STUDIES ON DRY ROOT ROT [*Rhizoctonia bataticola* (Taub.) Butler] OF CHICKPEA (*Cicer arietinum* L.)

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M.Sc. (Ag.)

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(PLANT PATHOLOGY)

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BY

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M.Sc. (Ag.)

THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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2016

DECLARATION

I, P**. SRINIVAS**, hereby declare that the thesis entitled **"STUDIES ON DRY ROOT ROT [*Rhizoctonia bataticola* (Taub.) Butler] OF CHICKPEA (*Cicer arietinum* L.)"** submitted to the **Professor Jayashankar Telangana State Agricultural University** for the degree of **Doctor of Philosophy in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Hyderabad. **(P. SRINIVAS)**

Date: **I. D. No. RAD/12-18**

CERTIFICATE

Mr. **P. SRINIVAS** has satisfactorily prosecuted the course of research and that thesis entitled **"STUDIES ON DRY ROOT ROT [*Rhizoctonia bataticola* (Taub.) Butler] OF CHICKPEA (*Cicer arietinum* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by his for a degree of any university.

(P. NARAYAN REDDY)

Date: Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON DRY ROOT ROT [*Rhizoctonia bataticola* (Taub.) Butler] OF CHICKPEA (*Cicer arietinum* L.)"** submitted in partial fulfilment of the requirements for the degree of "**DOCTOR OF PHILOSOPHY IN AGRICULTURE**" of the **Professor Jayashankar Telangana State Agricultural University**, Hyderabad is a record of the bonafide original research work carried out by Mr. **P. SRINIVAS** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	per cent
@	:	at the rate of
ΔAbs	:	change in absorbance
CD	:	Critical Difference
cm	:	centimetre
CRD	:	Completely Randomized Design
DRR	:	Dry root rot
cv.	:	Cultivar
DAS	:	Days after sowing
DAI	:	Days after incubation
e.g.	:	for example
et al.	:	and others
Fig.	:	Figure
g	:	Gram
h	:	Hour
i.e.,	:	that is
kg	:	Kilogram
kg $acre^{-1}$	:	Kilogram per acre
kg ha^{-1}	:	Kilogram per hectare
l	:	litre
m	:	Metre
m^2	:	Metre square
mg	:	milligram
ml	:	milliliter

mm	:	millimetre
MPa	:	Mega pascal
No.	:	Number
^{o}C	:	Degree Centigrade
POD	:	Peroxidase
PPO	:	Polyphenol oxidase
PAL	:	Phenylalanine ammonia lyase
PDA	:	Potato Dextrose Agar
PDI	:	Per cent Disease Incidence
pH	:	Hydrogen ion concentration
psi	:	pounds per square inch
RAPD	:	Randomly Amplified Polymorphic DNA
t	:	Tonne
viz.,	:	namely

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ABSTRACT

Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] is a soil borne fungal pathogen. Keeping in view the importance of the disease due to change in climatic conditions, studies were conducted on distribution of the disease, cultural, morphological, pathological and molecular diversity. Effect of temperature and moisture on the disease severity was studied. Various biochemical and physiological changes associated with the development of the disease in susceptible and moderately resistant genotypes under glass house conditions were conducted at ICRISAT, Hyderabad.

A survey was conducted in January, 2014 *rabi* cropping season in different chickpea growing locations of central (Madhya Pradesh and Maharashtra) and southern (Andhra Pradesh, Telangana and Karnataka) India. Due to diversified weather conditions and variation in sowing dates in different states, different crop growth stages i.e. from seedling (20 days old) to podding stage were observed and the disease was observed in all the stages. The maximum dry root rot incidence was observed in Telangana (18.28%) and the least in Maharashtra (5.38%). Disease occurrence was observed irrespective of cropping system, soil types and cultivars. Mostly the disease incidence was low in the irrigated fields compared to rainfed fields.

Disease severity rating of 2.5 to 9.0 was observed in the 68 isolates inoculated on BG 212 and their incubation period varied from 1.0 to 4.8 days. It was observed that with an increase in disease severity there was decrease in incubation period in all the isolates. Diversity in cultural characters *viz.*, radial growth ranged from 17.7 mm to 80.0 mm at 72h after incubation; colony colour of black, black with grey aerial mycelium, dark brown and grey colour; colony texture of appressed, fluffy and velvety were observed.

The morphological characters such as hyphal cell size varied from 9.38 x 3.80 µm (Rb10) to 14.88 x 7.50 µm (Rb 63) and sclerotial size varied from 54.86 x 45.49 µm (Rb 46) to 216.08 x 181.09 µm (Rb 59). Ratio between length and width of sclerotia varied from 1.00 (Rb 5) to 1.64 (Rb 23). The isolates categorized into irregular, round and ovoid groups based on shape of sclerotia, rough and smooth groups based on texture of sclerotia. Isolates took 1.7 (Rb1) to 4.3 days (Rb4, Rb23 and Rb 28) for sclerotial initiation and their intensity per microscopic field (10x) varied from 11.67 (Rb 63) to 70.67 (Rb 56).

The similarity coefficient ranged from 0.63 to 0.92 indicating that no any two or more isolates were 100% similar. The highest similarity coefficient (0.92) was between isolates Rb 2 and Rb 4. The results of the present study also indicated that, all the isolates were not necessarily showing the geographical linearity.

Influence of different temperature, osmotic potential and pH were tested on growth of *R. bataticola.* The maximum mycelial growth was observed at 35ºC followed by 30 and 25ºC. Among different osmotic potential levels of NaCl, KCl and dextrose, maximum mean radial growth was observed at -0.5 MPa *viz.*, 27.5 mm, 31.1 and 38.2 mm respectively while the minimum growth was observed in -1.0 (4.9 mm), -1.5 (2.1 mm) and -2.5MPa (10.7 mm) respectively. The growth was observed at all the levels of pH tested. Maximum radial growth was observed at pH 5.0 (67.3mm) followed by pH 6.0 (64.4) while the least was observed at pH 11.0 (14.6mm) followed by pH 10.0 (33.0mm).

The optimum temperature for dry root rot severity rating was at 35°C (8.5) followed by 30°C (7.9) followed by 25°C (7.0). In black soil, the disease severity rating was 9.0, 8.9, 8.1, 6.9, 5.3, 3.8 and 2.3 while in red soil, it was 9.0, 8.9, 8.7, 7.9, 6.5, 4.7 and 3.0 at 40, 50, 60, 70, 80, 90 and100% soil moisture, respectively. The disease severity decreased as the soil moisture increased in both the types of soil.

The susceptible genotype BG 212 was having fewer amounts of total sugars compared to moderately resistant genotypes *viz.,* ICCV 5530 and ICCV 8305. The total sugar was more in the control plants as compared to plants in sick soil, while in the sick soil, the amount of increase was more in 100% as compared to 60% soil moisture condition. Similar results were present in reducing and non reducing sugars.

Total phenol, Phenylalanine Ammonia Lyase, Polyphenol oxidase and Peroxidase were maximum at 30DAS in sick soil with 60% soil moisture in all the three genotypes. The amount of increase was more in the inoculated plants grown at 60% compared to 100% soil moisture condition. The amount of phenol and enzymes were more in the moderately resistant genotypes (ICCV 5530 and ICCV 8305) compared to susceptible genotype (BG 212)

Among the genotypes, the dry weight of the genotypes was more in the 100% soil moisture as compared to 60% moisture. With respect to the sick and control soil, it was observed that, severe disease in the sick soil made the disintegration of the roots and there by losing the weight of plants in sick soil at 60% compared to control.

Genotypes grown in 100% moisture in control soil had more transpiration and stomatal conductance while the least was noticed in sick soil at 60% soil moisture in contrast, leaf temperature was more in the genotypes grown in sick soil at 60% moisture and the least was noticed in control soil with 100% soil moisture.

Overall results showed that dry root rot present in all the locations of central and southern India and may become severe disease due to change in climate. The determination of changes in biochemical and physiology of the genotypes help in screening the resistant genotypes and also early detection of the disease in fields.

INTRODUCTION

Chapter I

INTRODUCTION

Chickpea (*Cicerarietinum*L.) is the premier pulse crop grown in more than 50 countries originated in south west Asia and is cultivated from ancient times both in Asia and European countries. It is the world's second most important food legume next to common bean.Asia accounts 89.20% of the chickpea area and 84.47% of production. The major chickpea producing countries, which contributed to about 90% of the global chickpea production, include India (67.4%), Australia (6.21%), Pakistan (5.73%), Turkey (3.86%), Myanmar (3.74%)and Iran (2.25%) (FAOSTAT,2013). In India, chickpea is grown in an area of 10.22million hectares with a production of 9.53million tonnes and productivity of 967kgha^{-1}. Madhya Pradesh, Chhattisgarh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh and Karnataka together contribute 95.71% of production and 90% of area in the country (Ministry of Agriculture, Govt. of India., 2013-14).

Chickpea belongs to Leguminaceae family usually grown after rainy season on conserved soil moisture during winter in the tropics; in spring in the temperate and Mediterranean regions. It is the important grain legume grown for proteinrich seeds for human consumption and to maintain the soil fertility by its nitrogen fixing capability.Chickpea seeds of *deshi* type are brown to black in colour with a rough surface whereas seeds of *kabuli* type are light coloured having smooth surface. The roots usually include a central strong tap root with numerous lateral root branches that spread out in all directions in the upper layers of the soil. Chickpea seeds contain 23% protein, 64% carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugar and 3% ash,minerals such as calcium (202 mg), phosphorous (312mg), iron (10.2mg), vitamin C(3.0 mg),calorific value (360 cal), small amounts of B complex, fiber (3.9g) and moisture (9.8g).Predominantlychickpea is being consumed as dhal or variety of snack foods, sweets and condiments. Husk and split beans are useful as livestock feed.Acidic liquid from glandular hairs of the plant contain 94% malic acid and 6% oxalic acid, has medicinal value and used in preparation of vinegar.

Chickpea cultivation is often subjected to significant yield losses due to insects and diseases ranging from 5-10% in temperate and 50-100% in tropical regions (Van Emden *et al.*, 1988). Currently chickpea is attacked by 172 pathogens *viz.*, 67 fungi, 3 bacteria, 22 viruses and mycoplasma, and 80 nematodes reported from 55 countries. Maximum number of pathogensinfecting chickpea (89) had been reported from India while in other countries, it varied from 1 to 40. The pathogens that are most widely distributed are *Ascochytarabiei* (35 countries), *Fusariumoxysporum*f. sp*ciceri* (32 countries), *Uromycescicerisarietini* (25 countries), bean leaf roll virus (23 countries), *Rhizoctoniabataticola* (21 countries), *Botrytis cinerea* (15 countries), *Sclerotiniasclerotiorum*(15 countries) and cucumber mosaic virus (9 countries) (Nene *et al.*, 1996).

Among the diseases of chickpea, dry root rotis emerging as the most destructive constraint to chickpea productivity and production, asthe disease is more prevalent during hot temperatureof 30 to 35°C and low soil moisture conditions (Taya*et al*., 1988; Pande and Sharma, 2010). Dry root rot caused by *Rhizoctoniabataticola* (Taub.) Butler [Pycnidial stage: *Macrophominaphaseolina*(Tassi) Goid] is a soil and seed borne necrotrophic fungal pathogen that has a global distribution, which can infect more than 284 plant species throughout the world including monocot and dicots(Farr*et al*., 1995).

In chickpea field, the onset of the disease appears as scattered drying of the plants. Affected plants are usually straw coloured, but in some cases the lower leaves and stems show brown discolouration. The tap root appears black, rotten and devoid of most of the lateral and fine roots. The dead root become quite brittle and shows shredding of bark. Dark minute sclerotial bodies can be seen on the roots exposed or inside the wood. When the dry stem of the collar region is split vertically, sparse mycelium or minute sclerotia can be seen in the pith (Nene *et al.*, 1991).

Successful survival and adaptation of *Rhizoctoniabataticola*to various geographic environments has been confirmed by several workers demonstrating morphological (Mayek-Pérez *et al*., 2001), pathogenic (Mayek-Pérez *et al*., 2001; Su *et al*., 2001) and genetic diversity(Vandemark*et al*., 2000; Mayek-Pérez *et al*., 2001; Su *et al*., 2001; Almeida *et al*., 2003; Jana *et al*., 2003; Aboshosha*et al*., 2007) in various crops.Techniques such as RAPD markers are useful for measuring genetic relationships and variations within and among populations of *Rhizoctoniabataticola*in different countries like Australia (Fuhlbohm, 1997), Brazil (Almeida *et al*., 2003) and India (Jana *et al*., 2003).Pathogenic variability in the target production area is a prerequisite for identifying genotypes with a stable resistance to the variable pathogen populations.

Environmental conditions like temperature, soil moisture and pH play an important role in the viability and growth of *Rhizoctoniabataticola*(Khan, 2007). *Rhizoctoniabataticola*is able to produce microsclerotia under relatively low water conditions while viability of microsclerotiais drastically reduced at high water potentials (Olaya*et al*., 1996).Temperature and soil moisture are the two important weather parameters influencing the dry root rot infection, colonization and development in chickpea. Better understanding the role of temperature and soil moisture will help in standardization of dry root rot resistance screening techniques which will assist breeders in developingbreeding strategy for dry root rot resistance over broader geographical areas.

Infection by pathogen brings changes in vital processes inside the plant leading to wide fluctuations in sugar content (Klement and Goodman, 1967). The estimation of these compounds help in understanding the extent of host resistance to the pathogen. Interaction between a plant and a pathogen results in the induction of numerous hostspecific biochemical responses, so that the plant could withstand attack from pathogens. These responses are based not only on preformed defenses, but also on induced mechanisms. The induced mechanisms are associated with local changes at the site of pathogen infection,

such as the hypersensitive response, which is one of the most efficient forms of plant defenses (Kortekamp and Zyprian, 2003). Phenols are the most widely distributed secondary plant products (Harborne, 1989) act asan important first line plant defense response against infection by its very rapid synthesis and their polymerization in the cell wall (Matern and Kneusel, 1988).

In addition to causing accumulation of antimicrobial compounds like phenolic compounds (Ortega *et al*., 2005), the hypersensitive response also leads to an increase in the activity of various oxidative enzymes. Phenylalanine ammonia lyase, polyphenol oxidase and peroxidase are enzymes involved in phenol oxidation and correlated with plant defense mechanisms (Karban and Kuc, 1999). Phenylalanine ammonia lyase is considered to be the principal enzyme of phenylpropanoid biosynthesis, which serves as a precursor of various secondary metabolites such as lignin, suberin, wallbound phenolics, flavanoidsetc(Chen *et al*., 2000; Gerasimova*et al*., 2005).Polyphenol oxidase is an enzyme with a wide distribution among plants and catalyzes the o-hydroxylation of monophenols to o-diphenols and their oxidation to o-diquinones (Vaughn and Duke, 1984) which are often more toxic to the microorganisms than the original phenolic compounds (Gandia-Herrero*et al.*, 2005). Peroxidase contributes to resistance by participating in the cell wall polysaccharides processes such as oxidation of phenols, suberization and lignification of host plant cells during the defense reaction against pathogenic agents (Breusegem*et al*., 2001; Lin and Kao,2001).

Transpiration is essential for plants to maintain turgidity, for uptake and translocation of solute and for reduction of temperature (Devlin and Witham, 2000). Plant diseases have been reported to affect transpiration, some diseases increased the rate of transpiration (Cruickshank and Rider, 1961; Gerwitz and Durbin, 1965) while others decreased transpiration (Mignucci and Boyer, 1979; Ellis *et al.,* 1981). Only in few cases, no effect of pathogen on transpiration has been reported (Spotts and Ferree, 1979). In general, plantsurface temperature is dependent on transpiration rate. At high transpiration rate, the leaf temperature isdecreases while at lower transpiration rate, this temperature increases. Surface leaf temperature is thus anindirect parameter to evaluate the overall physiological status of a plant (Chaerle*et al.*, 2004). As such, it has been largelyapplied to monitor plant water status. It is also powerful to identify stressed plants by both bioticand abiotic stresses (Leinonen*et al.*, 2004). Thus, this method has been applied to detect the presence of several plantdiseases both in field and laboratory indicating that infrared imaging could be used to detect vascular wiltdiseased plants (Wang *et al.,* 2012). However, the potential of these methods indetectionof disease progression in dry root rot has not been explored in chickpea. Thus, we used the chickpea-*Rhizoctoniabataticola*pathosystem to evaluate the potential of biochemical assay and infrared thermal imaging as a method to discriminate susceptible and moderately resistant plants.

Therefore, as the disease has become an emerging threat to chickpea production and very little information is available on dry root rot of chickpea system, proper understanding

of pathogen distribution and its variability, the environmental factors favouring disease development, the quantitative determination of the biochemical and physiology changes in chickpea will help in better understanding of dry root rot – chickpea system.The present study has been proposed with the following objectives:

1. To survey the occurrence and distribution of dry root rot incidence in major chickpeagrowing regions of central and southern India.
2. To study morphological, cultural, pathological and molecular variability among the collected isolates.
3. To study the environmental factors influencing dry root rot development in chickpea.
4. To study the biochemical changes associated with dry root rot development in chickpea.
5. To study the physiological changes associated with dry root rot development in chickpea.

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

A brief review of literature pertaining to occurrence and distribution of dry root rot disease, symptomatology, causal organism responsible for the disease and its cultural, morphological, pathological and molecular variability among the isolates, effect of temperature, moisture and pH on the development of the pathogen, various biochemical and physiological changes occurring with respect to disease development in plants were presented in this chapter. Wherever the literature on particular aspect of *Rhizoctonia bataticola* on chickpea was scanty, it was amply supplemented with and supported by other pathogens and crops.

2.1 Occurrence and distribution of disease

An association of *Fusarium* sp. and *Rhizoctonia* sp. with wilted chickpea was first reported by Narasimhan (1929). Subsequently, it was reported from Punjab by Luthra (1938), Madhya Pradesh (Sharma and Khare, 1969; Kotasthane *et al.,* 1979), West Bengal (Biswas and Guptha, 1981) and Haryana (Tripathi and Sharma, 1983). It has been reported from Egypt, Iran, Kenya, Lebanon, Mexico, Myanmar, Pakistan, Spain Srilanka, Sudan, Syria, Tanzania, Turkey, Uganda, USA and Zambia. It causes considerable yield losses that vary from 5-50% and may cause 100% losses in susceptible cultivars under favourable conditions (Nene *et al.,* 1996).

The incidence of *R. bataticola* (*Macrophomina phaseolina*) on *Cicer arietinum* was higher in sandy than clay soil. Low soil moisture was conducive to disease development (Taya *et al.*1988).

Pandey and Singh (1990) conducted the survey in Allahabad region of Uttar Pradesh and reported that the chickpea was infected by *Rhizoctonia bataticola* in 83% of villages with less than 25% disease intensity.

Rhizoctonia bataticola isolates of chickpea from several locations in Myanmar collected by Than *et al.* (1991) reported the possible occurrence of more than one type of isolate in a same field and the existence of closely related isolates at different places.

The incidence of dry root rot in chickpea caused by *Rhizoctonia bataticola* was studied in 20 locations in Uttar Pradesh and Uttaranchal, India during February to March of 1998-2000 and reported disease was widespread in Uttar Pradesh. It was severe in bold seeded cultivars (Prajapati *et al.*, 2003).

Survey conducted by Shubha and Gurha (2006) in different blocks of Bhundelkhand region of Uttar Pradesh found infection of *Rhizoctonia bataticola* on chickpea ranged from 5-22%.

Chickpea fields in Shimoga, Raichur and Bangalore districts in Karnataka were surveyed by Gurha and Trivedi (2008) for the prevalence of dry root rot. *R. bataticola* was the predominant pathogen infecting 60 to 70% of the plants in the fields of Gulbarga, Manchalapur, Eklaspur, Raichur and Tengri.

Intensive roving survey conducted by Manjunatha *et al.* (2011) during November to December, 2008 in the farmers' fields at Raichur, Gulbarga and Bidar districts in Karnataka. The maximum dry root rot incidence in chickpea was noticed in Gulbarga district (9.8%) followed by Raichur (7.6%) and the least (6.18 %) in Bidar. The overall incidence of dry root rot ranged from1-19% across the districts. This was mainly attributed due to variation in soil type (black and red soil) and cultivars grown (Annegiri and JG 11) under rain fed condition.

The survey for the occurrence and severity of chickpea dry root rot was made during crop season 2010-11 in Jammu and Kashmir. The highest incidence was up to 40% in Shangus and lowest up to 4.11% in Naina of Jammu and Kashmir. The incidence of dry root rot in chickpea caused by *R. bataticola* was observed in late October to mid November. The intensity of the disease was high in the month of February and March during late flowering and podding stage (Khan *et al.*, 2012).

A survey was conducted in 2010-2011 rabi cropping season to obtain information on the distribution and incidence of chickpea diseases with respect to soil type, cultivar used, seed treatment in Central and Southern parts of India (Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh). Local cultivars Annigeri (19.23%) followed by Harbora (12.82%) were most frequently grown by the farmers. However, among the improved varieties JG 11, JG 130, JG 16, JG 74 and Jawahar were most commonly grown covering 34.61% area. Dry root rot disease was found in all the sites surveyed where the incidence ranged from 8.9-10.3% irrespective of cultivar type and location. The local

variety occupied 48% area in Karnataka while in Andhra Pradesh it was 39%. Disease incidence was lower on improved (9%) as compared to local varieties (14%). Sixty three percent of the farmers practiced seed treatment with fungicides. The disease incidence in seed treated fields ranged from 6.63-12.40% compared with untreated 9.22-20.02% (Ghosh *et al.,* 2013).

In soils where soybean or susceptible hosts are planted successively year after year, *M. phaseolina* populations tend to increase. Cook *et al.* (1973) reported an increase in microsclerotia in a field planted on soybeans for three years from 80, 120 to 149 microsclerotia per gram of soil.

High levels of *M. phaseolina* colonization are positively correlated with poor seed quality and lower yields. After harvesting, roots loaded with microsclerotia start decaying and release the microsclerotia into the soil (Olaya *et al*., 1996). Inoculum survival in the soil depends on soil moisture and the soil microbial community (Collins *et al.*, 1991)

Macrophomina phaseolina poses greater problem in pigeon pea at high temperature (>30^{o}C) and dry weather. This disease was more prevalent in vertisols than alfisols. Rain after a prolonged dry spell predisposed plants to disease early (Bajpal *et al*., 1999).

Rajkrishan *et al.* (1999) studied the effect of edaphic factors and moisture regime on the incidence of *Macrophomina* root rot in sesame. Amongst the various soil types, sandy soil supported maximum disease (78.33%) as compared with clay soil (51.56%). Similarly, more disease incidence of 48.33% was observed when pots were irrigated after the interval of seven days as compared to pots receiving irrigation every day.

2.2 Disease symptomatology

According to Dastur (1935) only mature plants were affected, showing bronzing of the leaves on one or more of the lower branches. The colour of leaves later changed to yellow and then brown. The affected branches and leaf stalks of diseased plants were stiff and turned upwards and leaflets stand more or less vertically and shed prematurely. The terminal part of the tap root was black or brown and shriveled.

Singh *et al.* (1990) reported that roots inoculated with *Rhizoctonia bataticola* (*M. phaseolina*) caused disintegration of cortical tissues with mycelial and sceloritial bodies plugging the xylem vessels.

The dried plants due to disease generally appeared during flowering and podding time. These were scattered in the field. The seedlings can also get infected. Drooping of

petioles and leaflets was confined to those at top of the plant. Leaves and stem of affected plant usually straw coloured but in some cases, the lower portion of the stem was brown. When the plant was uprooted, lower portion of the tap root remains in the soil. It was devoid of lateral and finer roots. Dark minute sclerotial bodies can be seen on the roots exposed inside the wood (Nene *et al*., 1991).

2.3 Pathogen

Rhizoctonia bataticola (Taub.) Butler as a plant pathogen was recognized by Halsted (1890). Taubenhaus (1913) gave the name of the genus as *Sclerotium* because of absence of spores and the species name as *bataticola* because it was pathogenic to *Ipomea batatus* (L.) Lam. Briton Jones (1925) transferred the fungus to the genus *Rhizoctonia* based on the identification of cultures by Butler (1918). Ashby (1927) accepted *Macrophomina* and rejected the binomial *Macrophomina phaseoli* and proposed a new binomial *M. phaseolina* as the pycnidial stage of *R. bataticola* on the basis that *M. phaseoli* was the earliest applicable binomial. He was not aware that Tassi had earlier described *Macrophomina phaseolina*. Haigh (1930) suggested that *R. bataticola* be used for sclerotia isolate and pycnidial strains should be called *M. phaseolina*. Goidanich (1947) examined the original material of Tassi and compared it with *Macrophomina phaseoli, M. corchori, M. cajani, M. sesami, M. philippinensis, Dothiorella cajani* and *D. phaseoli*. He confirmed all of them were identical. He corrected the mistake made by Ashby. According to the International Code of Botanical Nomenclature, the binomial *Macrophomia phaseolina* was the valid name for the pycnidial stage of *R bataticola*.

Mycelia width varied from approximately 2-11µm and distance between two consecutive septa measured 46µm. However, the most important character regarding taxonomy and classification were the production size and composition of microsclerotia (Reichert and Hellinger, 1947).

Sclerotia within plant parts were black, smooth, hard and varied in size from 100 µm-1mm while in culture, it varied from 50-300µm. These descriptions were given by Common wealth Mycological Institute (CMI), Kew, England (1970).

During the sclerotial formation, 50–200 individual hyphal cells aggregate to give multicellular bodies called microsclerotia. The microsclerotia were black and variable in size from 50–150 µm depending on the available nutrients of the substrate on which the propagules were produced (Short and Wyllie, 1978).

2.4 Morphological variability of *Rhizoctonia bataticola*

2.4.1 Hyphal variability

Gupta *et al.* (2012) observed variation in size of hyphal width of 40 *Rhizoctonia bataticola* isolates from chickpea. 18 highly virulent isolates were classified under very thin (5.2-6.5 µm), 17 highly virulent and 2 virulent isolates under thin (6.6 -7.9 µm) and only three highly virulent isolates from Chhattisgarh and Gujrat exhibited (8.0 - 9.3 µm) thick hyphal width.

Sobti and Sharma (1992) reported that the width of hyphae varied from 4.16-8.48µm in *Rhizoctonia bataticola* isolates from groundnut.

Thirty six isolates of *M. phaseolina* of mung bean collected from different region of North, South, North East and Central India were studied for morphological variability. Typical right-angled branching of mycelium was found in isolate MP-1 and it was acute to right angled in isolates MP-2 and MP 3 (Devi and Singh, 1998).

Pathogen isolation was done from T9, PU 30, TPU 4, UK 17, UPU97-10 of black gram plants by Suryawanshi *et al.* (2008) from stem and leaves. The mycelium was superficial, hyaline to brown, septate and tree like in form.

2.4.2 Sclerotial variability

Sclerotial diameter of *Rhizoctonia bataticola* in gram varied from 98.1-119.2 µm (Ghosh and Sen, 1973). Hildebrand *et al.* (1945) observed the sclerotia were black, smooth varying from spherical to oblong to irregular shaped. The sclerotial shape of any isolate varied with substrate on which fungus was grown.

Twenty three isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from 10 different major chickpea growing states (Karnataka, Haryana, New Delhi, Punjab, Uttar Pradesh, Maharashtra, Jharkhand, Rajasthan, Madhya Pradesh and Chattisgarh) of India were highly variable in their morphological characters. The sclerotia formed in different isolates were dark brown to black and size varied from 40 - 600 µm. The isolates from Bangalore (Rb1) and Faridkot (Rb 5) produced the largest size sclerotia ranged from 200 – 600 µm and 100 – 400 µm respectively. Dumka (Rb19) isolate produced small sized sclerotia which ranged from 43.9- 81 µm. They observed most virulent isolate

(Rb1 from Bangalore, Karnataka) was fast growing and produced largest sclerotia. The isolates were highly variable in virulence (Aghakhani and Dubey, 2009).

Thirty isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from three major chickpea growing regions variable in their characters were categorized into three groups. The first group comprised of all isolates from Bidar except RB7 and exhibited light brown with fluffy growth and formed dark brown sclerotia centrally with clustering growth pattern. The second group comprised of all isolates from Raichur and Gulbarga except RG3 and RG7 and also an isolate from Bidar RB7 and showed brownish black with flat sclerotia which were uniformly distributed. Third group comprised of only RG3 and RG7 isolates from Gulbarga which showed flat colony and brown coloured sclerotia formed at the center of the culture (Manjunatha and Naik, 2011).

Forty isolates of *Rhizoctonia bataticola* from chickpea were studied by Gupta *et al.* (2012) observed sclerotial sizes varied form 72.7- 117.2 x 57.1-106.5 µm. Based on sclerotial size, the isolates were divided into three groups' *viz.* minute size (72.7 - 87.5 x 57.1-73.0 µm), medium size (87.6 - 103.2 x 73.5 - 90.0 µm) and bold (103.3 -117.2 x 90.1-106.5 µm) size of sclerotia groups. Bold size of sclerotia was observed in six highly virulent isolates belong to Madhya Pradesh and Maharashtra. Rest of the thirty two highly virulent and two virulent isolates placed in minute and medium size of sclerotial groups. The isolate from Betul, Madhya Pradesh produced maximum number of sclerotia (22.3 sclerotia per microscopic field) and isolate from Dhule, Maharashtra exhibited minimum number of sclerotia (8.1 sclerotia per microscopic field). The shape of sclerotia ranged from oblong (12 isolates) to round (28 isolates).

Morphological variability among thirty six isolates of *M. phaseolina* from mung bean collected from different region of North, South, North East and Central India were studied for by Devi and Singh (1998) and observed bigger sclerotia in isolate MP-2 (400 x 280 µm).

Seven isolates of *M. phaseolina* on maize collected from Ludhiana, Hyderabad, Delhi, Arabhavi, Udaipur, Bangalore and Coimbatore by Shekhar *et al*. (2006) observed Hyderabad isolate produced highest number of sclerotia (180.3 sclerotia 9 mm^{-1} disc) of bigger size (95.7 µm) compared to other isolates. On the basis of sclerotial morphology, isolates were divided into oblong shape having a regular edges and round with regular

edges. On the basis of disease expression, Hyderabad isolate was more virulent compared to other isolates.

The diversity patterns of 15 isolates from Mexico and 15 isolates from other countries of *M. phaseolina* on beans were analyzed based on morphological characteristics such as shape (round, ovoid and irregular) and production of microsclerotia. Microsclerotia were higher in most Mexican isolates than in non-Mexican isolates. However, no significant differences between Mexican and non-Mexican isolates were found in length, width and length/width ratios of microsclerotia (Fernandez *et al.*, 2006).

Macrophomina phaseolina isolation was done from T9, PU 30, TPU 4, UK 17, UPU97-10 cultivars of black gram by Suryawanshi *et al.* (2008) from stem and leaves. The typically dark black to brown colony observed on PDA medium. The mycelium was superficial, hyaline to brown, septate and tree like in form. The sclerotia produced were round, oblong and irregular in shape.

Variations in the 35 isolates of *M. phaseolina* collected from the major sesame producing regions in central China comprising of Hubei, Henan, Anhui, Jiangxi province were studied by Linhai *et al.* (2011). Sclerotia quantity and size had high variations. Pingyu and Xiaogan produced no sclerotia after 24 h of culture and sclerotium size of 7 days old culture varied between 0.10 mm (Wuxue) and 0.16 mm (Jiayu) with average of 0.13 mm.

Isolates of *M. phaseolina* were collected from semi arid regions of Maharashtra, Andhra Pradesh and Karnataka states of India. Variability in shape, length, width and length/width in morphological characters of microsclerotia were studied and observed AP 21isolate varied in length/width ratio (483.3/200.0 μm) while no significant differences were observed in others (Prasad *et al*., 2014).

Eleven isolates of *R. bataticola* from different pulse crops *viz.*, redgram, greengram, cowpea, soybean, blackgram showed variability in sclerotial intensity, shape and intensity of pigment synthesis. All isolates were more aggressive on the original host from which it was isolated than on other hosts (Sundravadana *et al.*, 2012).

From 14 Iranian provinces, fifty two *Macrophomina phaseolina* isolates were recovered from 24 host plant species. These isolates were studied for average size and time taken for microsclerotia formation. Isolates from Khorasan and Isfashan kashan had the

largest microsclerotia while isolates Yazd and Kerman had the smallest microsclerotia (Mahdizadeh *et al.*, 2011).

Iqbal and Mukhtar (2014) collected 65 specimens of charcoal rot caused by *M. phaseolina* in mungbean from Punjab and Khyber Pakhtunkhwa provinces of Pakistan. They classified nine isolates under large sized, 26 under small sized and remaining 30 isolates as medium sized sclerotia. Maximum sclerotial size was observed in isolates MP-20 and MP-3 showing 29.00 and 27.33 µm diameter respectively, while the isolates MP-39 and MP-28 were found to be the smallest in size. The average sclerotial size of isolates ranged from17.00 to 29.00 µm.

2.5 Cultural variability among *Rhizoctonia batatiocla*

2.5.1 Variability in radial growth

Twenty three isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from 10 different major chickpea growing states (Karnataka, Haryana, New Delhi, Punjab, Uttar Pradesh, Maharashtra, Jharkhand, Rajasthan, Madhya Pradesh and Chattisgarh) of India were highly variable in their cultural characters. After 48 hours of incubation, highest radial growth in Rb1 (Bangalore) followed Rb4 (Faridkot) was observed. Rb3 (New Delhi) and Rb5 (Faridkot) were next best with statistically similar colony diameters (83.5 mm). Least and similar colony diameter of 51.3 mm was recorded in Rb7 and Rb10 (Kanpur) isolates (Aghakhani and Dubey, 2009).

Thirty isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from three major chickpea growing regions were highly variable in their cultural characters. Among these, twelve isolates were fast in growth, another twelve were moderate and the remaining six isolates were slow in growth (Manjunatha and Naik, 2011).

Dhingra and Sinclair (1973) collected nine isolates of *M. phaseolina* from various parts of United States reported variation in growth rate and colony characters. Further, the growth rate was correlated with pathogenicity.

Lokesha and Benagi (2004) reported among four isolates of *M. phaseolina* in pigeon pea maximum growth was recorded in ICRISAT isolate which was significantly superior over other isolates (82.7 mm) followed by Bidar isolate (78.35 mm).

The radial growth was found maximum after 24 hours of incubation on PDA (40.3 mm) followed by potato sucrose agar (39.0 mm) and it completely covered the PDA plate by 48 hours of incubation (Chowdary and Govindaiah, 2007).

Iqbal and Mukhtar (2014) collected 65 isolates of charcoal rot caused by *M. phaseolina* in mung bean from Punjab and Khyber Pakhtunkhwa provinces of Pakistan were studied for cultural variability. Significant differences were noticed among 65 isolates with sixteen isolate rated as fast growing, 11 as slow growing and the rest as medium growing. Maximum colony diameter of 87.17 and 86.67 mm was observed in isolate MP- 7 (Dera Ghazi Khan) and MP-26 (Layyah) proved to be the fast growing, while isolates MP-8, MP-29 and MP-30 showed minimum radial growth and rated as slow growing.

2.5.2 Variability in colony texture

Twenty three isolates of *Rhizoctonia bataticola* (*Macrophomina phaseolina*) causing dry root rot of chickpea collected from 10 different major chickpea growing states (Karnataka, Haryana, New Delhi, Punjab, Uttar Pradesh, Maharashtra, Jharkhand, Rajasthan, Madhya Pradesh and Chattisgarh) of India were highly variable in colony texture. The majority of nineteen isolates produced suppressed mycelium whereas 4 isolates in which three from Kanpur and one from Rohtak produced aerial mycelium (Aghakhani and Dubey, 2009).

Thirty isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from three major chickpea growing regions were highly variable in their colony texture. These isolates were categorized into three groups. The first group comprised of all isolates from Bidar (except RB7) exhibited light brown with fluffy growth and formed dark brown sclerotia centrally with clustering growth pattern. The second group comprised of all isolates from Raichur, Gulbarga (except RG3 and RG7) and an isolate from Bidar (RB7) exhibited brownish black with flat colony and sclerotia which were uniformly distributed. Third group comprised of only RG3 and RG7 isolates from Gulbarga which showed flat colony and brown colour sclerotia formed at the center of the culture (Manjunatha and Naik, 2011).

Dhingra and Sinclair (1978) noticed fluffy growth in stem isolates, partially fluffy growth in root isolates and appressed growth in all other plant part isolates of *Macrophomina phaseolina* on soybean.

Thirty six isolates of *M. phaseolina* of mung bean collected from different region of North, South, North East and Central India and observed MP-1 isolate had cottony mycelial growth while MP-2 isolate showed 5 μm appressed mycelial growth (Devi and Singh, 1998).

The diversity patterns of 15 isolates from Mexico and 15 isolates from other countries of *M. phaseolina* on beans were analyzed based on presence or absence of aerial mycelium. They observed 15 isolates had intermediate and 15 isolates had poor aerial mycelium. Eight from Mexican isolates and 7 isolates of non Mexican isolates had intermediate aerial growth (Fernandez *et al.*, 2006).

Edraki and Banishashemi (2010) studied sixty isolates of *M. phaseolina* collected from different parts of Iran on various crops like soyabean, cucumber and sesame. Isolates were grouped in to four phenotypes *viz.*, fluffy with abundant sclerotia, fluffy with few sclerotia, partially fluffy and appressed growth.

Variability among the eleven isolates of *R. bataticola* from different pulse crops (redgram, greengram, cowpea, soybean, blackgram) were categorized into three groups *viz*., linear, fluffy and linear at the end with fluffy growth at the centre. All isolates were more aggressive on the original host from which it was isolated, which was shown by the variability in pathogenic characters (Sundravadana *et al*, 2012).

2.5.3 Colony pigmentation

Twenty three isolates of *Rhizoctonia bataticola* (*Macrophomina phaseolina*) causing dry root rot of chickpea collected from 10 different major chickpea growing states (Karnataka, Haryana, New Delhi, Punjab, Uttar Pradesh, Maharashtra, Jharkhand, Rajasthan, Madhya Pradesh and Chattisgarh) of India were highly variable in pigmentation of isolates. Pigmentation varied from white to dull white, creamy, grey and black. Black was prominent among the isolates (Aghakhani and Dubey, 2009).

Thirty six isolates of *M. phaseolina* of mung bean collected from different region of North, South, North East and Central India were studied for colony colour. MP-1 isolate had pale grey colonies while brown in MP-2 (Devi and Singh, 1998).

The diversity patterns of 30 isolates of *M. phaseolina* of beans 15 from Mexico and 15 from other countries were analyzed based on morphological characteristics such as

colour of colony (grey and black) and observed 22 isolates were grey, 4 isolates were white and 4 isolates were black in colour (Fernandez *et al.*, 2006).

Aboshosha *et al.* (2007) observed colonies of *Macrophomina* isolates of sunflower charcoal rot were mostly grey, dark grey, black and brown for fourteen stem isolates in frequencies of 4, 3, 5 and 1 on PDA and 4, 5, 2 and 2 on Czapek-Dox Agar while white colony was observed in only one isolate on both media.

2.6 Pathogenic variability

Jayanti and Bhatt (1993) sown pre-germinated seed of 21 chickpea varieties in soil inoculated with *R. bataticola* which caused seed rot within 24h in NEC874 and EG234. Bold 2375, BG209, JG62, JG315, ICC 3357 and JG1133 developed necrotic lesion in 3-5 cells deep at hypocotyl region within 7 days. RSG-44, AGC677, NEC41, GL269, JG74, ICC8983 and ICC 5003 developed only superficial necrosis along the hypocotyl region. BGM 416, BG 416, ICC 1376 and ICC 113314 were resistant. Resistant cultivars had a greater number of lateral roots during early growth phases.

Pande *et al.* (2004) screened 29 chickpea germplasm lines and 10 cultivars against dry root rot caused by *Rhizoctonia bataticola* in chickpea using paper towel method developed by Nene *et al.* (1981). Germplasm line ICC 14395, cultivar ICCV 2 and advanced breeding line (ICCX830203-BH-BH-11H) were found resistant to dry root rot, 22 moderately resistant, 19 susceptible and 3 highly susceptible were observed in the remaining lines. The disease severity in the two susceptible line BG 212 and ICC 12267 used as control was rated highest. The identified genotypes were used as source of resistance to dry root rot.

Pot culture experiment was conducted by Jayalakshmi *et al.* (2008) for screening 12 promising entries of chickpea (JG-11, GBS 964, GBS 963, GBC 2, GBM 6, GBM 2, GBM 10, BGD 103, GCP 107, GCP 101, A1, ICCV 10, L- 550 + 1 susceptible check JG-62) against dry root rot during rabi season, 2004-05 and 2005-06. Out of 12 genotypes tested against root rot disease, 4 resistant, 2 moderately susceptible, 3 susceptible and 3 highly susceptible to dry root rot disease were observed.

Forty isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea collected from four major chickpea growing states *viz*., Madhya Pradesh, Chhattisgarh, Gujarat and Maharashtra of central India were screened for virulence of the isolates on susceptible

chickpea variety BG 212 by blotter paper technique. The highly virulent isolates from Madhya Pradesh, Chhattisgarh, and Maharashtra were fast growing isolates and exhibited largest sclerotia. The sclerotial size and number of sclerotia of *R. bataticola* isolates had positive correlation with virulence (Gupta *et al,* 2012).

Lekhraj *et al.,* (2012) conducted pathogenic variability of *Macrophomina phaseolina* isolates collected on infected chickpea from 9 various parts of Rajasthan, Hissar and New Delhi. Pathogenicity of these isolates tested on 40 day old chickpea cv. C-235 showed there was no direct relationship between cultural characters and virulence of the isolates. The isolate BKN had scanty, suppressed growth was highly pathogenic. Isolates SGN, NHR, DUR, HAU, DEL and HNG having different types of colony growth were moderately pathogenic and the isolates CHR and UPR with different mycelium characters were less pathogenic. Similarly, there was no apparent correlation between the virulence and growth rate of the fungal colonies.

Fifty isolates of *Rhizoctonia bataticola* collected on chickpea from different agro climatic zones in India were evaluated for the degree variability in pathogenicity test. All the isolates proved pathogenic on chickpea cultivar BG 212. The dry root rot disease severity ranged from 4 to 9 (1-9 severity rating scale). The effect of isolates on severity was significant. The pathogenic and non pathogenic isolates were not concentrated in any one particular state/region (Sharma *et al,* 2012).

Among the 94 isolates of *R. bataticola* collected from different agro ecological zones, 8 isolates from Andhra Pradesh and Madhya Pradesh showed less aggressive on chickpea cultivar BG 212 (Sharma *et al*., 2012).

2.7 Genetic diversity in *Rhizoctonia bataticola* using RAPD marker

Jana *et al.* (2003) showed 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.

Universal rice primers were used to cluster 40 isolates of *M. phaseolina* from *soybean*, cotton and chickpea (Jana *et al*. 2005).

Thirty isolates of *Rhizoctonia bataticola* from chickpea were studied for molecular variability by Manjunatha (2009) using three primers OPO-10, OPO-12 and OPN-12. There were four isolates in Group-I, 16 isolates in Group-II and 10 isolates in Group-III.

However, in Group-II, three isolates (RG-18, RG-19 and RG-20) exhibited 100 per cent similarity. RB-29, RB-25 and RG-16 isolates from Bidar and Gulbarga respectively showed genetically diversity among thirty isolates.

Bayraktar and Dolar (2009) observed intra and inter specific polymorphism among fungal pathogens that cause root rot on chickpea by using 30 RAPD primers. UPGMA cluster analysis of RAPD datasets using Dice's coefficient differentiated all fungal isolates from each other and revealed considerable genetic variability between the isolates.

Genetic diversity of 23 isolates from chickpea and 4 from other host crops (mung bean, urd bean, bean leaf, ground nut) of *Rhizoctonia bataticola* representing 11 different states of India were studied by Aghakhani and Dubey (2009) using RAPD. The clusters generated by RAPD grouped 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 primers *viz.,* OPN 4, OPN 12 and OPN 20 clearly distinguished majority of the isolates into the area specific groups based on variability in virulence.

Fifty isolates of *Rhizoctonia bataticola* collected from different agro climatic zones in India were evaluated for the degree of subdivision in isolates level by Sharma *et al.,* (2012). Genetic characteristics were analyzed based on the sequence of rDNA- ITS region. The phylogenetic tree based on rDNA-ITS analysis showed maximum number of *R. bataticola* isolates were very diverse and did not depend on geographical origin. Both pathological and molecular data correlated with each other supported that the *R. bataticola* present in India were very diverse and independent to their origin.

M. phaseolina isolates of maize from Ludhiana, Hyderabad, Delhi, Arabhavi, Udaipur, Bangalore and Coimbatore analyzed through RAPD marker for genetic diversity by Shekhar *et al.* (2006). The UPGMA cluster analysis for 706 loci score permitted for identification of three main clusters. Similarity matrix and Jaccard's similarity coefficient between the isolates indicated that the maximum genetic variation was among isolates of Arabhavi and Coimbatore with 70.8% followed by Ludhiana and Coimbatore with 69.5%. The most closely related isolates were Hyderabad and Delhi with an affinity percentage of 65.5% followed by Udaipur and Bangalore isolates with 62.9% similarity.

Fourteen isolates of *Macrophomina phaseolina* collected from different groundnut growing regions in India were studied for their virulence and genetic diversity using PCR-

RAPD markers by Kumar and Gaur (2011). A total of 85 amplicon levels were obtained with 7 primers with an average of 12.1 bands per primer were available for analysis, of which 83 were polymorphic (97.74%). It was possible to discriminate all the isolates with any of the 7 primers employed. The UPGMA clustering of data indicated the isolates shared varied levels of genetic similarity within a range of 0.069 to 0.65 similarity coefficient index. Isolates Bikaner, Churu, Delhi, Hissar which were clustered in one group happened to be more virulent reflecting correlation of similarity with pathogenicity to some extent but there was practically no direct correlation between the virulence and RAPD genetic markers of all the isolates.

