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Cultural, Morphological and Pathological Variation in Indian Isolates of *Ascochyta rabiei*, the Chickpea Blight Pathogen

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Cultural, morphological and pathogenic variation in Indian isolates of *Ascochyta rabiei*, the causal agent of blight of chickpea, was investigated. Fungal isolates representative of seven agroclimatic regions in north western plain zones (NWPZ) of India showed variation in colony colour as mouse gray with green hue, light mouse gray with slate gray centre and gray with dark brown centre, when grown on chickpea dextrose agar (CDA). Conidiomatal color of the isolates varied from brown to slate gray and black. The number of conidiomata and conidia formed on CDA ranged from 49.7 to 90.7 and 5.5×10^4 to 3×10^5 cm^{-2}, respectively. The size of conidiomata and conidia of *A. rabiei* isolates varied from 274×232 μm to 156×116 μm, and from 14.0×6.2 μm to 10.7×4.6 μm, respectively. Fourteen *A. rabiei* isolates from the seven agroclimatic regions of NWPZ were evaluated for their virulence on 180 chickpea genotypes in controlled environment. Cluster analysis based on the disease rating on a 1-9 scale indicated higher similarity coefficient (> 0.65) between isolates from different agroecological regions, while few isolates from the same region had less similarity. The 14 isolates were grouped into eight pathotypes at > 0.5 similarity coefficient. Sixteen genotypes were identified as probable differentials to distinguish *A. rabiei* isolates.

Keywords : *Didymella rabiei*, pathotype, pathogen variability

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. (anamorph), is a devastating disease of chickpea in areas with persistent cool and humid weather (Nene and Reddy, 1987). The telomorph, *Didymella rabiei* (Kovatsch.) Arx is a bipolar heterothallic ascomycetes and requires the pairing of two compatible mating types (MAT-1 and MAT-2) for sexual reproduction (Armstong et al., 2001; Kaiser, 1997; Malhotra et al., 2003). Presence of a teleomorph stage in the life cycle contributes to heterozygosity within a population, resulting in new combinations of virulence genes and thus the development of new pathotypes of *A. rabiei*.

Deployment of host plant resistance (HPR) is one of the primary and economic options for disease management. In chickpea germplasm, HPR against AB is scarce and majority of the breeding efforts are localized because of the hyper variable virulence of *A. rabiei*. To characterize the extent of variability in *A. rabiei* isolates, experiments based on morphological characters, virulence and isozyme patterns, and DNA fingerprinting have been conducted in major chickpea growing countries (Pande et al., 2005). Based on the virulence, existence of different pathotypes of *A. rabiei* has been reported from India, Pakistan, Syria, Canada and several other chickpea growing countries (Malhotra et al., 2003; Nene and Reddy, 1987; Singh and Sharma, 1998). However, there is no clear relation was established between the pathogenic and genetic variability of *A. rabiei* (Pande et al., 2005). Since there is no uniformity in differential lines used in various studies, a definite comparison of *A. rabiei* isolates from different geographical regions of the world became difficult.

In the northwestern plain zones (NWPZ) of India, AB is a serious production constraint of chickpea and the disease often turns epidemic. As a result, there is a drastic reduction in area and productivity of chickpea, and most of the rice fallows where chickpea is grown traditionally are sown with other crops or left fallow during the post-rainy season. To re-establish/revive chickpea in these areas, it is imperative to identify stable and durable sources of resistance against the prevalent pathotypes of *A. rabiei*, if any. It is in this context, studies were initiated on the morphological, cultural and pathogenic variation among the *A. rabiei* isolates from NWPZ of India.

Materials and Methods

Fungal isolates. Fourteen cultures of *A. rabiei* were isolated from AB infected chickpea plants collected from different agroclimatic regions in NWPZ including foot hills, low hills, sub tropical and sub mountain zones of Himalayas in India (Fig. 1). Single spore isolates of

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individual cultures were maintained on chickpea dextrose agar (CDA, kabuli chickpea seed meal 40 g, dextrose 20 g and agar 20 g/l distilled water) at 4°C.

