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Morpho-pathogenic and molecular diversity among *Rhizoctonia bataticola* isolates causing dry root rot of chickpea in Madhya Pradesh, India

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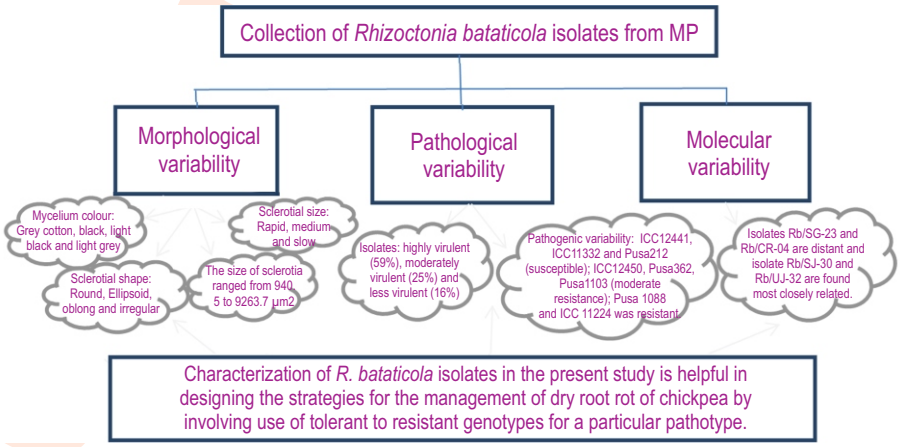
Abstract

Aim: The present study was conducted to identify the morphological, pathological and molecular variations in isolates of *Rhizoctonia bataticola*, causing dry root rot of chickpea collected from different parts of Madhya Pradesh, India.

Methodology: The isolation, purification and identification of *Rhizoctonia bataticola* isolates was done from dry root rot infected chickpea plants, collected from 23 districts of Madhya Pradesh, India. Total 32 isolates of pathogen were obtained and further used for morphological, cultural, pathological and molecular characterization. The acquired data were subjected to DARwin5 software analysis for variability studies. The DNA isolates of the pathogen were also obtained and analysed to study molecular variability by RAPD analysis and the bands produced were subjected to PAST software for cluster analysis.



Results: Thirty two isolates of *R. bataticola* with diverse origin showed a large variation in shape, size and initiation time of sclerotia. These isolates were pathogenic and grouped into three categories; highly virulent (59%), moderately virulent (25%) and less virulent (16%), under artificially inoculated conditions in sick soil method. The study of pathogenic variability revealed that ICC12441, ICC11332 and Pusa 212 were susceptible to all the isolates evaluated, whereas ICC12450, Pusa362, Pusa1103 showed moderate resistance against maximum isolates. Based on molecular characterization, the isolates were grouped into four clusters indicating that no two or more isolates were similar.

Interpretation: The isolates of *R. bataticola* of chickpea from different agro-climatic zones of Madhya Pradesh, possess variability in their morphological and cultural characteristics, which not limited to geographical boundaries. The present study would be extremely useful for dry root rot management, as well as, in identifying donors for resistance breeding programmes.

Key words: Chickpea, Diversity, Dry root rot, Pathogenicity, *Rhizoctonia bataticola*

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Introduction

Most diseases of chickpea are caused by fungi associated with seed and soil. Among soil-borne diseases, dry root rot is the major production constraint of chickpea in Madhya Pradesh (Ghosh *et al.,* 2013). Incidence of this disease is increasing day by day in soybean–chickpea cropping pattern, adopted in Madhya Pradesh and adjoining states of India. *Rhizoctonia bataticola* (Taub.) Butler [*Macrophomina phaseolina*] inciting dry root rot of chickpea is a crucial member of the genus that causes blight and root rot in many legumes (Hwang *et al.,* 2003). This disease appears as dispersed drying of plants with symptoms of yellowish colour plants, the leaves turn straw coloured, the tap root looks black, rotten and lack lateral and fine roots. The dead root becomes stiff and appears like a bark. Sometimes dark minute sclerotial bodies can be seen on the exposed or inside the roots. If dry stem at the collar region is split vertically, thin mycelium or little sclerotia can be seen in the pith (Nene *et al.,* 1981; Ghosh *et al.,* 2013). Environmental conditions like soil pH, less soil moisture and high temperature play an important role in the growth of *R. bataticola* (Khan, 2007). This pathogen is able to generate microsclerotia in scarcity of water whereas viability of microsclerotia is severely reduced in excess water (Olaya *et al.,* 1996). Globally chickpea is cultivated in 13.72 million ha area with an annual production of 14.25 million tons (FAOSTAT, 2019). India is the largest producer of chickpea in the world with the production of 11.08 million tons in 9.68 million hectare area. Madhya Pradesh is the largest producer of chickpea in India with a production of 2.73 million ton in 1.9 million ha area (AICRP, 2020-21). Climatic conditions play a predominant role in determining the course and severity of the disease epidemic. Yield losses in chickpea up to the tune of 40-50 percent can be seen in rain-fed areas. As Information on variability in *R. bataticola* of chickpea is limited, hence, this study was undertaken to understand this aspect in detail, for which a field survey was conducted to assess dry root incidence in chickpea covering 32 blocks from 23 districts and collected the pathogen isolates for this study.

Materials and Methods

Thirty-two isolates of *R. bataticola* were obtained from dry root rot infected chickpea plants collected from different agro-climatic regions of Madhya Pradesh covering 23 districts including Sehore, Bhopal, Vidisha, Jabalpur etc. (Table 1). The diseased plant samples were collected at flowering and early pod formation stage, as disease is generally observed at this stage. Diseased plant samples were brought in the laboratory and symptoms were recorded. Plants showing the symptoms of dry root rot were examined under the microscope for the presence of causal agent. During the field survey, disease intensity was calculated by square shaped bamboo sticks of 1 m^2 in different randomly selected areas in the field (Irullapan *et al.,* 2021).

Isolation purification and identification: Roots of diseased chickpea plant showing dry root rot symptoms were washed thoroughly with tap water, small pieces from infected roots were cut with the help of sterilized blade. These pieces were surface sterilized with 1:1000 $HgCl_2$ solution for 1 min followed by three changes in sterilized water to remove the traces of $HgCl_2$. The pieces were then transferred aseptically into petri plates containing Potato Dextrose Agarose medium. The inoculated petri plates were incubated at 28±1ºC and examined at frequent intervals to note the fungal growth developing from different pieces. The isolates were purified by single hyphal tip method. The growing mycelium was viewed under a microscope in inverted petri plate, containing thin layer of PDA and the mycelium tip was marked by glass marker pencil, a 5 mm disk was cut by cork-borer from marked area, containing mycelium tip thereafter it was transferred aseptically in another petri plate containing standard Hi-media PDA and incubated at 28±1ºC for studying cultural and morphological variability in the pathogen isolates.

