Morphological variability in the Indian isolates of *Ascochyta rabiei* causing blight in chickpea and evaluation of chickpea cultivars

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Abstract

Ascochyta *blight caused by* Ascochyta rabiei *is a serious disease of chickpea world wide. Twenty five Indian isolates of* A. rabiei *were evaluated for their morphological variability and 91 chickpea cultivars were screened for their reactions to* Ascochyta *blight. The populations of* A. rabiei showed *variations in various morphological characters like conidia size and density, colony colour and growth rate. Colonies of the isolates on artificial media were flat, submerged with sparse mycelium. The mycelium was pale cream at first but later turned greyish white or green to greenish dark. In Chickpea dextrose agar (CDA) medium, the isolate AR1 (Delhi) grew the largest followed by the isolates AR14 (Punjab), AR5 (Himachal Pradesh), AR9 (Jammu and Kashmir) and AR20 (Rajasthan) with statistically similar colony diameter at 20 days after incubation. Thus, the growth rate was significantly highest in isolate AR1 (Delhi) followed by the isolates AR14 (Punjab), AR5 (Himachal Pradesh), AR9 (Jammu and Kashmir), and AR20 (Rajasthan). More or less similar growth patterns of the isolates were observed on PDA medium with comparatively less growth as compared to CDA. The size of the conidia in all the isolates ranged from 9.19-12.51 x 3.36-4.32 µm whereas the density of conidia in* A. rabiei *isolates ranged from 0.5-7.2 x* 10^6 *conidia* ml^{-1}*. Three cultivars namely, ICC 76, ICC 3996 and ICC 15978were identified as highly resistant to* Ascochyta *blight. Thirty cultivars showed resistant and 14 cultivars showed moderately resistant reactions against the disease. These cultivars may be used for cultivation or resistant breeding programs.*

Keywords: Variability, morphological characters, screening, chickpea, *Ascochyta rabiei*

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world, India is the largest chickpea producing country in the world with a total production of 8.8 million tonnes and cultivated in an area of 9.6 million ha with an average yield of 920 $kgha^{-1}$ (FAOSTAT, 2015).

Ascochyta blight, a disease caused by *Ascochyta rabiei* (Pass.) Labr. is a major constraint, limiting chickpea productivity worldwide (Nene and Reddy, 1987; Kaiser and Muehlbauer, 1989; Chongo *et al*., 2003). The disease is devastating in areas where cool (15-25ºC) and humid weather (>150 mm rainfall) prevails during the crop season (Pande *et al*., 2005). The disease reduces grain yields and quality significantly, and in some circumstances yield losses for susceptible cultivars are as high as 100%. In India, the disease is very serious in north-western region, in the states of Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh and Rajasthan (Grewal and Pal, 1986). Recently, the disease sporadically occurred in Himachal Pradesh and Jammu and Kashmir and losses were recorded upto 5% (Project Coordinator Report, 2009-10). *Ascochyta* blight can infect all above-ground plant parts and can be found any time after crop emergence. *A. rabiei* is known for variation in its morphology (Grewal, 1984), pathogenicity (Porta-Puglia, 1992) and phytotoxin production (Hohl *et al*., 1991).

The cultivation of resistant chickpea varieties is the most effective and economical management strategy for *Ascochyta* blight since the application of fungicide is not economical (Gan *et al*., 2006). Breeding of chickpea for resistance to *Ascochyta* blight is, however, complicated by the frequent breakdown of host plant resistance, probably because of the variable nature of the pathogen (Singh and Reddy, 1991). The screening of cultivars of chickpea to determine the resistance sources to *Ascochyta* blight could

contribute significantly further, for virulence analysis and breeding for resistant varieties. Therefore, the present study was aimed to analyse the extent of variation in morphological characters present amongst the populations of the pathogen and also to evaluate the chickpea cultivars against *A. rabiei* to find out resistant sources for further utilization.

