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Cultural and Genetic Diversity of *Rhizoctonia bataticola* Isolates Causing Dry Root Rot of Chickpea

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A B S T R A C T

Cultural and genetic diversity of *Rhizoctonia bataticola* causing dry root of chickpea (*Cicer arietinum* L.) crop were studied during 2018-19 at VNMKV, Parbhani. In present studies, isolate Rb-6 , exhibited maximum mycelial growth of (90.00mm), followed by Rb-9 (89.50mm) and Rb-2 (88.50mm) indicated a great cultural variability amongst *R. bataticola* isolates, whereas, in case of genetic variability when a dendrogram generated based on UPGMA analysis of RAPD data grouped all of the 9 test isolates (*R. bataticola*) into two major clusters. The cluster I included 6 isolates *viz.*, Rb-1, Rb-2, Rb-3, Rb-4, Rb-5 and Rb-9 collected from the three zone of Marathwada region which showed 64.5 per cent genetic similarity. Cluster II comprised of only two isolates Rb-6 and Rb-7 with similarity coefficient of 85 per cent.

Introduction

Chickpea is an important *Rabi* crop sown in September – November and harvested in February. At present, it is grown in over 50 countries of Asia, Africa, America and Oceania in rainfed environments (Sharma *et al.,* 2015). The production of chickpea is largely constrained by *Fusarium* wilt (*Fusarium oxysporum* f. sp.*ciceris*); however, recent reports indicated that dry root rot (DRR) is emerging as a potential threat to chickpea production (Ghosh *et al.,* 2013, Pande *et al.,* 2010 and Sharma *et al.,* 2010). The dry root rot is caused by *Rhizoctonia bataticola* (Taub.) Butler. (Synonym: *Macrophomina phaseolina* (Maubl.) Ashby.) and it is an important component of the disease complex that causes root rots and seedling blight in many grain legumes when they are weakened by other stress factors (Hwang *et. al*. 2003). *R. bataticola* is a soil-inhabiting organism capable of infecting chickpea at any crop stage, but most

commonly infects chickpea at post-reproductive stage in dry and warm regions (Sharma and Pande, 2013). It can affect chickpea production, causes considerable yield losses that vary from 5to 50 per cent and may cause 100 per cent losses in susceptible cultivars under favourable condition (Pande *et al.,* 2012) and considerable yield losses which may be as high as 50 to 71 per cent (Veena*et.al*. 2014a). Considering the economic importance of disease, present investigation was planned to study the cultural and genetic diversity amongst *Rhizoctonia bataticola* isolates collected from Marathwada region of Maharashtra State.

Materials and Methods

Isolation of *Rhizoctonia bataticola*

Naturally diseased chickpea plants showing typical symptoms of dry root rot in standing chickpea crop fields, collected during survey of Marathwada region was brought to the laboratory, washed thoroughly with sterile distilled water, blot dried, cut with sharp sterilized blade into small bits (5mm) and subjected to tissue isolation (Tuite, 1969) on PDA. These bits were sterilized with 0.1 per cent aqueous solution of Mercuric chloride ($HgCl_2$) for two minutes, washed by giving three successive changes with sterile distilled water in glass Petri plates to remove traces of mercuric chloride and blot dried. These were inoculated separately (location-wise) and ascetically on autoclaved and cooled PDA medium in sterilized glass Petri plates under aseptic conditions of Laminar-air-flow cabinet (make: ACS, Bangalore) and were inoculated in BOD incubator (make: MAC, Delhi) at 28 ± 2^0C temperature. Within 2-3 days of incubation, blackish mycelia mat was developed and within next 7-8 days, microsclerotia were initiated in the plates. Applying hyphal tip and/or single spore/sclerotial isolation technique, the test pathogen was isolated aseptically on PDA medium, sub-cultured and the pure cultures of the test isolates obtained were maintained separately on PDA slant test tubes in refrigerator for further studies. After a week of incubation, the pure culture developed was again transferred aseptically by hyphal tip technique on PDA slant test tubes and incubated at 28 ± 2° C.

Pathogenicity test

Pathogenicity of *R. bataticola* test isolates was attempted by employing sick soil method. For the purpose, autoclaved and cooled potting mixture of soil : sand : FYM (2:1:1) was filled into black coloured nursery polybags (20 x 30 cm), disinfected with 5 per cent copper sulphate solution. The test isolates multiplied on sand: maize medium was inoculated @ 50g/kg potting mixture separately in these bags, mixed thoroughly in top 5-6 cm layer, watered lightly and maintained in screen house for two weeks, so as to proliferate the test pathogen and make the potting mixture sick with *R. bataticola*.

Surface sterilized (0.1% $HgCl_2$) healthy seeds of susceptible chickpea Cv. JG-62 were sown (10 seeds / bag) in these bags, watered lightly and maintained in the screen house. Three bags per test isolate were sown and maintained. The observations on seedling mortality were recorded at two weeks after sowing and based on per cent seedling mortality, pathogenic / non-pathogenic potential of the test isolates was determined. The test pathogen isolates were re-isolated aseptically on PDA plates, from artificially dry root rot diseased chickpea seedlings (pathogenicity test), compared their cultural and morphological characteristics with the original culture of *R. bataticola* isolates isolated from naturally dry root rot diseased chickpea plants to fulfill Koch's postulates.

