INVESTIGATION ON *Rhizoctonia bataticola* CAUSING ROOT ROT OF SOYBEAN

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation in the thesis entitled **"INVESTIGATION ON *Rhizoctonia bataticola* CAUSING ROOT ROT OF SOYBEAN"** or part thereof has neither been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

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This is to certify that thesis entitled **"INVESTIGATION ON *Rhizoctonia bataticola* CAUSING ROOT ROT OF SOYBEAN"** submitted in partial fulfilment of the requirement for the degree of **"Master of Science In Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by Pawan Dhondbaji Bansod under my guidance and supervision.

The subject of the thesis has been approved by the student's advisory committee.

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TABLE OF CONTENTS

Sr. no.	Particulars	Page
A	Declaration of student	i
B	Certificate	ii
C	Acknowledgement	iii-v
D	List of Tables	vii-viii
E	List of Figures	ix
F	List of Plates	x
G	List of Abbreviations	xi-xii
H	Thesis Abstract	xiii-xiv
I	INTRODUCTION	1-6
II	REVIEW OF LITRATURE	7-26
III	MATERIAL AND METHODS	27-48
IV	RESULTS AND DISCUSSION	49-81
V	SUMMARY AND CONCLUSIONS	82-85
VI	LITERATURE CITED	86-94
	Vita	95
	Appendix	96-101

A) LIST OF TABLES

Table	Title	Page
1.	*Rhizoctonia* root rot samples collected from major soybean growing area of Vidarbha, Maharashtra	28
2.	Details of *Rhizoctonia bataticola* isolates for molecular variability	29
3	Material used for detection of molecular variability of *Rhizoctonia bataticola*	30
4	List of RAPD primers used with their sequence	41
5	PCR reaction with for 2.5X of 25µlreaction for RAPD primer	42
6.	Constituent of PCR reaction for RAPD primer	42
7.	Steps used for PCR-RAPD reaction	43
8.	List of ISSR primers used with their sequence	44
9.	PCR reaction with for 2.5X of 25µlreaction for ISSR primer	44
10.	Constituent of PCR reaction for ISSR primer.	45
11.	Steps used for PCR-ISSR reaction	45
12.	List of ITS primers and Species specific marker used with their sequence	46
13.	Steps used for PCR - ITS primers and Species specific primer reaction	46
14.	Survey for incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha	50
15.	Isolation and designation of *Rhizoctonia bataticola* isolates collected from different locations	54
16.	Pathogenicity test of *Rhizoctonia bataticola*	56
17.	Grouping of *Rhizoctonia bataticola* isolates based on their pathogenic ability	57

18.	Morphological characters of *Rhizoctonia bataticola* isolates	59
19.	Growth of *Rhizoctonia bataticola*isolates on different media	63
20.	Per cent of polymorphism observed in RAPD primers	69
21.	Binary similarity coefficient of RAPD analysis against thirteen isolates of *Rhizoctonia bataticola*	72
22.	Per cent of polymorphism observed in ISSR primers	74
23.	Binary similarity coefficient of ISSR analysis against thirteen isolates of *Rhizoctonia bataticola*	77
24.	Evaluation of soybean genotypes against root rot under sick plot condition (*Kharif*,2018)	78
25.	Reaction of soybean genotypes against *Rhizoctonia* root rot	80

B) LIST OF FIGURES

Figures	Title	page
1.	A dendrogram of RAPD analysis against thirteen isolates of *Rhizoctonia bataticola*	71
2.	A dendrogram of ISSR analysis against thirteen isolates of *Rhizoctonia bataticola*	76

C) LIST OF PLATES

Plates	Title	page
1.	Geographical locations of *Rhizoctonia bataticola* isolates from major soybean growing area of Vidarbha	51
2	Symptoms of *Rhizoctonia* root rot	53
3.	Pathogenicity and symptoms caused by*Rhizoctonia bataticola*	55
4.	Growth of *Rhizoctonia bataticola*isolates on PDA	58
5.	Morphological character of *Rhizoctonia bataticola*: Acute angle and right angle branching pattern	60
6.	Sclerotia of *Rhizoctonia bataticola* isolates	61
7.	Growth of *Rhizoctonia bataticola*isolates on different cultural media	64
8.	ITS and Species specific marker banding pattern of primer	65
9.	RAPD banding pattern of primer OPA-1, OPA-7, OPA-9 and OPD-5	67
10.	RAPD banding pattern of primer OPB-2, OPB-4, OPC-4 and OPC-5	68
11.	ISSR banding pattern of primer UBC 808 and UBC 840	73
12.	Evaluation of diverse soybean entries against root rot under sick plot condition	79

D) Abbreviations

Abbreviations		Fullform
%	:	Per Cent
/	:	Per
@	:	At The Rate Of
ºC	:	Degree Celsius
µg	:	Micro Grams
µl	:	Micro Litre(S)
Cm	:	Centimetre(S)
CTAB	:	Cetyl Trimethyl Ammonium Bromide
dd H_2O	:	Double Distilled Water
Deptt.	:	Department
DNA	:	Deoxyribose Nucleic Acid
dNTPs	:	Deoxyribo Nucleoside Triphosphate
e.g.	:	Exampli gratia (for example)
EDTA	:	Ethylene Di- Amine Tetra Acetic Acid
et al.	*:*	Et Alia (And Associates)
etc.	:	Et Cetera
Fig.	:	Figure
G	:	Grams
ha	:	Hector
i.e.	:	That Is
ISSR	:	Inter Simple Sequence Repeat
Kbp	:	Kilo Base Pairs
M	:	Molar
Max.	:	Maximum
Mg	:	Milli Gram (S)
Min	:	Minutes
Ml	:	Milli Litre(S)
mM	:	Milli Molar
Mm	:	Milimeter
Ng	:	nano grams(s)
No.	:	Number

PCR	:	Polymerase Chain Reaction
PDA	:	Potato Dextrose Agar
PDB	:	Potato Dextrose Broth
ITS	:	Internal Transcribed Spacer
RAPD	:	Random Amplified Polymorphic DNA
Rpm	:	Revolution Per Minutes
Taq polymerase	:	Thermos Aquaticus DNA Polymerase Enzyme
TBE buffer	:	Tris Hydroxymethyl Aminomethane And Boric Acid Ethylene Diamine Tetra Acetic Acid Buffer
TE buffer	:	Tris Hydroxymethyl Aminomethane Ethylene Diamine Tetra Acetic Acid Buffer
Tris	:	Tris Hydroxymethyl Aminomethane
U	:	Unit
UPGMA	:	Unweighted Pair Group Method For Arithmetic Mean Average
UV	:	Ultra Violet
Viz.	:	Videlicet

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a)	**Title of the thesis**	:	**INVESTIGATION ON *Rhizoctonia bataticola* CAUSING ROOT ROT OF SOYBEAN**
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ABSTRACT

Rapid roving survey in the major soybean growing areas of Vidarbha revealed the prevalence of *Rhizoctonia* root rot at all the locations where disease incidence ranged from 2.33 to 39.33 per cent. Thirteen different isolates of *Rhizoctonia bataticola* were isolated from different regions of the Vidarbha. Pathogenic abilities of different isolates of *R.*

bataticola on soybean cultivar TAMS-38 a susceptible variety, confirmed the isolates of *Rhizoctonia bataticola* to be pathogenic. The isolates of *R. bataticola* were tentatively divided in five groups based on their pathogenic reaction as highly, strongly, moderately, weakly pathogenic and non pathogenic. The isolates differed in their morphological parameters like mean radial growth, colony colour, colony texture, and sclerotial formation. Potato Dextrose Agar media were the best media for the growth of *R. bataticola*.

Genetic variation was detected among 13 isolates of *R. bataticola* by using PCR-based RAPD and ISSR marker. Among 20 RAPD primers used for amplification, OPB-2 (88.88%) and 0PC-4 (88.88%) primers of RAPD marker and among 2 ISSR primers, UBC 808 (83.33%) primer showed highest polymorphism among isolates. The range of genetic similarity based on RAPD primers was 0.17-0.83 and based on ISSR primers was 0.12-0.83. The dendrogram by RAPD data revealed that, the thirteen isolates were differentiated into two major groups and the dendrogram by ISSR data showed that the isolates grouped into four major clusters.

Among fifteen soybean genotypes tested under sick plot condition six genotypes *viz*. AMS 38-24, AMS 358, AMS MB 5-18, AMS MB 5-19, AMS 77 and AMS 1001 were found highly resistance. Three genotypes AMS 475, AMS 353 and AMS 243 showed moderately resistance reaction. Only one genotype JS 93-05 has shown moderately susceptible reaction. Susceptible reaction showed by AMS 99-33, Bragg, JS 335 and Punjab 1. Only one genotype TAMS 38 was found highly susceptible reaction.

CHAPTER I

INTRODUCTION

1.1 Background Information

Rhizoctonia bataticola (Taub.) Butler (1925) = (*Sclerotium bataticola* Taub. (1913) (Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid. (1947) is a divers omnipresent ubiquitous soil-borne fungal pathogen, infecting more than 500 plant species. The pathogen causes different types of diseases *viz*. seed rot, seedling blight, root rot, charcoal rot, wilt, stalk rot, stem blight, fruit rot, seedling decay and leaf blight in crop plants (Dhingra and Sinclair, 1978). Fungus produces either microsclerotia (primary source of inoculums) or pycnidial and infects plants from seedling to maturity. The disease is difficult to manage as pycnideospore and sclerotia can survive in 2-15 years even in the absence of the host plant (Young *et al*., 1983 and Baird *et al*., 2003).

Soybean (*Glysine max* (L.) Merill) is the major oilseed crop in the world accounting for nearly 50% of total oilseed acreage as well production. It is unique crop of versatile nutritional attribute yielding both oil and protein. Soybean rank second in vegetable oil production of India after groundnut. Over the past decade, productivity trend of soybean indicate that yields achieve are not attained due to profound adverse effect of biotic and abiotic factors. Soybean suffers from many diseases while root rot caused by *Rhizoctonia bataticola* is the most widespread disease of soybean.

Soybean cultivation has taken a big stride in the country during the past few years. The area under soybean is merely spread in latitudinal belt of about 15 to 25 ˚N comprising the state of Madhya Pradesh, Maharashtra, Chhattisgarh, Andhra Pradesh and Karnataka (Agarwal *et al*., 2013). These states together contribute to about 98% of the total soybean production in the country. The area under soybean in India during *Kharif* (mansoon) 2018 was 108.39 lakh ha with production of 114.83 MT with average yield 1159 kg/ha. In Maharashtra, area sown under soybean is 36.39 lakh ha during *Kharif* (mansoon) 2018. The

estimated yield per ha and total production of soybean was 1054 kg/ha and 38.35 lakh MT, respectively (Anynomous, 2018; Soybean Proccess Association of India). The area under soybean in Vidarbha region of Maharashtra during *Kharif*, 2018 was 16.72 lakh ha.with production of 19.93 MT with productivity 11.91 q/ha. (Anynomous, 2018; Soybean Proccess Association of India).

Polygenic nature of root rot resistance, its interaction with moisture stress and temperature has compounded the breeding efforts for resistance root rot in soybean. Crop rotation, post harvest removal of infected plant debris, manipulation of planting dates, adequate and timely fertigation, planting density and crop protection are the measures to manage the disease. Bioagent and fungicide application to seed and soil has been practiced in some cases, it is neither economical nor environmentally safe (Abavi and Carroles, 1990). Host plant resistance is the most economical and practical alternative for efficient management of root rot.

Genetic variability and pathogenicity are the two key factors that influence the detection of resistance in the host plant and its management. Polykaryotic feature of *M. phaseolina* mycelium appears to make it highly variable in pathogenicity and cultural characters (Ali and Dennis, 1992; Dhingra and Sinclair, 1973 and Than *et al.,* 1991) interestingly, despite its wide host range, *Macrophomina* is monotypic genus. So far, microsclerotial size, cultural characteristic and pycnidial formation are most often considered character in variability studies of *M. phaseolina* (Mihail and Taylor, 1992; Cloud and Rupe, 1991 and Suriachandraselvan and Seetharam, 2000). Perhaps, evaluation of genetic diversity in pathogen isolates has been initial step towards understanding the population structure. Molecular techniques have reliable, highly suitable tool for identifying pathogen species and assessing genetic variation within collection and population. RAPD offers a promising, versatile and informative molecular tool to detect genetic variation within population plant pathogens (Chiocchetti *et al.,* 1999). Random Amplified Polymorphic DNA has been used to characterize genetic diversity of different isolates of *M.*

phaseolina (Almeida *et al.,* 2003). Variability in molecular characters is used for determining resistance cultivars (Thirumalaisamy *et al.,* 2006) and for the evaluation of the germplasm resistance lines (Shekhar *et al.*, 2006).

1.2 Importance of study

Among the various existing diverse climatic situation and environmental variations that the responsible for low yields, recurrence of diseases of crop plants are one of the most important factors. Due to cultivation of soybean in diversify climatic situation in various geographical areas it suffer from various diseases *viz*. Collar rot (*Sclerotium rolfsii*), Root rot and charcoal rot (*Rhizoctonia bataticola*), (*Macrophomina phaseolina*), Anthracnose (*Colletotrichum truncatum*), Yellow Mosaic (Mungbean Yellow Mosaic Virus), Purple seed stain (*Cercospora kukuchi*), Bactrial pustules (*Xanthomonas axonopodis pv. glycines*) etc. Sinclair and Shurlleff (1975) reported more than 100 pathogens known to affect soybean plant are prone to number of pathogen which reduce the quality and quantity of seed yield.

Due to infection of stem rot and root rot diseases a loss to the extent of 11 percent was reported by Mulroony (1988). Charcoal rot of soybean is an important disease under rainfed environment to cause yield loss about 6 to 33% (Mengistu *et al.,* 2011). Plant losses up to 77 per cent reported in soybean due to *Rhizoctonia bataticola* (Muthusamy and Mariappan, 1991). Yield losses 30 to 50 per cent due to *Macrophomina phaseolina* in soybean crop has been reported (Yang and Navi, 2005). *Rhizoctonia bataticola a*re reduces the yield of soybean by 2-21 % (Wrather and Koenning, 2006).

Simple technique that could be used to rapidly characterize *Rhizoctoinia bataticola* population in a particular area. However, evaluating genetic diversity information for *Rhizoctonia* management in the field requires powerful discriminating, selective and reliable criteria for genotypes the isolates (Sharma *et al*., 2004). In order to measure genetic variability more precisely, molecular markers provide an unbiased estimate of total genomic variation and have the potential to minimize error due to sampling variance (Spooner *et al.,* 1996). Furthermore, determination of

fungal genetic diversity based on molecular markers is reliable and independent technique to assess variability among pathogen.

Characterization of diverse population of *R. bataticola* could improve the knowledge of population biology and its interaction to contribute to the design of optimal breeding methods to produce soybean cultivars with stable and durable resistance to root rot. In addition to morphological physiological variation, genetic variability in plant pathogens may be great importance to minimize disease incidence. Breeding for disease resistance plant with durable resistance requires that the plant should be resistance to all pathotype of the all pathogen. Highly variable plant pathogens may prove difficult to combat the development of resistance plant genotypes. The DNA figure printing technique called Random Amplified Polymorphic DNA (Williams *et al.,* 1990) constitutes one of the methods for discrimination among strain of species. It compares favorably with strain of species and is often to deemed superior in resolution or efficacy to traditional traits. This will be valuable tools for epidemiology studies of these fungi and may be important for the developmentof selective pathogen management strategies.

1.3 Objectives of the study

1. To undertake survey to know the prevalence of *Rhizoctonia bataticola* in soybean growing region (area) of *Vidarbha*, Maharashtra.
2. To determine cultural, morphological and molecular variability in *Rhizoctonia bataticola.*
3. Quantification of resistance in soybean core genotypes in response to root rot under sick plot.

1.4 Scope and limitation

Looking the nature of pathogens involved, there is no alternative but to go for developing resistance varieties of soybean rather than excessive use of fungicide of management of this disease. As, field application of excessive fungicide, often in the case, pose increase in the risk of evaluation of resistance strain, ground water contamination, human health, in addition to affecting beneficial soil micro flora and long term soil

fertility, screening for breeding resistance cultivars should b strongly advocated.

An intensive rapid roving survey contributes to assess the distribution and incidence of plant pathogens prevalent in particular areas to check the variation occur within pathogens. Such survey collect data at a single point in time; it is difficult to measure changes in the plant population unless two or more surveys at done at different points in time. Such repetition is often expensive and time consuming, making frequent periodic surveys impractical.

In the recent year, it was observed that due to presence of pathogenic variability in the pathogen at one location results in the breakdown of resistance in several soybean genotypes. There are limited root rot resistant genotypes having good agronomical characters. In such solution, the resistance genotypes need to be used precisely in breeding programme. The past reports suggests that host plant resistance does not persist for long term as variety presumed resistance to root rot failed in near future either as a result of genetic breakdown or change in the virulence of pathogen (Saxena and Singh, 1987). Hence, there is great scope to established relation with pathogenic variability to find out the possible level of genetic diversity through molecular markers which could be applicable in the introduction of resistance breeding programme.

Before the RAPD data is employed for objective classification based on the fungal parameters (aggressiveness, pathogenicity, geographical and crop colonization) data from large number of primers is needed so that the most part of the genome is represented. Besides the used of more robust SSR and RFLP markers, RAPD markers could also be converted to the dependable SCARs.

1.5 Hypothesis

The importance of *R. bataticola* in soybean is likely to increase under climate change scenario with the increase in recurrence of droughts. Evaluation of genetic diversity in *R. bataticola* isolates from Vidarbha region has been initial step towards understanding the population pressure of the root rot pathogen. The result of present investigation demonstrate the applicability of RAPD and ISSR of genomic DNA in

analyzing genetic variation, which are particularly useful when combined with in vitro and in plants studies. Also, this study provide the details description of the diversity existing in the *R. bataticola* isolates in different area of Vidarbha region in Maharashtra that will be useful for breeders to identify the location specific *R. bataticola* resistance cultivars and facilitate the selection of germplasm to incorporate in advance breeding strategies for more or less tolerance to root rot in a given geographical region.

CHAPTER II

REVIEW OF LITERATURE

Literature pertaining to present investigation review critically and arrange under following major heads.

2.1 *Rhizoctonia bataticola* (*Macrophomina phaseolina*)

2.2 Occurrence and losses caused by *Rhizoctonia spp*.

2.3 Survey for incidence of *Rhizoctonia* root rot

2.4 Symptoms and pathogenicity

2.5 Variability among isolates of *Rhizoctonia* bataticola

2.5.1 Cultural and morphological variability

2.5.2 Genetic variability by Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR)

2.6 Screening for disease resistance against *Rhizoctonia* root rot

2.1 *Rhizoctonia bataticola* (*Macrophomina phaseolina*)

The genus *Rhizoctonia* was first established by De Candolle (1885). Members of the from genus of *Rhizoctonia* D.C. are considered as a complex mixture of filamentous fungi, having in common the possession of a non-spore imperfect state, usually referred to as the *Rhizoctonia* anamorph (Gonzalez *et al.,* 2006). Several species of *Rhizoctonia* genus have been recorded (Saksena and Vasteraja, 1961; Yamamato, 1962 and Hussain and Mckeen, 1963). Among them, *Rhizoctonia bataticola*, the important species caused seed root, root rot, charcoal rot and ashy blight in most of the cereal, pulses, oilseed, fiber crop, vegetables aromatic and medicinal plants, plantation and horticultural crop (Uppal, 1934; Likhite and Kulkarni, 1934 and Young 1949). The fungus *Rhizoctonia bataticola* is known to infect a wide range of host plants belonging to monocots and dicots with high aggressiveness and these attributes have made this fungus economically important pathogen.

Dhingra and Sinclair (1972) reported that *Macrophomina phaseolina (Rhizoctonia bataticol*a) produce and sclerotia on roots, stem,

petioles and pods of the soybean plant. Root isolates caused 80-100 per cent death of inoculated seedling within 10 days. Stem isolates produces fluffy growth while root isolates produce partially fluffy growth in vitro.

Meyer *et al.,* (1974) observed that *Macrophomina phaseolina* caused root rot in soybean seedling and stated that sclerotia and mycelia inoculum of *Macrophomina phaseolina* is effective in causing seedling disease under controlled and field conditions. The symptoms in field plots appeared first as reddish brown lesions at hypocotyls close to the cotyledons and then turned grey to black.

Vyas *et al.,* (1986) reported that root rot and collar rot diseases of soybean caused by *Rhizoctonia bataticola* and *Sclerotium rolfsii* is which reduces quality of soybean (*Glycine max* L.) in Central India.

2.2 Occurrence and losses caused by *Rhizoctonia* spp.

Tachibana *et al.*, (1971) stated that *Rhizoctonia solani* reduced soybean yield as much as 48% in small plots.

Kirkapatrick and Sinclair (1973) reported that *Macrophomina phaseolina* causes charcoal rot and root rot of soybean and reduced yield in USA and India.

Sinclair and Shurlleff (1975) observed that charcoal rot of soybean caused by *Macrophomina phaseolina* (Syn. *Rhizoctonia bataticola* (Tassi.) Butler and *Sclerotium bataticola* (Taub.) causing 77 per cent plant losses.

Agarwal and Sarbhoy (1976) reported that charcoal rot of soybean incited by *Macrophomina phaseolina* throughout various regions of country particularly in northern India and estimated loss 77 per cent.

Vishwadhar and Sarbhoy (1989) reported that *Rhizoctonia bataticola* (Taub.) Butler (Pycnidial stage -*Macrophomina phaseolina* (Tassi.) Goid causes seed / seedling rot, root rot, ashy stem blight and charcoal rot of soybean in India.

Muthusamy and Mariappan (1991) noted that charcoal rot of soybean is caused by *Macrophomina phaseolina* and its sclerotial stage *Rhizoctonia bataticola* causing the plant losses up to 77 per cent.

Eathington *et al.,* (1993) recorded the disease pressure in linois (US) and reported 0-63 incident of charcoal rot on soybean due to *Rhizoctonia bataticola*.

Doupnik (1993) estimated annual losses of 25.2 million bushels in soybean due to charcoal rot disease from 1989 to 1991 in the north central region of United State.