Materials and methods

Collection of samples, isolation and maintenance of cultures

Ascochyta blight infected chickpea plant samples were collected from chickpea growing states of Northern India namely, Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab, Rajasthan, Uttarakhand, Uttar Pradesh and Delhi and isolated 18 isolates of *A. rabiei* from the samples on chickpea dextrose agar (CDA) medium. The associated fungus, *A. rabiei* was isolated from stem, leaves and pods of chickpea on CDA medium following standard tissue isolation procedures. Diseased tissues were cut into 2-3 mm pieces (containing 1/3 diseased and 2/3 healthy portions), surface disinfested in 1% sodium hypochlorite solution for 1-2 min, washed three times with sterile distilled water (SDW), plated on the medium and incubated for 7 days at 20±1°C. The isolates were purified by single spore isolation. Throughout the study, the isolates were maintained by transferring periodically on CDA slants and stored at 4±1°C for further use. In addition, 7 pure cultures of the pathogen were procured from the ITCC, Division of Plant Pathology, New Delhi (AR1 and AR2), International Crops Research Institute for the Semi-Arid Tropics, Hyderabad (AR3, AR5, AR14 and AR21) and Punjab Agricultural University, Ludhiana (AR12). These isolates were further purified by single spore isolation and maintained in CDA slants for further use. Altogether, 25 isolates of *A. rabiei* were used in the present study (Table 1). The isolates (25) were identified by their morphological characters especially size, shape of pycnidia and conidia using calibrated compound microscope.

Morphological variability

The size of the conidia and its density of each isolate were measured using a calibrated compound microscope and haemocytometer, respectively. For each isolate, the size of 50 randomly selected conidia was measured and mean was taken for final observation. The spore density of each isolate of the pathogen was measured using haemocytometer. The inoculum suspension was prepared by adding sterile distilled water. A disc of 5 mm diameter of well sporulated culture growth on CDA medium was taken into 10 ml SDW. After thorough mixing, the conidia present in the suspension was counted using haemocytometer.

For cultural characters and growth rate, each isolate was inoculated in the centre of Petri plates (90 mm) in three replications containing CDA medium (15 ml/plate). A 5 mm diameter disc of 7 days old culture of the pathogen cut by a cork borer was used for inoculation. The pieces were placed on the centre of pre-poured Petri dish in inverted condition. The inoculated plates were incubated at 20±1ºC and growth diameter was measured at 10, 15 and 20 days after inoculation. After 20 days of inoculation the colony diameter and colour were observed. For comparison, the isolates were also cultured on potato dextrose agar (PDA) medium following the similar procedures. The growth rate day^{-1} was calculated based on colony diameter recorded at 20 days after incubation in both CDA and PDA media.

Screening of chickpea cultivars

The chickpea cultivars (91) collected from different sources (IARI, New Delhi; ICRISAT, Hyderabad) consisting of

Table 1. Details of *Ascochyta rabiei* isolates collected from different chickpea growing areas of the country and used in the present study

Isolate name	Place	State
AR1	IARI	Delhi
AR2	IARI	Delhi
AR3	Hisar	Haryana
AR4	Berthin	Himachal Pradesh
AR5	Dhaulakuan	Himachal Pradesh
AR6	Dhaulakuan	Himachal Pradesh
AR7	Dhaulakuan	Himachal Pradesh
AR8	Palampur	Himachal Pradesh
AR9	Jammu	Jammu and Kashmir
AR10	Samba	Jammu and Kashmir
AR11	Samba	Jammu and Kashmir
AR12	Gurdaspur	Punjab
AR13	Gurdaspur	Punjab
AR14	Ludhiana	Punjab
AR15	Ludhiana	Punjab
AR16	Ludhiana	Punjab
AR17	Ludhiana	Punjab
AR18	Ludhiana	Punjab
AR19	Ludhiana	Punjab
AR20	Sriganganagar	Rajasthan
AR21	Pantnagar	Uttarakhand
AR22	Pantnagar	Uttarakhand
AR23	Pantnagar	Uttarakhand
AR24	Pantnagar	Uttarakhand
AR25	Kanpur	Uttar Pradesh

cultivars those have already been used by various workers as differentials (Katiyar and Sood, 1985; Basandrai *et al*., 2005; Benzohra *et al*., 2011) were screened to identify the resistant sources during winter crop season, 2014 in net house conditions at IARI, New Delhi. A pot experiment was conducted under artificially inoculated conditions in three replications for each cultivar. The seeds (10-12) of each cultivar were sown in a plastic pot (10 cm dia) filled with sterilized soil (2 $kgpot^{-1}$). The soil was sterilized by using formalin solution (1%) for 15 days under polythene bags. After 15 days, the soil was removed from the polythene bags and turned 2-4 times for 2-3 days to remove the traces of formalin. The inoculum was prepared from 15 days old culture of Delhi isolate (AR1) of *A. rabiei* using sterile water. Test tubes containing sporulated cultures of AR1 were soaked with water for about 10 min and thereafter spores were harvested in water by inoculation needle and spore suspension was prepared after counting and adjusting the spore concentrations with the help of a haemocytometer at 10^5 conidia ml^{-1}. The spore suspension was sprayed on the 30 days old plants with hand atomizer. Prior to inoculation, the pots were heavily irrigated to maintain the humidity. The inoculated plants were covered with polythene sheets for 48 h. After 48 h, the polythene sheets were removed and regularly sterile distilled water was sprayed twice a day to maintain high humidity for infection.