The genomic DNA of 21 isolates of *Rhizoctonia bataticola* of soybean from Jabalpur were subjected for diversity analysis with 8 randomly selected decamer primers which amplified 64 RAPD marker loci. Out of these 64 bands, 29 bands (45.3%) were polymorphic. Similarity coefficient values for 21 accessions of *R. bataticola* in RAPDs marker was 0.69–0.98 (Pancheswar *et al*, 2012).

2.8 Influence of temperature on *Rhizoctonia bataticola*

Rhizoctonia bataticola on chickpea grew over a wide range of temperatures *viz.*, 10ºC to 45ºC. The optimum temperature for its growth was found to be 30ºC while the next best was 35ºC. Statistically the growth of the pathogen gradually decreased both below and above the optimum temperature (30ºC). Minimum growth was recorded at 10ºC (Khan *et al*, 2012).

The growth and sclerotial production of *M. phaseolina* causing root rot in mulberry at different temperatures *in vitro* were studied by Chowdary and Govindaiah (2007) recorded maximum growth of 90mm at 30 and 35ºC and minimum of 40 mm at 20ºC. Sclerotial production was observed in all the temperatures except at 20ºC.

Variability in *Rhizoctonia bataticola* isolates from roots, leaf and seeds of pulses *viz.*, blackgram, greengram, cowpea, soybean, and redgram were studied at different temperatures. It was observed that 15°, 20° and 25°C had recorded mean mycelial growth of 26.83, 34.23 and 48.51 mm respectively. The maximum mycelial growth of 74.76 and 74.20 mm was recorded at 30˚ and 35°C respectively. As the temperature increased from 10° to 30°C, the mycelial growth gradually increased and at 35°C there was gradual

decrease. Temperature was the specific limitation for the growth and its pathogenicity (Sundravadana *et al.,* 2012).

Lotfalinezhad *et al.* (2013) collected forty three isolates of *M. phaseolina* on different hosts (canola, cotton, melon, olive, pine, potato, safflower, soybean, sunflower, tomato and watermelon) from North, Northwest and Southwest of Iran. The optimum temperature for growth was 25ºC for 33 isolates and 33ºC for 10 isolates, when these isolates were incubated at 5, 10, 15, 20, 25, 30, 33, 35 and 40ºC. Isolates growth rate varied considerably at all temperatures but maximum variability occurred at 20 and 33ºC between isolates. They concluded environmental condition of the North and Northwest areas of Iran were similar but differ from those of the Southwest. High optimum temperature was detected for growth of southwest isolates as they were from warm area in contrast to other isolates.

2.9 Influence of temperature on disease development

In chickpea, seed exudates increased when incubated at 35ºC than 15 and 25ºC was reported by Singh and Mehrotra (1982). They also observed that increased seed exudation was a major factor contributed to increased pre-emergence damping off of gram seedling by *R. bataticola* at high temperatures.

Severity of dry root rot in chickpea with respect to temperature was studied by Sharma and Pande (2013) under controlled environment. Out of five temperature regimes *viz.*,15°C, 20°C, 25°C, 30°C and 35°C, optimum temperature for disease severity was 35°C with maximum disease severity of 9 on 1-9 severity rating scale. This was followed by 30°C, 25°C, 20°C and 15°C. The disease severity was very low (2 - 3 rating on 1-9 scale) at 20 and 25°C. The control plants did not show any symptoms at their respective temperatures. They confirmed that high temperature predisposed chickpea to *R. bataticola* infection, colonization and development.

Agarwal *et al.* (1973) reported that charcoal rot in soybean found to occur only at temperatures between 25º to 40ºC.

Ratnoo *et al*. (1997) reported the infection and development of ashy grey stem blight in cowpea caused by *M. phaseolina* was most favoured by high temperature 25 - 40ºC.

2.10 Effect of osmotic potential on radial growth

Olaya and Abawi (1996) studied the influence of water potential on growth of *Macrophomina phaseolina* in bean was determined at 30°C on potato dextrose agar

adjusted to different water potentials with KCl, NaCl or sucrose. Radial growth of *M. phaseolina* on PDA was maximum at water potential values between -1,220 and -1,880 J kg^{-1}. Growth was reduced at lower water potential values and completely inhibited at water potential between -8,270 and -12,020 J kg^{-1}. The influence of water potential adjusted by KCl, NaCl or sucrose on *M. phaseolina* followed a similar pattern, but growth of *M. phaseolina* was much greater in media adjusted with sucrose. Results of this study showed that *M. phaseolina* can grow and produce large quantities of sclerotia under relatively low water potentials.

Garcia *et al.* (2003) studied influence of osmotic potential on *in vitro* growth and morphology of four isolates of *Macrophomina phaseolina* in common bean was studied using potato glucose agar adjusted to different osmotic potentials with KCl, NaCl and sucrose. The three sources of osmotic potential reduced *M. phaseolina* growth under *in vitro* conditions. NaCl caused the highest negative effects. Concentrations of NaCl higher than 250 mM showed significant reduction of *M. phaseolina* growth in all isolates, while 1000 mM of NaCl inhibited growth completely. Sucrose did not cause significant reductions on *in vitro* growth of *M. phaseolina* at low concentration while growth was favoured. The high solute concentrations reduced the synthesis of mycelial pigments and the size and shape of microsclerotia.

The effect of osmotic potential on mycelial growth and sclerotial production of *Rhizoctonia solani* [Anastomosis Groups (AGs) 2-1 and 3] from potato were studied by Ritchie *et al.* (2006) on potato dextrose agar adjusted osmotically with sodium chloride, potassium chloride and glycerol. All isolates from AGs 2-1 and AG-3 exhibited fastest mycelial growth on unamended PDA and growth declined with decreasing osmotic potentials. Growth ceased between -3.5 and -4.0 MPa on osmotically adjusted media with slight differences between isolates. Sclerotia yield in AG 2-1 and AG-3 declined with decreasing osmotic potential and formation ceased between -1.5 and -3.0 MPa and -2.5 and -3.5 MPa respectively.

2.11 Effect of moisture on disease development

Dry root rot was observed in gram when exposed to temperature more than 30ºC. The disease was aggravated by dry soil conditions especially at flowering stage leading plants to sudden drying (Singh and Mehrotra, 1982).

Colonization of *M. phaseolina* on chickpea roots was high when subjected to moisture stress conditions compared to unstressed plants. Soils artificially inoculated

produced poor growth of plants both in low and high moisture compared to natural soil. Root colonization was greater at 40-50% moisture holding capacity as compared to 10-20% soil moisture holding capacity (Husain and Ghaffar, 1995).

Patel and Anahosur (2001) conducted an experiment with chickpea cultivars ICC 4951 (susceptible) and Bheema (resistant) in earthen pots filled up with artificially infected soil with *Macrophomina phaseolina* at four levels of soil moisture *viz.*, 25, 50, 75 and 100%. They observed *M. phaseolina* infection was maximum at 25% and minimum at 100% soil moisture. ICC 4951 developed higher disease than Bheema at 25% soil moisture. Both cultivars showed maximum disease incidence at the lowest soil moisture level compared to high moisture.

Role of soil moisture on disease severity of dry root rot in chickpea was studied by Sharma and Pande (2013) under controlled environment. The plants exposed to 40% moisture stress showed higher mortality as compared to 60%, 80% and 100%. It was observed that 40% soil moisture was insufficient for the normal growth of the plants as the plants grown in control showed the physiological stress (wilting of the plants due to lack of moisture). At 60% soil moisture, no physiological stress was found in control plants and dry root rot severity was maximum 35 days after imposing moisture stress. Disease progressed slowly at 80% and 100% soil moisture had least disease severity.

When cotton plants grown in soil temperatures of 20 - 40ºC were subjected to soil water stress, the severity of *Macrophomina phaseolina* was much greater in moisture stress condition than those provided with sufficient soil water (Ghaffar and Erwin, 1969).

Ratnoo *et al.* (1997) reported the infection and development of ashy grey stem blight in cowpea caused by *M. phaseolina* was most favoured by higher temperature 25-40ºC and low in flooded soil compared to dry soil of 40-60% soil moisture.

Role of edaphic factors on the incidence of dry root rot of sesame caused by *Rhizoctonia bataticola* were studied by Rajkrishan *et al.* (1999). Among various soil types, sandy soil supported maximum disease incidence (78.33%) as compared with clay soil (51.56%). More disease incidence of 48.33% was observed when pots were irrigated after the interval of seven days as compared to pots receiving irrigation every day.

Wokocha (2000) observed the disease incidence of dry root rot of soybean was significantly low when the soil moisture was 60 - 70% but it was 5.0% at 10 - 20% soil moisture.

2.12 Influence of pH on *Rhizoctonia bataticola*

Dry root rot caused by *Rhizoctonia bataticola* on chickpea could grow over a wide range of pH from 3.0 to 9.0. The optimum pH for its growth was found to be 5.5 followed by 6.0. However with increase up to 7.0 and thereafter it declined. The minimum growth of the pathogen was recorded at pH 3.0 (Khan *et al.*, 2012).

R. bataticola isolates collected from 19 districts of Madhya Pradesh on13 different crops were evaluated for responses to different pH levels *viz.*, 5.5, 6.0, 6.5, 7.0 and 7.5. Good growth was observed at pH 5.5 - 7.5, but pH 7.0 was optimum (Jha and Sharma, 2005).

Singh and Chohan (1982) reported pH 5.0-6.0 as the optimum for the mycelium development of *Macrophomina phaseolina* causing charcoal rot in muskmelon.

2.13 Biochemical changes due to pathogen and stress in host

2.13.1 Total sugars, reducing sugars and non reducing sugars

Carbendazim, carboxin, captan and thiram (0.2%) used as seed treatment, soil drench and seed treatment + soil drench significantly reduced the incidence of dry root rot of chickpea with a corresponding increase in carbohydrates in the plants (Rajider and Sindhan, 1998).

Cluster bean genotypes 116565 (moderately resistant) and 116676 (highly susceptible) were grown in sick and control soil with *Macrophomina phaseolina*. At 65 days after sowing, leaves and roots of sick and control plants were collected and analyzed. Total soluble sugars and reducing sugars decreased with pathogen inoculation (Joshi *et al.*, 2003).

2.13.2 Phenols

Carbendazim, carboxin, captan and thiram (0.2%) used as seed treatment, soil drench and seed treatment + soil drench significantly reduced the incidence of dry root rot

of chickpea with a corresponding increase in phenolic compounds in the plants (Singh and Sindhan, 1998).

Trichoderma viride and *T. harzianum* used as seed treatments, soil inoculum and soil inoculum + seed treatments proved effective in reducing the incidence of dry root rot (*Rhizoctonia bataticola*) in chickpea with increased levels of phenols (Singh *et al.,* 1998).

Cell wall protein extracted from *Macrophomina phaseolina* was tested against chickpea to elucidate elicitor properties. The chickpea seedlings exposed to cell wall protein showed enhanced synthesis of phenol relative to water treated control. Charcoal rot of chickpea was significantly reduced in seedlings following cell wall protein treatment. The results suggested cell wall protein of *Macrophomina* had elicitor property and effectively induced resistance in chickpea (Ratul *et al*., 2006).

Khirbat and Jalali (2003) conducted field experiment to investigate the effect of blight (*A. rabiei*) on the total phenol in chickpea cultivars E100Y (resistant) and H 208 (susceptible). The pathogen isolates from the cultivars H-208 and Pb 7 were used. Challenge inoculation with both isolates at 2 to 10 day intervals resulted in an increase in the total phenol content in the resistant cultivar and decrease in total phenol content in the susceptible cultivar.

Induction of some defense related enzymes and phenolics in roots of two different genotypes of chickpea cultivars which were susceptible (L 550) and resistant (ICCV 10) to wilt disease treated with salicylic acid, spermine, salicylic acid + spermine and *Fusarium oxysporum* f. sp. *ciceri* was investigated. Higher levels phenols were observed in roots of resistant cultivar than that of susceptible cultivar on treatment with elicitors and pathogen. The pathogen invasion was more in susceptible cultivar compared with resistant cultivar. Further, the invasion was restricted in roots of resistant cultivar treated with salicylic acid. These results suggest that accumulation of phenolics might have contributed to restrict the invasion of *F.oxysporum* f. sp. *ciceri* in resistant cultivar ICCV10 (Raju *et al.,* 2008).

Rathod and Vakharia (2011) conducted experiment to see the changes in total phenol content at different stages of infection of wilt disease in chickpea roots. The results indicated that total phenol content was significantly higher in root of all the cultivars obtained from sick plot. Total phenol in root tissues obtained from sick plot revealed higher amount of total phenol (0.83 mg g^{-1}fresh weight) as compared to the tissue received from

normal plot (0.65 mg g^{-1} fresh weight). Among the cultivars, GG-2 showed maximum amount of total phenol content (0.88 mg g^{-1} fresh weight) while susceptible cultivar JG-62 contained the lowest value of total phenol.

The maximum accumulation of phenolic acids in infected guar plants by *Macrophomina phaseolina* was revealed to be 23% higher than in control after 120 hours of infection in all the four cultivars. Phenolic acid accumulation in the compatible host pathogen combination presumes that it participated actively in the guar resistance to root rot (Sharma *et al.,* 2011).

Cluster bean genotypes 116565 (moderately resistant) and 116676 (highly susceptible) were grown in pots and inoculated with *Macrophomina phaseolina*. At 65 days after sowing, leaves and roots of inoculated and uninoculated plants were collected and analyzed. Total phenol content increased with pathogen inoculation and was higher in moderately resistant genotype than in highly susceptible genotype (Joshi *et al.,* 2003).

Bean seeds inoculated with both *Macrophomina phaseolina* and *Botrytis cinerea* had higher total phenol contents as compared to control seeds and the concentrations recorded an uptrend up to 20 days of storage after inoculation and thereafter started declining. Phenols were higher in relatively Giza-6 cultivar than in Local variety (22.0 mg g^{-1} and 11.23 mg g^{-1} fresh weight respectively). In case of inoculated seeds with *M. phaseolina* maximum phenols compounds content was attained in samples incubated at 35˚C for 20 days in both cultivars (Zahra, 2012).

2.13.3 Enzymes

Cell wall protein extracted from *Macrophomina phaseolina* was tested against chickpea to elucidate elicitor properties. The chickpea seedlings exposed to cell wall protein showed enhanced synthesis of phenylalanine ammonia lyase and peroxidase relative to water treated control. Charcoal rot of chickpea was significantly reduced in seedlings following cell wall protein treatment. The results suggested cell wall protein of *Macrophomina* had elicitor property and effectively induced resistance in chickpea (Ratul *et al*., 2006).

Cherif *et al.* (2007) showed induced resistance through activation of key enzymes *viz.,* peroxidase, polyphenoloxidase in phenylpropanoid and isoflavonoid pathways which play crucial role in the biological control and resistance of chickpea to pathogenic attacks.

Induction of some defense related enzymes in roots of two different genotypes of chickpea *viz.,* susceptible (L550) and resistant (ICCV10) to wilt disease treated with salicylic acid, spermine, salicylic acid + spermine and *Fusarium oxysporum* f. sp. *ciceri* was investigated. Higher levels of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were observed in roots of resistant cultivar than that of susceptible cultivar on treatment with elicitors and pathogen. However, no major changes were observed in susceptible cultivar after the treatments. The pathogen invasion was more in susceptible cultivar compared with resistant cultivar when observed under microscopic studies. Further, the invasion was restricted in roots of resistant cultivar treated with salicylic acid. These results suggest that induction of defense enzymes and accumulation might have contributed to restrict the invasion of *F.oxysporum* f. sp. *ciceri* in resistant cultivar ICCV10 (Raju *et al.,* 2008).

Phenylalanine ammonia lyase activity was also significantly increased by 37% after 96 or 120 hours of infection depending upon the cultivar in comparison to control. Enhanced phenylalanine ammonia lyase activity in the compatible host pathogen combination presumes that both participated actively in the guar resistance to root rot (Sharma *et al.,* 2011).

Changes in defense enzymes of guar leaves were compared by spray of fungal suspension and sorghum seed infection by *Macrophomina phaseolina*. Induction of infection in guar by either mode of infection showed a significant increase in the activity of phenylalanine ammonia-lyase. Result also showed increase in activities of all defense related proteins to varying degrees at different stages of infection. Further, extent of defense response in RGC 1031 was more than that of rest three cultivars, which proves this cultivar was more potent in fighting against the invading pathogen. Thus there was a possibility of gene transfer from RGC 1031 to other cultivars (Sharma *et al.,* 2012).

Induction of defense enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase content were studied by treating greengram plants with *Macrophomina phaseolina.* The accumulation was greater in treated plants as compared to untreated control. The induced systemic resistance was enhancing the disease resistance in greengram plants against leaf blight disease by the application of plant extracts and fungicides (Sundaramoorthy *et al.,* 2013).

Cluster bean genotypes 116565 (moderately resistant) and 116676 (highly susceptible) grown in pots were inoculated with *Macrophomina phaseolina*. At 65 days after sowing, roots of inoculated and uninoculated plants were collected and analyzed for biochemical parameters showed increased activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase in roots of both genotypes due to pathogen inoculation. The activity of these enzymes was higher in moderately resistant genotype than in highly susceptible genotype (Joshi *et al.*, 2003).

Oxidative enzymes in bean seeds infected with *Botrytis cinerea* and *M. phaseolina* increased gradually in both tested cultivars, but the increase in enzyme concentration in Giza-6 cultivar was more than in Libyan cultivar. The higher increase was obtained after 10 days from inoculation in both cultivars and continued for up to 20 days in incubation then these enzyme activities decreased in healthy and inoculated seeds of both cultivars. The increased peroxidase activity was observed in seed inoculation more than pholyphenol oxidase activity. The role of peroxidase in plant resistance had been attributed to its ability to oxidize important metabolites either of the pathogen or the host plant (Zahra, 2012).

The increased reactive oxygen species formation was associated with induction of defense response in the moth bean against *Macrophomina phaseolina*. Changes in peroxidase activity were determined in control and fungal pathogen inoculated plants of two varieties *viz.* RMO-40 and FMM-96. The peroxidase activity was higher in the pathogen inoculated plants as compared to control. Varietal differences were also observed as the peroxidase activity was comparatively higher in variety FMM-96 (moderately resistant) as compared to RMO-40 (susceptible variety). The data indicate hydrogen peroxide production and a distinct role of peroxidase in the defense response of moth bean plants against disease (Indu and Sharma, 2011).

2.14 Growth changes due to pathogen and stress in host

Greenhouse studies were undertaken to determine the impact of different soil conditions on seedling establishment, development of root rot and productivity of desi (Tyson) and kabuli (Sanford) chickpea plants sown in the presence of *R. solani* AG-4. The host responded to warm soils by increasing its growth rate and the pathogen by increasing its virulence. The kabuli cultivar Sanford showed greater susceptibility to root rot caused by *R. solani* than the desi cultivar Tyson. Shoot biomass of infected treatments for kabuli

was substantially lower than the controls while the biomass of Tyson was affected to a lesser degree (Chang *et al*, 2004).

Root biomass had been identified as the most promising plant traits in chickpea for terminal drought tolerance. ICCV-4958, H-208, HC-5, RSG-931 and CSJ-379 had wide adaptability to drought prone areas at national level were assessed for various root characteristics under two environments *i.e*. irrigated and rainfed. Biomass per plant of the root was higher in ICCV-4958 (6.7 g) and HC- 5 (5.6 g) under rainfed conditions. Similar observations were recorded for root/shoot ratio, dry weights of stem, leaf and total dry weight per plant. The moisture stress increased biomass partitioning towards the roots (Zaman-Allah, 2011).

Drought stress showed higher negative effects than *M. Phaseolina* on water relations at vegetative growth in common bean. It decreased dry weight of all vegetative structures. It increased charcoal rot disease in inoculated and moisture stress condition (Mayek *et al*, 2002).

2.15 Physiological changes due to pathogen and stress in host

Siddiqui (2004) conducted glass house studies to assess the effects of soil inoculants and wilt fungus, *Fusarium oxysporum* f. sp. *ciceri* on growth and transpiration of chickpea. Inoculation of *F. oxysporum* f. sp. *ciceri* reduced plant growth, transpiration and caused severe wilting. Application of soil inoculants alone or in combination increased chickpea growth and transpiration from 1st week onwards. However, *T. harzianum* alone had no effect on growth and transpiration of chickpea in plants without *F. oxysporum* f. sp. *ciceri* while *Rhizobium* and *G. fasciculatum* increased growth and transpiration from 1st week onwards. Use of *T. harzianum* alone caused a greater increase in growth and transpiration of *F. oxysporum* f. sp. *ciceri* inoculated plants than *Rhizobium*. Better growth and more transpiration of *F. oxysporum* f. sp. *ciceri*-inoculated plants were observed when all the three soil inoculants were applied together.

Physiological responses of tea plants to Phomopsis infection in term of transpiration rate and stomatal conductance were studied in susceptible TRI-2024 and tolerant TRI-QOES cultivars. Growth characteristics such as height, dry weight and plant strength of infected and healthy plants were also studied. The results revealed that all the growth characteristics and physiological responses were reduced significantly in infected plants

than healthy plants. The reduction was more prominent in susceptible cultivar than in tolerant ones (Ponmurugan and Baby, 2007).

Charcoal rot caused by *Macrophomina phaseolina* in soybean was a soil borne disease associated with hot dry weather. To separate the affects of disease from drought, four soybean cultivars *viz.,* DT97-4290, DPL 4546, R01-581F and LS980358 were grown in microplots with soil that was either infested or non-infested with *M. phaseolina*. Half of the plots were kept well watered and the other half under water stress. Canopy temperature and stomatal conductance of plants was measured periodically though out the season. Based on infared radiation, water stressed plants and infested plants had higher canopy temperatures than well watered or noninfested plants. Water stressed plants and infested plants had lower stomatal conductance than well watered or non-infested plants. These results suggest that infection with *M. phaseolina* may limit the water uptake in the plant before the onset of visible symptoms (Doubledee, 2010).

MATERIAL AND METHODS

Chapter III

MATERIALS AND METHODS

All the investigations were conducted under laboratory and glasshouse during 2013-14 and 2014-15 at Legumes Pathology laboratory, International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Patancheru, Hyderabad, India situated at 17.53^{o} North latitude, 78.27^{o} East longitude at an Altitude of 545 MSL.

3.1. Survey and collection of *Rhizoctonia bataticola* isolates

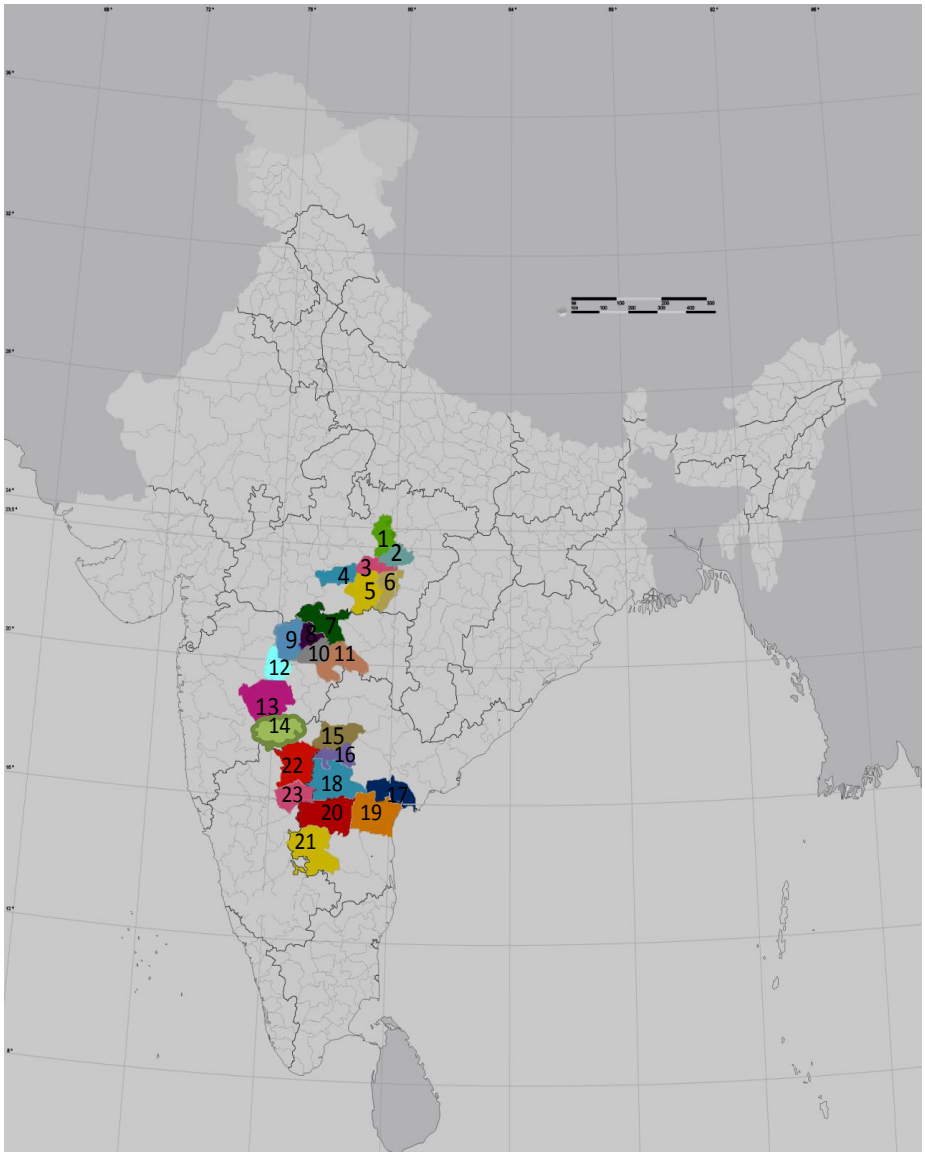
A roving survey was conducted to record the occurrence and distribution of dry root rot of chickpea in 23 districts of the five major chickpea growing states in central and southern parts of India *viz*. Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh and Telangana during *Rabi* 2013-14 (Fig 3.1). The districts in each region were selected randomly. The number of fields visited per district ranged from 20 to 30 and a distance of 15 - 20 km was allowed between sites, but the distance was greater where chickpea fields were far apart, resulting in a less number of sites visited in such districts. A total of 68 fields covering 23 districts were surveyed and information on soil type, cultivars grown, disease incidence and agronomic practices followed were recorded.

Four 1m^2 quadrants were randomly selected in each field and infected plants were counted in each quadrant. Based on infected and total number of plants, disease incidence was calculated. Chickpea plants showing the typical dry root rot symptoms were collected from surveyed areas, packed in labeled paper bags and brought to the laboratory for isolation of the pathogen.

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

3.2 Isolation and purification of *Rhizoctonia bataticola* isolates

Plants showing typical dry root rot symptoms were washed under running tap water and blot dried. Infected roots were cut into pieces of 5-6mm size and were surface sterilized by dipping in 0.8% sodium hypochlorite for 2 min. After thorough washing in sterile distilled water, the pieces were then transferred by using forceps on to sterilized potato dextrose agar (PDA) medium in Petri dishes and incubated at 25 ±2°C to obtain mycelial growth. After 48h of incubation, hyphal tips of the growing mycelium were marked on the



1. Damoh **2.** Jabalpur **3.** Narsingapur **4.** Hoshangabad **5.** Chindwara **6.** Seoni **7.** Amaravathi **8.** Akola **9.** Buldhana **10.** Wasim **11.** Yavatmal **12.** Jalna **13.** Beed **14.** Osmanabad **15.** Medak **16.** Rangareddy **17.** Guntur **18.** Mahboobnagar **19.** Prakasham **20**. Kurnool **21.** Anantapur **22.** Gulbarga **23.** Raichur

Fig 3.1. Map showing different districts surveyed for occurrence and distribution of dry root rot in different states of India

underside of the Petri dish with a glass marker by viewing through a light microscope. The hyphal tips from margins of resulting colonies were cut with the help of sterilized 2mm cork borer and transferred to Petri dish containing PDA. The cultures were purified by single sclerotial isolation and transferred to PDA slants. On the basis of morphological characters of mycelium and sclerotia, the isolates were identified as *Rhizoctonia bataticola.* The pure cultures of all the 68 isolates grown on PDA slants were stored at 4 ±1°C for further studies.

3.3 Variability among *Rhizoctonia bataticola* isolates

3.3.1 Morphological and cultural characterization of *Rhizoctonia bataticola*

All the 68 isolates of *Rhizoctonia bataticola* were characterized for morphological and cultural characteristics. The mycelial discs of 5 mm diameter were cut from the edge of a three days old culture and transferred aseptically to 80mm Petri dish containing 15ml PDA. These plates were incubated at 35°C with 12h photoperiod. Each treatment was replicated thrice.

The colonies of isolates were characterized for growth rate at 72h after incubation. Seven days old cultures were used to record texture, colour and presence or absence of aerial mycelium. At the seventh day after incubation length and width of hyphal cells, number of sclerotia/microscopic field (10x), sclerotia size and shape were recorded by using Q-capture image analyzer. All the cultures were observed daily for recording the time taken for sclerotial initiation.

3.3.2 Characterization of *Rhizoctonia bataticola* isolates for pathogenicity

Pathogenicity test for all the 68 isolates was performed in laboratory on a susceptible genotype BG 212 by paper towel technique (Nene *et al.,* 1981). Inoculum was prepared from the seven days old culture of *Rhizoctonia bataticola* grown on 100ml potato dextrose broth medium (PDB). The culture of each isolate was grinded in a blender by adding 50ml of sterile distilled water to each fungal mat. Seven day old seedlings of BG 212 grown in sterilized sand were uprooted, washed under running water and were inoculated by dipping in the inoculum of *Rhizoctonia bataticola* for 2 min. Seedlings inoculated with sterile deionized water served as control. Seven to ten inoculated seedlings were placed in paper towel with the shoot left outside, folded, moistened and placed in trays. Trays were transferred to incubators maintained at 35 ± 1°C with 12h photoperiod and regularly moistened with sterile deionized water for seven days. The inoculated seedlings were

observed daily for symptom expression and the data on incubation period was recorded as and when the symptom was observed. At seven days after inoculation, the data on disease severity was recorded using 1 to 9 disease severity rating scale developed by Nene *et al*. (1991). Those isolates which showed 1-5 rating on disease scale were considered the least virulent while those with 6 rating as virulent and 7-9 rating as highly virulent.

Table 3.1 Rating scale used to record disease severity of dry root rot in chickpea

Rating	Observation
1	No infection on roots
>1 - ≤3	Very few small lesions on roots
>3 - ≤5	Lesions on roots clear but small, new roots free from infection
>5 - ≤7	Lesions on roots many, new roots generally free from lesions
>7 - 9	Roots infected and completely discoloured

3.3.3 Molecular characterization of *Rhizoctonia bataticola*

Molecular variability among *R. bataticola* isolates from chickpea was studied by using RAPD marker. Of the 68 isolates, 50 isolates were selected representing all the districts as well as the variation among them in cultural and morphological characters were selected for the molecular variability study. Total genomic DNA from all the selected isolates was extracted by Sodium dodecyl sulphate (SDS)-lysis buffer method.

3.3.3.1 Materials for DNA isolation and RAPD profiling

i. Lysis buffer:

200mM tris base (pH 8.5) : 2.422g
250mM NaCl : 1.461g
25mM EDTA : 0.931g
0.5 % Sodium dodecyl sulphate (SDS) : 0.5g
Sterile milli Q water : 100ml

Tris base was first added to 50ml double distilled water and adjusted to pH 8.5. The above stated chemicals were added to 50ml double distilled water and both the solutions were mixed thoroughly. Finally the solution was autoclaved at 121.6ºC (15psi) for 20min.

ii. Ribonuclease solution

Ten milligrams of RNase-A powder was dissolved in 1ml of 10 mM Tris buffer (pH 7.5).

iii. Tris EDTA (TE) buffer:

1M Tris base (pH 8.0) :2.5 µl
0.5M EDTA (pH 8.0) : 0.5µl
Sterile MilliQ water : 250ml

All the above ingredients were added and mixed. Finally, the solution was autoclaved at a temperature of 121.6ºC (15lb pressure) for 20min.

iv. **Phenol: chloroform: isoamyl alchohol**

Phenol, chloroform and isoamyl alchohol were added in 25:24:1 ratio.

v. TBE buffer

Stock solution (50X TBE) was prepared by dissolving 109g of Tris and 55g of boric acid in 800ml distilled water and then 40 ml of 0.5M EDTA (pH 8.0) was added. The volume was made up to one liter with distilled water and sterilized by autoclaving. This was stored at 4°C.

Working solution (1X TBE) was prepared by taking 20ml of 50X TBE buffer and volume was made up to 1000ml by using distilled water.

vi. Ethidium bromide (10mg/ml)

A quantity of 100mg ethidium bromide was dissolved in 10ml of distilled water. The vessel containing this solution was wrapped in aluminum foil and stored at 4°C.

vii. Agarose gel

To prepare 1% agarose gel, one gram of agarose was added in 100ml of 1X TBE buffer and heated using microwave oven until agarose powder was completely dissolved.

To prepare 1% agarose gel, three gram of agarose was added in 200 ml of 1X TBE buffer and heated using microwave oven until agarose powder was completely dissolved.

viii. Orange loading dye

0.5 M EDTA (pH 8.0) : 10 ml
5 M NaCl : 1 ml

Glycerol : 50 ml

Distilled water : 39 ml

Orange dye powder (Orange G, Gurr CertistainR) was added to the above chemicals till the colour became sufficiently dark.

3.3.3.2 Fungal cultures

Mycelial discs of 5mm diameter were cut from the periphery of actively growing three day old cultures of 50 selected isolates and inoculated into conical flask (250ml) containing potato dextrose broth @100ml. Flasks were incubated at 25±2ºC for three days. After incubation, the fungal biomass was filtered through whatman no.1 filter paper and kept in -80ºC for further use.

3.3.3.3 Isolation of genomic DNA

The total genomic DNA of 50 selected isolates of *R. bataticola* was isolated from mycelia by employing the method of Raeda and Broda (1985) with minor modifications. For DNA extraction, 200mg of freeze dried mycelium was ground with the help of pestle and mortar in liquid nitrogen until fine powder of mycelium was obtained. The mycelium powder was transferred to 2 ml Eppendorf tube containing 1000µl of extraction lysis buffer and the resulting slurry was incubated at 60ºC for 20-25min in a water bath. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the incubated slurry, mixed gently and centrifuged at 10,000 rpm at 4ºC for 20min.The supernatant was transferred to a new sterile Eppendorf tubes and to which 3µl RNase solution (10mg/ml) was added and kept for incubation for 10min at 37ºC. To this, equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed gently. The tubes were centrifuged for 10min at 10,000rpm in 4ºC and the same step was repeated twice. The supernatant was pipetted out and to this Eppendorf tube ice cold 70 per cent ethyl alcohol and 3M sodium acetate (pH 5) were added. This mixture was kept overnight at -80ºC followed by centrifugation at 13000rpm for 10min at 4ºC to collect DNA in pellet form. Then the supernatant was pipetted out and the pellet was air dried in laminar air flow chamber. The pellet was resuspended in 20-100µl TE buffer (pH 8) and stored at -20ºC.

3.3.3.4 Assessment of DNA quality and quantity

Agarose gel of 1% was prepared and allowed to cool to 60˚C. After cooling, 5µl of ethidium bromide solution was added and the resulting mixture was poured into the gel casting tray for solidification. Before the gel solidified, an acrylic comb of desired well number was placed on the agarose solution to form wells for loading the samples. Each well was loaded with 5µl of sample aliquot having 3µl distilled water, 1µl orange dye and 1µl of DNA sample. The DNA samples in known concentration (lambda DNA of 50ng/µl, 100ng/µl and 200ng/µl) were also loaded on to the gel to estimate the DNA concentration of the experimental samples. The gel was run at 70V for 20min. After completing the electrophoresis run, DNA on the gel was visualized under UV light and photographed. If the DNA was observed as a clear and intact band, the quality was considered good, whereas a smear of DNA indicating poor quality was discarded and reisolated. The extracted DNA was quantified by using nanodrop to get desired concentration of 15-20ng/µl.

3.3.3.5 RAPD genotyping

RAPD was carried out for molecular characterization of the isolates as per the protocol described by Williams *et al.* (1990) with slight modifications.

A set of ten RAPD primers were selected to study the polymorphism among 50 selected isolates of *Rhizoctonia bataticola*. The primers were synthesized by Xceleris genomic, Xceleris Lab Ltd., Ahmedabad, India. Genomic DNA of all the isolates were diluted to 15-20ng/µl and used as template for amplification of DNA. The PCR reactions were performed in 0.5ml PCR tubes consisting of 0.5µl of 15ng/µl DNA template, 0.5µl of 2.5mM dNTPs, 3.0µl of 2.5mM $MgCl_2$, 1.5µl of primer (Table 3.2), 2.5µl of 10X PCR reaction buffer and 5U of *Taq* DNA polymerase. These ingredients were mixed in 16.7µl of nuclease free water. The reaction mixture was mixed thoroughly by using vortex and briefly centrifuged to avoid sticking of chemicals to wall.

PCR amplification was performed in a thermal cycler with the temperature profiles of 95ºC for 5min of initial denaturation, followed by 35 cycles of denaturation at 95ºC for 60sec, with constant annealing at 39°C for 60sec and extension at 72°C for 60sec with final extension at 72°C for 10min. After completion of the polymerase chain reaction, the products were stored at 4ºC until the gel electrophoresis was done.

Table 3.2 List of RAPD primers used for PCR amplification

S. No.	Primer Name	Primer Sequence (5'-3')
1	OPC-06	GAACGGACTC
2	OPG-15	ACTGGGACTC
3	OPP-14	CCAGCCGAAC
4	OPU-07	CCTGCTCATC
5	OPAA-04	AGGACTGCTC
6	OPAC-14	GTCGGTTGTC
7	OPA-03	AGTCAGCCAC
8	OPA-09	GGGTAACGCC
9	OPA-11	CAATCGCCGT
10	OPA-18	AGGTGACCGT

3.3.3.6 Separation of amplified products by agarose gel electrophoresis

Agarose gel of 1.5% was prepared and allowed to cool to 60˚C. After cooling, 10µl of ethidium bromide (0.5µg/ml) was added. The solution was mixed and poured into the gel casting platform after inserting the comb in the trough. While pouring, sufficient care was taken for not allowing the air bubbles to trap in the gel. The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoresis apparatus containing sufficient buffer (1X TBE) so as to cover the wells completely. The 20µl of amplified products were carefully loaded into the sample wells after adding orange dye with the help of micropipette. Electrophoresis was carried out at 60V until the tracking dye migrated to the end of the gel. Ethidium bromide stained DNA bands were viewed under UV transilluminator and photographed for documentation.

3.3.3.7 Scoring and analysis of the amplified fragments

The amplified profiles for all the primers were compared with each other and bands of DNA fragment were scored as 'l' for presence and '0' for absence, generating '0' and '1' matrix. Faint bands were not scored. Binary matrices were analyzed by NTSYS-PC (version 2.0; Exeter Biological Software, Setauket, NY) to calculate Jaccard's similarity coefficient for each pair wise comparison. Jaccard's coefficients were clustered to generate dendrogram using the SAHN clustering program selecting the unweighted pair group method with arithmetic average (UPGMA) algorithm in NTSYS PC (Rohlf, 1998).

3. 4 Effect of environmental factors on *Rhizoctonia bataticola* and dry root rot development

Effect of environmental factors such as temperature and moisture were studied under *in vitro* and *in vivo* conditions. Influence of pH on growth of pathogen was studied under *in vitro.* Five isolates *viz.* Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63 with disease severity rating more than 7 and representing 5 different states were selected to perform the experiments pertaining to effect of temperature on growth and disease development.

3.4.1 Effect of temperature on growth of *Rhizoctonia bataticola*

Effect of seven different temperatures *viz.* 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C were studied on growth of *Rhizoctonia bataticola* on PDA. Mycelial discs of 5mm diameter were cut from the edge of a 3 days old culture of five selected representative isolates grown in 25ºC were transferred to the center of 90mm Petri dish and incubated at different temperatures with 12h photoperiod. Each treatment was replicated three times in a completely randomized design. The average diameter of the fungal colony was recorded at 48h, 72h and 96h after incubation. Plants inoculated with deionized water served as contol.

3.4.2 Effect of temperature on disease development

Effect of different temperatures *viz.* 15˚C, 20˚C, 25˚C, 30˚C, 35˚C, 40˚C and 45˚C on severity of dry root rot in susceptible genotype BG 212 was studied by adopting paper towel technique. The experiment was conducted with same five representative isolates in completely randomized design (CRD) with three replications. Inoculation was done through root dip inoculation technique which was already explained in 3.3.2. In each replication, 7-10 plants/ paper towel were maintained. Disease severity was recorded at seven days after inoculation by using 1 - 9 disease severity rating scale. Plants inoculated with deionized water served as control.

3.4.3 Effect of different osmotic potentials on growth of *Rhizoctonia bataticola*

Among five selected isolates of *Rhizoctonia bataticola*, the most virulent isolate Rb 63 was used to study the effect of different osmotic potentials on growth of *Rhizoctonia bataticola* at different temperatures. The different osmotic potentials *viz.* -0.5MPa, -1.0MPa, -1.5MPa, -2.0MPa and -2.5MPa were obtained with KCl, NaCl and dextrose amended in PDA medium. These levels of osmotic potentials were obtained by using Wescor Psypro instrument. Unamended PDA medium served as control. Mycelia discs of 5mm diameter of Rb 63 were cut from 3 days old culture was transferred to Petri dishes

(80mm) containing PDA at different osmotic potential. Each treatment was replicated thrice. These Petri plates were incubated at different temperatures *viz.* 20°C, 25°C, 30°C, 35°C and 40°C. The average diameter of the fungal colony was recorded at 24h, 48h and 72h after incubation.

3.4.4 Effect of soil moisture on disease severity

i. Development of sick soil

The five representative isolates *viz*. Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63 were used for the study. Sand maize medium was prepared by mixing 90g sand and 10g maize granules in 250ml flask to which 10ml sterile distilled water was added. These flasks were autoclaved at 15lb pressure for 20min. Each flask was inoculated with a bit of actively growing fungal culture and incubated at 25ºC for 15 days. This fungus in sand maize medium was mixed with autoclaved black and red soils separately @50g/kg which were used to fill pots having 2kg capacity. The soil in pots was incubated for 4 days to allow the pathogen to multiply in soil. After 4 days, fifteen seeds of highly susceptible genotype BG 212 were sown in each pot at 2-3 cm depth and watered regularly. Healthy plants were removed after 30 days and diseased plants were chopped and incorporated into the soil. Again sowing was taken up in the same soil until >90% dry root rot incidence was observed. These sick pots were used for further studies.

ii. Determination of soil moisture

The soil moisture was determined using the gravimetric method on oven dry basis. In this complete saturation of soil sample with water was done and weight was recorded. This saturated soil was placed in hot air oven for drying at 100-110˚C until the weight remains constant. Samples were cooled slowly to room temperature and weighed again. The difference in weight was equivalent to amount of moisture in the soil. The available soil moisture in the soil was calculated by the following formula.

$$\text{Soil moisture per cent} = \frac{(\text{Saturated soil weight} - \text{oven dry soil weight}) \times 100}{\text{Oven dry soil weight}}$$

Different soil moisture levels *viz.* 40%, 50%, 60%, 70%, 80%, 90% and 100% were maintained by regular weighing and replacing the moisture deficit in each pot by watering soil in pots. Sterile deionized water was used for maintaining the soil moisture in each pot. Soil moisture effect on the severity of dry root rot in sick soil of five highly virulent isolates *viz.* Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63 representing 5 different states was

studied. Uninoculated soil maintained at same moisture levels served as control. The experiment was conducted at an optimum temperature of 35˚C. Each treatment was replicated thrice. Five surface sterilized BG 212 seeds were sown in each sick pot. At 45 days after sowing, the data on disease severity was recorded separately from the pots filled with black and red soils maintained at different soil moisture levels.

3.4.5 Effect of different pH levels on radial growth of *R. bataticola*

The five selected isolates *viz.* Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63 were grown on the PDA adjusted to different pH levels *viz.,* 3, 4, 5, 6, 7, 8, 9, 10, 11. The pH levels were obtained by adding 0.1N NaOH or 0.1N HCl to PDA before autoclaving and poured into 90mm Petri plates. The 5mm mycelial discs from seven day old culture of *R. bataticola* were transferred aseptically on to PDA in Petri plates. Three replications were maintained for each treatment. The average diameter of the fungal colony was recorded at 24h, 48h and 72h after incubation.

3.5 Study of biochemical changes associated with dry root development in chickpea

Three genotypes *viz.,* one highly susceptible genotype BG 212 and two moderately resistant genotypes ICCV 5530 and ICCV 8305 were selected for determining the biochemical changes with respect to dry root rot disease development. Pots filled with sick soil of isolate Rb 63 were used, maintained at two different soil moisture conditions *viz.,* 60% and 100% by gravimetric method. Seedlings were allowed to grow under glass house conditions up to 45 DAS. Root samples of seedlings were collected at three time point's *viz.,* 15, 30 and 45 DAS. Each treatment was replicated thrice with suitable controls.

3.5.1 Extraction of plant tissues

To determine phenols, total sugar, reducing and non-reducing sugars, the extraction of plant tissues was done in alcohol. For this purpose, root tissue of 1000 mg weight was taken and made into small pieces and plunged in boiling alcohol for 15 min. The pieces of the tissue were ground thoroughly in a mortar and pestle with 5 ml of 80% ethyl alcohol. The extract was passed through muslin cloth. The above step was repeated once again with the filtrate. The homogenate obtained after filtration was centrifuged at 10000 rpm for 15 min. The supernatant was collected and stored in a refrigerator at 4°C.

3.5.2 Determination of phenol

Phenol content in the roots of three genotypes of chickpea was determined with folin ciocalteu reagent method of Bray and Thorpe (1954).