Plant material. To erect a set of differential lines, 180 chickpea genotypes consisting of 145 genotypes used as differential lines in earlier studies or with reported field resistance to AB and 35 advanced breeding lines with proven disease resistance in field were selected. Seeds of all these genotypes/breeding lines were obtained from the legumes pathology, chickpea breeding and genetic resources units of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru.

Cultural and Morphological variation of *A. rabiei*. Seven isolates of *A. rabiei* viz. AB 1, AB 3, AB 4, AB 6, AB 7, AB 8 and AB 17, one each from different agro-climatic regions in NWPZ were observed for their morphological and cultural variations. Five mm diameter disc from actively growing cultures of *A. rabiei* were inoculated at the center of 90 mm diameter petri plates containing CDA. Inoculated plates were incubated at 20°C with 12 h photoperiod and observed for color of the colony, intensity of the mycelium, colony diameter (mm), number of conidiomata and conidia. For quantification of conidiomata, 5 mm disc was cut at a distance of 1 cm from the centre of a well sporulating culture on CDA, 10 days after incubation. The disc was observed under a Gallen kamp colony meter magnifying glass to count the number of conidiomata. For quantification of conidia, similar disc was macerated in minimal volume of water, diluted to 10 ml and the number of conidia was measured using a haemocytometer. The experiments were conducted in triplicates and repeated once.

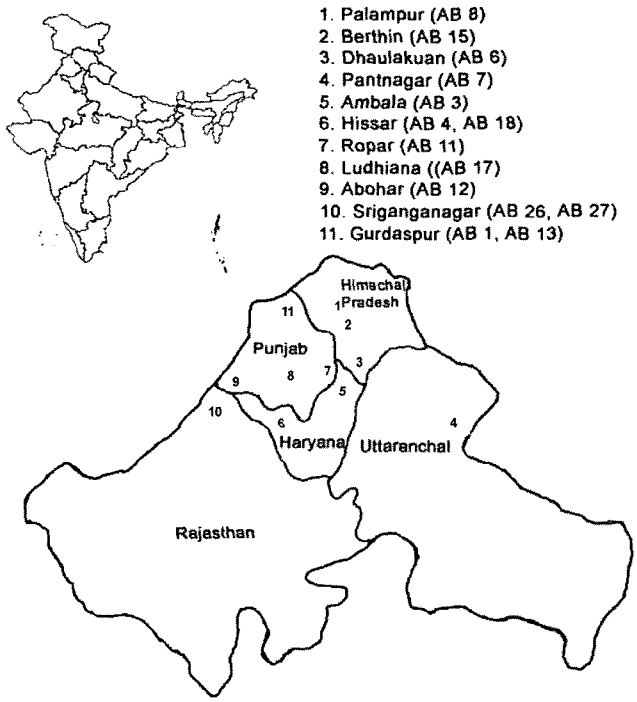

Fig. 1. Geographical locations from where *Ascochyta rabiei* isolates were collected in the north west plain zones of India.

Pathogenic variation of *A. rabiei* isolates. Fourteen *A. rabiei* isolates from seven agroclimatic regions in NWPZ were studied for their pathogenic variation on 180 chickpea genotypes and advanced breeding lines as follows.

Each of the 14 *A. rabiei* isolates were multiplied separately on sterile seeds of kabuli chickpea genotype ICCV 88901. Chickpea seeds were soaked overnight in water, autoclaved at 121°C for 25 min, and inoculated with 1 cm diameter actively growing culture of *A. rabiei* on CDA. Inoculated seeds were incubated for 8 days at 20°C and 12 h photoperiod. Profusely sporulated seeds were stirred in sterile distilled water to facilitate the release of conidia into water and filtered through a muslin cloth. The conidial concentration in the suspension was adjusted to 5×10^4 spores/ml and used as inoculum.