The differential reaction of the genotypes was recorded at 15 days after inoculation following a 1-9 scale (Nene *et al*., 1981) where 1- no lesions; 2- lesions on some plants usually not visible; 3- few scattered lesions usually seen after careful examination; 4-lesions and defoliation on some plants not damaging; 5-lesions common and easily observed on all plants but defoliation/damage not great; 6-lesions and defoliation common few plants killed; 7-lesions very common and damaging 25% plants killed; 8- all plants with extensive lesions causing defoliation and drying of branches 50% plants killed and 9-lesions extensive on all plants defoliation and drying of branches more than 75% plants killed. Based on the reactions, the cultivars were grouped as highly resistant (1), resistant (2-3), moderately resistant (4-5), susceptible (6-7) and highly susceptible (8-9).

Data analysis

The data of morphological characterization were analysed as per the procedure of completely randomized design (Gomez and Gomez, 1984). The colony diameter and growth rate for the isolates were statistically analyzed at 5% level by using Fisher's least significance difference test both in CDA and PDA media. The screening experiment in net house was conducted using completely randomized design. Disease severity grades were recorded for each plant with the average grade of plants in 3 pots representing one experimental unit.

Results and discussion

Identification of the pathogen

Based on morphological characteristics of the mycelium, pycnidia and conidia, the isolated fungus was identified as *A. rabiei*. The pycnidium was spherical or pear-shaped with an ostiole. The conidia were oval to oblong, hyaline, occasionally bi-celled, rounded at both ends under compound microscope. They developed on conidiophores and embedded in a mucilaginous mass of cream-pink to light tan. The fungus was flat, submerged with sparse mycelium on artificial media. Based on these characteristics, the isolates were identified as *Ascochyta rabiei* as described earlier by various workers (Basandrai *et al*., 2005, Pande *et al*., 2010 and Harveson *et al*., 2011).

Cultural and morphological variability

Twenty-five isolates of *A. rabiei* showed variable morphological characters in the present study. The different characters such as mycelial growth rate, colour of the colony, size of the conidia and spore concentrations were analyzed for morphological variability of *A. rabiei* isolates.

The cultural characters of the *A. rabiei* isolates were determined using two media such as CDA and PDA. Colonies of the isolates on artificial media were flat, submerged with sparse mycelium (Fig. 1). There were variations in colony colour in different isolates as the pathogen grew to advanced stages. The mycelium was pale cream at first but later turned greyish white or green to greenish dark and creamy white. However, most of the isolates were greyish white (Table 2). Similar variations in colony colour were also observed on PDA. The variations were observed in respect of colony diameter and growth rate among the isolates on PDA medium (Table 3). In CDA medium, the isolate AR1 (Delhi) grew the largest followed by AR14 (Punjab), AR5 (Himachal Pradesh), AR20 (Rajasthan) and AR9 (Jammu and Kashmir) with statistically similar colony diameter at 20 days after incubation (DAI). The isolate, AR11 (Jammu and Kashmir) showed the least colony diameter followed by the isolates, AR10 (Jammu and Kashmir), AR24 (Uttarakhand), AR12 and AR13 (Punjab) with statistically similar colony diameter. The growth rate was highly variable among the isolates. The isolate AR1 (Delhi) produced significantly highest

growth rate followed by the isolates AR14 (Punjab), AR5 (Himachal Pradesh), AR9 (Jammu and Kashmir) and AR20 (Rajasthan). The growth rates recorded in these isolates did not differ significantly. The isolates AR10, AR11 (Jammu and Kashmir) and AR24 (Uttarakhand) showed the least growth rate. More or less similar patterns of growth were recorded at 10, 15 and 20 DAI with the significantly highest growth in AR1 (Delhi) isolate.

