STUDIES ON THE VARIABILITY OF *Rhizoctonia solani* KUHN. INFECTING RICE, COWPEA AND AMARANTH

HARITHA J. KUMAR

2018-11-145

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2020

STUDIES ON THE VARIABILITY OF *Rhizoctonia solani* KUHN. INFECTING RICE, COWPEA AND AMARANTH

By

HARITHA J. KUMAR

2018-11-145

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

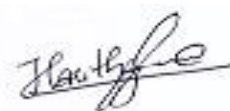**COLLEGE OF AGRICULTURE**

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"Studies on the variability of** *Rhizoctonia solani* **Kuhn. infecting rice, cowpea and amaranth."** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani
18-09-2020

Haritha J. Kumar
2018-11-145

CERTIFICATE

Certified that this thesis entitled **"Studies on the variability of *Rhizoctonia solani* Kuhn. infecting rice, cowpea and amaranth"** is a record of research work done independently by Ms. **Haritha J. Kumar** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani

Date: 18-09-20

Dr. Susha S. Thara

(MajorAdvisor, Advisory ommittee)

Assistant Professor (Plant Pathology)

College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. **Haritha J. Kumar**, a candidate for the degree of **Master of Science in Agriculture** with major in Plant Pathology, agree that the thesis entitled **"Studies on the variability of** *Rhizoctonia solani* **Kuhn. infecting rice, cowpea and amaranth."** may be submitted by Ms. **Haritha J. Kumar**, in partial fulfilment of the requirement for the degree.

Dr. Susha S. Thara
(Chairperson, Advisory Committee)
Assistant Professor
Dept. of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram -695522

Dr. Joy M
Associate Professor and Head
Dept. of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram -695522

Dr. Radhika N. S
Assistant Professor
Dept. of Plant Pathology
College of Agriculture, Padannakad
Kasaragod-671 314

Dr. Beena Thomas
Assistant Professor
Dept. of Plant Breeding & Genetics
College of Agriculture, Vellayani
Thiruvananthapuram- 695522

Acknowledgement

First and foremost, praises and thanks to the Almighty, for everything that happens to me…

With immense pleasure, I would like to express my sincere gratitude to **Dr. Susha S. Thara,** Assistant Professor, Department of Plant Pathology for the constructive guidance, constant inspiration, abundant encouragement, patient audience, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for providing a stress free situation by the open minded approach and for the care and affection bestowed on me throughout the study period.

I convey my heartfelt thanks to **Dr. Joy M,** Professor, Associate Professor and Head, Dept. of Plant Pathology, for his constructive criticism, inspiring professional guidance, valuable advices and whole hearted approach right from the beginning of the thesis work.

I gratefully acknowledge with thanks **Dr. Radhika N S,** Assistant Professor, Department of Plant Pathology, for the constructive comments, her generous support, creative suggestions and, affectionate approach at all the stages of research work.

I am extremely thankful to **Dr. Beena Thomas,** Assistant Professor , Plant Breeding & Genetics for the valuable support and suggestions.

I express my sincere thanks to **Dr. Umamahesshwaran K.,** Professor, Department of Plant Pathology for the valuable advices, ever- readiness, passionate approach and for the valuable support.

I am extremely thankful to **Dr. Aiysha R.** Assistant Professor, Department of Plant Pathology, for for her generous timely help, love and suggestions.

I owe my deepest gratitude to **Dr. Kiran A. G.** (Assistant professor in contract), Department of Plant Biotechnology for his valuable guidance and whole hearted approach towards my research work.

I am thankful to **Kerala Agricultural University** for the technical and financial assistance for carrying out my study and research work. I am so grateful to **Advanced Research Centre for Plant Disease Diagnosis (ARCPDD)** and **Department of Plant Pathology of College of Agriculture, Vellayani,** for the technical support provided for my successful completion of PG work.

I thankfully acknowledge **ICAR** for granting Junior Research Fellowship for my studies.

I express my sincere thanks to all the teachers of the department of Plant Pathology for their entire well wishes help and support.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Plate No.	Title	Page No.
1	Nature of symptoms of *R. solani* in rice, vegetable cowpea and amaranth collected from Thiruvananthapuram district	42-43
2	Nature of symptoms of *R. solani* in rice, vegetable cowpea and amaranth collected from Kollam district	42-43
3	Nature of symptoms of *R. solani* in rice,vegetable cowpea and amaranth collected from Pathanamthitta district	42-43
4	Isolates of *R. solani* from rice, vegetable cowpea and amaranth from different locations of Southern Kerala	44-45
5	Symptoms by artificial inoculation of *R. solani* isolates on 40 day old rice var. Uma at 5 days after inoculation	46-47
6	Symptoms on artificial inoculation of *R. solani* isolates on 25 day old vegetable cowpea var. Geethika at 7 days after inoculation	46-47
7	Symptoms on artificial inoculation of *R. solani* isolates on 30 day old amaranth var. Arun on 4 days after inoculation	46-47
8	Variation in colony colour, zonation, orientation of sclerotial formation of *R. solani* rice isolates on PDA at 7 days after inoculation (DAI)	48-49

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS USED

ºC	Degree Celcius
%	Per cent
&	And
μL	Microliter
μm	Micrometer
AG	Anastomosis Group
AUDPC	Area Under Disease Progress Curve
bp	Base pairs
cm	Centimetre
CRD	Completely Randomised Design
DAI	Days after inoculation
DAT	Days after transplanting
DI	Disease incidence
DNA	Deoxyribo nucleic acid
et al.	And coworkers
Fig.	Figure
g	Gram
h	Hour
L^{-1}	Per litre
KAU	Kerala Agricultural University
kb	Kilobase pair

kg	Kilogram
ml	Millilitre
min.	Minute
mm	Millimetre
mM	Millimolar
mg	Milligram
No.	Number
OD	Optical density
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PDI.	Percent disease incidence
RAPD	Random Amplified Polymorphic DNA
RLH	Relative Lesion Height
rpm	Rotations per minute
SCG	Self Compatibility Group
sec	Second
Sl.	Serial
sp	Species
TAE	Tris-acetate-EDTA
U	Unit
V	Volt
viz.	Namely

Introduction

1. INTRODUCTION

Rhizoctonia solani Kuhn (Teleomorph: *Thanatephorus cucumeris)* is a ubiquitous, diverse necrotrophic basidiomycete fungus having a wide host spectrum and cause significant damage to a great diversity of plants. The fungus is distributed throughout the world and can infect any plant part and produce different disease symptoms. This pathogen is generally regarded as an unspecialized pathogen which consists of indefinite races (Kotasthane *et al.,* 2004). It is the most widely recognized species of *Rhizoctonia* and was originally described by Julius Kuhn on potato in 1858. The pathogen does not produce any asexual spores and occasionally produces sexual spores (basidiospores). In nature, *R. solani* exists primarily as vegetative mycelium and sclerotia. It also survives in infected plant debris and other host plants.

R. solani is capable of causing sheath blight, web blight, damping-off, root rot, collar rot, stem canker, crown rot, bud and fruit rot and foliage blight on a variety of agriculturally important crops like rice, pulses and vegetables like amaranth. In Kerala rice, vegetable cowpea and amaranth possess very significant position. Rice (*Oryza sativa* L*.*) is the staple food of Kerala. It is a nutritious cereal crop and chief source of carbohydrate and protein. It also provides fibre and minerals. Vegetable cowpea (*Vigna ungiculata* subsp*. unguiculata* (L.) Verdcourt) is an important leguminous crop grown in Kerala. It is an excellent source of proteins, vitamins, minerals and dietary fibre. Amaranth (*Amaranthus tricolor* L) generally known as "poor man's spinach". It is considered as one of the cheapest and commercially cultivated short duration leafy vegetable in Kerala. It is excellent source micronutrients and fits well with the crop rotation practices of Kerala.

R. solani infection is a great threat to the successful cultivation of these crops. Humid climate and the heavy south-west monsoon of Kerala makes suitable for disease development (Gokulapalan *et al.,* 2000). Outbreak of these diseases is a recurrent problem in Kerala. Difficulty in management and absence of resistant cultivars lead to heavy yield loss of these crops. Control measures against the pathogen are complicated

and may be ineffective due to the wide host range and variability of the pathogen in different geographical locations.

Complex nature of *R. solani* and its adaptation to different ecological condition generates many recognizable strains. *R. solani* is varied in its pathogenicity, sclerotial morphology, cultural and microscopic characters. The pathogen is very diverse and possesses variation in many characters. Variability of the pathogen is the main reason for the difficulty in the control as the pathogenicity is complex and it contains many pathogenic races. There is a noticeable wide variation present in the pathogen, not only from different host but also from same hosts.

Many problems regarding the variability can be addressed through the molecular level studies. Molecular markers provide basis for studying the patterns, dispersal and colonization of pathogenic population. Use of molecular markers has given a boost to the analysis of accurate variation among different isolates of the pathogen. Evaluation of the genetic diversity in pathogen is considered as an initial step for understanding the population structure. With the use of various molecular technologies, genetic diversity analysis of population becomes feasible. Random amplified polymorphic DNA (RAPD) is used in numerous filamentous fungi in many fields of experimental mycology (Pollastro *et al.,* 2000).

RAPD analysis is a widely used technique because it is simple and used to differentiate strains present in the same species (Williams *et al.*1990). Through the variability studies, we were able to find out the population structure of the pathogen and able to provide suitable control measures of the pathogen especially for the development of location specific resistant cultivars. Nature and spread of the pathogen can be examined through the genetic variability studies. Management of the disease needs comprehensive knowledge about the races which is achieved through the variability studies of the pathogen. The variability among the population helps the pathogen to survive and adapt in various environment. Understanding the diversity is critical for

developing disease management practices and also for better understanding of the epidemiology.

To learn the present plant disease condition and to predict future management, it is essential to gain knowledge about the variation of the pathogen. The present study was envisaged with the objectives to study the cultural, morphological, pathogenic and molecular variability of *R. solani* affecting rice, cowpea and amaranth in Southern Kerala.

Review of Literature

2. REVIEW OF LITERATURE

Rhizoctonia solani is a ubiquitous necrotrophic soil borne pathogen producing different maladies *viz*., sheath blight, collar rot, root rot, wire stem, web blight and foliar blight in various crop plants (Lakshmanan *et al*., 1979; Kumari and Niza, 2005). It has very long survival period in the soil due to the production of sclerotial bodies and infection occurs through infection cushions or lobate appressoria on hosts (Basu *et al*., 2016; Ghosh *et al*., 2017).

2.1. SYMPTOMATOLOGY OF THE DISEASE

2.1.1 Collection of the Disease Sample

Akhtar *et al.* (2009) conducted survey to observe the occurrence and diversity of banded leaf and sheath blight incited by *R. solani* occurring in Jharkhand and reported the wide spread occurance of the disease. The disease severity was ranging from 30.30 to 80.46 per cent which shows economic importance of the disease. Ganeshmorthi and Dubey (2015) collected fifty isolates of *R. solani* causing wet root rot in chickpea and were categorized into three such as less virulent causing 20 per centisease incidence, moderately virulent causing 20 to 50 per cent and highly virulent causing 50 per cent and above based on the percent disease incidence.

Parashuram *et al*. (2017) conducted a purposive disease survey for studying the occurrence and spread of sheath blight disease in three different ecosystems of Chhattisgarh state during the vulnerable period from active tillering to panicle initiation stage. They have noticed that the disease incidence (DI) ranged from 20 to 90 per cent and classified into very high (>50%), high (31-50%), moderate (21-30%) and low (<20%). They have correlated the heavy incidence of the pathogen to the factors like high relative humidity, low temperature and water stagnation due to continuous rain.

2.1.2 Symptomatology of the Disease

R. solani causing sheath blight of rice was first reported in India by Paracer from Gurdaspur, Punjab and noted symptoms in early stage as circular or oblong lesions with

dark brown margins and mostly confined to the lower leaf sheaths at or near the water level. These characteristic symptoms can also be seen on the upper most leaf sheaths under constant humid weather conditions (Paracer and Chahal, 1963). Sclerotia present in the water float to the surface and get attached to the rice stems and infections occurs in the waterline. When the rice canopy is thick and the relative humidity is very high, it leads to the ideal microclimate for horizontal and vertical spread of the pathogen (Reissig *et al*., 1986).

Dasgupta (1992) narrated an account of the characteristics symptom of sheath blight as initial discoloured water soaked lesions of different shapes such as circular to oblong, ellipsoid to ovoid or irregularly elongated which later turns to discrete lesions on the sheath. These lesions are having pale greenish-grey to greenish white centre with narrow blakish to dark brown margin. These lesions coalesced leading to the girdling of leaf sheath, culm, boot and flag leaf thereby the tiller is succumbed to death.

Nayak and Sridhar (1979) observed the typical sheath blight symptom on the weed *Cynodon dactylon*. This weed served as the alternate host to rice plant and led to the perpetuation and spread of the pathogen. Environmental condition influences the dimensions and colour of the spots. Humid climate and the heavy south-west monsoon of Kerala favours disease development (Gokulapalan *et al.,* 2000).

Viswanathan and Viswambharan (1979) recorded the collar rot and web blight symptoms on cowpea. First visible symptoms were noted as water soaked lesions in the leaves which are followed by the rotting of collar region. When the disease advances, white cottony mycelial mat along with numerous creamy white sclerotia could be visible on the affected region. Finally yellowing of leaves followed by drying of the whole plant occurs. Shailbala and Tripathi (2007) gave an account of the web blight symptoms as it was most severe on the foliage, but the pathogen could also affect other parts like roots, stem, petioles and pods. Nature of the pathogen can be soil borne, air borne or seed borne. Initially small circular brown coloured spots appears and it advances later.

Concentric bands can also be seen surrounding the water soaked areas. White mycelial growth may be visible on the undersurface of leaves.

.   According to Nayar *et al*. (1996), symptoms of amaranth leaf blight appeared as light creamish specks which rapidly coalesce causing severe damage on the foliage. It was noted severely in post monsoon period of August – September and all stages of amaranth plant were found vulnerable to the pathogen. Gokulapalan *et al*. (2000) has described the infection of the pathogen on amaranth as the development of tiny asymmetric whitish cream spots, which enlarged to cause extensive translucent and light green lesions which finally lead to shot holes in the foliage.