Wrather (1995) narrated that the importance of charcoal rot caused by *Macrophomina phaseolina* which rank first among the five top most diseases of soybean causing huge annual losses. In USA alone the yield loss of soybean was 0.28M metric tons ($ 60.25M) due to this disease.

Wrather *et al.,* (1997) estimated that *Rhizoctonia* and *Pythium* root rot combinely caused yield reduction of 108,000 MT for the top 10 soybean producing countries during 1994. They also stated that during 2001, *Sclerotium rolfsii* causing root of soybean reduced the yield by 29.74 per cent from India.

Maglekar and Raut (1997) reported 30 per cent yield loss in soybean due to *Rhizoctonia* root / stem rot in Vidarbha region of Maharashtra.

Yang and Navi (2005) reported that *Macrophomina phaseolina* as a causal agent of soybean charcoal rot crop widely present in soil and observed plants during late august patches. The diseased plants were wilted and die prematurely. Symptoms observed up to 4^{th} or 5^{th} node which caused yield loses of 30-50 percent.

Wrather and Koenning (2006) estimated the disease effects on soybean yields in the United States during 2003 to 2005. They reported that *Rhizoctonia bataticola* reduced the yield of soybean from 2-21 per cent.

Koenning (2006) recorded the soybean disease loss in Southern United States for 2006 and reported that loss in yield due to *Rhizoctonia bataticola and Sclerotium rolfsii* i.e. 17.34 and 0.89 per cent, respectively.

Mengistu *et al.,* (2011) stated that the charcoal rot of soybean is an important disease under irrigated environment to cause yield loss about 6 to 33 per cent.

2.3 Survey for incidence *Rhizoctonia* root rot

Rhizoctonia bataticola (Taub.) Butler is one of the most damaging pathogens in the arid region and has a wide host range (Waller, 1976). In India, particularly form Andhra Pradesh, Bihar, Gujrat, Karnataka, Maharashtra, Madhya Pradesh, Rajsthan, Tamilnadu, Uttar Pradesh and West Bengal (Uppal, 1931; Likhite and Kulkarni, 1934; Deshpande *et al.,* 1969 and Philip *et al.,* 1969).

Gupta *et al.,* (1983) reported the incident of dry root rot of chickpea ranging from 3.2 to 20.6 per cent in 30 villages of northern Madhya Pradesh.

Syed Haque and Ghaffar (1995) reported that root rot of soybean caused by *Rhizoctonia bataticola* (*Macrophomina phaseolina*) and *Fusarium spp*. in Pakistan.

Abbas *et al.,* (2003) conducted a survey of soybean flied to determine the incidence and economic damage of charcoal rot on soybean and they reported 4.8 to 8.0 per cent infection in soybean due to root rot caused *by Rhizoctonia bataticola* in Iraq.

Erper *et al.,* (2008) determined the incidence and severity of root rot disease of bean, soybean, pea and faba bean in fields in 11 district of Samsun provinceduring 2001- 2002. The disease incident and severity was highest at 93.8 and 54.5 per cent in the bean growing area and the lowest at 64.0 % and 24.3 % in the faba bean growing area, respectively.

Inam-Ul-Haq *et al.,* (2012) surveyed 8 villages in Multan district (Pakistan) to recorded incidence of disease and losses of soybean

caused by root rot fungi. The root rot disease incidence was in the range 10-17% and losses ranged between 6.75–15.5 %.

Raja Mohan and Balabaskar (2012) conducted the field survey to assess the prevalence and incidence of dry root rot of groundnut in Cuddalore district of Tamilnadu, India and revealed the endemic nature of root rot of disease incidence with the maximum incidence to the disease 31.68 per cent registered in Vengatakuppam (MP18) location.

Sangeetha and Jahagirdar (2013) made roving survey during *Kharif* 2010 in major soybean growing area of northern Karnataka to assess the distribution and incidence of root rot/wilt. Per cent diseases incidence was ranged from 3.36 – 36.30 in different locations. Among the different talukas surveyed maximum disease incidence observed in Athani (19.08%) followed by Bailhongal (17.77%), Dharwad (8.34%), Kalaghatagi (5.90%) and least was observed in Raibag (3.36%).

Saifulla *et al.,* (2013) carried survey to know the severity of various diseases on chickpea during rabi season of 2010-2011 and recorded maximum dry root incidence in Bangalore urban areas (26.40%) followed by Chikmaglur (6.56%), Hassan (5-48%), Chamrajnagar (5.41%) and Devanagere (4.58%). The least disease incidence was in Chitradurga (0.40%) whereas Tumkur district was free from disease.

Mclaren *et al.,* (2014) surveyed soybean crops in Manitoba province of Canada for root rot disease and observed root rot in all soybean crops where root rot rating ranged from 1.9 to 6.8 with a mean of 4.6. Also root rot sever in low-laying area of some fields indicating that seed yield and quality may have been affected.

Nyandoro *et al.,* (2014) conducted the survey in August 2014 in southern Alberta of Canada to determined the incidence and severity of root rot in soybean fields and observed that root rot occurred in all locations with incidence averaging 34% and ranging from 18% to 73% and severity averaging 0.9 and ranging from 0.3 to 0.12 on a scale of 0-4.

Baste *et al.,* (2015) conducted the survey for root rot disease incidence in major soybean growing area of Vidarbha and per cent disease was recorded in the range of 10.62-36.48%.

2.4 Symptoms and pathogenicity

Sinclair and Shurlleff (1975) described the morphological characters of *Macrophomina phaseolina* and symptoms on the soybean plant as infected seedlings show reddish brown discoloration at emerging portion and in mature plants leaves turns yellow, wilt and remain attached to the plant.

Abawi and Coralles (1990) reported the *Macrophomina phaseolina* caused dark lesions on the epicotyls and hypocotyls on the soybean seedling and death followed by obstruction of xylem vessel resulted in wilting. In adult plants, the pathogens caused red to brown lesions on roots and stems and produced dark mycelia and black microsclerotia. The stem shows longitudinal dark lesions and the plant becomes defoliated and wilted.

According to Smith and Carvil (1997) charcoal rot disease symptoms usually appearing during midsummer in soybean under high ambient temperature (28 to 35˚ C) and low moisture or when unfavorable conditions causes stress in plants. The most diagnostic symptoms for charcoal rot disease in prematurely death of plants is the sloughing of cortical tissues from the lower stem and taproots and the speckled grey appearance of these infected tissues due to abundant microsclerotia formation in vascular, cortical and pith tissues.

Su *et al*., (2001) analyzed isolates of *Macrophomina phaseolina* obtained from plots cultivated with soybean, cotton, sorghum and maize and they found clear pattern of pathogenicity on the basis of crop patterns and suggested the host specialization of the fungus.

Pande *et al.*, (2004) observed that dry root rot of chickpea caused by *Rhizoctonia bataticola* generally appearing during late flowering and podding stage and the infected plants appeared completely dry. The root system of diseased plant shows extensive rotting with most of the

lateral roots are brittle and minute sclerotial bodies appear in the pith cavity and on the outer surface of the tap root.

Yang and Navi (2005) reported that *Macrophomina phaseolina* the causal agent of soybean charcoal rot showing symptoms as a pith of diseased plants had a brown discoloration in tap roots and lower stems. In discolored plants, microsclerotia of *M. phaseolina* were commonly observed in the epidermis, just beneath of the epidermis and inside taproots and lower stems of the wilted plants.

Jana *et al*., (2005) reported that *Macrophomina phaseolina* is a soil borne pathogen, infecting the plants through roots and moving up to the collar regions of the stems causing discoloration or charcoal rot of collar region and foliar chlorosis in soybean.

Shekhar *et al.*, (2006) reported that charcoal rot of maize caused by *Macrophomina phaseolina*is favored by high soil temperature ranging from 32˚C to 42˚C and low soil moisture. The pathogens over winter as sclerotia in soil and upon germination might penetrate roots and lower stem during growing season. Also a characteristic is sign is the presence of numerous, minutes and black sclerotia, particularly on the vascular bundles and outside the rind of the stalk.

Mengistu *et al*., (2011) reported the symptoms of charcoal rot on soybean which appeared after flowering, particularly at growth stages R5, R6 and R7. Diseased plants may wilt and prematurely die, with dead leaves remaining attached to petioles and petioles remaining attached to stems.

Sharma *et al*., (2012) performed pathogenicity test for 94 isolates of *Rhizoctonia bataticola* in laboratory on a susceptible cultivar BG 212 of chickpea caused by paper towel technique. All 94 isolates were highly pathogenic with disease severity ranging from 7 to 9 rating (1 to 9 scale), except two isolates [Jodhpur (RB 14) and Delhi isolates (RB16)] with a disease severity of 4.

Khan *et al.,* (2012) observed the incidence of dry root rot of chickpea caused by *Rhizoctonia bataticola* in late October to mid

November with maximum incidence in the month of February and March *i.e.* during the late flowering and podding stage. The symptoms of the disease were yellowing of the leaves within a dry or such leaves drop and plant showed completely dried symptoms within a week after the appearance of the first symptoms. If the plant were pull out from the soil and examined the basal stem and main root system of the diseased plant showed extensive rooting with most of the lateral roots decayed. The tissues were weekend and break off easily. In advance cases the sclerotia bodies scattered in the pith cavity and on the outer surface of the tap root.

Baste *et al*., (2015) conducted pathogenicity test of 9 isolates of *Rhizoctonia bataticola* causing root rot of soybean collected from Vidarbha region. The per cent mortality was observed upto 83.33% for different isolates. Artificially inoculated plants expressed typical symptoms of root rot. The infected plants loss turgidity and showed chlorotic symptoms.

2.5 Variability among isolates of *Rhizoctonia* bataticola

2.5.1 Cultural and morphological variability

Uppal *et al.,* (1936) studied to temperature requirement for growth of *Rhizoctonia bataticola* isolates and reported did not growth at 9˚C and 11˚C, respectively after two days, but slight growth of both isolates was observed after 5 days. They also recorded the optimum temperature between 30˚C to 35˚C for the growth of isolates.

Pearson *et al*., (1986) classified the isolates of *Macrophomina phaseolina* from maize stalk as a chlorate-resistance (possessing dense chlorate phenotype) and soybean isolates as chlorate-sensitive (possessing either feathery or restricted chlorate phenotype). On the basis of colony morphology studied on the medium containing potassium chlorate (analogue of nitrate) exhibited the apparent relationship between chlorate phenotype. Such host could be employed as a useful marker for identifying hot specific isolates of *Macrophomina phaseolina*.

Prameela Devi and Singh (1998) obtained thirty six isolates of *Macrophomina phaseolina* to study cultural variation and categorized into

three morphologically different groups. One representative isolated from each group *viz*. MP-1, MP-2 and MP-3 was selected for details studies. MP-1 isolates had cottony mycelia growth with pale grey colonies. The colony colour of the isolate was brown in MP-2 and showed 5 µm suppressed mycelia growth. The isolates were bigger in size and irregular in MP-2 (400 x 280 µm), oblong in MP-1 and round in isolates in MP-3. Typical right angle branching of mycelium found in isolates of MP-2 and MP-3.

Shekhar *et al*., (2006) divided seven isolates on the basis of pathogenic behavior and cultural characters of *Macrophomina phaseolina* from different agro-ecological zone of India. Consider colony colour and sclerotial morphology four groups *viz*. grayish white, blackish grey, dark black in centre periphery creamish and cottony white. On the basis of disease expression the most virulent Hyderabad isolates gave maximum disease (8.8%) whereas Coimbetore isolateswas least virulent one while working with charcoal rot of maize.

Aghakhani and Dubey (2009) reported twenty-three isolates of *Rhizoctonia bataticola* were highly variable in their morphological as well as cultural characters. The majority of the isolates (19) produced suppressed mycelium whereas 4 isolates produced aerial mycelium. The pigmentation of various colours as white to dull white, creamy, grey and black was prominent among the isolates. The isolates found variable in respect of their colony growth. After 48 hours of incubation colony growth ranged from 51.3 to 89.00 mm the sclerotia formed in different isolates were dark brown to black and highly variable in size (40 to 600 µm).

Sundravadana *et al.*, (2012) tested the growth of eleven isolates of *Rhizoctonia bataticola* in different solid media. Red gram root rot isolates recorded the maximum growth of 89.85 mm in PDA followed by red gram shoot (71.29 mm) and black gram seed isolates (70.29 mm). The maximum mycelia growth recorded by red gram shoot isolates in Richard's medium (69.8 mm), by red gram isolates in C'zapek's Dox agar medium (84.46 mm) and peptone sucrose agar (71.58 mm) Among different liquid culture broth mycelium dry wet of red gram root isolates was maximum in

all the five media testedviz., PD broth (75.13 mg), Richard's broth (58.5mg), oats broth (63.8 mg) C'zapek's Dox broth (69.48 mg) and peptone sucrose broth (65.41 mm) as compared to others.

Sharma *et al.*, (2012) collected *Rhizoctonia bataticola* populations from diverse agro-ecological zones. Different cultural and morphological parameters like colony colour, growth pattern, growth rate, mycelia characters, sclerotial initiation time, sclerotial intensity and morphology of the sclerotia, were assayed. The colony colour of *Rhizoctonia bataticola* varied from light black to light grey to grey. The shape of colony varied from redial to irregular and production of aerial mycelium was too low in most of the isolates.

Raja Mohan and Balabaskar (2012) studied cultural characters of thirteen isolates of the soybean root rot pathogen *M. phaseolina* and reported that all the isolates produced cottony white or dull white mycelia growth on PDA medium. The isolates MP1, MP11, Mp13 and MP18 significantly recorded the maximum (90mm) mycelia growth, while it was minimum (75.00 mm) in MP5. The biggest (105.10 µm) and the smallest (85.70 µm) sclerotia were produced by MP11 and MP5 isolates, respectively.

Mishra *et al*., (2014) collected twenty isolates of *Rhizoctonia solani* from rice, maize and green gram for studying their variability with respect to cultural, morphological characters and pathogenicity and reported the colony appearance of the isolates were sparse, sparse fluffy, cottony and cottony fluffy. Most of the isolates were sparse and light brown in colour. Out of three patterns of the radial growth namely fast, medium and slow, twelve isolates were medium growing where growth complete within (72 hrs), eight isolates were fast growing (48 hrs) g and two isolates were slow growing (96 hrs or more). Sclerotia of the isolates were light brown, brown, dark brown and black in colour and form as central, sub-central ring, peripheral ring, scattered and irregular manner. Majority of the isolates produced high number of sclerotia ranging from 40 to 60 within 3 to 10 days.

Sayyad *et al.,* (2015) cultured isolates of *M. phaseolina* on different media *viz*. oat meal agar, Richard's agar media, Asthana and Hawaker's media, C'zapek's Dox agar media, Host extract agar media and PDA to study their effect on cultural characteristics *viz*. colony characters, morphology, mycelia growth and sclerotial production. They observed excellent sclerotial production in PDA and host leaf extract agar, fair sclerotial production in C'zapek's Dox agar and Richard's agar media, good sclerotial production in Asthana and Hawaker's media and among these medium poor sclerotial production oat meal agar medium. All the culture media tested exhibited wide range of variations in the colony characteristics. The test pathogens also produced poor to abundant aerial mycelium with flat, submerged and scattered topography.

Srivastava (2017) investigated sixteen isolates and noticed that variation in rate of growth, colony characters, number and size of sclerotia of *M. phaseolina*.

Gade *et al.,* (2018) collected 40 isolates of *Rhizoctonia bataticola* from different agro climatic regions of India were analyzed for morphological and pathogenic variability. Thirty two isolates were rated as fast growing and the rest of the isolates as medium growing. Sixteen isolates were classified as large sized, 8 as small sized, and the remaining 16 isolates as medium sized on the basis of their sclerotial size. On the basis of disease expression, isolate Rb-29 (Kurundwad, Sangli) showed broad virulence range as it infected all the genotypes followed by isolate Rb-38 (Udaipur, Rajasthan) which infected eleven genotypes.

2.5.2 Genetic variability by Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Internal Transcribe Spacer (ITS)

It involve the single short random oligonucleotide sequence (called random primers) define cyclic amplification of DNA, which expose the polymorphism, distributed throughout the genome. The amplified fragments are called random amplified polymorphic DNA (Williams *et al.,* 1990)

Vandemark *et al.,* (2000) used a simple protocol for detecting amplified restriction fragment length polymorphism (AFLPs) to evaluate genetic diversity among isolates of *Macrophomina phaseolina* collected from different host and locations. Fifteen different selective primer pairs were evaluated. No significant differences were observed among the primer pairs, group based on the selective nucleotides, for several parameters that define primer utility. Relationship matrices generated with each group of AFLP primers were highly correlated (r^2> 0.92). Isolates were very diverse and could not clearly be grouped based on the geographic locations from which they were obtained. Genetic relationship among isolated were very robust. Relationships were they determined using both genetic similarity method of Nei and Li and the 'genetic distance' method of Skroch. They concluded that both methods were equally effective for determining intraspecific genetic relationship when the majority of markers were polymorphic.

Su *et al*., (2001) investigated variability among isolates of *Rhizoctonia bataticola* isolated from soybean corn, sorghum and cotton root tissue through RFLP and RAPD analysis. No variations were observed among isolates in restriction pattern of DNA fragments amplified by PCR, covering internal transcribed spacer (lTS) region 5.8 rRNA and part of 25 sRNA but variation were observed when ten random (RAPD) primers were used to amplify the total DNA of isolates.

Tarakanta *et al.*, (2003) showed that single RAPD primer A13 could be used to identify and discriminate several isolates of *M. phaseolina* and *Fusarium sp.* obtained from 20 hosts including soybean, cotton, chickpea and safflower.

Rajkumar (2004) observed that ten isolates of *R. bataticola* representing Dharwad and Bijapur region proved pathogenic to sorghum; molecular profiling using RAPD markers indicate a that the genetic differences among isolates and were species specific finger print to *R. bataticola (M. phaseolina)* was identified isolates were grouped using molecular and virulence data.

Jana *et al*., (2005) used single primer of simple sequence repeats (SSR) or microsatellite markers for the first time for the characterization of genetic variability of different populations of *Macrophomina phaseolina* obtained from soybean and cotton growth in India and USA. They calculated genetic similarity between the isolates and used cluster analysis to generate a dendogram showing relationship between isolates collected from two hosts. Fourty isolates clustered into three major groups corresponding to their hosts and geographical region. The SSR fingerprints (0.25 – 3.5 kb) generated using DNA from different populations of *Macrophomina phaseolina* of two hosts indicate that these repeat were interspersed with the genome of the pathogen. The variability found within closely related isolates of *Macrophomina phaseolina* indicate that such microsatellites were useful in population studies and represented a step towards identification of potential isolate diagnostic marker specific to soybean and cotton.

Shekhar *et al*., (2006) analyzed seven isolates of *Macrophomina phaseolina* incitant of maize charcoal rot through RAPD marker for genetic diversity by using 100 primers (OPA-E) and reported that the UPGMA cluster analysis for 706 loci identify three main clusters. Similarity matrix and Jaccard's similarity coefficient between the isolates indicate the maximum genetic variation among isolates of Arabhavi and Coimbtore (70.8%) followed by Ludhiana and Coimbtore (69.5%). The most closely related isolates were Hyderabad and Delhi with affinity percentage of 62.9 % similarity.

Purkayastha *et al.,* (2006) demonstrated genetic variation among the 59 isolates of *Macrophomina phaseolina* from cluster bean as well as other hosts using RAPD assay and PCR- RFLP of the ITS region, 5.8 S gene and part of 25S rDNA and reported the RAPD assay clearly distinguished the isolates on the basis of chlorate phenotype and host origin. Isolates from single host were generally similar to each other but differed distinctly from those from other hosts. Also, for the first time high degree of polymorphism in restriction pattern of the ITS region, including part of 25S rDNA had been reported in the charcoal rot of fungus by them.

Rajkumar *et al*., (2007) studied genetic diversity among 10 isolates of *Macrophomina phaseolina*, a causal agent of charcoal rot disease in sorghum using PCR-RAPD markers. From a set of 40 random primers tested, amplicon profiles of 15 were reproducible. A total 149 amplicon levels, with an average of 9.9 bands per primer, were available for analysis of which 148 were polymorphic (99.3%). UPGMA clustering of data indicated the isolates shared varied levels of genetic similarity within a range of 0.14 to 0.72 similarity coefficient index and grouping of isolates was not related to sampling location.

Monga *et al*., (2007) analyzed twenty five isolates of *Rhizoctonia bataticola* causal organism of cotton root rot using RAPD markers to study the relationship between molecular variability and pathogenicity. All the fifteen primers used generated scorable polymorphic bands for the isolates and classified the isolates into two broad groups. The group-1st consisted of isolate number 16 having the genetic similarity 0.59 and found to be pathogenic (83.3%). Group-2nd was further clustered into five sub-groups. The largest sub-groups were II b andII c which consisted of 9 isolates each with 0.79-1.0 genetic similarity coefficient. The other sub-group II a, II d and IIe consisted of two isolates each having similarity coefficient ranged from 0.63-1.0. However, no strict correlation was observed between isolates group based on pathogenicity, morphological feature and RAPD finger printing.

Aghakhani and Dubey (2009) determined genetic diversity among 27 isolates (23 from chickpea and 4 from other host crop) of *Rhizoctonia bataticola* representing 11 different state of India by random polymorphic amplified DNA (RAPD), internal transcribed spacer restriction fragment length polymorphism (ITS-RFLP) and ITS sequencing. The isolates showed variability in virulent test. They used unweighted group pair method with arithmetic average cluster analysis to group the isolates into distinct clusters. The cluster generated by RAPD group all the isolates into six categories at 40% genetic similarity. High level of diversity was observed among the isolates of different as well as same state. Some of the RAPD (OPN 4, OPN 12 and OPN 20) markers clearly distinguished

majority of the isolates into the area specific groups. The ITS I, 5.8rDNA and ITS II regions of 11 isolates representing different RAPD group were amplified with primers ITS 1and ITS 4 and digest with seven restriction enzymes. The restriction enzymes Dra I, Mbo I, Rsa I and Alu I were found to be suitable for differentiating the isolates into five categories by showing isolates specific ITS-RFLP patterns.