Pathogenicity test: The pathogenicity test of *R. bataticola*, isolated from dry root rot affected chickpea plant was conducted under pot culture condition. The fungus was multiplied in the laboratory on potato dextrose agar medium. To multiply inoculum of the fungus, a 5 mm disc of PDA containing mycelium of *R. bataticola* was cut and added in to 250 ml flask, containing 100 g soil-maize medium (90g+10g). These flask were incubated for 15 days to multiply inoculum at 28±1ºC. The earthen pots measuring 9" diameter were filled with 3 kg soil and 15-day-old fully grown inoculum of the fungus was added @ 3 flasks (300 g) in each pot. The pots were left for 7 days to allow the multiplication, as well as, invasion of fungus in the soil. The seeds of susceptible variety of chickpea (L550) were sown @ 10 seeds (surface sterilized in 5 min in 2.5 % sodium hypochlorite) in each pot. Germination was recorded after 10 days of sowing, while seedling mortality was recorded fortnightly starting 20 days after sowing. The dead plants were observed critically for the association of *R. bataticola*. Isolations were made from the affected plants and the culture was compared with original one.

Cultural characteristics: Cultural characteristics of all 32 isolates were studied on standard Hi-media PDA under *in-vitro* condition. Twenty ml of this medium was poured in each of the previously sterilized 90 mm diameter petri plates. Five mm disc was cut through sterilized corkborer from the margin of seven-day-old colony of the purified fungal culture growth and was placed in the center of each plate, incubated at 28±1º C for 7 days. Three replications were maintained for each isolate. The cultural characteristics were recorded after 24, 48, 72, 96 and 120 hr intervals. The type of mycelial growth was noted as fluffy, partially submerged and submerged along with the colour of mycelium light black, black, light grey, light brown and grey cottony. The growth rate was determined after 5-days of incubation.

Pathogenic variability: A set of 10 resistant and susceptible chickpea genotypes namely ICC 12441, ICC 11332, ICC 11224, ICC 12450, Pusa 362, BGD 112, Pusa 1103, Pusa 212, Pusa 1088 and BCP 17 were evaluated against isolates of the pathogen by blotter paper technique (Nene *et al.,* 1981). Seedlings of test

Table 1: Survey report from 32 district of Madhya Pradesh, India

Name of Block	Name of District	Name of Isolates	Mortality	Field area
Multai	Betul	Rb/BT-01	6-8 %	R
Ghoradongari	Betul	Rb/BT-02	5-8%	R
Phanda	Bhopal	Rb/BH-03	4-6%	R
Bijawar	Chhatarpur	Rb/CR-04	10-15%	R
Harrai	Chhindwara	Rb/CD-05	2-3%	I
Harrai	Chhindwara	Rb/CD-06	1-2%	R
Mohkheda	Chhindwara	Rb/CD-07	3-4%	I
Pathariya	Damoh	Rb/DM-08	5-7%	R
Dewas	Dewas	Rb/DW-09	8-10%	R
Guna	Guna	Rb/GN-10	4-5%	R
Piparia	Hosangabad	Rb/HS-11	9-12%	R
Indore	Indore	Rb/IN-12	3-5%	R
Sihora	Jabalpur	Rb/JB-13	1-2%	R
Khargone	Khargone	Rb/KH-14	10-13%	R
Mandsour	Mandsour	Rb/MD-15	5-6%	I
Gotegawn	Narsingpur	Rb/NR-16	5-8%	I
Panna	Panna	Rb/PN-17	8-10%	R
Bareli	Raisen	Rb/RI-18	6-8%	R
Narsingharh	Rajgarh	Rb/RJ-19	5-7%	I
Narsingharh	Rajgarh	Rb/RJ-20	4-5%	R
Rewa	Rewa	Rb/RW-21	7-9%	R
Sagar	Sagar	Rb/SG-22	1-2%	R
Kesoli	Sagar	Rb/SG-23	1-2%	R
Rahatgarh	Sagar	Rb/SG-24	4-5%	R
Satna	Satna	Rb/ST-25	3-5%	R
Sehore	Sehore	Rb/SE-26	4-6%	R
Sehore	Sehore	Rb/SE-27	5-6%	R
Ichhawar	Sehore	Rb/SE-28	8-9%	R
Kalapipal	Shajapur	Rb/SJ-29	7-8%	R
Sujalpur	Shajapur	Rb/SJ-30	3-4%	I
Tikamgarh	Tikamgarh	Rb/TK-31	8-9%	R
Tarana	Ujjain	Rb/UJ-32	10-11%	R
Total	32			

R = Rain fed area; I = Irrigated area

genotypes of chickpea were raised in small polythene bags (measuring 25×15 cm) filled with autoclaved mixture of sand and soil (2:1) and each bag was filled with 2 kg of mixture. Twenty seeds of each genotypes were surface sterilized and sown in each bag. Each isolate of the pathogen was multiplied on 100 ml PDA broth in 250 ml flask at 25±1˚C for 7 days. Mycelia mat were ground separately in 100 ml sterilized distilled water and placed in 250 ml beaker. Ten-day-old seedlings of the test varieties were uprooted, washed in running tap water and rinsed in sterilized distilled water. The entire root system of seedlings was dipped in the inoculums of each isolate separately for about 30 sec and the excess inoculum was removed. These seedlings were placed on sterilized blotter paper. The blotter paper was folded and moistened adequately, but not excessively with sterilized distilled water (one folded blotter paper with the seedlings of one test genotype). The inoculated seedlings were placed in a tray and incubated at 25±1˚C for 8 days blotters adequately every day. Control was maintained by dipping the seedlings in sterilized water. At the end of the incubation period, the seedlings were examined for the extent of root damage and scored in 1-9 scale for the disease.

DNA extraction: All the 32 isolates were grown in potato dextrose broth medium and mycelia were harvested by filtering through Whatman filter Paper No 1 and washed repeatedly with distilled sterile water to remove excessive salts adhering to it. DNA was extracted from 1 g mycelium followed by cetyl trimethyl ammonium bromide method (Murray and Thompson, 1980). The purity of DNA was checked by Nanodrop spectrophotometer at 260 nm. The 260/280 ratio was about 1.76, indicating high purity of DNA. Further, the isolated DNA was run in 1% agarose gel to check the quality and quantity of DNA.