2.2. ISOLATION, PURIFICATION OF PATHOGEN, PATHOGENICITY TESTING, AND VIRULENCE RATING

2.2.1. Isolation and Purification of the Pathogen

Mosaddeque *et al.* (2008) collected the infected sheaths of the rice plants and the samples were excised into small bits and then surface sterilized with $HgCl_2$ (0.1%) by placing in the solution for 30 sec for surface sterilization. Then the bits were transferred to sterile water and washed 3 times in it and dried properly. Then the bits were placed on the sterilized petriplate with solidified PDA and incubated for growing the pathogen. The mycelial tips with the typical taxonomical characters of *R. solani* which was growing out of the tissue were cut and placed in fresh PDA plates. Purity of the cultures was maintained by hyphal tip culture technique.

2.2.2. Pathogenicity Testing and Virulence Rating

Bhaktavalsalam *et al*. (1978) was successful in colonizing *R. solani* on typha (*Typha latifolia* L.) pieces. Using this typha leaf bit method, Surulirajan (2003) tested pathogenicity on Pusa Basmati-l with the isolates collected from different climatic zones. Among the nine isolates collected, Punjab isolate lIee -4500 was highly virulent showing 56.0 per cent disease incidence followed by ITee 4498 (Madras) showing 50.2 per cent disease incidence.

Using detached leaf technique, Neeraja *et al.* (2002) classified the 18 isolates of *R. solani* after analyzing the virulence spectrum on susceptible IR50 and tolerant Swarnadhan varieties as highly virulent, moderately virulent and avirulent. Using detached leaf technique, Takegami *et al*. (2004) developed a protocol for inoculating web blight symptom in bean plants. Swain *et al*. (2005) analyzed five isolates from different agro climatic regions and found that Bhubaneswar isolate *R. solani* (S1) was the most virulent. It produced bigger sized lesion length, higher number of sclerotia and larger sclerotia. It was found that isolate S5 and S2 were relatively less virulent.

Guleria *et al*. (2007) performed the detached leaf inoculation technique for inoculating on rice. Leaves from the second position from culm cv. PR116 was selected, and inoculated with one week old *R. solani* cultures. Moisture blotter sheet in a plastic tray was provided as the moisture chamber. Ends of the rice leaves were cut and were placed in the tray supported by clean sterile glass slides. The trays with leaves were then incubated in growth chamber. Majority of the isolates produced lesion length between 45.6 and 58.2 mm on detached rice leaves. According to Lore *et al*. (2007), maximum disease severity and incidence could occur at the booting stage and was the most susceptible stage following tillering stage both under pot and field conditions. Adipathi *et al.* (2013) observed typical rice sheath blight symptoms on artificial inoculation**.**

Singh and Singh (2012) analyzed the vertical and horizontal spread of sheath blight in six rice cultivars viz. Swarna, Pusa Basmati 1, Pusa 1121, Tetep, Vikramarya and Pankaj in an open field pot experiments with isolates RS1, RS2 and RS3. Swarna was the most susceptible and Tetep was found to be the most resistant. In this experiment the most virulent isolate was RS1 whereas isolate RS3 was found to be the least virulent. Isolate RS1 also produced maximum horizontal spread. Moni *et al.* (2016) also conducted artificial inoculation with five days old mycelial block and noted the symptom development on field conditions and the rice cultivar Purbachi produced the typical sheath bligh symptoms.

Gireesh (2016) mildly injured the leaves of thirty days old amaranthus seedlings by giving pin pricks on the upper surface for proving the pathogenicity. Mycelial bits of seven days old fungal culture were used for inoculating the foliage under proper humid condition. They found that RS1 was more virulent among the cultures.

Under glasshouse condition, Singh (2016) condcuted pathogenecity test on rice plants at maximum tillering stage using stem bit method. The symptoms on plants were showing the original field symptom and the pathogen was reisolated.

2.3 STUDYING THE CULTURAL VARIABILITY OF THE ISOLATES

Palo (1926) gave an account that brown pigment was the stable diagnostic character for *R. solani* and the pigmentation can be various shades of brown. Young colonies appeared nearly white while the older colonies appeared in different shades of brown. Maier and Stafferdt (1960) found that on carrot agar *R. solani* will appear as submerged growth but in potato dextrose agar medium both submerged and aerial growth can be found. Later, Chand and Logan (1983) noted some variation in the zonation and mycelial colour. Mycelial colour was showing variation from pale to light brown. In 1984, Dath investigated influence of cultural factors on the aggressiveness of *R. solani* rice isolates. The aggressiveness of the pathogen was higher in Elliott's, potato dextrose and onion agar but Sabouraud's and Oat meal agar was showing decrease in the aggressiveness. The pH of the medium and incubation temperature never influences the aggressive nature of the isolates.

There was significant variation observed in the cultural characteristics. Colonies were showing white colour when young and it later turned to buff or brown colour with effused growth and the culture was growing at a rate of 45 mm per day (Ahuja and Payak, 1985). Whereas Basu and Gupta in 1992 noted little variation in mycelial characters like texture, colour, serial growth of isolates. Later, Basu *et al.* (2004) observed aerial growth of *R. solani* that was luxuriant to abundant and colony colour was creamy to light brown.

Tiwari and Khare (1998) studied variability among 108 isolates of *R. solani* from diseased mung bean collected from different locations in Madhya Pradesh and noted variation in their rate of growth, type of growth, colony colour and time for sclerotial initiation. Meena *et al.* (2001) in their study noted the variability of mycelial characters. Madurai *R. solani* isolate was found to be growing faster and produced dark brown mycelium in the culture. Sunder *et al.* (2003) also reported that the colony colour was showing variation and it was ranging from brown, light brown to yellowish brown.

Mughal *et al.* (2017) observed colony colour variation of *R. solani* from greyish brown to dark brown. Guleria *et al.* (2007) in their study noted that most of the *R. solani* isolates showed the light yellowish brown color and were fast growing with raised and fluffy colonies except four which were exhibited moderate growth rate. Lal and Kandhari (2009) studied the cultural variations in terms of colony colour, growth pattern and initiation of sclerotia. Based on the colony pigmentation, the cultures were classified into culture showing light brown, yellowish brown, whitish brown, dark brown and very pale brown. Based on growth pattern, the isolates were categorized into abundant, moderate and slight. The isolates were showing fast, medium and slow growth. Fast and medium growing isolates were found to be more pathogenic. Time taken for initiation of sclerotia formation was ranging from three to five DAI.

Studies conducted by Khodayari *et al.* (2009) revealed that there can be two distinct groups in *R. solani* isolates ie, those with low growth rate and high growth rate. Goswami *et al.* (2010) indicated that the diversity among the *R. solani* isolates was not correlated with their origin. Similar results were shown by Kuiry *et al.* in 2013. They classified the cultures into positive, negative with respect to pigmentation and sclerotial secretion. Sclerotial initiations in the cultures were seen as very fast, fast, moderate and slow. Susheela and Reddy (2013) after studying *R. solani* isolates from rice and weeds proved that there are no consistent characters related to virulence and geographic origin.

Lal *et al.* (2014) observed the growth pattern of the isolates as abundant, moderate and scarce. They have divided the cultures into slow growing (40-59 mm), medium

growing (60-65 mm) and fast growing (>65 mm). Mishra *et al.* (2014) collected isolates of *R. solani* from different hosts viz, rice, maize and green gram and explored the cultural variability. Colony appearance was showing sparse, sparse fluffy, cottony and cottony fluffy; and colony pigmentation was light brown and brown. Radial growth was also showing variation such as fast (48h), medium (72h), slow growing (96h or more). The time required for sclerotial initiation ranged from 3 to 12 days. Manjunatha (2016) studied the cultural variability of 20 *R. solani* isolates and based on reverse surface colony colur, they classified the isolates into ivory, sand yellow and olive green.

Mughal *et al*. (2017) collected 27 isolates of *R. solani* from different locations of Kashmir valley.The isolates were belonging to five cultural groups designated as A, B, C, D and E on the basis of colour of mycelium and growth rate. There was no correlation found between morphological groups and anastomosis grouping of isolates. The colony colour was showing variation from grey, light brown to dark brown. Gopireddy *et al.* (2017) also noted significant variation among colony colour, abundance of mycelium, zonation and colony diameter. Based on the abundance of mycelium, they classified cultures into slight, moderate, abundant and cultures having no aerial mycelium. The cultures were also classified into five colour groups based on dominant spectral colour of Munsell colour chart as white, yellowish white, pale brown, brown and dark brown.

Desvani *et al.* (2018) explored the cultural variability of isolates from Central Java and observed colour of the colonies was changing from white to brown and produced micro and macro sclerotia within 3 to 5 days. Growth was rapid in majority of the isolates obtained. They proved that the virulence was not showing any relation with morphophology of the cultures. Divya *et al.* (2018) studied the cultural variability between the isolates obtained from different host viz. rice, soybean, moongbean, groundnut and maize. The rice isolates were showing submerged to subdued type growth whereas the isolates from other hosts produced strandy and subdued growth. There was variation in the shape and size. Rice and maize isolates produced pale yellow brown culture whereas it was grey for the rest. The radial growth was found significantly high

in rice isolates whereas maize isolates were having least radial growth. All these observed variations were having no correlation with their area of occurance.

Variability of 24 isolates of *R. solani* collected from different locations of Prayagraj (U.P.) was studied by Yaduman *et al.* in 2019. Growth of *R. solani* was observed in different colours like, light yellow, whitish yellow, pale yellow, light cream yellow, yellow and cream yellow.

2.4 STUDYING THE MORPHOLOGICAL VARIABILITY OF THE ISOLATES.

2.4.1 Microscopic Variability

Duggar (1915) reported that septae in young hyphae of *R. solani* were occurring at intervals of 100– 200 μm, whereas Bourn and Jenkins in1928 reported that septa was occurring at 50–225 μm interval**.** Peltier (1916) reported that hyphal width and different media are not related to each other.

Width of hyphae of the pathogen varied from 5 to 14 μm (Thomas, 1925; Richter and Schneider, 1953). Wei (1934) reported that hyphal width of *R. solani* was 4 – 6 μm. Vijayan and Nair in 1985 observed that width of the hyphae was ranging from 4.75 to 13.5 μm. Later, Thind and Aggarwal (2008) noted that the hyphal width was ranging from 7.2 to 12.1 μm.

Hyphae in the culture were modified to form moniliod cells. These cells were described as hyaline or brown coloured; barrel, pyriform, irregular or lobate shaped; numerous or rare in nature; loosely grouped or tightly clumped cells (Duggar, 1915; Dodge and Stevens, 1924; Palo, 1926)**.** Hyphae generally get swollen to give rise to branched moniloid cells which lead to the formation of sclerotia (Ou, 1985).

Parmeter (1970) gave an account that presence of constriction on branch at the point of origin and formation of a septum in the branch near its point of origin are the reliable taxonomic characteristics of *R. solani.* According to Ahuja and Payak (1985) *R. solani* can be morphologically characterized by hyphae of irregular to slender with diameter of 3-11 μm, septate, right angle branching near the distal septum, constriction

and formation of a septum near the point of origin, multinucleate cells with a prominent septal pore apparatus, nuclei numbering 3-16 normally 4 to 8, presence of monilioid cells and absence of clamp connections. Sneh *et al*. (1991) reported that right angle branching of hypha, constriction at the point of branching of the mycelium and also septum near the point of branching serve as important taxonomical character.

Chand and Logan (1983) noted that there was wide variation in hyphal diameter and based on this along with growth rate, they could separate AG-3 from AG-z type 1 isolates in Northern Ireland.

According to Yang *et al*. (1989), the inconsistency of macroscopic character of *R. solani* was due to the nature of the cell nucleus. The cell of *R. solani* can be uninucleate, binucleate and multinucleate. Elewa *et al*. (2000) collected isolates which were multinucleate in nature and also branched near the distal septum and septa were seen at the point of origin of branches. Taheri *et al.* (2007) observed barrel shaped moniliod cells in culture.

Zhou (2016) proved that *R. solani* of multinucleate nature can generate heterogeneous characteristics. Manjunatha (2016) explored morphological variation among *R. solani* isolates in Karnataka. There was wide variability in angle of branching, mycelial width and distance between two septation. The isolate RS-K-17 was showing highest degree of angle of branching (96.150) followed by RS-K-12 (93.840), RS-K-1 (93.740) and RS-K-9 (93.490) resepectively.

2.4.2 Sclerotial Variability

Sclerotia were initially grayish white later turns to brown to black, shape can be subglobose to slightly flattened and size vary from 0.5 to 5.0 mm (Matsumoto and Yamoto, 1935; Palo, 1926). In addition, sclerotia may be absent in some isolates (Meyer, 1965). Gangopadhyay and Chakrabarti (1982) reported that the sclerotia of *R. solani* isolates consisted of compact mass of cells and were initially greyish- white, later to brown or greyish-black on maturity.

Hyakumachi *et al.* (1988) described *R. solani* as a complex pathogen and it was showing great variation in terms of its sclerotial number and pathogenicity. Basu and Gupta (1992) observed significant variation in the production, distribution and size of sclerotia. Thakur *et al.* (1992) obtained 19 isolates of *R. solani* pathogen from different hosts and divided the isolates based on the characters of sclerotia. In this, 11 isolates were with microsclerotia (sclerotia < 1 mm dia), 3 isolates with macrosclerotia (sclerotia >1 mm) and remaining four isolates did not produce sclerotia.

Meena *et al.* (2001) noted a great variation in terms of sclerotial characters. Madurai isolate produced dark brown larger sclerotia where as Trichy isolate produced light brown small sclerotia. The number of sclerotia was higher for the Madurai isolate. Mishra *et al.* (2014) studied the morphological variability on the basis of various sclerotial characters of the isolates of *R. solani* cultured from rice, maize and green gram.

Sharma *et al.* (2005) collected 24 isolates of *R. solani* from soil, root and collar rot or foliage blight infected plants from several locations of North India. Seventeen isolates produced few to abundant, white to dark brown or black, small to larger sclerotia formed generally in the middle of the colony. Isolate 7 was having maximum sclerotial diameter (0.25–2 mm).