Reagents

i. Ethanol 80%

To prepare 80% ethanol, 80 ml of absolute ethanol was added with 20 ml of distilled water.

ii. Folin-ciocalteu reagent

One millilitre of 2 N folin-ciocalteu reagent was added to 1 ml of sterile distilled water.

iii. 20% $Na_2 CO_3$

Na_2CO_3 of 20 g was added with 100 ml of sterile distilled water.

iv. Catechol

100 mg of catechol was dissolved in 100 ml of water. This acted as standard solutions. Working standard was prepared from standard solution by diluting to get required concentration by serial dilution.

Procedure

One millilitre of each alcohol extract was taken in a test tube to which 1 ml of 1 N folin-ciocalteu reagent was added. After three minutes, 2 ml of sodium carbonate solution (20%) was added. The tubes were shaken well and heated in a hot water bath for exactly one minute and then cooled under running tap water. The blue colour complex absorbance was read at 650 nm in a spectrophotometer. The amount of phenols present in sample was calculated from a standard curve prepared from catechol. A standard curve was prepared to find out the concentration of phenols in the test sample and expressed as mg catechol $100mg^{-1}$ root sample.

3.5.3 Determination of total soluble sugars

Reagents

i. **Anthrone solution:** It was prepared by mixing 0.4 g of anthrone with 200 ml ice cold 95% H_2SO_4 in 250 ml volumetric flask. Required anthrone solution was prepared freshly before use.

ii. **Glucose solution:** 100 mg of glucose was dissolved in 100 ml of water. This acted as standard solutions. Working standard was prepared from standard solution by diluting to get required concentration by serial dilution.

iii. Blank solution: To prepare blank solution, 1 ml of distilled water was added to 4 ml of anthrone solution in a test tube.

Procedure

Total sugar content was estimated by anthrone method (Hedge and Hofreiter, 1962). Ethanol extract of 200 µl taken in test tube was placed in a water bath at 80°C to evaporate alcohol and then 1 ml of water was added to it to dissolve the sugars present in extract. To this, 4 ml of freshly prepared anthrone reagent was added and heated for 8 min in a boiling water bath and cooled rapidly. Then the reaction mixture appeared green in colour. The absorbance of the green coloured solution was measured at 630 nm using spectrophotometer. D-glucose was used as standard. The total sugar content was expressed in terms of mg $100mg^{-1}$ of fresh weight.

3.5.4 Determination of reducing sugar

Reagents

i. Dinitro salicylic acid reagent (DNS Reagent)

One gram of dinitro salicylic acid, 200 mg of crystalline phenol and 50 mg sodium sulphite were dissolved in 100 ml 1% NaOH contained in a 250 ml conical flask and stored at 4˚C. Since sodium sulphite deteriorates the reagent, it was added just before use.

ii. 40% Rochelle salt solution (potassium sodium tartrate)

To prepare 40% Rochelle salt solution, 40 g potassium sodium tartarate was added to 100 ml of water.

Procedure

Ethanol extract of 0.5ml was taken in test tube and the volume was made up to 3 ml with water in all the tubes. To this, 3 ml of DNS reagent was added and heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1 ml of 40% Rochelle salt solution was added. The intensity of dark red colour was measured at 510 nm. D-glucose was used as standard. The reducing sugar content was expressed in mg 100 mg^{-1} fresh weight (Miller, 1972).

3.5.5 Determination of non-reducing sugars

The quantity of non-reducing sugars was calculated by deducting the reducing sugar content from that of the total soluble sugars.

3.5.6 Determination of peroxidase activity

Using chilled pestle and mortar, 1 g of root sample was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.8) to which a pinch of polyvinyl pyrollidone (PVP) was added. The homogenate was centrifuged at 12000 rpm for 20 min at 4°C and the supernatant was used as the enzyme source for the assay of peroxidase activity.

Peroxidase activity was assayed using a slight modification of the method of Hartee (1955). The reaction mixture consisting of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2 was incubated at room temperature. At the start of enzyme reaction, the absorbance of mixture was set to zero at 420 nm in the spectrophotometer and the change in the absorbance was recorded at 30 s intervals for 2 min. Boiled enzyme preparation served as blank. The peroxidase activity was expressed as $\Delta_{Abs420}nm^{-1}min^{-1}g^{-1}$ on fresh weight basis (Hammerchmidt, 1982).

3.5.7 Determination of polyphenol oxidase (PPO)

Using chilled pestle and mortar, one gram of root sample was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 12000 rpm for 20 min at 4°C and the supernatant was used as the enzyme source for the assay. Polyphenol oxidase activity was assayed using the modified method of Mayer *et al.* (1965). The standard reaction mixture was prepared by adding 1.5 ml 0.1M sodium phosphate buffer (pH 6.5) and 200 µl of 0.01M catechol. To this reaction mixture finally enzyme extract of 200 µl was added. The reaction mixture was incubated at room temperature and the absorbance was set to zero at 495 nm. The change in the absorbance was recorded at 30 s intervals for 2 min and the polyphenol oxidase activity was expressed as $\Delta_{Abs495}nm^{-1}min^{-1}g^{-1}$ on fresh weight basis.

3.5.8 Assay of phenylalanine ammonia lyase (PAL)

One gram of root sample was homogenized in 5 ml of 25mM Tris HCl buffer (pH 8.8) using chilled pestle and mortar. The homogenate was centrifuged at 10000 rpm for 30 min at 4ºC. The supernatant was used for the assay of phenylalanine ammonia lyase activity.

Phenylalanine ammonia lyase activity was determined using the modified method of the conversion of L-phenylalanine to cinnamic acid at 290 nm (Dickerson *et al*., 1984). The reaction mixture contained 0.4 ml of 25mM Tris HCl buffer (pH 8.8), 0.5 ml of 50 mM L-phenylalanine and the enzyme extract of 0.1 ml. The blank reaction was taken with 0.4 ml of 25mM Tris HCl buffer (pH 8.8), 0.5 ml of 50 mM L-phenylalanine and 0.1 ml of heated enzyme extract. The reaction mixture and blank were incubated at 40°C for 60 min and the reaction was stopped by adding 60 µl of 5N HCl. The absorbance was read at 290 nm in the spectrophotometer. Phenyl alanine ammonia lyase activity was expressed in µmoles of cinnamic acid h^{-1} g^{-1} on fresh weight basis.

3.6. Physiological changes associated with dry root rot development in chickpea

Three genotypes *viz.,* one highly susceptible genotype BG 212 and two moderately resistant genotypes ICCV 5530 and ICCV 8305 were selected for determining the physiological changes with respect to dry root rot development in chickpea. Two different soil moisture conditions *viz.,* 60% and 100% were maintained by gravimetric method. Sick soil of Rb 63 isolate was used for this experiment. These soils were covered with plastic beads to avoid evaporation losses. Sampling was done at two time points *viz.*, 15 and 45 DAS. Each treatment was replicated thrice with suitable controls.

3.6.1 Leaf transpiration

Pots containing sick soil were maintained at 60 and 100% soil moisture levels by watering with sterile deionized water at one hour before conducting the experiment and the soil was covered with plastic beads to avoid evaporation. Pots were weighed with a 0.1 g precision weighing balance at 08:15 h and 16:15 h. Plant transpiration was estimated from the loss in weight of each pot per eight hours. Leaf transpiration was estimated at 15 and 45 days after sowing (DAS) expressed in g of H_2O $8h^{-1}$.

The plants were removed from the pots carefully without damaging roots at the end of transpiration measurement after 16:15 h. The plant parts were cut separately into shoot, root and leaves. These were kept in labeled paper bags and kept in 70ºC hot air oven for 48 h. Dry weights of individual parts were recorded separately.

3.6.2 Canopy temperature

Canopy temperatures of the genotypes were measured from thermal images obtained with an infrared flex Cam S (Infrared Solutions, Plymouth, MN, USA) with a sensitivity of 0.09ºC. The images were taken between 12:00PM and 2:00PM of the day at

15 and 45 days after sowing (DAS). Smart view 2.1.0.10 software (fluke thermography everett, WA, USA) was used to estimate the canopy temperatures.

3.6.3 Stomatal conductance

Variation in stomatal conductance in response to different moisture levels was determined at mid day from the upper leaf that was well exposed to sunlight. It was measured by leaf porometer (Decagon devices Inc., Pullman, WA, 99163, USA) at 15 and 45 DAS and expressed in mol m^{-2} s^{-1}.

3.7 Statistical analysis

Statistical analysis of all the laboratory experiments was carried out using Completely Randomized Design (CRD). The data was statistically analyzed (Gomez and Gomez, 1984) using the GENSTAT statistical package (version 13.0., Rothamsted Experiment Station, Herpenden Herts AL52JQ, UK). The data was subjected to angular transformation where ever necessary (data which had per cent values).

Data was subjected to analysis of variance (ANOVA) at significant levels ($P<0.05$ and $P < 0.01$) and treatment means were compared by critical difference (CD).

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

The present investigations on various aspects of dry root rot of chickpea caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] included survey on disease incidence, collection of pathogen isolates, diversity among isolates with respect to cultural, morphological, pathological and molecular characteristics, influence of environmental factors on disease development and biochemical and physiological changes associated with the disease development. The results obtained on these aspects are presented hereunder and discussed critically based on the previous information available on these aspects.

4.1 Occurrence and distribution of dry root rot of chickpea in major crop growing regions of central and southern India

A roving survey was conducted during January, 2014 in different chickpea growing areas of central (Madhya Pradesh and Maharashtra) and southern (Andhra Pradesh, Telangana and Karnataka) India to assess the status of dry root rot incidence and to collect diseased samples infected by *Rhizoctonia bataticola* under field condition. The data pertaining to survey is given in Table 4.1.

It is evident from the table that the mean maximum dry root rot incidence was observed in Telangana (18.28%) followed by Madhya Pradesh (18.10%) which however, were at par with each other. This was followed by Karnataka (7.85%), Andhra Pradesh (5.40%) and the least in Maharashtra (5.38%). There was no significant difference in disease incidence between Andhra Pradesh and Maharashtra. Out of 68 locations surveyed, the crop was cultivated in vertisols in 66 locations while in two locations *viz.*, Gandemala and Kurnool in Kurnool district of Andhra Pradesh, it was cultivated in alfisols. The highest disease incidence of 31.34% and 8.00% was recorded in Daroor village of Rangareddy district and Gandemala village of Kurnool district among vertisol and alfisol type locations, respectively.

In Madhya Pradesh, significantly highest disease incidence was noticed in Hardua village (28.0%) of Jabalpur district and the least in Bandol village (3.33%) of Seoni district

Table 4.1. Survey on occurrence and distribution of dry root rot of chickpea in central and southern India

S. No.	District	Location	Previous crop	Cultivar	Seed treatment	Irrigation facility	Percent disease incidence*
Madhya Pradesh							
1	Jabalpur	Tovaria	Sorghum	JG 11	No	No	10.00(18.06)
2		Hardua	Maize	JG 11	No	No	28.00 (31.91)
3		Bidagad	Rice	JG 62	Yes	Yes	7.33 (15.67)
4	Narsingapur	Barnagh	Rice	JG 11	No	Yes	22.67 (28.35)
5	Seoni	Bandol	Sorghum	JG 11	No	No	3.33 (10.14)
6	Chhindwara	Chaurai	Maize	KAK 2	No	No	24.00 (29.31)
7	Hoshangabad	Pipariya	Rice	JG 11	No	No	24.00 (29.31)
8	Damoh	Damoh	Sorghum	JG 62	No	No	25.33 (30.16)
						Mean	18.10(21.16)
Karnataka							
9	Gulbarga	Suntnoor	Greengram	Desi	No	No	6.67 (14.92)
10		Sarsamba	Sorghum	Desi	Yes	Yes	2.67 (9.26)
11		Sikhapur	Maize	Desi	Yes	No	3.33 (10.14)
12	Raichur	Devadurga	Maize	Desi	No	No	10.67 (19.04)
13		Raichur	Maize	Desi	No	No	15.33 (23.04)
						Mean	7.85(16.26)
Maharashtra							
14	Osmanabad	Edsi	Rice	Desi	No	No	2.67 (9.26)
15	Beed	Nagajhari	Soyabean	Desi	No	No	2.67 (9.26)
16	Jalna	Gauretaluka	Sorghum	Desi	No	No	2.67 (9.26)
17	Buldhana	Sindkhedgav	Kharif fallow	Desi	No	No	2.00 (6.55)
18		Kaparkeda	Soybean	JG11	No	No	4.00 (11.28)
19		Mekar	Soybean	Desi	No	No	23.33 (28.87)
20	Yavatmal	Vuttarana	Soybean	Desi	Yes	No	2.67 (9.26)
21	Wasim	Dongargiri	Soybean	Desi	No	No	2.00 (6.55)
22	Akola	Rajapur	Soybean	Improved desi	No	Yes	1.33 (5.42)
23		Pailpada	Soybean	Improved desi	No	No	3.33 (10.40)
24		Vurum	Soybean	Desi	No	No	8.00 (16.34)
25		Maulikhed	Soybean	Desi	No	No	11.33 (19.55)
26	Yavatmal	Lacina	Soybean	Wardha	No	Yes	2.00 (6.55)
27	Amaravathi	Velora	Soybean	Desi	No	No	5.33 (13.163)
						Mean	5.38(13.38)
Andhra Pradesh							
28	Anantpur	Egnoor	Maize	Desi	Yes	Yes	3.33 (10.39)
29		Chimalavagupalli	Sorghum	JG11	Yes	No	2.67 (7.43)
30		Pedakallu	Sorghum	JG11	No	No	1.33 (5.41)
31		Chinakallu	Rice	JG11	No	No	1.33 (5.42)
32	Guntur	Lam	Green gram	JG 11	No	No	6.67 (14.79)
33		Chilakaluripeta	Cotton	JG 11	No	Yes	10.00 (18.38)
34		Pedanandipadu	Black gram	JG 11	No	No	2.67 (9.27)

35	Kurnool	Nandyal	Kharif fallow	L550	No	No	27.33 (31.49)
36	Kurnool	Ayyaluru	Rice	MNK 1	No	Yes	1.33 (3.84)
37		Alur	Kharif fallow	JG11	No	No	4.00 (11.53)
38		Oruvagallu	kharif fallow	Desi	Yes	Yes	0.67 (2.71)
39		Chelkuru	Maize	Desi	No	yes	2.00 (6.55)
40		Rudipadu	kharif fallow	JG11	No	No	4.67 (12.41)
41		Devanoor	Pigeon pea	Desi	No	No	2.67 (7.43)
42		Medthur	Sorghum	Desi	No	No	22.00 (27.95)
43		Gandemala	Maize	JG11	No	No	8.00 (16.07)
44		Gospadu	Maize	JG11	Yes	Yes	1.33 (5.42)
45		Kollur	Cotton	JG11	Yes	Yes	2.00 (6.55)
46		Kurnool	Maize	JG11	No	No	2.67 (9.26)
47		Kolimigundla	Maize	JG 11	Yes	No	1.33 (5.41)
48		Chelpur	kharif fallow	Desi	No	No	2.00 (8.12)
49	Prakasam	Naguluppalapadu	kharif fallow	JG 11	No	No	9.33 (17.63)
50		Paduchuru	Kharif fallow	KAK 2	No	No	8.00 (16.07)
51		Marturu	kharif fallow	JG 11	No	No	3.33 (10.14)
						Mean	5.40(13.48)
	Telangana State						
52	Medak	Patancheru	Sorghum	JG 62	No	Yes	10.00 (18.37)
53		Patancheru	kharif fallow	JG 62	No	Yes	29.33 (32.77)
54		Patancheru	Sorghum	Desi	No	No	8.67 (16.64)
55		Patancheru	Sorghum	JG 62	No	No	27.33 (31.45)
56		Patancheru	Pearl millet	JG 62	No	Yes	25.33 (30.16)
57		Patancheru	kharif fallow	BG 212	No	Yes	24.00 (29.24)
58		Patancheru	Sorghum	JG 11	No	No	27.33 (31.45)
59		Patancheru	Sorghum	JG 11	No	No	19.33 (26.03)
60		Patancheru	Pigeonpea	ICCV2	No	No	14.67 (22.36)
61		Patancheru	Sorghum	JG 11	No	No	30.67 (33.17)
62		Patancheru	Pigeonpea	JAKI 9218	No	Yes	25.33 (30.16)
63		Patancheru	Sorghum	JG 11	No	No	18.67 (25.06)
64		Shankerpally	Sorghum	Desi	No	No	9.33 (17.62)
65	Rangareddy	Daroor	Ajowan	Desi	No	No	31.33 (33.98)
66	Mahbubnagar	Naraynapuram	Sorghum	Desi	No	No	3.33 (10.14)
67		Vundavelli	Maize	Desi	No	Yes	1.33 (5.42)
68		Rangapur	Sorghum	Desi	No	No	5.33 (13.29)
						Mean	18.28(25.29)
						CD	**5.73(6.08)**
						Mean CD	**1.11(1.08)**

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In Karnataka, maximum incidence was recorded in Raichur (15.33%) and the least incidence 2.67% in Sarsamba village of Gulbarga district. In Maharashtra, maximum incidence was in Mekar (23.33%) of Buldhana district and least in Rajapur (1.33%) of Akola district. In Andhra Pradesh, highest was found in Nandyal (RARS) with 27.33% incidence and least in Oruvagallu (0.67%) of Kurnool district. In Telangana state, the highest incidence (31.3%) was noticed in Daroor village of Rangareddy district and the least (1.34%) in Vundavelli village of Mahboobnagar district.

The per cent disease incidence recorded in each variety varied depending on the place of cultivation. Desi variety was cultivated in 26 locations had disease incidence ranging from 0.67% in Oruvagallu of Kurnool district to 31.33% in Daroor of Rangareddy district. In Karnataka, chickpea was occupied by desi variety in all the five locations surveyed where disease incidence varied from 2.67% to 15.33% while in Maharashtra it ranged from 2.00% (Sindkhedgav and Dongagiri) to 23.33% (Mekar). In Andhra Pradesh disease incidence in desi cultivar ranged from 0.67% (Oruvagallu) to 22.00% (Medthur) while in Telangana it was from 1.33% (Vundavalli) to 31.33% (Daroor) where the difference in disease incidence was significant.

JG 11 cultivated in 25 locations recorded the disease incidence ranging from 1.33% in Chinakallu of Anantapur to 30.67% in Patancheru of Medak district. Disease incidence varied from 3.33% (Bandol) to 28.00% (Hardua) in Madhya Pradesh and 1.33% (Gospadu, Kolimigundla, Chinakallu and Pedakallu) to 10.00% (Chilakaluripeta) in Andhra Pradesh. In Telangana, the disease incidence varied from 18.7 to 30.7 in different fields of Patancheru where JG 11 was cultivated and the difference in maximum and minimum disease incidence was significant. In Kaparkeda village of Maharashtra, the per cent disease incidence in JG 11 was 4.00.

JG 62 cultivated in six locations recorded disease incidence ranging from 7.33 (Bidagad of Jabalpur district) to 29.33% (Patancheru of Medak district). In Madhya Pradesh, the disease incidence varied from 7.33 (Bidagad) to 25.33 (Damoh) while in Telangana, it varied from 10.00 to 29.33% in different fields of Patancheru in JG 62 cultivated locations. The difference between maximum and minimum disease incidence was significant.

When compared to all the cultivars, improved desi had recorded minimum disease incidence which ranged from 1.33 (Rajapur) to 3.33% (Pailpada) in Akola district. KAK 2 which was cultivated in two locations had disease incidence varying from 8.00% in Paduchuru of Prakasam district to 24.00% in Chaurai of Chhindwara district. The cultivars BG 212, JAKI 9218 and ICCV 2 cultivated in different fields of Patancheru had percent disease incidence of 24.00, 25.33 and 14.67, respectively. Cultivars L 550, MNK 1 and Wardha cultivated in Nandyal, Ayyaluru and Lacina villages recorded percent disease incidence of 27.33, 1.33 and 2.00, respectively.

There was variation in disease incidence due to variation in location and type of the cropping system followed with chickpea. Sorghum - chickpea cropping system was observed in 18 locations where the percent disease incidence ranged from 1.33 Pedakallu village of Anatapur to 30.67 in Patancheru of Medak district. In sorghum - chickpea cropping system followed in Madhya Pradesh, Andhra Pradesh and Telangana, the percent disease incidence ranged from 3.33 (Bandol) to 25.33 (Tovaria), 1.33 (Pedakallu) to 22.00 (Medthur) and 3.33 (Narayanapuram) to 30.67 (Patancheru). The difference between maximum and minimum disease incidence was significant. The percent disease incidence in sorghum - chickpea cropping system was 2.67 in both Sarsamba and Gauretaluka of Karnataka and Maharashtra, respectively.

Maize - chickpea cropping system was observed in 12 locations where the percent disease incidence ranged from 1.33 in Vundavelli of Mahboobnagar to 28.00 in Hardua of Jabalpur district. In maize - chickpea cropping system, percent disease incidence varied from 24.00 (Chaurai) to 28.00 (Hardua) in Madhya Pradesh where the difference in disease incidence was at par while in Karnataka and Andhra Pradesh, it varied from 3.33 (Sikhapur) to 15.33 (Devadurga) and 1.33 (Kolimigundla and Gospadu) to 8.00 (Gandemla), respectively where the difference in disease incidence was significant.

Kharif fallow - chickpea cropping system was observed in 11 locations where the percent disease incidence was between 3.33 in Marturu village of Prakasam and 29.33 in Patancheru of Medak district. In kharif fallow - chickpea cropping system, the percent disease incidence varied from 0.67 (Oruvagal) to 27.33 (Nandyal) in Andhra Pradesh and the difference in disease incidence was significant while in Telangana it was 24.00 to 29.33 in Patancheru fields where the difference in disease incidence was non significant due to

monocropping and continuous build up of pathogen inoculum levels. In this system, the percent disease incidence was 2.00 in Sindkhedgav of Maharashtra.

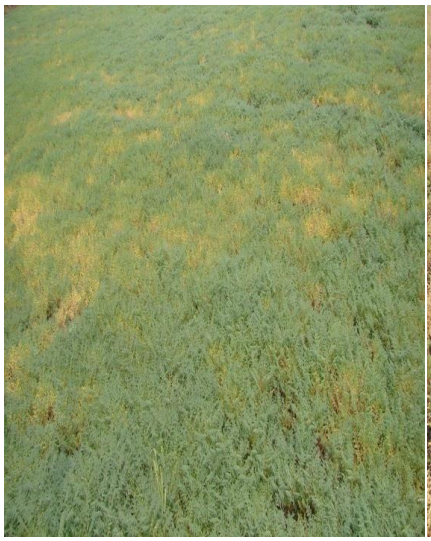
Soybean - chickpea cropping system was observed only in Maharashtra where the percent disease incidence varied from 1.33 (Rajapur) to 23.33 (Mekar) and the difference in disease incidence was significant. Rice - chickpea cropping system was observed in six locations where the percent disease incidence varied from 1.33 (Chinakallu and Ayyaluru of Anantpur and Kurnool district respectively) to 24.00 (Pipariya) and the difference in disease incidence was significant. In Madhya Pradesh where rice - chickpea cropping system was followed, the percent disease incidence varied from 7.33 (Bidagad) to 24.00 (Pipariya) and the difference in disease incidence was significant while in Maharashtra, it was 2.67 in Edsi.

The significant difference in percent disease incidence of pigeonpea - chickpea cropping system was observed, which varied from 2.67 in Devanoor of Andhra Pradesh to 25.33 in Patancheru of Telangana. Disease incidence of 6.67% was observed in Suntnoor and Lam of Karnataka and Andhra Pradesh respectively in greengram - chickpea cropping system where the difference in disease incidence was at par with other. In cotton - chickpea cropping system, the percent disease incidence varied from 2.00 (Kollur) to 10.00 (Chilakaluripeta) and the difference in disease incidence was significant. In chickpea followed by black gram, ajowan and pearlmillet - cropping systems, percent disease incidence was 2.67 (Pedanandipadu), 31.33 (Daroor) and 25.33 (Patancheru).

The per cent disease incidence recorded in different locations varied with respect to the cultivar and previous crop. Another important agronomic practise which generally play an important role in the management of soil borne pathogens during the initial stages of the crop growth is seed treatment. Out of 68 locations, seed treatment was practised in ten locations while maximum farmers from 58 locations did not practice seed treatment. The percent disease incidence varied from 0.67 (Oruvagal) to 7.33 (Bidagad) in locations where seed treatment was practised while it was from 1.33 to 31.33 (Daroor) in locations where no seed treatment was practised. Comparatively, in locations where seed treatment was practised had less disease incidence compared to locations where no seed treatment was practised. Disease incidence was 7.33% in Bidagad where seed treatment was done while it ranged from 3.33 to 28.00 in locations where no seed treatment was practised in Madhya P

Initial symptom of infected plant

Rotting of root system



Initial field symptoms in Hardua, Madhya Pradesh

Severely infected field in Patancheru, Telangana

Fig 4.1. Dry root rot symptoms on chickpea under field conditions observed during survey

radesh. In Karnataka, percent disease incidence was from 2.67 (Sarsamba) to 3.33 (Sikhapur) in the fields where seed treatment was done but it was from 6.67 (Suntnoor) to 15.33 (Devadurga) where no seed treatment was done. In Maharashtra, percent disease incidence of 2.67 was recorded from Vuttarana where seed treatment was done but it was 1.33 to 23.33 in locations where no seed treatment was practised. Disease incidence in Andhra Pradesh was from 0.67 to 3.33 in the fields where seed treatment was done but it was 1.33 to 27.33 in fields where no seed treatment was done. The difference in disease incidence was significant. None of the farmers in surveyed locations of Telangana had practised seed treatment.

Soil moisture plays an important role in the development of the crop. It also plays an important role in the survival and spread of the disease like dry root rot caused by *R. bataticola* which favours low moisture conditions. Knowledge on the irrigation facility and soil moisture status helps in determining the percentage of disease incidence. During survey it was observed that irrigation was given in only 18 locations and in rest of the locations, crop was cultivated under residual soil moisture or rainfall during crop growth. The percent disease incidence in the irrigated locations varied from 0.67 (Oruvagal) to 29.33 (Patancheru) where as in the locations where no irrigation facility was available there, it varied from 1.33 (Chinakallu, Pedakallu and Kolimigundla) to 31.33 (Daroor). Comparatively the disease incidence was less in irrigated fields over unirrigated ones. Disease incidence in Madhya Pradesh, Maharashtra, Andhra Pradesh, Telangana and Karnataka ranged from 7.33 (Bidagad) to 22.67 (Barnagh), 1.33 (Rajapur) to 2.00 (Lacina), 0.67 (Oruvagallu) to10.00 (Chilakaluripeta), 1.33 (Vundavelli) to 29.33 (Patancheru) and 2.67 (Sarsamba) in locations where irrigation was given while it was 3.33 (Bandol) to 28.00 (Hardua), 2.00 (Sindkhedgav and Dongagiri) to 23.33 (Mekar), 1.33 (Pedakallu, Chinakallu, Kolimigundla) to 27.33 (Nandyal), 3.33 (Narayanapuram) to 31.33 (Daroor) and 3.33 (Sikhapur) to 15.33 (Raichur) in locations where crop was cultivated under residual soil moisture respectively.

Similar results were also observed by Bajpal (1999) as hot and dry weather in vertisols favoured more disease development compared to alfisols. Decline in the viability and germination of *M. phaseolina* in sandy soils could be attributed to nutrient deprivation. In poor soil nutrient conditions, secondary microsclerotia are more likely to be smaller than primary microsclerotia and consequently a lower energy reserve thus reducing propagule

viability in the long term (Gangopadhyay *et al.,* 1982). Gurha and Trivedi (2008) observed dry root rot incidence in Gulberga and Raichur up to 60%. Ghosh *et al.* (2013) also observed disease incidence ranged from 8.9 to 10.3% irrespective of cultivar and locations. Local cultivars were most frequently grown by the farmers. Seed treatment was practised by 63% of the farmers where the disease incidence was low in their fields compared to fields in which no seed treatment was done. After harvesting, roots loaded with microsclerotia start decaying and release the microsclerotia into the soil (Olaya *et al*., 1996). Infected crops residues are one of the most important sources of inoculum and inoculum dispersal of *M. phaseolina* in the field and provide a mechanism of survival for long periods in the soil. Degradation of plant debris and relative longevity of *M. phaseolina* depends upon soil moisture and temperature (Baird *et al.,* 2003). *Macrophomina phaseolina* populations in soybean root debris or residue in the soil tend to increase over time. Root debris at or near the soil surface increases the *M. phaseolina* population more rapidly than buried residue, but surface residues are more directly exposed to environmental variations. Fluctuations in the population are inversely related to soil depth (Short *et al*., 1978). Irrigation throughout the soybean growth season reduced the population and colonization of *M. phaseolina* on roots compared with unirrigated cropping system, even though the propagules remain during the season in both systems and no symptoms in soybean plants were found in the irrigated field (Kendig *et al.,* 2000). Microsclerotia of *M. phaseolina* can be degraded and eliminated from the surface to 20 cm depth under paddy rice soil conditions. Such flooded conditions reduced the number of viable microsclerotia by 83% in two years (Zaki and Ghaffar, 1988). Substances found in flooded soils such as alcohols, volatiles and increased levels of CO_2 may had a detrimental effect on the inoculum (Wyllie *et al*., 1984).

4.2 Symptomatology and collection of pathogenic isolates of *Rhizoctonia bataticola*

During survey, due to diversified weather conditions and variation in sowing dates in different states, different crop growth stages *i.e.* from seedling (20 days old) to podding stage were observed. The crop was sown early in Karnataka and Andhra Pradesh during first fortnight of October while in Telangana in the second fort night of October. In Maharashtra sowings were done in the month of November whereas in Madhya Pradesh in the second fortnight of November to December. In Madhya Pradesh, seedlings infected with dry root rot appeared stunted without any lateral roots when uprooted and the tap root

was black in colour. In Maharashtra and Telangana, crop was in the vegetative to flowering stage. Symptoms on affected plants were observed as bronzing of the leaves on one or more of the lower branches, leaves became yellow to brown in plants showing advanced disease symptoms. In such plants, the affected branches and leaf stalks were stiff, turned upwards and the leaflets stand more or less vertically and were shed prematurely. The terminal part of the tap root and lateral roots became brown to black and shriveled. The tap root without any lateral roots was also observed. In Karnataka and Andhra Pradesh, the crop was in podding and harvesting stage. The disease was scattered in the field as dried plants. It was also observed that, the susceptibility of plant to this disease increased with age. Sometimes the apical leaves on the affected plants appeared chlorotic, when the rest of the plant was dry. The pods on affected plants were poorly developed and the number of pods per plant was less. Plants showing different symptoms at different stages of the crop growth (Fig 4.1) were collected and kept in paper bags for conducting further studies.

Similar symptoms were observed by Dastur (1935) as yellowing of leaves which later converted to brown in colour. The affected branches and leaflets were stiff and turned upwards, stand vertically and shed prematurely. Nene *et al.* (1991) observed infection in seedling stage. When the plants were uprooted, the lower portion of tap root remained inside the soil and devoid of most of lateral roots.

4.3 Isolation and identification of the fungus

Different isolates of *Rhizoctonia bataticola* were isolated from the samples collected from different chickpea growing areas of the country. The isolates were purified by single sclerotial isolation technique and were identified as *R. bataticola* based on morphological and cultural characters using the descriptions given by C.M.I (1970). The isolates were designated serially from Rb 1 to Rb 68.

The mycelium was initially white in colour which was later converted to dark brown to black in colour. Production of aerial mycelium was also observed in some isolates. The vegetative mycelium was characterized by the formation of barrel shaped cells and the formation of septum near the origin of branch of the mycelium. Branching occurred mostly at right angle to parent hyphae, but branching at acute angles was also observed. The hyphal cell size varied from 9.38 x 3.80 µm (Rb10) to 14.88 x 7.5 µm (Rb 63) and sclerotial size varied from 54.86 x 45.49 µm (Rb 46) to 216.08 x 181.09 µm (Rb 59). The

Table 4.2. Identity of the isolates along with their disease severity rate and incubation period

S.No	District	Village	Disease severity rating (1-9 rating scale)*	Incubation period*	Isolate code
	Madhya Pradesh				
1	Jabalpur	Tovaria	5.7	3.2	Rb 1
2		Hardua	8.3	1.7	Rb 2
3		Bidagad	4.8	3.7	Rb 3
4	Narsingapur	Barnagh	3.2	4.8	Rb 4
5	Seoni	Bandol	8.0	2.0	Rb 5
6	Chhindwara	Chaurai	7.0	2.5	Rb 6
7	Hoshangabad	Pipariya	7.8	2.0	Rb 7
8	Damoh	Damoh	6.7	2.2	Rb 8
	Karnataka				
9	Gulbarga	Suntnoor	7.8	2.0	Rb 9
10		Sarsamba	2.8	4.5	Rb 10
11		Sikhapur	7.3	2.2	Rb 11
12	Raichur	Devadurga	4.7	3.5	Rb 12
13		Raichur	7.2	2.0	Rb 13
	Maharastra				
14	Osmanabad	Edsi	5.8	2.7	Rb 14
15	Beed	Nagajhari	7.7	1.8	Rb 15
16	Jalna	Gaure Taluka	8.0	2.0	Rb 16
17	Buldhana	Sindkhedgav	8.2	1.7	Rb 17
18		Kaparkeda	7.5	2.0	Rb 18
19		Mekar	5.8	2.8	Rb 19
20	Yavatmal	Vuttarana	4.8	3.3	Rb 20
21	Wasim	Dongargiri	9.0	1.0	Rb 21
22	Akola	Rajapur	7.2	2.2	Rb 22
23		Pailpada	7.0	2.2	Rb 23
24		Vurum	7.2	2.3	Rb 24
25		Maulikhed	7.8	1.8	Rb 25
26	Yavatmal	Lacina	8.0	1.8	Rb 26
27	Amaravathi	Velora	8.7	1.3	Rb 27
	Andhra Pradesh				
28	Anantpur	Egnoor	7.7	2.0	Rb 28
29		Chimalavagupalli	5.2	3.3	Rb 29
30		Pedakallu	6.0	2.8	Rb 30
31		Chinakallu	4.5	3.7	Rb 31
32	Guntur	Lam (RARS)	3.8	4.3	Rb 32
33		Chilakaluripeta	7.5	2.0	Rb 33
34		Pedanandipadu	7.8	2.0	Rb 34
35	Kurnool	Nandyal (RARS)	9.0	1.0	Rb 35
36		Ayyaluru	6.0	2.8	Rb 36
37		Alur	5.8	2.8	Rb 37

S.No	District	Village	Disease severity rating (1-9 rating scale)	Incubation period	Isolate code
38	Kurnool	Oruvagallu	2.5	3.5	Rb 38
39		Chelkuru	3.8	4.2	Rb 39
40		Rudipadu	8.0	2.0	Rb 40
41		Devanoor	6.7	2.7	Rb 41
42		Medthur	6.8	2.2	Rb 42
43		Gandemala	7.2	2.2	Rb 43
44		Gospadu	4.2	3.8	Rb 44
45		Kollur	4.5	3.5	Rb 45
46		Kurnool	8.5	1.5	Rb 46
47		Kolimigundla	5.5	3.0	Rb 47
48		Chelpur	5.2	3.2	Rb 48
49	Prakasam	Naguluppalapadu	8.0	2.0	Rb 49
50		Paduchuru	7.2	2.2	Rb 50
51		Marturu	5.8	2.8	Rb 51
	Telangana State				
52	Medak	Patancheru	7.0	2.5	Rb 52
53		Patancheru	7.7	2.0	Rb 53
54		Patancheru	5.0	3.0	Rb 54
55		Patancheru	5.5	3.0	Rb 55
56		Patancheru	5.5	3.0	Rb 56
57		Patancheru	9.0	2.0	Rb 57
58		Patancheru	8.2	1.7	Rb 58
59		Patancheru	8.8	1.2	Rb 59
60		Patancheru	9.0	1.0	Rb 60
61		Patancheru	9.0	1.0	Rb 61
62		Patancheru	7.5	2.2	Rb 62
63		Patancheru	9.0	1.0	Rb 63
64		Shankerpally	6.7	2.2	Rb 64
65	Rangareddy	Daroor	8.8	1.2	Rb 65
66	Mahboobnagar	Naraynapuram	7.7	2.0	Rb 66
67		Vundavelli	3.3	4.7	Rb 67
68		Rangapur	4.8	3.3	Rb 68
		CD	0.97	0.66	

Table 4.3. Grouping of isolates based on disease severity and incubation period by artificial inoculation

Character	Type	MadhyaPradesh	Karnataka	Maharashtra	Andhra Pradesh	Telangana
Disease severity (1-9 rating scale)	>1-≤3		Rb 10		Rb 38	
	>3 -≤5	Rb 4,3	Rb 12	Rb 20	Rb32,39,44,31, 45	Rb 67,68,54
	>5 -≤7	Rb 1,8,6	Rb9,11,1 3	Rb14,19,23	Rb29,48,47,37, 51,30,36,41,42	Rb 55,56,64,52
	>7 -≤9	Rb 7,5,2		Rb 22,24,18,15, 25,16,26,17, 27,21	Rb 43,50,33,28,34, 40,49,46,35,	Rb62,53,66,5 8,59,65,57,60 ,61,63
Incubation period (days)	0-1.0				Rb 35	Rb 60,61,63
	1.1-2.0	Rb2,5,7	Rb 9,13	Rb15,16,17, 18,21,25,26, 27	Rb 28,33,34,40,46, 49	Rb50,53,57,5 8,59,65
	2.1-3.0	Rb6,8	Rb 11	Rb 14,19,22,23, 24	Rb30,36,37,41, 42,43,47,50,51	Rb52,54,55,5 6,62,64
	3.1-4.0	Rb 1,3	Rb 12	Rb 20	Rb 29,31,38,44,45, 48	Rb 68
	4.1-5.0	Rb 4	Rb 10		Rb 32,39	Rb67

shape of sclerotia varied from round, ovoid to irregular. The texture of sclerotia was either rough or smooth. The sclerotia were dark brown to black in colour.

The above observations were in accordance with the descriptions given by Short and Willie (1978) as branching occurs at right angle to parent hyphae but branching at acute angles was also common. Microsclerotia were formed from the aggregation of hyphae with 50 to 200 individual cells. The microsclerotia of *Macrophomina* were black in colour and size varied from 50 - 150 µm with respect to host and media used. Devi and Singh (1998) observed bigger sclerotia in isolate MP - 2 (400 x 280 µm) while working on *Macrophomina phaseolina* isolates in mungbean. He also observed typical right angled branching of mycelium in one of the isolate and acute to right angle branching in certain isolates.

4.4 Pathogenicity test

Pathogenicity of the 68 isolates of fungus obtained from different states was tested by artificial root dip inoculation using paper towels. The data pertaining to incubation period and disease severity are presented in the table 4.2 and 4.3.

Among 68 isolates, the maximum disease severity rating of 9 was observed in Rb 21, Rb 35, Rb 57, Rb 60, Rb 61 and Rb 63 inoculated plants while the least of 2.5 was observed in Rb 38. The incubation period ranged from 1.0 (Rb 21, Rb 35, Rb 60, Rb 61 and Rb 63) to 4.8 (Rb 4) days. It was observed that with an increase in disease severity there was decrease in incubation period across all the isolates tested.

Among Madhya Pradesh isolates, the disease severity rating varied from 3.2 (Rb 4) to 8.3 (Rb 2) and the incubation period varied between 1.7 (Rb 2) to 4.8 (Rb 4) days. In case of isolates collected from Karnataka, the disease severity rating varied from 2.8 (Rb 10) to 7.8 (Rb 9) while the incubation period fell between 2.0 (Rb 13) to 4.5 (Rb 10) days.

Across the isolates from Maharashtra, the disease severity varied from 4.8 (Rb 20) to 9.0 (Rb 21) and the incubation period ranged from 1.0 (Rb 21) to 3.3 (Rb 20) days. The disease severity rating from Andhra Pradesh isolates varied from 2.5 (Rb 38) to 9.0 (Rb 35) while the incubation period of those isolates ranged between 1.0 (Rb 35) to 4.3 (Rb 32) days. Among Telangana isolates, the disease severity varied from 3.3 (Rb 67) to 9 (Rb 61)

with a range of incubation period from the 1.0 (Rb 60, Rb 61 and Rb 63) to 4.7 (Rb 67) days.

Similarly, Pande *et al.* (2004) observed the maximum disease severity rating of 9 in the susceptible cultivar BG 212 when screened for source of resistance to dry root rot. Sharma *et al.* (2012) also proved the pathogenicity of 50 isolates of *Rhizoctonia bataticola* using chickpea cultivar BG 212 and reported the dry root rot disease severity ranging between 4 and 9 (on the basis of 1 to 9 severity rating scale). Their study also indicated that pathogenic and non pathogenic isolates were not concentrated in any one particular region /state.

4.5 Studies on variability in *Rhizoctonia bataticola*

4.5.1 Cultural and morphological variability in *Rhizoctonia bataticola*

Variability in the cultural and morphological characters of sixty eight isolates of *Rhizoctonia bataticola* were studied by growing on potato dextrose agar medium. The colony growth of the *R. bataticola* isolates measured at 72h after inoculation. The size of the hyphal cell and sclerotia was measured using Q-capture image analyzer software at 10x objective of the microscope. Observations on various cultural and morphological characters were recorded as described in material and methods and the results obtained are presented in Table 4.4 to 4.7 and Fig 4.2.

Colony diameter

The data on colony diameter presented in the table 4.4 revealed that there was a significant difference in the colony growth recorded among the isolates of *Rhizoctonia bataticola* which varied from 17.7 to 80.0 mm. Isolate Rb 14, Rb 17, Rb 22, Rb 26, Rb 49 and Rb 54 showed significantly highest colony growth (80 mm) while the least colony diameter was observed with the isolate Rb 20 (17.7 mm) Mean colony diameter among Madhya Pradesh, Karnataka, Maharashtra, Andhra Pradesh and Telangana isolates ranged from 41.0 mm (Rb 7) to 73.0 mm (Rb 1), 48.7 mm (Rb 12) to 72.5 mm (Rb 11), 17.7mm (Rb 20) to 80.0mm (Rb 14, 17, 22, 26), 36.3 mm (Rb 44) to 80.0mm (Rb 49) and 33.7mm (Rb 64) to 80.0mm (Rb 54) respectively.

Colonycolour.

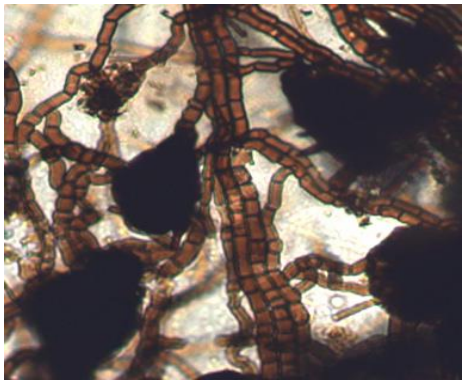
Table 4.4. Variability in cultural characteristics of *Rhizoctonia bataticola* isolates

S. No.	Isolate code	Radial growth* (mm)	Colony texture#	Pigmentation#	Aerial mycelium#
1	Rb 1	73.0	Appressed	Dark Brown	Absent
2	Rb 2	47.5	Velvetty	Dark Brown	Absent
3	Rb 3	60.0	Velvetty	Dark brown	Absent
4	Rb 4	57.7	Appressed	Dark brown	Present
5	Rb 5	72.0	Velvetty	Black	Present
6	Rb 6	61.0	Velvetty	Black	Present
7	Rb 7	41.0	Appressed	Dark brown	Absent
8	Rb 8	43.0	Appressed	Black	Absent
9	Rb 9	62.5	Fluffy	Black with grey aerial mycelium	Present
10	Rb 10	52.3	Appressed	Black	Absent
11	Rb 11	72.5	Appressed	Black	Present
12	Rb 12	48.7	Fluffy	Black with grey aerial mycelium	Present
13	Rb 13	52.7	Fluffy	Grey	Present
14	Rb 14	80.0	Velvetty	Grey	Present
15	Rb 15	66.7	Appressed	Grey	Present
16	Rb 16	68.5	Appressed	Black	Present
17	Rb 17	80.0	Fluffy	Black with grey aerial mycelium	Present
18	Rb 18	72.3	Velvetty	Black	Absent
19	Rb 19	64.7	Fluffy	Black	Present
20	Rb 20	17.7	Appressed	Grey	Present
21	Rb 21	71.0	Fluffy	Black with grey aerial mycelium	Present
22	Rb 22	80.0	Fluffy	Black with grey aerial mycelium	Present
23	Rb 23	75.8	Fluffy	Black with grey aerial mycelium	Present
24	Rb 24	74.0	Fluffy	Black with grey aerial mycelium	Present
25	Rb 25	71.0	Fluffy	Black	Present
26	Rb 26	80.0	Appressed	Black	Present
27	Rb 27	67.7	Fluffy	Black with grey aerial mycelium	Present
28	Rb 28	52.7	Velvetty	Grey	Present
29	Rb 29	62.5	Fluffy	Black	Present
30	Rb 30	66.7	Fluffy	Black with grey aerial mycelium	Present
31	Rb 31	72.3	Fluffy	Black with grey aerial mycelium	Present
32	Rb 32	64.0	Fluffy	Black with grey aerial mycelium	Present
33	Rb 33	73.2	Fluffy	Black with grey aerial mycelium	Present
34	Rb 34	44.7	Appressed	Black	Absent
35	Rb 35	53.7	Fluffy	Grey	Present
36	Rb 36	74.5	Appressed	Black	Absent
37	Rb 37	68.7	Fluffy	Black with grey aerial mycelium	Present
38	Rb 38	49.7	Fluffy	Black	Absent
39	Rb 39	74.3	Fluffy	Grey	Present

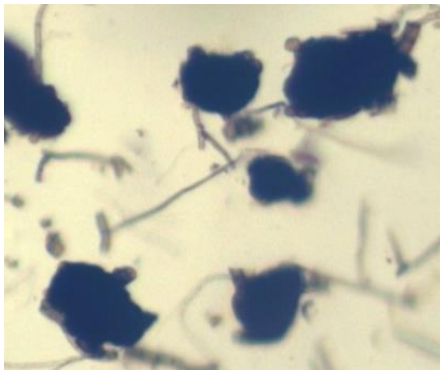
40	Rb 40	68.3	Appressed	Black	Absent
41	Rb 41	49.3	Fluffy	Grey	Present
S. No.	**Isolate code**	**Rradial growth* (mm)**	**Colony texture#**	**Pigmentation#**	**Aerial mycelium#**
42	Rb 42	57.7	Appressed	Black	Absent
43	Rb 43	72.3	Velvetty	Black with grey aerial mycelium	Present
44	Rb 44	36.3	Appressed	Black	Absent
45	Rb 45	71.7	Fluffy	Black with grey aerial mycelium	Present
46	Rb 46	62.7	Velvetty	Grey	Present
47	Rb 47	53.3	Appressed	Black	Absent
48	Rb 48	72.0	Appressed	Grey	Present
49	Rb 49	80.0	Fluffy	Black with grey aerial mycelium	Present
50	Rb 50	44.0	Velvetty	Black	Present
51	Rb 51	66.8	Appressed	Black	Absent
52	Rb 52	35.5	Appressed	Dark brown	Absent
53	Rb 53	57.7	Appressed	Dark Brown	Absent
54	Rb 54	80.0	Appressed	Black	Absent
55	Rb 55	73.7	Appressed	Black	Absent
56	Rb 56	46.8	Appressed	Dark brown	Absent
57	Rb 57	71.7	Appressed	Dark brown	Absent
58	Rb 58	65.7	Appressed	Dark brown	Absent
59	Rb 59	64.3	Appressed	Dark brown	Present
60	Rb 60	52.0	Appressed	Black	Absent
61	Rb 61	71.0	Fluffy	Black with grey aerial mycelium	Present
62	Rb 62	67.3	Fluffy	Black with grey aerial mycelium	Present
63	Rb 63	44.3	Appressed	Black	Absent
64	Rb 64	33.7	Velvetty	Black	Present
65	Rb 65	63.7	Appressed	Black	Absent
66	Rb 66	69.7	Appressed	Grey	Absent
67	Rb 67	72.3	Fluffy	Black with grey aerial mycelium	Present
68	Rb 68	73.7	Fluffy	Black with grey aerial mycelium	Present
	C.D.	1.85			

Appressed

Fluffy



Ovoid



Irregular

4.2. Morphological and cultural variability among the *Rhizoctonia bataticola* isolates

Based on visual observation on colony colour, the cultures were divided into four groups. Black colour colony was observed in 26 isolates while black with grey aerial mycelium was recorded in 20 isolates. Dark brown and grey colour was observed in 11 each respectively.