RAPD analysis: Four different decamer primers were used from RFu series-Random Fungal Primer Kit (RFu 'D'), Genie Pvt. Ltd., Bangalore, India to amplify the DNA (RFu 1: CCTGGGCCAG; RFu 2: CCTGGGCGAG; RFu 3: CCTGGGCTGG and RFu 4:

Table 2: Morphological and cultural characters of *Rhizoctonia bataticola* isolates from different districts

Name of Isolates	Average width (W) (µm)	Average length (L) (µm)	Size (W X L) µm2	Shape of sclerotia	Colony colour	Growth rate
Rb/BT-01	70.3	96	6748.8	Irregular	Light black	Medium
Rb/BT-02	55.8	69.5	3878.1	Round	Grey cottony	High
Rb/BH-03	38.5	54.3	2090.55	Round	Light brown	High
Rb/CR-04	59.3	93	5514.9	Oblong	Light brown	High
Rb/CD-05	48.9	64.6	3158.94	Oblong	Grey cottony	High
Rb/CD-06	51.2	62.8	3215.36	Round	Light brown	Low
Rb/CD-07	75.5	83	6266.5	Round	Light black	Medium
Rb/DM-08	68.3	80	5464	Round	Light cottony	High
Rb/DW-09	30.6	41.8	1279.08	Irregular	Light grey	Medium
Rb/GN-10	42.9	56.9	2441.01	Round	Light black	High
Rb/HS-11	51.4	73.5	3777.9	Round	Light grey	Low
Rb/IN-12	46.7	59.1	2759.97	Oblong	Black	High
Rb/JB-13	49.9	68.7	3428.13	Oblong	Black	Low
Rb/KR-14	28.6	41.6	1189.76	Ellipsoid	Light black	Medium
Rb/MD-15	79	95.5	7544.5	Oblong	Light grey	High
Rb/NR-16	62.7	82.1	5147.67	Round	Grey cottony	Medium
Rb/PN-17	53.4	70.9	3786.06	Oblong	Grey cottony	Medium
Rb/RI-18	32.3	43.3	1398.59	Round	Light black	High
Rb/RJ-19	49.5	68	3366	Irregular	Black	Medium
Rb/RJ-20	81.5	95.4	7775.1	Ellipsoid	Black	High
Rb/RW-21	84.6	109.5	9263.7	Round	Black	Medium
Rb/SG-22	52.9	68.4	3618.36	Oblong	Black	Low
Rb/SG-23	54.2	72.2	3913.24	Round	Light brown	Low
Rb/SG-24	40.2	59.7	2399.94	Irregular	Grey cottony	Medium
Rb/ST-25	57.9	86.7	5019.93	Irregular	Light brown	Low
Rb/SE-26	51.1	66.7	3408.37	Round	Grey cottony	Medium
Rb/SE-27	39.8	43	1711.4	Irregular	Light black	Medium
Rb/SE-28	48.5	64	3104	Irregular	Grey cottony	High
Rb/SJ-29	50.6	66.5	3364.9	Ellipsoid	Black	High
Rb/SJ-30	41.5	62.4	2589.6	Ellipsoid	Grey cottony	Medium
Rb/TK-31	34.5	53.2	1835.4	Ellipsoid	Grey cottony	High
Rb/UJ-32	27.5	34.2	940.5	Oblong	Black	High

CCTGGGCTAT. The RAPD-Bangalore mixture (25 ml) consisted of 30 ng template DNA, 0.16 ml (3 units) Taq polymerase and 4 mM of primer in 1X reaction buffer: Neno pure water (10.25 µl), Master mixture (12.5 µl), Primer (1.25 µl), DNA sample (1 µl). The amplification was performed in a thermal cycler at 94^{0}C for 2 min for initial denaturation, followed by 40 cycles at 92^{0}C for 1 min denaturation, annealing at 37^{0}C for 1 min and extension at 72^{0}C for 2 min with an elongation of 72^{0}C for 5 min. Amplification products were resolved by electrophoresis on agarose horizontal gel with 1 Kb marker in 1X TAE buffer stained with ethidium bromide and photographed under UV light.

Morphological and molecular variability assessment: The factorial and clusters analysis based on cultural and morphological variations of 32 isolates of *R. bataticola* was done by using DARwin5 software 5.0.158. Molecular variability of all 32 isolates of *R. bataticola* was analyzed using four primers. All the RAPD bands produced were subjected to cluster analysis based on the "PAST" software and dendrogram was generated.

Results and Discussion

Dry root rot is an important disease complex that causes root rots in food legumes. It is emerging as major constraint in the production of chickpea under the current scenario of changing climatic conditions related to increase in temperature and low rainfall (Ratul and Singh, 2018; Kumar, 2013). Due to high temperature (>30˚C) coupled with moisture stress at the time of crop maturity in central and southern India, the disease severity is more (Sharma and Pande, 2013; Deepa *et al.,* 2018; Chiranjeevi *et al.,* 2019). Total thirty two isolates of *R. bataticola* were collected from twenty three major chickpea growing districts of Madhya Pradesh. Maximum isolates of the fungus, obtained from DRR infected chickpea plants belonged to rainfed areas, exhibiting 10-15% mortality (Table 1). These isolates produced abundant mycelium on PDA cultures and later sclerotial bodies in inoculated Petri dishes. No pycnidial bodies (*M. phaseolina*) were formed in cultures. Based on the cultural and morphological variations, 32 isolates were placed in different groups.

Table 3: Mortality percentage and pathogenic behaviour of *Rhizoctonia bataticola* isolates

Name of isolates	Mortality percentage (Transform value)*	Pathogenic behaviour **
Rb/BT-01	76.66 (61.22)	HP
Rb/BT-02	66.67 (54.78)	P
Rb/BH-03	46.67 (43.07)	LP
Rb/CR-04	76.66 (61.22)	HP
Rb/CD-05	76.66 (61.22)	HP
Rb/CD-06	76.66 (61.92)	HP
Rb/CD-07	66.67 (54.78)	P
Rb/DM-08	63.34 (52.77)	P
Rb/DW-09	70 (57)	HP
Rb/GN-10	33.33 (35.22)	LP
Rb/HS-11	76.66 (61.22)	HP
Rb/IN-12	33.33 (35.22)	LP
Rb/JB-13	63.34 (52.77)	P
Rb/KR-14	76.66 (61.22)	HP
Rb/MD-15	70 (56.79)	HP
Rb/NR-16	66.66 (54.78)	P
Rb/PN-17	76.66 (61.22)	HP
Rb/RI-18	30 (33.21)	LP
Rb/RJ-19	70 (57)	HP
Rb/RJ-20	73.33 (59)	HP
Rb/RW-21	76.66 (61.22)	HP
Rb/SG-22	76.66 (61.22)	HP
Rb/SG-23	70 (57)	HP
Rb/SG-24	70 (57)	HP
Rb/ST-25	66.67 (54.78)	P
Rb/SE-26	76.66 (61.22)	HP
Rb/SE-27	70 (57)	HP
Rb/SE-28	70 (57)	HP
Rb/SJ-29	66.67 (54.78)	P
Rb/SJ-30	76.66 (61.22)	HP
Rb/TK-31	63.34 (52.77)	P
Rb/UJ-32	46.67 (43.07)	LP