Mughal *et al.* (2017) reported that *R.solani* isolates differed in pattern of sclerotial formation, size, colour and orientation. Size of sclerotia was ranging from 2 – 2.6 mm. Some isolates formed sclerotia at the centre and some produced towards periphery. Srinivas *et al.* (2002) collected ten *R. solani* f.sp. *sasakii* isolates from major maize growing areas. The isolates were divided into two groups based on the pattern of sclerotial production - one bearing peripheral sclerotia and other bearing scattered sclerotia in the culture. The cultures were showing sclerotial aggregation. *R.solani* isolate from Pantnagar produced sclerotia with higher weight (1.71 mg) than others. Texture of sclerotia varied from compact to loose with pits. Sharma *et al.* (2007) observed a great variability in terms of the number, size, weight, colour and pattern of sclerotia production

in an experiment involving 63 isolates collected from different maize agro-ecosystem of India.

Guleria *et al.* (2007) noted sclerotial variation in the *R. solani* isolates collected from rice. Sclerotial number per 5.0 mm culture block ranged between 2.1 and 11.2 and size of sclerotia was showing a variation from 1.31 to 2.08 mm. Most of the isolates produced dark brown sclerotia which was scattered in the colony. Kumar *et al.* (2008) studied sclerotial characters and observed variation in distribution pattern, colour, size and weight of sclerotia. Most of the isolates produced macro sized sclerotia distributed throughout the medium and average weight ranging from 0.04 –0.82 mg. Few isolates showed off white mycelium with sclerotia and were oriented near the inoculation point.

Lal and Kandhari (2009) studied the sclerotial variability in 25 *R. solani* isolates causing sheath blight of rice collected from different rice growing areas and observed that and sclerotia formation (central, peripheral or scattered), location (aerial or surface) and texture (smooth or rough) were showing significant variation. Based on the colour of the sclerotia, they divided the isolates into dark brown, dark yellowish brown, olive brown and light brown. Sclerotium was absent in the culture of Delhi isolate RS-22. The texture of sclerotia, was found to be smooth and rough and diameter of sclerotia was varying from 1.13- 2.03 mm. Jayaprakashvel and Mathivanan (2012) studied sclerotial variations of *R.solani* and found that colour, size, shape and distribution pattern of sclerotia varied among the isolates.

Adhipati *et al.* (2013) noted that some of the isolates formed dark brown macrosclerotia and were showing variation in its size and weight. They observed that majority surface sclerotia forming isolates were fast growers and embedded sclerotia forming cultures were slow growers. Size and weight of the sclerotia were noticeable as low (<0.35 mg / sclerotia), medium (0.35-0.70mg / sclerotia) and high (>0.70 mg / sclerotia). Kuiry *et al.* (2013) collected 67 isolates of *R. solani* from different hosts like rice, maize, sugarcane, weeds, cabbage, pointed gourd, water melon, potato and bean from different agro-ecological region of West Bengal. The cultures were showing

sclerotial secretion as positive and negative. Sclerotial initiations were noted as very fast, moderate and slow. The orientation of sclerotia were in the form of central, peripheral, peripheral and central, scattered whereas there were some isolates without producing any sclerotia in the culture. Sclerotial diameter was ranging between 995.32 x 1027.05 and 2720.07 x 2271.20 μm. Based on the position of the sclerotia, there can be aerial, surface and both are formed in the culture. Based on the texture of sclerotia, there can be smooth, smooth pitted, rough, rough pitted sclerotia. On the basis of the number of sclerotia, isolates were divided into no sclerotia, low, moderate, high and very high.

According to Susheela and Reddy (2013) *R. solani* isolates were highly variable in terms of sclerotial parameters without showing relationship with virulence, production system or geographic origin. Based on number of the sclerotia, Upadhyay *et al.* (2013) in their study classified the isolates into higher (>400), medium (>200 to 400) and lower (≤200) sclerotia formers.

Mishra *et al.* (2014) collected 24 isolates of *R. solani* from rice, maize and green gram and explored the variability in sclerotia. Sclerotia were expressing different shades of colours like light brown, brown, dark brown and black and were orientented as central, sub-central, peripheral, scattered and irregular. Sclerotia produced were ranging from 40 to 60 number and all isolates produced sclerotia in the culture within 3 to 10 days.They noted that rice isolate RRS1 produced sclerotia having highest weight (72 mg).

Ganeshamoorthi and Dubey (2015) collected 50 *R. solani* isolates causing wet root rot of chickpea from 10 different states of India and observed the variability in the isolates. Sclerotia formed in different isolates were light to dark brown and formed in variable patterns. The sclerotia were also varied in size also in number. The isolates were scattered mostly in the petri plates. Based on the sclerotial size they have divided isolates into six groups. There was a visible variation in the sclerotia as it was ranging from 0.4 to 2.6 mm.

Singh *et al.* (2015) analysed the cultural variability among 25 isolates of *R.solani* causing sheath blight in rice from Kerala, New Delhi, Punjab, Uttarakhand and Uttar

Pradesh of India and found that there was a great variability in *R. solani* population according to number, size, texture, colour, time taken for sclerotia formation, location and orientation of sclerotia in the culture. They have noted sclerotia were produced in the culture as central, peripheral and scattered; and found in different locations like in aerial, surface and embedded. They have categorized the isolates into poor (no sclerotia), fair (1-10), moderate (11-20), good (21-40), very good (41-60) and excellent which produced more than 60 sclerotia.

Moni *et al*. (2016) studied the sclerotial characters of *R. solani* isolates of rice plants. The number of sclerotia was showing variation from few to abundant. They have also observed other variation in the sclerotial characteristics like pattern of production, size and arrangement of sclerotia in the culture. Most of isolates produced flattened bottom with round to oval shaped sclerotia. Sclerotia were expressing brown to dark brown colour in almost every isolates. They have noted the presence of exuded droplet on the surface of sclerotia. Isolates produced sclerotia in different manner in the culture.

.   Gopireddy *et al*. (2017) observed a significant sclerotial variation in colour, location, pattern and number. They noted aerial and embedded type sclerotia. Sclerotia can be large or small with rough and smooth border. Sclerotia were light brown, brown, dark brown and deep dark brown. The pattern of the sclerotial arrangement can be more or less circular manner, concentrated towards periphery; irregularly scattered but more towards the centre of the colony; irregular very sparsely scattered and scattered irregularly all over the colony surface. RS 58 and RS 59 didn't produce sclerotia. Based on the clump formation, isolates were divided into more, moderate and less clump formers. They recorded the number of sclerotia as good, very good and excellent and texture can be smooth or rough.

Desvani *et al.* (2018) obtained 57 isolates from rice fields of Central Java and analysed the macroscopic characters. They noted variation in sclerotial color, size and distribution patterns. The sclerotia colour was diverse as white, pale brown and dark brown. The distribution pattern of sclerotia was showing large concentric rings, rare

concentric rings, concentrated at the edges and lots, concentrated at the edges and rare, wide sparsely, spread very rare, circular concentrated circle and circular concentrated sparse. Macroscopic characters were not affected by geographical origin and virulence.

Divya *et al.* (2018) studied the sclerotial morphology of five field isolates *R. solani* which were isolated from different host viz. rice, soybean, moongbean groundnut and maize. They observed various shades of dark brown sclerotia with varied shape and size. Rice isolates produced abundant number of sclerotia whereas soyabean isolates produced fewer sclerotia.

Sclerotia variability was studied by Yaduman *et al*. in 2019. Sclerotia were visible as thin and colour of the sclerotia was brown, dark brown as well as blackish brown. Different distribution patterns were also varied as central, peripheral and scattered. Number of sclerotia ranged between 53.67 - 64.00 and sclerotial weight ranged between 0.116 - 0.373 g.

2.4.3 Macroscopic Vegetative Compatibility between Isolates

Syminis *et al*. (1987) grouped macroscopic vegetative reactions observed between field isolates of *R. solani* representing different AGs into four types of reactions including as merging, merging line, barrage and barrage line. According to Yang *et al*. (1993), merging can be indicated as two colonies will merge each other without forming a distinguishable line of contact and merging line can be as a line of distinct demarcation between the colonies but there is no aerial hyphae present. Barrage reaction can be described as two colonies failed to mingle and there is a zone in which presence of mycelium is absent. Later, Yang *et al*. (1993) also defined tuft as a line of dense aerial mycelium. da Cunha *et al*. (2018) studied the vegetative compatibility reaction between combinations of *R. solani*. Most of the combinations showed fused reaction. There was no tuft reaction in the combinations.

2.4.4 Microscopic Hyphal Anastomosis Reactions between Isolates

According to Carling *et al. (*2002) *Rhizoctonia* spp. can be grouped to 14 anastomosis groups. AG serves as a genetic character and the exchange of nuclei resulting in the combining of different genotypes (Kataria *et al.,* 1991; Burpee and Martin, 1992).

Based on anastomosis (hyphal fusion), Richter and Schneider (1953) made the the first systematic grouping of *R. solani*. Parmeter *et al*. (1969) in their study grouped isolates of *R. solani* into four namely AG1, AG2, AG3 and AG4. Anderson (1982); Yokoyama and Ogoshi (1984) paired *R. solani* isolates in 2% water agar in 2-3 cm apart in a petridish. Fusion of the isolate indicated that they belong to same AGs. If fusion attraction or hyphal death does not occur, it was said that these isolates belongs to different AGs. They also tried to find out the existence of additional AGs and bridging isolates.

Vijayan and Nair (1985) classified 41 isolates of *R. solani* from Kerala into four morphological groups such as MG1, MG2, MG3 and MG4. They proved that MG1 was corresponding with AG1 of United States. It was found that all isolates of MG1 could able to infect rice and was producing typical sheath blight symptom. Isolates coming under MG2, MG3 and MG4 were asymptomatic on rice.

Based on pathogenicity and culture morphology, Ogoshi in 1987 subdivided AG-1 isolates into subgroups such as IA producing rice-sheath blight, IB causing web blight and IC which was identified in Japan on sugar beets and buckwheat. Carling *et al*. (1988) described different types of microscopic reactions as C0, C1, C2 and C3. In C0 microscopic hyphal reaction, there was no vegetative interaction and the intermingling hyphae of the two isolates neither recognise nor react to each other. In the category 1 (C1), there was a vegetative interaction which leads to hyphal contact and apparent connection of walls was seen without any membrane contact. In the case of category 2 (C2), there was a noticeable vegetative interaction which included wall connection and development of a pore plus death of anastomosing and adjacent cells. In category 3 (C3)

there was fusion of walls and membrane of the confronted pair in the combination. Jones and Belmar (1989) recovered AG-1 IB (web blight form) of *R. solani* from diseased foliage of soybean

Yang *et al*. (1991) described PDA coated slide method for performing anastomosis. Sterile microscope slides were thinly coated with 1.5 per cent water agar (WA). Isolates of both tester and sample placed on it at a distance separated by 2 cm. Slides were kept in petri dishes which contained 1.5 per cent WA to maintain humidity and incubated. The area where the mycelia from the tester and the sample isolates merged was stained with safranin O and 3 per cent KOH solutions and examined for the reactions.

Carling (1996) proved that some isolates of *R. solani* from different AG generally do not anastomose with each other. Banniza *et al.* (1996) supported the hypothesis, AG-1 is not a homogenous group based on the anastomosis behaviour. Mccabe *et al.* (1999) observed that hyphal anastomosis occur between tip cells from side branches and never between main runner hyphae. Singh *et al.* (1999) proved that there was no significant relationship exists between morphological characteristics with its anastomosis reactions. Isolates belonging to other anastomosis groups were found to be either non pathogenic or induced resistant type of hypersensitive reaction. Singh *et al*. (2002) characterized 46 isolates collected from Dehradun and Nagina in Uttar Predesh. All the isolates were coming under AG-1IA though they showed incompatible or compatible fusion anastomosis reactions with the tester belonging to AG-1IA. No such relationship exists between morphological characteristics of *R. solani* isolate with its same anastomosis behaviour (Singh *et al.* 1999).

Basu *et al.* (2004) paired the *R. solani* isolates in different combinations and noted different reaction types of hyphal contacts. Various reaction types observed was perfect fusion of hyphal cells, cell wall fusion followed by death of adjacent cells and the attachment of pairing hyphae with no fusion. There was no anastomosis detected in some combinations.

Moni *et al*. (2016) revealed that there was occurence of anastomosis reactions between all the isolates and the reference isolate AG1 IA as well as abundant self anastomosis reactions. During anastomosis reactions, there was an attraction between hyphal tips of the reference and test isolates before the actual contact. After the contact, 1–3 cells of the tips of reference and test isolates were then granulated, which can be called as killing reaction.

2.5. STUDYING THE PATHOGENIC VARIABILITY OF THE ISOLATES

Tu (1967) gave an account based on the correlation between the aerial mycelial growth of *R. solani* and their virulence pattern. They showed that strain which was rapid growing and with less aerial mycelium was more virulent. Dath (1984) proved that highly virulent isolate of *R. solani* produced more symptoms than less virulent one. Carling *et al.* (1987) reported that the isolates of AG-9 were found avirulent on cereal crops, forages, field pea and tomato, but highly virulent on cauliflower and moderately virulent on flax. Shahjahan *et al*. (1987) proved that growth rate and virulence were related with each other. According to Basu and Gupta (1992), there exists a positive correlation between the size of sclerotia and pathogenicity of the isolates. Isolates with large sclerotia were more aggressive than those isolates with smaller sclerotia or without sclerotia. Leach and Clapham (1992) reported that there existed a difference in virulence pattern among isolates of AG-5 on sugar beat and white lupine. Monga and Raj (1994) confirmed the existance of variation among the isolates of *R. solani* on the basis of host range and pathogenic variability.

According to Banniza *et al*. (1996) the diversity of *R. solani* isolates could be assessed using pathogenicity testing and virulence diversity. Acharya and Sengupta (1998) studied on collateral hosts of *R. solani* and reported that the weeds viz., *Cyperus rotundus, Cyperus difformis, Cynodon dactylon, Echinochloa colona, Setaria glauca, Panicum repens, Brachiaria* sp*., Commelina oblique* and *Amaranthus viridis* –produced symptoms similar to sheath blight and *R. solani* perennates in these plants in the absence of rice plants.