The pigmentation of the colony varied from dark brown (Rb 5, Rb 6 and Rb 8) to black (Rb 1, RB 2, Rb 3, Rb 4 and Rb7) in Madhya Pradesh isolates. Among Karnataka isolates, the pigmentation of the colony varied from grey (Rb 13), black with grey aerial mycelium (Rb 9 and Rb 12) and black (Rb 10 and Rb11). Across the Maharashtra isolates colony colour varied from grey (Rb 14, Rb 15, Rb 20), black with grey aerial mycelium (Rb 27, Rb 21, Rb 24, Rb 23, Rb 22 and Rb 17) to black (Rb 16, Rb 26, Rb 19, Rb 22 and Rb 18).

Among the Andhra Pradesh isolates, colony colour varied from grey (Rb 48, Rb 41, Rb 35, Rb 39, Rb 28 and Rb 46), black with grey aerial mycelium (Rb 32, Rb 30, Rb 37, Rb 45, Rb 31, Rb 33, Rb 49 and Rb 43) to black (Rb 34, Rb 51, Rb 36, Rb 44, Rb 47, Rb 42, Rb 40, Rb 38, Rb 2 and Rb 50).

Among Telangana isolates, pigmentation of the colony varied from black with grey aerial mycelium (Rb 62, Rb 61, Rb 67, Rb 68, Rb 52, Rb 53, Rb 59, Rb 58, Rb 57 and Rb 56), black (Rb 63, Rb 60, Rb 65, Rb 54, Rb 55 and Rb 64), brown (Rb 52, Rb 53, Rb 56, Rb 57, Rb 58 and Rb 59) to grey colour (Rb 66).

Colony texture

Isolates categorized into three groups on the basis of colony texture. Maximum of 30 isolates produced appressed colony while 27 isolates had fluffy texture. Only 11 isolates had produced velvety growth.

The colony texture of appressed (Rb 4, Rb 1, Rb 7 and Rb 8) and velvety (Rb 2, Rb 3, Rb 5 and Rb 6) were observed in Madhya Pradesh isolates. Among Karnataka isolates, colony texture varied from appressed (Rb 10 and Rb 11) to fluffy (Rb 9, Rb 12and Rb 13). Maharashtra isolates possessed three types of colony textures *viz.*, appressed (Rb 20, Rb 15, Rb 16 and Rb 26), fluffy (Rb 19, Rb 27, Rb 22, Rb 21, Rb 24, Rb 22, Rb 17 and Rb 23) and velvety (Rb 14 and Rb 18). Similarly all the three types were observed in Andhra Pradesh and Telangana isolates. Among Andhra Pradesh isolates, appressed texture was observed in Rb 34, Rb 51, Rb 48, Rb 36, Rb 44, Rb 47, Rb 4 and, Rb 40, while the fluffy

texture in Rb 41, Rb 38, Rb 35, Rb 29, Rb 32, Rb 30, Rb 37, Rb 45, Rb 31, Rb 33, Rb 39, Rb 49 and velvety texture was observed in Rb 50, Rb 28, Rb 46, Rb 43. Among Telangana isolates, mostly appressed texture was observed except in Rb 62, Rb 61, Rb 67, Rb 68 which had fluffy and only Rb 64 had developed velvety type texture.

Aerial mycelium

Maximum number of 42 isolates produced aerial mycelium and 26 isolates did not develop any aerial mycelium. No aerial mycelium was observed in most of the isolates that had appressed colony.

In Madhya Pradesh isolates mostly there was no aerial mycelial growth except in Rb 4, Rb 5 and Rb 6 while in Karnataka and Maharashtra isolates, aerial mycelial growth was observed in all except in Rb 10 and Rb 18 respectively.

Among Andhra Pradesh isolates, aerial mycelial growth was present in all the isolates except in Rb 34, Rb 51, Rb 36, Rb 44, Rb 47, Rb 42, Rb 40 and Rb 38 while in Telangana isolates, the aerial mycelial growth was observed only in Rb 64, Rb 62, Rb 61, Rb 67, Rb 68, Rb 59.

Hyphal cell

The hyphal cell size varied from 9.38 x 3.80 µm (Rb10) in Karnataka to 14.88 x 7.50 µm (Rb 63) in Telangana. When the data on length of hyphal cell was subjected to statistical analysis no significant difference was observed between isolates Rb 10, Rb 27, Rb 3, Rb 2, Rb 25 and Rb 24. Ratio between length and width of hyphal cell varied from 1.35 (Rb 27) to 2.96 (Rb 65).

Among Madhya Pradesh isolates, mean hyphal cell size varied from 9.55 x 5.14 µm (Rb 3) to 13.34 x 5.2 µm (Rb 1). The isolates Rb 6, Rb 5 and Rb 1 showed significant difference for mean hyphal cell length while there was no significant difference between isolates Rb 3 and Rb2. Ratio between the length and width varied from 1.57 (Rb 7) to 2.56 (Rb 1). There was significant difference between the isolates for length by width ratio except Rb 6 and Rb 8 which were at par with each other.

Among Karnataka isolates, mean hyphal cell size varied from 9.38 x 3.8 µm (Rb 10) to 14.14 x 5.53 µm (Rb 9). There was significant difference among the isolates for mean hyphal cell size. The length/width of hyphal cell varied from 1.8 (Rb 13) to 2.67 (Rb 9). There was significant difference among the isolates for length by width except in Rb 11 and Rb13 which were at par with each other.

Among Maharashtra isolates, hyphal cell size varied from 9.49 x 7.04 µm (Rb 27) to 14.58 x 6.84 µm (Rb 16). There was significant difference between the isolates Rb 16, Rb 17, Rb 14, Rb 20, Rb 21 and Rb 27 while there was no significant difference between Rb 19, Rb 14 and Rb 18. The length/ width of hyphal cell varied from 1.35 (Rb 27) to 2.75 (Rb 15). There was significant difference among the isolates except Rb 28 and Rb20, and Rb 17 and Rb16 which were at par with each other.

Among Andhra Pradesh isolates, mean hyphal cell size varied from 10.28 x 5.00 µm (Rb 51) to 14.29 x 6.44 µm (Rb 49). There was significant difference between the Rb 51, Rb30, Rb36, Rb 45 and Rb 48 while there was no significant difference between the Rb 40, Rb 48, Rb 41 and Rb 49. The length/ width varied from 1.56 (Rb 34) to 2.89 (Rb 47).

Among Telangana isolates, mean hyphal cell size varied from 10.35 x 6.6µm (Rb 52) to 14.88 x 7.5µm (Rb 63). There was significant difference between the Rb 52, Rb 53, Rb 55, Rb 65 and Rb 63 while there was no significant difference between the Rb 53, Rb 62, Rb 68 and Rb 54. The length/width of hyphal cell varied from 1.53 (Rb 60) to 2.96 (Rb 65). There was significant difference between the Rb 60, Rb 52, Rb 59, Rb 56, Rb 54, Rb57, Rb 62, Rb 63, Rb 53, Rb 55, Rb 67, Rb 68 and Rb 65 while there was no significant difference between the Rb 59 and Rb 61; Rb 57 and Rb 58.

Sclerotial size

The size of sclerotia varied from 54.86 x 45.49 µm (Rb 46) in Andhra Pradesh to 216.08 x 181.09 µm (Rb 59) in Telangana state. Ratio between length and width of sclerotia varied from 1.00 (Rb 5) to 1.64 (Rb 23).

The sclerotia size varied from 116.38 x 103.49 µm (Rb 6) to195.47x193.76 µm (Rb 1) among Madhya Pradesh isolates. There was significant difference between the isolates for size of sclerotia. Ratio between length and width of sclerotia varied from 1.00 (Rb 5) to 1.24 Among Karnataka isolates, the sclerotial size varied from 138.23 x 105.52 (Rb 9) - 150.22 x 142.06 µm (Rb 10). The length/ width varied from 1.06 (Rb 10) to 1.31 (Rb 9). There was significant difference among the isolates for length by width of sclerotia except in Rb 11 and Rb13 which were at par with each other.

The sclerotial size varied from 83.29 x 50.93 (Rb 23) to 155.43-143.78 µm (Rb 17) among Maharashtra isolates. There were significant differences between the isolates except (Rb4)

Table 4.5. Grouping of isolates based on cultural characteristics

Character	Type	Madhya Pradesh	Karnataka	Maharashtra	Andhra
Radial growth (mm)	<40			Rb 20	Rb 44
	40.01-50	Rb2,7,8	Rb 12		Rb34,38
	50.01-60	Rb 4	Rb 10,13		Rb 28,3
	60.01-70	Rb3, 6	Rb 9	Rb 15,16,19,27	Rb 9,30,32,
	70.01-80	Rb 5,1	Rb 11	Rb14,17, 18,21,22,26,23,24, 25	Rb 31,33,36 8,49
Colony texture	Appressed	Rb ,4,7,8	Rb 10,11	Rb 15,16,20,26	Rb34,36 7,48,51
	Velvetty	Rb2,3,5,6	Rb9,12,13	Rb14,18	Rb28,43
	Fluffy			Rb17,19,21,22,23,24 ,25,27	Rb29,30 7,38,39,
Pigmentation	Dark brown	Rb1,2,3,4,7			
	Black	Rb 5,6,8	Rb 10,11	Rb 16,18,19,25,26	Rb29,34 2,44,47,
	Grey		Rb 13	Rb 14,15,20	Rb 28,35,39
	Black with grey		Rb 9,12	Rb 17,21,22,23,24,27	Rb30,31 3,45,49

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Table 4.6. Variability in morphological characteristics of *Rhizoctonia bataticola* isolates

S. No.	Isolate Code	Hyphal cell		Sclerotia				
		Length x Width (µm)*	Length/ Width*	Length x Width (µm)*	Length/ Width*	Intensity per 10x microscopic field*	Initiation (Days)**	Shape
1	Rb 1	13.34x5.20	2.56	195.47x193.76	1.01	23.33	1.7	Round
2	Rb 2	9.62x4.62	2.08	183.42x174.45	1.05	33.67	3.3	Ovoid
3	Rb 3	9.55x5.14	1.86	121.59x118.04	1.03	11.67	3.3	Ovoid
4	Rb 4	10.67x5.46	1.95	140.23x113.25	1.24	33.33	4.3	Irregular
5	Rb 5	12.68x6.23	2.04	131.52x130.90	1.00	16.67	2.0	Round
6	Rb 6	11.79x7.19	1.64	116.38x103.49	1.12	38.33	3.0	Round
7	Rb 7	11.53x7.33	1.57	165.95x148.09	1.12	21.67	3.3	Round
8	Rb 8	10.9x6.73	1.62	139.68x130.36	1.07	19.67	3.3	Round
9	Rb 9	14.14x5.30	2.67	138.23x105.52	1.31	28.67	3.0	Round
10	Rb 10	9.38x3.80	2.47	150.22x142.06	1.06	23.33	3.3	Round
11	Rb 11	11.56x6.39	1.81	143.12x119.60	1.20	42.67	2.7	Irregular
12	Rb 12	12.46x6.29	1.98	148.43x138.50	1.07	13.67	2.7	Ovoid
13	Rb 13	10.78x6.00	1.80	139.32x114.86	1.21	39.67	2.0	Ovoid
14	Rb 14	12.52x5.27	2.38	129.03x106.96	1.21	57.33	2.0	Irregular
15	Rb 15	12.23x4.45	2.75	115.01x109.14	1.05	28.67	3.3	Irregular
16	Rb 16	14.58x6.84	2.13	150.52x137.52	1.09	31.67	3.3	Ovoid
17	Rb 17	13.25x6.23	2.13	155.43x143.78	1.08	34.33	2.0	Ovoid
18	Rb 18	12.87x6.38	2.02	120.31x117.18	1.03	51.67	2.3	Ovoid
19	Rb 19	12.52x5.74	2.18	96.35x79.11	1.22	34.67	2.3	Ovoid

20	Rb 20	11.79x5.81	2.03	108.65x105.24	1.03	41.33	3.3	Irregular
S. No.	**Isolate Code**	**Hyphal cell**		**Sclerotia**				
		Length x Width (µm)*	**Length/ Width***	**Length x Width (µm)***	**Length/ Width***	**Intensity per 10x microscopic field***	**Initiation (Days)****	**Shape**
22	Rb 22	10.64x6.16	1.73	109.84x104.35	1.05	18.67	2.0	Irregular
23	Rb 23	10.49x7.04	1.49	83.29x50.93	1.64	24.33	4.3	Ovoid
24	Rb 24	9.97x5.97	1.67	126.71x120.41	1.05	24.67	3.0	Irregular
25	Rb 25	9.81x6.71	1.46	151.13x112.1	1.35	30.67	3.0	Irregular
26	Rb 26	12.27x6.78	1.81	151.52x131.62	1.15	28.67	3.0	Irregular
27	Rb 27	9.49x7.04	1.35	146.80x146.02	1.01	28.33	2.0	Round
28	Rb 28	10.85x5.59	1.94	191.92x180.12	1.07	35.67	4.3	Irregular
29	Rb 29	10.97x5.25	2.09	103.83x95.01	1.09	19.00	3.0	Irregular
30	Rb 30	11.01x4.38	2.52	148.78x132.02	1.13	24.33	3.0	Irregular
31	Rb 31	11.16x4.93	2.27	109.68x106.44	1.03	31.33	3.0	Round
32	Rb 32	11.25x5.57	2.02	160.50x149.80	1.07	31.67	3.0	Round
33	Rb 33	11.38x4.67	2.44	129.89x115.05	1.13	32.67	3.3	Round
34	Rb 34	11.47x7.33	1.56	187.45x151.33	1.24	35.67	3.3	Ovoid
35	Rb 35	11.79x6.67	1.77	141.68x135.38	1.05	35.33	3.3	Ovoid
36	Rb 36	12.09x6.89	1.76	153.41x140.6	1.09	36.33	2.7	Round
37	Rb 37	12.72x5.21	2.44	128.37x118.98	1.08	33.67	2.7	Irregular
38	Rb 38	13.32x7.25	1.84	166.36x154.15	1.08	33.67	2.0	Round
39	Rb 39	13.44x7.69	1.75	119.28x99.50	1.20	35.33	3.0	Round
40	Rb 40	13.77x6.63	2.08	109.54x95.48	1.15	28.67	3.0	Ovoid
41	Rb 41	13.99x6.16	2.27	162.75x135.17	1.20	22.00	4.0	Round

42	Rb 42	11.4x6.60	1.73	129.64x124.59	1.04	17.67	3.0	Round
43	Rb 43	11.58x6.78	1.71	141.52x140.05	1.01	42.67	3.0	Round
S. No.	**Isolate Code**	**Hyphal cell**		**Sclerotia**				
		Length x Width (µm)*	**Length/ Width***	**Length x Width (µm)***	**Length/ Width***	**Intensity per10x microscopic field***	**Initiation (Days)****	**Shape**
45	Rb 45	13.05x6.10	2.14	132.81x129.42	1.03	26.00	2.0	Ovoid
46	Rb 46	12.26x5.18	2.37	54.86x45.49	1.21	20.67	3.0	Round
47	Rb 47	13.07x4.52	2.89	163.65x128.49	1.27	60.00	4.0	Irregular
48	Rb 48	13.94x5.19	2.69	97.79x89.92	1.09	28.67	4.0	Irregular
49	Rb 49	14.29x6.44	2.22	160.97x149.20	1.08	19.33	2.0	Ovoid
50	Rb 50	12.48x5.81	2.15	214.55x182.80	1.17	26.67	3.0	Ovoid
51	Rb 51	10.28x5.00	2.05	191.76x169.94	1.13	27.67	3.0	Round
52	Rb 52	10.35x6.60	1.57	162.32x148.81	1.09	23.33	4.0	Ovoid
53	Rb 53	11.38x5.30	2.15	118.05x114.63	1.03	43.67	2.0	Round
54	Rb 54	11.73x6.57	1.79	151.51x149.15	1.02	22.67	2.0	Round
55	Rb 55	12.29x5.43	2.27	188.59x169.28	1.11	26.33	3.0	Irregular
56	Rb 56	12.45x7.13	1.75	138.13x125.90	1.10	70.67	4.0	Ovoid
57	Rb 57	12.50x6.70	1.87	178.20x148.91	1.20	30.67	3.0	Ovoid
58	Rb 58	12.53x6.66	1.88	97.56x71.96	1.36	23.67	3.0	Round
59	Rb 59	12.65x7.49	1.69	216.08x181.09	1.19	28.00	3.0	Ovoid
60	Rb 60	13.38x8.76	1.53	165.75x129.55	1.28	38.00	4.0	Ovoid
61	Rb 61	13.81x8.11	1.70	167.63x141.64	1.18	23.67	3.0	Ovoid
62	Rb 62	11.41x5.90	1.94	125.79x116.98	1.08	38.67	3.0	Round
63	Rb 63	14.88x7.50	1.99	203.43x188.01	1.08	21.00	3.0	Ovoid

64	Rb 64	12.49x5 .13	2.43	135.11x1 26.78	1.07	14.00	3.0	Round
65	Rb 65	13.33x4 .51	2.96	129.55x1 23.52	1.05	34.67	3.7	Round
66	Rb 66	13.41x5 .91	2.27	125.65x1 24.44	1.01	34.67	3.0	Ovoid
S. No.	**Isolate Code**	**Hyphal cell**		**Sclerotia**				
		Length x Width (µm)*	**Length/ Width***	**Length x Width (µm)***	**Length/ Width***	**Intensity per10x microscopic field***	**Initiation (Days)****	**Shape**
68	Rb 68	11.66x4 .16	2.81	165.76x1 52.70	1.09	24.00	2.3	Round
	CD	0.59	0.03	2.26	0.05	2.80	0.55	

Table 4.7. Grouping of isolates based on morphological characteristics

Character	Type	Madhya Pradesh	Karnataka	Maharashtra	Andhra Pradesh	Telangana
Sclerotia shape	Irregular	Rb 4	Rb 11	Rb14,15,20,21,22,2,25,26	Rb28,29,30,37,47,48	Rb 55
	Ovoid	Rb 2, 3	Rb 12, 13	Rb 16, 17,18,19,23	Rb 34,35,40,45,49,50	Rb52,56,57,59,60,61,63,66
	Round	Rb 1, 5, 6, 7, 8	Rb 9, 10	Rb 27	Rb31,32,33,36,38,39,41,42,43,44,46,51	Rb53,54,58,62,64,65,67,68
Sclerotia texture	Smooth	Rb 2,3,5,6,7,8	Rb 10, 12, 13	Rb16,17,18,19,23,7	Rb28,29,30,37,41,43,5,46,48,49	Rb52,53,54,56,58,59,60,61,62,63,64,65,67,68
	Rough	Rb 1,4	Rb 9, 11	Rb14,15,20,21,22,24,25,26	Rb31,32,33,34,35,36,38,39,40,42,44,47,50,51	Rb 55,57,66
Sclerotia initiation (days)	1 to 1.99	Rb 1				
	2 to 2.99	Rb 5	Rb11,12,13	Rb14,17,18,19,22,27	Rb 36,37,38,45,49	Rb 53,54,67,68
	3 to 3.99	Rb 2,3,4,6,7,8	Rb 9,10	Rb15,16,20,21,24,25,26	Rb29,30,31,32,33,34,35,39,40,42,43,46,50,51,	Rb55,57,58,59,61,62,63,64,65,66
	4 or more			Rb 23	Rb 28, 41,44,47,48	Rb 52,56,60
Intensity per microscopic field (10x)	10 to 19.99	Rb 3,5,8	Rb 12	Rb 22	Rb 29,42,49	Rb 64
	20 to 29.99	Rb 1,7	Rb 9, 10	Rb 15, 23,24,26,27	Rb30,40,41,45,46,48,50,51	Rb52,54,55,58,59,61,63,67,68
	30 to 39.99	Rb 2,4,6	Rb 13	Rb 16,17,19,21,25	Rb28,31,32,33,34,35,36,37,38,39	Rb57,60,62,65,66
Character	**Type**	**Madhya Pradesh**	**Karnataka**	**Maharashtra**	**Andhra Pradesh**	**Telangana**
Intensity per microscopic field (10x)	40 to 40.99		Rb 11	Rb 20	Rb 43,44	Rb 53
	50or more			Rb 14,18	Rb 47	Rb 56
Hyphal size (µm)	9to 11	Rb 3,2,4,8	Rb 10,13	Rb27,25,24,23,22,21	Rb51, 28, 29	Rb52
	11 to 13	Rb 7,6,5,1	Rb 11, 12	Rb 20, 15, 26, 14, 19, 18	Rb 30,31, 32, 33, 42, 34, 43,	Rb53,62,68, 54,55,56,64,

					44, 35, 36, 46, 50, 37	57,58,59
	13 to 15		Rb 9	Rb 17,16	Rb45,47,38,39, 40,48,41,49	Rb65,60,66, 67,61,63
Hyphal length/ width	1 to 1.5			Rb 27,25,23		
	1.5 to 2.0	Rb 7, 8,6,3,4	Rb 13, 11, 12	Rb 24, 22,21, 26,	Rb34,43,42,39, 36,35,38,28,44	Rb60,52,59, 61,56,54,57, 58,62,63
	2.0 to 2.5	Rb 5, 2,	Rb 10	Rb 18, 20, 16, 17, 19, 14,	Rb32,51,40,29, 45,50,49,31,41, 46,33,37	Rb53,55,66, 67,64,
	2.5 to 3.0	Rb 1	Rb 9	Rb 15	Rb 30,48,47	Rb 68,65
Sclerotia size (µm)	50 to 100			Rb 23, 19	Rb 46, 48	Rb 57
	100 to 150	Rb 6,3,5,8,4	Rb 9,13,11,12	Rb20,22,21,15,18,24,14,27	Rb29,40,31,39, 37,42,44,33,45, 43,35,30	Rb52,64,53, 67,66,56
	150 to 200	Rb 7, 2, 1	Rb10	Rb 16,25,26,17	Rb36,32,49,41, 47,38,34,51,28	Rb59,60,62, 65,63,54,61
	>200				Rb50	Rb 55,68,58

Rb 20 and Rb 22, Rb 21 and Rb 15 which were at par with each other. The length/ width varied from 1.01 (Rb 27) to 1.64 (Rb 23). There was significant difference between the isolates of Rb 26 and Rb 14, Rb 25 and Rb 23 while others were at par with each other.

Among Andhra Pradesh isolates, the sclerotia size varied from 54.86 x 45.49 (Rb 46) to 214.55 x 182.8 µm (Rb 50). There was significant difference between the isolates Rb 46, Rb 48, Rb 29, Rb 40, Rb 39, Rb 37, Rb 45, Rb 43, Rb 30, Rb 36, Rb 32, Rb 47, Rb 38, Rb 34, Rb 51 and Rb 50 while others *viz,* Rb 40 and Rb 31, Rb 41 and Rb 47, Rb 51 and Rb 28 were at par with each other. The length/ width varied from 1.01 (Rb 43) to 1.27 (Rb 47). There was significant difference between the isolates Rb 43, Rb 28, Rb 33, Rb 39 and Rb 47 while others *viz,* Rb 49, Rb 38, Rb 48, Rb 29, Rb 36, Rb 33, Rb 30 and Rb 51were at par with each other.

Among Telangana isolates, the sclerotial size varied from 97.56 x 71.96 (Rb 58) to 216.08 x 181.09 µm (Rb 59). There was significant difference between the Rb 58, Rb53, Rb 66, Rb 65, Rb 64, Rb 56, Rb 54, Rb 52, Rb 60, Rb 57, Rb 55, Rb 63, Rb 67 and Rb 59 while there was no significant difference between the Rb 66 and Rb 62; Rb 60, Rb 68 and Rb 61. The length/ width varied from 1.01 (Rb 66) to 1.36 (Rb 58). There was significant difference between the Rb 66, Rb 64, Rb 61, Rb 60 and Rb 58 while there was no significant difference between the Rb 66, Rb 54, Rb 53, Rb 67 and Rb 65; Rb 62, Rb 63, Rb 52, Rb 68, Rb 56 and Rb 55.

Sclerotial texture

Based on sclerotial texture, the isolates were categorized into rough and smooth texture. Rough texture was observed in 25 isolates while smooth texture was observed in 43 isolates.

In Madhya Pradesh isolates, smooth texture was observed in all except Rb 1 and Rb 4. Among Karnataka isolates, rough texture of the sclerotia seen in Rb 9 and Rb 11 while smooth in Rb 10, Rb 12 and Rb 13.

Among Maharashtra isolates, the texture of the sclerotia was rough in Rb 22, Rb 14, Rb 24, Rb 26, Rb 22, Rb 15, Rb 21, Rb 20 and smooth in Rb 27, Rb 17, Rb 19, Rb 18, Rb 16, Rb 23. Among Andhra Pradesh isolates, the texture of the sclerotia varied from rough 14 isolates to smooth in 10 isolates. Among Telangana isolates, Mostly the texture of the sclerotia was smooth except in Rb 55, Rb 57 and Rb 66 which had rough texture.

Sclerotial shape

The isolates were categorized into irregular, round and ovoid groups based on shape of sclerotia. Irregular shaped sclerotia were observed in 17 isolates (Rb 22, Rb 29, Rb 30, Rb 24, Rb 55, Rb 26, Rb 15, Rb 48, Rb 25, Rb 21, Rb 4, Rb 37, Rb 28, Rb 20, Rb 11, Rb 14 and Rb 47) while ovoid shaped sclerotia were observed in 23 isolates (Rb 3, Rb 12, Rb 49, Rb 63, Rb 52, Rb 61, Rb 23, Rb 45, Rb 50, Rb 59, Rb 40, Rb 57, Rb 16, Rb 2, Rb 17, Rb 19, Rb 66, Rb 35, Rb 34, Rb 60, Rb 13, Rb 18 and Rb 56). Round shape of sclerotia was observed in 28 isolates (Rb 64, Rb 5, Rb 42, Rb 8, Rb 46, Rb 7, Rb 41, Rb 54, Rb 1, Rb 10, Rb 58, Rb 68, Rb 67, Rb 51, Rb 27, Rb 9, Rb 31, Rb 32, Rb 33, Rb 38, Rb 65, Rb 39, Rb 36, Rb 6, Rb 62, Rb 44, Rb 43 and Rb 53).

Sclerotial initiation

The time taken for initiation of sclerotia ranged from 1.7 (Rb 1) to 4.3 (Rb 4) days among Madhya Pradesh isolates but among Karnataka isolates, it ranged from 2 (Rb 13) to 3.3 (Rb 10) days. In Maharashtra isolates, sclerotial initiation was observed from 2.0 (Rb 22, Rb 27, Rb 17, Rb 14) to 4.3 (Rb 23) days. The isolates which produced sclerotia on second day differed significantly from those produced sclerotia on third and fourth day and vice- versa. Among Andhra Pradesh isolates, the time taken for sclerotial initiation was from 2.0 days (Rb 49, Rb 45, Rb 38) to 4.3 days (Rb28). Among Telangana isolates, the time taken for sclerotial initiation was from 2.0 (Rb 54, Rb 67, Rb 53) to 4.0 (Rb 52, Rb 60, Rb 56) days.

Sclerotial intensity

The number of sclerotia per microscopic field when observed through 10x objective varied from 11.67 (Rb 63) to 70.67 (Rb 56). Isolates Rb 3, Rb 12 and Rb 64; Rb 1, Rb 10, Rb 52, Rb 58, Rb 61, Rb 68, Rb 30, Rb 23, Rb 24, Rb 67 and Rb 45 were found at par with each other. Rb 53, Rb 18, Rb 14, Rb 47 and Rb 56 had highly significant difference with other.

The sclerotial intensity per microscopic field (10x) varied from 11.67 (Rb 3) to 38.33 (Rb 6) among Madhya Pradesh isolates. Among Karnataka isolates, the sclerotial intensity per microscopic field varied from 13.67 (Rb 12) to 42.67 (Rb 11). There was significant difference among the isolates for sclerotial intensity per microscopic field. Among Maharashtra isolates, the sclerotial intensity per microscopic field varied from 18.67 (Rb 22) to 57.33 (Rb 14). There was significant difference among the isolates of Rb 22, Rb 23, Rb 27, Rb17, Rb 20, Rb 18 and Rb 24 while others were at par with each other.

Among Andhra Pradesh isolates, the sclerotial intensity per microscopic field varied from 17.67 (Rb 42) to 60.00 (Rb 47). There was significant difference between the isolates Rb 42, Rb 46, Rb 30, Rb 5, Rb 32, Rb 35, Rb 44 and Rb 47 while others *viz*, Rb 45, Rb 50, Rb 51, Rb 48 and Rb 40; Rb 37, Rb 38, Rb 35, Rb 39, Rb 28 and Rb 34 were at par with each other while among Telangana isolates, the sclerotial intensity per microscopic field varied from 14.00 (Rb 64) to 70.67 (Rb 56). There was significant difference between the Rb 63, Rb 64, Rb 55, Rb 57, Rb 66, Rb 60, R 53 and Rb 56 while there was no significant difference between the Rb 54, Rb 52, Rb 61, Rb 58 and Rb 68; Rb 66 and Rb65; and Rb60 and Rb62.

Similar studies on cultural and morphological characters of *Rhizoctonia bataticola* were conducted by Sharma *et al.,* (2012) and Gupta *et al.,* (2012). Sharma *et al*., (2012) reported the variation among 94 isolates of *R. bataticola* collected from chickpea and concluded that the light black (52.12%) colony colour was more predominant. Different shapes of sclerotia *viz.,* oblong, ellipsoid, irregular and round type were also recorded. The isolates varied for length and width of sclerotia. However, the length/width ratio ranged from 1.1 to 1.8. Most of the isolates did not produce aerial mycelium except Madhya Pradesh and Andhra Pradesh isolates which produced very high aerial mycelium. Most of the isolates grew very fast and covered the plate within 96 h, while other isolates such as RB 21, RB 31, RB 7, RB 84 and RB 87 grew slowly and showed appressed growth. Sclerotial time of initiation varied from 36 to 48h after inoculation. Sclerotial intensity varied from very less to high and was moderate in majority of the isolates. These findings were in accordance with observations recorded in the present study.

Similarly, Gupta *et al.* (2012) when worked with forty isolates of *R. bataticola* of chickpea collected from Madhya Pradesh, Chhattisgarh, Gujarat and Maharashtra found the sclerotial size ranging from 103.3-117.2 x 90.1-106.5 µm (bigger size) to 72.7-87.5 x 57.1-73.5 µm (smaller size). Based on sclerotial morphology, they categorized the isolates into two groups' *viz.,* oblong shape with irregular edges and round with regular edges. These results were in agreement with the present work of difference in sizes and shapes of sclerotia and results reveal that the morphological characters of the *R. bataticola* vary with the isolate and age of the culture.

4.5. Correlation between cultural, morphological and pathological characters of *Rhizoctonia bataticola*

Correlation studies were done to know the effect of different parameters on the virulence of *Rhizoctonia bataticola* isolates. The results (Table 4.8) indicated that significant positive correlation was observed between colony texture and aerial mycelium (r=0.658), sclerotia texture and its shape (r=0.544), sclerotia length and width (r=0.925) while significant negative correlation was observed between time taken for sclerotial initiation and colony growth (r= -0.485), incubation period and disease severity(r= -0.974), hyphal width and hyphal length/width (r= -804).

With respect to disease severity, positive correlation was observed with colony growth, pigmentation, hyphal width and length, sclerotial length, width, length/ width, sclerotial shape, texture and its initiation, sclerotial intensity per microscopic field (10x) while negative correlation was observed with aerial mycelium, colony texture, hyphal length/ width but these were not significant.

Similar results were also observed by Sharma *et al.* (2012) in *R. bataticola* isolates of chickpea as significant positive relationship between time taken for sclerotial initiation-sclerotial intensity, disease severity- time taken for sclerotial initiation, isolates-disease severity, isolates-sclerotial intensity and disease severity-sclerotial intensity was observed. Hooda and Grover (1988) also reported relationship between sclerotial intensity and pathogenicity as more pathogenic isolates produced more sclerotia, but Manici *et al*. (1992) observed no such positive correlation. Simosa and Delgado (1991) observed negative correlation between cottony type and sclerotial productions.

4.5.2. RAPD analysis of *Rhizoctonia bataticola*

The molecular variability among different isolates of *R. bataticola* was analyzed by RAPD analysis using 10 random primers to characterize the population structure of *R. bataticola* in Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh and Telangana of India. The 10 random primers which produced consistent and reproducible RAPD patterns were used for analysis. All the 10 RAPD primers generated 158 polymorphic bands (Fig 4.3). When fingerprints of these isolates were compared, some bands common to all isolates were observed while others were unique to one or a few isolates. All the RAPD bands produced by 10 primers in the 50 isolates of *R. bataticola* (50 isolates were selected based on the

Table 4.8.Correlation matrix among morphological, cultural and pathological characteristics of *Rhizoctonia bataticola*

		1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15	16
Aerial mycelium	1	1.000															
Colony texture	2	**0.658**	1.000														
Radial growth	3	0.298	0.259	1.000													
Pigmentation	4	0.242	0.132	-0.014	1.000												
Sclerotia shape	5	-0.046	-0.012	0.093	-0.043	1.000											
Sclerotia texture	6	0.011	0.033	0.008	-0.117	**0.544**	1.000										
Disease severity	7	-0.005	-0.091	0.028	0.037	0.133	0.135	1.000									
Hyphallength/ width	8	0.100	0.029	0.092	0.086	-0.179	-0.216	-0.24	1.000								
Hyphal width	9	-0.107	-0.090	-0.008	-0.148	0.267	0.267	0.349	**-0.804**	1.000							
Hypal length	10	-0.002	-0.084	0.127	-0.026	0.064	-0.094	0.153	0.37	0.206	1.000						
Incubation period	11	-0.001	0.074	0.005	-0.035	-0.123	-0.149	**-0.974**	0.235	-0.329	-0.126	1.000					
Sclerotia initiation	12	-0.161	-0.336	**-0.485**	0.129	-0.008	0.112	0.063	-0.105	-0.028	-0.181	-0.057	1.000				
Sclerotia intensity (10x)	13	-0.035	-0.113	-0.002	0.073	0.083	0.111	0.025	-0.037	0.065	0.057	-0.046	0.117	1.000			
Sclerotia length x width	14	0.106	-0.012	-0.063	-0.033	-0.066	0.046	0.151	-0.099	0.189	0.118	-0.144	0.255	0.128	1.000		
Sclerotia length	15	-0.217	-0.148	-0.139	-0.156	0.047	0.016	0.091	-0.075	0.183	0.114	-0.063	-0.038	-0.086	0.091	1.000	
Sclerotia width	16	-0.265	-0.142	-0.115	-0.15	0.068	-0.008	0.043	-0.042	0.135	0.071	-0.012	-0.126	-0.172	-0.223	**0.925**	1.000

Bold values highly significant at 5% CD

differences between isolates with respect to cultural and morphological variation) were subjected to hierarchical cluster analysis based on the principle of UPGMA and a dendrogram was generated (Fig 4.4). The similarity coefficient ranged from 0.63 to 0.92 indicating that no any two or more isolates were 100% similar. The highest similarity coefficient (0.92) was between isolates Rb 2 and Rb 4. The UPGMA cluster analysis grouped the isolates into three major groups' *viz.,* group I, group II and group III based on high magnitude of genetic diversity among the isolates of *R. bataticola*. Group I consisted of 36 isolates. These were again sub grouped into cluster IA and cluster IB. Cluster IA consisted of 28 isolates. Of these, 7 isolates were from Madhya Pradesh, 5 isolates were from Karnataka, 8 isolates were from Maharashtra, 7 isolates were from Andhra Pradesh and one was from Telangana. Cluster IB consisted of one each from Maharashtra and Telangana and 6 from Andhra Pradesh. Group II consisted of 12 isolates. These were again sub grouped into cluster II A and cluster II B. Cluster II A consisted of two isolates. Of these, one was from Maharashtra and the other was from Telangana. Cluster II B consisted of 10 isolates. Of which, Rb 15 was from Maharashtra and Rb 48 was from Andhra Pradesh. The remaining eight isolates were from Telangana. Group III consisted of two isolates *viz.,* Rb 26 and Rb 32 from Maharashtra and Andhra Pradesh, respectively.

The results indicated that, all the isolates of Madhya Pradesh and Karnataka were present under same group IA. The Maharashtra isolates were distributed across all the three groups and were found in IA, IIA and III, while the Andhra Pradesh isolates were grouped under all the three groups, but were found in IA, IB, IIB and III. The Telangana isolates were found in IA, IB, IIA and IIB groups. The results of the present study also indicated that, all the isolates were not necessarily showing the geographical linearity.

Similar observations were noted by Aghakhani and Dubey (2009) in genetic diversity studies conducted in 27 isolates (23 from chickpea and 4 from other host crops) of *R. bataticola* representing 11 different states of India by RAPD. The clusters generated by RAPD grouped all the isolates into six categories at 40% genetic similarity.

The results of study conducted by Manjunatha (2009) were also in agreement with present study representing high level of diversity among the isolates of *Rhizoctonia bataticola* from chickpea of different as well as same state. Thirty isolates were categorized into three groups with 4, 16 and10 in group I, II and III respectively by using three primers OPO-10, OPO-12 and OPN-12. However, the three isolates (RG-18, RG-19 and RG-20) of

group II exhibited 100 per cent similarity. Isolates RB-29 and RB-25 from Bidar and isolate RG-16 from Gulbarga found genetically divergent among isolates studied.

4.6 Influence of environmental factors on dry root rot development in chickpea

4.6.1 Effect of temperature on colony growth of *Rhizoctonia bataticola*

Temperature was known to have profound effect on the growth of fungal organism. Present studies were taken up to know the optimum, minimum and maximum temperature requirements for the growth of pathogen. Five isolates (Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63) representing 5 different states were selected to conduct the study. All the isolates were grown at seven different temperatures *viz.*, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC**.** The data on colony growth was presented in the table 4.9.

At 48h after inoculation, among the seven different temperatures tested, the maximum colony growth was observed in Rb 22 (13.0mm) and no growth was observed in Rb 63 at 15ºC whereas the maximum colony growth was observed in Rb 63 (38.3mm) and the least in Rb 2 (27.7 mm) at 20ºC. Maximum colony growth of 54.0, 77.0 and 15.0 mm in Rb 22 and the least of 44.0, 62.3 and 3.7 mm in Rb 2 was recorded at 25, 30 and 40ºC respectively. In contrast to this, maximum colony growth was observed in Rb 40 (85.3mm) and the least in Rb 2 (71.3 mm) at 35ºC. The difference between isolates at different temperatures was significant except in Rb 13 and Rb 63 at 30 ºC.

At 72h after inoculation (Fig 4.5), the maximum colony growth of 25.3 and 67.0 mm in Rb 22 and the least growth of 17.3 and 60.3 mm was observed in Rb 13 at 15 and 20ºC respectively while at 25ºC, maximum colony growth was observed in Rb 13 (84.7mm) and the least in Rb 2 (67.0 mm). At 30ºC, maximum colony growth was observed in Rb 13, Rb 40, Rb 22 (90 mm) whose difference is at par with each other and the least in Rb 2 (82.0mm). At 35ºC, colony growth reached its maximum (90mm) in all the isolates which were at par with each other. At 40ºC, maximum colony growth was observed in Rb 22 (20.7mm) and the least in Rb 2 (8.3mm). There was significant difference between isolates at 15, 20, 25 and 40ºC except in Rb 13 and Rb 40.