Seedlings of the test genotypes along with a susceptible check pb 7 (ICC 4991) were raised in $40 \times 30 \times 5$ cm plastic trays filled with sand and vermiculite mixture (10:1), in greenhouse at 25 ± 3°C and 12-13 h photoperiod. Ten-day-old seedlings were transferred to a growth room maintained at 20 ± 1°C with ~1500 lux light intensity for 12 h a day. The seedlings were inoculated by foliar spray of the inoculum using hand-operated atomizers. Inoculated plants were allowed to partially dry for 30 min to avoid dislodging of the spores and thereafter, 100% RH was provided 24 h a day up to 4 days after inoculation (DAI) and 6-8 h a day up to 14 DAI. The experiment was conducted in triplicates with eight plants in each replication and repeated once.

Ascochyta blight severity was recorded 14 DAI using a slightly modified 1-9 scale (Jan and Wiese, 1991), where 1 = no symptoms, 2 = minute lesions prominent on the apical stem/leaf tip, 3 = lesions up to 5 mm size and slight drooping of the apical stem, 4 = lesions obvious on all plant parts, and clear drooping of apical stem, 5 = lesions obvious on all plant parts, defoliation initiated, and slight to moderate breaking and drying of branches, 6 = lesions as in 5, defoliation, broken, dry branches common, some plants killed, 7 = lesions as in 5, defoliation, broken, dry branches very common, up to 25% of the plants killed, 8 = symptoms as in 7 but up to 50% of the plants killed, 9 = symptoms as in 7 but up to 100% of the plants killed. Based on the disease rating, each genotype was categorized as asymptomatic (disease score 1.0), resistant (disease score 1.1 to 3.0), moderately resistant (disease score 3.1 to 5.0),

susceptible (disease score 5.1 to 7.0) and highly susceptible (disease score 7.1 to 9.0).

Data analysis. The virulence index of individual isolate was calculated as (No. of genotypes in each category × respective disease rating) / total no. of genotypes. Disease reaction of individual isolate on selected 16 genotypes was coded in a binary form (1 and 0) representing the susceptible (disease score > 5.0) and resistant (disease score ≤ 5.0) reactions. A similarity matrix was constructed using Jaccard's coeeficient in SIMQUAL programme of numerical taxonomy and multivariate analysis system (NTSYS-pc) software (Rohlf, 1998). The resulting similarity data was used to construct a dendrogram using the unweighted pair group method with arithmetic averages in the SAHN programme within NTSYS-pc software.

Results

Cultural and morphological variation of *A. rabiei*. Colonies of *A. rabiei* isolates had varied shades of gray and white with distinct coloration in the center. The colony of isolate AB 4 was light mouse gray with slate gray centre whereas, isolate AB 1 developed colony with dark brown centre (Fig. 2). Pycnidial color was black in isolates AB 6, AB 7, AB 3 and AB 17, brown in isolates AB 4 and AB 1, and slate gray in AB 8. Mycelium of the majority isolates was off white and varied in its intensity at the margins of the colonies. However, in isolates AB 8 and AB 1 ash coloured mycelium was observed even in the centre. Colony diameter of the seven test isolates on CDA varied from 55.3 to 65.0 mm with AB 8 being the maximum. The number of conidiomata and conidia varied from 42.3 (AB 8) to 90.7 (AB 6) cm^{-2}, and 3.0×10^5 (AB 4) to 0.55×10^5 (AB 3) cm^{-2}, respectively. There was no positive correlation between the number of conidiomata and conidia produced per unit area. The isolates also varied in conidioma size (273.5 × 231.5 μm to 156.0 × 116.0 μm) and pycnidiospore size (14.0 × 6.2 μm to 10.7 × 4.6 μm) (Table 1).