Identification of the pathogen

On the basis of symptoms expressed (both on naturally and artificially diseased) on chickpea plants, pathogenicity test, cultural and morphological characteristics and microscopic characteristics, the test pathogen was identified and further confirmed by comparing the description of *R. bataticola* given by Barnett and Hunter (1972).

Cultural variability

Nine test isolates of *R.bataticola* (Taub.) Butler, were aseptically inoculated separately on autoclaved and cooled PDA plates and incubated at 28 ± 2° C. For each test isolate, a triplicate set of PDA plates was maintained. Observations on cultural characteristics *viz*., colony diameter, colony colour, growth rate, colony texture, elevation and margin were recorded after seven days of incubation.

Molecular variability

Molecular variability among 9 isolates of *R. bataticola* was analyzed by RAPD molecular markers. Standard protocols were used for the isolation of DNA and RAPD analysis.

Extraction of genomic DNA

The extraction of fungal genomic DNA from 9 isolates of *Rhizoctonia bataticola* was carried out by following a protocol described by Lee *et al.,* (1988) and Wu *et al.,* (2001) with some modifications. The liquid culture of each isolate was raised in conical flask. PDB (Potato Dextrose Broth) 100ml was inoculated with 5mm bit of culture disc cut from edge of 5 days old culture of each isolate grown in petridish.

The inoculated broth was incubated at 28 ± 2^0C for 7 days. The mycelia mat was filtered through Whatman no. 1 filter paper and dried at room temperature. The dry mycelium was transferred to sterile mortar and pestle and ground with quartz sand powder and glass wool. Sufficient extraction buffer was added to the mortar so that quartz sand powder / mycelia mixture became saturated.

A mixture of buffer saturated phenol/ chloroform/ iso-amyl alcohol (25:24:1) was added per 0.5 gm of starting tissue and the solution was mixed thoroughly. The mixture was then transferred into several eppendorf tubes and centrifuged at 16000 rpm for 5 min at room temperature to pellet tissue debris and the glass wool pellet to the bottom of tube. The aqueous phase was transferred to a new tube. The DNA in each tube was precipitated with 0.6 volume of isopropanol by incubating the mixture at room temperature for 10 min. The DNA was recovered by centrifugation.

The pellet was rinsed with 75 % ethanol, air dried briefly and re-suspended in 200 µl TE buffer containing RNase A @ 20µg/ml. The extracted DNA was resolved on 0.8 % Agarose gel. The quantification was done by spectrophotometer and stored at -20^0C until further use.

Quantification of DNA

Spectrophotometer was used for quantitative and qualitative analysis of the DNA of the test isolates. Five µl of DNA sample was added in Cuvette carrying 0.995 µl of sterile H_2O and absorbance was measured at 280 nm wave length.

Similarly, the purity of DNA was checked by measuring the ratio of OD at A260/A280 nm. The quantification of DNA was calculated by using following formula.

$$\text{DNA } (\mu g/\mu l) = \frac{\text{OD at 260 nm} \times \text{dilution factor}}{1000} \times 50$$

Sr. No.	PCR Components	Required Concentration	Volume / reaction
1	PCR Buffer (10X)	1X	2.5 µl
2	$MgCl_2$ (25 mM)	1.5 mM	1.5 µl
3	dNTP mix. (10 mM)	200 µM	2.0 µl
4	Primer (10 µM)	0.4 µM	1.0 µl
5	*Taq* DNA Polymerase (3 U/µl)	1.25 U	0.5 µl
6	Template DNA	30 ng	1.0 µl
7	Nuclease free water		16.5 µl
	Total	-	**25.0 µl**

RAPD analysis of *R. bataticola* isolates

The PCR protocol for RAPD reaction was optimized with various PCR components and thermal cycler programme as detailed above.

Random primers *viz.*, OPA and OPB (Operon Technologies, USA) were used for RAPD analysis of nine fungal cultures of *Rhizoctonia bataticola* species. Master mixture (24.0 µl) containing all the above reactants, except template DNA were dispensed in autoclaved PCR tubes (0.2 ml). Genomic DNA of each pathogen of *Rhizoctonia bataticola* species was added to the individual tubes containing the master mixture. The contents of each tube were mixed by tapping with fingers followed by a brief spin to collect contents at the bottom of the tube. The tubes were placed in Thermocycler and subjected to PCR according to the protocol given below.

Standardized PCR protocols for amplification of DNA

A PCR master mix in sterile distilled water with all of the above mentioned compounds in required quantities were prepared and amplifications were done through Thermal Cycler, using following PCR conditions.

Sr. No.	Steps	Temperature		Time
1	Initial Denaturation	94°C		4 min
2	Denaturation	94°C	39 cycles	1 min
3	Annealing	35°C		1 min
4	Primer Extension	72°C		1.5 min
5	Final Extension	72°C		10 min
6	Final hold	4°C		Forever

The amplified RAPD PCR product was separated on 1.2 % agarose gel, stained with ethidium bromide and visualized under Gel Documentation System. The polymorphism was detected by comparing RAPD fingerprinting pattern of all *Rhizoctonia bataticola* strains.

