE) buffer [30]. The gels were stained with ethidium bromide (0.1 µg/ml) and visualized under UV light. Size markers were 1 kb ladder and 100bp ladder form Invitrogen (France).

PCR products of the ITS regions were sequenced with universal ITS1 and ITS4 primers by Beckman Coulter Genomics (England).

RESULTS

Sixteen isolates were obtained from chickpea presenting typical ascochyta blight symptoms, from fields in 6 regions of north western Algeria with different agroclimatic conditions. They were maintained as monosporic cultures and characterised.

Koch's postulate was checked to confirm the responsibility of each isolate in the disease. Each isolate was inoculated (10^6 conidia/ml) on two chickpea plants belonging to line ILC 263 (susceptible). All plants showed typical symptoms of *A. rabiei* ten days after inoculation. Re-isolation of the fungus from diseased plants confirmed that all isolates were the causal agent of ascochyta blight and Blastn analysis of the sequenced fungal ITS rDNA region showed that all the sixteen isolates belonged to *A. rabiei*.

Cultural and Morphological Variation of *A. rabiei*: The 16 *A. rabiei* isolates were first characterised by the observation of morphological and cultural traits. As shown in Table 2, clear differences were seen between isolates, notably with the three criteria coloration of colony, size of conidia and mycelial growth.

Mycelium of most isolates was olive green or mouse gray and varied in its intensity at the margins of the colonies. Some colonies of *A. rabiei* isolates had various shades of gray and white with distinct coloration in the center and the colony of isolate R2 was brown with a dark brown center (Table 2). Cirrhe colour was dirty white in isolates C2 and M1, brown in isolates M2, B1, B2, A2, A3, C1 and C3 and light brown for others isolates.

The colony diameter of the 16 isolates on CSMDA after 10 days of culture varied from 30.75mm for B2 to 54.5 for C1 being the maximum (Table 2).

Table 2: Morphological and cultural characteristics of *Ascochyta rabiei* isolates from North-western Algeria Growth and morphology parameters were recorded after 10 days of growth on CSMDA medium at 22 ±2 °C and values are means of six replications ±SE

				Conidia size (µm)	
Isolate	Colony colour	Cirrhe colour	Colony diameter (mm)	Width	Length
C1	Mouse gray	Brown	54,5 ±0,77	4,72 (3,12-5,7)	10,51 (5,98-14,2)
C2	Olive green	Dirty white	44,66 ±0,52	4,91 (3,4-5,6)	11,17 (6,2-13,8)
C3	Olive gray	Brown	40,67±1,08	5,54 (2,6-6,7)	12,14 (7,8-15,6)
A1	Olive green	Salmon	32±0,63	5,24 (2,4-6,4)	10,87 (6,8-15,6)
A2	Light green	Brown	33,08±0,20	4,97 (3,3-6)	10,73 (6,2-15)
A3	Mouse gray	Brown	33,82±1,44	4,36 (2,6– 6,5)	10,47 (7,54-13,78)
B1	Olive green	Brown	48,58±1,36	4,15 (2,8-6)	10,39 (6,2-16)
B2	Olive green	Brown	30,75±0,42	4,59 (3-6,4)	9,59 (6,4-14)
M1	Olive green	Dirty White	42±1,22	4,02 (2,85-5,72)	10,17 (5,98-13,2)
M2	Mouse gray	Brown	35,34±0,41	3,98 (2,86-5,98)	10,11 (5,98-16,12)
R1	Mouse gray	Light brown	32,17±0,26	4,45 (3,12-5,98)	10,14 (6-14,3)
R2	Dark brown	Light brown	33,42±0,49	4,64 (3,6-5,8)	10,99 (6-17,2)
R3	Dark brown	Light brown	41,74±0,52	4,54 (3,6-6)	10,31 (6,2-14,2)
Z1	Brown	Light brown	38,17±0,52	4,49 (3-5,98)	11,53 (6,24-13,6)
Z2	Mouse gray	Light brown	34,26±0,76	4,45 (3,12-5,72)	11,35 (5,2-15,08)
Z3	Olive green	Light brown	38±0,32	4,67 (3,64-5,8)	11,16 (6-15,5)