Neeraja *et al*. (2002) analyzed the virulence spectrum of *R. solani* isolates collected from different rice growing regions of India. Virulence analysis was examined on susceptible variety IR50 and tolerant Swarnadhan varieties. Based on the isolate reactions towards the pathogen, isolates were grouped as highly virulent, moderately virulent and avirulent cultures. Singh and Singh (2003) studied the population dynamics of *R. solani* and found that all the 46 isolates exhibited varying degree of virulence on cultivar Pusa Basmati 1 and based on disease severity (lesion length) caused by these isolates, isolates were divided into highly virulent, moderately virulent, less virulent and avirulent.

Swain *et al.* (2005) in their study found that isolate Bhubaneswar *R. solani* (S1) was more virulent as it produced bigger lesions and more number of sclerotia. Isolate S5 and S2 were comparatively less virulent in nature. Reddy and Susheela (2005) studied 35 isolates of *R. solani* collected from different agroecological regions. Based on the virulence spectrum, isolates were classified into highly virulent (70-100%), virulent (50-60%), moderately virulent (30-49%) and least virulent (10-29%). Xiao *et al*. (2008) collected 55 *R. solani* samples in rice from five distinctive ecological regions. They found that the pathogenicity among the strains was significantly different. Kumar *et al*. (2008) analyzed virulence diversity of *R. solani* isolates under glasshouse conditions. Most of the isolates were found to be moderately virulent, some were highly virulent and a few were less virulent in nature.

Goswami *et al.* (2010) analysed the pathogenicity and host range of five selected isolates of different cluster groups. They found that the isolate JES-16 as avirulent, SYL-30 was low virulent with narrow host range, DIN-8 and GAZ-18 were virulent with wide host range and GAZ-9 as highly virulent isolate with wide host range. Susheela and Reddy (2013) evaluated the pathogenicity of *R. solani* in the susceptible cultivar of rice (IR 50) on its maximum tillering stage. Based on virulence, they classified isolates into different groups such as highly virulent (70-90%), virulent (50-69%), moderately virulent (30-49%) and least virulent (10-29%).

Adhipathi *et al*. (2013) carried out investigation for the identification of resistant genotypes of rice against sheath blight disease and virulence diversity among the isolates of the pathogen. Twelve *R. solani* isolates were collected and characterized based on virulence diversity on 10 different rice varieties. The disease progress and severities were analysed using Area Under Disease Progress Curve (AUDPC) value on the basis of lesion length. They found Sarju-52 as the resistant and Pusa Basumati-1 as the susceptible. The resistant varieties will serve as potential source of resistance for the breeding programmes to develop resistant varieties in rice. The virulence pattern of the pathogen will help in the evaluation of pathogenic races and to identify disease susceptibility and resistant genotypes.

Similarly an experiment was conducted to screen varieties of rice for resistance to sheath blight disease and observed that 17 varieties were moderately resistant, 12 were susceptible and one variety showed resistance (Biswas *et al.,* 2011).

Mishra *et al*. (2014) conducted the cross infectivity of the isolates collected from rice, maize and greengram. Cross infection was found to be positive in all the three hosts with variability in their pathogenicity. Four isolates of rice, two each of maize and green gram isolates were found to be more aggressive and produced higher incidence of disease which could be utilized in resistant breeding programmes of their respective hosts. Cluster analysis showed four separate groups for the rice and maize isolates of *R. solani*, however the green gram isolates clustered together with maize isolates.

Srinivas *et al.,* (2014) reported that rice isolate of *R. solani* could infect 14 species belonging to three different families *i.e.,* Amaranthaceae, Graminaceae and Leguminaceae.

Ganeshmoorthi and Dubey (2015) assessed pathogenic variability in *R. solani* isolates.The pathogenic behaviour of the isolates of *R. solani* varied significantly among chickpea, urdbean and mungbean crops under artificial inoculation conditions. The pathogenic behaviour of the isolates was differentiated into less, moderately and highly virulent. Irrespective of hosts, all isolates of *R. solani* were found to be

pathogenic on chickpea, urdbean and mungbean. AG3 was identified as a highly virulent isolate on all three pulse crops. The isolates obtained from chickpea roots were also proved pathogenic on aerial parts of the mungbean and urdbean.

Similarly, Singh (2016) conducted the cross infectivity test under glasshouse conditions. Sixty day-old rice plants were artificially inoculated with *R. solani* of rice and maize; and similar treatment was repeated with maize plants. It was found that the *R. solani* of maize was more aggressive and virulent when compared to *R. solani* of rice. Disease severity was found maximum (30.56%) when rice plants were inoculated with maize isolates of *R. solani*, while it was 9.51 per cent when inoculated with rice isolates of *R. solani*. Whereas in maize plants treated with maize isolates of *R. solani*, the disease severity was 11.66 per cent, whereas 8.33 per cent when inoculated by rice isolates of *R. solani*. Ghosh *et al*. (2017) identified pathogenicity determinants genes and processes crucial for *R. solani* pathogenesis in rice.

Manjunatha (2016) observed the pathogenic variability of 20 *R. solani* isolates on susceptible cv. TN-1. They observed that the size of the lesion was ranging from 0.15 - 3.15 cm^2. The disease severity was showing a range from 17.26 - 33.86 per cent among the isolates. All the isolates were found to be highly virulent (>50 % DI) as per the per cent disease incidence.

Singh *et al*. (2018) examined cross infectivity of 62 Rhizoctonia isolates maintained on maize host and then inoculated on rice and sugarcane. The reaction was assessed as poor, average and strong. All isolates produced symptoms of banded leaf and sheath blight on maize and were also found to be cross infective on rice and sugarcane but there was significant variability in the pathogenicity and symptoms expression. This experiment gave an account about the ability of the pathogen to adapt diverse areas.

Divya *et al.* (2018) conducted an experiment for finding the virulence potential of field isolates of *R. solani* from different hosts such as ground nut, rice, maize, mung bean and soyabean; and were teated on different rice varieties *viz.,* Swarna, Maheshwari, TN-

1, and HMT. All these isolates from different host behaved differently in their virulence reaction.

2.6. STUDYING THE MOLECULAR VARIABILITY OF THE ISOLATES

Random Amplified Polymorphic DNA (RAPD) provides a very informative molecular tool for detecting genetic variation among of plant pathogens in population (Chiocchetti e*t al*., 1999). RAPD markers served a great potential for analysis of population (Welsh and McClelland, 1990; Williams *et al.*, 1990). Duncan *et al.* (1993) used RAPD technique for analyzing the variation present in several anastomosis groups.

Toda *et al.* (1999) analysed the genetic relatedness among 41 isolates of *R. solani* belonging to 11 AGs using 3 RAPD primers. Considerable variability was observed among the isolates coming under different AG types. They proved that there was no genetic relation regarding the geographical origin or host plants among the isolates. Neeraja *et al.*, (2002) assessed the genetic variability in sheath blight fungus using 18 isolates collected from different rice growing regions of India using RAPD markers. They got the similarity values of RAPD profiles ranged from 0.41 to 0.85 with an average of 0.66 among all the isolates. The percentage polymorphism detected per primer varied from 79.2 to 100 per cent. Cluster analysis of the isolates using unweighted paired group method with arithmetic averages were able to distinguish between *R. solani* isolates, at the same time the virulent and avirulent isolates was also easily distinguished.

Genetic variability can be used for developing resistant cultivars (Thirumalasamy e*t al.,* 2006). Guleria *et al*. (2007) analyzed the genetic diversity among the 19 *R. solani* isolates obtained from various regions of Punjab using10 inter simple sequence repeats (ISSR) and eight RAPD markers. The size of amplified DNA bands ranged from 0.25– 3.0 to 0.5– 4.0 kb with ISSR and RAPD markers respectively. Combined data of these analysis resulted in the formation of five clusters with 49–89 per cent genetic similarity. They found that RAPD markers detected more genetic variability compared to ISSR markers. RAPD analysis yielded 67 polymorphic bands. Xiao *et al*. (2008) collected 55 *R. solani* samples in rice from five distinctive ecological regions and analyzed the genetic

variations. RAPD analysis was able to classify these strains into 8 groups at a similarity level of 0.941.

Banerjee *et al*. (2012) assessed the molecular variability in 22 isolates of sheath blight fungus from four different ecological regions of West Bengal, ranging from coastal, alluvial, red lateritic to terai belts using RAPD markers. A total of 267 reproducible and scorable polymorphic bands ranging approximately as low as 200 bp to as high as 1500 bp were generated with eight RAPD primers. The similarity values of RAPD profiles ranged from 0.41 to 0.94. Isolates collected from similar agro-ecological location were mostly clustered together. They indicated high genetic variability of pathogen population collected from different regions of West Bengal.

Susheela and Reddy (2013) analysed the variability in 35 isolates of *R. solani* causing sheath blight of rice and weeds using PCR-RAPD analysis. The similarity values of RAPD profiles were ranging from 0.37 to 0.85. The percentage polymorphism detected per primer varied from 76.9 to 100%. These results along with the cultural and morphological characteristics, and aggressiveness proved the high intra group variability of the cultures. Prasad *et al*. (2014) studied the genetic variability using RAPD markers with eight isolates of *R. solani* from wet root rot of chickpea collected from Haryana, Rajasthan and Delhi and the isolates differed in their banding pattern.

Lal *et al.* (2014) analyzed the genetic variability of *R. solani* isolates using ten RAPD markers and 4 major clusters were obtained for cluster analysis. The range of genetic variability was found to be varying from 17-77 per cent. Maximum similarity (77%) value was found between RS-14 and RS-15 collected from Kerala. Lowest similarity level (17%) was obtained between isolates RS-22 (Delhi) and RS-4 (Punjab). Mostly the isolates obtained from same geographical area showed similar genetic characters.

Yugander *et al*., (2015) gave an account about the main advantages of RAPD as its speed and simplicity. Main utility is closely related strains can be easily distinguished without the prior knowledge about the nature of polymorphic regions. Singh *et al*. (2015)

analysed the genetic diversity among 25 isolates of *R. solani* cultures and they obtained a great genetic diversity among the fungal population. 80 PCR bands were detected among 25 isolates and the number of alleles per locus was varying from 1 to 7. Primer OPW-13 and OPA-04 yielded highest PCR products whereas primer UBC-310 and OPB-08 yielded the least. The similarity coefficient among *R. solani* population was ranging from 0.53 to 0.94. Manjunatha (2016) analysed the genetic variability in 20 isolates of *R. solani* causing sheath blight of rice and weeds using ten RAPD markers. The Polymorphism Information Content (PIC) value ranged from 0.82 to 0.88 which give an account that intra group variability exists among the isolates. There was cent per cent polymorphism present in the analysis indicating a good level of variations among isolates.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Studies on the variability of *Rhizoctonia solani* Kuhn. infecting rice, cowpea and amaranth." was undertaken in the Department of Plant Pathology, College of Agriculture, Vellayani during the year 2018-2020. Details of materials used and the methods that were followed are presented in this chapter.

3.1. SYMPTOMATOLOGY OF THE DISEASE

3.1.1. Collection of Disease Sample

Rice, vegetable cowpea and amaranth plants showing sheath blight, web blight, collar rot and leaf blight symptoms of *R. solani* infection were collected from the three southern districts *viz.*, Thiruvananthapuram, Kollam and Pathanamthitta were used for the study. Two locations each from these three districts were used for sample collection. Hotspot areas of the disease were selected as locations of collection of diseased samples. Locations selected were Vellayani, Chirayinkeezh from Thiruvananthapuram; Kottarakkara, Pooyapally from Kollam; and Ranni and Thiruvalla fom Pathanamthitta. The diseased samples of rice, vegetable cowpea and amaranth from these six locations were collected.

Detailed observations such as source of diseased sample, the stage of the crop affected, disease incidence and per cent disease index were recorded for each of the disease affected plant samples collected from different locations. Besides, geographical information (longitude/latitude) of the location was also collected.

For assessing the Disease Incidence, the number of infected plants and total number of plants assessed were recorded. Disease incidence was calculated using the formula given by Mayee and Datar (1986).

Disease incidence= $\dfrac{\text{Number of infected plants}}{\text{Total number of plants in field}} \times 100$

The disease severity of sheath blight disease was rated by calculating the Relative Lesion Height (RLH) given by Sharma *et al.,* (1990). The RLH was calculated as

27

$$RLH = (\text{Lesion height/Plant height}) \times 100$$

Based on the RLH, the plants showing sheath blight was scored according to 0 - 9 scale of Standard Evaluation System for Rice (IRRI, 2002) (Table 1).

Table 1. Disease scale for scoring sheath blight disease of rice

Scale based on RLH	Description
0	No infection observed
1	Lesions limited to lower 20 per cent of the plant height
3	Lesions limited to lower 20 to 30 per cent of the plant height
5	Lesions limited to lower 31 to 45 per cent of the plant height
7	Lesions limited to lower 46 to 65 per cent of the plant height
9	Lesions limited to more than 65 per cent of the plant height

Disease severity of web blight samples of vegetable cowpea were rated by the scale given by Mathew and Gupta (1996). Ten samples were randomly selected from the affected field and scored using 0-5 disease scale (Table 2).

Table 2. Disease scale for scoring web blight disease of vegetable cowpea

Grade	Description
0	Healthy
1	1-20 per cent of leaf area infected
2	21-40 per cent of leaf area infected
3	41-60 per cent of leaf area infected
4	61-80 per cent of leaf area infected
5	81-100 per cent of leaf area infected

Collar rot severity of vegetable cowpea sample was assessed by the scale given by Haware and Nene (1980). The samples are score based on the 0-4 scale (Table 3).

Table 3. Disease scale for scoring collar rot disease of vegetable cowpea

Scale	Description
0	No visible symptoms
1	1-25% - Water soaked lesion at collar region and rotting
2	25-49%- Lesion progression and rotting
3	50-74%- Horizontal and vertical spread and rotting
4	75-100% - Advanced stage of rotting, yellowing of foliage and collapse of plant

Disease severity of leaf blight amaranth samples was rated following the score used by Gireesh (2016). Ten disease samples were randomly selected and leaf was scored using 0-9 scale (Table 4).