At 96h after inoculation, maximum colony growth of 35.3 and 85.3 mm was recorded in Rb 22 while the least was observed in Rb 40 (20.3mm) and Rb 13 (74.7mm) at 15 and 20ºC respectively. At 25ºC, 30ºC and 35ºC, all the isolates had covered the entire 90

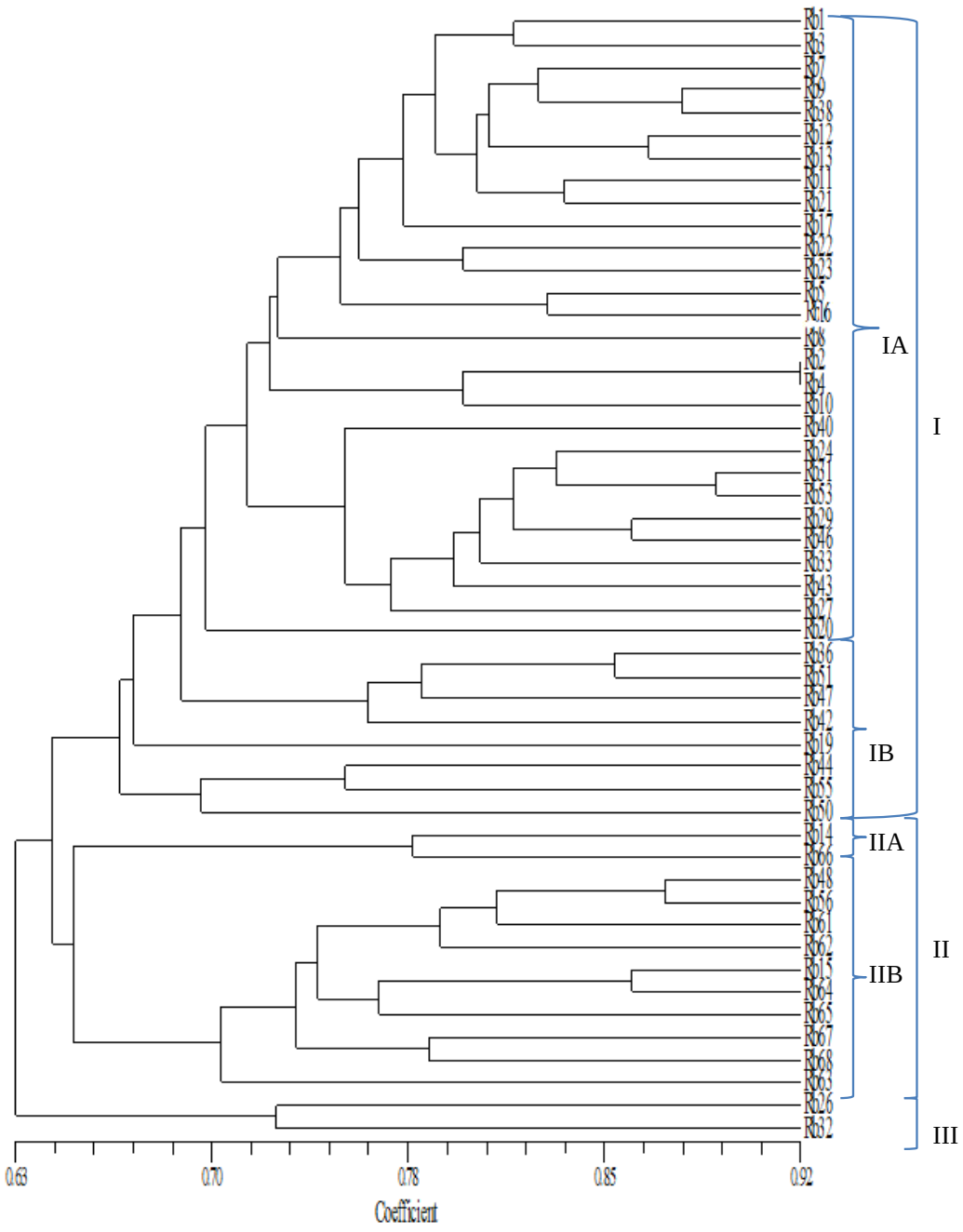

Fig 4. 4. UPAGMA dendrogram of 50 isolates of *Rhizoctonia bataticola* based on RAPD marker

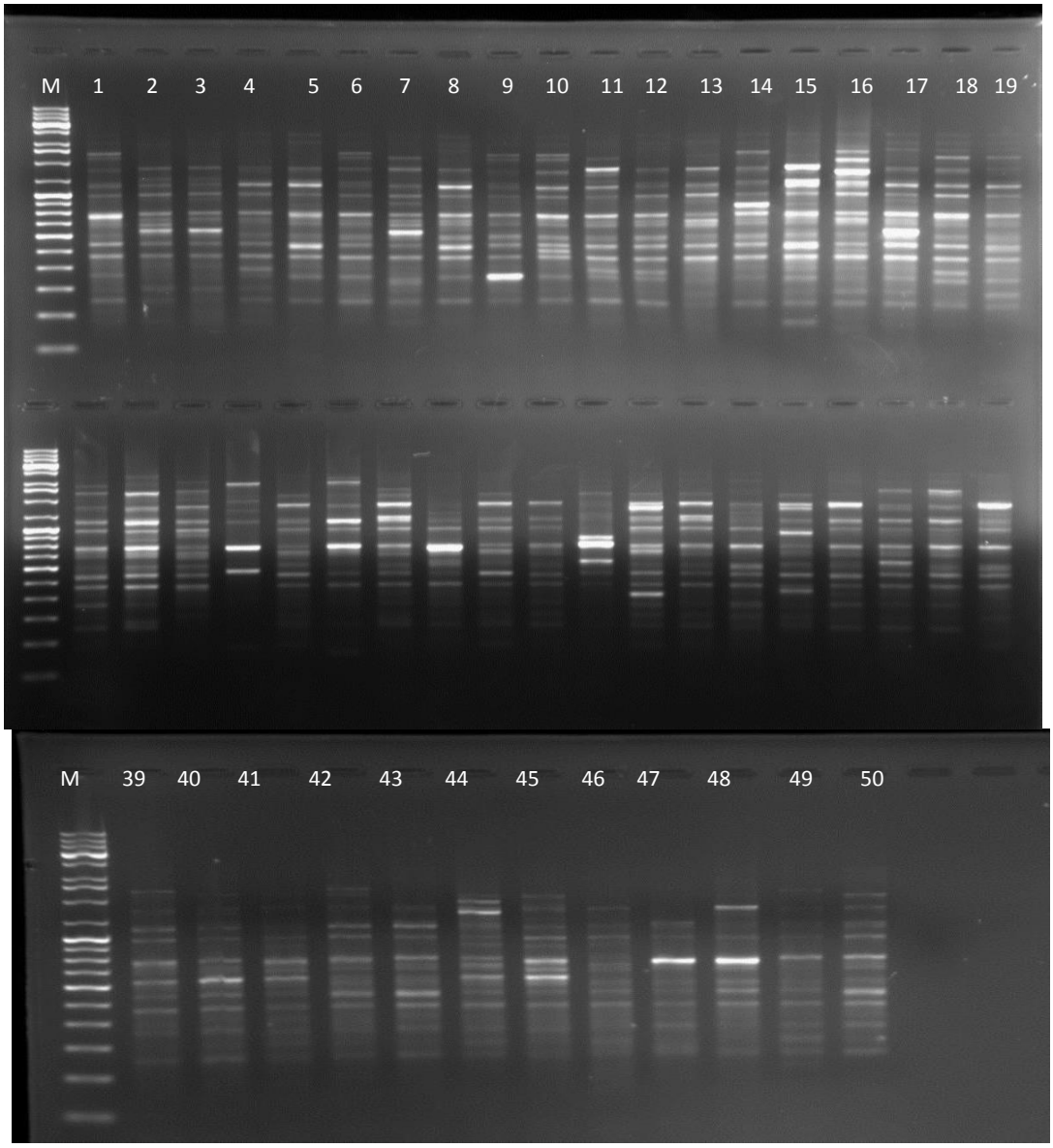

Fig 4.3. RAPD banding profile of *Rhizoctonia bataticola* isolates with primer OPC 06

1-Rb 1, 2-Rb 2, 3-Rb 3, 4-Rb 4, 5-Rb 5, 6-Rb 6, 7-Rb 7, 8-Rb 8, 9-Rb 9, 10-Rb 10
11-Rb 11, 12-Rb 12, 13-Rb 13, 14-Rb 14, 15-Rb 15, 16-Rb 16, 17-Rb 17, 18-Rb 20
19-Rb 21, 20-Rb 23, 21-Rb 25, 22-Rb 26, 23-Rb 27, 24-Rb 29, 25-Rb 31, 26-Rb 32
27-Rb 33, 28-Rb 35, 29-Rb 36, 30-Rb 38, 31-Rb 42, 32-Rb 43, 33-Rb 44, 34-Rb 45
35-Rb 46, 36-Rb 47, 37-Rb 48, 38-Rb 50, 39-Rb 51, 40-Rb 53, 41-Rb 55, 42-Rb 56
43-Rb 61, 44-Rb 62, 45-Rb 63, 46-Rb 64, 47-Rb 65, 48-Rb 66, 49-Rb 67, 50-Rb 68

mm Petri plate. The difference between the isolates at 25, 30 and 35ºC was at par with each other. Maximum colony growth was observed in Rb 22 (24.0mm) and the least in Rb 2 (10.7mm) at 40ºC. No growth was observed till 96h after inoculation in any isolate and medium also became dry.

The maximum colony growth was observed 72h after inoculation in all five isolates at 35ºC. After 96h of incubation, all the isolates had covered Petri plates at 25ºC and 30ºC. The sclerotial initiation was started after 48 hours at 30ºC and 35ºC. The sclerotial initiation started 72h after inoculation at 25ºC and it was observed at 96h after incubation in 20ºC. At 15ºC, it was observed that the growth was very slow and sclerotial initiation was observed after 144 hours after inoculation.

The optimum growth of the fungal isolates was found in 35ºC (79.3, 90.0 and 90.0 mm at 48, 72 and 96 hours after inoculation respectively). The next best temperature was 30ºC with colony growth of 71.3, 88.1 and 90.0 mm at 48, 72 and 96 hours after inoculation respectively) followed by 25ºC, 20ºC and 15ºC. There was very meager growth in 40ºC (10.4, 15.0 and 17.5mm at 48, 72 and 96 hours after inoculation respectively) and no growth was observed at 45ºC. Among means of the isolates, there was significant difference between the isolates at three time intervals except at 96 hours after inoculation between Rb 2 and Rb 40.

The above results were supported by Khan *et al.* (2012) as they also observed pathogen growth over a wide range of temperature from 10^0C to 45^0C, but the optimum temperature for its growth was found to be 30^0C. The next best temperature for its growth was recorded 35^0C. Statistically the growth of the pathogen gradually decreased both at below 30^0C and above 35^0C. Patel and Patel (1990) also reported 35°C to be optimum temperature for growth and sclerotial formation of *M. phaseolina* in sesame.

4.6.2 Effect of temperature on development of disease

There was very significant relation between the temperature and the development of the disease. The disease severity on the BG 212 cultivar was observed after seven days of incubation after inoculation at different temperatures. The average disease severity at different temperatures was given in the Table 4.10.

Maximum disease severity rating was recorded in Rb 2 and Rb 40 (1.7) and no symptom was observed in Rb 63 (1.0) inoculated plants at 15ºC. In contrast, maximum disease severity rating was recorded in Rb 63 (3.4) and the least in Rb 22 (2.9) inoculated

Table 4.9. Colony diameter of *Rhizoctonia bataticola* isolates at different temperatures under *in vitro* condition

48 HAI (mm)*						
Temperature (˚C)	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**
15	4.0	3.3	13.0	6.7	0.0	5.4
20	27.7	32.7	37.0	31.7	38.3	33.5
25	44.0	53.7	54.0	51.3	50.0	50.6
30	62.3	71.3	77.0	75.0	71.0	71.3
35	71.3	82.0	80.0	85.3	77.7	79.3
40	3.7	9.3	15.0	13.7	10.3	10.4
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	30.4	37.7	39.4	36.5	35.3	
72 HAI (mm)						
Temperature (˚C)	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**
15	20.0	17.3	25.3	17.7	18.3	19.7
20	64.0	60.3	67.0	61.0	61.7	62.8
25	67.0	84.7	82.0	80.0	71.7	77.1
30	82.0	90.0	90.0	90.0	88.3	88.1
35	90.0	90.0	90.0	90.0	90.0	90.0
40	8.3	14.7	20.7	18.3	13.0	15.0
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	47.3	52.3	53.6	50.0	49.0	
96 HAI (mm)						
Temperature (˚C)	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**
15	25.3	21.3	35.3	20.3	24.3	25.3
20	80.3	74.7	85.3	78.3	75.0	78.7
25	90.0	90.0	90.0	90.0	90.0	90.0
30	90.0	90.0	90.0	90.0	90.0	90.0
35	90.0	90.0	90.0	90.0	90.0	90.0
40	10.7	17.3	24.0	20.0	15.3	17.5
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	55.2	57.0	59.2	55.2	54.9	

Factors	**CD**		
	48 HAI	**72 HAI**	**96 HAI**
Temperature	0.64	0.54	0.55
Isolate	0.54	0.46	0.47
Temperature x Isolate	1.42	1.21	1.23

HAI – Hours after inoculation * mean of three replications

Table 4.10. Disease severity (1-9 rating) of *Rhizoctonia bataticola* isolates on BG 212 at different temperatures

Isolates	Temperatures (˚C)							
	15	20	25	30	35	40	45	Mean
Rb 2	1.7	3.1	6.7	7.4	8.3	9.0[#]	9.0[#]	6.5
Rb 13	1.3	3.0	7.2	8.2	8.7	9.0[#]	9.0[#]	6.6
Rb 22	1.3	2.9	7.0	7.9	8.3	9.0[#]	9.0[#]	6.5
Rb 40	1.7	3.2	6.5	8.0	8.1	9.0[#]	9.0[#]	6.5
Rb 63	1.0	3.4	7.4	8.2	9.0	9.0[#]	9.0[#]	6.7
control	1.0	1.0	1.0	1.0	1.0	1.0[##]	1.0[##]	1.0
Mean	1.4	3.1	7.0	7.9	8.5	9.0	9.0	

Factors	**C.D.**
Temperature (T)	0.2
Isolates (I)	0.17
T x I	0.45

[#]Mortality of plants due to combined effect of physiological wilting and *Rhizoctonia bataticola*

[##]Plants showed physiological wilting

* mean of three replications

plants at 20ºC. Maximum disease severity rating of 7.4 in Rb 63 inoculated plants and the least in Rb 40 (6.5) and Rb 2 (6.7) which were at par with each other in inoculated plants at 25ºC. At 30ºC, maximum disease severity was observed in Rb 63 and Rb 13 (8.2) and the least in Rb 2 (7.4). At 35ºC, maximum disease severity was observed in Rb 63 (9.0) and least was observed in Rb 40 (8.1) inoculated plants. At 40 and 45ºC, mortality of plants was due to combined effect of physiological wilting and *Rhizoctonia bataticola* isolates. In control, plants were shriveled at 40ºC whereas at 45ºC there was complete physiological wilting. It was also supported by the scarce and absence of mycelial growth in Petriplates at 40 and 45ºC respectively in all the isolates.

The optimum temperature for dry root rot development was 35°C as maximum disease severity rating of 8.5 was observed irrespective of the isolate. This was followed by 30°C (7.9) followed by 25°C (7.0). It was observed that 20°C and 15°C had helped in the development of the lesions but could not develop further. The disease severity was very low 1.4 rating at 15°C while it was 3.1 at 20°C.

Among Rb 2, Rb 13, Rb 22 and Rb 40 isolates, there was no significant difference while Rb 63 was virulent isolate compared to others. The plants had showed symptoms on the tap root leaving the lateral roots unaffected at 25°C. At 30, 35, 40, 45°C, there was complete blackening of the roots and the reisolation from the roots showed the presence of *Rhizoctonia batatiocla*. The uninoculated plants did not show any symptoms except at 40 and 45°C which showed physiological wilting and death of plants by complete drying respectively.

Similar observations were recorded by Sharma and Pande (2013) as disease incidence of dry root rot was significantly affected by high temperature. Out of five temperature levels *viz.*, 15°C, 20°C, 25°C, 30°C and 35°C tested, chickpea predisposed to dry root rot early and severity was more at 35°C. Singh and Mehrotra (1982) observed increased levels of seed exudates when incubated at 35ºC than at 15 and 25ºC which contributed to increased pre-emergence damping off in gram seedling by *R. bataticola*. This study clearly demonstrated high temperature (35ºC) was the climate change predisposing chickpea to *R. bataticola* infection, colonization and development of disease.

4.6.3 Effect of osmotic potential and temperatures on growth of *Rhizoctonia bataticola*

Osmotic potential was recognized as an important parameter in the growth of pathogenic fungi. Soil fungi respond to fluctuations in the osmotic potential and temperature by changes in the metabolic activity and growth rate. Growth rate of isolate Rb

Table 4.11. Colony growth of *Rhizoctonia bataticola* at different levels of osmotic potentials and temperatures

	NaCl						KCl						Dextrose				
Temp (ºC)	20	25	30	35	40	Mean	20	25	30	35	40	Mean	20	25	30	35	4
OP (-MPa)	24 hours after incubation(mm)*																
Control	19.0	25.0	35.3	41.3	5.3	25.2	19.0	25.0	35.3	41.3	5.3	25.2	19.0	25.0	35.3	41.3	5.
0.5	12.0	23.0	38.6	44.5	19.5	27.5	15.0	21.5	42.5	49.5	27.0	31.1	17.0	29.5	51.5	75.5	17
1.0	0.0	0.0	7.5	16.8	0.0	4.9	0.0	12.0	20.8	31.5	0.0	12.9	15.5	27.5	47.5	64.2	29
1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	0.0	2.1	8.0	16.5	34.5	53.5	20
2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.0	13.5	23.0	41.5	14
2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	13.5	22.2	7.
Mean	5.2	8.0	13.6	17.1	4.1		5.7	9.8	16.4	22.1	5.4		11.4	20.4	34.2	49.7	15
	48 hours after incubation(mm)																
Control	38.3	50.0	62.3	71.3	10.3	46.4	38.3	50.0	62.3	71.3	10.3	46.4	38.3	50.0	62.3	71.3	10
0.5	27.5	42.0	67.3	74.0	23.5	46.9	38.8	57.0	72.0	77.0	45.0	57.9	48.2	72.0	77.5	80.0	20
1.0	7.0	9.5	16.5	30.0	0.0	12.6	15.0	26.0	54.5	61.7	0.0	31.4	43.0	64.5	76.0	80.0	43
1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	15.5	30.0	0.0	11.7	25.5	45.5	72.5	80.0	39
2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.5	42.5	71.5	73.5	37
2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	30.0	52.5	67.5	13
Mean	12.1	16.9	24.4	29.2	5.6		15.3	24.3	34.1	40.0	9.2		31.8	50.8	68.7	75.4	27

	72 hours after incubation(mm)																
Control	61.7	71.7	80.0	80.0	13.0	61.3	61.7	71.7	80.0	80.0	13.0	61.3	61.7	71.7	80.0	80.0	13.
0.5	46.0	64.7	80.0	80.0	23.5	58.8	72.0	77.0	80.0	80.0	45.0	70.8	79.0	80.0	80.0	80.0	20.
1.0	14.0	12.0	23.3	39.3	0.0	17.7	27.0	47.0	74.3	77.5	13.0	47.8	75.0	80.0	80.0	80.0	43.
1.5	0.0	0.0	0.0	0.0	0.0	0.0	11.0	28.0	34.3	52.5	0.0	25.2	52.0	76.5	80.0	80.0	39.
2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	48.5	67.5	80.0	80.0	37.
2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.0	60.5	65.0	80.0	13.
Mean	20.3	24.7	30.6	33.2	6.1		28.6	37.3	44.8	48.3	11.8		56.7	72.7	77.5	80.0	27.

Factors	CD		
	24	48	72
Source (S)	0.16	0.21	0.18
Temperature (T)	0.21	0.27	0.23
Osmotic potential (OP)	0.23	0.29	0.26
S x T	0.36	0.46	0.41
S x OP	0.39	0.50	0.44
T x OP	0.51	0.65	0.57
S x T x OP	0.88	1.12	0.99

63 was studied at different osmotic potentials *viz.*, -0.5, -1.0, -1.5, -2.0 and -2.5 MPa incubated at five different temperatures *viz.*, 20ºC, 25ºC, 30ºC, 35ºC and 40 ºC**.** The mean mycelium growth at 24 h, 48 h and 72 h after inoculation was presented in the Table 4.11 and Fig 4.6.

At 24h after inoculation, the growth of *Rhizoctonia bataticola* on PDA amended with NaCl, KCl and dextrose at five different temperatures showed maximum mean colony growth at 35ºC *viz.*, 17.1, 22.1 and 49.7 mm followed by 30ºC of 13.6, 16.4 and 34.2 mm respectively. The least growth was observed at 40ºC of 4.1 and 5.4mm whereas in dextrose amended PDA least growth was observed at 20ºC (11.4mm). Similar observations were also recorded at 48 and 72 h after inoculation except in dextrose where least growth was observed at 40ºC.

At 24 h after inoculation, among different osmotic potential levels of NaCl, KCl and dextrose, maximum mean colony growth was observed at -0.5 MPa *viz.*, 27.5 mm, 31.1 and 38.2 mm respectively where there was significant difference between them while the minimum growth was observed in -1.0 (4.9 mm), -1.5 (2.1 mm) and -2.5MPa (10.7 mm) respectively. At 48 h after inoculation among different osmotic potential levels of NaCl, KCl and dextrose, maximum colony growth was observed in -0.5 MPa of 46.9 and 57.9 mm respectively in NaCl and KCl amended medium whereas in dextrose amended medium it was maximum at -1.0 MPa (61.4 mm) followed by -0.5 MPa (59.5 mm). There was significant difference between the means of different salts. Similar observations were also recorded at 72 h after inoculation.

In NaCl amended PDA medium, maximum colony growth of 19.0 and 25.0 mm was recorded at 24 h after inoculation in control at 20 and 25ºC followed by -0.5 MPa of 12.0 and 23.0 mm respectively. In contrast, maximum colony growth at 30, 35 and 40ºC was observed at -0.5 MPa of 38.6, 44.5 and 19.5 mm respectively followed by control. No growth was observed from -1.0 to 2.5 MPa at 20, 25 and 40ºC up to 24h after inoculation. The difference between maximum and minimum at same temperature and difference between the temperatures at same osmotic potential was significant. Similar type of observations was only recorded at 48 h after inoculation. At 72 h after inoculation in NaCl amended PDA medium, maximum colony growth of 80 mm was observed at both 30 and 35ºC in -0.5 MPa and control which were at par with each other. While maximum colony

growth of 61.7 and 71.7 mm at 20 and 25ºC was observed in control medium respectively followed by -0.5 MPa of 46.0 and 64.7 mm respectively. Maximum colony growth at 40ºC recorded in -0.5 MPa (23.5 mm) followed by control (13.0 mm). There was no growth in the medium at -1.5, -2.0 and -2.5 MPa irrespective of temperature up to 72h after inoculation in NaCl amended PDA medium.

Growth at 24 h after inoculation in KCl amended PDA medium showed maximum of 19.0 and 25.0 mm at 20 and 25ºC in control followed by -0.5 MPa osmotic potential of 15.0 and 21.5 mm respectively. Maximum colony growth of 42.5, 49.5 and 27.0 mm at 30, 35 and 40ºC was observed at -0.5 MPa respectively followed by control. No growth was observed in -1.0 MPa medium at 20 and 40ºC. The difference between maximum and minimum at same temperature and difference between the temperatures at same osmotic potential was significant. At 48h after inoculation, maximum colony growth was observed at 20, 25, 30, 35 and 40ºC in -0.5MPa of 38.8, 57.0, 72.0, 77.0 and 45.0mm respectively followed by control. Growth was observed in the -1.5MPa osmotic potential of 13.0, 15.5 and 30.0mm at 25, 30 and 35ºC. There was no growth at 40ºC in -1.0 and -1.5MPa. No growth was observed at 2.0 and 2.5MPa irrespective of temperature at 48h after inoculation in KCl amended PDA medium. There was significant difference between the temperatures within same osmotic potential.

At 72h after inoculation, maximum colony growth was observed at 30 and 35ºC of 80 mm at 0.5 MPa and control which were at par with each other while maximum colony growth at 20, 25 and 40ºC was observed in -0.5 MPa medium of 72.0, 77.0 and 45.0 mm respectively. There was no growth in the medium at -2.0 and -2.5 MPa irrespective of temperature up to 72h after inoculation. There was significant difference between the temperatures within same level of osmotic potential and within same temperature at different osmotic potential except at 30 and 35ºC at -0.5MPa and control.

Growth at 24h after inoculation in dextrose amended PDA medium showed maximum colony growth of 19.0mm at 20ºC in control followed by -0.5MPa osmotic potential of 17.0mm. Maximum colony growth at 25, 30 and 35ºC was observed at -0.5MPa of 29.5, 51.5 and 75.5mm respectively while at 40ºC it was observed at -1.0MPa of 29.0mm. No growth was observed on -2.5MPa medium at 20ºC. The difference between maximum and minimum colony growth at same temperature and difference between the temperatures at same osmotic potential was significant. At 48 h after inoculation, maximum

colony growth was observed at 20, 25, 30 and 35ºC in -0.5MPa *viz.*, 48.2, 72.0, 77.5 and 80.0 while at 40ºC it was recorded in -1.0MPa of 43.5mm. There was significant difference between the temperatures and osmotic potential except at 35ºC in -0.5, -1.0 and -1.5MPa.

At 72h after inoculation, mycelia growth completely covered the Petriplate (80 mm) at 30 and 35ºC in all osmotic potential levels except in -2.5MPa (67.5mm) at 30ºC. Maximum colony growth of 79.0 mm at 20ºC was observed in -0.5 MPa medium followed by -1.0 MPa (75.0mm). The Petriplate was completely covered in -0.5 and -1.0 MPa at 25ºC followed by -1.5MPa (76.5mm). The maximum growth of 43.5 mm was observed in -1.0 MPa at 40ºC. At 72h after inoculation, there was significant difference between the temperatures except at 30 and 35ºC in all osmotic potentials; also at 25ºC in -0.5 and -1.0MPa levels.

The variable response was recorded in growth of *Rhizoctonia bataticola* in different osmotic potentials amended with dextrose, KCl and NaCl at different temperatures *viz.*, 20, 25, 30, 35 and 40°C. It was observed that the increase in the osmotic potential and temperature had positive effect on the growth of the fungus up to certain level and then onwards, negative effect on the growth of the fungus at higher osmotic potentials (-2.0 to -2.5MPa at 40ºC). Exosmosis of the fungus and death was observed at higher osmotic levels amended with NaCl and KCl.

Olaya *et al.* (1996) conducted similar experiment to know the influence of osmotic potential on growth of *Macrophomina phaseolina* from soyabean at 30ºC on PDA adjusted to different osmotic potential with KCl, NaCl and sucrose. Maximum colony growth was recorded between -1220 to -1880 J Kg^{-1} and medium amended with sucrose favoured good growth. Garcia *et al.* (2003) also studied influence of osmotic potential on growth of *Macrophomina phaseolina* from common bean on potato glucose agar adjusted to different osmotic potentials with KCl, NaCl and sucrose. Concentrations of NaCl higher than 250 mM showed significant reduction of *M. phaseolina* growth while 1000 mM of NaCl completely inhibited the growth of pathogen. Sucrose did not cause significant reduction on

growth at low concentrations. On the contrary, colony growth was favoured by sucrose. The above observations were in accordance with the results obtained.

4.6.4 Effect of soil moisture on disease development

Soil moisture plays a vital role in the development of plants. It also influences the growth of the soil microorganisms. In order to determine the effect of the soil moisture on development of dry root rot disease in chick pea, highly susceptible cultivar BG 212 was grown in inoculated pots with black and red soils maintained at different moisture levels by gravimetric method. Disease severity and disease incidence at 45 DAS in different soil moisture levels of black and red soils was presented in Table 4.12.

The plants grown under 40% and 50% soil moisture showed higher stress by drooping and dead. It was considered that 40% and 50% soil moisture level was insufficient for the normal growth of the plants as the plants grown in control (pathogen free soil) also showed physiological stress (wilting symptoms due to lack of moisture). No physiological stress was found in control pots of both black and red soils from 60% soil moisture.

In black soil the disease severity rating was 9.0, 8.9, 8.1, 6.9, 5.3, 3.8 and 2.3 while in red soil, it was 9.0, 8.9, 8.7, 7.9, 6.5, 4.7 and 3.0 at 40, 50, 60, 70, 80, 90 and100% soil moisture respectively. The disease severity decreased as the soil moisture increased in both the types of soil. There was significant difference between them.

Among the selected isolates, Rb 63 showed highest disease severity of 6.7 in black soil while 7.5 in red soil. This was followed by Rb 2 of 6.5 and 7.2 in black and red soils respectively. The least disease severity of 5.9 was observed with Rb 40 in black soil and 6.2 with Rb 13 in red soil respectively. There was significant difference between the disease severities.

The disease progressed slowly with respect to the increase in soil moisture. The initial symptoms of yellowing were observed 12 DAS in 40% followed by 13 DAS in 50% soil moisture in inoculated soil. But they also showed wilting in control. While in 60% soil moisture the dry root symptoms yellowing and upward turning of leaflets was started after 17 days after sowing. In 40 to 60 per cent soil moisture they were mostly lacking the lateral roots and root system became completely black. Red soil had high significant impact on disease incidence compared to black soil as the symptoms in red soil started earlier than black soil. At 70 and 80% soil moisture content, dry root rot symptoms were observed on

19 and 21DAS in red and black soils. At 90 and 100% soil moisture, initial symptoms were observed 25 and 26 DAS in red and black soils. Disease progressed slowly in 100% soil moisture as disease incidence was 25.78% and 33.11% at 45DAS in black and red soil respectively.

Table 4.12. Disease severity (1-9rating) of *Rhizoctonia bataticola* isolates at various soil moistures levels in black soil and red soil

Isolates and severity rating (1-9 scale)*												
Soil moisture (%)	**Black soil**						**Red soil**					
	Rb 2	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**
40	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
50	9.0	8.3	9.0	9.0	9.0	8.9	9.0	8.7	9.0	9.0	9.0	8.9
60	8.3	7.7	8.5	7.5	8.7	8.1	9.0	7.7	8.7	9.0	9.0	8.7
70	7.3	6.3	7.3	5.9	7.7	6.9	8.3	6.7	7.3	8.3	8.7	7.9
80	5.7	6.0	4.7	4.5	5.7	5.3	7.3	5.3	5.7	6.7	7.7	6.5
90	4.0	4.0	3.3	3.2	4.3	3.8	4.7	3.7	5.0	4.3	5.7	4.7
100	2.3	2.1	2.2	2.3	2.7	2.3	3.0	2.3	3.3	3.0	3.3	3.0
Mean	6.5	6.2	6.3	5.9	6.7		7.2	6.2	6.9	7.0	7.5	

* mean of three replications

Factors	**C.D.**
Soil type (S)	0.12
Isolate (I)	0.18
Sx I	0.26
Soil moisture (M)	0.22
S xM	0.31
Ix M	0.49
S x I x M	0.69

In the black soil, the disease severity rating was maximum at 60% moisture condition 8.7 in Rb 63 followed by Rb 22 (8.5) and Rb2 (8.3), the difference between them was at par with each other. The least 7.5 and 7.7 was observed in Rb 40 and Rb13 inoculated plants they were at par with each other. The maximum disease severity rating of 7.7 at 70% in Rb 63 pots followed by Rb 2 and Rb 22 i.e. 7.3 the difference between them was at par with each other while the least rating of 5.9 was observed in Rb 40 and significantly different from others. At 80% soil moisture, the maximum disease severity rating of 6.0 recorded in Rb 13 pots and least Rb 40 (4.5). The difference between these isolates was significant while with other isolates it was at par. The maximum disease severity in 90% moisture was 4.3 in Rb 63 and least of 3.2 in Rb 40. The difference between these isolates was significant. The disease severity in 100% was maximum 2.7 in Rb 63 and least 2.1 were observed in Rb 13. The difference in between isolates was at par with other.

In the red soil, the disease severity rating in 60% moisture condition was 9.0 in Rb 2, Rb 40 and Rb 63 and 8.7 in Rb 13 which were at par with each other and least of 7.7 observed in Rb 13 which was significantly different from others. The disease severity rating in 70% was maximum of 8.7 in Rb 63and the least of 6.7 in Rb 13. The difference between them was significantly different. The maximum disease severity rating in 80% soil moisture was 7.7 in Rb 63 and least of 5.3 in Rb 13. The maximum disease severity in 90% soil moisture was 5.7 in Rb 63 and least of 3.7 in Rb 13. The difference between the maximum and minimum was significant. The maximum disease severity rating in 100% was 3.3 in Rb 63 and least of 2.3 in Rb 13. Disease progressed slowly at 90% and 100% in both black and red soil.

Similar observations were recorded by Ratnoo *et al.*, (1997) observed that disease development was low in flooded soil compared to drier soil (40 to 60% moisture). Sharma and Pande (2013) also observed difference in the dry root rot incidence with change in soil moisture content. The plants grown in control showed the physiological stress at 40% moisture as compared to 60%, 80% and 100%. 40% soil moisture was insufficient for the normal growth of the plants. At 60% soil moisture, no physiological stress was found in control plants and dry root rot incidence was 100%. Symptoms of dry root rot on aerial plant parts were found to be directly related with the disease severity on roots. Blackening of the roots initiated 5 days after maintaining the moisture stress at 40% and 60%. Roots were apparently free from infection at 80% and 100%. These results supported pathogen acts differently with respect to different moisture levels.

4.6.5 Influence of pH on growth of *Rhizoctonia bataticola*

pH plays an important role in providing nutrients and growth of the organisms. pH levels alter the growth behaviour. Present studies were taken up to know the optimum, minimum and maximum pH levels suitable for growth of pathogen. The five fungal isolates colony growth on potato dextrose agar medium at pH levels ranging from 3.0 to 11.0 was given in the Table 4.13.

At 24h after inoculation maximum mean colony growth was observed at pH 5.0 (67.3 mm) followed by pH 6.0 (64.4 mm) while the least was observed at pH 11.0 (14.6 mm) followed by pH 10.0 (33.0 mm). There was significant difference between the pH levels at 24h after inoculation. Similar observations were also observed at 48h after inoculation where there was significant difference between the pH levels except pH 4.0 (75.0 mm) and pH 7.0 (74.6 mm) which were at par with each other. At 72h after inoculation, maximum colony growth of 90.0mm was observed at 5.0, 6.0 and 7.0 pH levels which were at par with other. In the remaining pH levels there was significant difference between them where the least growth was observed at pH 11 of 53.5 mm.

Among selected *Rhizoctonia bataticola* isolates, maximum colony growth was observed in Rb 22 (48.3 mm) followed by Rb 40 (46.0 mm) while the least was observed in Rb 2 (38.8 mm). The similar trend was followed at 48h and 72h after inoculation. There was significant difference among the isolates at 24, 48 and 72h after inoculation.

At 24h after inoculation at different pH levels, maximum colony growth of 43.0 mm was observed at pH 3.0 in Rb 22. In pH 4.0, maximum colony growth was observed in Rb 40 (54.7 mm) and least in Rb 2 (42.0 mm). There was significant difference between the isolates at pH 4.0. Maximum colony growth at pH 5.0 was observed in Rb 22 and Rb 13 (68.7 mm) which were at par with other while the least was observed in Rb 40 and Rb 63 (65.3 mm) followed by Rb 2 (66.3 mm) which were at par with other. At pH 6.0, maximum colony growth was observed in Rb 13 (66.3 mm) and least in Rb 63 (56.3 mm) where there was significant difference between them. Except in Rb 40 at pH 11 (21.0 mm), maximum colony growth was observed in Rb 22 at pH 7.0 (60.3 mm), 8.0 (43.0 mm), 9.0 (43.3 mm) and 10.0 (43mm) while the least was observed in Rb 2 at pH 7.0 (50.7 mm), 8.0 (35.0 mm), 9.0 (33 mm), 10.0 (22.7mm) and 11.0 (11.0 mm). There was significant difference between the maximum and minimum colony growth of isolates at different pH levels at 24h after inoculation. Similar trend was observed at 48h after inoculation.

Table 4.13. Influence of different pH levels on colony growth (mm) of *Rhizoctonia bataticola* isolates

pH	24 HAI						48 HAI						72 HAI					
	Rb 2	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**	**Rb 2**	**Rb 13**	**Rb 22**	**Rb 40**	**Rb 63**	**Mean**
3.0	28.7	35.7	43.0	35.7	31.0	35.8	58.7	62.7	68.3	61.7	61.0	62.9	78.3	84.3	87.0	83.0	81.3	83.2
4.0	42.0	44.3	52.7	54.7	46.7	48.4	69.0	74.3	78.3	78.3	71.0	75.0	82.3	85.7	90.0	90.0	85.3	87.0
5.0	66.3	68.7	68.7	65.3	65.3	67.3	87.7	88.7	88.7	84.3	84.3	87.4	90.0	90.0	90.0	90.0	90.0	90.0
6.0	61.7	66.3	65.0	64.7	56.3	64.4	76.3	82.0	82.0	79.3	72.7	79.9	90.0	90.0	90.0	90.0	90.0	90.0
7.0	50.7	55.7	60.3	54.7	51.7	55.4	73.0	74.0	77.7	73.7	74.7	74.6	90.0	90.0	90.0	90.0	90.0	90.0
8.0	35.0	40.3	43.0	39.0	38.0	39.3	63.0	68.3	73.7	70.3	67.0	68.8	81.3	87.0	90.0	90.0	84.7	87.1
9.0	33.0	36.7	43.3	41.0	35.3	38.5	58.7	67.3	73.0	72.3	61.7	67.8	77.7	87.3	85.0	87.0	81.7	84.3
10.0	22.7	31.3	40.3	37.7	25.7	33.0	44.7	57.7	69.3	62.3	50.3	58.5	75.0	77.0	87.0	76.7	74.7	78.9
11.0	8.7	10.7	18.0	21.0	15.0	14.6	17.0	26.3	37.3	46.3	31.7	31.7	38.3	53.8	61.8	60.0	61.7	53.5
Mean	38.8	43.3	48.3	46.0	40.6		60.9	66.8	72.0	69.8	63.8		78.1	82.8	85.6	84.1	82.2	

Factors	CD		
Hours after incubation	24	48	72
pH	0.59	0.63	0.51
Isolate	0.44	0.47	0.38
pH x Isolate	1.32	1.40	1.15

* mean of three replications

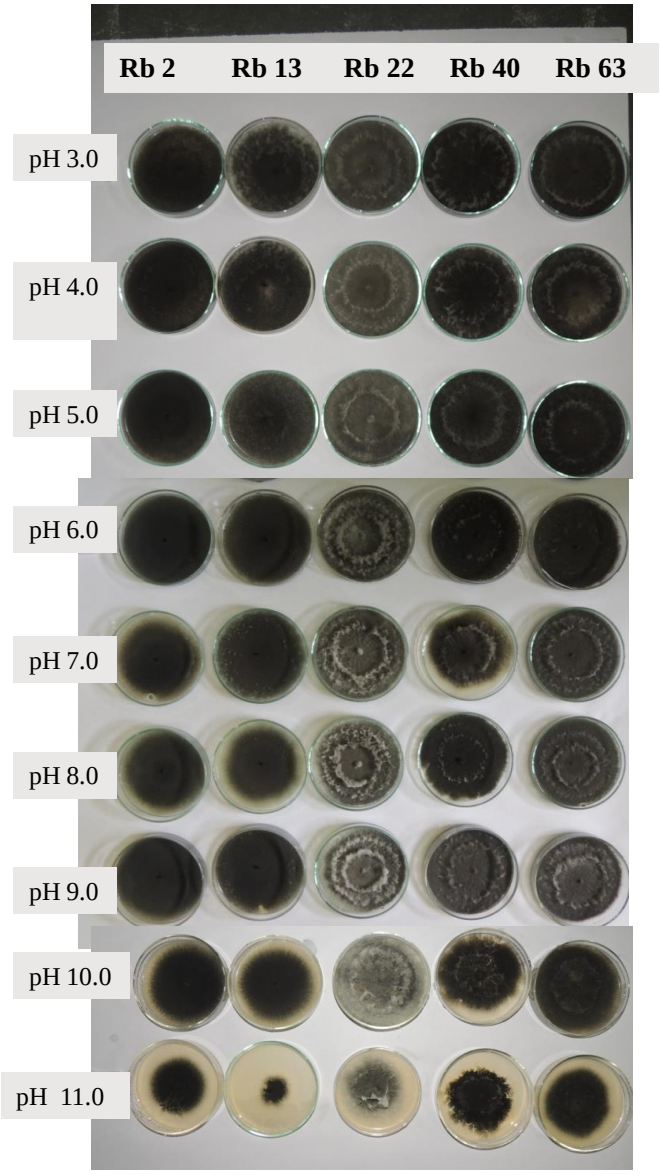

Fig 4.7. Effect of pH on colony growth of *Rhizoctonia bataticola* isolates at 72 hours after inoculation

After 72 h after inoculation (Fig 4.7), maximum colony growth at pH 3.0 was observed in Rb 22 (87.0 mm). At pH 4.0 and 8.0, maximum colony growth was observed in Rb 22 and Rb 40 (90.0 mm) and least in Rb 2 (82.3 mm and 81.3 mm) respectively. At pH 5.0, 6.0 and 7.0, maximum colony growth of 90.0 mm was observed in all the isolates. At pH 9.0,

maximum colony growth was observed in Rb 13 (87.3 mm) and least in Rb 2 (77.7 mm). At pH 10.0, maximum colony growth was observed in Rb 22 (87 mm) and least in Rb 63 (74.7 mm). At pH 11.0, maximum colony growth was observed in Rb 22 (61.8 mm) and least in Rb 2 (38.3 mm).

Similar results were also observed by Khan *et al.* (2012) while studying wide range of pH from 3.0 to 9.0 on *Rhizoctonia bataticola* in chickpea observed optimum pH for its growth was 5.5 followed by 6.0 with increase upto to 7.0 and thereafter it declined. The minimum growth of the pathogen was recorded at pH 3.0. *R. bataticola* isolates from 19 districts of Madhya Pradesh collected by Jha and Sharma (2005) from 13 different crops observed good growth at pH 5.5-7.5, but pH 7.0 was optimum when they tested at different pH levels *viz.,* 5.5, 6.0, 6.5, 7.0 and 7.5. Chowdary and Govindaiah (2007) also observed similar observations.

4.7 Biochemical changes associated with dry root development in chickpea

Infection by pathogens brings about a lot of changes in biochemical processes in the host plant.The amount of total sugars, reducing sugars and non reducing sugars, phenols, activity of enzymes *viz.* peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were altered. Amount of variation in the healthy and diseased plants of three genotypes were studied at two different moisture levels to understand the inbuilt resistance in the plants. It is crucial to know the changes in metabolites at various stages of plant- pathogen interaction. Keeping these points in view, sampling was done at three time points 15, 30 and 45DAS and the results obtained after following standard protocol were presented in respective tables.

The genotypes showed difference in the development of the disease with respect to the soil moisture and the cultivars in inoculated and uninoculated soil. The BG212 genotype showed disease severity of 9.0 and 3.7 at 60 and 100% soil moisture. Whereas, in the genotypes ICCV 5530 and ICCV 8305 showed the disease severity of 4.6 and 4.2 at 60% soil moisture and 2.2 and 2.0 at 100% soil moisture at 45 DAS. No symptoms were observed in the uninoculated at both 60 and 100% moisture levels.

4.7.1 Total Sugars

Sugars in the plants act as a biochemical markers to analyse disease severity which varies in host cell according to fungal attack. Sugars were precursors for synthesis of

phenols, phytoalexins, lignin and callose. Hence, they play an important role in defence mechanism of plants (Klement and Goodman, 1967). The estimation of these compounds help in understanding the extent of host resistance to the pathogen. The amount of total sugars was estimated by following anthrone method (Hedge and Hofreiter, 1962) as described in materials and methods. The results were presented in the table 4.14 and Fig 4.8.

Significantly maximum total sugar were found in 100% soil moisture (10.82 mg/100 mg of fresh weight) compared to 60% (8.89 mg/100 mg of fresh weight). Plants grown in inoculated soil had significantly low total sugar (8.28 mg/100 mg of fresh weight) content than plants in uninoculated (11.44 mg/100 mg of fresh weight). Among the genotypes, maximum total sugars was present in ICCV 5530 (11.99 mg/100 mg of fresh weight) followed by ICCV 8305 (10.60 mg/100 mg of fresh weight) and least was observed in BG 212 (6.98 mg/100 mg of fresh weight). There was significant increase in the total sugar content from 15 DAS (4.82 mg/100 mg of fresh weight) to 30DAS (9.56 mg/100 mg of fresh weight) and maximum was recorded at 45 DAS (15.20 mg/100 mg of fresh weight). There was significant difference between the factors. In interaction between moisture and time, maximum total sugar was observed at 45DAS (17.45 mg/100 mg of fresh weight) and the least (4.20 mg/100 mg of fresh weight) was observed at 100% soil moisture at 45 and 15 DAS respectively. In interaction between moisture and soil condition, maximum total sugar was present in uninoculated at 100% soil moisture of 12.78 mg/100 mg of fresh weight while the least was observed at 60% in inoculated soil of 7.69 mg/100 mg of fresh weight.

In interaction between moisture and genotype, maximum total sugar was recorded at 100% soil moisture followed by 60% in all the genotypes. The maximum total sugar was observed in ICCV 5530 of 13.27 mg/100 mg of fresh weight while the least was observed in BG 212 of 6.51 mg/100 mg of fresh weight.

In interaction between time point and genotype, it was observed that there was increase in total sugar with increase in time in all the genotypes. The maximum total sugar

Table 4.14. Sugars in chickpea genotypes grown in inoculated and unioculated soils at different moisture levels

Factor			Genotypes and sugar content (mg 100 mg^{-1} fresh weight)								
			Total sugars			Reducing sugars			Non reducing sugars		
Soil moisture (%)	Time point (DAS)	Pathogen in soil	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305
60	15	Uninoculated	4.07	6.24	5.30	2.33	3.13	2.77	1.74	3.11	2.53
		Inoculated	4.42	6.73	5.91	3.10	4.85	4.19	1.33	1.88	1.71
	30	Uninoculated	7.45	10.11	9.21	4.56	5.71	5.11	2.89	4.40	4.11
		Inoculated	5.87	8.69	8.43	3.36	5.30	5.06	2.51	3.44	3.38
	45	Uninoculated	12.62	19.21	16.67	7.68	11.72	10.17	4.94	7.49	6.49
		Inoculated	4.60	13.26	11.26	2.99	8.56	7.11	1.61	4.70	4.15
100	15	Uninoculated	3.45	4.65	4.26	2.10	2.88	2.68	1.34	1.77	1.58
		Inoculated	3.73	4.72	4.37	2.47	3.12	2.88	1.26	1.60	1.49
	30	Uninoculated	8.62	15.34	13.21	5.98	10.25	9.25	2.64	5.09	3.96
		Inoculated	6.31	10.84	10.62	3.75	6.63	6.41	2.56	4.21	4.20
	45	Uninoculated	16.34	25.52	23.65	9.97	16.08	15.98	6.36	9.44	7.67
		Inoculated	6.30	18.56	14.36	3.97	11.88	9.48	2.33	6.68	4.88

Factor	CD		
Soil moisture (M)	0.05	0.03	0.02
Time (T)	0.06	0.04	0.02
Pathogen in soil (S)	0.05	0.03	0.02
Genotype (G)	0.06	0.04	0.02
M x T	0.08	0.05	0.03
M x S	0.07	0.04	0.03
T x S	0.08	0.05	0.03
M x G	0.08	0.05	0.03
T x G	0.10	0.06	0.04
S x G	0.08	0.05	0.03
M x T x S	0.12	0.07	0.04
M x T x G	0.14	0.09	0.05
M x S x G	0.12	0.07	0.04
T x S x G	0.14	0.09	0.05
M x T x S x G	0.20	0.13	0.08

was present in ICCV 5530 of 19.14mg/100 mg of fresh weight at 45 DAS while the least was observed in BG 212 of 3.92 mg/100 mg of fresh weight at 15 DAS.

In interaction between soil condition and genotype, it was observed that maximum total sugar was present in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum total sugar was observed in ICCV 5530 of 13.51 mg/100 mg of fresh weight in uninoculated soil while the least was observed in BG 212 of 5.21 mg/100 mg of fresh weight in inoculated soil.

In susceptible genotype BG 212, total sugars recorded was 3.45 mg/100 mg of fresh weight at 15 DAS and increased to 16.34mg/ 100 mg of fresh weight at 45 DAS in the uninoculated soil in 100% soil moisture. In moderately resistant genotype ICCV 5530, the lowest (4.65 mg/ 100mg of fresh weight) and the highest (25.52 mg/100 mg of fresh weight) amount of total sugar was recorded in uninoculated soil with 100% soil moisture at 15 and 45 DAS respectively.

In moderately resistant genotype ICCV 8305, the lowest (4.26 mg/100 mg of fresh weight) and the highest (23.65 mg/100 mg of fresh weight) amount was recorded at 15 and 45DAS in the inoculated and uninoculated soil respectively with 100% soil moisture.