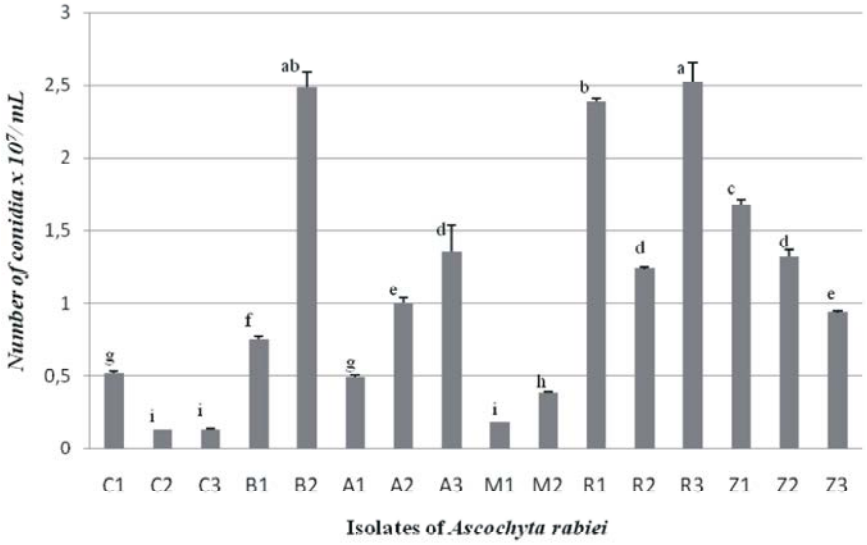

Fig. 2: Sporulation of *Ascochyta rabiei* isolates
Spore production was assessed after 10 days of culture on CSMDA medium. The values are means of six replications + SE

Conidial size also shows variation in length and width between the studied isolates. Conidia had a length ranging from 5.2 to 17.2 µm and a width ranging from 2.4 to 6.7 µm.

Values for sporulation of the 16 isolates are shown in Figure 2. The number of conidia varied from 0.13 x 10^7/ml for C2 and C3 to 2.52 x 10^7/ml for isolate R3. Sporulation varies greatly between the 16 isolates and the effect of strain is highly significant (Table 3). When mean values for sporulation were calculated for isolates from the same region, it seems that there is also an effect of the region of origin. Isolates from Mascara (C1, C2, C3) and Mostaganem (M1, M2), produced low amounts of spores with mean values of 0.242 x 10^7 sp/ml and 0.279 x 10^7 sp/ml respectively, whereas those from Ain Temouchet (A1, A2, A3), Ain Defla (Z1, Z2, Z3), Sidi Belabbes (B1, B2) and Relizane (R1, R2, R3) produced consistently higher amounts with mean values of 0.947 x 10^7 sp/ml, 1.309 x 10^7 sp/ml, 1.616 x 10^7 sp/ml and 2.049 x 10^7 sp/ml respectively. The effect of region is highly significant at $p<0,001$ and allowed classification into 4 groups (Table 4).

Table 3: Analysis of variance for sporulation of *Ascochyta rabiei* isolates after 10 days of incubation at 20 ±2 °C on CSMDA medium.

Total variation	[a]df	[b]MS	F value
Isolate	15	4,08	912,12*
Error	80	0,004	

[a]df: degree of freedom

[b] MS: mean square

*significant at 0,05

Table 4: Classification of the regions into homogeneous groups according to the mean value of sporulation of the isolates

		Homogenous groups (P = 0.01)			
Region	N	1	2	3	4
Relizane	18	2,0490[a]			
Sidi Belabbes	12		1,6160[b]		
Ain Defla	18		1,3091[b]		
Ain Témouchent	18			0,9465[c]	
Mostaganem	12				0,2793[d]
Mascara	18				0,2566[d]