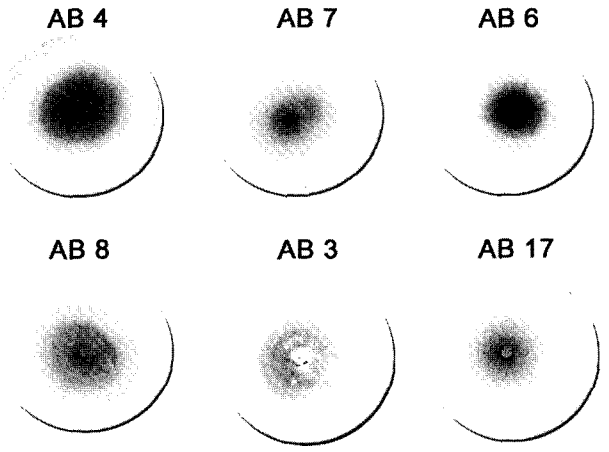

Fig. 2. Variation in the colony morphology of different *Ascochyta rabiei* isolates grown on chickpea dextrose agar for 10 days at 20°C and 12 h photoperiod.

Pathogenic variation in *A. rabiei*. The fourteen *A. rabiei* isolates differed in their virulence pattern against 180 chickpea lines in controlled environment, with AB 13

Table 1. Morphological and cultural characteristics of isolates of *Ascochyta rabiei* representing seven agroclimatic regions in northwestern plain zones of India[a]

Isolate	Colony color	Conidio-mata color	Colony diameter	Mycelium		Conidio-mata/cm^2	Conidia × $10^5/cm^2$	Conidiomata size (μm)	Conidia size (μm)
				Color	Intensity				
AB 1	Mouse gray greenish hue and dark brown center	Brown	58.3	Ash colored centre and dull white margins	+++	82. 3	0.58	217.5 × 215.5	10.7 × 5.3
AB 3	Mouse gray brownish with green hue	Black	56.7	Off white	+++	46.0	0.55	156.0 × 116.0	10.7 × 4.6
AB 4	Light mouse gray with slate gray centre	Brown	63	Off white	++	77. 7	3.01	263.0 × 226.5	13.6 × 6.4
AB 6	Mouse gray with greenish brown hue	Black	61.3	Off white	+++	90. 7	0.71	260.0 × 199.5	13.5 × 5.8
AB 7	Mouse gray with light green hue	Black	57.3	Off white	++	57. 7	0.84	273.5 × 231.5	13.5 × 5.8
AB 8	Light reddish mouse gray	slate gray	65.0	Ash	+++	42. 3	1.83	217.5 × 176.5	14.0 × 6.2
AB 17	Light mouse gray with greenish hue	Black	55.3	Off white	+++++	49. 7	0.97	259.5 × 187.5	11.7 × 4.7

[a] Five mm discs of actively growing individual cultures were inoculated on chickpea dextrose agar and incubated at 20 ± 1°C. All the observations were recorded 10 days after inoculation and are the mean of six replications.

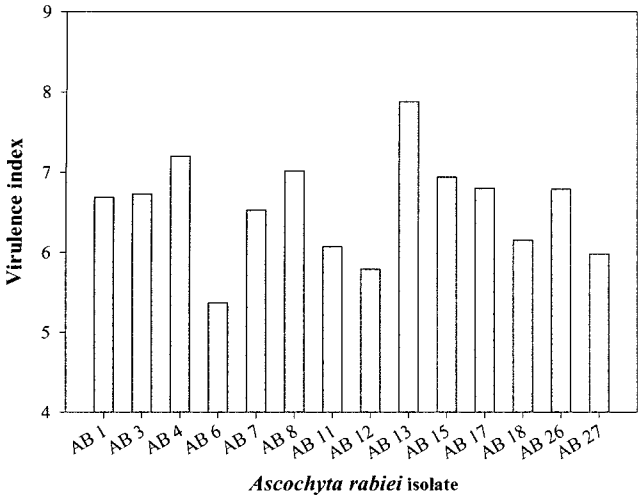

Fig. 3. Virulence index of fourteen isolates of *Ascochyta rabiei* against 180 chickpea lines.

having the maximum virulence index of 7.9 and AB 6 the minimum of 5.4 (Fig. 3). None of the chickpea lines were asymptomatic against any of the test isolates and differed in their response to individual isolate (Table 2). Of the 180 test lines inoculated with AB 6 and AB 27, 10 and 7 lines showed a resistant reaction. None of the test genotypes were resistant against the five isolates AB 4, AB 17, AB 26, AB 1 and AB 13 whereas, only one genotype showed resistant reaction to isolates AB 8, AB 15, AB 3 and AB 18.