*Average of three replications, Transform value in brackets; * 50 and less then= Less pathogenic, 51 to 69 = Pathogenic, 70 and above = Highly Pathogenic; ** HP = Highly Pathogenic, P = Pathogenic, LP = Less Pathogenic

Table 4: Clustering based on UPGMA analysis of different *Rhizoctonia bataticola* isolates based on their morphological and cultural characters

Group	Sub-group	Cluster	Group number	Isolate
		1	22, 13, 19, 27, 14	Rb/SG-22, Rb/JB-13, Rb/RJ-19, Rb/SE-27, Rb/KH-14
	IA	2	24, 9, 30, 17, 25,	Rb/SG-24, Rb/DW-09, Rb/SJ-30, Rb/PN-17,
I		1		Rb/ST-25, Rb/BT-01
		1	29, 12,	Rb/SJ-29, Rb/IN-12,
	IB		32, 20,	Rb/UJ-32, Rb/RJ-20
		2	28, 5, 31, 15, 4	Rb/SE-28, Rb/CD-05, Rb/TK-31, Rb/MD-15, Rb/CR-04
		1	18, 10,	Rb/RI-18, Rb/GN-10,
	IIA		3, 2	Rb/BH-03, Rb/BT-02
		2	8	Rb/DM-08
II		1	26, 16, 23, 6, 11	Rb/SE-26, Rb/NR-16, Rb/SG-23, Rb/CD-06, Rb/HS-11
	IIB	2	21, 7	Rb/RW-21, Rb/CD-07

The isolates were grouped into three categories on the basis of 5 days mycelia growth rate (low growth rate: below 80 mm, medium growth rate: 80-85 mm and high growth rate: a bow 85 mm). Six isolates were found with low growth rate, 12 isolates were with medium growth rate and 14 isolates were observed with high growth rate (Table 2). The isolates were also characterized on the basis of colony colour and were grouped in five groups namely grey cottony (31%) then black (25%), light black (19%), light brown (16%) and light grey (9%) (Table 2). All isolates showed large variations in sclerotia shape, size and initiation time of sclerotia. More variation was found in shape of sclerotia like (a) Round (b) Ellipsoid (c) Oblong and (d) Irregular (Fig. 1).

The size and shape of sclerotia is standard in *R. bataticola* isolates and have implications on the pathogenic behavior. In the present study it was found that the isolates having large size of sclerotia (>3000 μm^2) are pathogenic to highly pathogenic, as compared to isolates having small size sclerotia. Similarly, the isolates having round shapes sclerotia are less pathogenic as compared to *R. bataticola* isolates having ellipsoid, oblong and irregular sclerotia. The isolates having irregular shape sclerotia were highly pathogenic (Table 3). Initiation time of sclerotia formation of different isolates was measured after 24 hr interval. Some isolates were recorded to form sclerotia rapidly after 48 hr of inoculation (31.25% of total isolates) whereas after 72 hr maximum 87.5% of isolates produced sclerotia and after 96 hours, only 12.5% isolates formed sclerotia. the maximum isolates belonged to rain-fed areas of Madhya Pradesh and dry root rot was also high in these areas (Table 1). Sharma and Pande (2013) also observed that dry root rot of chickpea is mainly found in rain-fed chickpea and accelerated with the depletion of soil-moisture in field. Significant variations in morphological and cultural characteristics were noted in colony colour, growth rate, growth pattern in mycelium of different isolates, the sclerotial initiation time, their size and shape varied considerably in the isolates. Five groups of colony colours were observed *viz.* light black, black, light grey, light brown, grey cottony.

Euclidean distances were calculated and a phylogenetic tree was constructed for all the morphological characters of *R. bataticola* isolates. The dendrogram of isolates indicated major two distinct groups (I and II). The group I are further divided into two groups (IA and IB) based on the cultural and morphological variations (Fig 2). Group IA contains 11 isolates, Group IB contains 09 isolates, Group IIA contains 05 isolates and Group IIB contains 07 isolates based on the cultural and morphological variations (Table 4). A data matrix plot based on the cultural and

Table 5: Pathogenic response of ten genotypes against 32 isolates obtained from different districts

Cultivars Isolates	ICC 12441	ICC 11332	ICC 11224	ICC 12450	Pusa 362	BGD 112	Pusa 1103	Pusa 212	Pusa 1088	BCP 17
Rb/BT-01	9 (S)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)
Rb/BT-02	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	5 (R)	9 (S)	3 (R)	9 (S)
Rb/BH-03	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	3 (R)	5 (R)	9 (S)	7 (S)	9 (S)
Rb/CR-04	9 (S)	7 (S)	9 (S)	9 (S)	7 (S)	5 (R)	9 (S)	7 (S)	9 (S)	9 (S)
Rb/CD-05	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)	9 (S)
Rb/CD-06	9 (S)	7 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)
Rb/CD-07	9 (S)	9 (S)	7 (S)	5 (R)	9 (S)	9 (S)	5 (R)	7 (S)	7 (S)	9 (S)
Rb/DM-08	9 (S)	9 (S)	9 (S)	9 (S)	3(R)	7 (S)	9 (S)	7 (S)	3 (R)	9 (S)
Rb/DW-09	9 (S)	9 (S)	9 (S)	3 (R)	7 (S)	9 (S)	5 (R)	9 (S)	7 (S)	7 (S)
Rb/GN-10	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	9 (S)
Rb/HS-11	9 (S)	9 (S)	9 (S)	5 (R)	9 (S)	3 (R)	9 (S)	7 (S)	3 (R)	5(R)
Rb/IN-12	9 (S)	9 (S)	9 (S)	3 (R)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)
Rb/JB-13	9 (S)	9 (S)	5 (R)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)	9 (S)
Rb/KR-14	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	5 (R)	9 (S)	9 (S)	7 (S)
Rb/MD-15	9 (S)	9 (S)	9 (S)	3 (R)	5(R)	9 (S)	5 (R)	9 (S)	7 (S)	9 (S)
Rb/NR-16	9 (S)	9 (S)	5 (R)	9 (S)	3(R)	9 (S)	5 (R)	7 (S)	5 (R)	7 (S)
Rb/PN-17	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)
Rb/RI-18	9 (S)	9 (S)	3 (R)	9 (S)	5(R)	9 (S)	3 (R)	9 (S)	9 (S)	9 (S)
Rb/RJ-19	9 (S)	9 (S)	9 (S)	9 (S)	5(R)	7 (S)	9 (S)	9 (S)	7 (S)	9 (S)
Rb/RJ-20	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)
Rb/RW-21	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	7 (S)	7 (S)	9 (S)	7 (S)	9 (S)
Rb/SG-22	9 (S)	9 (S)	9 (S)	9 (S)	7 (S)	7 (S)	9 (S)	7 (S)	7 (S)	9 (S)
Rb/SG-23	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	5 (R)	7 (S)	9 (S)	9 (S)
Rb/SG-24	9 (S)	9 (S)	7 (S)	9 (S)	5(R)	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)
Rb/ST-25	9 (S)	9 (S)	9 (S)	3 (R)	9 (S)	3 (R)	7 (S)	9 (S)	3 (R)	7 (S)
Rb/SE-26	9 (S)	9 (S)	3 (R)	9 (S)	3(R)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)
Rb/SE-27	9 (S)	9 (S)	9 (S)	5 (R)	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)
Rb/SE-28	9 (S)	9 (S)	9 (S)	5 (R)	5(R)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)
Rb/SJ-29	9 (S)	9 (S)	7 (S)	9 (S)	9 (S)	9 (S)	5 (R)	7 (S)	7 (S)	7 (S)
Rb/SJ-30	9 (S)	9 (S)	9 (S)	7 (S)	7 (S)	9 (S)	7 (S)	7 (S)	9 (S)	9 (S)
Rb/TK-31	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)	7 (S)	5 (R)	9 (S)	9 (S)	9 (S)
Rb/UJ-32	9 (S)	9 (S)	9 (S)	9 (S)	9 (S)	7 (S)	9 (S)	7 (S)	7 (S)	9 (S)