Data scoring and analysis

The RAPD PCR amplicons were scored as present (1) or absent (0). Data matrices were generated and used to plot dendrogram by using Jaccard's similarity coefficient by using software NTSYS-pc.2

Results and Discussion

Isolation of the pathogen

Applying tissue isolation technique, the test fungus was isolated successfully from naturally dry root rot diseased roots and stems of chickpea plants, on autoclaved and cooled Potato Dextrose Agar plates. After 2-3 days of incubation, black mycelial mat on PDA plates was developed and after 7-8 days of incubation, microsclerotia were developed and identified as *R. bataticola* based on morphological and cultural characters using the descriptions given by C.M.I. (1970). The isolates were designated as: Rb-1, Rb-2, Rb-3, Rb-4, Rb-5, Rb-6, Rb-7, Rb-8 and Rb-9. The cultures of these isolates were purified by hyphal tip / single spore isolation technique and their pure cultures on PDA slant tubes were maintained separately and mass multiplication of the test isolates were done by using Sand: Maize and PDB medium for further *invitro* studies.

Overall, the test pathogen developed initially, white mycelium, which later turned dark brown to black. Production of aerial mycelium was also observed in some isolates. Branching occurred mostly at right angle to parent hyphae, but branching at acute angles was also observed. The microsclerotia were produced within 7-8 days of incubation, which were dark brown to black, round, ovoid to irregular shaped. Observations under Research microscope (Labomed Vision 2000, at 400X), revealed dark black coloured septate mycelium and numerous black coloured microsclerotia.

These observations on isolations and characterizations of *Rhizoctonia bataticola* on Potato Dextrose Agar medium are in consonance with the earlier reports of Sharma and Pande, (2013) isolated a pathogenic isolate of *R. bataticola*, from naturally infected chickpea plant at ICRISAT, Patancheru and purified using mono-sclerotia and maintained on PDA slants at 5^0C in refrigerator.

Veena *et al.,* (2014a) isolated a virulent isolate of *Rhizoctonia bataticola* from infected chickpea plants by using tissue segment method and identified the pathogen based on its mycelial and sclerotial characters and pathogenicity test proved by soil inoculation method and present studies also are in line with several workers (Aghakhani and Dubey 2009 a &b, Edraki and Banihashemi 2010, Sharma *et al.,* 2015, Shrinivas 2016, Sundravadana *et al.,* 2012).

Pathogenicity test

Pathogenicity of the *R. bataticola* test isolates was proved by applying sick soil method in polybag / pot culture, under screen house conditions, by sowing the seeds of susceptible chickpea cultivar JG-62. The test isolates induced the symptoms such as pre-emergence seed rot, post-emergence seedling mortality, yellowing and drooping of the leaves, microsclerotia production on stems and roots, rotting and shredding of roots and stems etc. Further, this test also revealed constant association of *R bataticola* with dry root rot disease of chickpea.

Re-isolation of artificially dry root rot diseased chick pea plants specimens also consistently yielded *R. bataticola* typical colony growth on PDA plates. Also, their cultural and morphological characteristics were exactly identical with *R. bataticola* pure cultures of the isolates obtained from

naturally dry root rot diseased chickpea plants specimens. Thus, by applying Koch's postulates, pathogenicity of *R. bataticola* test isolates was conclusively proved.

Similarly, pathogenicity of *R. bataticola/ M. phaseolina*, causing dry root rot / charcoal rot was attempted by sick soil method and proved successfully the pathogenic association of *R. bataticola/ M. phaseolina,* in various oilseed crops, pulses cereals, vegetables etc, earlier by several workers and the present study was in agreement with the reports of Katariya and Gaur(2007) isolated nine isolates of *R. bataticola* and tested pathogenicity of these isolates both in sterilized and unsterilized soil in Chickpea variety C-325.

Sharma *et al.,* (2012a) isolated the 50 isolates of *R. bataticola* collected during different years (2004 to 2010) from different chickpea growing regions of India. They reported that all the isolates proved to be pathogenic on chickpea cultivar BG 212.

There was significant variation in virulence of the pathogen, but there was no clear pattern of distribution linked to the virulence of the isolates. Veena *et al.,* (2014a) isolated a virulent isolate of *Rhizoctonia bataticola* from infected chickpea plants by using tissue segment method and identified the pathogen based on its mycelial and sclerotial characters and pathogenicity test proved by soil inoculation method.

Identification of the pathogen

Based on typical symptomatology of naturally / artificially dry root rot diseased chickpea plants, morpho-cultural characteristics, microscopic observations and pathogenicity test, the test pathogen was identified as *R. bataticola*, the cause of dry root rot of chickpea as described by Butler (1918) and Sneh *et al.,* (1991).

Variability among *R. bataticola* isolates

Cultural variability

Results (Table 1, PLATE I and Fig. 1) revealed that all of the 9 test isolates of *R. bataticola,* exhibited very meager variability in respect of their cultural characteristics, except colony growth. After seven days of incubation, the colony growth / diameter (mm) of the test isolates arranged from 79.00 to 90.00 mm and the growth rate was slow, medium and fast.