Fiers *et al.*, (2011) characterized the genetic diversity of 73 French strain *Rhizoctonia solani* collected from the different potato growing area in France by pathogenic analyses of the internal transcribed spacer sequence of ribosomal RNA. The determination of AGs of *Rhizoctonia solani* based on the sequencing of this ITS region showed three different AGs among the collection (60 Ag PT, 8 AG 2-1 and 5 AG 5)

Mamta Sharma *et al.*, (2012) collected 94 isolates of *Rhizoctonia bataticola* infected chickpea plants from agro climatic region of India to find out the diversity in *Rhizoctonia bataticola* populations. They analyzed the samples with amplified fragment length polymorphism (AFLP). Five AFLP primer combination provided total of 121 fragments. All fragments were found polymorphic with an average polymorphic information content value of 0.213. The dendogram based on AFLP analysis showed maximum number of *Rhizoctonia bataticola* isolates were very diverse and did not depend on geographical origin.

Pancheshwar *et al*., (2012) isolated the genomic DNA from 21 isolates of *Rhizoctonia bataticola* and subjected to amplification with RAPD markers for diverse analysis. Randomly selected 8 decamer primer amplified 64 RAPD marker loci. Out of the 64 bands, 29 bands (45.3%) were polymorphic and remaining 35 bands (54.6%) were monomorphic. The range of genetic diversity among *Rhizoctonia bataticola* isolates was 0.69 to 0.98. The cluster analysis showed 2 distinct groups among 21 isolates. They also suggested that any *Rhizoctonia bataticola* accessions under study did not made any group in respect to their geographical region.

Prasad *et al*., (2013) collected eight isolates of *Rhizoctonia solani* Kuhn, the incitant of wet root rot of chickpea from Haryana, Rajasthan and Delhi and assessed their genetic variability using RAPD

markers. Isolates are different in their RAPD banding pattern. The similarity value of RAPD profiles ranged from 0.12 (Hanumangarh-Karnal) to 0.79 (Gurgaon-Delhi and Hissar- Karnal) with an average of 0.34 among the isolates. They used ten random primers to fingerprint the individual isolates and using unweighted pair group method with arithmetic average distinguished *R. solani* isolates into different fingerprint groups.

Mishra *et al*., (2015) did molecular characterization of *Rhizoctonia solani* (Kuhn) isolates of three hosts, *viz*. rice, maize and green gram using Random Amplified Polymorphic DNA (RAPD) and reported that these markerswere found suitable for resolving the genetic polymorphism hidden among 22 isolates of *Rhizoctonia solani*. The polygenic analysis based on RAPD revealed three clusters comprising two major clusters of maize and rice and one minor cluster with four outliers of the isolates. The two green gram isolates, PRS21 and PRS 22, were clustered with maize isolates.

Prameela Devi T. *et al.,* (2016) studied genetic diversity of *Rhizoctonia bataticola* from different 19 crops using RAPD and ISSR markers. ISSR markers were found more efficient than RAPD marker to the correlate the genetic diversity with the grouping of isolates according to geographical regions.

Belkar *et al.,* (2016) collected forty isolates of *Rhizoctonia bataticola* out of that twenty isolates from Madhya Pradesh, Rajasthan and Maharashtra and assessed their genetic variability using RAPD markers. They noticed that randomly selected 20 decamer primer amplified 149 RAPD marker loci. Out of these 149 bands, 111 bands (74.49%) were polymorphic. Similarity coefficient value for 20 accession of *R. bataticola* in RAPD marker were 0.36 – 0.99.

Mane and Walunj (2018) studied the genetic diversity among of the eight isolates of *Rhizoctonia bataticola* using 10 ISSR primers. Among them 9 primers produced 37 scorable bands. Average level of polymorphism was 81.08%. The similarity matrix indicated that eight isolates of *Rhizoctonia bataticola* exhibited 37.5 to 80.8 percent similarity coefficient.

2.6 Screening of genotypes for disease resistance against *Rhizoctonia* root rot

Dreksha *et al*., (1974) evaluated one hundred and sixty three genotypes of mungbean to charcoal rot by paper towel technique and reported that only one genotype (11160a) was moderately resistance.

Lewis and Papavizas (1977) found variation among soybean cultivars of susceptibility to *Rhizoctonia solani* with Delmar and Hood being the least susceptible among eight cultivars evaluated.

Cardoso *et al.,* (1978) evaluated 39 cultivars and plant introduction (pls) of soybean for resistance against *Rhizoctonia solani*, anastomosis group (AG 4) and found all entries as susceptible as Chippewa 64, the susceptible check.

Singh and Mehrotra (1982) tested chickpea varieties under field conditions in infected soil against *R. bataticola* in Haryana and reported that cultivar BG-203, G-543 and Hare chhole were resistance to *R. Bataticola.*

Pearson *et al.*, (1984) measure rate of *Macrophomina phaseolina* colonization in entire root system below the cotyledonary node in nine soybean cultivars. They observed significance difference among genotypes and noted that the method could be used to identified tolerance and resistance mechanism in soybean to *M. phaseolina.*

Muyolo *et al*., (1993) evaluated 15 soybean cultivars for resistance to two isolates of *Rhizoctonia solani* (AG 2-2 and AG-4) and reported a resistance root reaction and a partially resistance hypocotyls reaction for Asgrow 7986, Cenrtannial, Pella, RA 606 and Vickery.

Smith and Carvil (1997) screened 24 soybean cultivars in a field highly infested with *M. phaseolina* for resistance and susceptible to the pathogen based microsclerotia densities in the lower stem and taproot (LSTR) at growth stage R 7. Four cultivars namely Asgrow 4715, Delta Pineland 3478, Hamilton and Jackson II were found resistance to pathogen.

Bradly *et al.* (2001) assessed ninety ancestral soybean lines, maturity groups (MGs) 000 to X and 700 commercial cultivars, MGs II to IV for resistance to *Rhizoctonia solani* causing root and hypocotyls rot of soybean under green house condition. They reported that most of the ancestral lines and cultivars were susceptible; however, 21 of the ancestral lines and 20 of the commercial cultivars were partially resistance. Out of 21 ancestral lines, CNS, Mandarin and Jackson were in the pedigree of cultivars as a partial resistance to *Rhizoctonia solani*.

Iqbal *et al*., (2003) examined seventy-one germplasm accession of urdbean to identify source of genetic resistance against charcoal rot disease caused by *Macrophomina phaseolina* by paper towel technique. Six genotypes (45718, 45719, 45721, VH 9440034-1 and VH 9440034-7) were highly resistance, 7 were resistance and 10 were moderately resistance. Sixteen genotypes were tolerant whereas rests of the accessions were susceptible or highly susceptible and GL 91059 were moderately resistance.

Amer (2005) evaluated eight soybean cultivars (Giza 21, Giza 22, Giza 35, Giza 82, Giza 83, Crowford, Holladay and Toama) against *Rhizoctonia solani* using agar plates and potted plant technique. In agar plate assay all soybean cultivars were found moderately susceptible, where as in potted assay, the reactions were resistant or moderately resistance to root rots.

Jain *et al*., (2005) evaluated more than 50 soybean germplasm and breeding lines for resistance to *Rhizoctonia* root rot during 2000 and 2001 in Jabalpur, Madhya Pradesh, India. Only 10 accession exhibited high degree of resistance for *Rhizoctonia* root rot, while 15 were categorized as moderately resistance. Eight and fourteen line showed susceptible and highly susceptible to *Rhizoctonia* root rot, respectively.

Ansari (2007) ascertained to response of 126 elite genotypes to charcoal rot under field conditions in Madhya Pradesh, India, during *Kharif*, 2004 and 2005. No genotypes was found immune (0.0% mortality) to the disease. Ten genotypes (JS 335, G 213, Birsa Sova-1, GS 1, GC 175320, G-9, G-688, NRC-37, Dsb 6-1 and RSC 14) were highly resistance

(1.0% mortality), whereas 60 lines were moderately resistance (1.1-10.0% mortality). Thirty two genotypes were moderately susceptible (10.1- 25% mortality) while other genotypes were susceptible (25.1- 50.0% mortality) to the disease.

Khan and Shuib (2007) tested twenty-nine germplasm accession of mungbean to know the source of genetic resistance against charcoal rot disease caused by *Macrophomina phaseolina* by paper towel technique. Two genotypes (NCM 252-10, 40536) were highly resistance, 5 (40504, NCM 257-5, 40557, NCM 251-4, 6368-64-72) resistance and 6 moderately resistance. Three genotypes were tolerant whereas rests of the accession were susceptible or highly susceptible.

Shrirao *et al.,* (2009) evaluated soybean genotypes against *Rhizoctonia* root rot and reported that out of 16 entries four entries were highly resistance (JS-72-44, JS-71-05, Monetta and JS 335), three as moderately resistance (JS-75-46, Bragg and VLS-58), three were showed susceptible reaction (PK-472, MACS-58 and Panjab-1) and five highly susceptible (JS-71-280, PK- 0472, VLS-2, KHsb-2 and Shivalik) against *Rhizoctonia* root rot.

Patil *et al.*, (2011) screened eighteen soybean genotypes against *Rhizoctonia* root rot disease under natural and sick plot conditions. Moderately resistance reaction was observed in JS-335, JS-93-05, AMS-353 and MACS-450 whereas seven genotypes were moderately susceptible. Also five genotypes *viz*. TAMS-98-21, Pk-472, MAUS-47, AMS-99-24, MAUS-81 and TAMS-38 showed susceptible and highly susceptible reaction, respectively against *Rhizoctonia* root rot.

Mengistu *et al*., (2011) evaluated 27 maturity group (MG) III, 29 Early MG IV, 34 Late MG IV and 59 MG V genotypes of soybean against *M. phaseolina* between 2006 and 2008 in a non irrigated, no- till field that has been artificially infested for three years. Significant variation was in root colonization among genotypes and years, indicating the value of screening genotypes over multiple years. Based on the colony forming unit index (CFUI) there was no genotype that was consistently immune to charcoal rot each year, However, six genotypes (one genotype in MG III,

one in Late MG IV, and four in MG V) were identified as moderately resistant. Some of the commercial and public genotypes were resistance to *M. phaseolina* at level equal to or greater than the stand DT97-4290, a moderately resistance cultivar.

Baste *et al.,* (2015) evaluated 40 genotypes of soybean against *R. bataticola* in natural field and sick plot field condition and reported that ten entries *viz*. AMS-39-2-7, AMS-104, AMS-102, AMS-77-3-6, AMS-353, AAMS-1001, AMS-115, AMS-MB-5-19, AMS-38-24 and AMS-358 were found absolutely resistance.

Ingle *et al.,* (2016) screened 30 advance lines of soybean along with five check in protected and unprotected condition and reported that four entries *viz*. NRC-94, JS-20-69, JS-20-71 and KDS-699 were shown resistance high yield reaction to *Rhizoctonia* root rot while NRC-92, KDS-705, KDS-378 and JS-20-41 exhibit susceptible high yield tolerance reaction to disease consortia.

CHAPTER III

MATERIAL AND METHOD

The present investigation entitled “Investigation on *Rhizoctonia bataticola* causing root rot of soybean” was carried out during academic year, 2018-2019 and material and methods was adopted during the present investigation are given herein.

All in vitro studies on root rot pathogen of soybean were conduct in the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The molecular analysis of collected isolates of *R. bataticola* was conducted in Bhabha Atomic Research Centre, Mumbai. The field screening of different soybean germplasm against root rot pathogen was conducted at *Rhizoctonia* sick plot of Regional Research Centre, Amravati, during *Kharif,* 2018.

3.1 Material

The material used during the present investigation is as below.

3.1.1 Glassware

Several types of glassware*viz.,* Petri plates, reagent bottles, conical flasks (*viz.,* 100 ml, 250 ml, 500 ml, 1000 ml and 2000 ml), test tubes, micropipettes, microtips, beakers, slides, cover slips, glass rods, measuring cylinders (10 ml, 100 ml, 250 ml and 1000 ml) were used.

3.1.2 Equipment

During present investigations, following laboratory equipments *viz.,* Autoclave, Hot Air Oven, Laminar Air Flow, BOD incubator (Remi), Research microscope, stereoscopic microscope, digital weighing balance (Wensar HBT 516), Centrifuge machine (Eppendorf 5810R), PCR machine (Eppendorf GR), Nano-Drop machine, Gel electrophoresis (Genexy, Scie-Plas), Gel Documentation Unit, vortex (Spinix), Deep freezer -20^{0}C (Blue Star), freezer, digital camera.

3.1.3 Seeds

The soybean genotype used in the study was obtained from Regional Research Centre, Amravati.

3.1.4 Miscellaneous material

Inoculation needle, single and double distilled water, spirit lamp, spirit, scalpel, cotton, plastic pots, dissection needle, trey, rubber bands, mortar pestles, scissors etc. were used in present study.

3.1.5 Collection of disease samples

Rhizoctonia root rot infected plant samples of soybean were collected from major soybean growing area of Vidarbha region of Maharashtra state during *Kharif*, 2018. (Table 1)

Table 1. *Rhizoctonia* root rot samples collected from major soybean growing area of Vidarbha, Maharashtra

STATE	DISTRICT	TALUKA	VILLAGE
Maharashtra	Washim	Mangrulpir	Godhani
		Mangrulpir	Bhapur
	Nagpur	Hingna	Hingna
		Hingna	Waddhamna
	Buldhana	Chikhali	Girola
	Yavatmal	Mahagaon	Bori Ijara
	Wardha	Shelu	Hamdapur
		Shelu	Dahegaon
	Amaravati	Chandurbazar	Kurha
		Chandurbazar	Virul Purna
		Amaravati	Amaravati (RRC)
	Akola	Balapur	Vyala
		Akola	Washimba

3.1.6 Culture media

Following media was used during laboratory studies of fungi.

Name of medium	Composition	Quantities
Potato Dextrose Agar (PDA)	Potato (peeled and sliced) Dextrose Agar Distilled water	200g 20g 20g 1000ml

3.1.7 Molecular variability

Different *Rhizoctonia bataticola* isolates, material, instrument and chemicals used for molecular variability are given below (Table 2.)

Table 2. Details of *Rhizoctonia bataticola* isolates for molecular variability study

State	District	Village	Isolates No.
Maharashtra	Amaravati	Kurha	R-7
		Virul Purna	R-8
		Amaravati	R-9
	Akola	Vyala	R-10
		Washimba	R-11
	Buldana	Girola	R-3
	Nagpur	Waddhamna	R-12
		Hingna	R-2
	Wardha	Hamdapur	R-5
		Dahegaon	R-6
	Washim	Bhapur	R-13
		Godhani	R-1
	Yavatmal	Bori Ijara	R-4

Table 3. Material used for detection of molecular variability of *Rhizoctonia bataticola*

Sr. No.	Name of chemicals
1. DNA Extraction	
1	Agarose
2	B-mercaptoethanol
3	Boric acid
4	Cetyl Trimethyl Ammonium Bromide (CTAB)
5	Chloroform
6	Ethyline Diamine Tetra Acetic Acid Disodium Salt (EDTA Na_2)
7	Etidium bromide
8	Ethanol
9	Phenol
10	Isomyl alcohol
11	Isopropanol
12	Sodium acetate
13	Ribonuclease A
14	Tris-HCl
2. PCR Amplification	
1	dNTP mix (10mM)
2	Gel loading dye (6X)
3	$MgCl_2$ 25Mm
4	Oligonucleotide RAPD primers
5	Sterile double distilled water
6	Taq DNA polymerase (5U/µl)
7	10X Taq buffer supply with Taq
8	Template DNA
3. Agarose gel electrophoresis	
1	Agarose
2	Boric acid
3	EDTA Na_2
4	Tris base HCl
5	Methanol

6	Ethidium bromide
7	Tris buffer
8	DNA ladder 1 kb
4. Software's used	
1	MICaps
2	Nano Drop 2000
3	NTSYS 2.02

3.1.8 Different solutions, buffer and reagents used for study

Different solutions, buffers and reagents used to perform present study are listed below along with their composition.

3.1.8.1 Extraction of DNA

- Extraction buffer [2 % w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, 0.4 % β-mercaptoethanol (added at the time of use)]. (The extraction buffer was autoclaved before addition of β-mercaptoethanol)
- Phenol : Chloroform : Isoamyl alcohol (25:24:1)
- Chloroform : Isoamyl alcohol (24:1)
- 100 % chilled Isopropanol
- Wash buffer : 96 % and 70 % ethanol
- TE buffer (10mM Tris and 1mM EDTA, p^H 8.0) Autoclaved before use.
- 1 M NaCl (solution was sterilized by autoclaving).
- 1 M Tris HCl (p^H 8.0)
- 0.5 M EDTA Na_2 (p^H 8.0)

3.1.8.2. PCR Amplification

Various reagents used for the RAPD, ISSR and ITS amplification are listed below:

- 10 ng/ µl Oligonucleotide RAPD, ISSR and ITS primers
- 5U/µl *Taq* DNA polymerase
- 10 X *Taq* buffer

- 25 mM $MgCl_2$
- 10 mM dNTP

3.1.8.3 Agarose gel electrophoresis

- 0.5X TBE (0.9 M Tris base, 0.9 M Boric acid, 0.10 M EDTA)
- 6X Gel loading dye (10 mM Tris-Cl p^H 7.6, 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 60 % glycerol in water).
- Ethidium bromide (0.5 mg/ml)

3.2 Methods

3.2.1 Glassware cleaning

Borosil and corning glassware were used for the all experimental studies, wherever required and were kept in the cleaning solution containing 60g of potassium dichromate and 60 ml of concentrated sulphuric acid (H_2SO_4) in one liter of water for a day. Then they were cleaned by washing with detergent powder followed by rinsing several time in tap water.

3.2.2 Sterilization

All the glassware was sterilized in an autoclave at 1.04kg/cm3 pressure for 20 minutes. All the media were sterilized for 15 minutes at 1.04kg/cm3 pressure and soil used for experiment was sterilized by using formaldehyde chemical for seven days.

3.2.3 Surface sterilization of plant parts

Plant materials were surface sterilized using 0.1 per cent mercuric chloride solution for 30 second and then washing with sterile water thrice.

3.2.4 Precaution to eliminate contamination

All isolation and inoculation work of microbial culture was carried out in laminar flow. The laminar air flow was sterilized by glowing ultraviolet lamp half an hour before use.

3.2.5 Survey for incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha

An rapid roving field survey was conducted to know the incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha, Maharashtra state *viz*. Amravati, Akola, Buldana, Washim, Nagpur, Wardha, Yavatmal during *Kharif*, 2018. In Amravati, the survey was conducted at Daryapur, Chandur bazaar, Amravati taluka. In Akola, survey was conducted in Akola, Murtizapur and Balapur taluka. From Buldana district, place like Chikhali taluka were selected. From Washim, Risod and Washim talukas were selected for the survey. In Nagpur, survey was conducted in Hingna taluka whereas in Wardha district survey was conducted in Wardha and Seloo taluka and from Yavatmal district, root rot samples were collected from Mahagaon taluka.

Root rot infected samples were collected from the fields of the above different places. Minimum samples were taken from one location. Each sample was taken in paper bag and labeled. The collected samples were further analyzed and used for isolation and detection of root rot pathogen.

3.2.6 Determination of *Rhizoctonia* root rot disease incidence

The diseased plants associated with root rot infecting fungi causing root rot was noted for the determination of disease incidence. In a field, one meter area mark at place randomly, and then number of diseased and total number of plants counted. Two fields selected from each village and the per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

3.2.7 Isolation of plant pathogenic *Rhizoctonia bataticola* from diseased plant parts

For the purpose of isolation and obtaining pure culture of the pathogen, standard tissue isolation technique was followed. Disease root

and stem portions were cut into small bits and washed well in tap water. These bits surface were sterilized with1:1000 sodium hypochloride solution for 30 second. Bits were washed thoroughly in sterilized distilled water three times to remove traces of sodium hypochloride and then aseptically transferred to sterile Petri plates containing potato dextrose agar media. The plates were incubated at room temperature (27 ± 2^0C) and as soon the emergence of mycelia from the infected portion, the hyphal tips were transferred to other plates for obtaining pure culture and isolates obtained were designated as R-1 to R-13.

3.2.8 Purification of fungal pathogenic cultures

3.2.8.1 Hyphal tip isolation

This method was used for maintaining pure culture of *R. bataticola.* Hyphal tip isolation was done on water agar plates. Dilute mycelia suspension was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess was aseptically drained. Single mycelia bits were then marked under the microscopic field with ink on the glass surface of the plates and it was allowed to grow. Such plates were incubated at (27 ± 2^0C) and hyphae coming from each end cell of mycelia bit was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants under aseptic condition and incubated at temperature of (27 ± 2^0C) for 10 days.

3.2.9 Identification of pathogen

The identification of *Rhizoctonia bataticola* isolates was done on the basis of morphological characters described by Aghakhani and Dubey, 2009 as well as on molecular basis by using ITS-1 and ITS-4 molecular markers (Aghakhani and Dubey, 2009) and species specific marker (Prameela Devi *et al*., 2016)

3.2.10 Maintenance of fungal culture

The receptive fungal pathogen cultures were maintained on PDA at room temperature by adopting subsequent sub culturing at periodically, regular intervals. Seven day old culture used for further studies.

3.2.11 Mass multiplication

Thirteen isolates of *Rhizoctonia bataticola* purified by hyphal tip method were multiplied by separately on sorghum grain medium in laboratory. Sorghum grains 200g + water 50ml was filled in 500ml conical flask and were autoclaved at 1.05 kg/cm2 for 15 minutes. The flasks were allowed to cool and inoculated with pure culture. The inoculated flask were inoculated at room temperature (27 ± 2^0C) for two weeks and shaken at every alternate day during incubation period. Sufficient quantity of inoculum was prepared and mixed in pots containing sterilized soil.

3.2.12 Preparation of sick soil for pots

The field soil + FYM (1:1) were sterilized for seven days by using formaldehyde chemical. Incubated flasks with full growth of fungus were then added in sterilized soil in 1:9 proportions (inoculum +soil). The plastic pots 12 cm diameter were filled the above mixture. The pots were watered and inoculated for 7 days to multiply pathogen in soil.