R= Resistant; S= Susceptible

morphological variations based on all the morphological characters was subjected to PCoA for estimating differentiation among the isolates. The scatter plot based on these components disclosed a pattern of mainly four groups, which were distinctively separated on the basis of shape of sclerotia and growth rate (Fig. 3). The results suggest that the highest colony of grey cottony mycelium (31%) was more predominant then black, light black, light brown and light grey. Also, isolates showed large variations in sclerotia shape, size and initiation time of sclerotia. The variations in shape of sclerotia were round, ellipsoid, oblong and irregular type. The variation in growth rate and colony characters of *R. bataticola* causing dry root rot in chickpea has also been reported from different studies, which support the present findings (Dhingra and Sinclair, 1973; Manjunatha and Naik, 2011; Aghakhani and Dubey, 2009a; Sharma *et al.,* 2012 a,b). Prasad *et al.* (2014) also found that isolates of *R. bataticola* collected from semi-arid regions of Maharashtra, Andhra Pradesh and Karnataka states of India had variability in shape, length, width and length/width in morphological characters of micro-sclerotia. Variations in the sclerotia in 35 isolates of *R. bataticola* collected from the major sesame producing regions in Central China comprising Hubei, Henan, Anhui, Jiangxi province has also been reported by Linhai *et al.* (2011). All thirty-two isolates were artificially inoculated and were found pathogenic.

The isolates were highly pathogenic (59%), pathogenic (25%) and less pathogenic (16%), when inoculated with soil sick method (Fig. 4). The infected plants lost turgidity and showed chlorotic symptoms. The leaves and stems of infected plants turned straw colour. Tap root became dark and rotting extended to entire root system. Under favorable conditions, minute sclerotia developed on root. Morphology of re-isolated fungus confirmed the identity of *R. bataticola*. The results of pathogenic variability were highly variable in virulence with disease severity ranging from 7 to 9 rating (Manjunatha and Saifulla, 2021) (Table 5). Out of ten chickpea genotypes, ICC11332, ICC12441 and Pusa 212

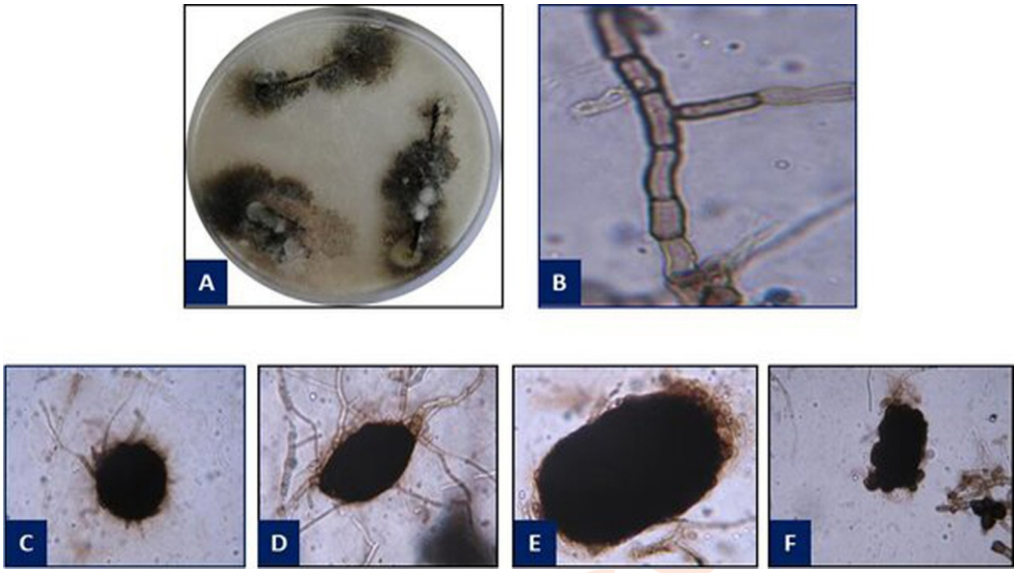

Fig. 1: Culture and different shapes of sclerotia (*Rhizoctonia bataticola*): (A) Culture; (B) Mycelium of *R. bataticola*; (C) Round; (D) Ellipsoid; (E) Oblong and (F) Irregular.