Of the 180 chickpea lines, 63 genotypes differed significantly in their response to different isolates of *A. rabiei*, of which 15 were selected for precise differentiation of *A. rabiei* isolates. These genotypes include ICC 12, ICC 607, ICC 2165, ICC 3918, ICC 4200, ICC 4475, ICC 5124, ICC 6306, ICC 7002, ICC 13754, ICC 14911, ICCX 810800 and ICCX 910028-39ABR-BP-10ABR-BP, ILC 3870 and FLIP 82-258 (Table 3). The dendrogram resulting from the reaction of 14 isolates on 16 genotypes, including 15 selected promising genotypes and susceptible check ICC 4991, showed that the level of similarity among different isolates of *A. rabiei* varied from 0.16-0.80. Both the isolates AB 17 and AB 18 were clustered at 0.8 level of similarity followed by AB 1 and AB 26 at 0.76 level of similarity, and AB 3 and AB 13 at 0.68 level of similarity. Rest of the isolates were clustered at less than 0.57 level of similarity (Fig. 4). Virulence pattern of *A. rabiei* isolates on selected genotypes matched with few of the isolates reported from other parts of the world (Table 4).

An effort was made to authenticate the validity of diversity in the proposed differential lines. On the basis of reaction of these 16 lines against 14 isolates, ICC 13754 and ICC 4991 were clustered at 0.86 level of similarity, and ICC 12 and ICC 2165 at 0.80 level of similarity. Other genotypes were cluseted into different groups up to a least similarity coefficient of 0.19, indicating the diverse reaction of these genotypes to the test isolates (Fig. 5).

Discussion

Ascochyta rabiei isolates from different agroecological regions of India varied in their cultural and morphological characters including size of pycnidia and pycnidiospores, and sporulation. Similar variations have been reported in Italian and Turkish (Porta-Puglia, 1992), Indian (Nene and Reddy, 1987; Singh and Pal, 1993; Singh and Sharma, 1998), Syrian (Haware, 1987; Reddy and Kabbabeh, 1985), and American isolates (Jan and Weise, 1991). However, it is likely that morphological variations can provide only the preliminary variation in *A. rabiei* isolates, since these variations did not correlate with the geographical origin and pathogenic variations in several of the earlier studies (Pande et al., 2005). Randomly amplified DNA (RAPD) analysis of *A. rabiei* isolates from Pakistan indicated that isolates from the same host plant were genetically different, whereas isolates from different plants and cultivars were grouped in the same cluster (Sarwar et al., 2000).

The isolates tested in the present study showed variations

Table 2. Summary of the reaction of 180 chickpea test lines against 14 different isolates of *Ascochyta rabiei*[a]

Disease reaction	Number of genotypes in each category against individual *A. rabiei* isolate													
	AB 1	AB 3	AB 4	AB 6	AB 7	AB 8	AB 11	AB 12	AB 13	AB 15	AB 17	AB 18	AB 26	AB 27
asymptomatic	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Resistant	0	1	0	10	2	1	5	5	0	1	0	1	0	7
Moderately resistant	44	40	18	95	47	39	70	84	8	43	32	74	41	78
Susceptible	66	83	74	50	81	60	66	67	65	65	71	66	84	63
Highly susceptible	63	43	79	22	48	70	36	20	103	69	56	32	53	29

[a] Ten-day-old seedlings of 180 genotypes were separately inoculated with fourteen isolates of *A. rabiei* (5×10^4 conidia ml^{-1}) and incubated at 20 ± 1°C at 100% RH. The observations of disease severity were based on a 1-9 rating scale and individual genotypes were categorized as follows: 1 = asymptomatic; 1.1-3 = resistant; 3.1-5 = moderately resistant; 5.1-7 = susceptible; 7.1-9 = highly susceptible. Data was not obtained in few of the test lines against individual isolate.