Based on colony growth / diameter and growth rate the test isolates were categorized as Fast, Medium and slow growing. The fast growing isolates with colony growth of 81.00-90.00 mm were Rb-1, Rb-2, Rb-3, Rb-5, Rb-6, Rb-7, Rb-8 and Rb-9; medium growing isolates with colony growth of 71.00-80.00 mm was Rb-4 and none of the isolate belonged to the category slow growing.

The colonies developed by the test isolates were circular shaped and the colony colour exhibited varied from blackish grey (Rb-1, Rb-4 and Rb-5), charcoal black (Rb-3 Rb-7), grayish white (Rb-2, RB-6 and Rb-8) and light black (Rb-9).

The colony textures of the test isolates were categorized into three groups such as appressed, velvety and fluffy. Among 9 isolates, isolates (Rb-1, Rb-7 and Rb-9) produced oppressed colony while isolates (Rb-2, Rb-4 and Rb-8) had fluffy texture (Rb-1, Rb-7 and Rb-9), whereas remaining isolates (Rb-3, Rb-5 and Rb-6) had produced velvety growth.

The colony elevation was mostly raised in majority of the isolates (Rb-1, Rb-2, Rb-4, Rb-6 and Rb-8); whereas, it was flat in the isolates *viz.,* Rb-3, Rb-5, Rb-7 and Rb-9. The colony margin in most of the isolates was wavy (Rb-2, Rb-4, Rb-5, Rb-7 and Rb-8); whereas, it was smooth in the isolates *viz.,*

Rb-1, Rb-3, Rb-6 and Rb-9.

Present results obtained on cultural variability among the isolates of *R. bataticola / M. phaseolina,* causing dry root rot of chickpea are in agreement with those findings of several earlier workers. Monga *et al.,* (2004, 2007) reported morpho-cultural and pathogenic variation among *Rhizoctonia* spp. (*R. bataticola* and *R. solani*), causing root rot of cotton. Aghakhani and Dubey, (2009a), reported all the twenty three isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea were highly variable in their cultural characters. After 48 hours of incubation, highest radial growth in Rb1 (Bangalore) followed Rb4 (Faridkot) was observed. Rb3 (New Delhi) and Rb5 (Faridkot) were next best with statistically similar colony diameters (83.5 mm). Least and similar colony diameter of 51.3 mm was recorded in Rb7 and Rb10 (Kanpur) isolates. Sharma *et al.,* (2012a, b) studied pathogenic and morpho-cultural variability among 94 isolates of *R. bataticola* causing dry root rot of chickpea and reported all test isolates as highly pathogenic to chickpea and also varied in morpho-cultural characteristics. Manjunatha and Naik, (2011) collected thirty isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea from three major chickpea growing regions were highly variable in their cultural characters. Among these, twelve isolates were fast in growth, another twelve were moderate and the remaining six isolates were slow in growth. Similar findings are also observed in line with the several other workers viz. Garg and Kumar (2012), Sharma *et al.,* (2012a) and Sundravadana *et al.,* (2012).

Molecular variability of *R. bataticola* isolates

Standardization of RAPD Protocol

The RAPD-PCR protocol described by Williams *et al.,* (1990) was used with some modifications, to produce RAPD fingerprinting profile of nine fungal pathogens of *Rhizoctonia bataticola* (Table 2, PLATE II and Fig. 2)

The PCR amplification reaction was optimized by varying concentration of PCR components. Amplification reaction was carried out in 25 µl reaction mixtures containing 30 ng of genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 mMdNTPs, 0.4 µM primers and 1.25 U of *Taq.*DNA polymerase. PCR amplification was performed in master cycler gradient Eppendorf PCR Machine. The program consisted of an initial denaturation at 94^oC for 4 min, followed by 39 cycles of 94^oC for 1 min. 35^oC for 1 min and 72^oC for 1.5 min, followed by final extension at 72^oC for 10 min. Finally a product was hold at 4^oC. PCR amplified product was separated by electrophoresis in 1.2 % agarose gel in 1X TAE buffer stained with Ethidium bromide and visualized under gel documentation system.

Diversity analysis using RAPD marker

The genomic DNA of all fungal pathogen was subjected for PCR amplification by using RAPD primers from Operon tech, USA. Initially 10 primers were screened with nine pathogen of *Rhizoctonia bataticola* among which five primers were produced larger no. and reproducible amplicons and further those were employed for molecular characterization of nine *Rhizoctonia bataticola* isolates.

The average sizes of amplicons generated by all RAPD primer were found in the range between 100 bp to 8.0 kb. The RAPD-PCR amplification result showed that five RAPD primers generated total 72 bands,out of these, 51 bands were found polymorphic and 21 bands were reported as monomorphic with an average of 4.20 bands per primers among nine fungal pathogens of *Rhizoctonia bataticola*.

The primer OPA-02, OPA-03, OPA-07, OPA-09 and OPA-13, were more found informative as they could generate total number of bands range 13 to 16. The primer OPA-07 has generated lowest number of bands i.e. 13 (Table 2 & Fig. 2). The primer OPA-03 has produced higher number of amplicons i.e. 96, followed by OPA-13 and OPA-02 which has produced 91 and 82 amplicons respectively, while the primer OPA-09 has produced minimum number of amplicons i.e. 62.