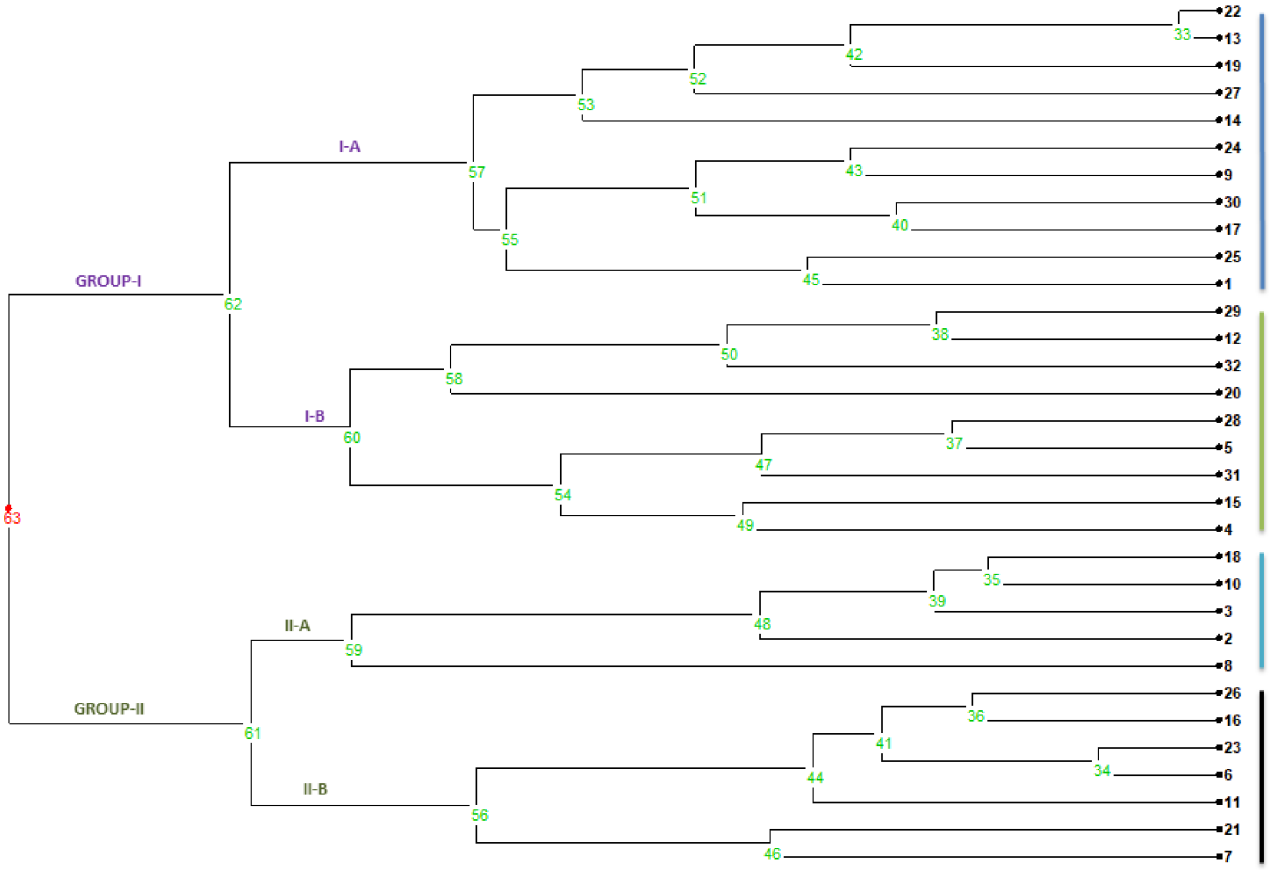

Fig. 2: Dendrogram generated from an unweighted pair group method analysis (UPGMA) cluster analysis based on all the morphological and cultural characters of *Rhizoctonia bataticola* isolates.

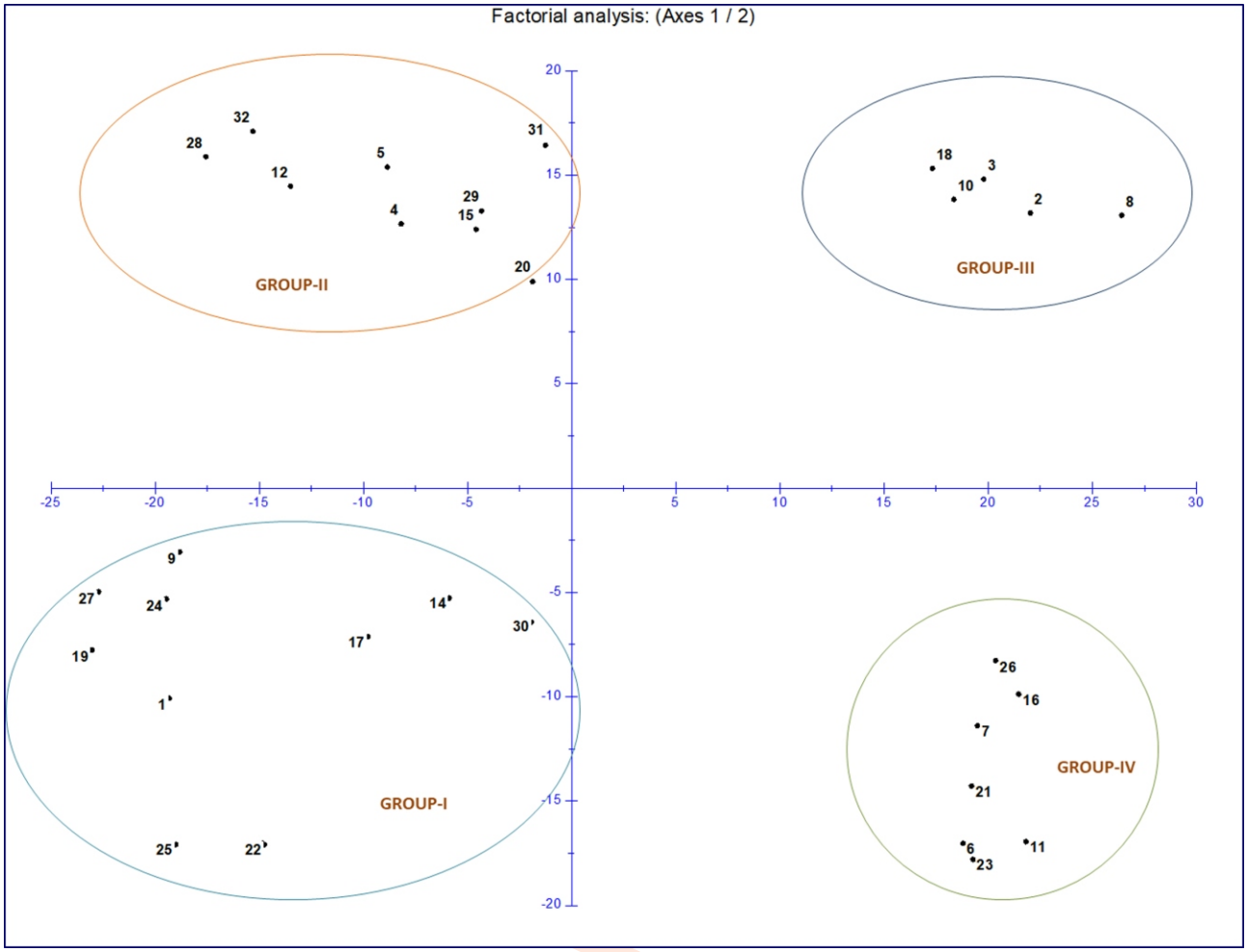

Fig. 3: Factorial analysis based on all the morphological and cultural characters of *Rhizoctonia bataticola* isolates.