Table 3. Differential response of selected chickpea genotypes/breeding lines against various isolates of *Ascochyta rabiei*[a]

Genotype	Disease score on a 1-9 rating scale													
	AB 1	AB 3	AB 4	AB 6	AB 7	AB 8	AB 11	AB 12	AB 13	AB 15	AB 17	AB 18	AB 26	AB 27
ICC 12	5	7	8	5	6	3	9	6	9	7	8	9	5	4
ICC 607	6	7	7	5	7	4	9	5	9	5	8	6	7	6
ICC 2165	5	7	9	5	6	7	9	6	9	4	6	7	5	5
ICC 3918	5	5	7	4	7	5	5	5	7	5	6	7	6	4
ICC 4200	7	5	6	6	5	5	6	5	5	8	6	4	6	5
ICC 4475	5	3	7	4	5	8	4	5	6	9	6	5	5	5
ICC 5124	9	6	5	4	9	9	5	3	–[b]	5	6	–	7	3
ICC 6306	5	6	4	4	6	6	3	6	9	7	6	7	5	5
ICC 7002	6	7	5	3	5	–	4	7	9	5	8	–	9	5
ICC 13754	6	6	7	4	6	7	6	4	9	6	6	9	6	7
ICC 14911	7	5	5	4	5	7	7	5	6	8	6	6	6	6
ICCX 810800	7	5	9	4	5	5	3	7	6	6	5	5	6	4
ICCX 910028-39ABR-BP-10ABR-BP	5	5	4	4	6	9	4	5	5	–	4	4	8	5
ILC 3870	5	8	8	4	5	5	5	4	7	5	5	5	5	5
FLIP 82-258	6	6	5	4	5	5	4	5	7	7	5	5	5	5
Pb 7 (ICC 4991)[c]	9	9	9	9	9	9	9	9	9	9	9	9	9	9

[a] Ten-day-old seedlings were separately inoculated with 14 *A. rabiei* isolates (5×10^4 conidia ml^{-1}) and incubated at 20 ± 1°C at 100% RH. The observations of disease severity were based on a 1-9 rating scale at 14 days after inoculation.
[b] Data not recorded
[c] Susceptible check