3.2.13 Pathogenicity test

The seeds of susceptible soybean cultivar TAMS-38 were used for studying the pathogenicity and infective capacity of isolates. The seed were sterilized with 0.1 per cent mercuric chloride for 1 minutes followed by three subsequent washing with sterile water to remove the traces of mercuric chloride. In each pot, 10 seeds were sown. One set of pots of sterilized soil, without inoculum was kept as control. The pots were watered as and when required and observations on the occurrence of root rot were recorded. On the basis of occurrence and symptoms, the isolates were identified as pathogenic. After proving the pathogenicity, re-isolation was made and compared with original culture.

3.2.14 Grouping of isolates

Thirteen isolates of *Rhizoctonia bataticola* were tested by sick soil method for their virulence against susceptible variety TAMS- 38. The per cent root rot was recorded on the basis of healthy and root rot infected plants.

The isolates of *Rhizoctonia bataticola* were tentatively divided into five groups on the basis of virulence as follows.

Category	Per cent mortality due to root rot
1. Non pathogenic	0 %
2. Weakly pathogenic	1-20 %
3. Moderately pathogenic	21-50 %
4. Strongly pathogenic	51-70 %
5. Highly pathogenic	> 70

(Pawar, 2010)

3.2.15 Morphological study

Morphological variation studies among thirteen isolates of *Rhizoctonia bataticola* was carried out. Basic cultural characteristics were studied following the method of Aghakhani and Dubey (2009).

3.2.15.1 Radial growth and sclerotial development on PDA

Autoclaved media was poured in the plates and on solidified medium the isolates were inoculated separately. After seven days growth 5 mm fungal disc were cut with help of flamed sterilized cooled cork borer. Three discs of each isolates were transferred on solidified PDA plates, one disc per plates. Inoculated plates were incubated at room temperature (27±2˚C).radial mycelium growth measurements of each isolates were recorded on 3rd and 5th day of inoculation. The colonies were measured in two mark directions at right angle to each other, passing through the centre of the colony and average colony diameter was worked out. Sclerotial formation and colony characters were recorded on 4th day of inoculation.

3.2.15.2 Measurement of sclerotial size

Research microscope was used to measure the sclerotial diameter. Value of one part ocular micrometer was calibrated under low magnification (10x) of microscope and then diameter of twenty random sclerotia of each isolates was measured. Finally mean sclerotial diameter

of each isolates was worked out. Shape of sclerotia of each isolates was also recorded.

3.2.16 Cultural study

The growth characters of *R. bataticola* were study on four different solid media *viz*. Potato dextrose Agar (PDA), C'zapek's Dox agar, Sabouraud's agar and peptone agar. All media were sterilized at 1.04 kg/cm^2 pressure for 15 minutes. 20 ml of each of the medium was poured in 90mm Petri plates. This Petri plates were inoculated with 5mm disc cut from periphery of actively growing culture and incubated at (27±2˚C). Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petri plates in any one of the medium. The colony diameter was recorded accordingly. The data on the radial mycelia growth was analyzed statistically.

The preparation method of each medium used in furnished below

1) Potato dextrose agar (Tuite, 1969)

Potatoes were peeled and cut into small slices and gently washed. 200 gm of sliced potato added into 500 ml of water and allowed to boil till it gets soft. These boiled potatoes slices passed through muslin cloth, filtrate was collected into 2000 ml capacity beaker and final volume was made to 1000 ml by adding distilled water. The filtrate was allowed to boil, gently added 20 gram of dextrose followed by 20 gram of agar-agar with intermediate shaking for 3-5 minutes. The medium thus prepared was poured in 500 ml flask and plugged by non absorbent cotton and wrapped with paper over it. The PDA media was autoclaved at 1.04 kg/cm^2 for 15 minutes.

2) C'zapek's Dox agar (Tuite, 1969)

Agar agar was melted in 500 ml of distilled water. All the other ingredients were mixed in 400 ml of distilled water. The two solutions were mixed thoroughly. The volume was made up to 1000 ml by adding distilled water and 1.04 kg/cm^{2}for 15 minutes.

3) Sabouraud's agar

Agar- agar was melted in 400 ml distilled water. All other ingredients were dissolved in 400 ml distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water. This was sterilized at 1.04 kg/cm^2 pressure for 15 minutes.

4) Peptone agar

All the ingredients were dissolved in 1000 ml distilled water and mix thoroughly. The volume was made up to 1000 mi by adding distilled water and sterilized at 1.04 kg/cm^2 pressure for 15 minutes.

3.3 Molecular variability analysis

In present investigations, 13 isolates of *Rhizoctonia bataticola* were used to detect variations using Random Amplified Polymorphic DNA (RAPD), Interspecific Simple Sequence Repeat (ISSR) and Internal Transcript Spacer (ITS) markers.

3.4 Preparation of mycelium mat

The pure culture of thirteen *Rhizoctonia bataticola* isolates were mass multiply by transferring mycelium tissue into 100 ml of PDB and kept for incubation inside BOD incubator at 27 ± 2^{0}C for next seven days without disturbing the flasks. The mycelium mat from broth was transferred to blotting paper and allowed to air dry for 20 min at room temperature. This mycelial mat was used for isolation of genomic DNA.

3.5 Extraction of DNA

Genomic DNA was isolated from the eight selected isolates by the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications.

3.5.1 Reagents required

1.1M Tris HCl (p^H 8.0)

The 30.285 gram Tris HCl dissolved in 150 ml of distilled water and volume was made up to 250 ml with distilled water. The buffer was autoclaved and stored at room temperature.

2. 0.5M Na_2 EDTA

The 37.224 gm dissolved Na_2 EDTA in 150 ml of distilled water by adding NaOH pellets till the p^H of the buffer came to 8.0 and the total volume of buffer was made up to 200 ml with distilled water. The buffer was autoclaved and stored at room temperature.

3. Extraction buffer

Tris HCl 50 Mm (p^H 8.0), NaCl 500 mM, Na_2EDTA 50 mM, p^H 8.0 and 1 per cent (v/v) β-Mercaptoethanol (Added immediately before use).

4. Phenol : Chloroform : Isoamyl alcohol (25:24:1)

The 250 ml of phenol, 240 ml chloroform and 10 ml of isoamyl alcohol was mixed together.

5. Chloroform : Isoamyl alcohol (24:1)

The 480 ml chloroform and 20 ml of isoamyl alcohol was mixed together.

6. Isopropanol

7. Sodium acetate

8. Ethanol 96 % & 70 %

9. TE 10:1, p^H 8.0 (10 mM Tris; 1mM Na_2 EDTA p^H 8.0)

2 ml 1M Tris, p^H 8.0 and 0.4 ml of 0.5 M Na_2 EDTA, p^H 8.0 was added to 197.6 ml of sterile distilled water.

3.5.2 Procedure for Genomic DNA Extraction

The seven days old mycelium mat was transferred on sterilized blotter paper, air dried to remove moisture and media adhering to the mat. The dried mycelium mat was used for DNA isolation. Approximately, two gram of air dried fungal mat was quickly frozen in liquid nitrogen (-196^{0}C) and crushed into powder form with the help of sterilized mortar and pestle. The powder was immediately homogenized by adding pre-warmed (65^{0}C) extraction buffer [2 % w/v, CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-Cl, 0.4 % β – mercaptoethanol (added at the one of

use)], 30 µl Ribonuclease A (RNAse A) and transferred to 1.5 ml eppendorf tubes. The content of tube was shaken vigorously for one minute for uniform mixture of suspension. These tubes were incubated in a heating block at 65^0C for one hour with gentle shaking at every 10 minutes and kept in ice box for 5 minutes. Tubes containing homogenate were centrifuged at 12000 rpm for 10 min at 4˚C temperature. Then supernatant was transferred into new 1.5 ml eppendorf tubes without disturbing the pellet of cell debris. Then 700 µl of Phenol:chloroform:Isoamyl alcohol (25:24:1) was added and mixed gently. Centrifugation was carried out at 12000 rpm for 10 min at 4˚C temperature. Then supernatant was transferred into new 1.5 ml eppendorf tubes and added 700 µl Chloroform:Isoamyl alcohol (24:1). Centrifuged was carried out at 12000 rpm for 10 min at 4˚C temperature.

The upper aqueous phase was transferred into new 1.5 ml eppendorf tubes with a wide bore pipette. 0.6 volume of chilled isopropanol was added and mixed by inversions. CTAB-DNA complexes formed a fibrous network. After mixed with isopropanol centrifuged at 10000 rpm at 4˚C temperature for 10 minutes. After centrifugation a pellet was formed at the bottom of the eppendorf tubes. The supernatant was decanted and the pellet was dissolved 300 µl of sterile water. Then each tube 7 µl of 3M sodium acetate and 600 µl of chilled ethanol (96%) were added and kept at -20^0C for 15 minutes. Then again centrifuged at 10000 rpm for 10 minutes at 4^0C temperature and supernatant was discarded. Then pallet 300 µl of 70 % ethanol was added and the pallet was allowed to suspend for a while. Then samples were centrigufed at 10000 rpm for 5 minutes at 4^0C and supernatant was decanted and pallet was air dried for around 30 to 45 minutes. Then pallet was dissolved in 100 - 200 µl of sterile water. The pellets were allowed to dissolve completely overnight at 4^0C without agitation. Then store at -20^0C until needed.

3.5.3 Quantification of DNA

A 2 µl sample is pipetted onto the end of a fiber optic cable (the receiving fiber) and a second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the

gap between the fiber optic ends. The gap is controlled to both 1 mm and 0.2 mm paths. A pulsed xenon flash lamp provides the light source and a linear CCD array is used to analyze the light after passing through the sample. The instrument is controlled by computer based software, and the data is logged in an archive file on the PC. Dilute all the DNA samples to 10 ng/ µl concentration for further PCR amplification.

3.5.4 Genetic diversity analysis by Random Amplified Polymorphic DNA (RAPD)

The 20 RAPD primers were selected to study genetic diversity among *Rhizoctonia bataticola* isolates. The list of 20 RAPD primers used for amplification of genomic DNA with their sequence are given in Table 5. The RAPD primers used by various workers were reviewed from available literature.

Table 4. List of RAPD primers used with their sequences

Sr. No.	Primer Screened	Sequence	Annealing Temp. (^{0}C)
1	OPA-1	CAGGCCCTTC	36
2	OPA-2	TGCCGAGCTG	36
3	OPA-3	AGTCAGCCAC	36
4	OPA-5	AGGGGTCTTG	36
5	OPA-6	GGTCCCTGAG	36
6	OPA-7	GAAACGGGTG	36
7	OPA-8	GTGACGTAGG	36
8	OPA-9	GGGTAACGCC	36
9	OPA-10	GTGATCGCAG	36
10	OPB-1	GTTTCGCTCC	36
11	OPB-2	TGATCCCTGG	6
12	OPB-3	CATCCCCCTG	36
13	OPB-4	GGACTGGAGT	36
14	OPB-5	TGCGCCCTTC	36
15	OPC-1	TTCGAGCCAG	36
16	OPC-2	GTGAGGCGTC	36
17	OPC-3	GGGGGTCTTT	36
18	OPC-4	CCGCATCTAC	36
19	OPC-5	GATGACCGCC	36
20	OPD-5	TGACGCGACA	36

The PCR was carried out in small reaction tubes 200 µl containing a reaction volume typically of 25 µl (Table 5 and 6) that was inserted into a thermal cycler (Eppendorf) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. PCRs were run on the programmable thermal cycler given in Table 7.

Table 5. PCR reaction mix for 2.5x of 25 µl reaction

Sr. No.	Master Mix	2.5x
1	10x *Taq* buffer	2.5 µl
2	$MgCl_2$ (25mM)	1.5 µl
3	dNTPs (10mM)	2.0 µl
4	*Taq* polymerase (5U/µl)	0.2 µl
5	Sterile water	3.8 µl
	Total Volume	10 µl

Table 6. Constituents of PCR reaction

Sr. No.	PCR Reaction	Quantity
1	Master Mix vol.	10.00 µl
2	$MgCl_2$ (25mM)	0.5 µl
3	Primer (10 ng/ µl)	3.0 µl
4	Template DNA (10ng/ µl)	5.0 µl
5	Sterile water	6.5 µl
	Total Reaction Volume	25 µl

3.5.5 Procedure for PCR reaction

i. Sterile PCR tubes were numbered and placed on PCR stand.

ii. At first 5 µl of DNA was added to each PCR tube followed by master mix.

iii. Add 0.5 µl of $MgCl_2$.

iv. Then 3 µl primer for RAPD was added in each PCR tube.

v. Then 6.5 µl sterile added.

vi. The samples were mixed by brief centrifugation to bring down the content of tube.

vii. PCR's were run on the programmable thermal cycler with the following reactions. (Prameela Devi *et al.,* 2016)

Table 7. Steps used for PCR- RAPD reaction

Name of step	Temperature (^{0}C)	Time
Initial Denaturation	95^{0}C	5 min
44cycles -Denaturatio	95^{0}C	30sec
44cycles - Annealing	36^{0}C	30 sec
44cycles - Extension	72^{0}C	1 min
Final Extension	72^{0}C	10 min

After completion of the cycles the samples were kept at 4^{0}C till electrophoresis.

3.5.5 Separation of RAPD amplified products by agarose gel electrophoresis

Electrophoresis of RAPD-PCR amplified analysis on agarose gel was carried out in 0.5x TBE buffer in horizontal gel electrophoresis. Cleaned and dried electrophoresis assembly was used for gel electrophoresis. The gel tray was wiped and cleaned with methanol. It was set to prepare the gel 1.5% agarose gel solution was prepared in 0.5x TBE buffer. Ethidium bromide was added to the lukewarm liquefied gel solution as staining agent. PCR products were loaded in the well on the gel along with the 3.5 µl ladder as 100bp marker in the14th well. 3 µl 6x loading dye was loaded along with each PCR product in the wells. Then the gel was run for one and half hours at 100 volts. After completion of 5 cm run, the gel was observed and captured under UV light with the help of Bio-Rad Gel documentation system.

3.5.6 Genetic diversity analysis by Interspecific Simple Sequence Repeat (ISSR)

Two ISSR primers were selected to study genetic diversity among *Rhizoctonia bataticola* isolates. The detailed of ISSR primers given in Table 8.

Table 8. List of ISSR primers used with their sequences

Sr. No.	Primer Name	Primer sequence (5′-3′)	Annealing Temperature (^{0}C)
1	UBC 808	AGAGAGAGAGAGAGAGC	50.0
2	UBC 840	GAGAGAGAGAGAGAGAYT	50.0

The PCR was carried out in small reaction tubes, containing a reaction volume typically of 25 µl (Table 9 and 10) that was inserted into a thermal cycler (Eppendorf tube 200µl) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. PCRs were run on the programmable thermal cycler given in Table 11.

Table 9. PCR reaction mix for 2.5x of 25 µl reaction

Sr. No.	Master Mix	1x
1	10x *Taq* buffer	2.5 µl
2	$MgCl_2$ (25mM)	1.5 µl
3	dNTPs (10mM)	2.0 µl
4	*Taq* polymerase (5U/µl)	0.2 µl
5	Sterile water	3.8 µl
	Total Volume	10.00 µl

Table 10. Constituents of PCR reaction

Sr. No.	PCR Reaction	Quantity
1	Master Mix vol.	10.00 µl
2	$MgCl_2$ (25mM)	0.5 µl
3	Primer (10 ng/ µl)	1.0 µl
4	Template DNA (10ng/ µl)	5.0 µl
5	Sterile water	8.5 µl
	Total Volume	25.00 µl

Table 11. Steps used for PCR-ISSR

Name of step	Temperature (^{0}C)	Time (Min.)
Initial Denaturation	95	5
44cycles -Denaturatio	95	30 Sec.
44cycles - Annealing	55*	30 Sec.
44cycles - Extension	72	1 min.
Final Extension	72	10

* At first 5 cycle of annealing temperature of 55^0 C with decreasing -1^0 C remaining 50^0 C given in the Table 11.

After completion of the cycles keep the samples at 4^0C till electrophoresis.

3.5.7 Internal Transcribed Spacer (ITS) and Species specific marker amplification

Molecular identification of *Rhizoctonia bataticola* was evaluated by using ITS primers and species specific marker (Aghakhani and Dubey, 2009, Prameela devi et al., 2016) (Table 12).

Table 12. List of ITS primers& Species specific primer used with their sequences

Sr. No.	Oligo Name	Primer Sequence (5′-3′)
1	ITS 1	TCCGTAGGTGAACCTGCGG
	ITS 4	TCCTCCGCTTATTGATATGC
2	MpkFI	CCGCCAGAGGACTATCAAAC
	MpkRI	CGTCCGAAGCGAGGTGTATT

3.5.7.1 Procedure for PCR reaction

Sterile PCR tubes were numbered and placed on PCR tube stand. At first 5 µl of DNA was added to each PCR tube followed by master mix given in table 11 and 12. The samples were mixed by vertex of tube. PCRs were run on the programmable thermal cycler given in table 15. PCR products were separated by electrophoresis in 1.5 per cent agarose gel run in 0.5x TBE.

Table 13. Steps used for PCR-ITS& Species specific primer reaction

Name of step	Temperature (^{0}C)	Time
Initial Denaturation	95^{0}C	5 min.
35 cycles -Denaturatio	95^{0}C	30 sec.
35 cycles - Annealing	55^{0}C	30 sec.
35 cycles - Extension	72^{0}C	1 min.
Final Extension	72^{0}C	10 min.

3.5.8 Data analysis

The gel image of RAPD and ISSR analysis were captured and visualized under light in gel documentation system (Biorad).

i. Data was scored as the presence (1) or absence (0) of individual band for each isolate.

ii. The similarity index was calculated as

$$\text{Similarity Index} = \frac{2\,N_{AB}}{N_A + N_B}$$

Where,

N_{AB} : is the number of bands shared by individuals A and B and

N_A and N_B : Total number of bands in individual A and B, respectively.

i. The data was used to generate similarity coefficient using simple matching coefficient based on RAPD and ISSR bands scoring. The similarity coefficients betweeneach pair of accession were then used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (Prameela Devi *et al.,* 2016).

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3.6 Screening of soybean genotypes for resistance against root rot

In this study, 15 entries along with check were received from Regional Research Centre, Amaavati. Inoculum of *Rhizoctonia bataticola* isolated from soybean plants, were prepared separately by growing on sterilized sorghum sand medium for soil ingestatioin. Experiment was conducted on field at RRC, Amravati under sick plot condition. Different genotypes were tested by soil inoculation method for their reaction against *Rhizoctonia bataticola* isolate.

Mass multiplied culture of *Rhizoctonia bataticola* are mixed with the most soil and incorporated in the field. The genotypes were sown (3 rows of 3m length) on dated 04/07/2018. Second inocultation of culture was undertaken on dated 23/08/2018 and third inoculation was undertaken on dated 16/09/2018 near the periphery of the root zone and observation recorded monthly of root rot incidence.

3.6.1 Details of layout

Fifteen genotypes were sown in non-replicated design during *Kharif,* 2018. The experimentation was conducted as per following details.

Spacing : 45 x 5 cm

Plot size : 3 rows of 3 meter length

Genotypes : 15

Method of sowing : Dibbling

The observations regarding infection of plant were recorded by 0-9 scale and on the basis of per cent disease incidence genotypes classified as follows. (Technical Proceeding of AICRP on soybean, Annual report, 2005-06 pp 1-36).

Rating	Reaction	Infection presence
0	Absolutely Resistance	0 %
1	Highly Resistance	0.01-11.11 %
3	Moderately Resistance	12.22-33.33 %
5	Moderately Susceptible	34.44-55.55 %
7	Susceptible	56.66-77.77 %
9	Highly Susceptible	78.88-100.0 %

The disease incidence of root rot was recorded for each genotype.

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

CHAPETR IV

RESULTS AND DISCUSSION

Sclerotial fungus *Rhizoctonia bataticola* (Taub.) Butler, is an important root inhibiting pathogen with wide host range and is responsible for causing losses to more than 500 cultivated and wild plant species. Considering the economic importance of the pathogen present investigation carried out to study molecular and morphological variability of *Rhizoctonia bataticola* isolates collected from major soybean growing area of Vidarbha region of Maharashtra and screening of soybean genotypes against *Rhizoctonia* root rot disease to identify resistance source. The data generated during the period investigation is presented in this chapter.

4.1 Survey for incidence of *Rhizoctonia* root rot and collection of disease sample

An intensive rapid roving field survey was conducted to assess the distribution an incidence of *Rhizoctonia* root rot and collect the infected plant samples in major soybean growing areas of Vidarbha *viz*. Amaravati, Akola, Washim, Buldana, Yavtmal, Wardha and Nagpur during *Kharif*, 2018. The soybean growing areas of the above mentioned district were surveyed as explained in material and methods and the data generated are presented in Plate 1 and Table 14.

The survey indicated that the disease was presented throughout the soybean growing areas of Vidarbha. The disease incidence was noticed in all the location surveyed with the range from 2.33 to 39.33 per cent. Among the location surveyed, maximum root rot incidence observed in Vyala village (39.33 %) of Akola district and least in Hamdapur (2.33%) of Wardha district. Almost all surveyed area noticed that JS 335 cultivar sown on farmer’s field and found variations in disease incidence.

Table 14. Survey for incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha

District	Taluka	Village	Veriety	% incedence of rizoctonia root rot
Washim	Mangrulpir	Godhani	JS 335	7.33
	Mangrulpir	Bhapur	JS 335	10.40
Nagpur	Hingna	Hingna	JS 335	4.36
	Hingna	Waddhamana	JS 335	7.68
Buldhana	Chikhali	Girola	JS 335	8.07
Yavatmal	Mahagaon	Bori Izara	JS 335	14.34
Wardha	Seloo	Hamdapur	JS 335	2.33
	Seloo	Dahegaon	JS 335	18.26
Amaravti	Chandurbazar	Kurha	JS 335	13.67
	Chandurbazar	Virul Purna	JS 335	21.26
	Amaravati	Amaravati (RRC)	JS 335	9.31
Akola	Balapur	Vyalla	JS 335	39.33
	Akola	Washimba	JS 335	8.66

In Amaravati district, root rot incidence observed in the range of 9.31 to 21.26% whereas Akola district, root rot pressure recorded in the range of 8.66 to 39.33% which is higher in comparison to other district surveyed. In Nagpur distirct, maximum root rot incidence was recoreded at waddhamna (7.68%) and minimum in Hingana (4.36%). In washim district, the maxzimum root rot incidence observed in Bhapur (10.40%) and least in Godhani (7.33%). In Wardha district, servey was conducted in Seloo Taluka where maximum root rot incidence observed in Dahegaon(18.26%) and minimum incidence in Hamdapur (2.33%). In Buldhana district, root rot

Plate 1. Geographic location of *Rhizoctonia bataticola* isolates from Vidarbha region of Maharashtra

incidence observed (8.07%) in girola village and in bori Ijara village of Yavatmal district root rot incidence observed 14.34%.