Based on the growth rate the isolates were grouped into three categories as fast, medium and slow growing isolates. Eight isolates, *viz.,* AR1 (Delhi), AR3 (Haryana), AR5 (Himachal Pradesh), AR9 (Jammu and Kashmir), AR14 (Punjab), and AR20 (Rajasthan), AR21 and AR22 (Uttarakhand) were grouped into the category of fast growing by providing more than 2.5 mm growth rate day^{-1}. Eleven isolates originated from Delhi (AR2), Himachal Pradesh (AR4, AR6, AR7 and AR8), Punjab (AR15, AR16, AR17, AR18 and AR19) and Uttarakhand (AR23) provided 2.0-2.5 mm growth rate day^{-1}were grouped into the medium growing category, while 6 isolates from Jammu and Kashmir (AR10 and AR11), Punjab (AR12 and AR13), Uttarakhand (AR24) and Uttar Pradesh (AR25) provided less than 2 mm growth rate day^{-1}were considered as slow growing (Table 4).

The variable growth amongst the isolates of the pathogen was also observed in PDA medium. More or less similar patterns of the growth were observed at different intervals of incubation with a few exceptions as isolate AR5 (Himachal Pradesh) provided the significantly highest growth diameter at 10 and 15 DAI, but ranked second at 20 DAI after AR1 which was the second in rank at 10 DAI

Table 2. Colony diameter of different isolates of *Ascochyta rabiei* on chickpea dextrose agar medium at 20±1°C along with their colour variations

Isolate	State	Colony diameter (mm)* at days of incubation			Growth rate (mm day^{-1})	Colour
		10	15	20		
AR1	Delhi	40.2 ^{a}	57.5 ^{a}	70.0 ^{a}	3.5 ^{a}	Greyish white
AR2	Delhi	30.3 ^{ef}	43.0 ^{efgh}	50.7 ^{defg}	2.5 ^{defg}	Greyish white
AR3	Haryana	32.3 ^{def}	45.5 ^{def}	54.0 ^{cd}	2.7 ^{cd}	Greyish green
AR4	Himachal Pradesh	30.3 ^{ef}	38.3 ^{jk}	49.7 ^{cde}	2.5 ^{cde}	Greenish dark
AR5	Himachal Pradesh	36.8 ^{b}	50.0 ^{b}	61.7 ^{b}	3.0 ^{b}	Greyish white
AR6	Himachal Pradesh	29.8 ^{f}	41.2 ^{ghi}	49.7 ^{efgh}	2.5 ^{efgh}	Greenish dark
AR7	Himachal Pradesh	31.7 ^{def}	38.0 ^{jk}	44.5 ^{ij}	2.2 ^{ij}	Greenish dark
AR8	Himachal Pradesh	24.8 ^{gh}	33.5 ^{lm}	42.0 ^{jk}	2.1 ^{jk}	Greyish white
AR9	Jammu and Kashmir	32.5 ^{de}	44.5 ^{de}	60.8 ^{b}	3.0 ^{b}	Greyish dark
AR10	Jammu and Kashmir	20.0 ^{k}	27.3 ^{q}	36.2 ^{lm}	1.8 ^{lm}	Creamy white
AR11	Jammu and Kashmir	19.5 ^{k}	27.7 ^{q}	35.3 ^{m}	1.8 ^{m}	Greyish dark
AR12	Punjab	23.7 ^{hi}	32.3 ^{mn}	37.8 ^{lm}	1.9 ^{lm}	Greenish dark
AR13	Punjab	22.5 ^{ij}	31.7 ^{mno}	37.0 ^{lm}	1.9 ^{lm}	Greenish dark
AR14	Punjab	34.8 ^{bc}	48.7 ^{bc}	63.7 ^{b}	3.2 ^{b}	Greenish dark
AR15	Punjab	20.3 ^{jk}	29.5 ^{opq}	39.0 ^{kl}	2.0 ^{kl}	Greyish white
AR16	Punjab	31.0 ^{ef}	40.5 ^{hij}	47.0 ^{hi}	2.4 ^{hi}	Greyish white
AR17	Punjab	32.6 ^{cde}	46.0 ^{cd}	50.3 ^{efgh}	2.5 ^{efgh}	Greyish white
AR18	Punjab	32.2 ^{def}	41.7 ^{fghi}	47.8 ^{gh}	2.4 ^{gh}	Greenish dark
AR19	Punjab	26.7 ^{g}	35.8 ^{kl}	48.7 ^{fgh}	2.4 ^{fgh}	Greyish white
AR20	Rajasthan	33.5 ^{cd}	44.3 ^{def}	61.3 ^{b}	3.0 ^{b}	Greyish white
AR21	Uttarakhand	32.2 ^{def}	43.5 ^{defg}	54.8 ^{c}	2.7 ^{c}	Greyish white
AR22	Uttarakhand	23.7 ^{hi}	39.2 ^{ij}	51.7 ^{cdef}	2.6 ^{cdef}	Greyish white
AR23	Uttarakhand	23.8 ^{hi}	33.7 ^{lm}	41.7 ^{jk}	2.0 ^{jk}	Greyish white
AR24	Uttarakhand	21.2 ^{jk}	28.5 ^{pq}	36.3 ^{lm}	1.8 ^{lm}	Greyish dark
AR25	Uttar Pradesh	20.8 ^{jk}	30.7 ^{nop}	39.0 ^{kl}	1.9 ^{kl}	Greyish White