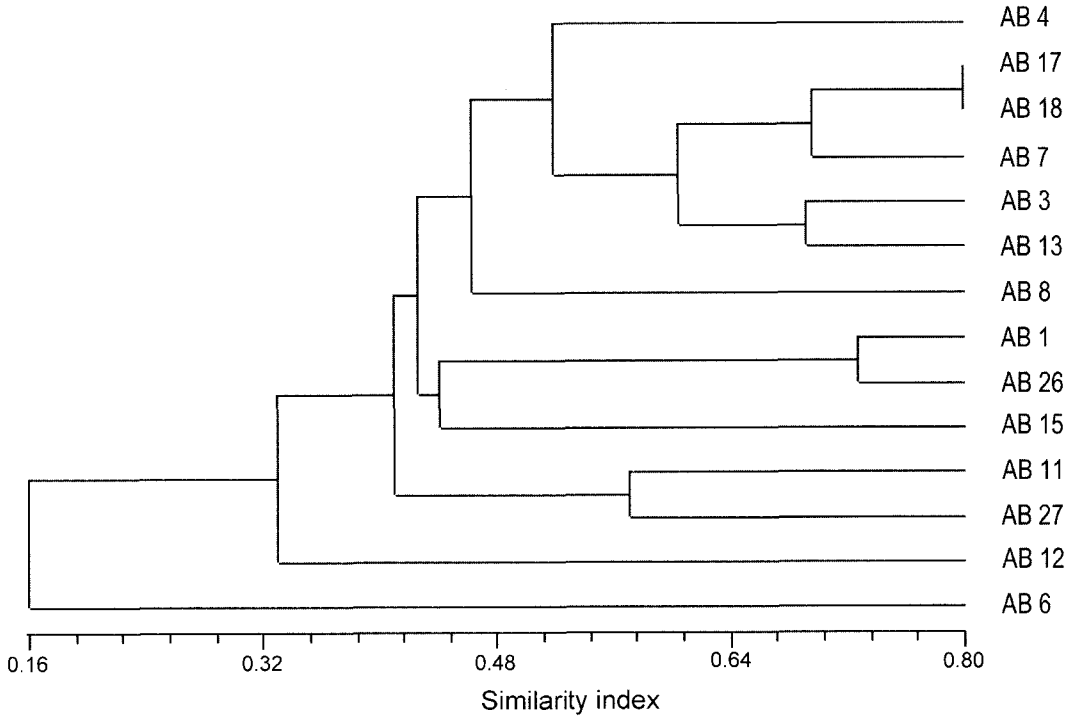

Fig. 4. Dendrogram showing the clustering of the virulence of *Ascochyta rabiei* isolates on 180 chickpea lines.

in their virulence on selected chickpea lines. In India, variations in pathogenicity among a collection of 268 *A. rabiei* isolates, have been observed by Vir and Grewal (1974). Teleomorph of *A. rabiei* has been reported from

Table 4. Comparison of the virulence pattern of *Ascochyta rabiei* isolates against selected chickpea lines with the reported pathotypes/ races

Isolates	Selected chickpea lines	Similarities of virulence pattern with reported pathotype/race	Reference
AB 3 and AB 4	ILC 72, ILC 191, ILC 202, ILC 484, ILC 3279, Calia and Principe	Italian race 4 and 5	Porta-Puglia, 1992
AB 6	P 1341-1, P 1801, C 235, EC 26435, V 135, ILC 1929, ILC 201 and ILC 249	isolate L86	Singh and Pal, 1993
AB 6 and AB 12	C 727, RC 32, CM 72, ILC 195 and ILC 200	2, 10, 12, 18 and 6 from Pakistan	Sajjad and Qureshi, 1992
AB 6, AB 3 and AB 18	ILC 72. ILC 200, ILC 215, ILC 482, ILC 1929, ILC 2956, ILC 3856, ICC 4475, ICC 9199 and ICC 12004	isolates R 3, D and U	Mmbaga, 1997
AB 15 and AB 6	ICC 1067, ICC 1467, ICC 1472, ICC 2165, G 543, NEC 138-2 and C 235	race 5	Amberdar and Singh, 1996
AB 15, AB 18, AB 26 and AB 13	F 8, ICC 1903, ILC 249 and ILC 3279	Syrian race 6	Reddy and Kabbabeh, 1985