The result of the present investigation are in accordance to Sangeetha and Jahagirdar (2013) who did roving survey during *Kharif* 2010 in major soybean growing areas of northern Karnataka to assess the distribution and incidence of root rot and reported that per cent disease incidence was range in 3.36 to 36.30 from different locations. The chance of maximum disease incidence depends upon favorable conditions, susceptible host and optimum inoculum in soil. Soybean root rot is major threat when weather conditions are conducive. The variation in the extent of the disease incidence could be due to difference in weather conditions, temperature (Gupta *et al.,* 1983) and prevalence of the pathogen differing in their virulence as observed in the present study.

4.2 Symptomatology

4.2.1 Symptoms of *Rhizoctonia* root rot

The infection started at the collar region of plants as water soaked areas and decaying of the root system takes place. The infection was found to spread to the roots of the plant and caused decay, which ultimately toppled and collapsed. These infected plants could be easily pulled out from the soil and exhibited brown discoloration of roots followed by rotting of roots. In addition, the extensive sloughing off of affected bark and shredding of roots (Plate 2) was also observed. In advanced stage, the aerial portion of the plants decayed completely. Similar symptoms also reported by erlier workers (Mengistu *et al.,* 2011 and Khan *et al.,* 2012).

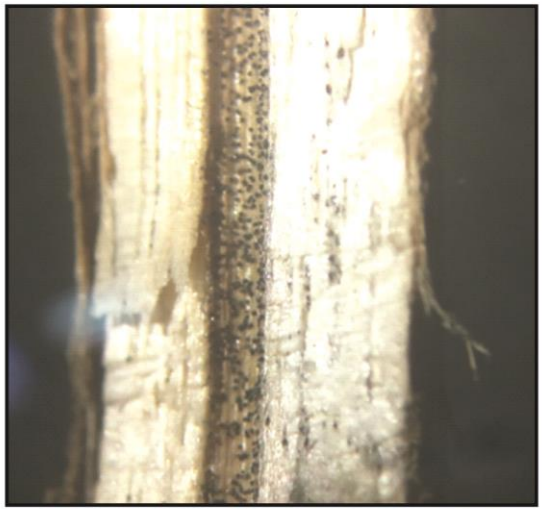
4.3 Isolation, Purification and Identification

4.3.1 Isolation

The standard tissue isolation technique was followed to obtain *Rhizoctonia* culture from infected plant parts showing root rot symptoms. Potato dextrose agar was used as basal medium for isolation of the fungus.Thus thirteen different isolates of the fungus were isolated from soybean

Root rot infected soybean Plants in field

Typical symptoms of soybean root rot under field conditions



Sclerotia on infected stem

Plate 2. Symptoms of *Rhizoctonia* root rot

roots. Isolation and designation details of *Rhizoctonia bataticola* isolates collected from different locations are furnished in Table 15.

Table 15. Isolation and designation of *Rhizoctonia bataticola* isolates collected from different locations.

STATE	DISTRICT	VILLAGE	Isolated designation
Maharashtra	Washim	Godhani	R-1
		Bhapur	R-13
	Nagpur	Hingna	R-2
		Waddhamana	R-12
	Buldhana	Girola	R-3
	Yavatmal	Bori Izara	R-4
	Wardha	Hamdapur	R-5
		Dahegaon	R-6
	Amaravati	Kurha	R-7
		Virul Purna	R-8
		Amaravati (RRC)	R-9
	Akola	Vyalla	R-10
		Washimba	R-11

4.3.2 Purification and identification.

The pure culture was obtained using single hyphal tip technique and was maintained on PDA slants for further studies. The pure culture thus obtained was identified as *Rhizoctonia bataticola* on the basis of morphological characters described by Aghakhani and Dubey, 2009 as well as on molecular basis by using ITS-1 and ITS-4 molecular markers (Aghakhani and Dubey, 2009) and species specific marker, Plate 8. (Prameela Devi *et al.,* 2016)

4.4 Pathogenicity

The pathogenicity test of 13 isolates of *Rhizoctonia bataticola* isolated from the different parts of the Vidarbha region was tested by using cultivar TAMS 38. The results are presented in Table 16 and Plate 3.

The result indicates that amongst all the thirteen isolates of *Rhizoctonia bataticola* proved to be pathogenic to cultivar TAMS 38. Isolates R-6 collected from Dahegaon village of Wardha district recorded highest per cent mortality (66.66%) in tested cultivar TAMS 38. Next in order

Plate 3. Pathogenicity of *Rhizoctonia bataticola isolates*

as R-7 (Kurha), R-4 (Bori Ijara) and R-12 (Waddhamna) recorded 57.14 and 50% mortality, respectively. Minimum per cent of mortality (16.66%) recorded in isolates R-8, R-10 and R-13. On the basis of per cent mortality, these thirteen isolates were grouped as highly pathogenic (1), strongly pathogenic (2), moderately pathogenic (7) and weakly pathogenic (3).

Table16. Pathogenicity test of *Rhizoctonia bataticola* against variety TAMS 38

Sr. No.	Isolates	Total no. of seeds sown	Total no. of seeds germinated	No. of plants infected	Days to initiate symptoms	Per cent mortality due to pathogen (45 DAS)	Place
1.	R-1	10	6	2	41	33.33	Godhani
2.	R-2	10	7	3	45	42.85	Hingna
3.	R-3	10	5	2	49	40.00	Girola
4.	R-4	10	6	3	44	50.00	Bori Ijara
5.	R-5	10	5	2	48	40.00	Hamdapur
6.	R-6	10	6	4	43	66.66	Dahegaon
7.	R-7	10	7	4	44	57.14	Kurha
8.	R-8	10	6	1	49	16.66	Virulpurna
9	R-9	10	7	2	47	28.57	Amaravati
10	R-10	10	6	1	42	16.66	Vyala
11	R-11	10	5	2	48	40.00	Washimba
12	R-12	10	6	3	50	50.00	Waddhamna
13	R-13	10	6	1	48	16.66	Bhapur
14.	Control	10	9	0	0	0	

The present study showed that all the 13 isolates of *Rhizoctonia bataticola* proved to the pathogenic to cultivar TAMS 38. Similar result also noted by Belkar *et al.,* (2016).

4.5 Grouping of *Rhizoctonia bataticola* isolates based on their pathogenic ability

The data based on pathogenic variability in different isolates of *Rhizoctonia bataticola* are given in Table 17. The isolates of *Rhizoctonia bataticola* were tentatively divided in five groups based on their pathogenic ability on cultivar TAMS 38 (Table 17).

Grouping of *Rhizoctonia bataticola* isolates from different cultivated crops have been reported by Aghakhani and Dubey (2009) and Gade *et al.,* (2018).

Table 17. Grouping of *Rhizoctonia bataticola* isolates based on their pathogenic ability

Sr. No.	Category	Per cent mortality due to root rot	Isolates
1.	Non-pathogenic (NPI)	0	Nil
2.	Weakly pathogenic (WPI)	1-20	R-8, R-10, R-13
3.	Moderately pathogenic (MPI)	21-50	R-1, R-2, R-3, R-4, R-5, R-9, R-11, R-12
4.	Strongly pathogenic (SPI)	51-70	R-6, R-7
5.	Highly pathogenic (HPI)	>70	Nil

4.6 Morphological variability in *Rhizoctonia bataticola* isolates.

Variations were observed in the morphological parameters among thirteen isolates of *R. bataticola* collected from different areas of Vidarbha region of Maharashtra. Data with regard to colony characteristics are presented in Table 18 and Plate 4.

Differences among *R. bataticola* isolates collected from different places were observed on the basis of mean radial growth. After 48 hours of incubation the individual mean radial growth of the isolates ranged from 61.03 to 90.00 mm with highest in R-8, R-9, R-12, R-13, R-6, R-4, R-1 Isolates and R-2, R-10 and R-11 were next in order with statistically similar colony diameter (81.00 mm) followed by R-3 (75.13 mm), R-7

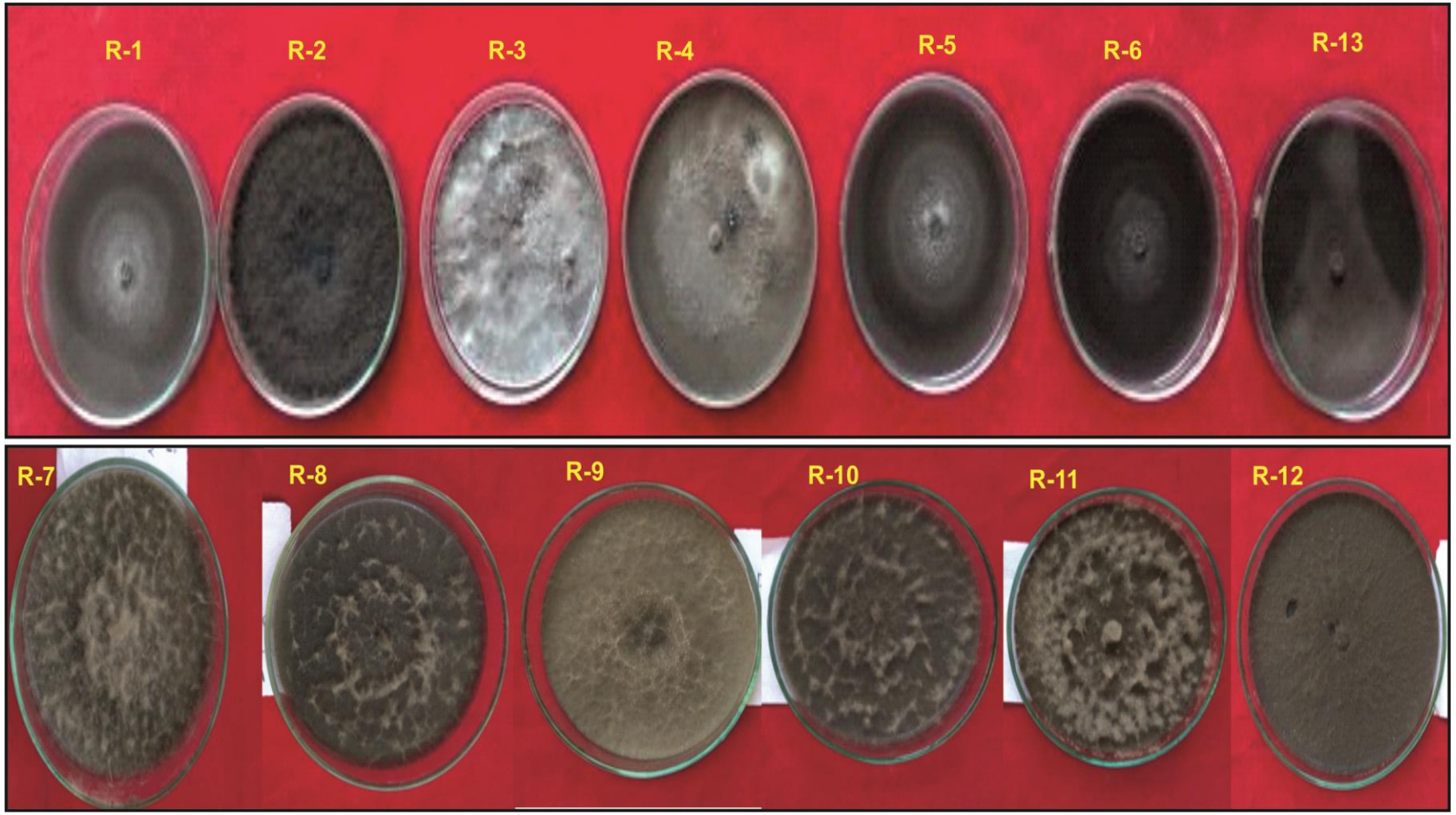


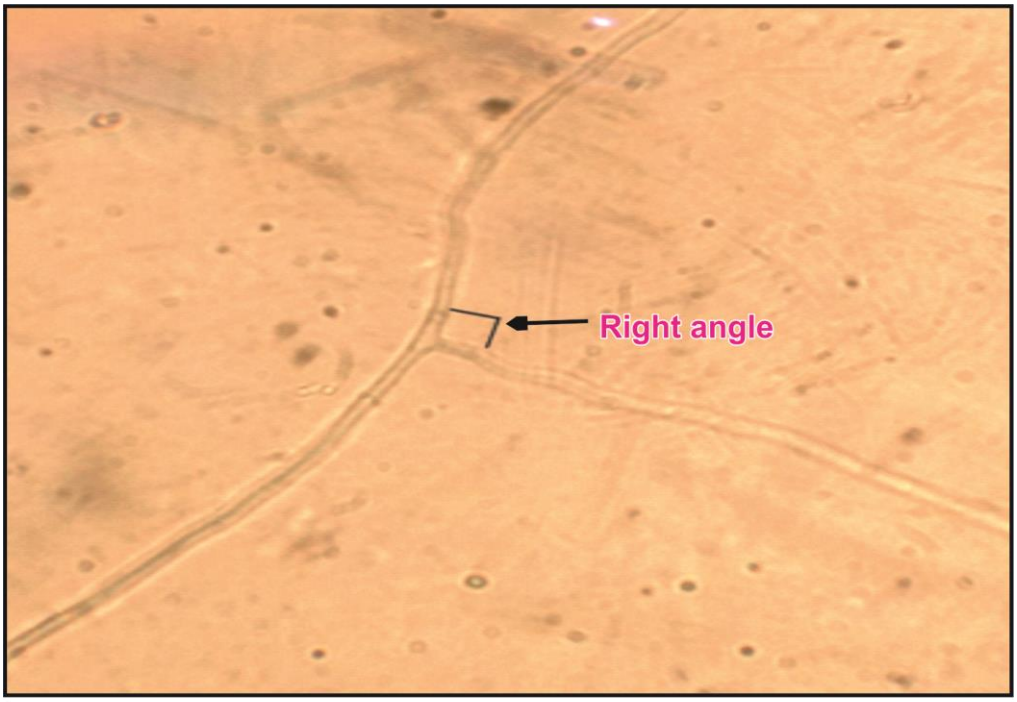
Plate 4. Growth of *Rhizoctonia bataticola* isolates on PDA

Table 18 Morphological characters of *Rhizoctonica bataticola* isolates on Potato dextrose agar medium

Sr. No.	Character	Isolates												
		R-1	**R-2**	**R-3**	**R-4**	**R-5**	**R-6**	**R-7**	**R-8**	**R-9**	**R-10**	**R-11**	**R-12**	**R-13**
1	**Colony diameter (mm)**	83.10	80.40	75.13	84.30	61.03	88.63	73.97	90.00	90.00	81.49	81.00	90.00	90.00
2	**Colour of colony (Reverse)**	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black
3	**Colour of colony**	Grayish Black	Charcoal Black	Grayish White	Grayish White	Blackish Gray	Charcoal Black	Charcoal Black	Charcoal Black	Grayish Black	Charcoal Black	Charcoal Black	Charcoal Black	Blackish Gray
4	**Colony appearance**	++	++	++++	+++	++	++	+++	+++	++++	+++	+++	++	++
5	**Mycelium**	Sub-Aerial	Sub Aerial	Aerial	Aerial	Sub Aerial	Suppressed	Sub Aerial	Sub Aerial	Aerial	Sub Aerial	Sub Aerial	Suppressed	Sub Aerial
6	**Branching pattern**	Right Angle	Right Angle	Acute Angle	Acute Angle	Acute Angle	Right Angle	Acute Angle	Right Angle	Right Angle	Right Angle	Right Angle	Acute Angle	Right Angle
7	**Sclerotia formation**	Ample	Ample	Ample	Ample	Ample	Sparse	Ample	Ample	Ample	Ample	Ample	Ample	Sparse
8	**Shape of sclerotia**	Round	Round	Oblong	Round	Round	Round	Round	Round	Round	Oblong	Round	Round	Oblong
9	**Mean sclerotial size (µm)****	125.83	36.12	57.57	61.15	51.14	36.53	67.36	124.07	95.79	70.44	80.06	79.38	83.49

++ : less feathery, +++ : more feathery, ++++ : maximum feathery

** mean of 20 observations for each isolate



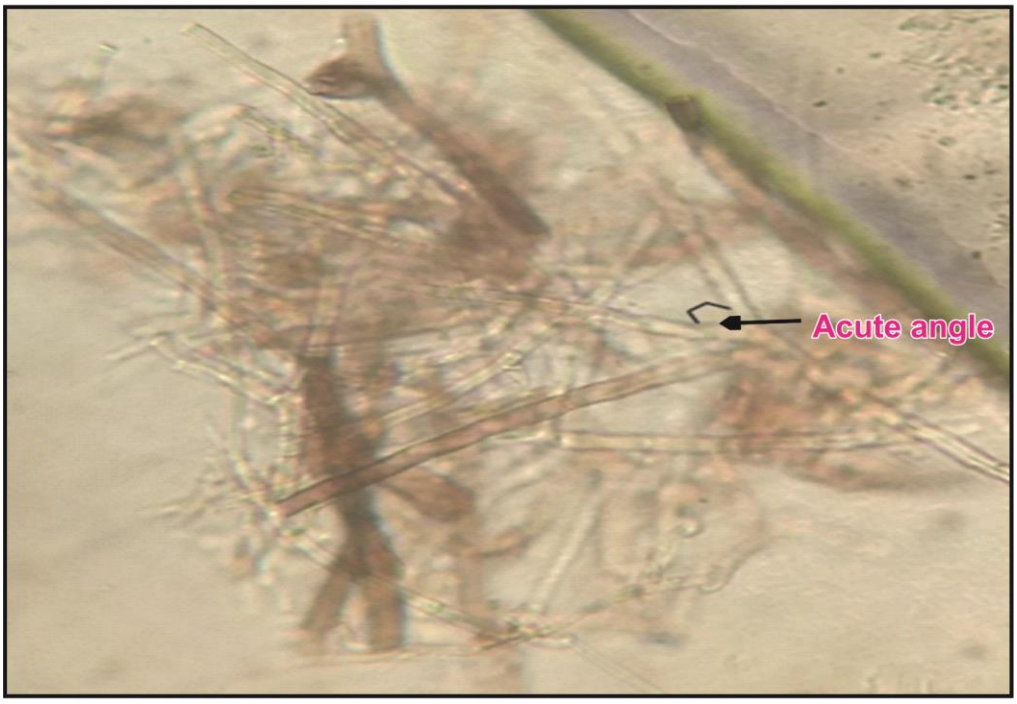


Plate 5. Morphological character of *Rhizoctonia bataticola*; Right angle and acute angle branching pattern

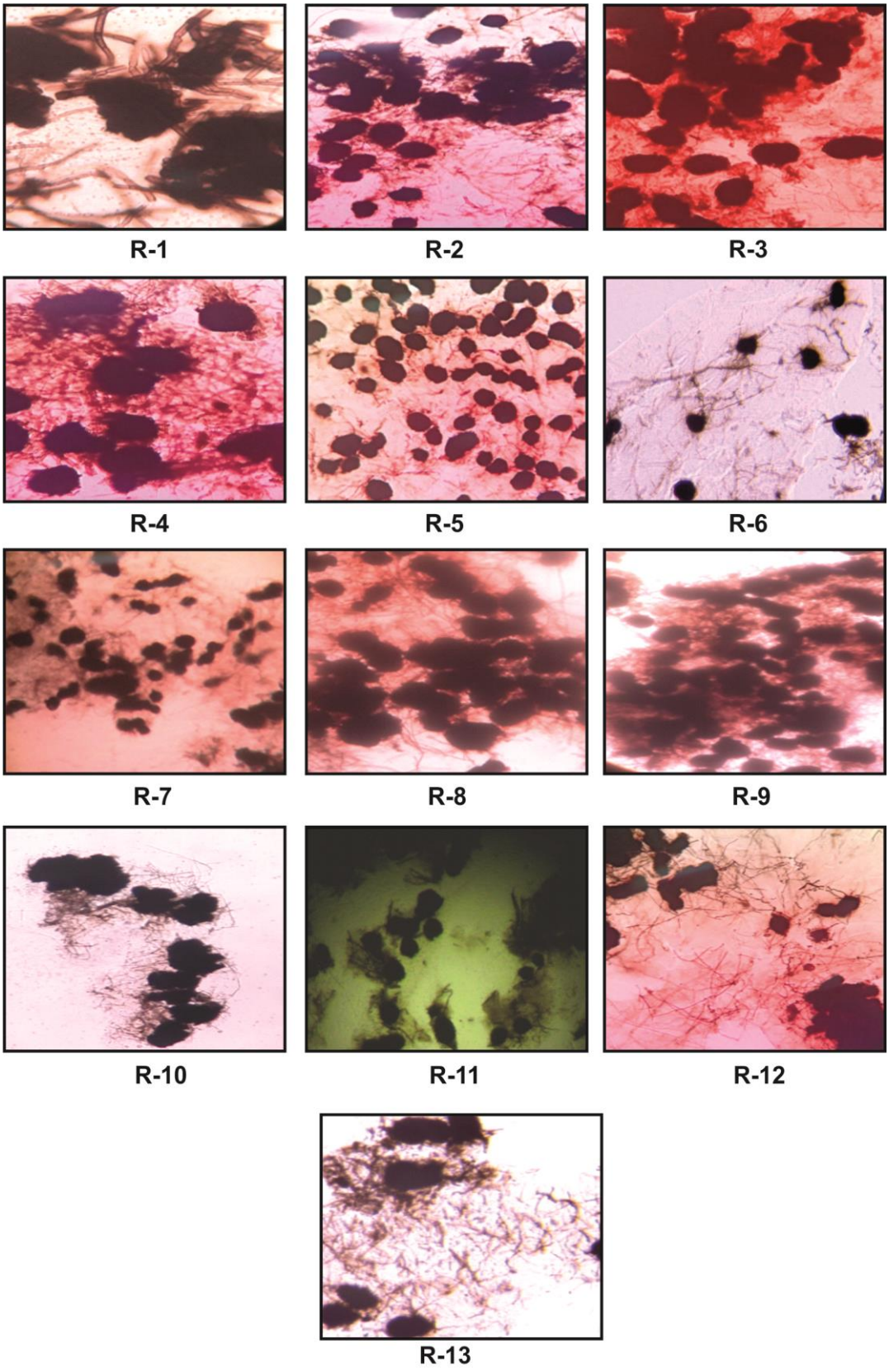


Plate 6. Sclerotia of *Rhizoctonia bataticola* isolates

(73.97 mm) least diameter 61.03 mm were recorded in R-5 (Plate 4.) Based on colony colour, the cultures grouped into four group *i.e.* grayish white (R-3 and R-4); blackish gray (R-5 and R-13); charcoal black (R-2, R-6, R-7, R-8, R-10, R-11, R-12) and grayish black (R-1 and R-9). Although, from reverse side all isolates showed blackish colour.