N=number of samples

Table 5: Disease scoring (1 to 9) of *Ascochyta rabiei* isolates on 11 differential chickpea genotypes and their mating type. Values represent means obtained in 2 replicates, with 4 plants in each replicate, + SE. Mating type was determined by PCR with specific MAT 1-1 or 1-2 primers

	Lines											
Isolate	ILC 9393c	ILC 263	ILC 8068	ILC 7795	ILC 249	ILC 483	ICC 3996	ILC 484	ILC 1929	ILC 482	ILC 3279	Mean	Mating type (1 or 2)
M1	3,1±0,6	6±0,0	5,3±0,5	3,1±0,4	4±0,0	5±0,5	2,9±0,6	3,8±0,9	7±0,5	3±0,0	4±0,8	4,28[hi]	1
M2	6,3±1,2	9±0,0	5,8±0,9	9±0,0	7,3±0,7	7,3±0,7	7,5±0,8	7±0,0	9±0,0	7±0,0	7±0,0	7,45[a]	1
B1	5,3±0,5	7±0,5	5±0,0	7,8±0,5	5,3±0,7	5±0,0	4,6±0,5	5,3±0,5	5,9±0,4	6,9±0,6	3±0,5	5,53[cd]	1
B2	2,8±0,5	7±0,0	4±0,0	6±0,0	3,8±0,5	4,1±0,4	3±0,5	3±0,0	5,4±0,7	4,8±0,5	4,9±0,6	4,42[hi]	1
C1	5,6±0,7	5,4±0,9	4,6±0,7	6±1,5	5,6±0,7	3±0,0	4±0,5	5,3±0,7	9±0,0	7±0,5	4,6±0,7	5,47[cd]	1
C2	5±1,1	8,1±0,6	5,5±0,8	5,4±0,7	5,8±0,5	5,3±0,9	3,6±0,7	4,8±0,9	8±0,5	7,1±0,6	3,6±0,9	5,65[c]	2
C3	7±0,0	9±0,0	6,8±0,5	7,4±0,7	7,4±1,4	6,6±0,7	6±0,0	6,8±0,5	9±0,0	7,3±0,5	6,4±1,2	7,23[a]	2
Z1	3±0,5	6,3±0,5	4,1±0,6	5,8±0,5	4,1±0,4	4,8±0,9	4,4±0,7	4±0,0	6±0,0	3,9±1,1	3,1±1,0	4,49[ghi]	1
Z2	2,5±0,5	7,5±1,1	3,5±0,8	6±0,0	3±0,5	4±0,0	3,9±0,4	5,5±0,5	5,9±0,4	4,9±0,8	3,8±0,8	4,58[fgh]	2
Z3	5,1±0,4	6,3±0,5	4,3±0,5	5±0,8	2,3±0,5	5,1±0,3	3,1±0,4	2,8±0,5	5,9±0,6	3±0,0	4,6±0,7	4,31[hi]	2
A1	3±0,0	6,1±0,4	4,1±0,4	5,3±0,7	4±0,5	3,3±0,5	5±0,5	3,8±0,5	5,4±0,5	4±0,0	3±0,0	4,26[i]	2
A2	4,6±0,7	7±0,0	5±0,5	4,5±0,8	6±0,8	4,6±0,7	4±0,5	3,8±0,4	7,1±0,4	4,8±0,6	4±0,5	5,03[e]	1
A3	6±0,5	6±0,5	3±0,0	4±0,5	3,1±0,6	6±0,0	4±0,0	5±0,0	5,6±0,6	5,6±0,7	4,6±0,5	4,82[ef]	2
R1	5,3±0,7	9±0,0	2±0,5	5,3±0,9	4,1±0,6	4±0,5	3,6±0,7	3,8±0,5	8±0,0	3,8±0,9	3,1±0,8	4,72[fg]	1
R2	4,4±1,1	9±0,0	5±1,1	5±0,8	5,6±0,5	3±0,0	3,9±0,6	5,5±0,8	8,1±1,2	5,4±1,0	3,6±0,9	5,32[d]	1
R3	7,1±0,6	6,1±1,2	6,3±0,5	4±0,5	6,5±0,9	6,8±0,7	6,4±1	7±0,5	8,4±0,9	6±0,0	4,8±0,9	6,30[b]	1
Mean	4,75[d]	7,17[a]	4,63[d]	5,59[b]	4,86[d]	4,86[d]	4,37[e]	4,8[d]	7,1[a]	5,27[c]	4,26[e]		

[A,b,…n] letters from [a] to [n] indicate comparison of mean for the main effect of lines and isolates. Lines or isolates with the same letter are in the same group.