Overall all five primers were generated total 409 amplicons with an average of 81.80amplicons per primers (Table 2 and Fig. 2). Out of 409 amplicons, 220 amplicons were found polymorphic, they showed 53.78 % polymorphism. Similarly, 189 amplicons were found monomorphic. They showed, 46.22 % monomorphism and the average number of monomorphic bands per primer were 4.20.

Cluster analysis of RAPD DNA fingerprint

The data obtained by RAPD markers was analyzed by NTSYS pc2.02i and dendrogram was depicted by using Jaccard's similarity coefficient. The genetic similarity matrix obtained by Jaccard's similarity coefficient of the *Rhizoctonia bataticola* isolates (Table 3, Fig. 3& 4). Dendrogram generated based on UPGMA analysis of RAPD data grouped all these isolates in to two major clusters (Figure No.4). These clusters were formed on the basis of genus as well as species level. The group I, cluster I, comprised, maximum of six fungal pathogens together which showed 64.5 % genetic similarity viz., Rb-1, Rb-2, Rb-3 Rb-4, Rb-5 and Rb-9.

The cluster I comprised fungal isolates of Rb-2 and Rb-4 showed that higher similarity each other i.e. 85 %.Whereas the group II, Cluster II comprised three isolates together showing 72.2 %similarity with each other viz., Rb-6, Rb-7 and Rb-8. Theses cluster comprised fungal isolates of Rb-6 and Rb-7 showed that higher similarity each other i.e. 85 %. The cluster I and II comprised of nine isolates showed that 62 % similarity each other.

Thus, the present study on molecular variability exclusively indicated very negligible diversity among the population of *R. bataticola* prevailed in Marathwada region surveyed, of the Maharashtra state, which may be due to close similarity between the three agro-climatic zones surveyed, during present study.

Using various molecular technique, genetic variability among and between the isolates of *R. bataticola,* causing dry root rot in chickpea and other many crops have been reported earlier by many workers.

Aghakhani and Dubey (2009b) studied genetic diversity among 27 isolates (23 from chickpea and 4 from other host crops) of *R. bataticola* representing 11 different states of India, by random amplified polymorphic DNA (RAPD) internal transcribed spacer restriction fragment length polymorphism (ITS-RFLP) and ITS sequencing.

The isolates showed virulence and UPGMA cluster analysis, grouped the isolates into six categories at 40% genetic similarity, indicating high level of diversity. Manjunatha (2009) studied thirty isolates of *Rhizoctonia bataticola* from chickpea for molecular variability by using three primers OPO-10, OPO-12 and OPN-12. There were four isolates in Group-I, 16 isolates in Group-II and 10 isolates in Group-III.

Table.1 Cultural variability among the isolates of *R. bataticola*

Sr.No.	Isolates	Col. Dia.* (mm) /	Growth rate	Colour	Colony texture	Elevation	Margin
1	Rb_1	84.00	(++++)	Blackish Grey	Appressed	Raised	Smooth
2	Rb_2	88.50	(++++)	Grayish White	Fluffy	Raised	Wavy
3	Rb_3	81.00	(++++)	Charcoal black	Velvetty	Flat	Smooth
4	Rb_4	79.00	(+++)	Blackish Grey	Fluffy	Raised	Smooth
5	Rb_5	85.00	(++++)	Blackish Grey	Velvetty	Flat	Smooth
6	Rb_6	90.00	(++++)	Grayish White	Velvetty	Raised	Smooth
7	Rb_7	84.50	(++++)	Charcoal black	Appressed	Flat	Wavy
8	Rb_8	86.00	(++++)	Grayish White	Fluffy	Raised	Wavy
9	Rb_9	89.50	(++++)	Light black	Appressed	Flat	Wavy

*Average of a triplicate set of PDA plates per isolates, Growth Rate = Fast : ++++ (81.00-90.00 mm), Medium : +++ (71.00-80.00 mm), Slow : ++ (<70.00 mm)

Table.2 RAPD primers and the number of total polymorphic bands produced in isolates of *Rhizoctonia bataticola*

Primer	Sequence (5'-3')	Total No. of Bands	Total No. of Polymorphic Bands	Total No. of Monomorphic Bands	Total No. of fragment amplified	Polymorphic Percentage (%)
OPA-02	TGCCG AGCTG	15	10	5	82	**66.67**
OPA-03	AGTCA GCCAC	16	12	4	96	**75.00**
OPA-07	GAAAC GGGTG	13	10	3	78	**76.92**
OPA-09	GGGTA ACGCC	14	11	3	62	**78.57**
OPA-13	CAGCA CCCAC	14	08	6	91	**57.14**
Total		**72**	**51**	**21**	**409**	**354.30**
Mean		**14.40**	**10.20**	**4.20**	**81.80**	**70.86**

Table.3 The similarity matrix representing coefficient of similarity among 9 isolates of *R. bataticola* based on RAPD marker