Isolates were also assigned in three groups, on the basis of mycelial growth and colony texture as maximum feathery, more feathery and less feathery. Isolates R-3 and R-9 showed maximum feathery growth whereas R- 4, R-7, R-8, R-10 and R-11 showed more feathery growth and remaining all isolates showed growth less feathery. The majority of isolates (8) produced sub aerial mycelium whereas (3) isolates produced aerial mycelium and (2) isolates produced suppressed mycelium with right angle or acute angle branching pattern (Plate 5) (Meena Shekhar *et al.,* 2006). All isolates produced sclerotia in abundant form except isolates R-6 and R-13. *R. bataticola* isolates R-3, R-10 and R-13 had oblong shape sclerotia with irregular edges whereas the remaining ones had round shaped sclerotia with regular edges sclerotia (Plate 6).

Significant variations were also observed among these isolates regarding the size of their sclerotia. The sclerotia formed in different isolates were dark brown to black and highly variable in size (36.12 to 125.83 µm) throughout the study. It was observed that the isolate R-1 produced largest sclerotia of 125.83 µm followed by isolates R-8, R-9, R-13, R-11, R-12, R-10, R-7, R-4, R-3, R-5 and R-6 while the smallest sclerotia of 36.12 µm was noticed in R-2 isolate. Isolates were assigned into three categories according to the size of sclerotia viz. sclerotial size in range of small size (>60 µm) (R-2, R-3, R-5, R-6), medium size (60-80 µm) (R-4, R-7, R-10, R-12) large size above 80 µm(R-9, R-11, R-13, R-1 and R-8).

In the present studies, 13 isolates of *R. bataticola* belongs to different soybean areas of Vidarbha region of Maharashtra showed variation in different morphological traits *viz*. radial growth, sclerotia size, colony colour. Colony appearance and the variation in morphology might be due to difference in temperature, moisture, soil type and other edaphic

factor of various places (Iqbal and Mukhtarar, 2014). Purkaystha et al. (2006) grouped *M. phaseolina* isolates into three mycelial phenotypes. In the present investigation, on the basis of colony characters, the isolates were grouped into different categories. Each group had the isolates from different area of the Vidarbha. Earlier workers while working on *R. bataticola* associated with different crop plants also grouped the isolates into different categories based on the colony characters on medium (Dhingra and Sinclair, 1973;; Meena Shekhar *et al*., 2006 and Gade *et al.,* 2018).

4.7 Cultural studies of *Rhizoctonia bataticola*

4.7.1. Effect of different solid media on radial mycelial growth of *R. bataticola* isolates

Significant variations were observed in growth pattern of collected isolates on different media. Data with regard to colony growth are presented in Table 19 and Plate 7.

Table 19. Growth of *R. bataticola* on different media

Sr. No.	Isolates	Potato Dextrose Agar (mm)	C'zapek's Dox Agar (mm)	Sabouraud's Agar(mm)	Peptone Agar (mm)
1.	R-1	83.2	77.1	63.4	56.2
2.	R-2	80.1	73.6	63.8	54.2
3.	R-3	75.8	65.3	59.9	54.4
4.	R-4	61.1	63.5	60.9	69.3
5.	R-5	88.6	80.0	63.5	48.3
6.	R-6	73.7	78.3	64.6	57.0
7.	R-7	89.9	68.3	65.6	60.3
8.	R-8	89.8	76.3	69.3	54.6
9.	R-9	81.3	66.6	72.2	80.1
10.	R-10	81.2	75.3	60.4	59.8
11.	R-11	81.0	68.4	58.4	66.4
12.	R-12	89.8	62.6	71.4	53.0
13.	R-13	89.5	83.3	69.4	56.1
	Mean	81.93	72.20	64.83	59.20
	F- test	Sig	Sig	Sig	Sig
	SE(m)	0.17	0.17	0.87	0.23
	CD(p=0.01)	0.68	0.66	3.40	0.89

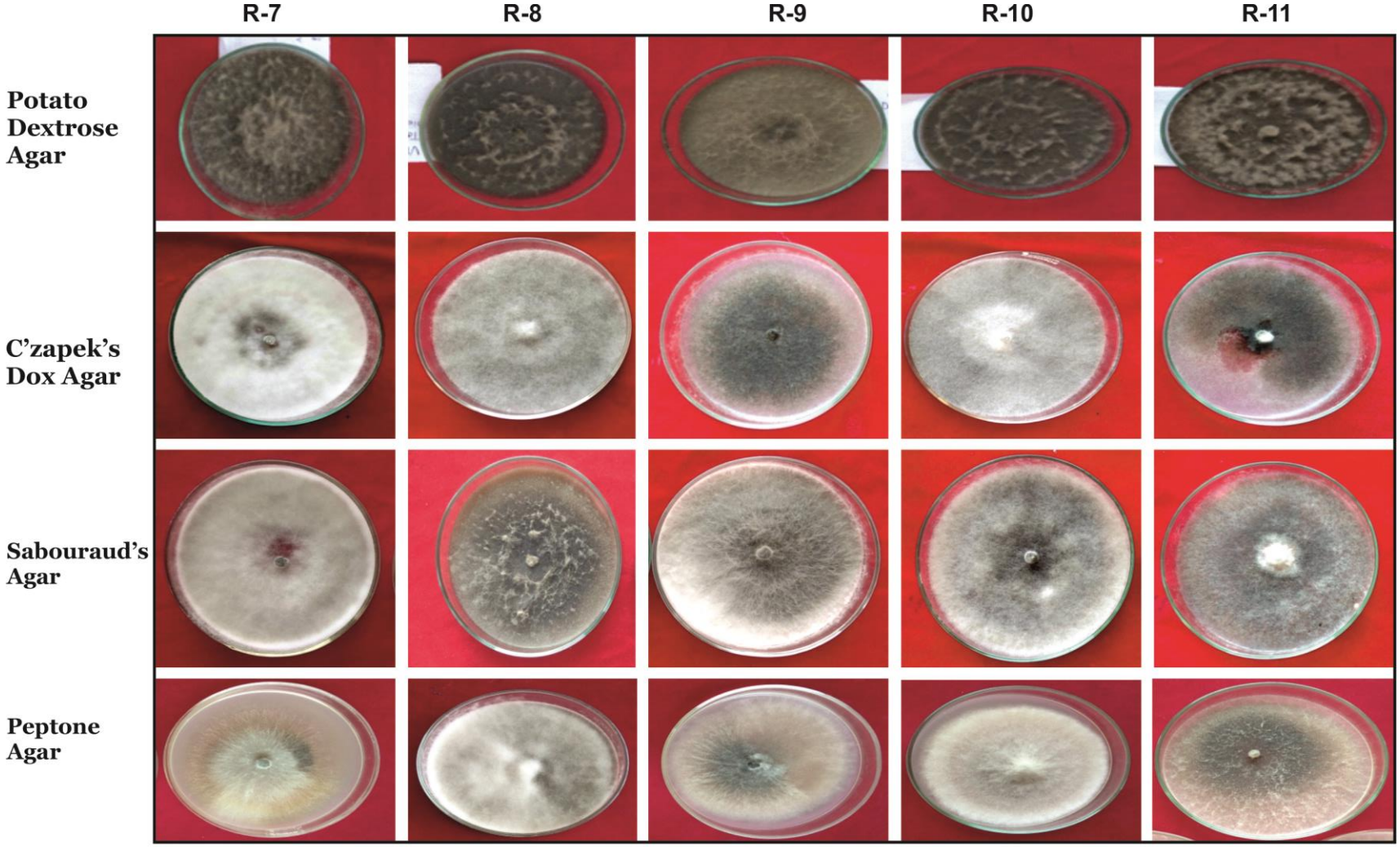
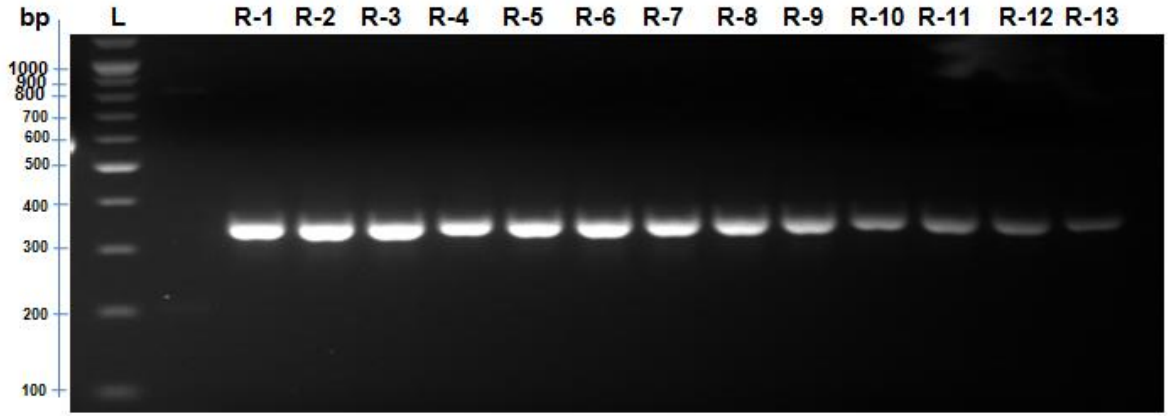
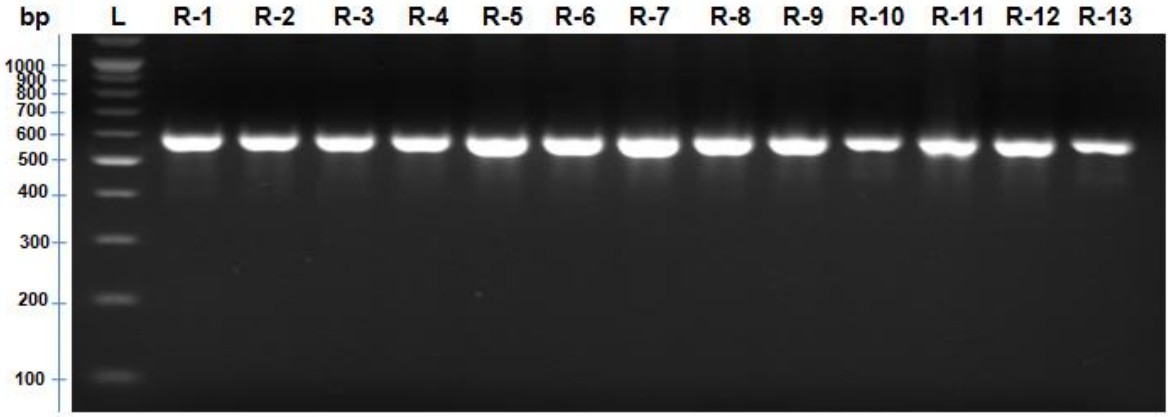


Plate 7. Growth of *Rhizoctonia bataticola* on different cultural media



Species specific marker : MpkFI and MpkRI



ITS 1 and ITS 4

Plate 8. Identification and confirmation of *Rhizoctonia bataticola* by using Species specific marker and ITS marker

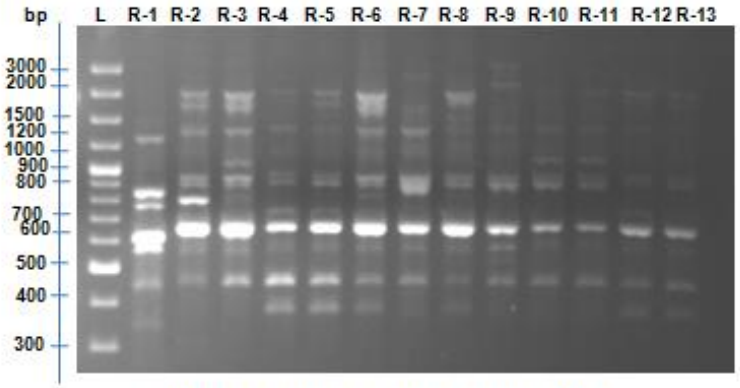
Four culture media were tested to understand cultural behavior of thirteen isolates of *Rhizoctonia bataticola* and to identify the best growth supporting medium. The results are presented in Table 19 (Plate 7). Significance differences were observed in respect different media among thirteen isolates of *R. bataticola*. Among media, Potato dextrose agar media found to support maximum growth mean (81.93 mm) followed by C'zapek's Dox agar (72.20 mm) and Sabouraud's Agar media (64.83 mm) while minimum growth was noted in peptone agar media (59.20 mm). Similar results noted by Sundravadana *et al*., (2012) and Raja Mohan and Balabhaskar, (2012).

4.8 Molecular variability among isolates of *Rhizoctonia bataticola*

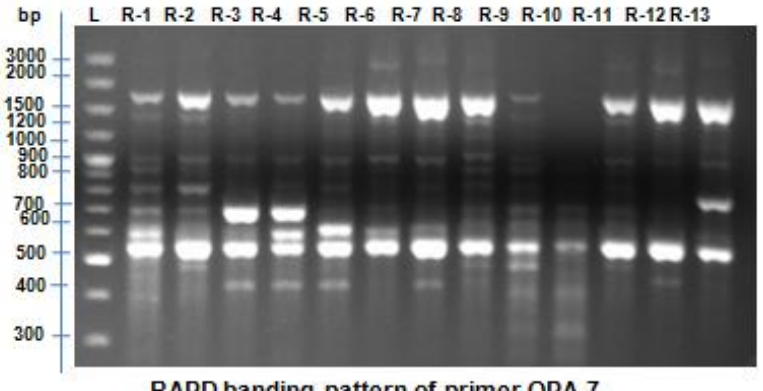
4.8.1 Random Amplified Polymorphic DNA analysis

The isolates of *Rhizoctonia bataticola* were obtained from different areas of Vidarbha region of Maharashtra. The thirteen isolates *viz.,* Kurha, Virulpurna, Amaravati, Vyala, Washimba, Godhani, Bhapur, Girola, Bori ijara, Hamdapur, Dahegaon,Waddhamna, Hingna were selected for the analysis. The *Rhizoctonia bataticola*species specific marker MpkFI (CCGCCAGAGGACTATCAAAC) and MpkRI (CGTCCGAAGCGAGGTGTATT) and ITS primer pair ITS-1 (TCCGTAGGTGAACCTGCGG) and ITS-4 (TCCTCCGCTTATTGATATGC) were used for molecular confirmation of the isolates. All the isolates of *Rhizoctonia bataticola* yielded the 350 bp band with the species specific marker and 650 bp band with ITS marker, hence confirm the culture of *Rhizoctonia bataticola* were as per the result of Aghakhani and Dubey 2009 and Prameela Devi *et al.,* 2016).

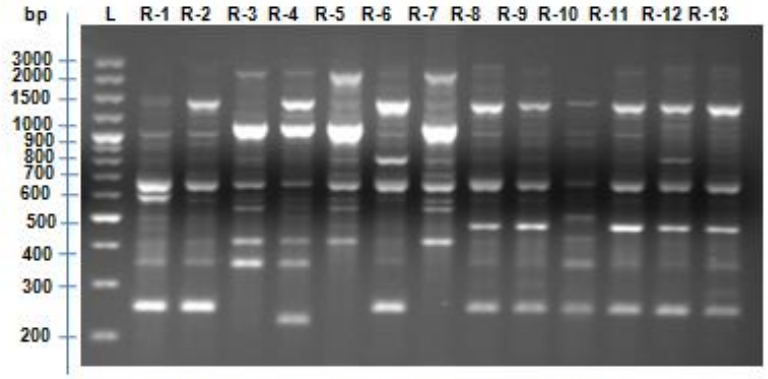
Genetic variation detected among 13 isolates of *R. bataticola* using 20 RAPD Primers (Table 20; 9 & 10 Plate). Among 20 primers used for amplification, OPB-2 and OPC-4 (88.88 %) showed highest polymorphism among isolates. A total of 165 amplicons level resulted from 20 primers and were available for analysis. Information on banding pattern for all the primers was used to determine genetic variation between isolates and to construct a dendrogram.



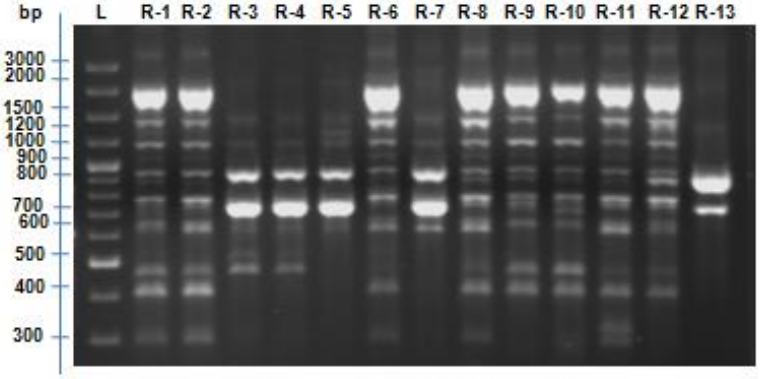
RAPD banding pattern of primer OPA-1



RAPD banding pattern of primer OPA-7

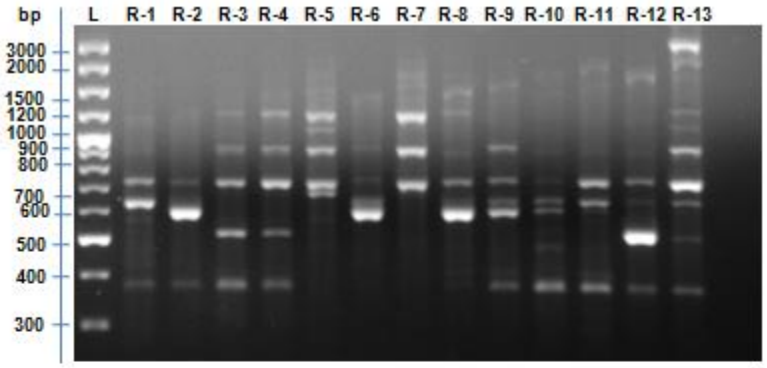


RAPD banding pattern of primer OPA-9

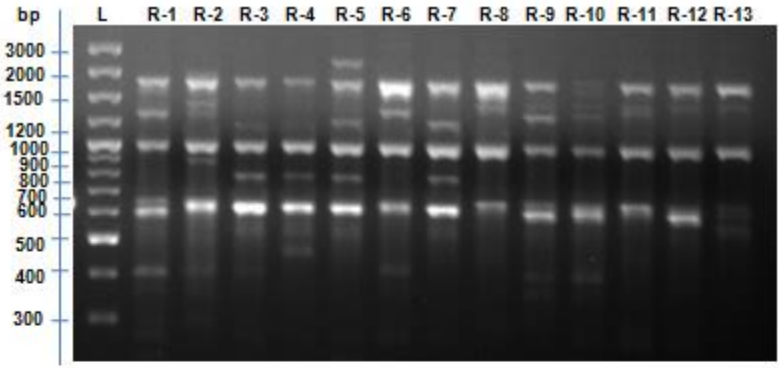


RAPD banding pattern of primer OPD-5

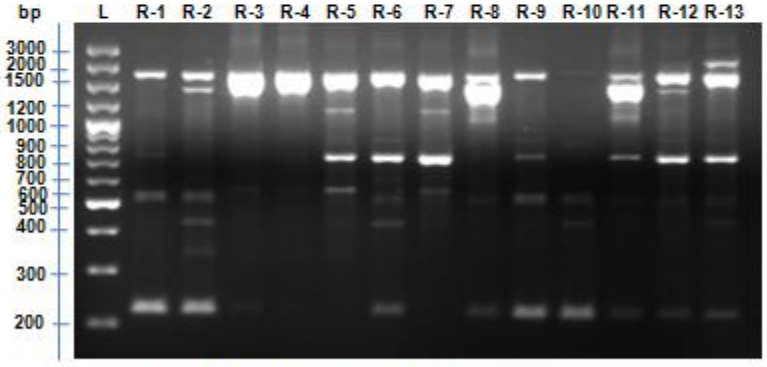
Plate 9. RAPD banding pattern by using different primers



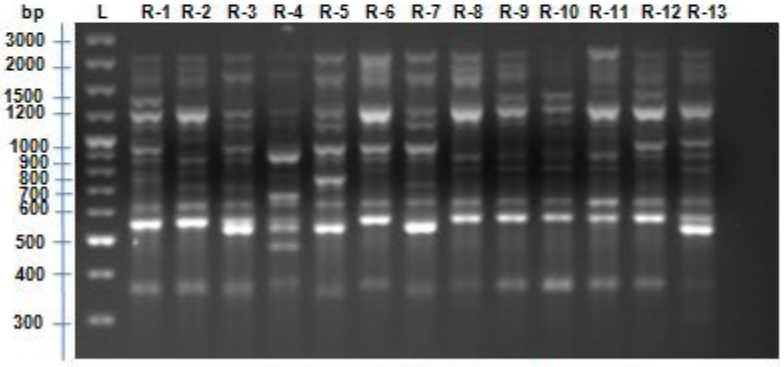
RAPD banding pattern of primer OPB-2



RAPD banding pattern of primer OPB-4



RAPD banding pattern of primer OPC-4



RAPD banding pattern of primer OPC-5

Plate 10. RAPD banding pattern by using different primers

Table 20. Per cent polymorphism observed in RAPD primers

Sr. No.	Primer	Bands	Polymorphic bands	Per cent of Polymorphism
1	OPA-1	11	9	81.81
2	OPA-2	10	7	70.00
3	OPA-3	5	2	40.00
4	OPA-5	10	5	50.00
5	OPA-6	8	5	62.50
6	OPA-7	11	8	72.72
7	OPA-8	4	1	25.00
8	OPA-9	11	8	72.72
9	OPA-10	7	6	85.71
10	OPB-1	3	1	33.33
11	OPB-2	9	8	88.88
12	OPB-3	5	2	40.00
13	OPB-4	9	7	77.77
14	OPB-5	6	3	50.00
15	OPC-1	10	7	70.00
16	OPC-2	10	4	40.00
17	OPC-3	5	2	40.00
18	OPC-4	9	8	88.88
19	OPC-5	15	9	60
20	OPD-5	7	6	85.71
	Total	**165**	**108**	**65.45**

Based on Jaccard's coefficient (Table 21 and Fig.1) a genetic similarity matrix was constructed to assess the genetic relatedness among the genetic relatedness among the isolates of *R. bataticola.* Genetic similarity coefficient of 13 isolates of *R. bataticola* basedon RAPD analysis is given in the Table 20. The range of genetic similarity based on RAPD primers was 0.17-0.83. Isolate R-5 had high value of similar coefficient

(0.83) to isolate Rb-7 followed by isolate R-4 to R-3 and R-8 to R-11 (0.66).This is indication of similarity between these isolates and have high similarity index as compared to others. Also, isolate R-5 had lower value of similarity coefficient (0.17) to isolate R-10 whichindicates least similarity between them.