4.7.2 Reducing sugars

Sugars with reducing property (arising out of the presence of a potential aldehyde or keto groups) are called reducing sugars. Some of the reducing sugars are glucose, galactose, lactose and maltose. The amount of reducing sugars was altered due to infection by the pathogen and changes were estimated by following dinitro salicylic acid method (Miller, 1972) and the results are presented in table 4.12. The significantly maximum reducing sugars were present in 100% soil moisture (6.99 mg/100 mg of fresh weight) compared to 60% (5.43 mg/100 mg of fresh weight). Plants grown in inoculated soil had low reducing sugars (5.28 mg/100 mg of fresh weight) content than plants in uninoculated (7.13 mg/100 mg of fresh weight). Among the genotypes, maximum reducing sugars were present in ICCV 5530 (7.51mg/100 mg of fresh weight) followed by ICCV 8305 (6.76 mg/100 mg of fresh weight) and least amount were observed in BG 212 (4.35 mg/100 mg of fresh weight). There was significant increase in the reducing sugars content from 15 DAS (3.04 mg/100 mg of fresh weight) to 30DAS (5.95 mg/100 mg of fresh weight) and maximum was recorded at 45 DAS (9.63mg/100 mg of fresh weight). There was significant difference between the factors. It was observed that maximum reducing sugars were observed at 45DAS in 100% soil moisture of 11.23mg/100 mg of fresh weight while the

least were observed in 100% soil moisture at 15 DAS of 2.69mg/100 mg of fresh weight in interaction between moisture and time point. Maximum reducing sugars were observed in uninoculated at 100% soil moisture of 8.35mg/100 mg of fresh weight and the least were observed at 60% soil moisture in inoculated soil of 4.95mg/100 mg of fresh weight in interaction between moisture and soil condition.

In interaction between moisture and genotype, more amount of reducing sugars were recorded at 100% soil moisture than 60% soil moisture in all the genotypes. The maximum reducing sugars were observed in ICCV 5530 of 8.47mg/100 mg of fresh weight while the least in BG 212 of 4.00 mg/100 mg of fresh weight at 60% soil moisture.

Increase in reducing sugars was observed with increase in time in all the genotypes in interaction between time point and genotype. The maximum reducing sugars were recorded in ICCV 5530 of 12.06 mg/100 mg of fresh weight at 45 DAS while the least in BG 212 of 2.50 mg/100 mg of fresh weight at 15 DAS.

In Interaction between soil condition and genotype, maximum reducing sugars were observed in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum reducing sugars was present in ICCV 5530 of 8.29mg/100 mg of fresh weight in uninoculated soil while the least amount in BG 212 of 3.27mg/100 mg of fresh weight in inoculated soil.

In susceptible genotype BG 212, highest amount of 9.97 mg/100 mg of fresh weight of reducing sugars was recorded at 45 DAS in uninoculated soil with 100% soil moisture while the least amount of 2.1 mg/ 100 mg of fresh weight was observed in inoculated soil with 60% soil moisture after 15 DAS.

In moderately resistant genotype ICCV 5530, maximum amount of 16.08 mg/ 100mg of fresh weight was observed in uninoculated soil with 100% soil moisture at 45 DAS and the least amount of 2.88 mg/100 mg of fresh weight was recorded in uninoculated soil with 100% soil moisture at 15 DAS.

The higher amount of 15.98 mg/100 mg of fresh weight of reducing sugars was recorded at 45 DAS in uninoculated soil with 100% soil moisture in moderately resistant genotype ICCV 8305 while the least amount was recorded in 100% soil moisture at 15 DAS in uninoculated with 2.68 mg/100 mg of fresh weight (Fig 4.9).

4.7.3 Non reducing sugars

Biochemical resistance or susceptibility of the plants against any disease depends mainly on pre-existing preformed substances by pathogen in host. Nutritional status prior to infection determines the severity of the disease. The amount of non reducing sugars was estimated by deducting reducing sugars from total sugars and the results were presented in table 4.12. Significantly maximum amount of non reducing sugars observed in 100% soil moisture (3.84mg/100 mg of fresh weight) compared to 60% (3.47 mg/100 mg of fresh weight). Plants grown in inoculated soil had low non reducing sugars (3.00mg/100 mg of fresh weight) content than plants in uninoculated (4.31mg/100 mg of fresh weight). Among the genotypes, maximum amount of non reducing sugars present in ICCV 5530 (4.49mg/100 mg of fresh weight) followed by ICCV 8305 (3.85 mg/100 mg of fresh weight) and least in BG 212 (2.63mg/100 mg of fresh weight). There was significant increase in non reducing sugars from 15 DAS (1.78 mg/100 mg of fresh weight) to 45 DAS (5.56mg/100 mg of fresh weight). In interaction between moisture and time, it was observed that maximum amount of non reducing sugars was observed at 45DAS in 100% soil moisture of 6.23mg/100 mg of fresh weight while the least in 100% soil moisture at 15 DAS of 1.51mg/100 mg of fresh weight. Interaction between moisture and soil condition, it was recorded that maximum non reducing sugars was present in uninoculated at 100% soil moisture of 4.43mg/100 mg of fresh weight while the least at 60% in inoculated soil of 2.75mg/100 mg of fresh weight.

In interaction between moisture and genotype, maximum non reducing sugars was present at 100% soil moisture followed by 60% in all the genotypes. Significantly maximum non reducing sugar was observed in ICCV 5530 of 4.80mg/100 mg of fresh weight while the least in BG 212 of 2.50 mg/100 mg of fresh weight at 60% soil moisture.

Increase in amount of non reducing sugars was observed with increase in time in all the genotypes in interaction between time point and genotype. The maximum non reducing sugars was present in ICCV 5530 of 7.08mg/100 mg of fresh weight at 45 DAS and the least in BG 212 of 1.42 mg/100 mg of fresh weight at 15 DAS.

In interaction between soil condition and genotype, it was observed that maximum non reducing sugars was observed in plants grown in uninoculated compared to plants grown in inoculated soil in all the genotypes. The maximum non reducing sugars was

recorded in ICCV 5530 of 5.22mg/100 mg of fresh weight in uninoculated soil while the least was observed in BG 212 of 1.93mg/100 mg of fresh weight in inoculated soil.

In susceptible genotype (BG 212), higher amount of 6.36 mg/100 mg of fresh weight of non reducing sugars was recorded at 45 DAS in uninoculated soil with 100% soil moisture while the least 1.26 mg/ 100 mg of fresh weight in uninoculated soil with 100% soil moisture at 15 DAS.

In moderately resistant genotype (ICCV 5530), maximum amount (9.44 mg/ 100mg of fresh weight) was observed in uninoculated soil with 100% soil moisture at 45 DAS and minimum of 1.60 mg/100 mg of fresh weight recorded in inoculated soil at 100% soil moisture at 15 DAS.

In another moderately resistant genotype (ICCV 8305) the higher amount (7.67 mg/100 mg of fresh weight) of non reducing sugars was recorded at 45 DAS in uninoculated soil with the 100% soil moisture while the least amount was recorded at 100% soil moisture at 15 DAS in inoculated soil with 1.49 mg/100 mg of fresh weight (Fig 4.10).

A similar trend of higher amounts of reducing, non reducing and total sugars were observed in resistant genotypes than susceptible ones. Sugars play an important role in the inhibition of pectinolytic and cellulolytic enzymes which were essential for pathogen (Bateman *et al,* 1965). Similarly Joshi *et al.* (2003) also reported decreased levels of total soluble sugars and reducing sugars with pathogen inoculation in leaves and roots of inoculated plants as compared to uninoculated plants incluster bean genotypes grown in uninoculated and inoculated soil with *Macrophomina phaseolina* at 65 DAS.

4.7.4 Total Phenol

It is known that several fungal products such as proteins, gluco-proteins or oligosaccharides can trigger the defence mechanisms in plants. In the presence of pathogen, plants develop a vast array of metabolic defence responses sequentially activated by a

Table 4.15. Phenols in chickpea genotypes at different moisture levels in inoculated and uninoculated soils with *Rhizoctonia bataticola*

Factors	Genotypes and phenol
	(mg catechol 100 mg^{-1} root

			sample)		
Soil moisture (%)	Time point (DAS)	Pathogen in soil	BG 212	ICCV 5530	ICCV 8305
60	15	Uninoculated	0.89	1.36	1.00
		Inoculated	1.10	2.01	1.39
	30	Uninoculated	2.38	4.36	3.20
		Inoculated	2.76	5.92	4.19
	45	Uninoculated	1.85	3.22	2.43
		Inoculated	2.09	4.27	3.23
100	15	Uninoculated	0.80	1.25	0.95
		Inoculated	0.94	1.64	1.22
	30	Uninoculated	2.14	3.61	2.77
		Inoculated	2.44	4.62	3.35
	45	Uninoculated	1.74	2.82	2.24
		Inoculated	1.96	3.65	2.75
Factors			CD		
Soil moisture (M)			0.01		
Time (T)			0.01		
Pathogen in soil (S)			0.01		
Genotype (G)			0.01		
M x T			0.02		
M x S			0.02		
T x S			0.02		
M x G			0.02		
T x G			0.03		
S X G			0.02		
M x T x S			0.03		
M x T x G			0.04		
M x S x G			0.03		
T x S x G			0.04		
M x T x S x G			0.05		

omplex multi component network that may be local and/or systemic. The induction of phenols might be due to the activation of the shikimic acid pathway, through which the aromatic amino acids, phenylalanine and tyrosine are formed and channelled for the synthesis of phenolics. Phenols are secondary metabolites; constituent of lignin may

contribute to enhance the mechanical strength of the host cell wall and may also inhibit fungal growth as they are fungitoxic in nature. In the present study amount of total phenols in different genotypes grown in various moisture levels was estimated by following Folin Ciocalteu reagent method (FCR) of Bray and Thorpe (1954) and the results were presented in table 4.15 and Fig 4.11.

The significantly high phenol content was recorded in 60% soil moisture (2.65mg/100 mg of fresh weight) than 100% (2.27 mg/100 mg of fresh weight). Plants grown in inoculated soil had more phenol content (2.75mg/100 mg of fresh weight) than plants in uninoculated (2.17mg/100 mg of fresh weight). Among the genotypes, maximum phenol content was present in ICCV 5530 (3.23mg/100 mg of fresh weight) followed by ICCV 8305 (2.39 mg/100 mg of fresh weight) and least in BG 212 (1.76mg/100 mg of fresh weight). There was significant increase in the phenol content from 15 DAS (1.21 mg/100 mg of fresh weight) to 30DAS (3.48 mg/100 mg of fresh weight) and decreased at 45 DAS (2.69mg/100 mg of fresh weight). In interaction between soil moisture and time, it was observed that maximum amount of phenol content was observed at 30DAS in 60% soil moisture of 3.80mg/100 mg of fresh weight while the least was observed at 100% soil moisture at 15 DAS of 1.13mg/100 mg of fresh weight. In interaction between moisture and soil condition, it was recorded that maximum phenol content was present in inoculated soil at 60% soil moisture of 3.00mg/100 mg of fresh weight while the least was in uninoculated soil with 100% soil moisture of 2.04mg/100 mg of fresh weight.

Significantly maximum phenol content was present at 60% soil moisture followed by 100% in all the genotypes in interaction between moisture and genotype. The maximum phenol content was observed in ICCV 5530 of 3.52mg/100 mg of fresh weight while the least was present in BG 212 of 1.67 mg/100 mg of fresh weight at 60 and 100% soil moisture respectively.

Interaction effect between time point and genotype showed that increase in phenol content with increase in time in all the genotypes upto 30DAS and then there was decline. The maximum phenol content was observed in ICCV 5530 of 4.63mg/100 mg of fresh weight at 30 DAS while the least was observed in BG 212 of 0.93 mg/100 mg of fresh weight at 15 DAS.

In interaction effect between soil condition and genotype, maximum phenol content was noted in plants grown in inoculated soil than in uninoculated soil in all the genotypes. The maximum phenol content was recorded in ICCV 5530 of 3.69mg/100 mg of fresh weight in inoculated soil and the least in BG 212 of 1.63mg/100 mg of fresh weight in uninoculated soil.

In susceptible genotype BG 212, and moderately resistant genotypes (ICCV 5530 and ICCV 8305), total phenol content was maximum at 30DAS of 2.76, 5.92, and 4.19 mg/100mg fresh weight of root tissue respectively in inoculated soil with 60% soil moisture while the least amount of 0.80, 1.25 and 0.95 mg/100mg fresh weight of root tissue was observed in uninoculated soil at 100% soil moisture at 15 DAS respectively.

Similar observations of increased levels of phenols at different stages of infection were observed by Rathod and Vakharia (2011) in wilt disease of chickpea. Total phenol content was significantly higher in root of all the genotypes obtained from inoculated plot. The highly susceptible genotypes had lower concentration of total phenol than others. Sharma *et al.* (2011) also revealed maximum accumulation of phenolic acids in infected guar plants by *Macrophomina phaseolina* than in uninoculated after 120 hours of infection in all genotypes of guar. Phenolic acid accumulation in the compatible host-pathogen combination presumes that phenol participated actively in the guar resistance to root rot. Sapru and Mahajan (2010) concluded that accumulation of phenol in susceptible genotype was not sufficient to resist the infection in mung bean infected by *Rhizoctonia bataticola.*

4.7.5 Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia-lyase played an important role in the biosynthesis of various defence chemicals in phenylpropanoid metabolism. PAL activity was an extremely sensitive indicator of stress conditions and fungal challenge elevates level of the flux through phenylpropanoid pathway, thereby supplying the carbon skeletons for secondary products such as phenolics which are the precursor molecules of lignin. The variations in

Table 4.16. Effect of different moisture levels on enzyme activity in chickpea genotypes grown in uninoculated and inoculated soils

Factors			Peroxidase (ΔAbs_{420} nm min^{-1} g^{-1} fresh sample)			Polyphenol oxidase (ΔAbs_{495} nm min^{-1} g^{-1} fresh sample)			Phenylalanine ammonia lyase (µmol cinnamic acid hr^{-1} g^{-1} sample)		
Soil moisture (%)	Time point (DAS)	Pathogen in soil	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305
60	15	Uninoculated	0.20	0.71	0.25	0.02	0.10	0.04	0.42	0.62	0.48
		Inoculated	0.21	0.74	0.25	0.02	0.12	0.07	0.47	0.72	0.52
	30	Uninoculated	1.19	1.42	1.34	0.05	0.15	0.12	0.82	1.56	1.27
		Inoculated	1.34	1.50	1.43	0.07	0.20	0.13	1.52	2.27	1.98
	45	Uninoculated	0.95	1.33	1.33	0.04	0.13	0.10	0.51	0.85	0.70
		Inoculated	0.59	1.48	1.41	0.01	0.14	0.08	0.72	1.07	1.06
100	15	Uninoculated	0.17	0.61	0.20	0.01	0.04	0.04	0.35	0.50	0.39
		Inoculated	0.18	0.62	0.24	0.02	0.05	0.03	0.36	0.56	0.44
	30	Uninoculated	0.73	1.25	1.03	0.04	0.12	0.06	0.36	1.32	1.09
		Inoculated	1.26	1.38	1.28	0.07	0.13	0.08	1.40	1.96	1.54
	45	Uninoculated	0.47	1.06	0.73	0.03	0.08	0.03	0.31	0.51	0.49
		Inoculated	1.04	1.31	1.10	0.03	0.09	0.06	0.53	1.23	0.70

Factors	CD		
Soil moisture (M)	0.001	0.001	0.003
Time (T)	0.002	0.001	0.003
Pathogen in soil (S)	0.001	0.001	0.003
Genotype (G)	0.002	0.001	0.003
M x T	0.002	0.002	0.005
M x S	0.002	0.001	0.004
T x S	0.002	0.002	0.005
M x G	0.002	0.002	0.005
T x G	0.003	0.002	0.006
S x G	0.002	0.002	0.005
M x T x S	0.003	0.002	0.007
M x T x G	0.004	0.003	0.008
M x S x G	0.003	0.002	0.007
T x S x G	0.004	0.003	0.008
M x T x S x G	0.006	0.004	0.011

PAL activity was determined in chickpea genotypes using the modified method of the conversion of L-phenylalanine to cinnamic acid at 290 nm (Dickerson *et al*., 1984) and the results were presented in table 4.16.

Significantly PAL activity in 60% soil moisture (0.98µmol cinnamic acid/hr/g fresh sample) was higher than 100% soil moisture (0.78µmol cinnamic acid/h/g fresh sample). Plants grown in inoculated soil had more PAL activity (1.06µmol cinnamic acid/h/g fresh sample) than in uninoculated (0.70µmol cinnamic acid/h/g fresh sample). Among the genotypes, maximum PAL activity was present in ICCV 5530 (1.10µmol cinnamic acid/h/g fresh sample) followed by ICCV 8305 (0.89µmol cinnamic acid/h/g fresh sample) and least was observed in BG 212 (0.65µmol cinnamic acid/h/g fresh sample). There was significant increase in the PAL activity from 15 DAS (0.49µmol cinnamic acid/h/g fresh sample) to 30DAS (1.42µmol cinnamic acid/hr/g fresh sample) and decreased at 45 DAS (0.72µmol cinnamic acid/hr/g fresh sample). In interaction effect between moisture and time showed higher amount of PAL activity at 30DAS in 60% soil moisture of 1.57µmol cinnamic acid/h/g fresh sample while the least was observed in 100% soil moisture at 15 DAS of 0.43µmol cinnamic acid/hr/g fresh sample. Maximum PAL activity was recorded in inoculated soil at 60% soil moisture of 1.15µmol cinnamic acid/h/g fresh sample while the least was observed at 100% in uninoculated soil of 0.59µmol cinnamic acid/h/g fresh sample in interaction between moisture and soil condition.

In interaction between moisture and genotype, maximum PAL activity was present at 60% soil moisture than 100% in all the genotypes. The maximum PAL activity was recorded in ICCV 5530 of 1.18µmol cinnamic acid/h/g fresh sample while the least in BG 212 of 0.55µmol cinnamic acid/h/g fresh sample at 60 and 100% soil moisture respectively.

PAL activity increased with time in all the genotypes upto 30DAS and then there was decline in interaction between time point and genotype. The maximum PAL activity was observed in ICCV 5530 of 1.78µmol cinnamic acid/h/g fresh sample at 30 DAS while the least in BG 212 of 0.40µmol cinnamic acid/h/g fresh sample at 15 DAS.

Maximum PAL activity was observed in plants grown in inoculated soil than in uninoculated soil in all the genotypes in interaction between soil condition and genotype. The maximum PAL activity noted from ICCV 5530 of 1.30µmol cinnamic acid/h/g fresh

sample in inoculated soil while the least was observed in BG 212 of 0.46µmol cinnamic acid/h/g fresh sample in uninoculated soil.

In susceptible genotype (BG 212), significantly maximum PAL activity was recorded at 30DAS of 1.52µmol cinnamic acid/h/g fresh sample in 60% soil moisture in inoculated soil while the least (0.32 µmol cinnamic acid/h/ g fresh sample) in uninoculated with 100% soil moisture at 45 DAS. In moderately resistant genotype (ICCV 5530), maximum activity of 2.27µmol cinnamic acid/h/g fresh sample of PAL activity was observed in inoculated soil with 60% soil moisture at 30DAS while the least (0.51µmol cinnamic acid/h/g fresh sample) activity noted in uninoculated at 100% soil moisture at 45DAS. In moderately resistant genotype (ICCV 8305), highest level (1.98 µmol cinnamic acid/h/ g fresh sample) of PAL activity was observed in inoculated soil with 60% soil moisture at 30DAS while the least (0.39 µmol cinnamic acid/h/ g fresh sample) activity in uninoculated soil with 100% soil moisture at15 DAS (Fig 4.12).

Similar results were observed in same pathogen but in Guar crop as PAL activity was significantly increased from 96 to 120 hours after infection depending upon the genotype in comparison to uninoculated. Enhanced PAL activity in the compatible host-pathogen combination presumes that PAL participated actively in the guar resistance to root rot (Sharma *et al.*, 2011). Sundaramoorthy *et al.* (2013) while studying the disease resistance in green gram against leaf blight caused by *Macrophomina phaseolina* by the application of plant extracts and fungicides reported that PAL was greater in treated plants as compared to untreated uninoculated. The increase in PAL activity has frequently been mentioned as a defence reaction of plants to pathogen attack, showing significant increases after infection by pathogens or wounding (Cui *et al.*, 2000, Logemann *et al*., 2000).

4.7.8 Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) is a nuclear encoded, plastid copper containing enzyme, which catalyzes the oxygen dependent oxidation of phenols to quinones. Because of conspicuous reaction products and induction by wounding and pathogen attack, PPO has frequently been suggested to participate in plant defence against pests and pathogens. The activity of PPO was found to be increasing in challenged plants compared to the unchallenged ones. PPO activity was assayed using the modified method of Mayer *et al.* (1965) and the results were presented in table 4.16.

Plants grown in inoculated soil had high PPO activity (0.08 ΔAbs_{495} nm/ min/g fresh sample) than plants in uninoculated (0.07 ΔAbs_{495} nm/ min/g fresh sample). Significantly maximum PPO activity observed in 60% soil moisture (0.09 ΔAbs_{495} nm/ min/g fresh sample) than 100% (0.06 ΔAbs_{495} nm/ min/g fresh sample). Among the genotypes, maximum PPO activity present in ICCV 5530 (0.11 ΔAbs_{495} nm/ min/g fresh sample) followed by ICCV 8305 (0.07 ΔAbs_{495} nm/ min/g fresh sample) and least was in BG 212 (0.03 ΔAbs_{495} nm/ min/g fresh sample). There was significant increase in the PPO activity from 15 DAS (0.05 ΔAbs_{495} nm/ min/g fresh sample) to 30DAS (0.10 ΔAbs_{495} nm/ min/g fresh sample) and decreased at 45DAS (0.07 ΔAbs_{495} nm/ min/g fresh sample). Interaction between moisture and time showed maximum amount of PPO activity at 30DAS in 60% soil moisture of 0.12 ΔAbs_{495} nm/ min/g fresh sample while the least in 100% soil moisture at 15DAS of 0.03 ΔAbs_{495} nm/ min/g fresh sample. Maximum PPO activity was observed in inoculated soil in 60% soil moisture of 0.09 ΔAbs_{495} nm/ min/g fresh sample while the least was observed in 100% in uninoculated soil of 0.05 ΔAbs_{495} nm/ min/g fresh sample in interaction between moisture and soil condition and the interaction was not significant.

In interaction between moisture and genotype, maximum PPO activity was observed in 60% soil moisture followed by 100% in both ICCV 5530 and ICCV 8305 while least was in BG212 at 60 and 100% (0.03 ΔAbs_{495} nm/ min/g fresh sample) which was at par with each other. The maximum PPO activity was observed in ICCV 5530 of 0.14 ΔAbs_{495} nm/ min/g fresh sample followed by ICCV 8305 in 60% soil moisture.

In interaction between time point and genotype, it was noted that maximum PPO activity was observed with increase in time in all the genotypes up to 30DAS and then there was decline. The maximum PPO activity was observed in ICCV 5530 of 0.15 ΔAbs_{495} nm/ min/g fresh sample at 30 DAS while the least was observed in BG 212 of 0.02 ΔAbs_{495} nm/ min/g fresh sample at 15 DAS.

Significantly maximum PPO activity was observed in plants grown in inoculated soil compared to plants grown in uninoculated soil in all the genotypes in interaction between soil condition and genotype. The maximum PPO activity was observed in ICCV 5530 of 0.12 ΔAbs_{495} nm/ min/g fresh sample in inoculated soil while the least in BG 212 of 0.03 ΔAbs_{495} nm/ min/g fresh sample in uninoculated soil.

In susceptible genotype (BG 212), polyphenol oxidase activity was maximum at 30 DAS (0.07 ΔAbs_{495} nm/ min/g fresh sample) in inoculated soil at 60 and 100% soil moisture. The critical difference between them was at par with each other. The least (0.01 ΔAbs_{495} nm/ min/g fresh sample) PPO activity was observed in inoculated soil at 60% soil moisture at 45 DAS and 100% soil moisture at 15 DAS. These were at par with each other. In moderately resistant genotype (ICCV 5530) highest (0.20 ΔAbs_{495} nm/ min/g fresh sample) PPO activity was observed in inoculated soil in 60% soil moisture at 30 DAS while the least (0.04 ΔAbs_{495} nm/ min/g fresh sample) activity of PPO was observed in uninoculated soil at 100% soil moisture at 15 DAS. In moderately resistant genotype (ICCV 8305) highest level (0.13 ΔAbs_{495} nm/ min/g fresh sample) of PPO was observed in inoculated soil with 60% soil moisture at 30DAS while the lowest activity of 0.03 ΔAbs_{495} nm/ min/g fresh sample was recorded in uninoculated and inoculated soil at the 60and 100% soil moisture at 45 and 15 DAS respectively (Fig 4.13).

Similar results of increased PPO activity were observed by Joshi *et al.* (2003) in *Macrophomina phaseolina* infected roots of both moderately and susceptible genotypes of cluster bean at 65 days after sowing. The activity of PPO was higher in moderately resistant genotype than in highly susceptible genotype. Cherif *et al.,* (2007) observed pre-treatment of chickpea seedlings with selected *Rhizobium* isolates before challenge inoculation with *Fusarium oxysporium* fsp *ciceri* increased significantly the levels of PPO and concluded higher accumulation of PPO may play a crucial role in resistance of chickpea against pathogenic *Fusarium oxysporium* fsp *ciceri* attack. Sundaramoorthy *et al.* (2013) while studying the disease resistance in green gram plants against leaf blight disease caused by *Macrophomina phaseolina* by the application of plant extracts and fungicides reported that PPO was greater in treated plants as compared to untreated uninoculated.

4.7.9 Peroxidase (POD)

Most phenols occur in plant tissues in less toxic forms and change into more toxic forms by the action of phenol oxidase enzymes such as polyphenoloxidase and peroxidase. Increase in the activity level of these enzymes was reported to be associated with infected tissues (Bateman *et al.,* 1965). The products of peroxidase enzyme in the presence of a hydrogen donor and hydrogen peroxide have antimicrobial activity and even antiviral activity. Besides these, higher intercellular peroxidase levels may also play an integral role in the lignification of cell walls, which assist in the resistance of the plant to penetration by

fungal pathogen (Retig, 1974). Peroxidase activity was assayed using the modified method of Hammerchmidt, 1982) and the results are presented in table 4.16.

Significantly maximum POD activity observed in 60% soil moisture (0.98 ΔAbs_{420} nm/ min/g fresh sample) followed by 100% (0.81 ΔAbs_{420} nm/ min/g fresh sample). Plants grown in inoculated soil had significantly high POD activity (0.96 ΔAbs_{420} nm/ min/g fresh sample) than plants in uninoculated (0.83 ΔAbs_{420} nm/ min/g fresh sample). Among the genotypes, maximum POD activity present in ICCV 5530 (1.12 ΔAbs_{420} nm/ min/g fresh sample) followed by ICCV 8305 (0.88 ΔAbs_{420} nm/ min/g fresh sample) while least activity noted in BG 212 (0.69 ΔAbs_{420} nm/ min/g fresh sample). There was significant increase in the POD activity from 15 DAS (0.36 ΔAbs_{420} nm/ min/g fresh sample) to 30DAS (1.26 ΔAbs_{420} nm/ min/g fresh sample) and decreased at 45DAS (1.06 ΔAbs_{420} nm/ min/g fresh sample). There was significant difference between time points. In interaction between moisture and time, it was recorded that maximum amount of POD activity was observed at 30DAS in 60% soil moisture of 1.37 ΔAbs_{420} nm/ min/g fresh sample while the least was noted in 100% soil moisture at 15DAS of 0.34 ΔAbs_{420} nm/ min/g fresh sample. In interaction between moisture and soil condition, significantly maximum POD activity was present in inoculated soil at 60% soil moisture of 0.99 ΔAbs_{420} nm/ min/g fresh sample while the least activity in uninoculated soil with 100% soil moisture of 0.69 ΔAbs_{420} nm/ min/g fresh sample.

In interaction between moisture and genotype, maximum POD activity was noted in 60% soil moisture followed by 100% in all genotypes. POD activity was significantly high in ICCV 5530 of 1.20 ΔAbs_{420} nm/ min/g fresh sample while the least was observed in BG 212 of 0.64 ΔAbs_{420} nm/ min/g fresh sample in 100% soil moisture.

In interaction between time point and genotype, it was observed that maximum POD activity was recorded with increase in time in all the genotypes up to 30DAS and then there was decline. The maximum POD activity was recorded in ICCV 5530 of 1.39 ΔAbs_{420} nm/ min/g fresh sample at 30 DAS while the least was observed in BG 212 of 0.19 ΔAbs_{420} nm/ min/g fresh sample at 15 DAS.

In interaction between soil condition and genotype, POD activity was maximum in plants grown in inoculated soil compared to plants grown in uninoculated soil in all the genotypes. The maximum POD activity was recorded in ICCV 5530 of 1.17 ΔAbs_{420} nm/

min/g fresh sample in inoculated soil while the least activity in BG 212 of 0.62 ΔAbs_{420} nm/ min/g fresh sample in uninoculated soil.

In susceptible genotype (BG 212), POD activity was maximum at 30DAS in inoculated soil (1.34 ΔAbs_{420} nm/ min/g fresh sample) at 60% soil moisture while the least (0.17 ΔAbs_{420} nm/ min/g fresh sample) activity of POD was observed in uninoculated soil at 100% soil moisture at 15 DAS. In moderately resistant genotype ICCV 5530 highest (1.50 ΔAbs_{420} nm/ min/g fresh sample) activity of POD was observed in inoculated soil at 60 per cent soil moisture after 30 DAS. The least (0.61 ΔAbs_{420} nm/ min/g fresh sample) accumulation of POD was observed in uninoculated soil at 100% soil moisture after 15 DAS. In moderately resistant genotype (ICCV 8305) highest level (1.43 ΔAbs_{420} nm/ min/g fresh sample) of POD was observed in inoculated soil at 60 per cent soil moisture after 30 DAS. The least activity of 0.20 ΔAbs_{420} nm/ min/g fresh sample was recorded in uninoculated soil at 100% soil moisture at 15 DAS (Fig 4.14).

Similar results of increased POD activity in *Macrophomina phaseolina* infected roots of cluster bean genotypes were observed at 65 days after sowing by Joshi *et al.* (2003). The activity of POD was higher in moderately resistant genotype than in highly susceptible genotype. Cherif *et al.,* (2007) also observed pre-treatment of chickpea seedlings with selected *Rhizobium* isolates before challenge inoculation with *Fusarium oxysporium* fsp *ciceri* increased significantly the levels of POD and concluded higher accumulation of POD may play a crucial role in resistance of chickpea against pathogenic *Fusarium oxysporium* fsp *ciceri* attack. Sundaramoorthy *et al.* (2013) reported that defence enzyme POD was greater in treated plants as compared to untreated uninoculated green gram plants against leaf blight disease caused by *Macrophomina phaseolina* by the application of plant extracts and fungicides.

Ravi and Sharma (2011) concluded that reactive oxygen species are produced during the moth bean-*Macrophomina phaseolina* interaction and play a role in the defence response of the moth bean plant against the fungal pathogen *Macrophomina phaseolina*. Varietal differences are also observed, overall, one week old plants of var. FMM-96 which is moderately resistant showed better response in terms of peroxidase activity and H_2O_2 production than RMO - 40, a susceptible variety.

4.8 Growth changes associated with dry root development in chickpea

The effect of dry root rot at different moisture levels was studied on various plant parts *viz.* root dry weight, shoot dry weight, leaf dry weight, total dry biomass (Fig 4.15). The results obtained were presented in table 4.17.

4.8.1 Root dry weight

The roots are the beginning of the vascular system pipeline that moves water and minerals from the soil up to the leaves. These are affected due external moisture stress and diseases. The experiment was conducted to know the affect of *Rhizoctonia bataticola* and soil moisture on the root growth of chickpea.

Plants grown in inoculated soil had significantly low root dry weight (0.35g) than plants in uninoculated (0.48g). Among the genotypes, maximum root dry weight present in ICCV 5530 (0.49g) followed by ICCV 8305 (0.44g) and least was observed in BG 212 (0.32g). There was significant increase in the root dry weight from 15 DAS (0.24g) to 45 DAS (0.59g). The maximum root dry weight observed in 100% soil moisture (0.54g) compared to 60% (0.29g). There was significant difference between them.

In interaction between soil condition and genotype showed maximum root dry weight in plants grown in uninoculated compared to plants grown in inoculated soil in all the genotypes. The maximum root dry weight was observed in ICCV 5530 of 0.58g in uninoculated soil while the least was observed in BG 212 of 0.26g in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum root dry weight was noted in plants grown in uninoculated followed by plants grown in inoculated soil at 45DAS (0.71g) while the root dry weight was at par with each other at 15 DAS (0.24g) .

In interaction between time point and genotype, it was recorded that maximum root dry weight was observed with increase in time in all the genotypes. The maximum root dry weight was observed in ICCV 5530 of 0.75g at 45 DAS while the least was observed in BG 212 of 0.18g at 15 DAS.

In interaction between moisture and soil condition, it was observed that maximum root dry weight was observed in 100% soil moisture followed by 60% in all the genotypes. Significantly more dry weight was observed in uninoculated of 0.62g while the least of 0.25g was observed in 60% soil moisture in inoculated soil.

Table 4.17. Effect of 60 and 100% soil moisture levels on dry weights of chickpea genotypes in inoculated and uninoculated soils

Factors			Root dry weight (g)			Shoot dry weight (g)			Leaf dry weight (g)			Total dry biomass (g)		
Soil moisture (%)	Timepoint (DAS)	Pathogen in soil	BG212	ICCV5530	ICCV8305	BG212	ICCV5530	ICCV8305	BG212	ICCV5530	ICCV8305	BG212	ICCV5530	ICCV8305
60	15	Uninoculated	0.16	0.22	0.24	0.12	0.20	0.27	0.15	0.22	0.30	0.43	0.65	0.81
60	15	Inoculated	0.15	0.22	0.24	0.12	0.20	0.26	0.14	0.22	0.28	0.41	0.63	0.78
60	45	Uninoculated	0.34	0.56	0.47	0.35	0.65	0.81	0.34	0.41	0.42	1.02	1.63	1.70
60	45	Inoculated	0.18	0.36	0.35	0.24	0.53	0.76	0.19	0.35	0.39	0.61	1.24	1.50
100	15	Uninoculated	0.20	0.25	0.39	0.17	0.22	0.33	0.18	0.27	0.31	0.55	0.73	1.03
100	15	Inoculated	0.20	0.23	0.37	0.16	0.22	0.32	0.17	0.25	0.31	0.53	0.70	1.00
100	45	Uninoculated	0.80	1.27	0.85	0.61	0.89	1.11	0.58	0.86	0.92	1.99	3.02	2.87
100	45	Inoculated	0.52	0.79	0.64	0.52	0.77	1.05	0.46	0.67	0.87	1.51	2.22	2.56

Factors	CD			
Pathogen in soil (S)	0.001	0.005	0.004	0.007
Genotype (G)	0.001	0.006	0.005	0.009
Time (T)	0.001	0.005	0.004	0.007
Soil moisture (M)	0.001	0.005	0.004	0.007
S x G	0.002	0.009	0.007	0.012
T x S	0.002	0.007	0.005	0.010
T x G	0.002	0.009	0.007	0.012
M x S	0.002	0.007	0.005	0.010
M x G	0.002	0.009	0.007	0.012
M x T	0.002	0.007	0.005	0.010
T x S x G	0.003	0.012	0.009	0.018
M x S x G	0.003	0.012	0.009	0.018
M x T x S	0.002	0.010	0.008	0.014
M x T x G	0.003	0.012	0.009	0.018
M x T x S x G	0.004	0.018	0.013	0.025

In interaction between moisture and genotype, it was recorded that maximum root dry weight was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum root dry weight was observed in ICCV 5530 of 0.63g while the least was observed in BG 212 of 0.21g.

Maximum root dry weight was observed at 45DAS in 100% soil moisture of 0.81g while the least was observed at 60% at 15 DAS of 0.21g in interaction between moisture and time. Maximum root dry weight was observed in uninoculated at 100% soil moisture of 0.62g while the least at 60% in inoculated soil of 0.25g in interaction between moisture and soil condition.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated soil had more weight of 0.80g and the least weight was noticed at 15DAS in inoculated soil at 60% soil moisture (0.15g). In ICCV 5530, highest root weight was recorded at 100% moisture in uninoculated soil of 1.27g at 45 DAS and the least weight was noticed in 60% uninoculated and inoculated soil which were at par each other i.e 0.22g at 15 DAS. In ICCV 8305, highest root weight was recorded in 100% moisture in uninoculated soil of 0.85g at 45 DAS while the least was noticed in 60% inoculated and uninoculated soils, which were at par each other i.e 0.24g at 15DAS (Fig 4.16).

From the results, it was observed there was no significant difference in the dry weight of the roots among same moisture conditions at different type of soil during 15 DAS. The dry weight of the genotypes was more in the 100% soil moisture as compared to 60% moisture in the 15 DAS. The dry weight of the roots at 45 DAS had significant differences. It was more in the 100% compared to the 60% uninoculated. With respect to the inoculated and uninoculated soil, it was observed that, severe disease in the inoculated soil made the disintegration of the roots and there by losing the weight of roots. It was observed more root weight in the 100% soil moisture compared to the 60% in inoculated soil.

4.8.2 Shoot dry weight

Plants grown in inoculated soil had low shoot dry weight (0.43g) than plants in uninoculated (0.48g). Among the genotypes, maximum shoot dry weight present in ICCV 8305 (0.61g) followed by ICCV 5530 (0.46g) and least was observed in BG 212 (0.29g).

There was significant increase in the shoot dry weight from 15 DAS (0.22g) to 45 DAS (0.69g). Significantly maximum shoot dry weight observed in 100% soil moisture (0.53g) compared to 60% (0.38g).

In interaction between soil condition and genotype, it was recorded that maximum shoot dry weight was observed in plants grown in uninoculated compared to inoculated soil in all the genotypes. The maximum shoot dry weight was noted in ICCV 8305 of 0.63g in uninoculated soil while the least in BG 212 of 0.26g in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum shoot dry weight was present in plants grown in uninoculated soil compared to plants grown in inoculated soil at 45DAS (0.74g) while the shoot dry weight was at par with each other at 15 DAS (0.21 and 0.22g).

In interaction between time point and genotype, increase in shoot dry weight with increase in time was observed in all the genotypes. The maximum shoot dry weight was noted in ICCV 8305 of 0.93g at 45 DAS while the least was in BG 212 of 0.14g at 15 DAS.

In interaction between moisture and soil condition, significantly maximum shoot dry weight was present in 100% soil moisture followed by 60% in all the genotypes. The maximum shoot dry weight was observed in uninoculated soil of 0.55g while the least of 0.35g was observed at 60% soil moisture in inoculated soil.

In interaction between moisture and genotype, it was observed that maximum shoot dry weight was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum shoot dry weight was observed in ICCV 8305 of 0.70g while the least was observed in BG 212 of 0.21g.

In interaction between moisture and time point, it was observed that maximum shoot dry weight was observed at 45DAS in 100% soil moisture of 0.82g while the least was observed at 60% at 15 DAS of 0.20g.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated soil had more weight of 0.61g and the least weight was noticed at 15DAS in inoculated and uninoculated soil at 60% soil moisture (0.12g) which was at par with each other. In ICCV 5530, highest root weight was recorded at 100% moisture in uninoculated soil of 0.89g at

45 DAS and the least weight was noticed in 60% uninoculated and inoculated soil which were at par each other i.e 0.20g at 15 DAS. In ICCV 8305, highest root weight was recorded in 100% moisture in uninoculated soil of 1.11g at 45 DAS while the least was noticed in 60% inoculated soil of 0.26g at 15DAS (Fig 4.17).

4.8.3 Leaf dry weight

Plants grown in inoculated soil had low leaf dry weight (0.36g) than plants in uninoculated (0.41g). Among the genotypes, maximum leaf dry weight present in ICCV 8305 (0.47g) followed by ICCV 5530 (0.41g) and least was observed in BG 212 (0.28g). There was significant increase in the leaf dry weight from 15 DAS (0.23g) to 45 DAS (0.54g). There was significant difference between the time points. The maximum leaf dry weight observed in 100% soil moisture (0.49g) compared to 60% (0.28g). There was significant difference between them.

In interaction between soil condition and genotype, it was observed that maximum leaf dry weight was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum leaf dry weight was observed in ICCV 8305 of 0.49g in uninoculated soil while the least was observed in BG 212 of 0.24g in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum leaf dry weight was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil at 45DAS (0.59g) while the leaf dry weight was at par with each other at 15 DAS (0.23 and 0.24g).

In interaction between time point and genotype, it was observed that maximum leaf dry weight was observed with increase in time in all the genotypes. The maximum leaf dry weight was observed in ICCV 8305 of 0.65g at 45 DAS while the least was observed in BG 212 of 0.16g at 15 DAS.

In interaction between moisture and soil condition, it was observed that maximum leaf dry weight was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum leaf dry weight was observed in uninoculated soil was 0.52g at 100% soil moisture while the least of 0.26g was observed at 60% soil moisture in inoculated soil.

In interaction between moisture and genotype, it was observed that maximum leaf dry weight was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum leaf dry weight was observed in ICCV 8305 of 0.60g while the least was observed in BG 212 of 0.20g.

In interaction between moisture and time point, it was observed that maximum leaf dry weight was observed at 45DAS in 100% soil moisture of 0.73g while the least was observed at 60% at 15 DAS of 0.22g.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated soil had more leaf weight of 0.58g and the least weight was noticed at 15DAS in inoculated and uninoculated soil at 60% soil moisture (0.14 and 0.15g) which were at par with each other. In ICCV 5530, highest leaf weight was recorded at 100% moisture in uninoculated soil of 0.86g at 45DAS and the least weight was noticed in 60% uninoculated and inoculated soil which were at par each other i.e 0.22g at 15 DAS. In ICCV 8305, highest leaf weight was recorded in 100% moisture in uninoculated soil of 0.92g at 45 DAS while the least was noticed at 60% inoculated soil of 0.28g at 15DAS (Fig 4.18).

4.8.4Total dry biomass

Plants grown in inoculated soil had low total plant dry weight (1.14g) than plants in uninoculated (1.37g). Among the genotypes, maximum total plant dry weight recorded in ICCV 8305 (1.53g) followed by ICCV 5530 (1.35g) and least was observed in BG 212 (0.88g). There was significant increase in the total plant dry weight from 15 DAS (0.69g) to 45 DAS (1.82g). There was significant difference between the time points. The maximum total plant dry weight observed in 100% soil moisture (1.56g) compared to 60% (0.95g). There was significant difference between them.

In interaction between soil condition and genotype, it was observed that maximum total plant dry weight was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum total plant dry weight was observed in ICCV 8305 of 1.60g in uninoculated soil while the least was observed in BG 212 of 0.77g in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum total plant dry weight was observed in plants grown in uninoculated soil compared to plants

grown in inoculated soil at 15 and 45DAS. Maximum total plant dry weight was recorded as 2.04g at 45 DAS in uninoculated while the least was at 15 DAS (0.68g) in inoculated soil.

In interaction between time point and genotype, it was observed that maximum total plant dry weight was observed with increase in time in all the genotypes. The maximum total plant dry weight was observed in ICCV 8305 of 2.16g at 45 DAS while the least was observed in BG 212 of 0.48g at 15 DAS.

In interaction between moisture and soil condition, it was observed that maximum total plant dry weight was observed at 100% soil moisture followed by 60% in uninoculated and inoculated soil. The maximum total plant dry weight was observed in uninoculated soil of 1.70g at 100% soil moisture while the least of 0.86g was observed at 60% soil moisture in inoculated soil.

In interaction between moisture and genotype, it was observed that maximum total plant dry weight was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum total plant dry weight was observed in ICCV 8305 of 1.87g while the least was observed in BG 212 of 0.62g.

In interaction between moisture and time point, it was observed that maximum total plant dry weight was observed at 45DAS in 100% soil moisture of 2.36g while the least was observed at 60% at 15 DAS of 0.62g.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated soil had more total plant dry weight of 1.99g and the least weight was noticed at 15DAS in inoculated soil at 60% soil moisture of 0.41g. In ICCV 5530, highest total plant dry weight was recorded at 100% moisture in uninoculated soil of 3.02g at 45DAS and the least weight was noticed in 60% inoculated soil of 0.63g at 15DAS. In ICCV 8305, highest total plant dry weight was recorded in 100% moisture in uninoculated soil of 2.87g at 45 DAS while the least was noticed at 60% inoculated soil of 0.78g at 15DAS (Fig 4.19).

The stress plants and infected plants had poor growth compared to healthy and irrigated plants. Drought stress showed higher negative effects coupled with *M. Phaseolina* attack in vegetative growth stage in common bean. It decreased leaf area and dry weight of all vegetative structures significantly (Mayek *et al*, 2002).

4.9 Physiological changes associated with dry root development in chickpea

Physiology parameters *viz.,* transpiration, leaf temperature and stomatal conductance at two time points were recorded. The results obtained were presented in table 4.18.

4.9.1Transpiration

Plants grown in uninoculated soil had high transpiration loss (25.0g) than plants in inoculated soil (19.0g). Among the genotypes, maximum transpiration loss present in ICCV 8305 (24.0g) followed by BG 212 (20.09g) and least was observed in ICCV 5530 (20.9g). There was significant increase in the transpiration loss from 15 DAS (16.3g) to 45 DAS (27.7g). There was significant difference between the time points. The maximum transpiration loss observed in 100% soil moisture (26.3g) compared to 60% (17.7g). There was significant difference between them.

In interaction between soil condition and genotype, it was observed that maximum transpiration loss was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum transpiration loss was observed in ICCV 8305 of 26.7g in uninoculated soil while the least was observed in BG 212 of 17.7g in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum transpiration loss was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil at 15 and 45DAS. Maximum transpiration loss of 32.6g was observed at 45 DAS while least was observed in 15DAS of 15.3g.

In interaction between time point and genotype, it was observed that maximum transpiration loss was observed with increase in time in all the genotypes. The maximum transpiration loss was observed in ICCV 8305 of 30.7g at 45 DAS while the least was observed in BG 212 of 17.4g at 15 DAS.

In interaction between soil moisture and soil condition, it was noted that maximum transpiration loss was observed at 100% soil moisture followed by 60% in all the genotypes. The maximum transpiration loss was observed in uninoculated soil was 29.5g at 100% soil moisture while the least of 15.0g was observed at 60% soil moisture in inoculated soil.