Table 4. Disease scale for scoring leaf blight disease of amaranth

Grade	Description
0	No infection
1	1-10 per cent area infected
3	11-25 per cent of leaf area infected
5	26-50 per cent of leaf area infected
7	51-75 per cent of leaf area affected
9	More than 76 per cent of leaf area affected

After that, samples collected from different hosts ware subjected to Percentage Disease Severity or Percentage Disease Index (PDI) calculation. PDI was calculated using the formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of grades on each leaf}}{\text{Number of leaves assessed}} \quad X \quad \frac{100}{\text{Maximum grade used}}$$

-

3.1.2. Symptomatology of the Disease

Nature of symptoms of the disease affected plant samples of rice, vegetable cowpea and amaranth collected from different locations were studied.

3.2. ISOLATION, PURIFICATION OF PATHOGEN, PATHOGENICITY TESTING, AND VIRULENCE RATING

3.2.1. Isolation and Purification of the Pathogen

The collected disease specimens were taken to the laboratory and subjected to pathogen isolation. The entire procedure was performed within laminar air flow chamber which was prior sterilized by UV light and 70 per cent alcohol. The samples showing characteristic symptoms were washed thoroughly in tap-water followed by neat cut into small bits of 2-3 mm size along with some healthy tissue with a sterilized blade. The bits were surface sterilized in 0.1 per cent mercuric chloride for 30 sec and then subjected to 3 consecutive wash in sterile water for 1 min in order to remove traces of the chemical. The sterilized bits were kept in a sterile blotting paper for removing the excess moisture. These bits were aseptically transferred to solidified sterile potato dextrose agar (PDA) medium amended with 0.03 g streptomycin sulphate in sterile petriplates (Singh, 2016). The plates were sealed using cling film or parafilm and incubated at room temperature ($28\pm2\ ^{0}$C).

The petri dishes were observed for the growth of pathogen from next day onwards. After attaining sufficient growth all isolates were purified by hyphal tip method (Parmeter *et al.,* 1969) and pure cultures were obtained on PDA slants. Maintenance of the culture was performed under refrigeration at 15°C and periodical subculturing was performed. Virulence of the pathogen was maintained by inoculating the pathogen on healthy plant for every two months.

3.2.2. Pathogenicity Testing and Virulence Rating

Pathogenicity of the isolates screened through artificial inoculation to the respective hosts. Pathogenicity screening was performed under laboratory condition. For the pathogenicity screening of rice isolates, susceptible rice variety Uma was selected. Rice stalks at active tillering stage were excised (15 cm length) from the field. Selected healthy rice stalks were washed under tap water. After washing, stalks were kept on 20.0 cm diameter petriplate. With the aid of moist cotton humidity of the chamber was maintained. Pin pricks were given in the leaf sheath followed by inoculation. From the fully grown seven days old culture of fungal isolate, mycelial disc of 3 mm size along with sclerotia was placed inside the sheath of stalk with the help of sterile forceps. Rice stalks inoculated with 3 mm PDA disc alone on sheath served as control. Thin layer of moistened cotton was placed over the inoculated regions in order to maintain humidity (Pillai, 1990).

For the virulence screening of vegetable cowpea isolates, fully expanded trifoliate leaves (var. Geethika) detached from the stem were taken and evaluation as per detached leaf method was followed (Takegami, 2004). The collected leaves were washed and dried; then placed over a moist paper towel arranged in pans. Leaves were mildly injured by giving pin pricks on the upper surface. Then the injured portions were inoculated with 3mm sized mycelial bits of seven days old culture along with sclerotia on it. Uninoculated trifoliate leaved were also maintained. Thin layer of moistened cotton was placed on the inoculated area for maintaining the humidity.

For the pathogenicity screening of amaranth isolates, 30 days old seedlings (var. Arun) raised on the pots were selected. The leaves wiped with 70 per cent alcohol were slightly injured on the aerial portion. Inoculation was performed with the aid of mycelial bits from seven days old fungus culture. Thin layer of moistened cotton was placed over the inoculated region in order to maintain humidity. Plants inoculated with PDA disc alone served as control for each isolates. Inoculated plants were covered with perforated polythene bags to ensure humidity and aeration. This would provide the microclimate for

the initiation of symptom development. The plants were incubated at room temperature (28 ± 2^0C) and periodical observations were taken on symptom development (Gireesh, 2016).

The inoculated plants showing the characteristic disease symptoms were re-isolated for proving the Koch's postulates. Cultural comparisons, nature and type of symptom development were performed accordingly.

Virulence rating of the pathogen was assessed using the disease score chart of respective hosts showing disease symptoms. Per cent disease severity or Percentage Disease Index (PDI) was calculated according to Wheeler (1969). Observations such as number of days taken for symptom development, number of lesions and lesions size were recorded. For the assessment of disease severity of sheath blight of rice, web blight and collar rot of vegetable cowpea and leaf blight of amaranth, scale given by IRRI (2002), Mathew and Gupta (1996), Haware and Nene (1980) and Gireesh (2016) respectively were used.

3.3 STUDYING THE CULTURAL VARIABILITY OF THE ISOLATES

All the isolates from the rice, vegetable cowpea and amaranth collected were cultured in PDA medium in petridishes at $28 \pm 2\ ^0C$ and cultural characters were studied. Cultural characters like colony color, reverse colony colour, growth margin, texture of growth, type of growth, pigmentation in the media, days taken for producing sclerotia, colony diameter after 72 h, zonation, days for full plate coverage, rate of growth, abundance of mycelium in PDA were recorded. All isolates were inoculated in potato dextrose broth (PDB) for the observation of pigmentation and mycelia weight.

Colony colour and reverse colony colour were determined using Munsell' s soil colour chart (Munsell Color Company, Inc., 1954) at 10 days after incubation. The culture and key cards were placed side by side for comparison of the color (Burpee *et al.*, 1980).

32

Growth margin of the isolates was categorized as regular, slightly irregular and irregular. For recording the type of growth, colony diameter after 72 h of incubation was observed and categorized as fast, medium and slow. Colony diameter showing 85-90 mm was considered as fast grower, colony diameter showing 80-85mm was considered as medium grower and less than 80 mm was considered as slow grower. Texture of growth was categorized into appressed and fluffy based on the nature of their mycelial growth.

The colony diameter of each isolate was measured and recorded at regular intervals of 24 h. Three replications were maintained for each isolate. Growth rate of the isolate was calculated by observing the colony growth per day measured in cm.

Abundance of the mycelium in the culture was categorized into four major categories such as slight, moderate, abundant and no mycelium (Burpee *et al*., 1980). In slight category, aerial mycelium does not obscure surface mycelim, in moderate category aerial mycelium obscure surface mycelium and does not touch the cover of petridish and finally in abundant category, aerial mycelium obscure surface mycelium and touches the cover of petridishes.

To study the mycelial weight of each isolate, 100 ml of PDB was poured in 250 ml conical flask, plugged and sterilized. Mycelial discs of 3 mm diameter were cut from the margin of the seven day old culture of each isolate of the pathogen and transferred to the conical flask containing the sterilized medium under aseptic conditions. The flaks were incubated at room temperature (28 ±2 ℃). The mycelial mat was removed aseptically and washed thoroughly with sterile distilled water and dried in sterilized blotters. The mycelial weight of each isolate was taken using digital electronic balance. Pigment production of the pathogen and its intensity were recorded in PDA medium and PDB. The intensity of pigment production was recorded as low (+), medium (++) and high (+++).

2.4 STUDYING THE MORPHOLOGICAL VARIABILITY OF THE ISOLATES.

Morphological variability of different isolates were studied using macroscopical and microscopical characters. Macroscopic characters including sclerotial characters such

as size, texture, shape, number, orientation, position, colour, clump formation of scerotia and exudation or honeydew secretion on the sclerotia were studied. Microscopic characters such as mean hyphal width, presence of constriction at the point of branching, formation of septum near the point of origin of branch/adjacent to branch, shape of moniliod cells, presence of septa in the mycelium and presence of clamp connection. Mycelial compatibility between the isolates were also studied macroscopically and microscopically. Macroscopic compatibility was assessed by analyzing vegetative compatibility between the isolates through dual culture technique (da Cunha *et al.*, 2018). Microscopic compatibility was assessed by hyphal anastomosis (MacNish *et al.,* 1993).

Microscopic characters were recorded by observing the morphology of fungal hyphae. For the same, 2 per cent agar media was prepared and sterilized. The sterilized molten media were aseptically transferred into sterile petridish. Cellophane papers were cut into round shape (9 cm) and sterilized it. The sterilized cellophane papers were placed on the surface of the solidified media with the help of sterile forceps. Mycelial disc (3 mm diameter) of the pathogen from a seven days old culture was inoculated at the central position of the cellophane paper. The petridishes were sealed with cling film and incubated at room temperature (28 ± 2^0C). After incubation of 5 days, square shaped pieces (1cm X 1cm) of cellophane paper were cut along with the fungal mycelia with the help of sterilized scalpel. Later these bits were stained with 1-2 drops of 0.1 per cent lactophenol cotton blue and covered with cover slip and sealed. This was observed under Zeiss / Axio Lab Stereo Microscope, Germany for the assessment of different microscopic characters. Measurement of mean hyphal width was performed at 400 X magnification. Values of five replications were taken. The cumulative values of the hyphal width were divided by its number of replication. Presence of moniliod cells and shape of the moniliod cells were noted. Moniliod cells are histological change of the hyphae formed in addition to the ordinary hyphae and observed in different shapes and may be hyaline or brown in colour.

For studying the sclerotial characters 3 mm sized culture disc of the pathogen placed in sterile solidified PDA medium and incubated for 15 days. The colour of the

sclerotia was recorded with Munsell's soil colour chart (Munsell Colour Company, 1954). A single sclerotium was kept on a sterilized glass slide and the colour was compared by placing the colour chart side by side against white background under sunlight (Burpee *et al*., 1980).

Sclerotial weight and size were recorded by taking the 3 mm sized mycelial discs bearing sclerotial bodies from five random sites in petri plate. Sclerotia from these random sites were collected and weighed in electronic balance and average weight of single sclerotium was calculated. With the help of vernier calipers, the diameter of the sclerotium in mm was recorded. Based on mean diameter, size of the sclerotia was grouped into large and small. By counting the number of sclerotia present in each culture plate of the isolates, cultures were classified based on the production of the sclerotia as excellent, very good, good and no sclerotia at all.

Single slerotium placed in a clean sterile glass slide was observed under binocular stereo zoom microscope (LEICA, USA). Shape and texture of the sclerotium were recorded. Based on the texture, sclerotium was grouped into rough border and smooth border. Position of the sclerotia in the petriplate was observed and recorded as aerial and submerged. In aerial, sclerotia produced within the aerial mycelium and in submerged type, sclerotia formed within the medium.

The orientation of the sclerotial formation in the medium was recorded and the isolates were divided into different groups based on the character. Sometimes, sclerotia may be aggregated to form clumps. The presence of clump formation in the media was also recorded. Based on this, isolates were classified into more clump, moderate clump and less clump formation. The cultures often produce honey dew or exudation on the sclerotia or in the culture and were also recorded.

Vegetative compatibility among isolates was tested with macroscopic pairing of the culture in dual culture technique. The isolates were paired in all combinations. The pairing of isolates was done in the petridishes containing 2 per cent agar medium. Culture disc of 3 mm diameter from the edge of actively growing cultures of two isolates were

placed on the agar medium with a sterile cork borer and separated by a distance of 3 cm. After incubation of 7 days at room temperature (28 ± 2^0C), the pairing of the isolates was evaluated and combinations were grouped into fused or merge, tuff or tuft, merging line, barrage, barrage line (Syminis *et al.,* 1987; Yang *et al.,*1993; MacNish e*t al.,*1997; da Cunha *et al.,* 2018)

In the merge or fused type, the two cultures would come together with no evidence of demarcation. In the tuff or tuft category there was an area of distinct demarcation between the cultures that were occupied by band of hyphae raised above the general level of mycelium above the agar medium. In the merging line type, there was a line of distinct demarcation with no band of aerial hyphae raised above the general mycelial level. In the barrage reaction type, two colonies failed to mingle, leaving a zone in which no obvious mycelium grows. In the barrage line also two colonies failed to mingle, but a clear line can be seen with no mycelia between the cultures. Three replicates for all these combinations were maintained.

For the evaluation of hyphal anastomosis, modified cellophane technique was used. Glass slides (75 X 25 mm) were cleaned and sterilized. Two per cent agar medium (water agar) was sterilized and poured aseptically into petri plate forming thin layer on the petri plates. The thin layer of solidified agar media (3 cm X 1 cm) were cut with sterile scalpel and placed above the glass slide. Inoculum blocks of *R. solani* (3 mm dia.) were encised with sterile cork borer and placed above the thin layer of agar medium. Inoculum blocks were separated by a distance of 2 cm. Sterile cellophane papers were cut into small pieces (1 cm X 1 cm) and placed aseptically between the inoculum blocks. The glass slides were then incubated at 28±2ºC for 12-48 h till the mycelium of the paired isolates came in close vicinity. 1-2 drops of 0.1 per cent lactophenol cotton blue added to another sterile glass slide. After the incubation period, the cellophane paper was placed on the stain drop. The glass slides were microscopcally examined under Zeiss / Axio Lab Stereo Microscope, Germany at 100X and 400X magnifications.All the isolates were paired in all combinations and were examined for hyphal fusion and other hyphal interactions. Hyphal interactions were classified into C0, C1, C2 and C3. In C0, there

was no reaction at all and intermingling hyphae neither recognise nor react to each other. In C1 there was a vegetative interaction leading to hyphal contact and apparent connection of walls but no membrane to membrane contact (Contact fusion). In C2 reaction, wall connection and development of a pore along with the death of anastomosing and adjacent cells observed (imperfect fusion). C1 and C2 were vegetative incompatibility reactions and described as unsuccessful attempts to anastomose. In C3, there was a fusion of wall and membrane (Perfect fusion). The C3 reaction was a successful anastomosis reaction and isolates showing C3 reaction are said to be vegetatively compatible (Matsumoto *et al.,* 1932; Carling *et al.*, 1988).