A dendrogram was generated by unweighted pair group method with UPGMA" sub programme of “NTSYS-pc”. The dendrogram for pooled datashowed two major clusters (Fig 1). The isolates R-10, R-9, R-6, R-2, R-1, R-11, R-8 and R-12 were found in one cluster; isolates R-4, R-3, R-7 and R-5 were found in second cluster. Remaining isolate R-13 was not making any group in dendrogram analysis.It is difficult to distinguish these species using traditional morphological and cultural differences. To understand better, the existence of variation among the isolates of *R. bataticola*, PCR based technique, RAPD (Random Amplified Polymorphic DNA) was used in the present investigation. The suitability of RAPD was used to select the variations among the isolates of *R. bataticola.*In the present investigation, OPA series primers were used to see the genetic distance between isolates and to construct dendrogram. Of the 13 primers used for amplification, OPB-2 and OPC-4 showed highest polymorphism (88.88%) followed by OPA-10 and OPD-5 (85.71%) polymorphism and OPA-1, OPA-7, OPA-9 and OPB-4 showed more than 70 per cent polymorphism 13 among isolates.

The dendrogram by RAPD data revealed that, the selected thirteen isolates were differentiated into two major clusters, I and II. In the present investigation, the results revealed that irrespective of geographical locations, the isolates are different. Similar study were conducted by Meena Shekhar *et al.,* (2006) in which they analyzed seven isolates of incitant of maize charcoal rot through RAPD marker for genetic diversity. They observed that the most closely related isolates were Hyderabad and Delhi within affinity percentage of 75.5 followed by Udaipur and Bangalore isolates with 62.9 per cent similarity. Rajkumar *et al.,* (2007) also studied the genetic variability in ten isolates of *M. phaseolina* using PCR-RAPD markers.

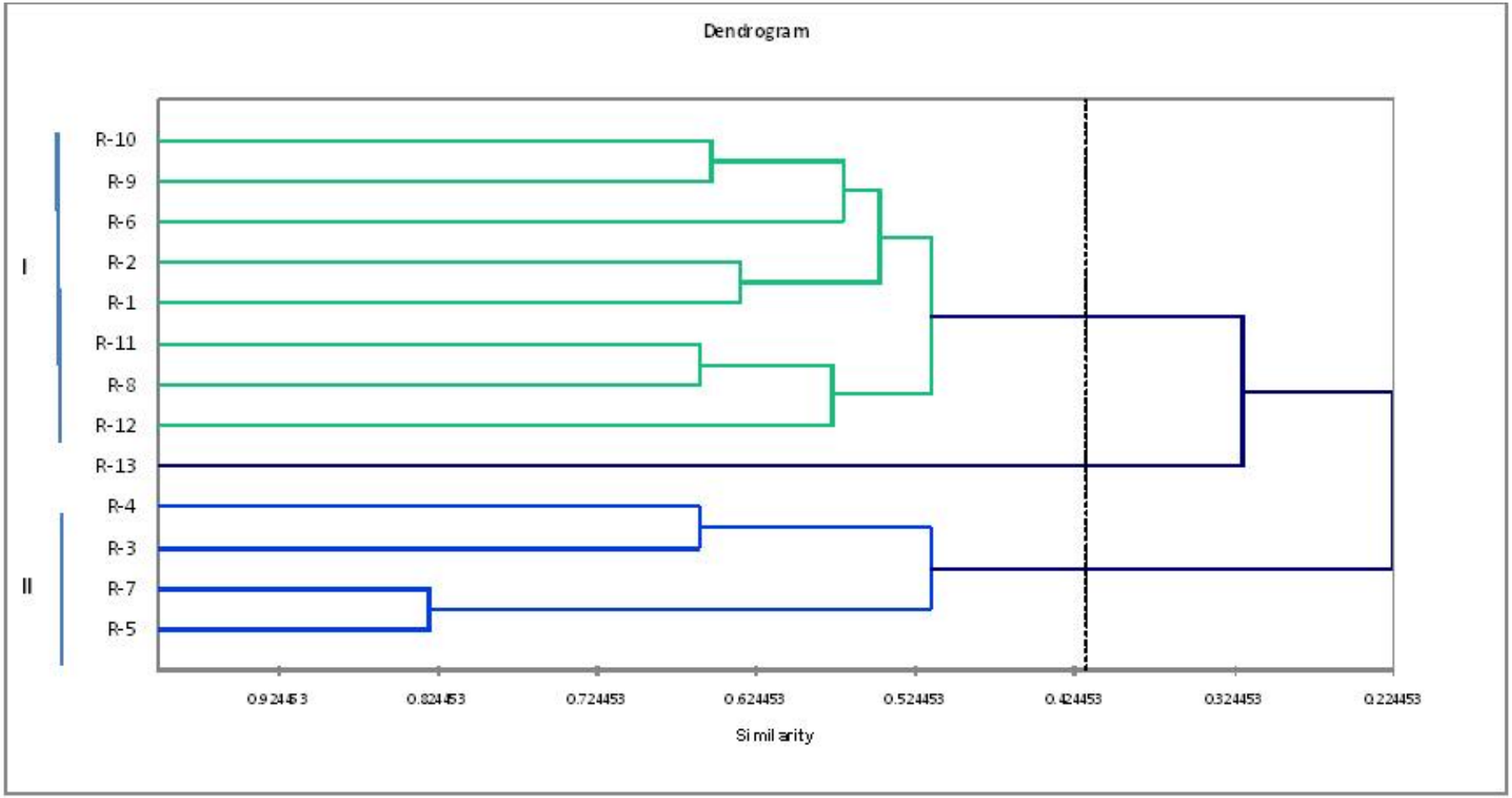
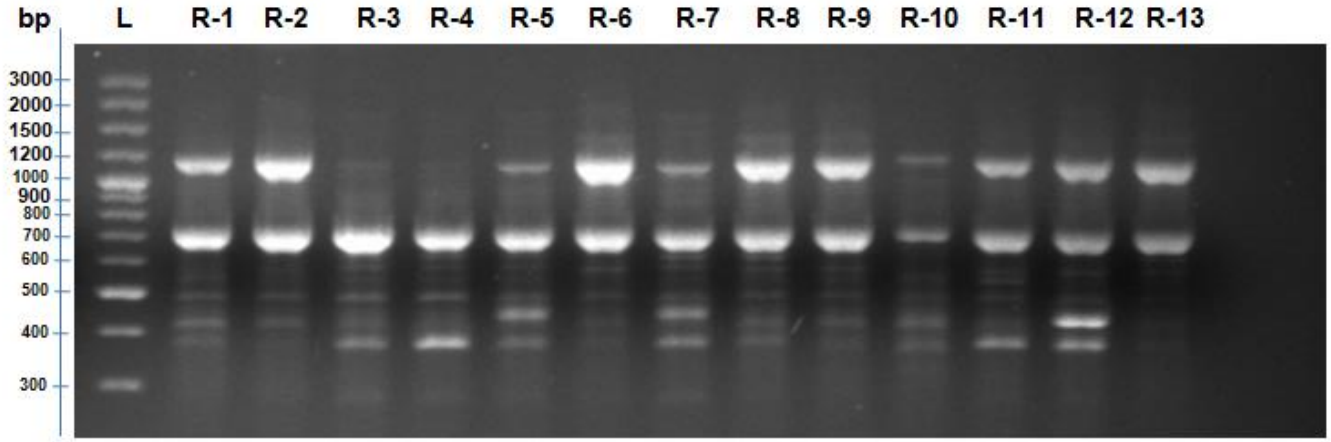


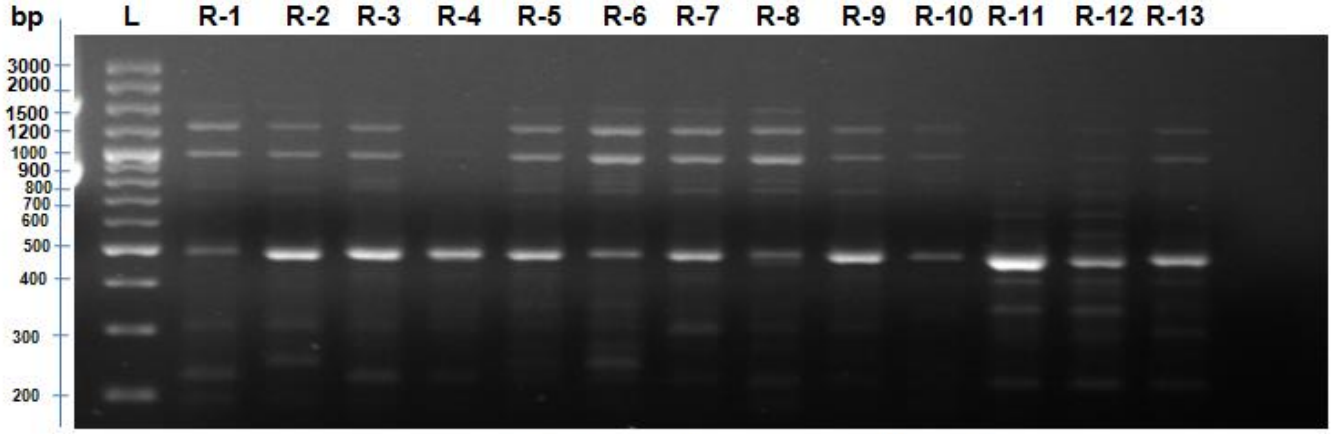
Fig 1. A dendrogram of RAPD analysis against thirteen isolates of *R. bataticola*

Table 21.Binary similarity coefficient of RAPD analysis against thirteen isolates of *R. bataticola*

Isolates	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10	R-11	R-12	R-13
R-1	1												
R-2	0.635	1											
R-3	0.323	0.277	1										
R-4	0.232	0.229	0.660	1									
R-5	0.225	0.205	0.574	0.419	1								
R-6	0.509	0.588	0.182	0.174	0.221	1							
R-7	0.277	0.235	0.620	0.448	0.830	0.234	1						
R-8	0.431	0.585	0.209	0.235	0.265	0.549	0.281	1					
R-9	0.500	0.544	0.197	0.205	0.233	0.566	0.246	0.654	1				
R-10	0.529	0.612	0.206	0.179	0.174	0.574	0.185	0.510	0.653	1			
R-11	0.448	0.545	0.206	0.214	0.208	0.569	0.221	0.660	0.611	0.472	1		
R-12	0.443	0.459	0.229	0.219	0.264	0.474	0.261	0.554	0.542	0.439	0.600	1	
R-13	0.250	0.264	0.189	0.213	0.224	0.344	0.203	0.348	0.348	0.254	0.343	0.403	1



Banding pattern of primer UBC 808



Banding pattern of primer UBC 840

Plate 11. ISSR banding pattern by using different primers

UPGMA data clustering revealed that, the isolates shared vary levels of genetic similarity within range of 0.14 to 0.72 similarity coefficient index and it was suggestive that grouping of isolates was not related to sampling location in anyway. Monga *et al.,* (2007) made studies on 25 isolates of *R.bataticola* and obtained similar results.

4.8.2 Internal Simple Sequence repeat (ISSR) analysis

Table 22. Per cent of polymorphism observed in ISSR primers

Sr. No.	Primer	Bands	Polymorphic bands	% of Polymorphism
1	UBC 808	6	5	83.33
2	UBC 840	9	4	44.44
	Total	15	9	63.88

A total of 15 bands were produced for *Rhizoctonia bataticola* isolates and out of them 9 bands were found to be polymorphic. Maximum polymorphisms were obtained with UBC 808 (83.33%) followed by UBC 840 (44.44%). The data obtained from ISSR analysis of 13 isolates of *Rhizoctonia bataticola* with two primers was subjected to UPGMA analysis. A dendrogram was prepared using the similarity coefficient of ISSR marker. The dendrogram showed the presence of differences among *R. bataticola* isolates.

Based on Jaccard's coefficient, a genetic similarity matrix was constructed to assess the genetic relatedness among the genetic relatedness among the isolates of *R.bataticola.* Genetic similarity coefficient of 13 isolates of *R. bataticola* basedon ISSR analysis is given in the Table 22 and Plate 11. The range of genetic similaritybased on RAPD primers was 0.12-0.83. Isolate R-11 had high value of similar coefficient (0.83) to isolate Rb-12 followed by isolate R-1 to R-3 and R-11 to R-13 (0.80).This is indication of similarity between these isolates and have high similarity index as compared to others. Also, isolate R-2 had lower value of

similarity coefficient (0.17) to isolate R-11 whichindicates least similarity between them.

A dendrogram was generated by unweighted pair group method with “UPGMA" sub programme of “NTSYS-pc”. The dendrogram for pooled data showed four major clusters (Fig 2). The isolates R-7 and R-5 were found in one cluster and isolates R-12, R-11, and R-13 were found in second cluster. The isolate R-9, R-8, R-10, R-3, R-1, and R-4 were found in third cluster. Isolates R-6 and R-2 were found in forth cluster. To understand better, the existence of variation among the isolates of *R. bataticola*, PCR based technique, ISSR (Internal Simple Sequence Repeat) was used in the present investigation. The suitability of ISSR was used to select the variations among the isolates of *R. bataticola.* In the present investigation UBC series primers were used to see the genetic distance between isolates and to construct dendrogram of the 13 primers used for amplification, UBC 808 showed highest polymorphism (83.33%) followed by UBC 840 (44.44%) polymorphism among 13 isolates.Similar study were conducted by Mane *et al.,* (2018) in which they analyzed eight isolates of *Rhizoctonia bataticola* incitant of chickpea root rot through ISSR marker for genetic diversity. UPGMA data clustering revealed that, the isolates shared vary levels of genetic similarity within range of 37.5 to 80.8 similarity coefficient index and it was suggestive that grouping of isolates was not related to sampling location in anyway. Prameela Devi *et al.,* (2016) also studied genetic diversity of *Rhizoctonia bataticola* from different 19 crops using RAPD and ISSR markers. Five ISSR primers tested for amplification DNA of *R. bataticola* Unweighted Pair-Group Method using arithmetic means (UPGMA) clustering of the data showed that isolates did clearly differentiate to the specific group according to the geographical regions. ISSR markers were found more efficient than RAPD marker to the correlate the genetic diversity with the grouping of isolates according to geographical regions.

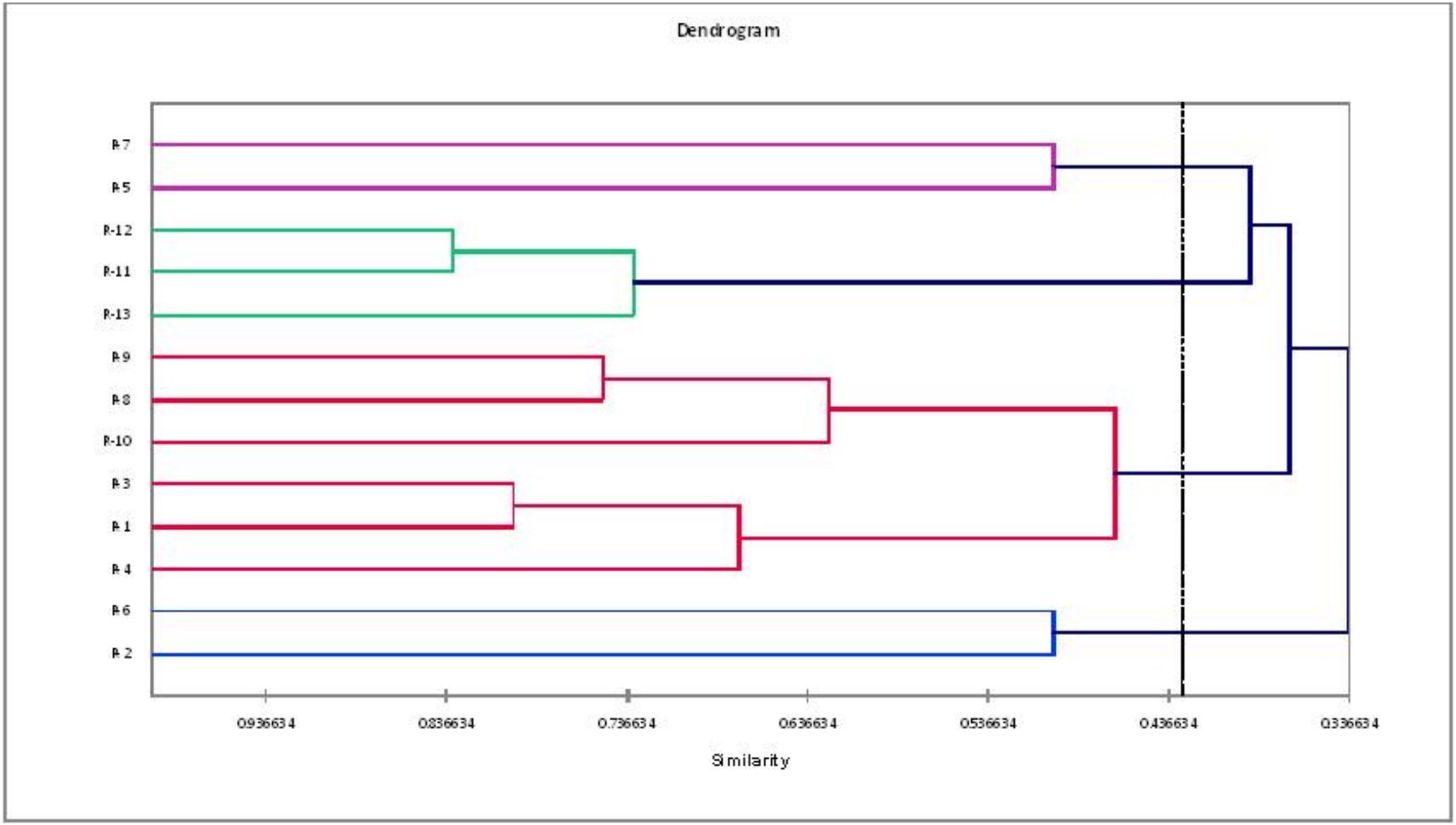


Fig 2. A dendrogram of ISSR analysis against thirteen isolates of *R. bataticola*

Table 23.Binary similarity coefficient of ISSR analysis against thirteen isolates of *R. bataticola*

Isolates	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10	R-11	R-12	R-13
R-1	1												
R-2	0.500	1											
R-3	0.800	0.333	1										
R-4	0.600	0.167	0.750	1									
R-5	0.429	0.286	0.286	0.333	1								
R-6	0.429	0.500	0.286	0.143	0.250	1							
R-7	0.500	0.143	0.333	0.400	0.500	0.286	1						
R-8	0.800	0.333	0.600	0.400	0.286	0.500	0.600	1					
R-9	0.600	0.400	0.400	0.200	0.143	0.600	0.400	0.750	1				
R-10	0.600	0.400	0.400	0.200	0.333	0.333	0.400	0.750	0.500	1			
R-11	0.429	0.125	0.286	0.333	0.429	0.429	0.500	0.500	0.333	0.333	1		
R-12	0.571	0.250	0.429	0.286	0.375	0.571	0.429	0.667	0.500	0.500	0.833	1	
R-13	0.286	0.143	0.143	0.167	0.286	0.500	0.333	0.333	0.400	0.167	0.800	0.667	1

4.9 Screening of soybean genotypes to quantify resistance against root rot

Fifteen soybean genotypes were screened for their resistance against *Rhizoctonia* root rot. All genotypes different in their response to root rot. The disease reaction of various genotypes ranged from 0 to 9 grades on basis of Plant Disease Index (PDI).

Table 24. Evaluation of soybean genotypes against root rot under sick plot condition (*Kharif*, 2018)

Sr.No.	Genotypes	Per cent of root rot incidence	Reaction
1.	TAMS 38	90.06	Highly susceptible
2.	AMS 38 -24	07.14	Highly resistance
3.	AMS 358	10.33	Highly resistance
4.	AMS 475	13.80	Moderately resistance
5.	AMS MB 5-18	04.00	Highly resistance
6	AMS MB 5-19	02.43	Highly resistance
7	AMS 77	05.12	Highly resistance
8	AMS 1001	07.89	Highly resistance
9	AMS 353	21.73	Moderately resistance
10	AMS 99-33	56.16	Susceptible
11	Bragg	60.00	Susceptible
12	AMS 243	23.33	Moderately resistance
13	JS 93-05	41.73	Moderately susceptible
14	JS 335	56.87	Susceptible
15	Panjab 1	61.66	Susceptible

It is evident from Table 24 and Table 25, Plate 12 that out of fifteen genotypes screened six *viz*. AMS 38-24, AMS 358, AMS MB 5-18, AMS MB 5-19, AMS 77 and AMS 1001 were found highly resistance.Three genotypes *i.e.* AMS 475, AMS 353 and AMS 243 was showing moderately resistance reaction against the pathogen. Only one genotype JS 93-05 were found moderately susceptible whereas four genotypes namely AMS

Plate 12. Evaluation of diverse soybean entries against root rot under sick plot condition

99-33, Bragg, JS 335 and Panjab 1 were susceptible and only TAMS 38 showed highly susceptible reaction.