* Mean of three replications

The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

and statistically similar with AR5 (Himachal Pradesh) at 15 DAI. The colony diameter ranged from 20.5-52.5 mm at 20 DAI. The isolate AR1 (Delhi) provided the highest colony diameter followed by AR5 (Himachal Pradesh) and AR9 (Jammu and Kashmir). The isolate AR15 (Punjab) provided the lowest colony diameter followed by AR11 (Jammu and Kashmir) and AR6 (Himachal Pradesh) and the colony diameter recorded in these two isolates did not differ significantly. In PDA medium, the significantly highest growth rate was observed in AR1 (Delhi) followed by AR5 (Himachal Pradesh) and AR9 (Jammu and Kashmir) with statistically similar growth rate (Table 3). Based on their growth rate at 20 DAI, only one isolate AR1 (Delhi) with >2.5 mm growth rate day^{-1} was identified as fast grower on PDA medium. Two isolates namely, AR5 (Himachal Pradesh) and AR9 (Jammu and Kashmir) with growth of 2.0-2.5 mm day^{-1} were characterized as medium grower (Table 5). The remaining 22 isolates of *A. rabiei* originated

Table 3. Colony diameter of different isolates of *Ascochyta rabiei* on Potato dextrose agar medium at 20±1°C

Isolate	State	Colony diameter (mm)* at days of incubation			Growth rate (mmday^{-1})
		10	15	20	
AR1	Delhi	22.7 ^{b}	38.7 ^{a}	52.5 ^{a}	2.6 ^{a}
AR2	Delhi	17.5 ^{efghi}	27.0 ^{cd}	37.5 ^{c}	1.9 ^{c}
AR3	Haryana	16.8 ^{fghij}	22.8 ^{fgh}	32.0 ^{ef}	1.6 ^{ef}
AR4	Himachal Pradesh	19.2 ^{cdef}	26.7 ^{cde}	32.0 ^{ef}	1.6 ^{ef}
AR5	Himachal Pradesh	25.7 ^{a}	36.5 ^{a}	44.8 ^{b}	2.2 ^{b}
AR6	Himachal Pradesh	12.8 ^{m}	18.2 ^{j}	22.0 ^{ij}	1.1 ^{ij}
AR7	Himachal Pradesh	12.8 ^{m}	18.0 ^{j}	23.7 ^{hi}	1.2 ^{hi}
AR8	Himachal Pradesh	16.8 ^{fghij}	20.8 ^{ghij}	23.5 ^{hi}	1.2 ^{hi}
AR9	Jammu and Kashmir	20.3 ^{cd}	31.2 ^{b}	43.2 ^{b}	2.2 ^{b}
AR10	Jammu and Kashmir	17.3 ^{efghi}	25.3 ^{def}	31.2 ^{f}	1.6 ^{f}
AR11	Jammu and Kashmir	15.7 ^{ijk}	19.3 ^{hij}	21.2 ^{ij}	1.1 ^{ij}
AR12	Punjab	19.7 ^{cde}	27.2 ^{cd}	31.3 ^{f}	1.6 ^{f}
AR13	Punjab	17.5 ^{efghi}	20.3 ^{ghij}	22.8 ^{hij}	1.1 ^{hij}
AR14	Punjab	18.8 ^{defg}	31.2 ^{b}	35.5 ^{cd}	1.8 ^{cd}
AR15	Punjab	18.8 ^{ghij}	18.7 ^{ij}	20.5 ^{j}	1.0 ^{j}
AR16	Punjab	14.5 ^{jklm}	19.0 ^{ij}	23.6 ^{hi}	1.2 ^{hi}
AR17	Punjab	13.3 ^{lm}	18.5 ^{j}	22.5 ^{ij}	1.1 ^{ij}
AR18	Punjab	16.7 ^{ghij}	26.7 ^{cde}	33.5 ^{def}	1.7 ^{def}
AR19	Punjab	16.5 ^{ghij}	20.5 ^{ghij}	25.3 ^{h}	1.3 ^{h}
AR20	Rajasthan	21.2 ^{bc}	28.5 ^{bcd}	34.2 ^{de}	1.7 ^{de}
AR21	Uttarakhand	18.7 ^{defg}	29.0 ^{bc}	37.2 ^{c}	1.9 ^{c}
AR22	Uttarakhand	14.2 ^{klm}	20.0 ^{efg}	25.3 ^{h}	1.3 ^{h}
AR23	Uttarakhand	15.5 ^{ijkl}	23.0 ^{fg}	22.5 ^{ij}	1.1 ^{ij}
AR24	Uttarakhand	18.2 ^{defgh}	22.3 ^{fghi}	28.0 ^{g}	1.4 ^{g}
AR25	Uttar Pradesh	16.0 ^{hijk}	20.7 ^{ij}	22.5 ^{ij}	1.1 ^{ij}