Table 6: Analysis of variance for symptom scores in chickpea genotypes after 14 days of inoculation by *Ascochyta rabiei* isolates

Source of variation	[a]df	[b]MS	F value
Isolate	15	89,09	252,55**
Genotype	10	129,78	367,88**
Isolate x Genotype	150	7,55	21,40**
Error	1232	0,35	

[a]df: degree of freedom

[b]MS: mean square

**significant at 0,05 levels

Table 7: *Ascochyta rabiei* pathotype groups characterised by inoculation of 3 differential chickpea lines

Isolate	Line ILC 1929	Line ILC 482	Line ILC 3279	Mean	Pathotype
M1	7,3±0,82	2,7±0,82	3,3±0,82	4,4^{de}	I
M2	9±0,00	7±0,00	6,7±0,82	7,6^{a}	III
B1	7,3±0,82	7,3±0,82	2,3±1,03	5,7^{bc}	II
B2	7±0,00	3±0,00	2,7±0,82	4,2^{de}	I
C1	9±0,00	6,7±0,82	3,3±0,82	6,3^{b}	II
C2	8±1,67	6,7±0,82	3±0	5,9^{bc}	II
C3	9±0,00	7,3±0,82	7±0,00	7,8^{a}	III
Z1	8,7±0,82	3±1,12	1,3±0,82	4,3^{de}	I
Z2	7±0,00	4,7±0,82	3,7±1,03	5,1^{cd}	I
Z3	8±1,67	2,3±1,03	1,3±0,82	3,9^{e}	I
A1	6,7±0,82	3,3±0,82	3±0,82	4,3^{de}	I
A2	8,7±0,82	5±0,00	3,3±0,82	5,6^{bc}	I
A3	7,3±1,51	7±0,00	4±1,10	6,1^{b}	II
R1	9±0,00	3,3±0,82	3±0,00	5,1^{cd}	I
R2	9±0,00	5±0,00	2,7±0,82	5,6^{bc}	I
R3	9±0,00	6±1,67	3±0,00	6,0^{bc}	II
Mean	8,1^{a}	4,9^{b}	3,3^{c}		

An inverse correlation between mycelium growth and sporulation was observed with a R value of – 0.27 at P = 0.05. Vigorously growing strains such as C1, C2 or B1 had a lower sporulation rate than strains with slower growth such as B2.

Pathogenicity of *A. rabiei* Isolates: The pathogenic characterisation of *A. rabiei* isolates was performed with a set of 11 differential chickpea genotypes (Flip 9393 C, ILC 263, ILC 8068, ILC 7795, ILC 249, ILC 483, ILC 3996, ILC 484, ILC 1929, ILC 482 and ILC 3279). These cultivars were chosen because of their frequent use to test the aggressiveness of isolates. Artificial inoculation of *Ascochyta rabiei* isolates on this differential host range led to the production of typical symptoms namely lesions on leaves and stems and stem girdling. The symptom scores obtained from notations on individual plants 14 days after inoculation are shown in Table 5.

It can be seen that the isolates varied greatly in aggressiveness depending on the cultivars Analysis of variance showed that the effects of plant genotype and fungal isolate as well as their interaction were highly significant for these two parameters (Table 6). The Newman-Keuls test at the 5% and 1% allows establishing homogeneous groups for the isolates.

The symptom score mean values for genotype and isolate (Table 5) showed that genotype ILC 263 was the most susceptible line and ILC 3279 the most resistant line. Isolate M2 was the most aggressive (index 7.45) against all accessions. Isolates C3 and R3 also showed high aggressiveness with symptom scores of 7.23 and 6.40 respectively. Otherwise A1 and M1 were the least aggressive *Ascochyta* isolates (4.26 and 4.28 respectively).