	Rb-1	Rb-2	Rb-3	Rb-4	Rb-5	Rb-6	Rb-7	Rb-8	Rb-9
Rb-1	1.000								
Rb-2	0.583	1.000							
Rb-3	0.681	0.597	1.000						
Rb-4	0.625	0.847	0.639	1.000					
Rb-5	0.722	0.778	0.681	0.708	1.000				
Rb-6	0.611	0.611	0.569	0.597	0.667	1.000			
Rb-7	0.597	0.625	0.611	0.611	0.681	0.847	1.000		
Rb-8	0.514	0.653	0.583	0.556	0.569	0.708	0.722	1.000	
Rb-9	0.681	0.792	0.694	0.694	0.764	0.736	0.722	0.722	1.000
Avg. Similarity	**0.668**	**0.721**	**0.673**	**0.698**	**0.730**	**0.705**	**0.713**	**0.670**	**0.756**

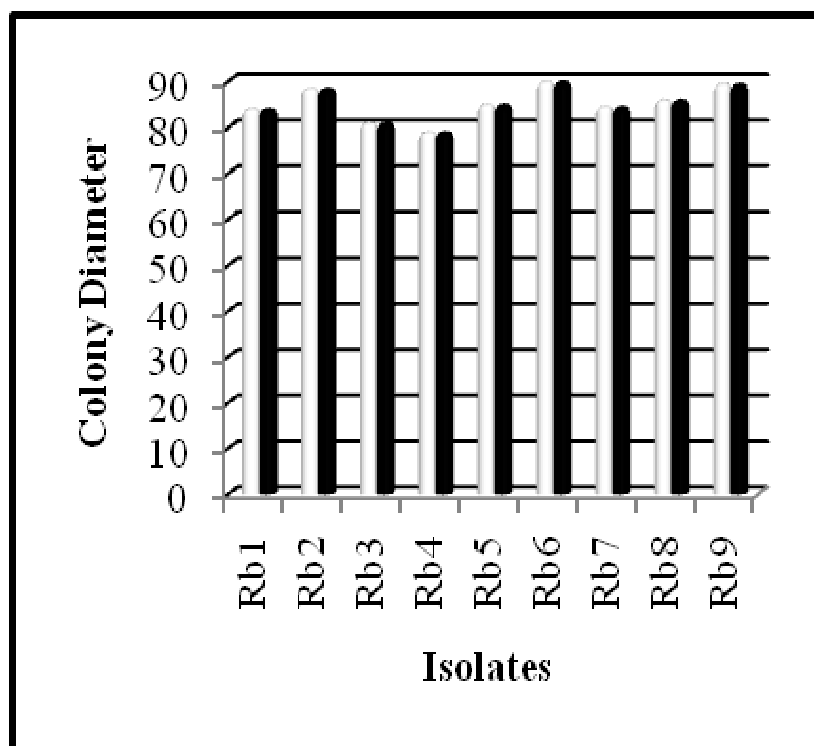


Figure.1 Cultural variability among the isolates of *R.bataticola*

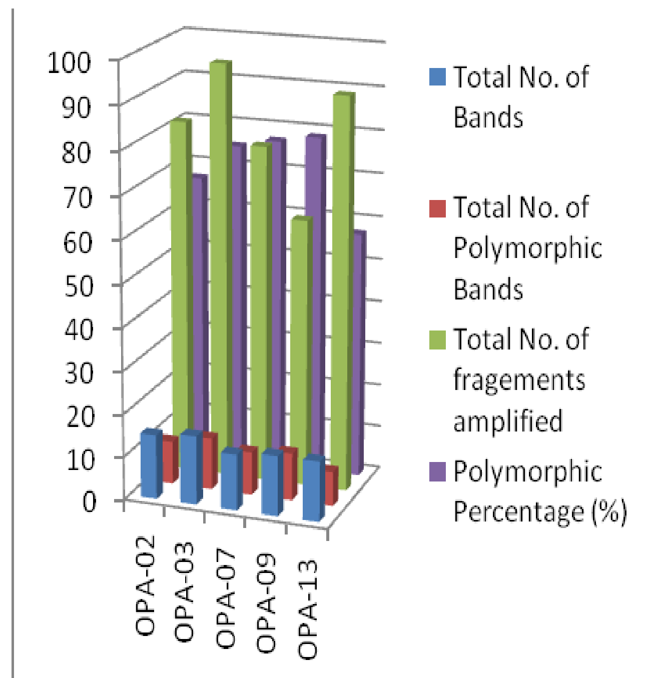


Figure.2 Graphical representation of comparative analysis of RAPD primer and polymorphic percentage