Table 4.18. Effect of 60 and 100% soil moisture levels on transpiration, stomatal conductance and leaf temperature of chickpea genotypes in inoculated and uninoculated soils

Factor			Transpiration ($g\ H_2O\ 8h^{-1}$)			Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)			Leaf temperature (˚C)		
Soil moisture (%)	Time point (DAS)	Pathogen in soil	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305	BG 212	ICCV 5530	ICCV 8305
60	15	Uninoculated	17.30	12.00	16.00	0.96	0.67	0.89	30.40	31.20	29.60
60	15	Inoculated	14.30	11.00	13.70	0.79	0.61	0.76	32.70	31.80	30.60
60	45	Uninoculated	23.70	26.70	27.00	0.62	0.70	0.71	36.60	34.50	35.50
60	45	Inoculated	13.30	16.30	21.70	0.35	0.43	0.57	37.50	36.50	37.60
100	15	Uninoculated	20.70	17.00	21.10	1.15	0.94	1.17	28.10	27.10	28.30
100	15	Inoculated	19.30	14.70	18.70	1.07	0.82	1.07	28.80	28.80	28.00
100	45	Uninoculated	36.30	39.30	42.70	0.96	1.03	1.12	32.10	32.30	29.60
100	45	Inoculated	24.00	30.00	31.30	0.63	0.79	0.82	33.60	32.90	33.20

Factor	CD		
Pathogen in soil (S)	0.10	0.004	0.21
Genotype (G)	0.12	0.005	0.26
Time (T)	0.10	0.004	0.21
Soil moisture (M)	0.10	0.004	0.21
S x G	0.17	0.008	0.37
T x S	0.14	0.006	0.30
T x G	0.17	0.008	0.37
M x S	0.14	0.006	0.3
M x G	0.17	0.008	0.37
M x T	0.14	0.006	0.3
T x S x G	0.25	0.011	0.52
M x S x G	0.25	0.011	0.52
M x T x S	0.20	0.009	0.42
M x T x G	0.25	0.011	0.52
M x T x S x G	0.35	0.015	0.73

In interaction between moisture and genotype, maximum transpiration loss was rcorded at 100% soil moisture followed by 60% in all the genotypes. The maximum transpiration loss was observed in ICCV 8305 of 28.4g while the least was observed in ICCV 5530 of 16.5g.

In interaction between moisture and time point, maximum transpiration loss was observed at 45DAS in 100% soil moisture of 33.9g while the least was observed at 60% at 15 DAS of 14.1g.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated soil had transpired more of 36.3g and the least transpiration loss of 13.3g was noticed in inoculated soil at 60% soil moisture at 45DAS. While in ICCV 5530 highest transpiration loss was recorded in 100% moisture in uninoculated soil with 39.3g after 45 DAS and the least transpiration loss was noticed in 60% inoculated soil of 11.0g at 15DAS. In ICCV 8305, highest transpiration was recorded in 100% moisture in uninoculated soil with 42.7g after 45 DAS and the least was noticed in 60% moisture in inoculated soil of 13.7g at 15 DAS (Fig 4.20).

Inoculation of pathogen resulted in clogging of xylem vessels by mycelia, spores and tyloses. Crushing of vessels by proliferating adjacent parenchyma cells was also observed, which hamper the translocation of water of the infected plants. The leaves of infected plants transpire more water than the roots and stem can transport resulting in wilting symptoms (Agrios, 2005). That is why growth and transpiration were reduced in *F. oxysporum f. sp. ciceri* infected plants. Drought stress showed higher negative effects than *M. Phaseolina* on water relations, vegetative growth in common bean. It decreased transpiration rate and leaf area (Mayek *et al.*, 2002). Ponmurugan and Baby (2007) conducted under greenhouse condition to study the physiological changes in tea plants due to Phomopsis infection. Physiological responses of tea plants to infection in term of transpiration rate studied in susceptible TRI-2024 and tolerant TRI-QOES genotypes. In addition, growth characteristics such as height, dry weight and plant strength. The results revealed that all the growth characteristics and physiological were reduced significantly in infected plants rather than healthy plants. However, the reduction was more prominent in susceptible genotype than in tolerant ones.

4.9.2 Stomatal conductance

Plants grown in uninoculated soil had high stomatal conductance (0.91) than plants in inoculated soil (0.73 mol m^{-2} s^{-1}). Among the genotypes, maximum stomatal conductance present in ICCV 8305 (0.82 mol m^{-2} s^{-1}) followed by BG 212 (0.82 mol m^{-2} s^{-1}) and least was observed in ICCV 5530 (0.75 mol m^{-2} s^{-1}). There was significant decrease in the stomatal conductance from 15 DAS (0.91 mol m^{-2} s^{-1}) to 45 DAS (0.73 mol m^{-2} s^{-1}). There was significant difference between the time points. The maximum stomatal conductance observed in 100% soil moisture (0.97 mol m^{-2} s^{-1}) compared to 60% (0.67 mol m^{-2} s^{-1}). There was significant difference between them.

In interaction between soil condition and genotype, it was observed that maximum stomatal conductance was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil in all the genotypes. The maximum stomatal conductance was observed in ICCV 8305 of 0.97 mol m^{-2} s^{-1} in uninoculated soil while the least was observed in ICCV 5530 of 0.66 mol m^{-2} s^{-1} in inoculated soil.

In interaction between soil condition and time point, it was observed that maximum stomatal conductance was observed in plants grown in uninoculated soil compared to plants grown in inoculated soil at 15 and 45DAS. Maximum stomatal conductance of 0.96 mol m^{-2} s^{-1} was observed at 15 DAS while least was observed in 45DAS of 0.60 mol m^{-2} s^{-1}.

In interaction between time point and genotype, significant decrease in stomatal conductance was noted with increase in time in all the genotypes. The maximum stomatal conductance was in BG 212 of 0.99 mol m^{-2} s^{-1} at 15 DAS while the least was observed in BG 212 of 0.64 mol m^{-2} s^{-1}at 45 DAS.

In interaction between soil moisture and soil condition, maximum stomatal conductance was recorded at 100% soil moisture followed by 60% in all the genotypes. The maximum stomatal conductance was in uninoculated soil of 1.06 mol m^{-2} s^{-1} at 100% soil moisture while the least of 0.59 mol m^{-2} s^{-1} was observed at 60% soil moisture in inoculated soil.

Maximum stomatal conductance was observed at 100% soil moisture followed by 60% in all the genotypes in interaction between moisture and genotype. The maximum stomatal conductance was observed in ICCV 8305 of 1.05 mol m^{-2} s^{-1} while the least was observed in ICCV 5530 of 0.60 mol m^{-2} s^{-1}.

In interaction between moisture and time point, maximum stomatal conductance was noted at 15DAS in 100% soil moisture of 1.04 mol m^{-2} s^{-1} while the least was at 60% at 45 DAS of 0.56 mol m^{-2} s^{-1}.

Among the genotypes, BG 212 grown in 100% moisture in uninoculated had more stomatal conductance of 1.15mol m^{-2} s^{-1} at 15 DAS and the least was noticed at 45DAS in inoculated soil with 60% soil moisture of 0.35mol m^{-2} s^{-1}. While in ICCV 5530, highest stomatal conductance was recorded in 100% moisture in uninoculated soil with 0.94 mol m^{-2} s^{-1} at 15 DAS and the least stomatal conductance was noticed at 60% moisture in inoculated soil of 0.43 mol m^{-2} s^{-1} at 45DAS. In ICCV 8305 highest stomatal conductance was recorded in 100% moisture in uninoculated soil with 1.17mol m^{-2} s^{-1} at 15 DAS and the least was noticed in 60% moisture inoculated soil of 0.57 mol m^{-2} s^{-1} at 45 DAS (Fig 4.21).

Drought stress showed higher negative effects than *M. Phaseolina* on water relations, vegetative growth in common bean. It increased charcoal rot and stomatal resistance due to damage caused to the root system (Mayek *et al.,* 2002).

The above results were In accordance with the work done by Doubledee (2010). Above ground level disease symptoms in soybean by *Macrophomina phaseolina* were difficult to distinguish from those of drought. To separate the affects of disease from drought, four soybean genotypes *viz.*, DT97-4290, DPL 4546, R01-581F and LS980358 were grown in infested or non-infested with *M. phaseolina*. Half of the plots were kept well watered and the other half were allowed to water stress. At flowering, plants in infested and non-irrigated plants had lower stomatal conductance than those in infested irrigated or non-infested irrigated or non-irrigated plants. These results suggest that infection with *M. phaseolina* may limit the water uptake in the plant, before the onset of visible symptoms.

4.9.3 Leaf temperature

Infra-red thermography is a very powerful method to monitor changes in plant water status *in vivo*. Thus it is being largely used in agriculture for a wide range of applications, from scheduling irrigation to evaluating fruit maturity (Vadivambal *et al.,* 2011). However, the usefulness of thermal imaging has not been sufficiently explored in disease resistance breeding. Here, infra-red thermography used in detection of chickpea

plants infected by the dry root rot pathogen *Rhizoctonia bataticola* and the discrimination between susceptible and moderately resistant genotypes at a very early stage of the interaction.

Plants grown in uninoculated soil had low leaf temperature (31.3ºC) than plants in inoculated soil (32.7ºC). Among the genotypes, maximum leaf temperature present in BG 212 (32.5ºC) followed by ICCV 5530 (31.9ºC) and least was observed in ICCV 8305 (31.6ºC). There was significant increase in the leaf temperature from 15 DAS (29.6ºC) to 45 DAS (34.3ºC). There was significant difference between the time points. Significantly high leaf temperature observed in 60% soil moisture (33.7ºC) compared to 100% (30.2ºC).

In interaction between soil condition and genotype, it was observed that maximum leaf temperature was observed in plants grown in inoculated soil compared to plants grown in uninoculated soil in all the genotypes. The maximum leaf temperature was observed in BG 212 of 33.1 ºC in inoculated soil while the least was observed in ICCV 8305 of 30.8 ºC in uninoculated soil.

In interaction between soil condition and time point, it was observed that maximum leaf temperature was observed in plants grown in inoculated soil compared to plants grown in uninoculated soil at 15 and 45DAS. Maximum leaf temperature of 35.2 ºC was observed at 45 DAS while least was observed in 15DAS of 29.1 ºC.

In interaction between time point and genotype, it was observed that leaf temperature was observed with increase in time in all the genotypes. The maximum leaf temperature loss was observed in BG 212 of 35.0 ºC at 45 DAS while the least was observed in ICCV 8305 of 29.1 ºC at 15 DAS.

In interaction between soil moisture and soil condition, it was observed that maximum leaf temperature was observed at 60% soil moisture followed by 100% in all the genotypes. The maximum leaf temperature was observed in inoculated soil was 34.5 ºC at 60% soil moisture while the least of 29.6 ºC was observed at 100% soil moisture in uninoculated soil.

In interaction between moisture and genotype, it was observed that maximum leaf temperature was observed at 60% soil moisture followed by 100% in all the genotypes. The

maximum leaf temperature was observed in BG 212 of 34.3 ºC while the least was observed in ICCV 8305 of 33.3 ºC.

In interaction between moisture and time point, it was observed that maximum leaf temperature was observed at 45DAS in 60% soil moisture of 36.4 ºC while the least was observed at 100% at 15 DAS of 28.2 ºC.

Among the genotypes, BG 212 grown in 60% moisture in inoculated soil had more temperature 37.5ºC and the least temperature was noticed at 15DAS in uninoculated soil with 100% soil moisture of 28.1ºC. While in ICCV 5530, highest temperature was recorded in inoculated soil at 60% moisture with 36.5ºC after 45 DAS and the least temperature was noticed in uninoculated soil at 100% moisture i.e. 27.1ºC at 15DAS. In ICCV 8305, highest temperature was recorded in inoculated soil at 60 per cent moisture with 37.6ºC at 45 DAS and the least was noticed in inoculated soil at 100% i.e. 28.0ºC at 15DAS (Fig 4.22 and 4.23).

The above results were in accordance with the work done by Doubledee (2010) on soybean infected by *Macrophomina phaseolina* to distinguish disease from those of drought. Water stressed plants and infested plants had higher canopy temperatures (based on infared radiation expressed as Crop Water Stress Index) than well watered or non-infested plants. These results suggest that infection with *M. phaseolina* may limit the water uptake in the plant, before the onset of visible symptoms. Changes in stomatal conductance, transpiration rate and superficial leaf temperature had more effect on susceptible wilted plants upon infection by *F. oxysporum* reported by Wang *et al.* (2013). This reinforced the close relationship between vascular wilt and drought. The increase in leaf temperature and associated stomatal closure might be related to the vessel plugging induced by the intensive fungal growth within xylem vessels and by the attempt of plant defence that blocked xylem cells. Independently of the causal effect leading to stomatal closure and transpiration rates, this temperature increase was only detected in susceptible accessions since the surface leaf temperature of resistant pea accessions was maintained at uninoculated level (Nicolas and Diego, 2015).

Fig 4.19. Effect of 60 and 100% soil moisture levels on total dry weight of chickpea genotypes in inoculated and uninoculated soils

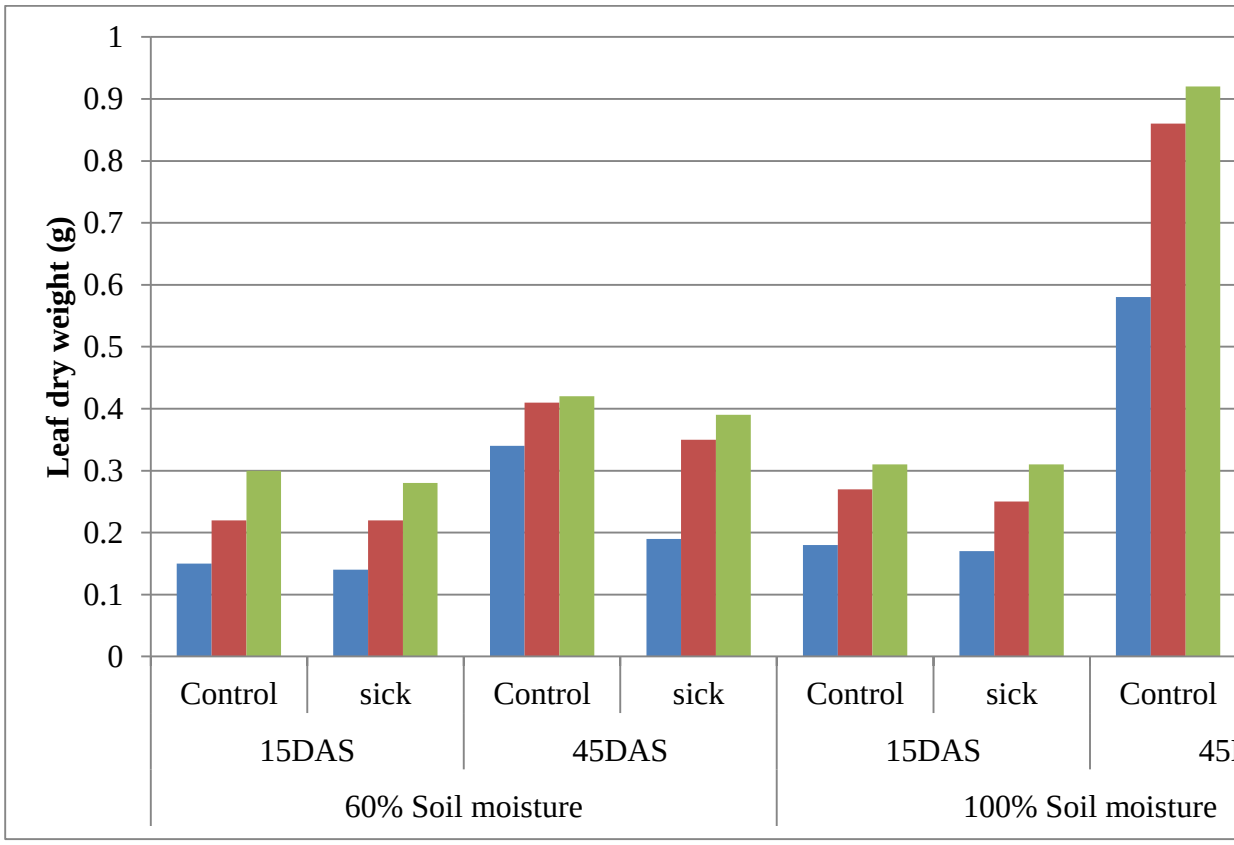

Fig 4.18. Effect of 60 and 100% soil moisture levels on leaf dry weight of chickpea genotypes in inoculated and uninoculated soils

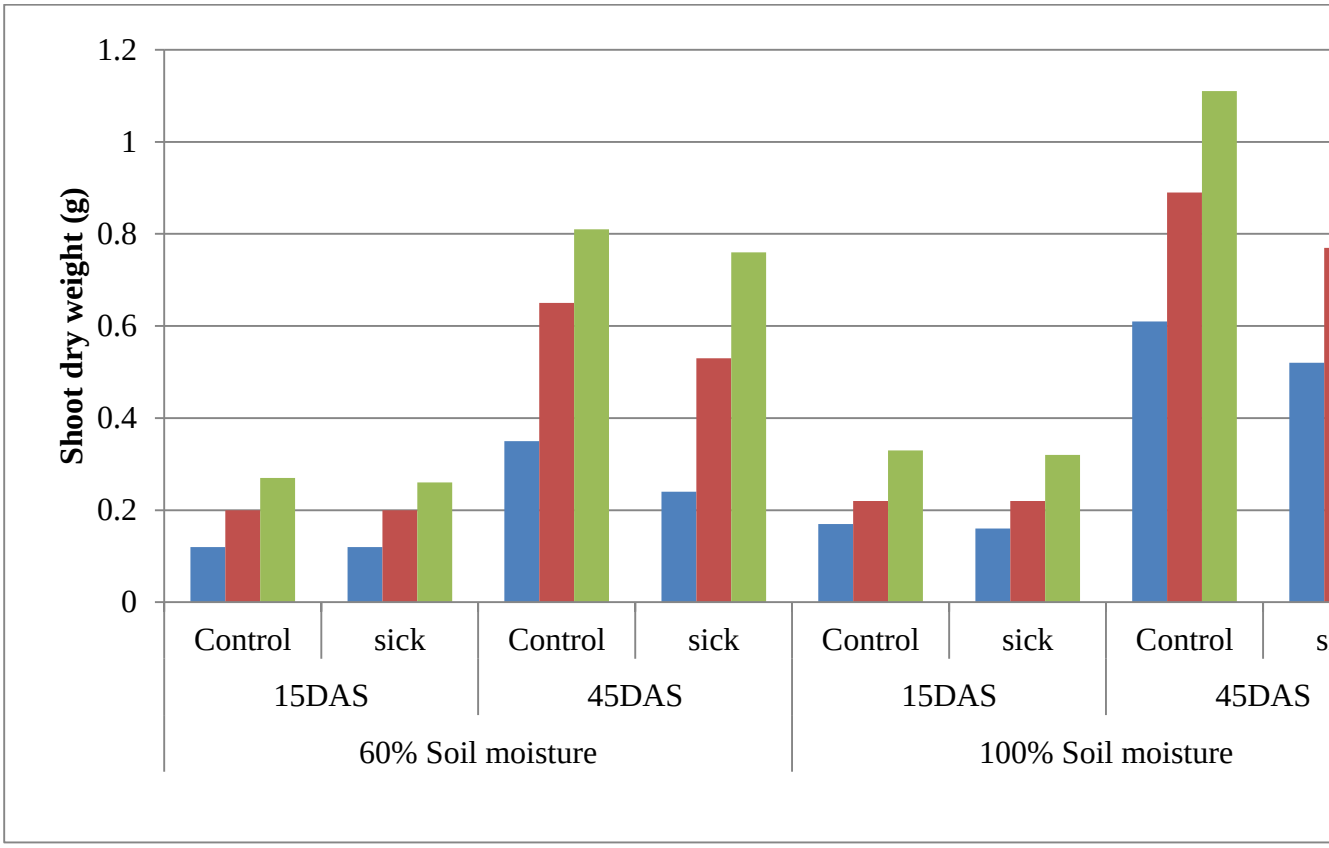

Fig 4.17. Effect of 60 and 100% soil moisture levels on shoot dry weight of chickpea genotypes in inoculated and uninoculated soils

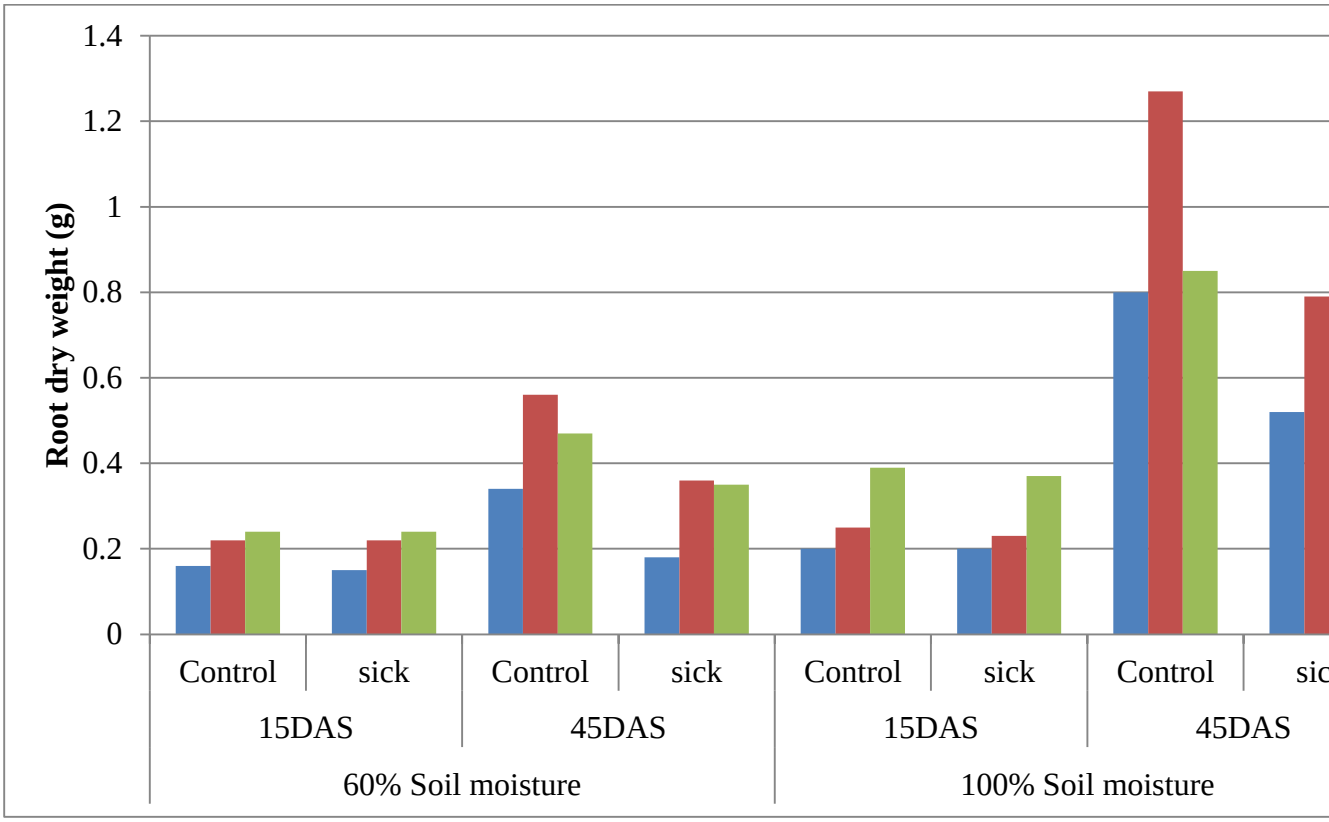

Fig 4.16. Effect of 60 and 100% soil moisture levels on root dry weight of chickpea genotypes in inoculated and uninoculated soils

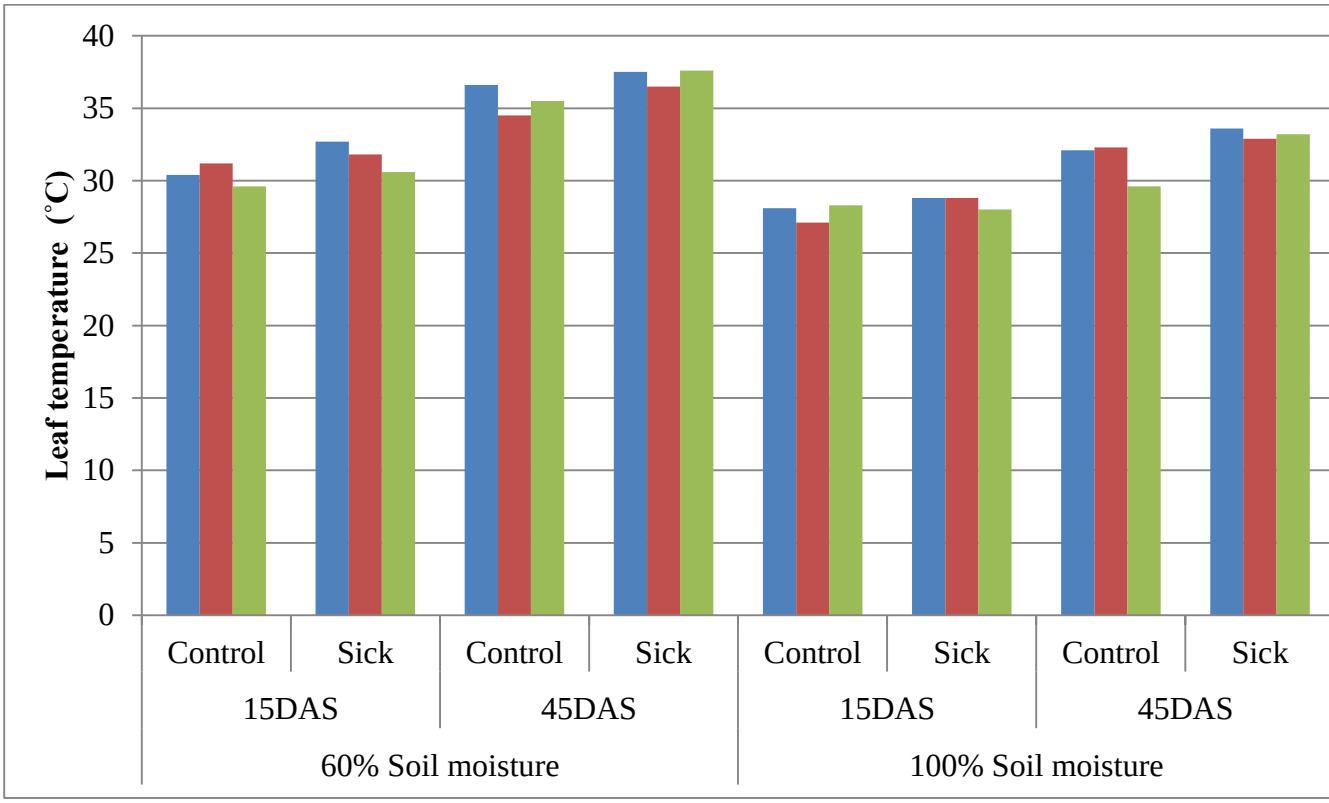

Fig 4.22. Effect of 60 and 100% soil moisture levels on leaf temperature of chickpea genotypes in inoculated and uninoculated soils

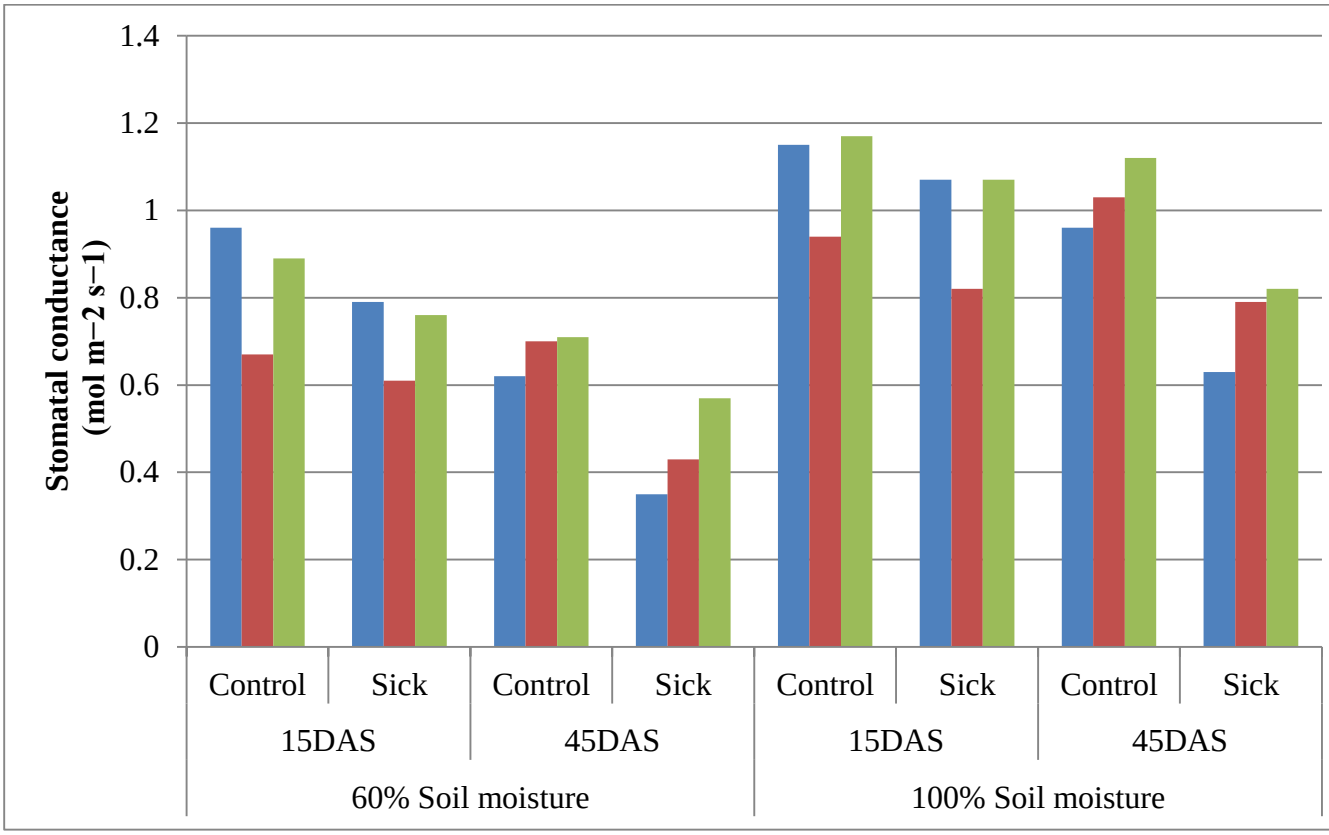

Fig 4.21. Effect of 60 and 100% soil moisture levels on stomatal conductance of chickpea genotypes in inoculated and uninoculated soils

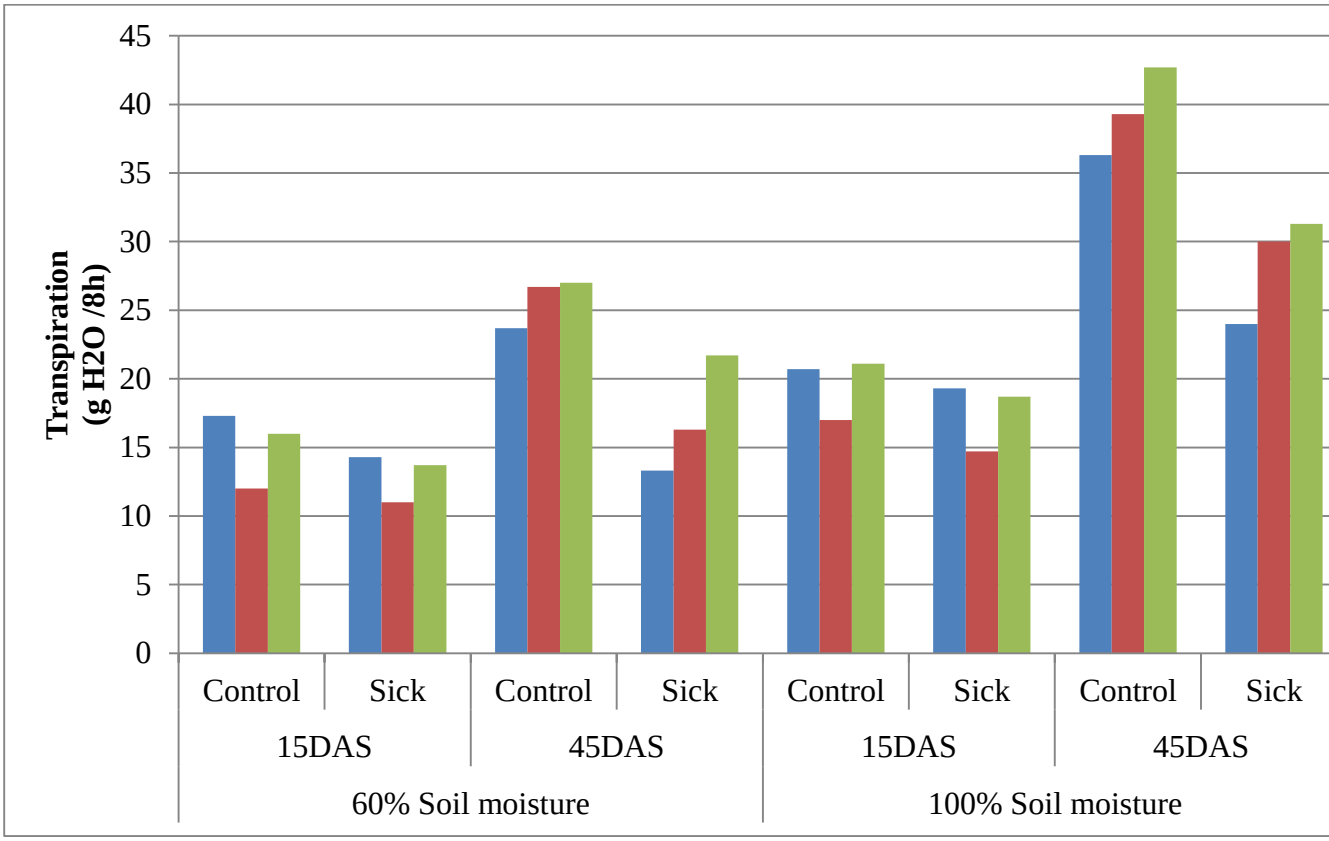

Fig 4.20. Effect of 60 and 100% soil moisture levels on transpiration of chickpea genotypes in inoculated and uninoculated soils

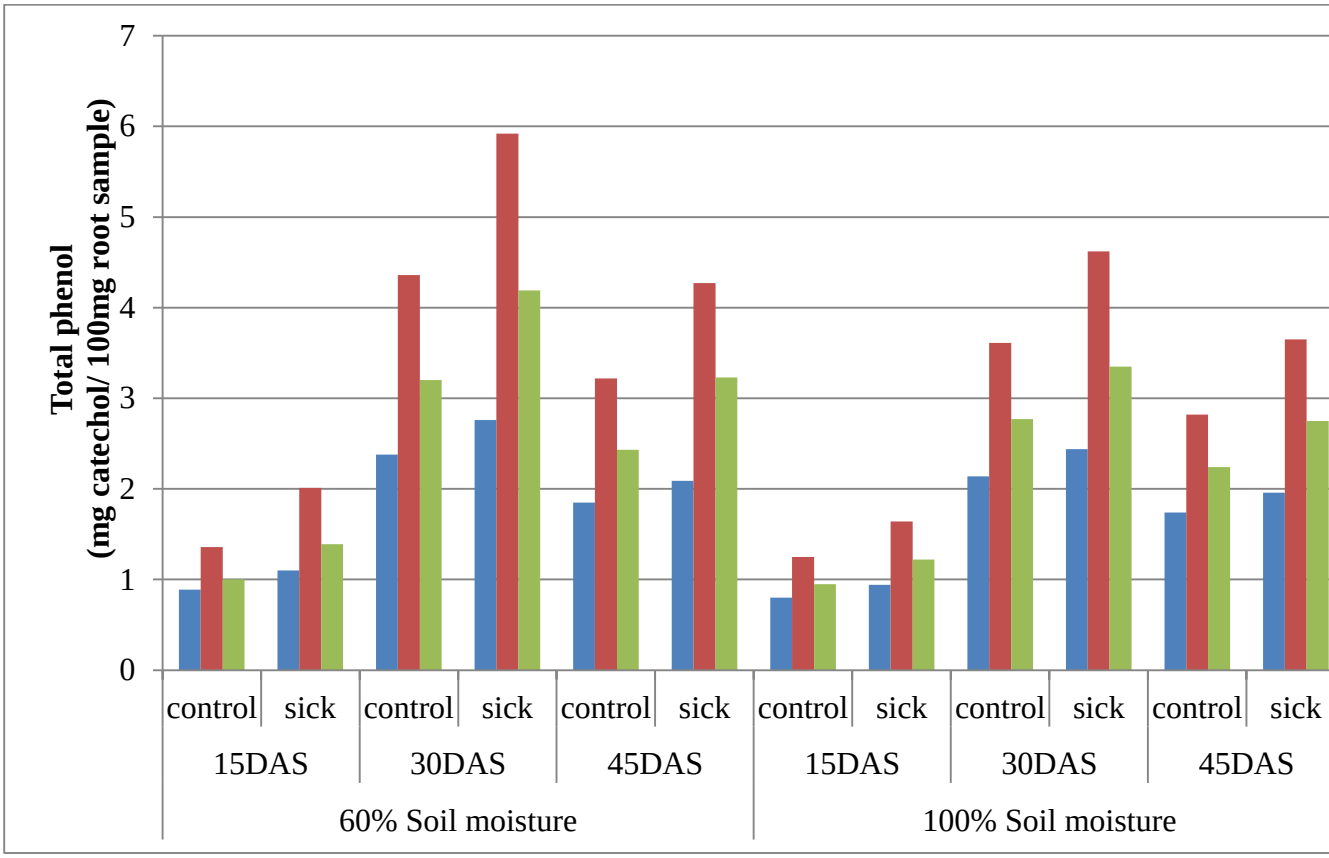

Fig 4.11.Effect of different moisture levels on total phenols in chickpea genotypes grown in sick and control soils

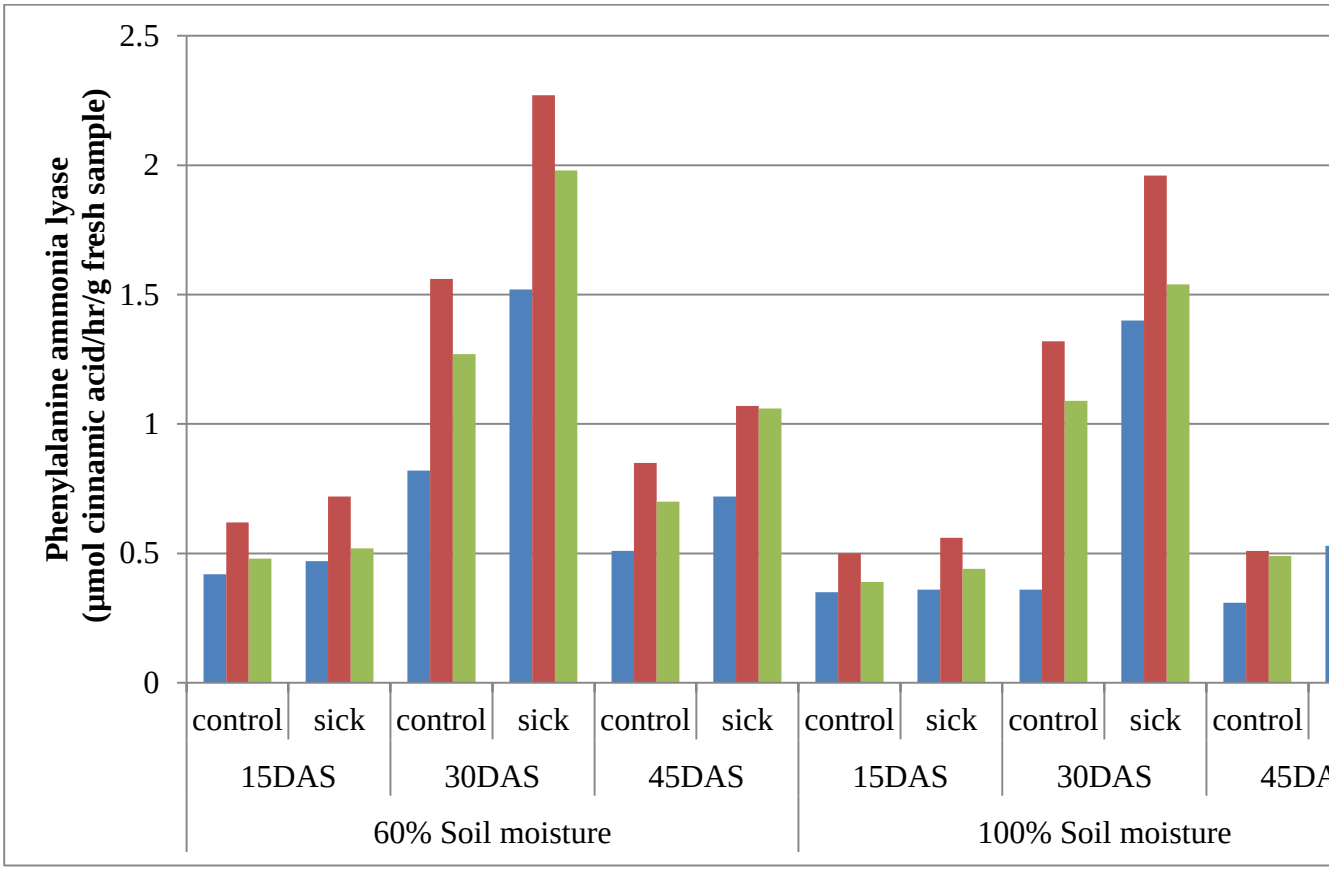

Fig 4.12. Effect of different moisture levels on phenylalanine ammonia lyase in chickpea genotypes grown in inoculated and uninoculated soils

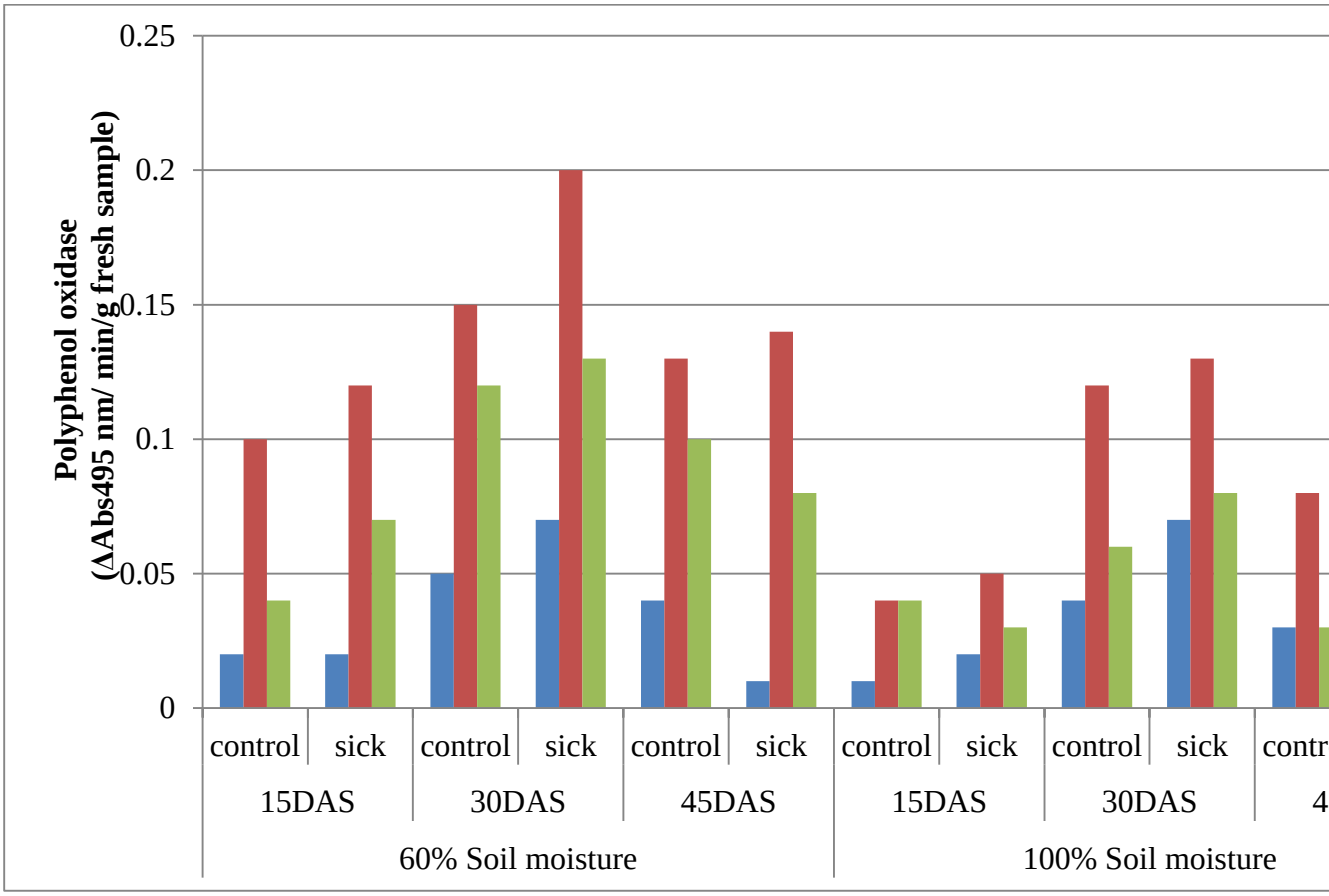

Fig 4.13. Effect of different moisture levels on polyphenol oxidase activity in chickpea genotypes grown in inoculated and uninoculated soils