Table 25. Reaction of soybean genotypes against *Rhizoctonia* root rot

Rating	Disease Reaction	Genotypes	Total
0	Absolutely resistance	Nil	0
1	Highly resistance	AMS 38-24, AMS 358, AMS MB 5-18, AMS MB 5-19, AMS 77, AMS 1001	6
3	Moderately resistance	AMS 475, AMS 353, AMS 243	3
5	Moderately susceptible	JS 93-05	1
7	Susceptible	AMS 99-33, Bragg, JS 335, Panjab 1	4
9	Highly susceptible	TAMS 38,	1

Development of efficient screening technique and identification of resistance donors in the past decade led to the development of varieties resistance to root rot. Shrirao *et al*., (2009) evaluated soybean genotypes against *Rhizoctonia* root rot and reported that out of 16, four entries were highly resistance (JS-72-44, JS-71-05, Monetta and JS 335), three as moderately resistance (JS-75-46, Bragg and VLS-58). Three genotypes were showed susceptible reaction (PK-472, MACS-58 and Panjab-1) to *Rhizoctonia* root rot. Similarly, Patil *et al.,* (2011) screened eighteen soybean genotypes against *Rhizoctonia* root rot disease under natural and sick plot conditions. Moderately resistance reaction was observed JS 335, JS 93-05, AMS 353 and MACS-450 whereas seven genotypes were moderately susceptible while under present study JS 335 susceptible. This change in reaction might be due to virulence of the pathogen in different area. Also five genotypes *viz*. TAMS 98-21, PK-472, MAUS-47, AMS 99-24 and TAMS showed susceptible and highly susceptible reaction, respectively to *Rhizoctonia* root rot.

The present finding also correspond to the research of Belkar *et al.,* (2016) screened thirteen seven soybean genotypes against

Rhizoctonia root rot under sick soil conditions. Out of seven genotypes viz. AMS 100-1, AMS 131-1, AMS 77-3-16, AMS 1003, AMS 475 AMS 3923 and JS 9305 showed moderately reaction while two genotypes AMS 0542-G and TAMS 38 showed susceptible reaction against *Rhizoctonia* root rot. Pancheshwar *et al.,* (2016) also screened 77 cultivars out of them none of the entries were found resistance and moderately resistance against *Rhizoctonia* root rot. Only two entries viz. JS 97-52 and JS 93-05 reacted as a moderately susceptible under disease grade 5 while JS 335 showed susceptible reaction as disease grade 7.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation was carried out during academic year 2018-2019 at Department of Plant Pathology, Dr. Panajabrao Deshmukh Krishi Vidyapeeth, Akola, Bhabha Atomic Research Centre, Mumbai and Regional Research Centre, Amravati which included different studies *viz*., survey for incidence of *Rhizoctonia* root rot among major soybean growing area of Vidarbha, isolation, pathogenicity, identification, symptomatology, morphological, cultural,molecular characterization of pathogen and screening experiment against pathogen of soybean genotypes. The salient features of the findings are summarized below.

An rapid roving field survey was conducted to assess the distribution and incidence of *Rhizoctonia* root rot and to collect the root rot infected plant samples in major soybean growing area of Vidarbha *viz*. Amaravati, Akola, Washim, Buldana, Yavatmal, Wardha and Nagpur. The disease incidence was recorded all the locations surveyed in the range 2.33 to 39.33 per cent. Among the locations surveyed, maximum root rot incidence was observed in Vyala (39.33 %) of Akola district and least in HamdapurVillage (2. 33 %) of Wardha district. Symptoms due to *R. bataticola* initiated at the collar region of plants as water soaked areas and the affected tissues soon turned into a soft, watery mass, and caused decay, which ultimately resulted in the wilting of the plants. Thirteen different isolates of the *R. bataticola* fungus were isolated from soybean roots by using the standard tissue isolation technique.

The pathogenicity test of 13 isolates of *Rhizoctonia bataticola* isolated from the different parts of the Vidarbha region was tested by using cultivar TAMS 38. Among 13 isolates all proved to be pathogenic with susceptible cultivar TAMS-38. Two isolate *viz*. R-6and R-7 were strongly pathogenic with 51-70 per cent root rot incidence whereas isolates *viz*. R-1, R-2, R-3, R-4, R-5, R-9, R-11 and R-12 were moderately pathogenic with average of 21-50 per cent root rot.

Morphologically thirteen isolates of *R. bataticola* showed significant variations. After 48 hours of incubation the individual average radial growth of the isolates ranged from 61.03 to 90.00 mm with highest in R-7, R-8, R-9, R-12, R-6, R-3 and R-4 Isolates. Based on colony colour, the cultures were grouped into four group i.e. grayish white, blackish gray, charcoal black and grayish black Although from reverse side all isolates showed blackish colour.

Isolates were also grouped into three groups, on the basis of mycelial growth pattern and colony texture as maximum feathery, more feathery and less feathery. The majority of the isolates (8) produced sub-aerial mycelium whereas 3 isolates produced aerial mycelium and 2 isolates produced submerged mycelium with right angle or acute angle branching pattern. The isolates also varied in amount of formation of sclerotia as ample and sparse. The sclerotia formed in different isolates were dark brown to black and highly variable in size (36.12-125.83 µm). Four different solid media were used to study their effect on growth of *R. bataticola* isolates. Mean of radial growth 81.93 mm was recorded on potato dextrose agar followed by 72.20 mm with C'zapek's Dox agar medium.

Molecular variation was detected among 13 isolates of *R. bataticola* using 20 RAPD primers and 2 ISSR primers. Among 20 primers of RAPD used for amplification, OPB-2 and OPC-4 showed highest polymorphism (88.88%) followed by OPA-10 and OPD-5 (85.71%) among 13 isolates whereas two primers of ISSR used for amplification UBC 808 showed maximum polymorphism (83.33%) followed by UBC 840 showed 44.44% polymorphism. A total of 165 amplicons levels resulted from 20 primers of RAPD whereas 15 amplicons level resulted from 2 primers of ISSR. Based on Jaccard's coefficients, the range of genetic similaritybased on RAPD primers was 0.17-0.83 and 0.12-0.83 based on ISSR primers.The dendrogram by RAPD data revealed that thethirteen isolates were differentiated into two major clusters I and II. The isolates R-10, R-9, R-6, R-2, R-1, R-11, R-8 and R-12were found in one cluster; isolates R-4, R-3, R-7 and R-5 were found in second cluster. Remaining isolate R-13

was not making any group in dendrogram analysis. The dendrogram by ISSR data revealed that thirteen isolates were differentiated into four major clusters I, II, III and IV. The isolates R-1, R-3, R-4, R-8, R-9 and R-10 were found in one cluster, R-2 and R-6 were found in second cluster, third cluster included isolates R-5 and R-7, and isolates R-11, R-12 and R-13 included in forth cluster.

Fifteen soybean genotypes were screened for their resistance against *Rhizoctonia* root rot. None of the genotypes found immune to Rhizoctonia root rot, however six genotypes were found highly resistance.

Conclusions

The following conclusions can be drawn from the present investigation

1. Roving survey in the major soybean growing area of India Vidarbha revealed the prevalence of *Rhizoctonia* root rot at all the locations of surveyed where disease incidence ranged from 2.33 to 39.33 per cent.

2. Under pathogenic test, among 13 isolates all proved pathogenic to susceptible cultivar TAMS-38. Two isolates viz. R-6, R-7 were strongly pathogenic whereas six isolates viz. R-1, R-2, R-3, R-4, R-5, R-9, R-11 and R-12 were moderately pathogenic and remaining three isolates viz. R-8, R-10 and R-13 were weakly pathogenic. However, none of the isolate proved, to be non pathogenic.

3. Isolates R-1, R-2, R-5, R-7, R-8, R-10 R-11and R-13 produced sub-aerial mycelium whereas isolates R-3, R-4 and R-9 produced aerial and isolates R-6 and R-12 produced submerged mycelium with right angle or acute angle branching pattern.

4. Among different solid media maximum growth was recorded on potato dextrose agar followed by C'zapek's Dox agar medium.

5. PCR-based RAPD analysis revealed that OPB-02 and OPC-4 showed highest per cent polymorphism. The dendrogram by RAPD data indicated that the thirteen isolates differentiated into two major clusters I

and ll. The results revealed that Irrespective of geographical locations the isolates are differed.

6. PCR-based ISSR analysis revealed that isolates formed four major clusters with two primers tested. UBC 808 primer showed highest polymorphism followed by UBC 840. The dendrogram by ISSR data revealed that thirteen isolates differentiate into four major clusters i.e. I, II, III and IV. ISSR markers were found a much more efficient tool to differentiate variability among the different isolates of *Rhizoctonia bataticola*.

7. 0ut of thirty fifteen soybean genotypes tested six were found to be highly resistant.

Finally it can be concluded that all the isolates collected from different area of Vidarbha proved to be highly pathogenic and the basis of their pathogenic reaction there is no much variation amongst the isolate tested. The number of alleles at a locus and their frequency of distribution are responsible for polymorphism thereby genetic variation in population. RAPD and ISSR markers target different portion of the genome resulted the difference in discrimination among the isolates. The genetic variation usually depends on the number of polymorphic bands obtained with each marker.

CHAPTER VI

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APPENDIX I

a) Potato dextrose agar (Tuite, 1969)

Peeled and sliced potatoes: 200 g

Dextrose (C6H1206): 20 g

Agar-agar: 20 g

Distilled water (to make up): 1000 ml

b) Czapek's Dox agar (Tuite, 1969)

Sucrose ($C_{12}H_{22}0_{11}$): 30 g

Sodium nitrate ($NaN0_3$): 2 g

Potassium dihydrogen phosphate (KH_2PO_4) 1 g

Magnesium sulphate (Mgs04 $7H_2O$): 0. 5g

Potassium chloride (KCI): 0. 5g

Ferrous sulphate ($FeSO_4.7H_2O$): 0. 01g

Agar-agar: 20 g

Distilled water (to make up): 1000 ml

c) Sabouraud's agar

Dextrose ($C_6H_{12}O_6$): 20 g

Peptone: 10 g

Agar-agar: 20 g

Distilled water (to make up): 1000 ml

d) Peptone agar

Peptone: 5 g

Yeast: 2 g

Agar-agar: 20 g

Distilled water (to make up): 1000 ml

Appendix II

		R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10	R-11	R-12	R-13
OPA-1_1	1500	1	0	0	0	0	0	1	1	1	0	1	1	0
OPA-1_2	1350	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-1_3	1200	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-1_4	1000	0	0	1	1	0	0	0	0	0	0	1	0	0
OPA-1_5	900	1	1	1	1	1	1	1	1	1	1	1	1	0
OPA-1_6	850	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-1_7	800	0	0	0	0	0	0	0	0	0	0	0	1	1
OPA-1_8	620	1	1	1	1	1	1	1	1	1	1	1	1	0
OPA-1_9	600	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-1_10	450	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-1_11	400	1	1	0	0	0	1	1	1	1	1	0	0	0
OPA-2_1	2000	1	1	1	0	1	1	1	1	1	1	1	1	1
OPA-2_2	1500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-2_3	1200	0	0	1	0	1	0	1	0	0	0	0	0	0
OPA-2_4	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-2_5	900	1	1	0	0	0	1	0	1	1	1	1	1	1
OPA-2_6	800	0	0	0	1	0	0	0	0	0	0	0	0	1
OPA-2_7	640	0	1	0	0	0	0	0	0	0	0	0	1	0
OPA-2_8	500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-2_9	390	0	0	0	0	1	0	0	1	1	1	1	1	1
OPA-2_10	350	0	0	1	0	1	0	1	0	0	0	1	1	0
OPA-3_1	500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-3_2	600	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-3_3	750	0	0	0	0	1	1	0	1	1	0	0	1	1
OPA-3_4	1300	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-3_5	1500	0	0	0	0	0	0	0	0	0	0	0	1	0
OPA-5_1	2000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-5_2	1500	1	1	1	1	0	0	0	0	0	0	0	1	0
OPA-5_3	1350	0	0	1	1	1	0	1	0	0	0	0	0	0
OPA-5_4	1200	1	1	0	0	1	1	1	1	1	1	1	1	1
OPA-5_5	1100	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-5_6	750	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-5_7	600	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-5_8	400	1	1	1	1	1	1	1	1	1	1	1	1	1

OPA-5_9	320	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-5_10	270	1	1	1	0	1	1	1	0	0	1	0	0	0
OPA-6_1	1500	1	0	0	0	1	0	1	1	0	0	0	1	1
OPA-6_2	1000	0	0	0	0	0	1	0	0	0	0	1	0	1
OPA-6_3	600	1	1	1	1	1	1	1	1	0	0	1	1	1
OPA-6_4	560	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-6_5	530	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-6_6	400	0	1	0	0	0	1	0	1	1	1	1	1	1
OPA-6_7	360	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-6_8	270	1	0	1	1	1	0	1	0	0	0	0	0	0
OPA-7_1	1700	1	1	1	1	1	1	1	1	1	0	1	1	1
OPA-7_2	1000	1	1	0	0	0	0	0	0	0	0	0	0	0
OPA-7_3	900	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-7_4	800	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-7_5	700	0	0	1	1	0	0	0	0	0	0	0	0	0
OPA-7_6	600	1	0	0	1	0	1	0	0	0	0	0	0	0
OPA-7_7	520	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-7_8	500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-7_9	490	0	1	0	0	0	0	0	1	1	0	1	0	0
OPA-7_10	400	0	0	1	1	1	0	1	0	0	0	0	1	0
OPA-7_11	200	1	0	0	0	0	0	0	0	1	1	0	0	0
OPA-8_1	2000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-8_2	1200	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-8_3	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-8_4	900	0	0	1	1	1	0	1	0	0	0	0	0	0
OPA-9_1	1500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-9_2	1200	0	1	0	1	0	1	0	1	1	0	1	1	1
OPA-9_3	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-9_4	900	0	0	1	1	1	0	1	0	0	0	0	0	0
OPA-9_5	650	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-9_6	550	0	0	1	1	1	0	1	0	0	0	0	0	0
OPA-9_7	470	0	0	0	0	0	0	0	1	1	0	1	1	1
OPA-9_8	400	1	1	1	1	1	0	1	0	0	0	0	0	0
OPA-9_9	350	1	1	1	1	0	0	0	0	0	1	0	0	1
OPA-9_10	260	1	1	0	0	0	1	0	1	1	1	1	1	1
OPA-9_11	230	0	0	0	1	0	0	0	0	0	0	0	0	0
OPA-10_1	1800	1	1	1	1	1	1	1	1	1	1	1	0	1

OPA-10_2	1000	0	0	0	0	1	1	1	1	1	0	1	1	1
OPA-10_3	750	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA-10_4	650	0	0	0	0	0	0	0	0	0	0	0	0	1
OPA-10_5	600	0	0	0	0	0	0	0	0	0	0	1	0	0
OPA-10_6	500	0	0	1	1	1	0	1	0	0	0	0	0	0
OPA-10_7	370	0	1	0	1	1	0	1	1	1	1	1	1	0
OPB-1_1	1500	0	0	0	0	1	0	0	0	0	0	0	0	0
OPB-1_2	1100	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-1_3	550	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-2_1	2000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-2_2	1500	0	0	0	0	1	0	0	0	0	0	0	0	0
OPB-2_3	1200	0	0	1	1	1	0	1	1	0	0	0	0	1
OPB-2_4	900	0	0	1	1	1	0	1	0	1	0	0	0	1
OPB-2_5	750	1	1	1	1	1	0	1	1	1	0	1	1	1
OPB-2_6	640	1	0	0	0	0	1	0	0	1	1	1	1	1
OPB-2_7	600	0	1	0	0	0	1	0	1	1	1	0	0	0
OPB-2_8	500	0	0	1	1	0	0	0	0	0	0	0	1	1
OPB-2_9	380	1	1	1	1	0	0	0	1	1	1	1	1	1
OPB-3_1	2000	0	0	1	1	1	0	1	1	0	0	0	0	0
OPB-3_2	1300	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-3_3	1100	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-3_4	650	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-3_5	500	1	1	0	0	0	1	0	0	0	0	1	1	1
OPB-4_1	2500	0	0	0	0	1	0	0	0	0	0	0	0	0
OPB-4_2	1800	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-4_3	1240	1	1	0	0	0	1	0	0	1	1	0	0	0
OPB-4_4	1200	0	0	0	0	1	0	1	0	0	0	0	0	0
OPB-4_5	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-4_6	800	0	0	1	1	1	0	1	0	0	0	0	0	0
OPB-4_7	650	0	1	0	0	0	1	0	1	1	1	1	0	0
OPB-4_8	620	0	0	1	1	1	0	1	0	0	1	0	1	0
OPB-4_9	600	1	0	0	0	0	0	0	0	1	0	0	0	0
OPB-5_1	1500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-5_2	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPB-5_3	800	1	1	1	0	0	0	0	0	1	1	0	0	0
OPB-5_4	700	0	0	0	1	0	0	0	0	0	0	0	0	1
OPB-5_5	600	1	1	1	1	1	1	1	1	1	1	1	1	1

OPB-5_6	500	0	0	0	0	0	0	0	0	0	0	0	0	1
OPC-1_1	1500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-1_2	900	0	1	0	0	1	1	1	1	1	1	0	1	1
OPC-1_3	600	0	0	0	0	0	0	0	0	0	0	0	1	0
OPC-1_4	550	0	0	0	0	0	0	0	0	0	0	0	0	1
OPC-1_5	500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-1_6	450	1	1	1	1	1	1	1	1	1	1	1	1	0
OPC-1_7	390	0	0	0	0	0	0	0	1	0	0	0	0	0
OPC-1_8	350	1	1	0	0	0	1	0	0	1	1	1	1	0
OPC-1_9	300	0	0	0	0	0	0	0	0	0	0	0	0	1
OPC-1_10	250	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_1	1200	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_2	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_3	900	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_4	750	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_5	700	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_6	580	0	0	0	1	0	0	0	0	0	0	0	0	0
OPC-2_7	520	0	0	1	1	1	0	1	0	1	0	0	0	0
OPC-2_8	450	1	1	0	0	0	0	0	1	1	1	1	1	1
OPC-2_9	350	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-2_10	260	1	1	1	1	1	1	1	1	1	1	1	0	0
OPC-3_1	2000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-3_2	1500	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-3_3	1300	0	0	0	0	0	0	0	0	1	0	0	0	1
OPC-3_4	1200	0	0	0	0	0	0	0	0	0	0	1	0	0
OPC-3_5	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-3_6	900	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-3_7	800	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-4_1	2000	0	0	0	0	0	0	0	0	0	0	0	0	1
OPC-4_2	1800	1	1	0	0	0	1	0	1	1	1	1	1	1
OPC-4_3	1400	0	1	0	0	0	0	0	1	0	0	1	1	0
OPC-4_4	1200	0	0	0	0	1	0	1	0	0	0	0	0	0
OPC-4_5	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-4_6	700	0	0	0	0	1	1	1	0	1	0	1	1	1
OPC-4_7	530	0	0	1	1	1	0	1	0	0	0	0	0	0
OPC-4_8	440	0	1	0	0	0	1	0	0	0	1	0	0	1
OPC-4_9	230	1	1	0	0	0	1	0	1	1	1	1	1	1

OPC-5_1	2000	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_2	1800	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_3	1600	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_4	1500	0	0	0	1	0	1	0	1	1	1	1	1	1
OPC-5_5	1300	1	0	0	0	0	0	0	0	1	1	0	1	0
OPC-5_6	1200	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_7	900	1	0	1	0	1	1	1	0	0	0	0	1	1
OPC-5_8	800	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_9	700	0	0	0	0	1	0	0	0	0	0	0	0	0
OPC-5_10	650	0	0	0	1	0	0	0	0	0	0	0	0	0
OPC-5_11	600	1	1	1	1	1	1	1	1	1	1	1	1	1
OPC-5_12	550	1	1	1	1	0	1	0	1	1	1	1	1	1
OPC-5_13	540	0	0	1	1	1	0	1	0	0	0	0	0	1
OPC-5_14	480	0	0	0	1	0	0	0	0	0	0	0	0	0
OPC-5_15	360	1	1	1	0	1	0	1	0	0	0	0	0	0
OPD-5_1	2000	1	1	0	0	0	1	0	1	1	1	1	1	0
OPD-5_2	900	1	1	1	1	1	1	1	1	1	1	1	1	1
OPD-5_3	790	1	1	0	0	0	1	0	1	1	1	1	1	0
OPD-5_4	700	0	0	1	1	1	0	1	0	0	0	0	0	1
OPD-5_5	480	1	1	1	1	0	1	0	1	1	1	1	1	0
OPD-5_6	400	1	1	0	0	0	1	0	1	1	1	1	1	0
OPD-5_7	300	1	1	0	0	0	1	0	1	0	0	1	0	0
UBC 808_1	1200	1	1	0	0	1	1	1	1	1	1	1	1	1
UBC 808_2	700	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC 808_3	500	1	1	1	1	1	0	0	0	0	0	0	0	0
UBC 808_4	450	0	0	0	0	1	0	1	0	0	0	0	0	0
UBC 808_5	410	1	1	1	0	0	1	0	1	1	1	0	1	0
UBC 808_6	390	1	0	1	1	1	0	1	1	0	1	1	1	0
UBC-840_1	1200	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC-840_2	1000	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC-840_3	800	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC-840_4	700	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC-840_5	500	1	1	1	1	1	1	1	1	1	1	1	1	1
UBC-840_6	390	0	0	0	0	0	0	0	0	0	0	1	1	1
UBC-840_7	310	0	0	0	0	1	1	0	0	0	0	1	1	1
UBC-840_8	250	0	1	0	0	0	1	0	0	0	0	0	0	0
UBC-840_9	230	1	0	1	1	0	1	1	1	1	0	1	1	1