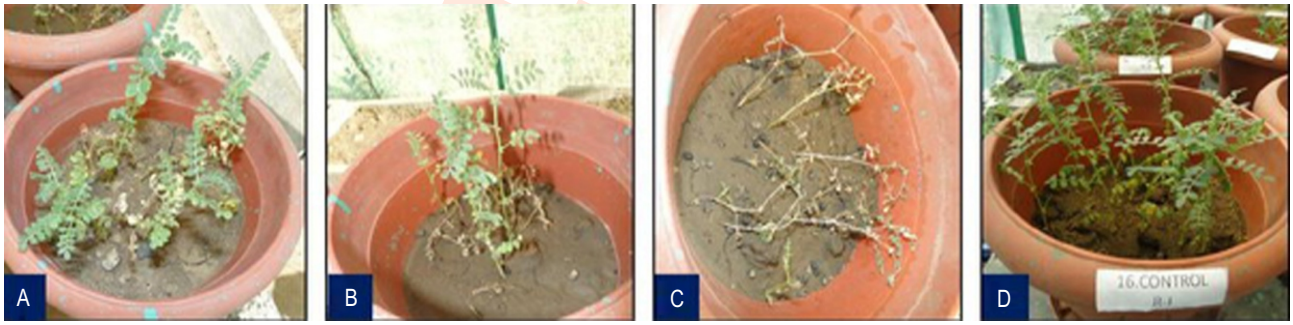

Fig. 4: Pathogenicity of isolates; (A) Less pathogenic; (B) Pathogenic; (C) Highly pathogenic and (D) Control.

were sensitive to all the isolates of *R. bataticola*. The genotype BGD112 was found resistant to one isolate, Pusa 1088 exhibited resistance to six isolates, Pusa 1103 and Pusa 362 were resistant to eight isolates, ICC11224 was resistant to four isolates and genotype ICC12450 was resistant to eight isolates (Fig. 5). Pathogenic variations in *R. bataticola* have also been reported form different parts of the country (Aghakhani and Dubey, 2009b; Lekhraj *et al.,* 2012).

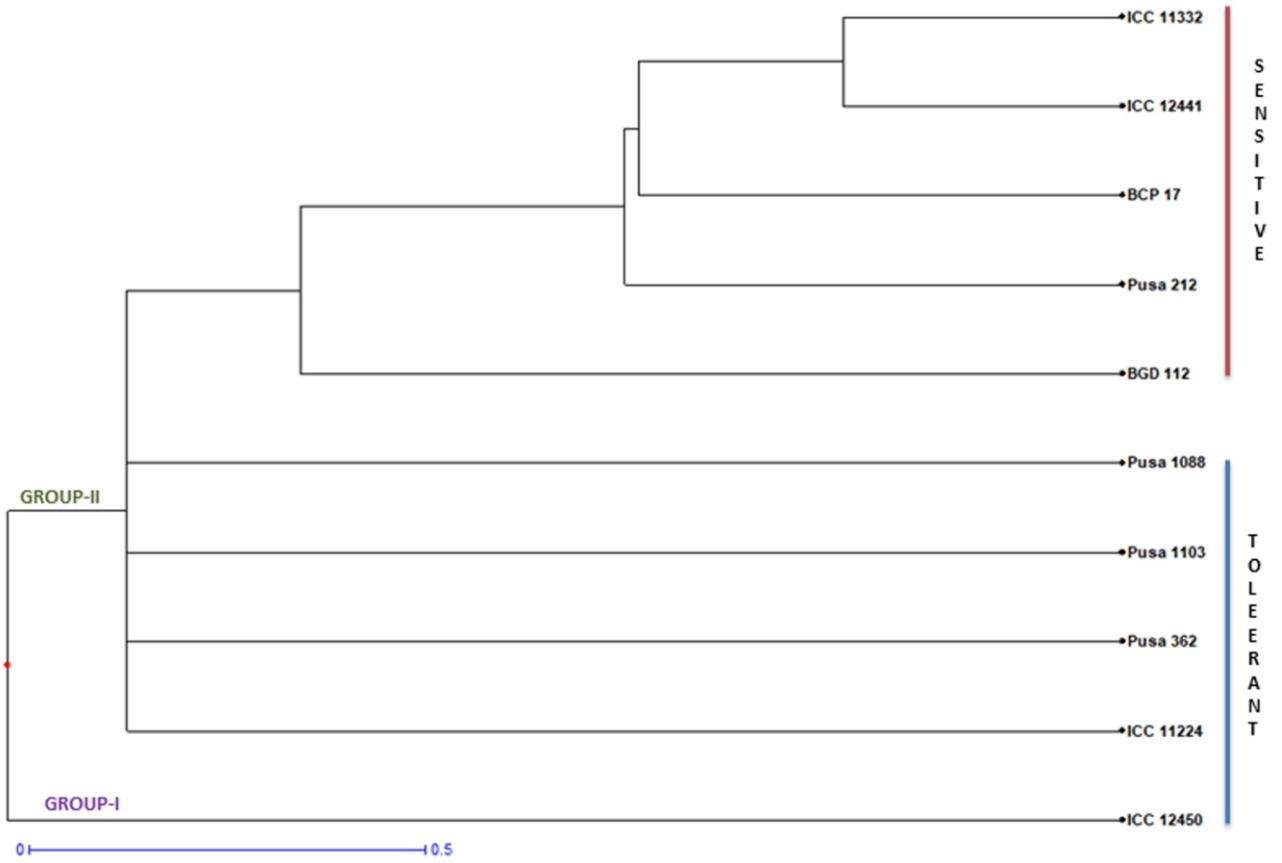

Fig. 5: Dendogram generated from an unweighted pair group method analysis (UPGMA) cluster analysis based on pathogenic response of ten genotypes against 32 Isolates.

A study made by Lekhraj *et al.*, (2012) suggested positive relationship between sclerotial intensity and pathogenicity, as more pathogenic isolates produced more sclerotia, but Sharma *et al.* (2016) observed no such positive correlation among them. Sharma *et al.* (2012a) has also proved the pathogenicity of *R. bataticola* isolates and their study suggest that pathogenic and non-pathogenic isolates were not concentrated in any one particular region/state. Genotypes ICC12441, ICC11332, and Pusa 212 were susceptible to all the isolates evaluated (disease score 7-9) and BCP17 was found resistant to Rb/HS-11 (5 score). Molecular variability of all 32 isolates of *R. bataticola* was analysed to characterize the population structure of chickpea dry root rot in Madhya Pradesh using four primers RFu-1, RFu-2, RFu-3 and RFu-4. All four RAPD primers generated polymorphic bands. When fingerprints of these isolates were observed, some bands common to all isolates were observed while others were unique to one or a few isolates. All the RAPD produced bands were further subjected to cluster analysis based on the "PAST" software and dendrogram was generated. The neighbour-joining analysis grouped the isolates into four major groups *viz.,* Group I, Group II, Group III, and Group IV based on high magnitude of genetic diversity among the isolates of *R. bataticola* (Fig. 6). Group-I consists maximum of 16 isolates, Group-II consists of 10 isolates, and Group-III consisted of 5 isolates. Group-IV consists of only 01 isolate. The highest similarity coefficient ranged from 0.0 to 1.0 indicating that no two or more isolates were 100% similar.