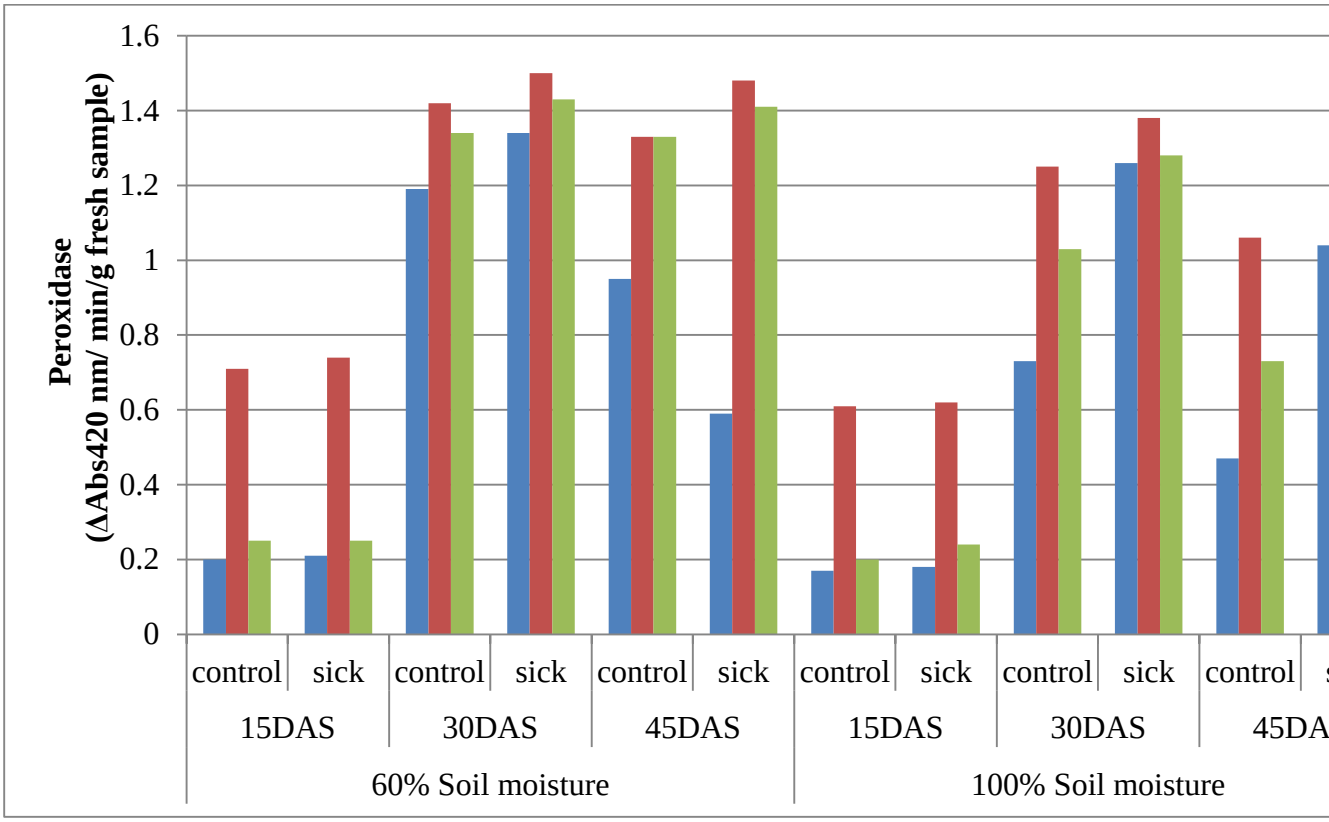

Fig 4.14. Effect of different moisture levels on peroxidase activity in chickpea genotypes grown in inoculated and uninoculated soils

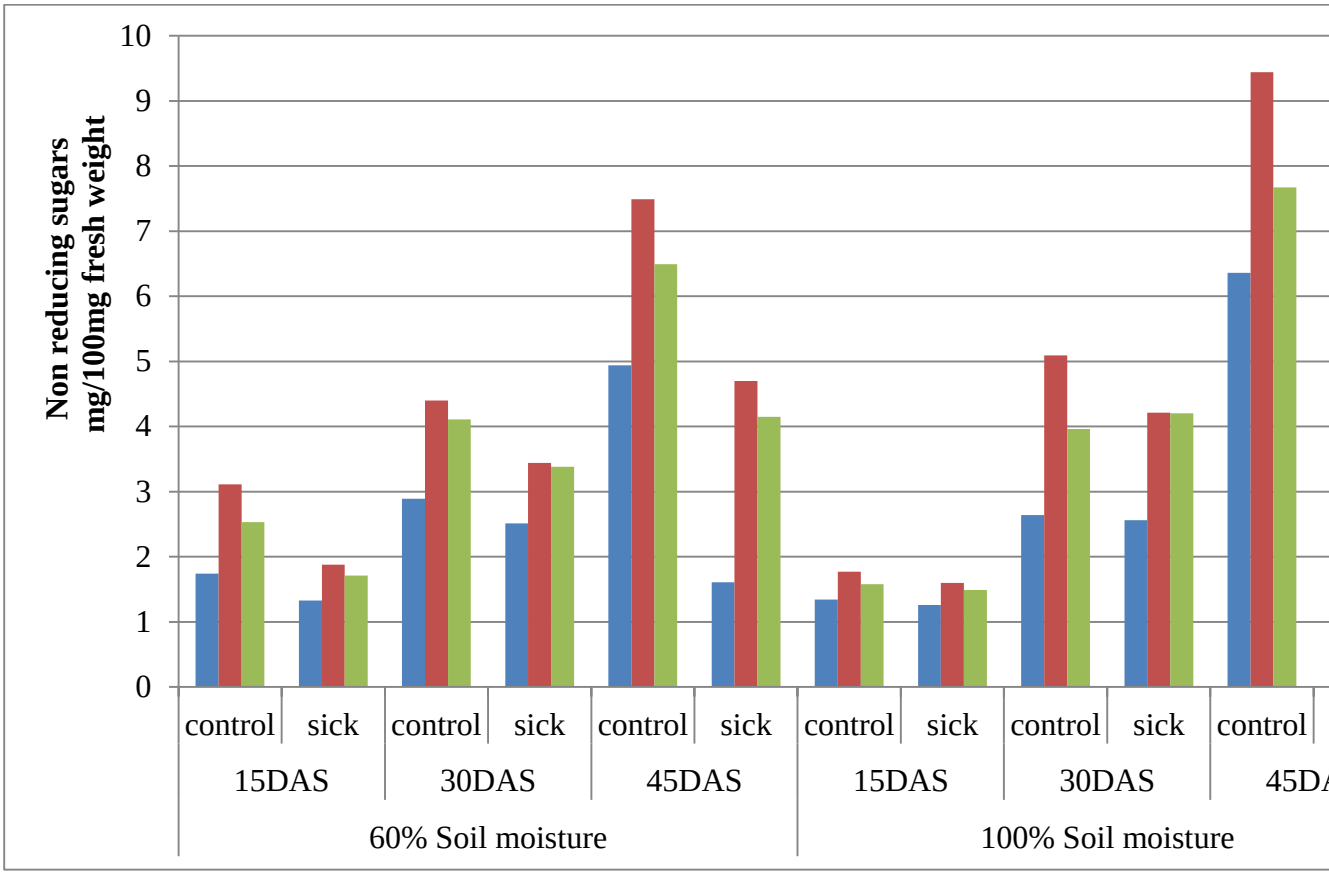

Fig 4.10. Non reducing sugar in chickpea genotypes grown in inoculated and uninoculated soils at different moisture levels

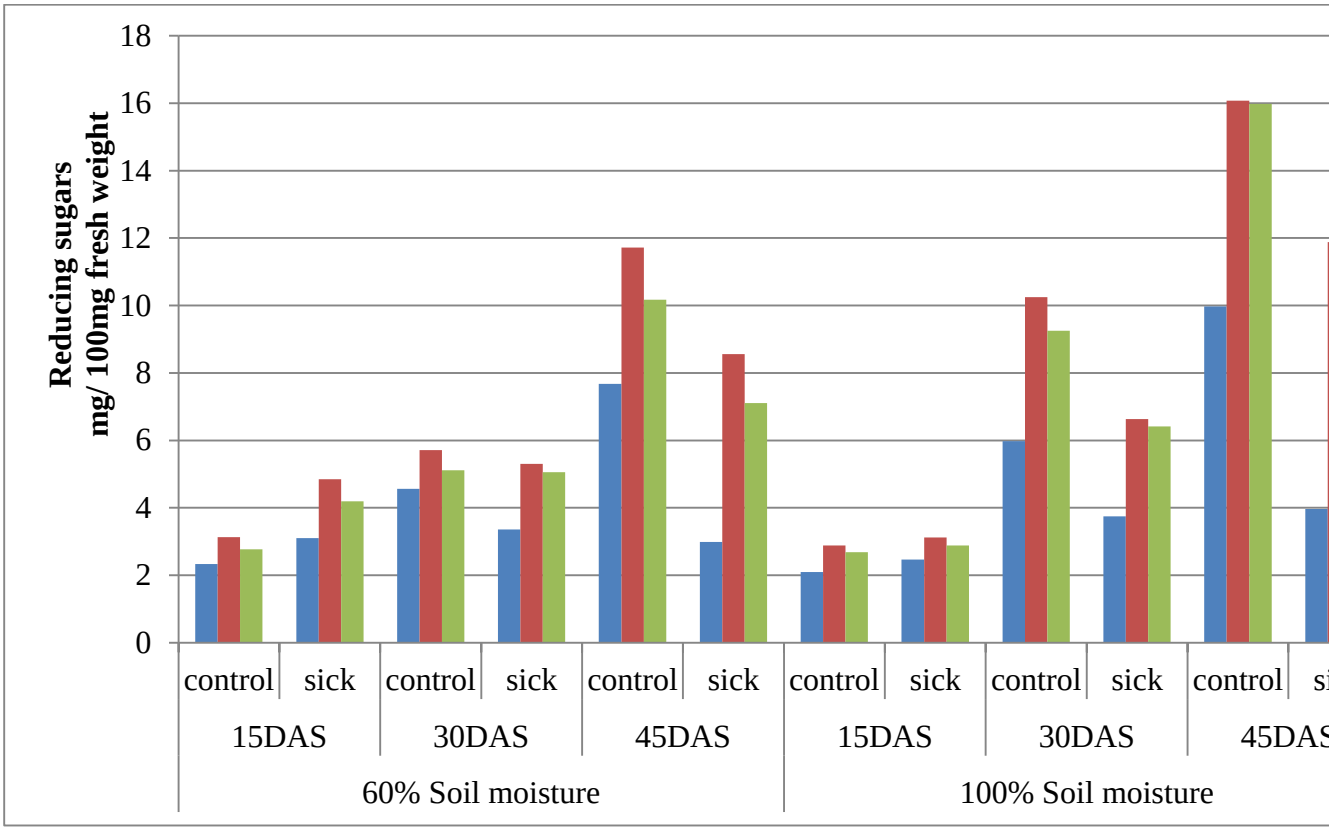

Fig 4.9. Reducing sugar in chickpea genotypes grown in inoculated and uninoculated soils at different moisture levels

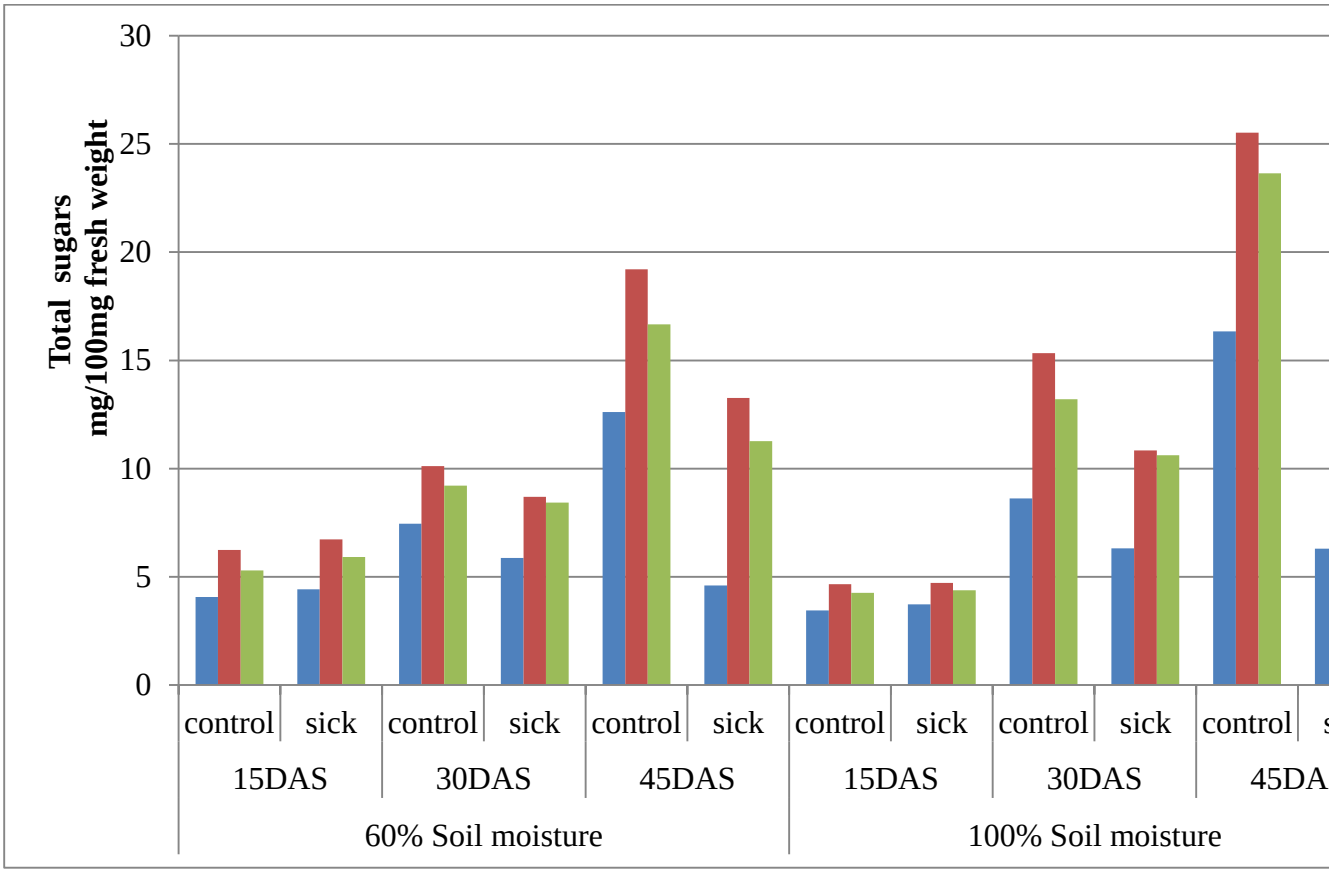

Fig 4.8. Total sugar in chickpea genotypes grown in inoculated and uninoculated soils at different moisture levels

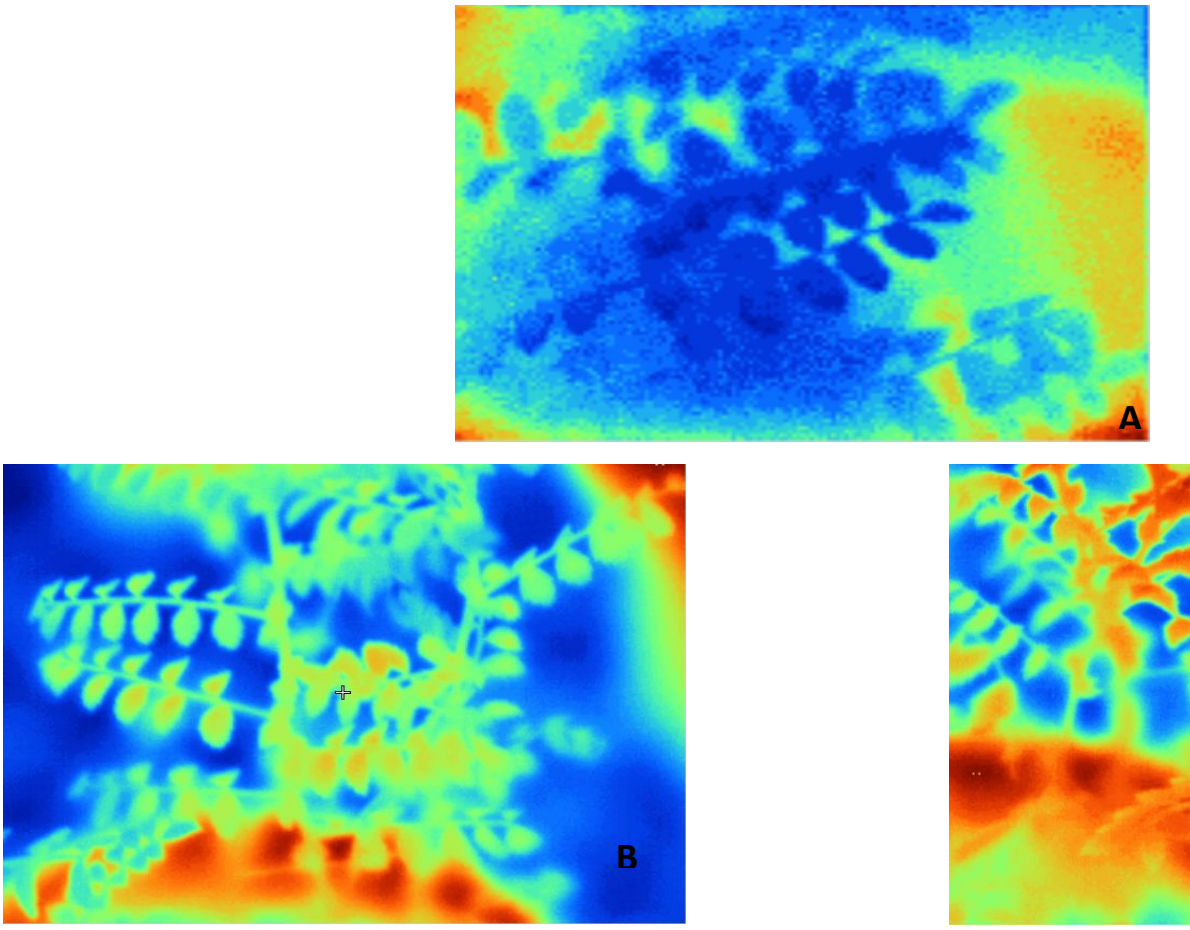

Fig 4.23. Infrared images of control (A), moderately resistant (B) and susceptible (C) genotypes at 45 DAS at 60% soil moisture

SUMMARY AND CONCLUSIONS

Chapter V

SUMMARY AND CONCLUSIONS

The present investigation included survey on disease incidence of dry root rot, collection of pathogen isolates, diversity among isolates with respect to cultural, morphological, pathological and molecular variability, influence of environmental factors on growth and disease development, biochemical and physiological changes associated with the dry root rot disease development in chickpea caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid]. The results obtained in these investigations are summarized below.

A roving survey was conducted during January, 2014 in 68 different chickpea growing locations of central (Madhya Pradesh and Maharashtra) and southern (Andhra Pradesh, Telangana and Karnataka) India to assess the status of dry root rot incidence. Mean maximum dry root rot incidence was observed in Telangana (18.28%) followed by Madhya Pradesh (18.10%), Karnataka (7.85%), Andhra Pradesh (5.40%) and the least in Maharashtra (5.38%). Out of 68 locations surveyed, the crop was cultivated in vertisols in 66 locations except in Gandemla and Kurnool in Kurnool district of Andhra Pradesh, it was cultivated in alfisols. In Madhya Pradesh maximum incidence was noticed in Hardua village (28.0%) of Jabalpur district and the least in Bandol village (3.33%) of Seoni district. In Karnataka, maximum incidence was noticed in Devadurga (15.33 %) and the least incidence was 2.67% from Sarsamba village of Gulbarga district. In Maharashtra, maximum incidence was in Mekar (23.33 %) of Buldhana district and least was in Rajapur (1.33 %) of Akola District. In Andhra Pradesh, highest was found in Nandyal (RARS) with

27.33 % incidence and least in Oruvagalu (0.67%) of Kurnool District. In Telangana state, the highest incidence (31.3%) was noticed in Daroor village of Rangareddy district and the least (1.34 %) was in Vundavelli village of Mahboobnagar district.

The percent disease incidence recorded in each variety varied depending on the place of cultivation. Desi variety was cultivated in 26 locations had disease incidence ranging from 0.67 in Oruvagal of Kurnool district to 31.33 in Daroor of Rangareddy district. JG 11 was cultivated in 25 locations recorded the disease incidence ranging from 1.33 in chinakallu in Anantapur to 30.67 in Patancheru, Medak district. Variety JG 62 grown in six locations recorded disease incidence ranging from 7.33 in Bidagad village in Jabalpur district to 29.33 in Patancheru in Medak district. The cultivar BG 212 cultivated in Patancheru, Medak recorded highest disease incidence of 24.00. ICCV 2 was cultivated in the Patancheru in Medak district recorded 14.67. Improved desi had recorded the disease incidence of 1.33 in Rajapur to 3.33 in Pailpada of Akola district. JAKI 9218 grown in Patancheru, Medak recorded 25.33 percent disease incidence. KAK 2 was cultivated in two locations had disease incidence varying from 8.00 in Paduchuru in Prakasam district to 24.00 in Chaurai in Chhindwara district. L550, MNK 1 and Wardha were cultivated in Nandyal, Ayyaluru and Lacina villages recorded 27.33, 1.33 and 2.00, respectively.

Sorghum chickpea cropping system was observed in 18 locations where the disease incidence ranged from 1.33 Pedakallu village of Anatapur to 30.67 in Patancheru of Medak district. Maize chickpea cropping system was observed in 12 locations where the disease incidence ranged from 1.33 Vundavelli village of Mahboobnagar to 28.00 in Hardua of Jabalpur district. Kharif fallow chickpea cropping system was observed in 11 locations where the disease incidence ranged from 3.33 Marturu village of Prakasam to 29.33 in Patancheru of Medak district. Soyabean chickpea cropping system was followed in Maharashtra where the disease incidence varied from 1.33 (Rajapur) to 23.33 (Mekar) and the difference in disease incidence was significant. In rice chickpea cropping system locations, the disease incidence varied from 1.33 (Chinakallu and Ayyaluru of Anantpur and Kurnool district respectively) to 24.00 (Pipariya) and the difference in disease incidence was significant. In pigeonpea chickpea cropping system in Telangana, the disease incidence varied from 14.67 (Patancheru) to 25.33 (Patancheru). In black gram chickpea, ajowan chickpea and pearlmillet chickpea cropping systems, disease incidence was 2.67 (Pedanandipadu), 31.33 (Daroor) and 25.33 (Patancheru) respectively.

Out of 68 locations, seed treatment was done in ten locations while maximum farmers of 58 locations were not practicing seed treatment. The disease incidence in the seed treated locations varied from 0.67 in Oruvagal of Kurnool to 7.33 in Bidagad of Jabalpur district. Where as in the locations where seed treatment was not practiced the disease incidence varied from 1.33 to 31.33.

Irrigation was given in 18 locations while maximum farmers in 50 locations cultivated the crop under residual soil moisture or rainfall during crop growth. The disease incidence in the irrigated locations varied from 0.67 in Oruvagal of Kurnool to 29.33 in Patancheru of Medak district where as in the locations where irrigation facility was not there, the disease incidence varied from 1.33 (Chinakallu, Pedakallu and Kolimigundla) to 31.33 (Daroor).

During survey, due to diversified weather conditions and variation in sowing dates in different states, different crop growth stages i.e. from seedling (20 days old) to podding stage were observed. The crop was sown early in Karnataka and Andhra Pradesh during first fortnight of October while in Telangana in the second fort night of October. In Maharashtra sowings were done in the month of November whereas in Madhya Pradesh in the second fortnight of November to December. In Madhya Pradesh, seedlings infected with dry root rot appeared stunt without any lateral roots when uprooted and the tap root was black in colour. In Maharashtra and Telangana, crop was in the vegetative stage to flowering stage. Symptoms on affected plants were observed as bronzing of the leaves on one or more of the lower branches, leaves became yellow to brown in plants showing advanced disease symptoms. In such plants, the affected branches and leafstalks were stiff, turned upwards and the leaflets stand more or less vertically and were shed prematurely. The terminal part of the tap-root and lateral roots became brown to black and shriveled. The tap root without any lateral roots was also observed. In Karnataka and Andhra Pradesh, the crop was in podding and harvesting stage. The disease was scattered in the field as dried plants. It was also observed that, the susceptibility of plant to this disease increased with age. Sometimes the apical leaves on the affected plants appeared chlorotic, when the rest of the plant was dry. The pods on affected plants were poorly developed and the number of pods per plant was less.

The mycelium was initially white in colour which was later converted to dark brown to black in colour. Production of aerial mycelium was also observed in some

isolates. The vegetative mycelium was characterized by the formation of barrel-shaped cells and the formation of septum near the origin of branch of the mycelium. Branching occurred mostly at right angle to parent hyphae, but branching at acute angles was also observed. The hyphal cell length varied from 9.38x3.8 µm (Rb10) to 14.88x7.5 µm (Rb 63). The sclerotial size varied from 54.86x 45.49 µm (Rb46) to 216.08x 181.09 µm (Rb 59). The shape of sclerotia varied from round, ovoid to irregular. The texture of sclerotia was either rough or smooth. The sclerotia were dark brown to black in colour.

Among 68 isolates the maximum disease severity rating of 9 was observed in Rb 21, Rb 40, Rb 57, Rb 60, Rb 61 and Rb 63 while the least disease severity rating of 2.5 was observed in Rb 38. The incubation period ranged from 1.0 (Rb 21, Rb 40, Rb 60, Rb 61 and Rb 63) to 4.8 (Rb 4) days. It was observed that with an increase in disease severity there was decrease in incubation period in all the isolates.

Significant difference in the radial growth among the isolates of *Rhizoctonia bataticola* ranging from 17.7mm to 80.0 mm at 72h after incubation. Isolate Rb 14, Rb 17, Rb 22, Rb 26, Rb 49 and Rb 54 showed significantly highest colony growth (80mm). The least colony diameter was observed in the isolate Rb 20 (17.7mm).

Black colour colony was observed in 26 isolates while black with grey aerial mycelium was recorded in 20 isolates. Dark brown and grey colour was observed in 11 each respectively.

Isolates assigned into three groups on the basis of colony texture into appressed, fluffy and velvety. Maximum of 30 isolates produced appressed colony while 27 isolates had fluffy texture. Only 11 isolates had produced velvety growth.

Maximum number of 42 isolates produced aerial mycelium and 26 isolates did not develop any aerial mycelium. No aerial mycelium was observed in most of the isolates that had appressed colony.

The hyphal cell size varied from 9.38x3.80µm (Rb10) to 14.88x7.50µm (Rb 63). There was no significant difference between isolates Rb 10, Rb 27, Rb 3, Rb 2, Rb 25 and Rb 24. Ratio between the length/width of hyphal cell varied from 1.35 (Rb 27) to 2.96 (Rb 65).

The length of sclerotia varied from 54.86x45.49µm (Rb 46) to 216.08x181.09µm (Rb 59). Ratio between length and width of sclerotia varied from 1.00 (Rb 5) to 1.64 (Rb 23).

Based on sclerotial texture, the isolates were grouped into two groups *viz.*, rough and smooth texture. Rough texture was observed in 25 isolates while smooth texture was observed in 43 isolates. The isolates were categorized into irregular, round and ovoid groups based on shape of sclerotia. Irregular shaped sclerotia were observed in 20 isolates while round shaped sclerotia were observed in 27 isolates and ovoid shape of sclerotia was observed in 27 isolates.

Isolates took 1.7 (Rb1) to 4.3 days (Rb4, Rb23 and Rb 28) for sclerotial initiation on PDA medium. In 13 isolates sclerotia had initiated forming by second day. Maximum of 32 isolates had initiated sclerotia production after 2 to 3days, while 11 isolates had initiated sclerotial formation after 3days. The number of sclerotia per microscopic field when observed through 10x objective varied from 11.67 (Rb 63) to 70.67 (Rb 56).

All the 10 RAPD primers generated 158 polymorphic bands. When fingerprints of these isolates were compared, some bands common to all isolates were observed while others were unique to one or a few isolates. All the RAPD bands produced by 10 primers in the 50 isolates of *R. bataticola* (50 isolates were selected based on the differences between isolates with respect to cultural and morphological variation) were subjected to hierarchical cluster analysis based on the principle of UPGMA and a dendrogram was generated. The similarity coefficient ranged from 0.63 to 0.92 indicating that no any two or more isolates were 100% similar. The highest similarity coefficient (0.92) was between isolates Rb 2 and Rb 4. All the isolates of Madhya Pradesh and Karnataka fell under same group IA. The Maharashtra isolates were distributed across all the three groups and were found in IA, IIA and III, while the Andhra Pradesh isolates were also fell under all the three groups, but were found in IA, IB, IIB and III. The Telangana isolates were found in IA, IB, IIA and IIB groups. The results of the present study also indicated that, all the isolates were not necessarily showing the geographical linearity.

The maximum radial growth was observed at 35ºC and 72h after incubation in all five isolates. After 96h of incubation, all the isolates had covered petriplate at 25ºC and 30ºC. The sclerotial initiation was started after 48 hours at 30ºC and 35ºC. The sclerotial

initiation started at 72h after incubation 25ºC and it was observed at 96h after incubation in 20ºC. In 15 ºC, it was observed that the growth was very slow and sclerotial initiation was observed after 144 hours after incubation.

The optimum growth of the fungal isolates was found at 35ºC (79.3, 90.0 and 90.0 mm at 48, 72 and 96 hours after incubation respectively). The next best temperature was 30ºC with radial growth of 71.3, 88.1 and 90.0 mm at 48, 72 and 96 hours after incubation respectively) followed by 25ºC, 20ºC and 15ºC. There was very meager growth at 40ºC (10.4, 15.0 and 17.5mm at 48, 72 and 96 hours after incubation respectively) and no growth was observed at 45ºC. Among means of the isolates, there was significant difference between the isolates at three time intervals except at 96 hours after incubation between Rb 2 and Rb 40.

The optimum temperature for dry root rot severity was at 35°C with maximum disease severity rating of 8.5 irrespective of the isolate. This was followed by 30°C with disease severity rating of 7.9 followed by 25°C with disease severity rating of 7.0. It was observed that 20°C and 15°C had helped in the development of the lesions but could not develop further. The disease severity was very low 1.4 rating at 15°C while it was 3.1 at 20°C.

Among Rb 2, Rb 13, Rb 22 and Rb 40 isolates, there was no significant difference while Rb 63 was virulent isolate compared to others. The plants had showed symptoms on the tap root leaving the lateral roots unaffected at 25°C. At 30, 35, 40, 45°C, there was complete blackening of the roots and the reisolation from the roots showed the presence of *Rhizoctonia batatiocla*. The uninoculated plants did not show any symptoms. The plants at 40 and 45°C showed physiological death by complete drying of plant parts both uninoculated and inoculated plants.

At 24h after incubation, the growth of *Rhizoctonia bataticola* on PDA amended with NaCl, KCl and dextrose incubated at five different temperatures showed maximum mean radial growth at 35ºC *viz.*, 17.1, 22.1 and 49.7mm followed by 30ºC of 13.6, 16.4 and 34.2mm respectively. The least growth was observed at 40ºC of 4.1 and 5.4mm whereas in dextrose amended PDA least growth was observed at 20ºC (11.4mm). Similar observations were also recorded at 48 and 72h after incubation except in dextrose where least growth was observed at 40ºC.

In black soil the disease severity rating was 9.0, 8.9, 8.1, 6.9, 5.3, 3.8 and 2.3 while in red soil, it was 9.0, 8.9, 8.7, 7.9, 6.5, 4.7 and 3.0 at 40, 50, 60, 70, 80, 90 and100% soil

moisture respectively. The disease severity decreased as the soil moisture increased in both the types of soil. There was significant difference between them.

Among the selected isolates, Rb 63 showed highest disease severity of 6.7 in black soil while 7.5 in red soil. This was followed by Rb2 of 6.5 and 7.2 in black and red soils respectively. The least disease severity was observed in Rb 40 in black soil and 6.2 in Rb 13. There was significant difference between the disease severities.

At 24h after incubation maximum mean radial growth was observed at pH 5.0 (67.3mm) followed by pH 6.0 (64.4) while the least was observed at pH 11.0 (14.6mm) followed by pH 10.0 (33.0mm). There was significant difference between the pH levels at 24h after incubation. Similar observations were also observed at 48h after incubation where there was significant difference between the pH levels except pH 4.0 (75.0mm) and pH 7.0 (74.6mm) which were at par with each other. At 72h after incubation, maximum radial growth of 90.0mm was observed at 5.0, 6.0 and 7.0 pH levels which were at par with other. In the remaining pH levels there was significant difference between them where the least growth was observed at pH 11 of 53.5mm.

Among selected *Rhizoctonia bataticola* isolates, maximum radial growth was observed in Rb 22 (48.3mm) followed by Rb 40 (46.0mm) while the least was observed in Rb 2 (38.8mm). The similar trend was followed at 48h and 72h after incubation. There was significant difference among the isolates at 24, 48 and 72h after incubation.

The susceptible genotype BG 212 was having less total sugars compared to moderately resistant genotypes *viz.*, ICCV 5530 and ICCV 8305. There was significant increase in the amount of the total sugars in all the genotypes grown in control soil with increase in age of the crop in both 60 and 100% soil moisture. There was an increase in the amount of the total sugars in sick soil with increase in age of the crop in both 60 and 100% soil moisture in sick soil and then there was decrease in the 45 DAS in inoculated soil. The amount of total sugar was more in the 100% soil moisture compared to 60% soil moisture in control soil. The total sugar was more in the healthy plants as compared to plants in sick soil. The amount of increase was more in the inoculated plants grown at the 100% as compared to the 60% soil moisture condition. The plants were more prone to the disease in the 60% inoculated condition and this suggests the plant was utilising more total sugars for the synthesis of several pathways to restrict the growth of pathogen.

There was an increase in the amount of reducing sugars in all the treatments with increase in age of the crop in both 60 and 100% soil moisture in control soil. There was an increase in the amount of the reducing sugars in BG 212 in both 60 and 100% soil moisture in sick soil up to 30 DAS and there was decrease by 45 DAS in sick soil. The amount of reducing sugar was more in 100% soil moisture compared to 60% soil moisture in control. The reducing sugar was more in the healthy plants as compared to plants in sick pots. The amount of increase was more in the sick plants grown at the 100% as compared to the 60% soil moisture condition. The susceptible genotype BG 212 was having less reducing sugars compared to moderately resistant genotypes (ICCV 5530 and ICCV 8305).

There was an increase in the amount of the non reducing sugars in all the treatments with increase in age of the crop in both 60 and 100% soil moisture in control soil. There was an increase in the amount of the non reducing sugars in BG 212 with increase in age of the crop in both 60 and 100% soil moisture in sick soil and there was decrease in the 45 DAS in sick soil at 60% soil moisture. The amount of non reducing sugar was more in the 100% soil moisture compared to 60% soil moisture in control. The non reducing sugar was more in the healthy plants as compared to plants in sick soil. The amount of increase was more in the sick soil grown at 100% as compared to 60% soil moisture condition. The susceptible genotype (BG 212) was having less non reducing sugar compared to moderately resistant genotype (ICCV 5530 and ICCV 8305).

In susceptible genotype BG 212, total phenol content was maximum at 30DAS (2.76 mg/100mg fresh weight of root tissue) in sick soil with 60% soil moisture while the least amount of 0.80 mg/100mg fresh weight of root tissue was observed in control soil at 100% soil moisture at 15 DAS. In moderately resistant genotype (ICCV 5530) highest amount of total phenol (5.92mg/100mg fresh weight of root tissue) was observed in sick soil at 60% soil moisture at 30 DAS while the least amount of 1.25 mg/100mg fresh weight of root tissue was noted in control soil at 100% soil moisture at 15 DAS. Moderately resistant genotype ICCV 8305 maximum amount of total phenol (4.19 mg/100mg fresh weight of root tissue) was observed in sick soil at 60% soil moisture at 30 DAS while the least amount of total phenol (0.95 mg/100mg fresh weight of root tissue) was recorded in the control soil with 100% soil moisture at 15 DAS.

There was an increase in the amount of PAL in all the treatments with increase in age of the crop in both 60 and 100% soil moisture in sick and control soil at 15 and 30 DAS and there was decrease at 45 DAS. The amount of PAL activity was more in 60% soil moisture compared to 100% soil moisture in sick and control soil. The PAL was more in the diseased plants as compared to healthy plants upto 30 DAS. The amount of increase was more in the inoculated plants grown at 60% compared to 100% soil moisture condition. The plants were more prone to the disease in the 60% inoculated condition and this suggests the plants had more PAL activity in them as a defence mechanism against pathogen. The susceptible genotype (BG 212) was having less PAL activity compared to moderately resistant genotype (ICCV 5530 and ICCV 8305). But, these moderately resistant genotypes resistance mechanism was broken as there was decrease in the amount of the PAL as compared to healthy plants in which there was gradual decrease.

In susceptible genotype (BG 212), polyphenol oxidase activity was maximum at 30 DAS (0.07 ΔAbs_{495} nm/ min/g fresh sample) in sick soil at 60 and 100% soil moisture. The critical difference between them was at par with each other. The least (0.01 ΔAbs_{495} nm/ min/g fresh sample) PPO activity was observed in sick soil at 60% soil moisture at 45 DAS and 100% soil moisture at 15 DAS. These were at par with each other. In moderately resistant genotype (ICCV 5530) highest (0.20 ΔAbs_{495} nm/ min/g fresh sample) PPO activity was observed in sick soil in 60% soil moisture at 30 DAS while the least (0.04 ΔAbs_{495} nm/ min/g fresh sample) activity of PPO was observed in control soil at 100% soil moisture at 15 DAS. In moderately resistant genotype (ICCV 8305) highest level (0.13 ΔAbs_{495} nm/ min/g fresh sample) of PPO was observed in sick soil with 60% soil moisture at 30DAS while the lowest activity of 0.03 ΔAbs_{495} nm/ min/g fresh sample was recorded in control and sick soil at the 60and 100% soil moisture at 45 and 15 DAS respectively.

In susceptible genotype (BG 212), POD activity was maximum at 30DAS in sick soil (1.34 ΔAbs_{420} nm/ min/g fresh sample) at 60% soil moisture while the least (0.17 ΔAbs_{420} nm/ min/g fresh sample) activity of POD was observed in control soil at 100% soil moisture at 15 DAS. In moderately resistant genotype ICCV 5530 highest (1.50 ΔAbs_{420} nm/ min/g fresh sample) activity of POD was observed in sick soil at 60 per cent soil moisture after 30 DAS. The least (0.61 ΔAbs_{420} nm/ min/g fresh sample) accumulation of POD was observed in control soil at 100% soil moisture after 15 DAS. In moderately resistant genotype (ICCV 8305) highest level (1.43 ΔAbs_{420} nm/ min/g fresh sample) of

POD was observed in sick soil at 60 per cent soil moisture after 30 DAS. The least activity of 0.20 ΔAbs_{420} nm/ min/g fresh sample was recorded in control soil at 100% soil moisture at 15 DAS.

Among the genotypes, BG 212 grown in 100% moisture in control soil had more weight of 0.80g and the least weight was noticed at 15DAS in sick soil at 60% soil moisture (0.15g). In ICCV 5530, highest root weight was recorded at 100% moisture in control soil of 1.27g at 45 DAS and the least weight was noticed in 60% control and sick soil which were at par each other i.e 0.22g at 15 DAS. In ICCV 8305, highest root weight was recorded in 100% moisture in control soil of 0.85g at 45 DAS while the least was noticed in 60% sick and control soils, which were at par each other i.e 0.24g at 15DAS.

From the results, it was observed there was no significant difference in the dry weight of the roots among same moisture conditions at different type of soil during 15 DAS. The dry weight of the genotypes was more in the 100% soil moisture as compared to 60% moisture in the 15 DAS. The dry weight of the roots at 45 DAS had significant differences. It was more in the 100% compared to the 60% control. With respect to the sick and control soil, it was observed that, severe disease in the sick soil made the disintegration of the roots and there by losing the weight of roots. It was observed more root weight in the 100% soil moisture compared to the 60% in sick soil.

Among the genotypes, BG 212 grown in 100% moisture in control soil had more weight of 0.61g and the least weight was noticed at 15DAS in sick and control soil at 60% soil moisture (0.12g) which was at par with each other. In ICCV 5530, highest root weight was recorded at 100% moisture in control soil of 0.89g at 45 DAS and the least weight was noticed in 60% control and sick soil which were at par each other i.e 0.20g at 15 DAS. In ICCV 8305, highest root weight was recorded in 100% moisture in control soil of 1.11g at 45 DAS while the least was noticed in 60% sick soil of 0.26g at 15DAS.

Among the genotypes, BG 212 grown in 100% moisture in control soil had more leaf weight of 0.58g and the least weight was noticed at 15DAS in sick and control soil at 60% soil moisture (0.14 and 0.15g) which were at par with each other. In ICCV 5530, highest leaf weight was recorded at 100% moisture in control soil of 0.86g at 45DAS and the least weight was noticed in 60% control and sick soil which were at par each other i.e 0.22g at 15 DAS. In ICCV 8305, highest leaf weight was recorded in 100% moisture in

control soil of 0.92g at 45 DAS while the least was noticed at 60% sick soil of 0.28g at 15DAS.

Among the genotypes, BG 212 grown in 100% moisture in control soil had more total plant dry weight of 1.99g and the least weight was noticed at 15DAS in sick soil at 60% soil moisture of 0.41g. In ICCV 5530, highest total plant dry weight was recorded at 100% moisture in control soil of 3.02g at 45DAS and the least weight was noticed in 60% sick soil of 0.63g at 15DAS. In ICCV 8305, highest total plant dry weight was recorded in 100% moisture in control soil of 2.87g at 45 DAS while the least was noticed at 60% sick soil of 0.78g at 15DAS.

Among the genotypes, BG 212 grown in 100% moisture in control soil had transpired more of 36.3g and the least transpiration loss of 13.3g was noticed in sick soil at 60% soil moisture at 45DAS. While in ICCV 5530 highest transpiration loss was recorded in 100% moisture in control soil with 39.3g after 45 DAS and the least transpiration loss was noticed in 60% sick soil of 11.0g at 15DAS. In ICCV 8305, highest transpiration was recorded in 100% moisture in control soil with 42.7g after 45 DAS and the least was noticed in 60% moisture in sick soil of 13.7g at 15 DAS.

Among the genotypes, BG 212 grown in 100% moisture in control had more stomatal conductance of 1.15mol m^{-2} s^{-1} at 15 DAS and the least was noticed at 45DAS in sick soil with 60% soil moisture of 0.35mol m^{-2} s^{-1}. While in ICCV 5530, highest stomatal conductance was recorded in 100% moisture in control soil with 0.94 mol m^{-2} s^{-1} at 15 DAS and the least stomatal conductance was noticed at 60% moisture in sick soil of 0.43 mol m^{-2} s^{-1} at 45DAS. In ICCV 8305 highest stomatal conductance was recorded in 100% moisture in control soil with 1.17mol m^{-2} s^{-1} at 15 DAS and the least was noticed in 60% moisture sick soil of 0.57 mol m^{-2} s^{-1} at 45 DAS.

Among the genotypes, BG 212 grown in 60% moisture in sick soil had more temperature 37.5ºC and the least temperature was noticed at 15DAS in control soil with 100% soil moisture of 28.1ºC. While in ICCV 5530, highest temperature was recorded in sick soil at 60% moisture with 36.5ºC after 45 DAS and the least temperature was noticed in control soil at 100% moisture i.e. 27.1ºC at 15DAS. In ICCV 8305, highest temperature was recorded in sick soil at 60 percent moisture with 37.6ºC at 45 DAS and the least was noticed in sick soil at 100% i.e. 28.0ºC at 15DAS.

Conclusions

- Dry root rot of chickpea distributed in all the locations of central and southern parts of India.
- Presence of variability among the isolates with respect to cultural, morphological, pathological and molecular characters was observed..
- Optimum temperature of 35˚C with 60% soil moisture favourable for the pathogen growth and disease development.
- It was observed that the increase in the osmotic potential and temperature had positive effect on the growth of the fungus up to -0.5MPa and then onwards, negative effect on the growth of the fungus in KCl and NaCl amended media while in dextrose amended media growth was observed up to -2.5MPa at 35˚C.
- At higher osmotic potentials (-2.0 to -2.5MPa at 40ºC), ex-osmosis of the fungus and death was observed in media amended with NaCl and KCl.
- The susceptible genotype had fewer amounts of total sugars, reducing and non reducing sugars compared to moderately resistant genotypes. The sugars were more in the healthy plants as compared to infected plants. In the inoculated soil, the amount of increase was more in 100% as compared to 60% soil moisture condition.
- Total phenol, phenylalanine ammonia lyase, polyphenol oxidase and peroxidase were maximum at 30DAS in inoculated soil with 60% soil moisture in all the three genotypes. The amount of increase was more in the inoculated plants grown at 60% compared to 100% soil moisture condition. The amount of phenol and enzymes were more in the moderately resistant genotypes compared to susceptible genotype.
- Dry weight of the genotypes was more in the 100% soil moisture as compared to 60% moisture. With respect to the inoculated and uninoculated soil, it was observed that, severe disease in the inoculated soil made the disintegration of the roots and there by losing the weight of plants in inoculated soil at 60% compared to control.
- Genotypes grown in 100% moisture in uninoculated soil had more transpiration and stomatal conductance while the least was noticed in inoculated soil at 60% soil moisture in contrast, leaf temperature was more in the genotypes grown in

inoculated soil at 60% moisture and the least was noticed in inoculated soil with 100% soil moisture.

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