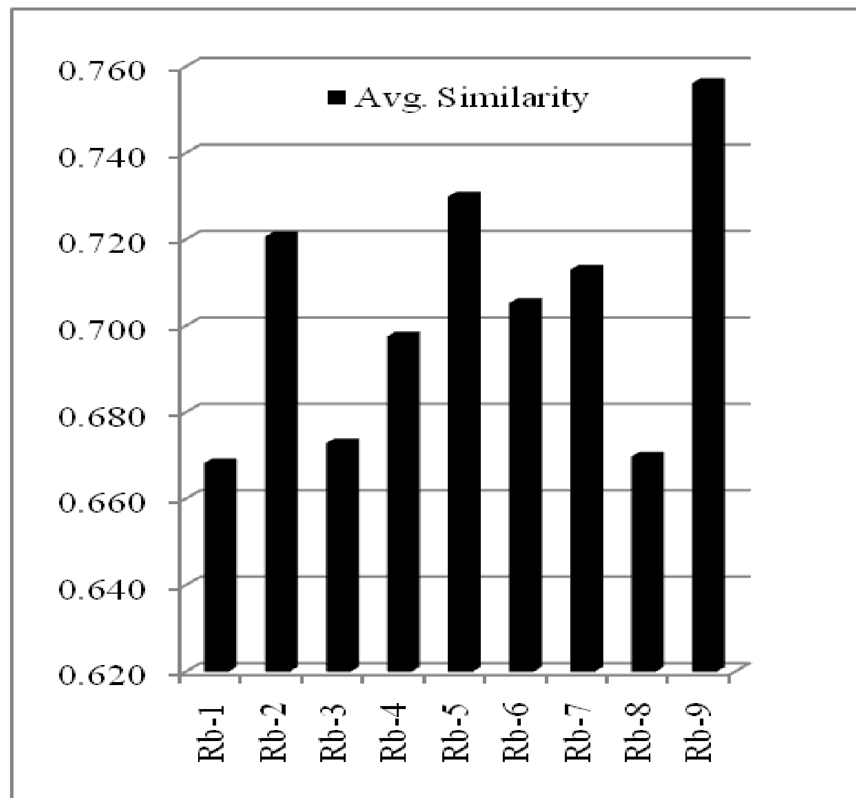


Figure.3 Average similarity of nine isolates of*R.bataticola* based on UPGMA analysis

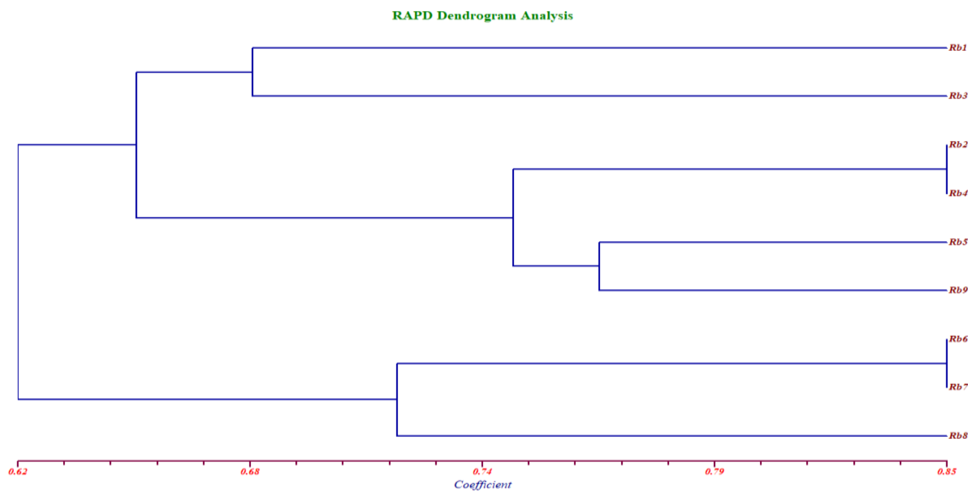


Figure.4 Dendrogram generated based on RAPD primer data through UPGMA analysis

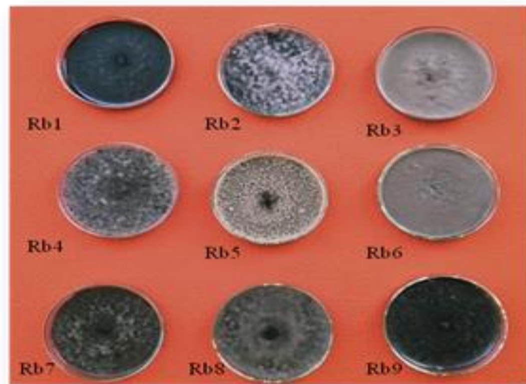
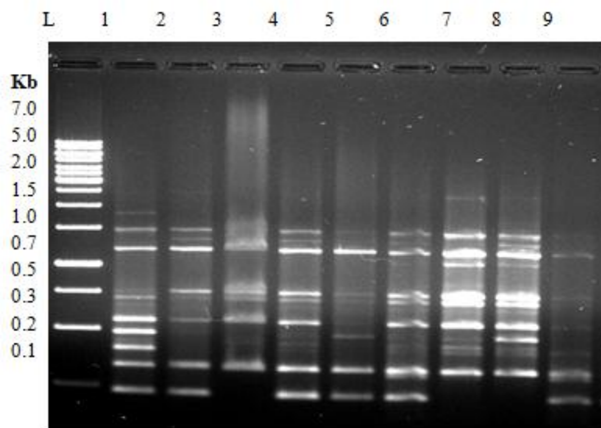
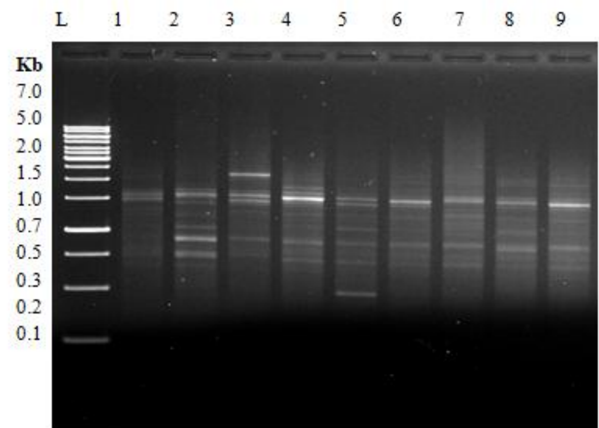