3.5. STUDYING THE PATHOGENIC VARIABILITY OF THE ISOLATES

The ability of different isolates to cause infection in amaranth plants was evaluated through a pot culture experiment in completely randomized design (CRD) with 18 treatments and 3 replications. The treatments include isolates of *R. solani* from rice, cowpea and amaranth from two locations of three districts of Southern Kerala. For conducting cross infectivity test, Arun variety of amaranth from Instructional farm, College of Agriculture, Vellayani was used. These seeds were broadcasted in nursery beds. Thirty days old seedlings were transplanted into small grow bags. The grow bags (15 X 12 cm) was filled with potting mixture. For artificial inoculation, amaranth leaves were sterilized with 70 per cent alcohol. Mild pin pricks were given on the upper surface of the leaves. Inoculation was done in the evening and inoculated plants were sprayed with water in the next morning. Culture discs of 3 mm diameter was taken from 7 day old culture and were placed above the pin pricked portions. Thin layer of moist cotton was used for covering the inoculated portions in order to provide humidity. One set of uninoculated seedling was maintained as control. The seedlings were covered with perforated polythene covers and tied at the bottom to ensure the humid condition suitable for the expression of symptom. The plants were observed regularly for the symptom development. Observations like production of symptom on amaranth, days taken for symptom development, number of lesions and lesion size were recorded. Percentage disease index were calculated on 7 days after inoculation.

Disease severity of the different isolates based on above mentioned criteria was rated by the score chart used by Gireesh (2016).

Percentage Disease Index (PDI) was calculated by the formula given by Wheeler (1969).

$$PDI = \frac{Sum\ of\ grades\ on\ each\ leaf}{Number\ of\ leaves\ assessed} \times \frac{100}{Maximum\ grade\ used\ or\ 9}$$

3.6. STUDYING THE MOLECULAR VARIABILITY OF THE ISOLATES

Genetic relatedness among 18 isolates of *R. solani* isolated from rice, vegetable cowpea and amaranth were assessed using Random Amplified Polymorphic DNA analysis (RAPD).

For isolating the DNA, 2-3 mycelial bits of 3 mm diameter inoculated in PDB and incubated at room temperature (28 ± 2^0C). The mycelial mat was harvested after 7 days of incubation. Mycelial mat was washed with sterile distilled water repeatedly and blot dried. Total genomic DNA was isolated from the mycelia of all isolates of *R. solani* using DNeasy plant minikit (QIAGEN: Cat.No. 69104). The protocol for DNA isolation was followed as per the manufacturer of the kit. Mycelial mat (≤100 mg wet weight) was distrupted using liquid nitrogen in a sterile mortar and pestle. To this distrupted sample, 400µl buffer AP1 and 4 µl RNase A were added. Lysate was transferred to an eppendorf tube and vortexed. Then the lysate was incubated for 10 min at 65^0C. After incubation, 130 µl of buffer P3 was added to the eppendorf tube and mixed properly with a micropipette and incubated for 5 min on ice. Then the lysate was centrifuged for 5 min at 14000 rpm. The lysate was then pipetted into a QIAshredder spin column placed in a 2 ml collection tube and centrifuged for 2 min at 14000 rpm. The flow-through was then transferred a new eppendorf tube without disturbing the pellet if present. 1.5 times volume of buffer AW1 was added to the above eppendorf tube and mixed with micropipette. 650 µl of the mixture was transferred into a DNeasy Mini spin column

placed in a collection tube and centrifuged for 1 min at 8000 rpm. The flowthrough was discarded and the step was repeated with remaining sample. The spin column was placed into another collection tube and 500 µl of buffer AW2 was added. This was centrifuged for 1 min at 8000 rpm. The flow through obtained was discarded. Another 500 µl of AW2 buffer was added to the spin column and centrifuged for 2 min at 14000 rpm. The spin column was removed and placed in a fresh collection tube. 100 µl of AE buffer was added to the spin column for elution and incubated for 5 min at room temperature (15-25^0 C). This was centrifuged for 1 min at 8000 rpm. The last step was repeated once. The isolated DNA was stored at -20^0 C.

The quality of DNA was measured using spectrophotometer (BioSpectrometer, eppendorf) and absorbance was read at 260 and 280 nm. For analyzing the quality of DNA, 10 µl extracted DNA sample was diluted in 1000 µl of distilled water. The quality of DNA obtained was calculated as ratio of O.D. 260 to O.D. 280 value (OD-Optical Density). Four primers (OPW-13, OPA-04, OPA-02, OPA-19, OPA-20) were selected for RAPD analysis (Singh *et al.,* 2015) (Table 5). The primers that gave reproducible and scorable amplifications were used in the analysis of genetic variability of the isolates.

Table 5. List of random oligonucleotide primers selected for PCR amplification

Sl. No.	Primer Name	Primer Sequence (5'-3')
1	OPW-13	CACAGCGAACA
2	OPA-04	AATCGGGCTG
3	OPA-19	CAAACGTCGG
4	OPA-20	GTTGCGATCC

The PCR amplification reactions were carried out in a 25 µl reaction mixture containing 10X PCR Taq buffer - 2.5 µl, 2.5 mM MgCl$_2$ - 2.5 ml, 0.4 mM dNTPs (dATP,dGTP, dCTP and dTTP) - 1 µl, DNA - 4µl, Taq polymerase - 0.3µl (5U/ µl), 0.4µM random primer-4µl (SIGMA-ALDRICH) and 10.7µl sterile distilled water.

The PCR amplifications were performed by using thermal cycler (Veriti 96 well Thermal Cycler, Applied Biosystems) programmed for initial DNA denaturation at 94 ^{0}C for 5 min followed by 38 cycles at 94°C for 1 min, 30°C for 1 min, 72°C for 2 min and a final cycle of extension was performed at 72°C for 10 min. All the amplified DNA products were resolved by agarose gel electrophoresis.

Agarose gel of 1.2 per cent was prepared in 1X TAE buffer containing 2 µl ethidiumbromide (EtBr). The solution was poured immediately into the gel casting tray. Care was taken to avoid air bubbles in the gel. The comb was inserted into the gel and the solution was allowed to polymerize. The inserted comb was gently removed from the gel after polymerization. The gel plate was then placed in horizontal electrophoretic apparatus filled with 1 X TAE buffer. Two µl of 6X loading dye was mixed with 10 µl of PCR products, loaded and electrophoresis was performed at 65V power supply with 1X TAE as electrophoresis buffer for about 1-2 h. The molecular standard used was 1 kb DNA ladder. The gels were then visualized and image was documented using Gel documentation system (Molecular Imager Gel DOC TM XR+ with Image Lab TM Software, BIO-RAD) under UV light.

Each amplified band was considered as RAPD marker and recorded in all isolates. Data was entered using a binary matrix in which all observed bands were listed. The characters state "1" was given if this band could reproducibly detect in RAPD analysis. The character state "0" was assigned if band was lacking or it was not possible to determine its presence with certainty. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc (version 2.0). Jaccard's coefficients were clustered to generate dendrograms by using the SAHN clustering programme, selecting the unweighted pair-group method with arithemetic average (UPGMA) algoritm in Numerical Taxonomy and Multivariate Analysis System (NTSYS) for displaying genetic relatedness among the isolates.

Results

4. RESULTS

The results of the investigations carried out to determine the morphological, cultural, pathogenic and genetic variation among the isolates of *R. solani* collected from rice, vegetable cowpea and amaranth from different locations of Southern Kerala are presented in this chapter.

4.1. SYMPTOMATOLOGY OF THE DISEASE

4.1.1. Collection of Disease Sample

Rice, vegetable cowpea and amaranth plants infected by *R. solani* were collected during April to September, 2019 from different locations of Vellayani and Chirayinkeezh in Thiruvananthapuram district; Kottarakkara and Pooyapally in Kollam district; Ranni and Thiruvalla from Pathanamthitta district. The samples were numbered as TRR1, TRR2, KRR1, KRR2, PRR1 and PRR2 from rice, TRC1, TRC2, KRC1, KRC2, PRC1 and PRC2 from vegetable cowpea and TRA1, TRA2, KRA1, KRA2, PRA1 and PRA2 from amaranth. (Plate 1-3). Geographical coordinates of the location, crop stage, and plant part affected and types of symptom were recorded. Percent disease index (PDI) and disease incidence (DI) were assessed as per 3.1 and the results are depicted in Table 6.

PDI ranging from 11.11 to 99.99 per cent and DI ranging from 18-92 per cent were recorded from rice field. Rice plants collected from Pooyapally showed the highest PDI and disease severity of 99.99 and 92 per cent respectively. However, the lowest PDI (11.11%) and DI (18%) were observed in Chirayinkeezh.

During the sampling survey, vegetable cowpea fields infected by web blight disease showed PDI ranging from 33.33 to 77.77 per cent and DI was ranging from 32 to 88 per cent. It was also found that Vellayani recorded highest PDI (77.77%) and DI (88%). Meanwhile, Pooyapally showed the lowest PDI (33.33%) and Kottarakkara recorded the least DI (32%).

While conducting the field screening for disease severity of amaranth leaf blight, PDI was ranging from 11.11% to 77.77% whereas DI was ranging from 30 to 98%.

Among the fields surveyed, Ranni was showing the highest PDI (77.77%) and DI (86%). However, amaranth fields of Thiruvalla recorded the lowest PDI (11.11%).

Rice plants showing sheath blight symptoms were collected from fields at active tillering stage and harvesting stage. Likewise, cowpea plants with web blight symptoms were also collected at seedling, vegetative, pod development and harvesting stage. Samples of leaf blight infected amaranth leaves were collected when the plants were at vegetative stage, maturation stage and harvesting stage.

4.1.2. Symptomatology of the Disease

Symptoms of the diseased specimens were studied and nature of symptoms was recorded. The fungal pathogen *R. solani* showed considerable variation in the symptoms produced in the crops under study. *R. solani* infection in rice was seen on leaf as well as on lower leaf sheath. Foliar infection was manifested as banded blight and blighted lesions were visible on the leaf sheath. The symptoms on the leaf sheath can be described as oval or irregular, discoloured mostly greyish water soaked lesions with light brown, dark brown or purple coloured margins. The symptoms were mostly visible near the waterline and gradually the lesions extended horizontally and vertically. Brown mustard like sclerotia was seen in the infected parts of leaf sheath as well as leaf.

In vegetable cowpea plants brownish black lesions appear at the collar region leading to girdling of the stem. Initial stages of web blight appeared as small circular greyish brown spots on the foliage surrounded by water soaked areas. Later, the spots enlarged in to lesions with oblong to irregular shape and it may cover the entire leaf lamina leading to severe blighting. The affected leaves were clubbed together resulting in the webbing of leaves. On high humid conditions, mycelial mat were seen on the leaves along with yellowing and shedding of leaves. Sclerotial bodies were produced in the infected plant parts.

TRR1

TRC1

TRA1

Location 1 - Vellayani

TRR2

TRC2

TRA2

Location 2 - Chirayinkeezh

Plate 1. Nature of symptoms of *R. solani* in rice, vegetable cowpea and amaranth collected from Thiruvananthapuram district

Location 3 - Kottarakkara

Location 4 – Pooyapally

Plate 2. Nature of symptoms of *R. solani* in rice, vegetable cowpea and amaranth collected from Kollam district
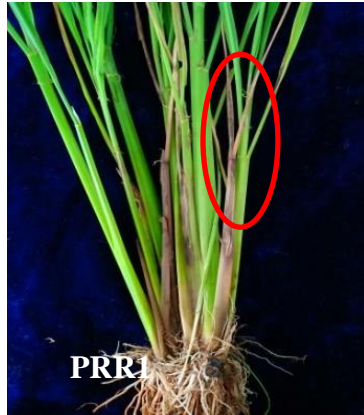
Location 5 - Ranni
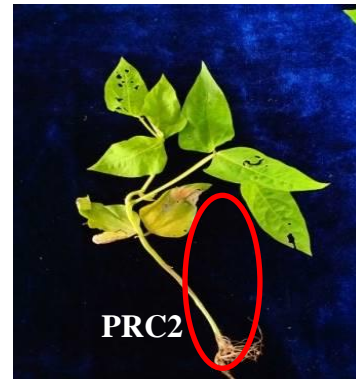
Location 6 - Thiruvalla

Plate 3. Nature of symptoms of *R. solani* in rice, vegetable cowpea and amaranth collected from Pathanamthitta district

Table 6. Nature of symptom and severity of *R. solani* infection on rice, vegetable cowpea and amaranth on natural infection by different isolates

Isolate	Nature of symptom	PDI (%)	DI (%)	Crop and stage affected	Plant parts affected
TRR1	Grey lesions with confined dark brown margin	33.33	25	Rice - Active tillering	Sheath and leaf
TRR2	Grey water soaked lesion with greyish brown margin	11.11	18	Rice - Active tillering	Sheath and leaf
KRR1	Greyish water soaked lesion with light brown margin	11.11	30	Rice - Active tillering	Sheath and leaf
KRR2	Grey lesions with purplish brown margin	99.99	92	Rice - Active tillering	Sheath and leaf
PRR1	Greyish lesion with brown margin	77.77	80	Rice -Harvesting	Sheath and leaf
PRR2	Greyish water soaked lesion with dark brown margin	55.55	85	Rice - Active tillering	Sheath and leaf
TRC1	Greyish irregular to oblong lesion with greenish brown margin on leaf and shedding of leaves	77.77	88	Cowpea - Vegetative	leaf
TRC2	Greyish irregular shaped lesion covering the leaf lamina	77.77	72	Cowpea - Vegetative	leaf
KRC1	Greyish brownish lesion with dark brown margin	44.44	32	Cowpea - Harvesting	leaf
KRC2	Brownish black lesion at collar region resulting in the rotting	33.33	75	Cowpea - Vegetative	Collar region
PRC1	Greyish irregular shaped lesion with brown margin, webbing of leaves	77.77	87	Cowpea - Pod development	leaf
PRC2	Greenish greyish irregular lesions with brown margin on the leaf and rotting at the collar region	33.33	88	Cowpea-Seedling	leaf
TRA1	Irregular greyish cream spots on the leaf	55.55	88	Amaranth -Maturation	leaf
TRA2	Irregular greenish grey spot with light margin and shot hole symptom	55.55	98	Amaranth - Harvesting stage	leaf
KRA1	Greyish irregular spots with shot hole symptom	77.77	30	Amaranth - Maturation stage	leaf
KRA2	Greenish grey spots with shot hole symptoms	77.77	35	Amaranth - Vegetative stage	leaf
PRA1	Greyish irregular translucent spots with shot hole symptoms	77.77	86	Amaranth - Harvesting stage	leaf
PRA2	Greenish irregular spots	11.11	40	Amaranth - Vegetative stage	leaf

Leaf blight symptoms in amaranth plant appeared as greenish, grey or cream coloured spots or lesions with irregular shape. Adjacent lesions gradually coalesced and enlarged under high humid conditions. In severe cases, shot hole symptoms appeared on the leaves. Papery white appearance on leaves can also found.