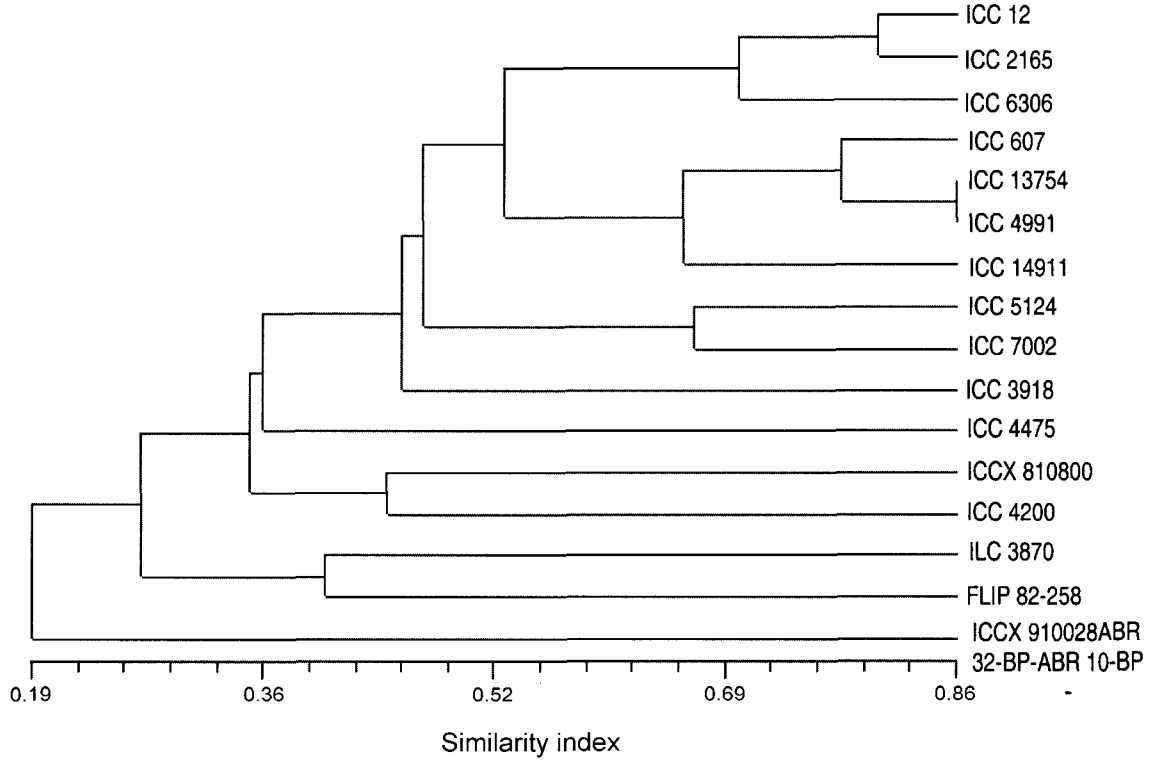

Fig. 5. Dendrogram showing the clustering of differential reaction of selected 16 chickpea lines to fourteen isolates of *Ascochyta rabiei*.

different chickpea growing countries including Canada, Australia and Turkey, and is likely to occur in the vast chickpea growing areas of India thus contributing to pathogenic diversity (Malhotra et al., 2003). It is difficult to precisely compare the virulence pattern of these isolates with those studied earlier, since several of these studies differed in environment, age of the seedlings and disease rating scale (Malhohtra et al., 2003; Porta-Puglia, 1992; Singh and Sharma, 1998). To a limited extent, these isolates were comparable to isolates from other countries as mentioned in Table 4. These comparisons might also be due to the seed transmission of *A. rabiei*, since a large proportion of the germplasm collections of these countries were from India. These results are also substantiated by Morjane et al. (1997), who reported higher levels of *A. rabiei* diversity within rather than between different

locations and also different genotypes from a particular location. Chongo et al. (2004) based on RAPD analysis reported that *A. rabiei* isolates from Australia, Syria, USA and India were similar to the major pathotypes of *A. rabiei* in Canada.

Presence of different races or pathotypes of a fungal pathogen necessitates the development of durable form of host plant resistance and also a continuous monitoring for a shift in the pathogen communities. Sixteen chickpea lines were identified as a set of potential differential lines to study the virulence pattern of *A. rabiei*. The diversity of these genotypes with respect to susceptibility to different *A. rabiei* isolates suggested that they can be used for differentiation of isolates from different locations. Interestingly, these lines originated in different countries, ICC 12, ICC 607, ICCX 81080 and ICCX 910028-39ABR-BP-10ABR-BP in India, ICC 2165 in Mexico, ICC 3918, ICC 4200, ICC 4475, and ICC 7002 in Iran, ICC 6306 and ICC 14911 in USSR, ICC 5124 in Israel, ILC 3850 in Bulgaria, and FLIP 82-258 in Syria. This set consisted of both desi and kabuli lines and also these lines had an entirely different pedigree compared to each other. Hence, they can be successfully exploited to study the pathogenic variation of *A. rabiei* from a large number of chickpea growing areas in India and other countries.

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