The highest similarity coefficient (0.72) was between the isolates Rb/SJ-30 and Rb/UJ-32, the second-highest similar isolates were Rb/CD-07 and Rb/NR-16 at similarity coefficient (0.71). A large variation in *R. bataticola* population was found from all over Madhya Pradesh. The results of the present study also indicated that all the isolates were not necessarily showing the geographical linearity which suggests that no similar isolates of *R. bataticola* were found from the adjoining areas. Similar observations were noted by Aghakhani and Dubey (2009b) in genetic diversity studies conducted in 27 isolates (23 from chickpea and 4 from other host crops) of *R. bataticola* representing 11 different states of India by RAPD. The results of study conducted by Manjunatha and Saifulla (2021) were also in agreement with the present study representing high level of diversity among the isolates of *R. bataticola* from chickpea of different, as well as, same state. Successful survival and adaptation

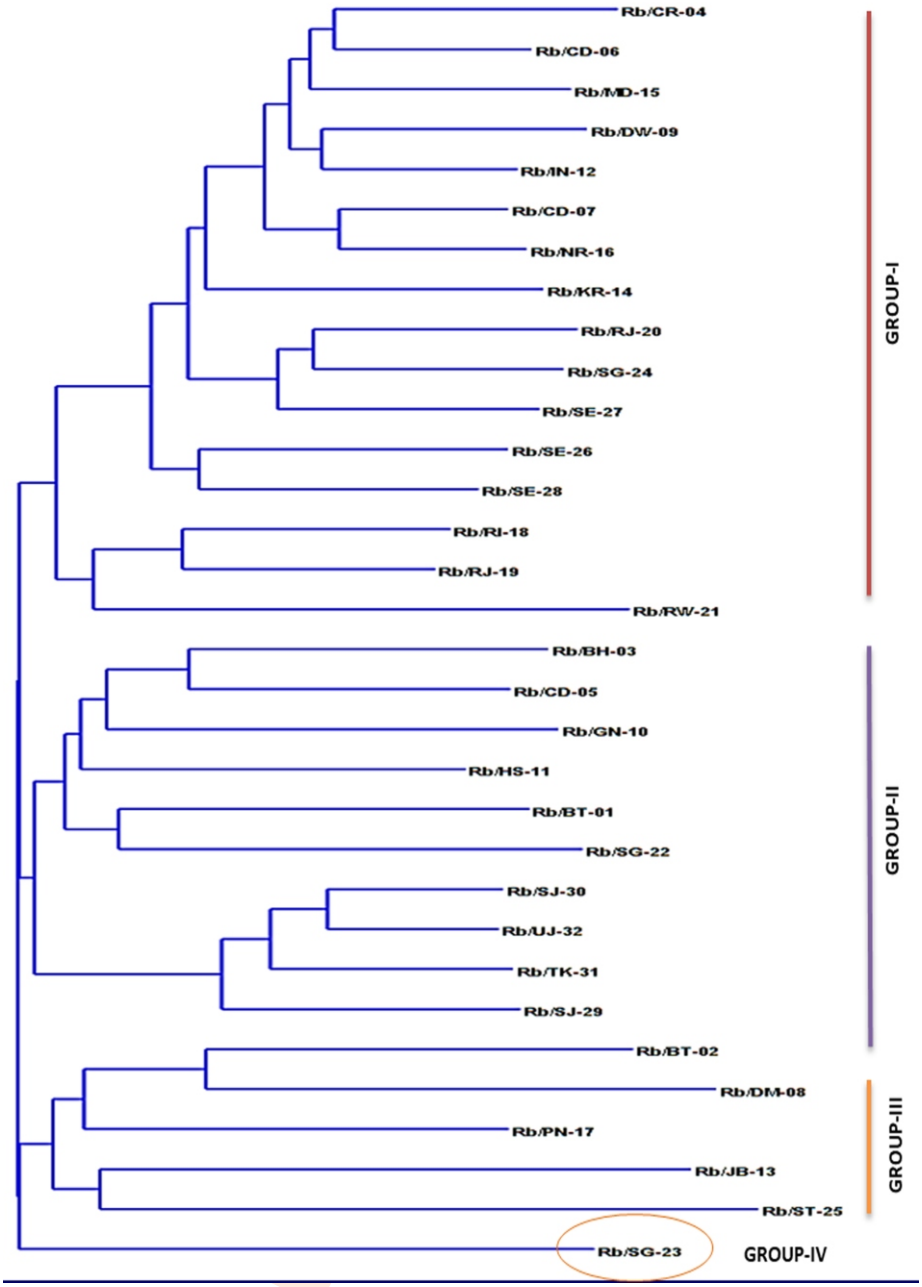

Fig. 6: Dendrogram generated from an unweighted pair group method analysis (UPGMA) cluster analysis based genotypic characters of *Rhizoctonia bataticola* isolates.

of *R. bataticola* to various geographic environments has been confirmed by several workers demonstrating morphological (Mayek-Perez *et al.,* 2001), pathogenic (Su *et al.,* 2001) and genetic diversity (Almeida *et al.,* 2003; Jana *et al.,* 2003; Aboshosha *et al.,* 2007) in various crops.

Therefore, as the disease has become an emerging threat to chickpea production and limited information is available on characterization of *R. bataticola* isolates causing dry root rot of chickpea in Madhya Pradesh. Due to short season and pre dominant rain-fed condition, the crop is vulnerable to dry root rot disease which causes major yield loss of chickpea in the state. The present study is an effort to identify the morphological and pathogenic diversity of dry root rot of chickpea collected from different parts of Madhya Pradesh. The study identified different pathotypes of *R. bataticola* in Madhya Pradesh and their

pathogenic behavior on different genotypes of chickpea. These findings may be used to design integrated management strategy for a specific area. If there is existence of pathogenic to highly pathogenic isolates of *R. bataticola*, in a particular area, then the chickpea genotypes showing tolerance/resistance to dry root rot can be used with other components of integrated management.

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Add-on Information

Authors' contribution: D.R. Saxena, M. Saxena, N. Tiwari, T. Kumar: Conceptualization, supervision, data analysis paper writing, editing and review; **R. Kumbhkar:** Conduction of experiment and data recording and **A. Chauhan:** Collection of isolates .

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