4.2. ISOLATION, PURIFICATION, PATHOGENICITY TESTING, AND VIRULENCE RATING OF THE PATHOGEN

4.2.1. Isolation and Purification of the Pathogen

The pathogens were isolated on PDA medium from the diseased samples of rice, vegetable cowpea and amaranth plants collected by following the standard procedure. Eighteen isolates of *R. solani* were isolated from the samples collected from the three districts in southern Kerala. These isolates were numbered same as that of sample number. The isolates were purified by following hyphal tip method. The purified cultures were maintained by periodic subculturing on PDA slants and used for further studies (Plate 4).

4.2.2. Pathogenicity Testing and Virulence Rating

The pathogenicity of the isolates was confirmed by following Koch's postulates. The pathogenicity and virulence of the isolates obtained from rice, vegetable cowpea and amaranth were assessed by artificial inoculation on respective hosts. Observations like nature of symptom, days taken for symptom development, number of lesions and lesion size were recorded. Artificial inoculation of all isolates on respective host produced symptoms within two to three days.TRR2, KRR1 and KRR2 of the rice isolates produced symptoms on two days after inoculation (DAI). Rest of the rice isolates produced symptoms on three DAI. Among the vegetable cowpea isolates, KRC1, PRC1 and PRC2 produced symptom on two DAI. Rest of the cowpea isolates produced symptoms on three DAI. TRA2 and PRA2 isolates of *R. solani* produced symptoms on two DAI and on three DAI in amaranth plants.
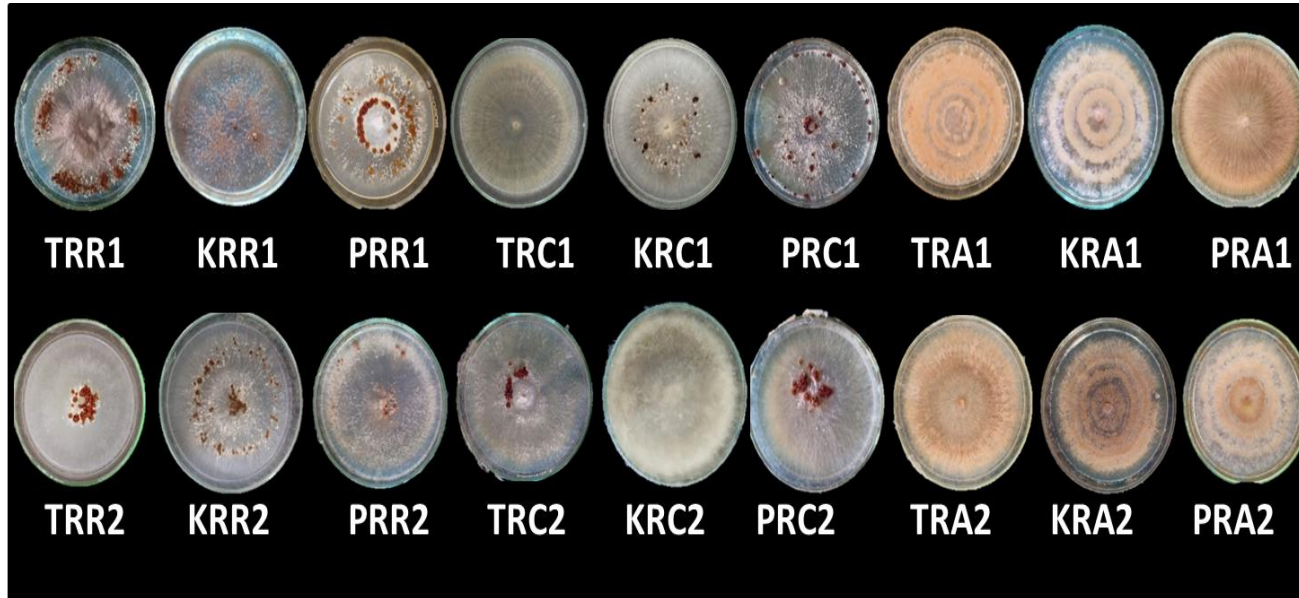
Plate 4. Isolates of *R. solani* from rice, vegetable cowpea and amaranth from different locations of southern Kerala

Lesion number on artificial inoculation ranges from 2-7, 1-4 and 2-4 in rice, vegetable cowpea and amaranth respectively. Size of the lesion produced by the pathogen in rice plants ranged from 0.2-3.1 x 0.1-2 cm. However, in vegetable cowpea plants, size of lesion ranged from 0.1-5.2 x 1.1 to 2.8 cm and in amaranth it was ranging from 0.1-2.5 x 0.1-2 cm. Nature of symptom development was recorded on 4, 5 and 7 DAI on amaranth, rice and vegetable cowpea respectively. Pathogenicity tests showed that all the 18 isolates under study were virulent and able to cause infection. Reisolation of the pathogen from artificially inoculated plants yielded *R. solani* culture identical to the original culture.

Nature of the symptoms produced showed considerable variations in the respective hosts on artificial inoculation. Greyish water soaked lesions were observed in the leaf sheath of rice. A difference in the colour of lesion margin was also observed and it varied from light brown, purple or dark brown. The lesion enlarged gradually resulting in the coalescing of nearby lesions forming bigger patches. Greenish, grey and brown irregular lesions could be seen on vegetable cowpea. Such lesions were mostly accompanied by chlorosis of the leaf lamina. Greenish or greyish straw coloured spots which later coalesced to form blighting and shot hole symptom was observed on artificial inoculation on amaranth (Plate 5-7).

Virulence rating of the pathogen was also done in the respective hosts. It was assessed by analyzing PDI using the formula given by Wheeler (1969) and using the disease score chart of respective hosts. The disease severity of sheath blight of rice, web blight of vegetable cowpea, collar rot of vegetable cowpea and leaf blight of amaranth were analyzed with the scale given by IRRI (2002), Mathew and Gupta (1996), Haware and Nene (1980) and Gireesh (2016) respectively. All the 18 isolates produced symptom in the respective host but varied in the degree of pathogenicity (Table 7). By assessing the lesion number, days taken for symptom development, lesion size and PDI, virulence of the isolate was evaluated in respective hosts. PDI of rice isolates on artificial inoculation was ranging from 10 to 30 per cent.
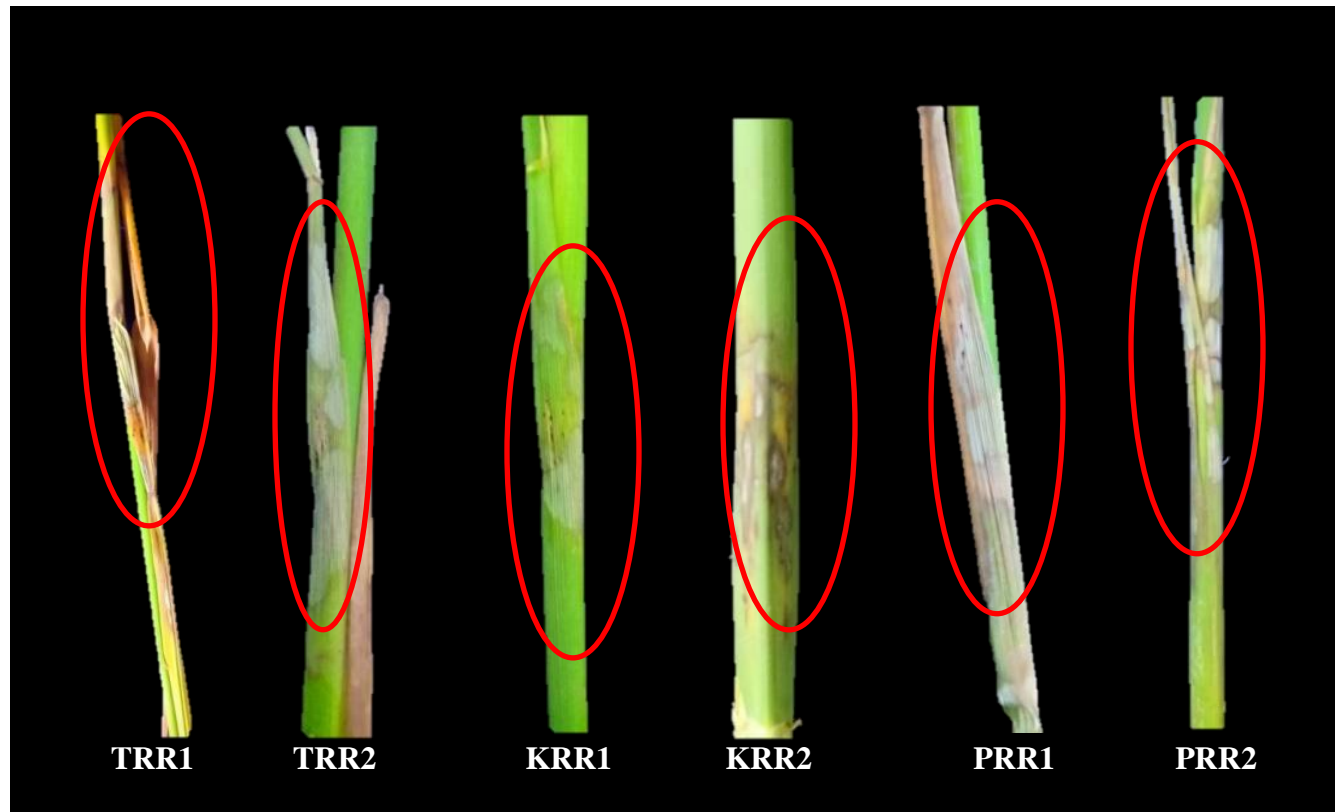
Plate 5. **Symptoms by artificial inoculation of *R. solani* isolates on 40 day old rice var. Uma at 5 days after inoculation**
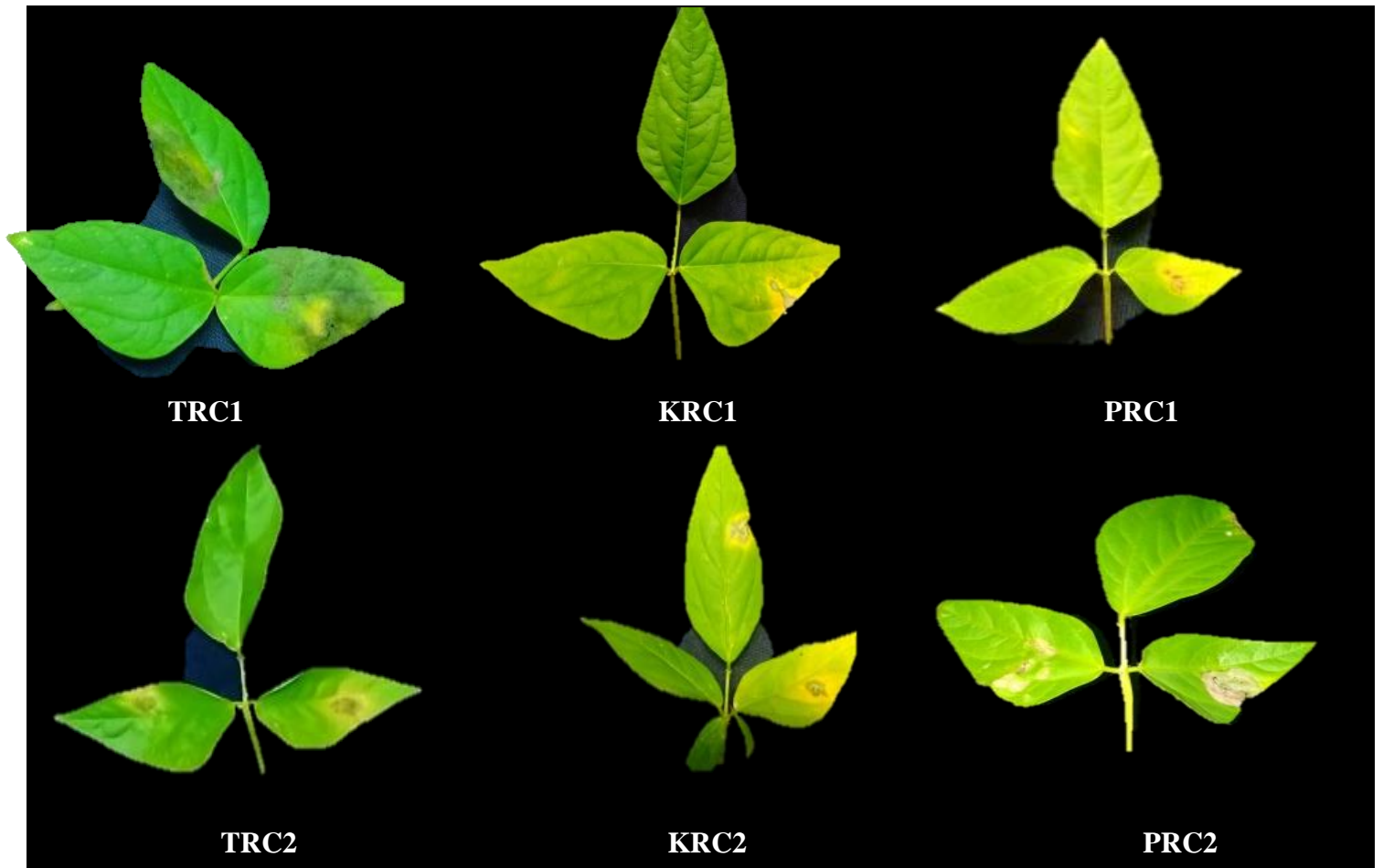
Plate 6. Symptoms on artificial inoculation of *R. solani* isolates on 25 day old vegetable cowpea var. Geethika at 7 days after inoculation
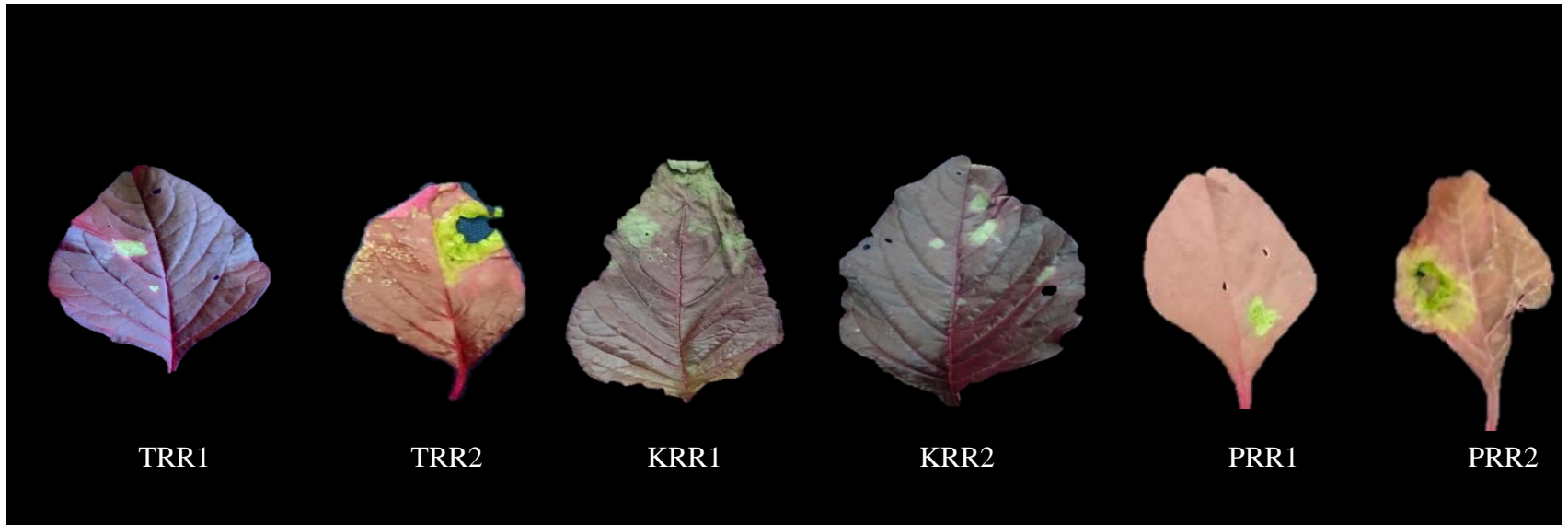
Plate 7. Symptoms on artificial inoculation of *R. solani* isolates on 30 day old amaranth var. Arun on 4 days after inoculation