*Mean of three replications
The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

Table 4. Grouping of *Ascochyta rabiei* isolates based on growth rate of the colony on CDA medium at 20 days after incubation

Group	Growth rate (mm/day)	Number of isolates	Isolates
Fast growth	>2.5	8	AR1, AR3, AR5, AR9, AR14, AR20, AR21 and AR22
Medium growth	2.0-2.5	11	AR2, AR4, AR6, AR7, AR8, AR15, AR16, AR17, AR18, AR19 and AR23
Slow growth	<2.0	6	AR10, AR11, AR12, AR13, AR24 and AR25

Table 5. Grouping of *Ascochyta rabiei* isolates based on growth rate of the colony on PDA medium at 20 days after incubation

Group	Growth rate (mm/day)	Number of isolates	Isolates
Fast growth	>2.5	1	AR1
Medium growth	2.0-2.5	11	AR5 and AR9
Slow growth	<2.0	6	AR2, AR3, AR4, AR6, AR7, AR8, AR10, AR11, AR12, AR13, AR14, AR15, AR16, AR17, AR18, AR19, AR20, AR21, AR22, AR23, AR24 and AR25

from different states such as Delhi (AR2), Haryana (AR3), Himachal Pradesh (AR4, AR6, AR7 and AR8), Jammu and Kashmir (AR10 and AR11), Punjab (AR12, AR13, AR14, AR15, AR16, AR17, AR18 and AR19), Rajasthan (AR20), Uttarakhand (AR21, AR22, AR23 and AR24) and Uttar Pradesh (AR25) with growth of <2 mm day^{-1} were characterized as slow grower. The CDA medium was superior to PDA medium for *A. rabiei* as it supported for fast growth might be due to presence of chickpea as one of the ingredients in the medium, which provided additional nutrients for growth of the pathogen.

The pycnidium of *A. rabiei* was spherical or pear-shaped with a single opening called an ostiole. The conidia were oval to oblong, straight to slightly bent at one or both ends, hyaline, occasionally two-celled, rounded at both ends under compound microscope (Fig. 2). They developed on short conidiophores (stalks) embedded in a mucilaginous mass of cream-pink to light tan. The size of the conidia in all the isolates ranged from 9.19-12.51 x 3.36-4.32 µm (Table 6). The maximum conidial size was observed in isolate, AR5 (12.51 x 4.17 µm) followed by the isolates AR7 (12.50 x 3.90 µm) and AR25 (12.37 x 4.23 µm). Whereas, the minimum conidia size was found in isolate AR22 (9.19 x 3.45 µm) followed by the isolates AR3 (9.27 x 3.50 µm) and AR12 (9.66 x 3.39 µm). The conidia density for each isolate ranged from 0.5-7.2 x 10^6 conidia/ml (Table 6). The lowest spore density was found in isolate AR2 (0.5 x 10^6 conidia/ml) followed by AR1 (0.7 x 10^6 conidia/ml) and AR7 (0.8 x 10^6 conidia/ml). The highest conidia density was in isolate AR4 (7.2 x 10^6 conidia/ml) followed by AR18 (6.3 x 10^6 conidia/ml) and AR5 (4.7 x 10^6 conidia/ml) (Table 6).