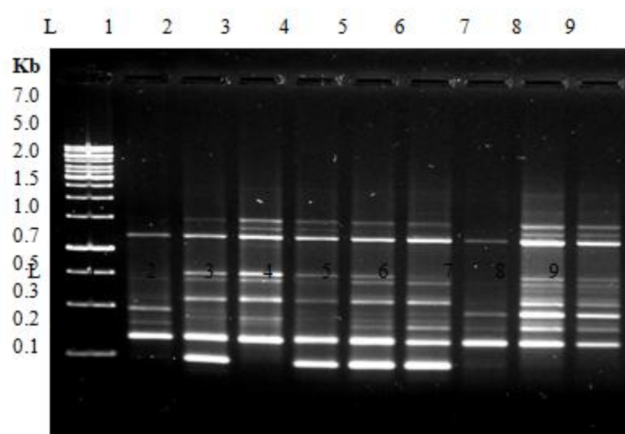
Plate.1 Cultural variability among the test isolates of *R.bataticola*



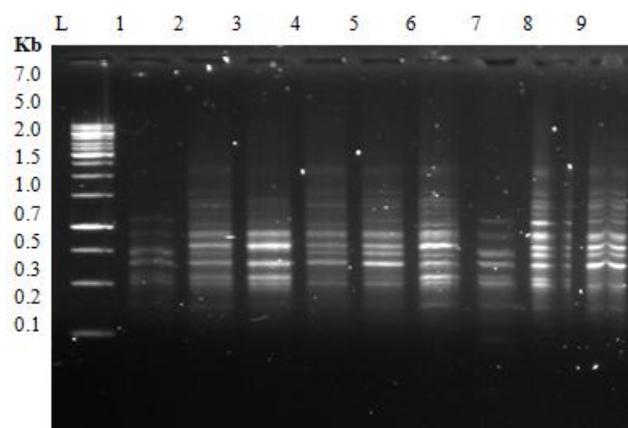
Primer.OPA-02 Lane L, Marker (1Kb DNA ladder)



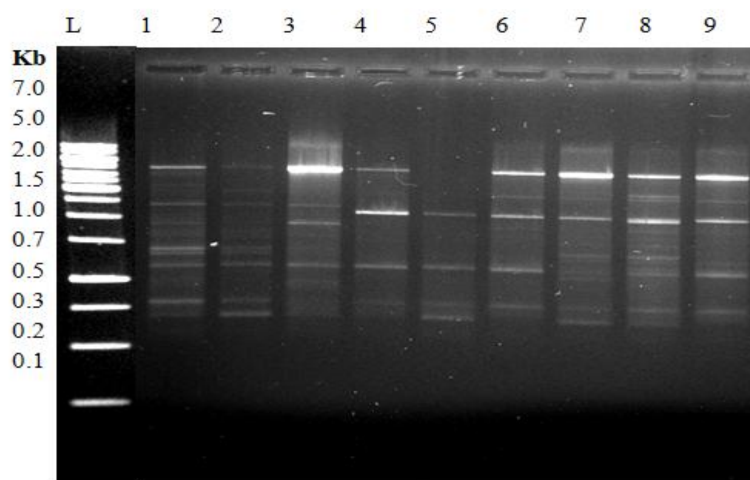
RAPD fingerprint profile of 9 pathogen isolates of *Rhizoctoniabataticola* by using primer OPA-07.



RAPD fingerprint profile of 9 pathogen isolates of *Rhizoctoniabataticola*by using primer OPA-02, OPA-03: Lane L, Marker (1Kb DNA ladder); Lanes 1 –9 isolates of *Rhizoctoniabataticola*



RAPD fingerprint profile of 9 pathogen isolates of*Rhizoctoniabataticola* by using primer OPA-13: Lane L, Marker (1Kb DNA ladder); Lanes 1 –9 isolates of *Rhizoctoniabataticola*



Primer.OPA-07 Lane L, Marker (1Kb DNA ladder)

However, in Group-II, three isolates (RG-18, RG-19 and RG-20) exhibited 100 per cent similarity. RB-29, RB-25 and RG-16 isolates from Bidar and Gulbarga respectively showed genetically diversity among thirty isolates. Similar results of molecular/genetic variability among isolates of *Rhizoctonia bataticola* were reported by several workers viz. Agle(2018), Belkar and Gadhe (2016), Monga *et al.,* (2004), Pancheshwar *et al.,* (2012), Shriniwas (2016) and Sundravandana *et al.,* (2011).

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