In our investigation most of the chickpea lines were moderately resistant, with mean values below 5 but above 4.

The three ICARDA lines ILC 1929, ILC 482 and ILC 3279 were respectively susceptible, intermediate and resistant by their mean values. ILC 3279 was confirmed as the most resistant genotype with resistant disease reaction to all isolates except isolate M2 and C3.

This set of 3 differential chickpea genotypes (ILC 1929, ILC 482, ILC 3279) was used to determine the pathotypes of the algerian *A. rabiei* isolates, as described by Udupa *et al*. [18]. As shown in table 7, they could be classified into pathotypes I, II and III. Accordingly, nine isolates belonged to pathotype I, five isolates to pathotype II and two isolates to pathotype III. These two pathotype III isolates (M2 and C3) which have overcome resistance in ILC 3279 were also the most aggressive by mean values.

Characterization of Mating Types: Primers specific for mating type Mat1-1 and Mat1-2 were used in multiplex PCR experiments. Gel electrophoresis revealed the presence of 490 bp and 700 bp amplicons for MAT1-2 and MAT1-1, respectively (data not shown) and isolates could be unambiguously assigned to one of the two mating types. This analysis revealed that both mating types occur in northwestern Algeria (Table 5) and that the MAT1-1 genotype seemed more common than MAT1-2, in a ratio of 3:1.

DISCUSSION

The control of ascochyta blight is mainly dependent on the use of resistant cultivars due to the high cost of fungicide applications and their effect on environment in many countries. Identification of new sources of resistant requires knowledge of cultural, morphological pathogenic and genetic variation in pathogen. This study reveals the levels of variation in morphology, growth and aggressiveness among 16 isolates of *A. rabei* from 6 different provinces in northwestern Algeria.

Despite the limited number of isolates, significant differences in pigmentation and growth on CSMDA medium were observed, even among isolates from a same region (Table 2).

Colony diameter of the sixteen test isolates on CSMDA medium varied, in agreement with other studies which demonstrated the variability of the mycelial growth of *A. rabiei* [31, 32].

Conidial size was also variable and was comparable to data obtained with australian [11] and indian isolates [31].

The same variability was observed for sporulation where the effect of isolate was statistically significant, indicating a genetic determinism for this trait. The origin of the isolates seemed to have some influence on sporulation. As can be seen in Figure 2 2, isolates from Mascara (C1, C2, C3) and Mostaganem (M1, M2) had much lower spore production than isolates from Relisane (R1, R2, R3) and Ain Defla (Z1, Z2, Z3). When mean values for sporulation were calculated for all isolates from one region, they differed among the regions. ANOVA revealed a significant effect of region at $P<0.001$(data not shown) and Newman-Keuls test showed that they could be classified into 4 groups (Table 4). An inverse correlation was observed between radial growth of the mycelium and spore production; isolates such as C1 and B1 with a high growth rate were among those with lowest sporulation. Our data are in agreement with others such as Grewal [33] who reported similar correlations between growth and sporulation for *A. rabiei* isolates from various regions in India. Such a negative correlation is indicative of a trade-off between vegetative growth and reproduction when limited resources are invested either in growth of mycelium or production of spores [34].

Similar variability in morphological and cultural characters among *A. rabiei* isolates have been reported from various countries [33, 35]. However, these variations did not correlate with the geographical origin and pathogenic variations in several of the earlier studies [36].

In addition to morphology and growth, a high variability of aggressiveness and pathogenicity has been reported for *A. rabiei* [13, 16, 17, 33, 37, 38]. While some these authors characterised the pathogenic groups in races according to their virulence on different chickpea cultivars, others stated the difference in aggressiveness rather than in virulence [16, 39].