The present results are in accordance with the earlier observations that the cultural and morphological characters including size of conidia and conidia density were found to be variable in different isolates of *A. rabiei* originated from Syria (Haware, 1987), USA (Jan and Weise, 1991),

Table 6. Conidial size and density in different isolates of *Ascochyta rabiei*

Isolates	State	Conidia size length x width (µm)*	Conidia density (no./ml)
AR1	Delhi	10.32 x 3.75	0.7 x 10^6
AR2	Delhi	10.72 x 3.83	0.5 x 10^6
AR3	Haryana	9.27 x 3.50	1.1 x 10^6
AR4	Himachal Pradesh	11.48 x 4.32	7.2 x 10^6
AR5	Himachal Pradesh	12.51 x 4.17	4.7 x 10^6
AR6	Himachal Pradesh	10.75 x 3.80	2.1 x 10^6
AR7	Himachal Pradesh	12.50 x 3.90	0.8 x 10^6
AR8	Himachal Pradesh	10.88 x 3.42	3.2 x 10^6
AR9	Jammu and Kashmir	10.18 x 3.51	1.0 x 10^6
AR10	Jammu and Kashmir	11.57 x 3.63	3.7 x 10^6
AR11	Jammu and Kashmir	11.55 x 4.16	4.0 x 10^6
AR12	Punjab	9.66 x 3.39	3.6 x 10^6
AR13	Punjab	10.62 x 4.13	4.0 x 10^6
AR14	Punjab	10.76 x 3.36	2.2 x 10^6
AR15	Punjab	11.20 x 3.90	4.0 x 10^6
AR16	Punjab	10.20 x 3.50	1.9 x 10^6
AR17	Punjab	11.10 x 3.94	4.3 x 10^6
AR18	Punjab	11.92 x 4.15	6.3 x 10^6
AR19	Punjab	12.17 x 4.17	4.2 x 10^6
AR20	Rajasthan	11.08 x 3.64	3.9 x 10^6
AR21	Uttarakhand	9.84 x 3.41	2.5x 10^6
AR22	Uttarakhand	9.19 x 3.45	4.1 x 10^6
AR23	Uttarakhand	10.55 x 3.56	3.1 x 10^6
AR24	Uttarakhand	11.16 x 3.80	3.7 x 10^6
AR25	Uttar Pradesh	12.37 x 4.23	3.5 x 10^6

* Average of 50 conidia

Table 7. Grouping of chickpea cultivars based on disease reactions against *Ascochyta rabiei* (AR1) at 15 days after inoculation in net house conditions.

Reactions	Grade	Cultivars (no.)	Cultivar names
Highly resistant	1	3	ICC 76, ICC 3996 and ICC 15978
Resistant	2-3	30	C 30, DKG 1030, GL 24021, GL 26054, H00 108, ICC 607, ICC 1467, ICC 1903, ICC 3918, ICC 4324, IC 4475, ICC 5124, ICC 7000, ICC 7002, ICC 12955, ICC 13754, ICC 14911, ICCV 04512, ICCV 05530, NNAB 1, NNAB 4, NNAB 5, NNAB 8, NNAB 11, NNAB 14, NNAB 15, NNAB 16, NNAB 18, NNAB 20 and NNAB 21.
Moderately resistant	4-5	14	C 29, GPF 2, ICC 12, ICC 1527, ICC 1591, ICC 2165, ICC 4200, ICC 5127, ICC 6306, ICC 11879, ICC 12512, ICC 12967, NNAB 17 and NNAB 22.
Susceptible	6-7	9	BG 1053, BG 5023, DKG 876, DKG 964, DKG 986, ICC 4935, ICCV 96026, NNAB 3 and NNAB 7.
Highly susceptible	8-9	35	BG 276, BG 362, BG 1088, BG 5028, DKG 10, DKG 933, DKG 972, HC 5, HC 11, ICC 1460, ICC 1913, ICC 2232, ICC 4958, ICC 4973, ICC 4991, ICC 5568, ICC 7196, ICC 8294, ICC 12963, ICC 17258, IG 72933, ILWC 292, JG 62, NNAB 2, NNAB 6, NNAB 9, NNAB 10, NNAB 12, NNAB 13, NNAB 19, Pusa 256, Pusa 261, Pusa 362, Pusa 372 and Pusa 1103.

Italy and Turkey (Porta-Puglia, 1992) and India (Basandrai *et al*., 2005). This is the first study in which the isolates of the pathogen originated from northern parts of India, included for morphological variability considering all the morphological features together.

Screening of chickpea cultivars

The inoculated plants showed typical symptoms of *Ascochyta* blight initially as minute lesionson leaves and stems. The lesions increased in size and often girdle the stems, weaken and broke the branches and petioles finally killed all plant parts above the lesions. The pathogen produced fruiting bodies (pycnidia) that become visible as tiny, black, raised spots, often arranged in concentric rings within those lesions. The pathogen destroyed all the susceptible cultivars which except control (uninoculated) plants which remained disease free throughout the experiment.

Out of 91 cultivars evaluated, three cultivars showed highly resistant reactions, 30 cultivars showed resistant reactions, 14 cultivars showed moderately resistant reactions, nine cultivars showed susceptible reactions and 35 cultivars

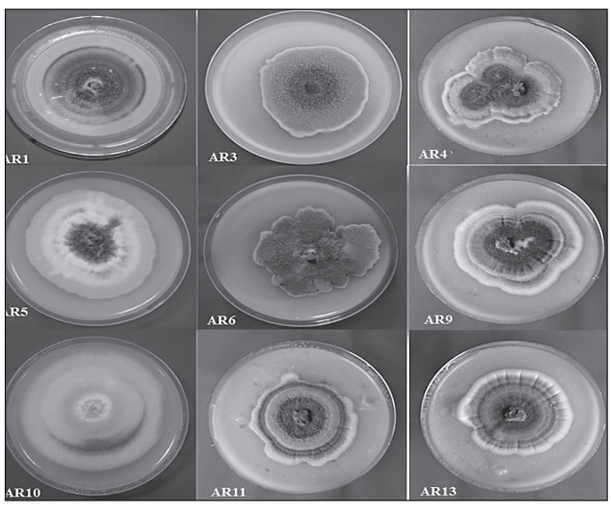

Figure 1. Colony growth patterns of *A. rabiei* isolates on chickpea dextrose agar at 20 days after incubation

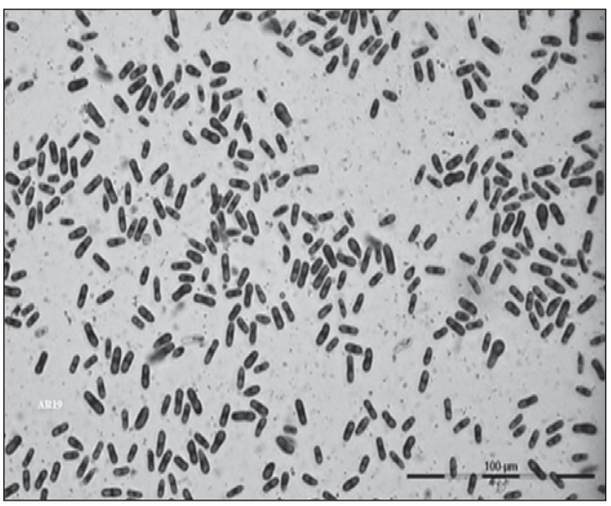

Figure 2. Conidia of representative isolate of *Ascochyta rabiei* AR19 Punjab

showed highly susceptible reactions against the pathogen (Table 7). Only few cultivars were highly resistant which indicated the conducive environmental conditions for disease development during screening. Among the resistant cultivars, only three cultivars, *viz.,* ICC 76, ICC 3996 and ICC 15978 were identified as highly resistant which can be used directly in breeding programs as sources of resistance against ascochyta blight. The numbers of cultivars under highly susceptible and resistant categories were more or less equal. Majority of cultivars were comprised of highly susceptible and resistant followed by moderately resistant and susceptible cultivars. Iqbal *et al*., (2002) also found only seven lines resistant during screening of 356 chickpea germplasm accessions whereas, none of the lines was found highly resistant. Ahmad *et al*., (2013) also observed that the most of the genotypes showed susceptible reactions against *Ascochyta* blight.

The isolates of *A. rabiei* originated from different states of India varied in their cultural and morphological characters. The chickpea cultivars showed variability in respect of resistant levels and resistant cultivars were identified for further use in cultivation or resistant breeding program.

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