Our study revealed a high variability for disease reaction when the 16 isolates were inoculated on 11 chickpea genotypes. According to the 1 to 9 disease rating, seven chickpea genotypes were resistant with disease scores of 1 to 5 and four genotypes were susceptible with disease scores from 5.1 to 9. Eight among the 16 isolates can be considered as aggressive with mean disease scores above 5 and among these three were highly aggressive with mean values higher than 6. Two of these highly aggressive isolates, isolates C3 and M2 from fields in Mascara and Mostaganem respectively, can be classified as pathotype III due to their ability to cause disease in chickpea line ILC3279. . Our results showed that the predominant pathotype in north western Algeria was pathotype I. Pathotype I is also dominant in Turkey, followed by pathotype III [24]. Although in our present work inoculations of 11 chickpea lines were done differently and symptoms scored on individual plants, the susceptibility of line ICC-3996 to isolates C3 and M2 indicates that both isolates might even belong to pathotype IV as described for *A. rabiei* in Syria by Atik *et al*. [40]. However, Tuerkkan and Dolar [24] considered lines infecting ICC-3996 as pathotype III. Due to lack of data it is not possible to say if pathotype III in north-west Algeria occurs since a long time already or if it has appeared more recently and could be expected to spread more in the future, as it has been observed in Syria where a genetic shift towards more virulent strains over time and space was reported [40]. However, the occurrence of mating types 1-1 and 1-2 in Northwest Algeria and notably in the Mascara region where the pathotype III isolate C3 was detected, could favour spreading of the highly aggressive genotype.

Chickpea line ILC 3279 was distributed to farmers during the 1986-1987 campaign of a program of the Algerian government to stimulate intensification of crops. Our results show that this line can no longer be considered to be resistant in all circumstances. Notably in the Mascara and Mostaganem regions chickpea fields should be surveyed in order to avoid the spread of this pathotype to other regions. First experiments on fungicide efficiency have shown that fortunately pathotype III is very sensitive to chlorothalonil and difenoconazole (Mahiout, unpublished results).

Our results also show that the most aggressive isolates such as C3 and M2 produced only low amounts of spores whereas less aggressive isolates like B2 and R1 were sporulating abundantly. However, this tendency was not generalised, since the highly aggressive isolate R3 also produced high amounts of spores. In contrast to our observation, Grewal [33] and Kaur [41] reported that relatively fast growing and less sporulating *A. rabiei* isolates were less virulent than the abundantly sporulating isolates. In other studies, no relationships were observed between virulence of isolates, their geographical origin and morphological characteristics such as spore size, colony colour and radial growth *in vitro* [42].

It has been reported that the degree of pathogenicity of different isolates of *A. rabiei* in India was well correlated with the amount of phytotoxins they produced [41]. It would be interesting to study toxin production by the various Algerian isolates and to assess a putative correlation with aggressiveness.

At ICARDA sources of resistance to *Ascochyta* blight such as line ILC-3279 which showed high level resistance in several countries have been described [26]. It was well established that *A. rabiei* possesses variability and that pathotypes present in Pakistan and India are more aggressive than those prevalent in the Mediterranean region [43]. However, our results showed that some algerian isolates were not less aggressive because some of them were able to cause disease on the resistant line ILC 3279. *Ascochyta rabiei* has a sexual stage and mating types 1-1 and 1-2 were identifed in our study for isolates from north-western Algeria. MAT1-1 was more common than MAT1-2. The reason of its unequal distribution is not known. This finding is in line with the study of Nourollahi *et al*. [44] in which the majority of iranian isolates belonged to Mat1-1 (64.08%) with the remainder (35.92%) in the western Iranian province. Both mating types have been detected in many countries including Italy, Portugal, Egypt, Morocco, Greece, Turkey, Tunisia, Spain, USA and Canada [9, 29, 45, 46]. The occurrence of both mating types in Algeria suggests that sexual reproduction might take place on crop residues and that the teleomorph might be an alternative source of inoculum. In addition, genetic recombination could easily lead to the appearance of new and more aggressive pathotypes.

These newly described highly aggressive pathotypes from Algeria should be included in national breeding programs and used for screening resistant genotypes originating from ICARDA and various other sources.

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