Table 7. Days for initiation of symptom, number and size of lesion by 18 *R. solani* isolates on artificial inoculation

Isolate	Nature of symptom	Days for symptom development	Number of lesion	Size of lesion (lx b) in cm	PDI (%)
TRR1	Greyish lesion with brown margin	3	4	0.4-2.4 X 0.8-1.2	30.00
TRR2	Greyish water soaked lesions	2	2	0.1-2.5 X 0.1-1.8	10.00
KRR1	Greyish water soaked coalesced lesions	2	5	1.5-2.2 X 0.8-1	26.66
KRR2	Greyish water soked lesion with light brown margin	3	7	0.2-3.1 X 0.1-1.3	19.16
PRR1	Greyish bigger water soaked lesions	3	7	0.5-2.6 X 0.3-1.0	21.66
PRR2	Greyish water soaked lesion with brown margin	2	7	0.1-2.5 X 0.1-2	20.83
TRC1	Greenish lesions , chlorosis	3	3	2-3.2 X 1.4-2.8	33.33
TRC2	Greyish irregular lesions, chlorosis	3	1	0.4-2.5 X 0.8-2	22.22
KRC1	Greyish brownish lesion with brown margin, chlorosis	2	2	2.1-5.2 X 1.5-2.5	44.44
KRC2	Greyish brown lesion, chlorosis	3	2	2.1-3.2 X 1.5-2.7	33.33
PRC1	Greyish irregular shaped lesion with brown margin, chlorosis	2	3	0.1-1.2 X 0.8- 1.1	22.22
PRC2	greyish irregular lesions with brown margin, chlorosis	2	4	0.4-2.5 X 0.2-2.2	22.22
TRA1	Irregular greyish creamy spots	3	2	0.5-1.2 X 1.7-2.1	22.22
TRA2	Irregular greenish spots coalesced and shot hole	2	4	0.1-2.5 X 0.1-1.8	55.55
KRA1	Greyish irregular spots	3	4	0.3-1.0 X 0.2-0.8	33.33
KRA2	Greenish grey spots	3	3	02-0.6 X 0.2-0.4	33.33
PRA1	Irregular greenish spots	2	2	0.1-0.6 X 0.1-04	11.11
PRA2	Greenish irregular coalesced spots and shot hole	3	3	0.1-2.5 X 0.1-2	33.33

In vegetable cowpea isolates, PDI was ranging from 22.22 to 44.44 per cent and in amaranth isolates, it ranged from 11.11-55.55 per cent. TRR1 (Vellayani isolate) was more virulent among rice isolates having PDI of 30 per cent and produced symptom on two DAI. Among vegetable cowpea isolates, KRC1 (Kottarakkara) was more virulent having PDI of 44.44 per cent and produced symptom on two DAI. TRA2 (Chirayinkeezh) was more virulent among amaranth *R. solani* isolates having PDI of 55.55 per cent and produced symptom on two DAI. Least virulent culture among rice isolates was TRR2 (PDI of 10%). Among the vegetable cowpea isolates, TRC2, PRC1 and PRC2 produced a PDI of 22.22 per cent. PRA1 (Ranni) showed the least PDI (11.11%) among amaranth isolates.

4.3. STUDYING THE CULTURAL VARIABILITY OF THE ISOLATES

Eighteen isolates from different hosts like rice, vegetable cowpea and amaranth showed variation in their cultural characters like colony colour,reverse colony colour, margin, texture, growth rate, pigmentation in the media, colony diameter, zonation, days taken for full plate coverage, dry weight of mycelium and days for sclerotial initiation (Table 8) (Plate 8-10).

4.3.1. Colony Colour

Based on the pigmentation of dominant spectral colour of Munsell's soil colour chart, colony colour was assessed. Initially the colony colour of the isolates was white and then changes to different shades of colour. The colony colour showed a variation from olive yellow to light brown in the culture. The various colony colour expressed by the culture were as follows: Olive yellow (2.5 Y6/6), Light yellowish brown (2.5 Y 6/4), Yellowish brown (10YR 5/4), Dark yellowish brown (10 YR 4/4), Light olive brown (2.5 Y 5/4), Olive brown (2.5 Y 6/6), brownish yellow (10 YR 6/6) and Light brown (7.5 YR 6/4). Most of the culture were shown light yellowish brown (TRC2, KRC1, TRA1 and KRA1) followed by yellowish brown (PRR1, PRC2 and PRA2), olive yellow (TRR1, KRC2), dark yellowish brown (KRR1 and PRA1), brownish yellow (TRA2 and KRA2), light brown (TRR2 and TRC1) and light olive brown (KRR2 and PRR2) and PRC1 (olive

brown). Maximum number of rice isolates exhibited light olive brown (2). Light yellowish brown is the colour expressed by maximum number of vegetable cowpea isolates (2) whereas amaranth isolates expressed light yellowish brown (2) and brownish yellow.

4.3.2. Reverse Colony Colour

Reverse colony colour of cultures was also evaluated according to Munsell's soil colour chart. The reverse colony colour showed a variation from dark yellowish brown to dark brown. The variations of reverse colony colour was as follows: dark yellowish brown (10 YR 4/4), light olive brown (2.5 Y 5/4), dark olive brown (10 YR 3/3), dark greyish brown (10 YR 4/2), dark reddish brown (5 YR 3/4) and dark brown (10 YR 3/3). Most of the colours expressed by the *R. solani* isolates were dark yellowish brown (PRR1, PRC2, TRA1, KRA1, KRA2, PRA1 and PRA2) followed by dark olive brown (TRR2, KRR2, TRC2 and KRC1), light olive brown (TRC1, KRC2 and PRR2), dark brown (TRR2 and TRA2), dark greyish brown (KRR1) and dark reddish brown (PRC1). Maximum number of rice, vegetable cowpea and amaranth isolates showed dark olive brown, light olive brown to dark olive brown and dark yellowish brown respectively.

4.3.3 Pigmentation

Based on the pigmentation in the media, cultures were divided into low pigmented, medium pigmented and high pigmented in PDA and PDB. Out of the 18 cultures, seven cultures were high pigmented (TRR1, TRR2, KRR1, KRR2, PRR1, PRR2, and PRC2) and 11 cultures were medium pigmented (TRC1, TRC2, KRC1, KRC2, PRC1, TRA1, KRA1, KRA2, TRA2, and PRA2) in PDA. Most of the rice cultures were highly pigmented. Most of the vegetable cowpea cultures were medium pigmented except PRC 2 which was highly pigmented. All amaranth cultures were medium pigmented in both PDA nd PDB. (Plate. 11-12).

4.3.4. Growth Margin

According to pattern of growth margin, the cultures were designated as regular, slightly irregular and irregular. Out of 18 cultures, 11 cultures were having regular margin (TRR1, TRR2, KRR1, KRR2, PRR1, PRR2, TRC 49 C1, PRC2, TRA2 and PRA1), four were having slightly irregular margin (TRA1, KRA1, KRA2 and PRA2) and three were having irregular margin (TRC2, KRC1 and KRC2). All the rice isolates were having regular margin. Vegetable cowpea isolates were showing regular (TRC1, PRC1 and PRC2) and irregular margin (TRC2, KRC1, KRC2). Most of amaranth isolates were having slightly irregular margin except TRA2 and PRA1 which were having a regular margin.

4.3.5. Texture of Growth

Under artificial conditions, growth pattern of cultures varied from appressed to fluffy. All the isolates from rice, vegetable cowpea and amaranth were showing appressed growth in the culture except the isolate KRC2 (Pooyapally). KRC2 produced fluffy mycelial growth in the culture. In fluffy type, aerial mycelium obscured the surface mycelium and mostly touches the cover of the petri plate and there is abundance of mycelium visible in the culture. But in the appressed type, there is no abundance of mycelium present in the culture and the mycelial growth is flattened.

4.3.6. Colony Diameter and Growth Rate

Colony diameter of the rice, vegetable cowpea and TRA2 and PRA1 of amaranth isolates were 90 mm after an incubation of 72 h. Other amaranth cultures were showing colony diameter of 85 mm (TRA1), 60 mm (KRA1), 46 mm (KRA2) and 54 mm (PRA2) respectively at 72 h (Plate 14). Based on the colony diameter and growth rate of the isolates on media, they were categorised into fast, medium and slow. All rice and vegetable cowpea isolates of *R. solani* were showing fast growth in culture. TRA2 and PRA1 isolates of amaranth were fast growers. Among the rest of the isolates of amaranth, TRA1 was medium grower and KRA1, KRA2 and PRA1 were slow growers. KRR1 was showing the highest growth rate of 5.9 cm/24 h among rice isolates. Among vegetable

cowpea isolates, PRC2 was showing the highest growth rate (4.6 cm in 24 h. Likewise, PRA1 was showing maximum growth rate of 4cm/24 h among amaranth isolates. The isolates KRR2 (4.8 cm in 24 h), TRC1 (3.4 cm in 24 h) and PRA2 (1.7 cm in 24 h) were showing the lowest growth rate among rice, vegetable cowpea and amaranth isolates respectively (Plate 13).

4.3.7. Zonation

Zonation was present in all amaranth *R. solani* isolates except PRA1 (Ranni). TRA2 (Chirayinkeezh) of amaranth culture was having slight zonation in their culture. Zonation was absent in all rice and vegetable cowpea isolates except TRC1 (Vellayani) where a slight zonation was observed.

4.3.8. Days Taken for Full Plate Coverage

All the isolates under study completed full growth (90 mm) within 2-7 DAI at temperature (28 ± 2 ^{0}C). All the rice isolates of *R.solani* took 2 days to completely cover the petri plate and the vegetable cowpea isolates took 2-3 days for covering the petri plate. However, the amaranth isolates completed full growth within a period of 2-7days. Amaranth isolates KRA1 (Kottarakkara) and KRA2 (Pooyapally) were the slowest growing isolates and they completed the full growth in petri plate on 7 DAI.

4.3.9. Dry Weight of Mycelium

Dry weight of mycelium was recorded after sieving out the culture media and drying the mycelial mat for a period of 4 h at a temperature of 60^0C in oven. The dry weight of mycelium was ranging from 2.10 (KRA1) to 7.10g (PRR1). In rice isolates, dry weight ranged from 2.67 to 7.10 g. Dry weight of vegetable cowpea isolates was ranging from 4.63-6.29 g. In amaranth isolates, dry weight of mycelium was ranging from 2.10 to 4.0 g. Dry weight of mycelium was found higher in Ranni rice isolate PRR1 (7.10g), Thiruvalla vegetable cowpea isolate PRC2 (6.29g) and amaranth isolate PRA1 (4g). Dry weight of mycelium was lowest in KRA1 (2.10g), KRC2 (4.63g) and KRR2 (2.67g) for amaranth, vegetable cowpea and rice isolates respectively.

a. Front view of petriplate

b. Rear view of petriplate

| TRR1 | TRR2 | KRR1 | KRR2 | PRR1 | PRR2 |
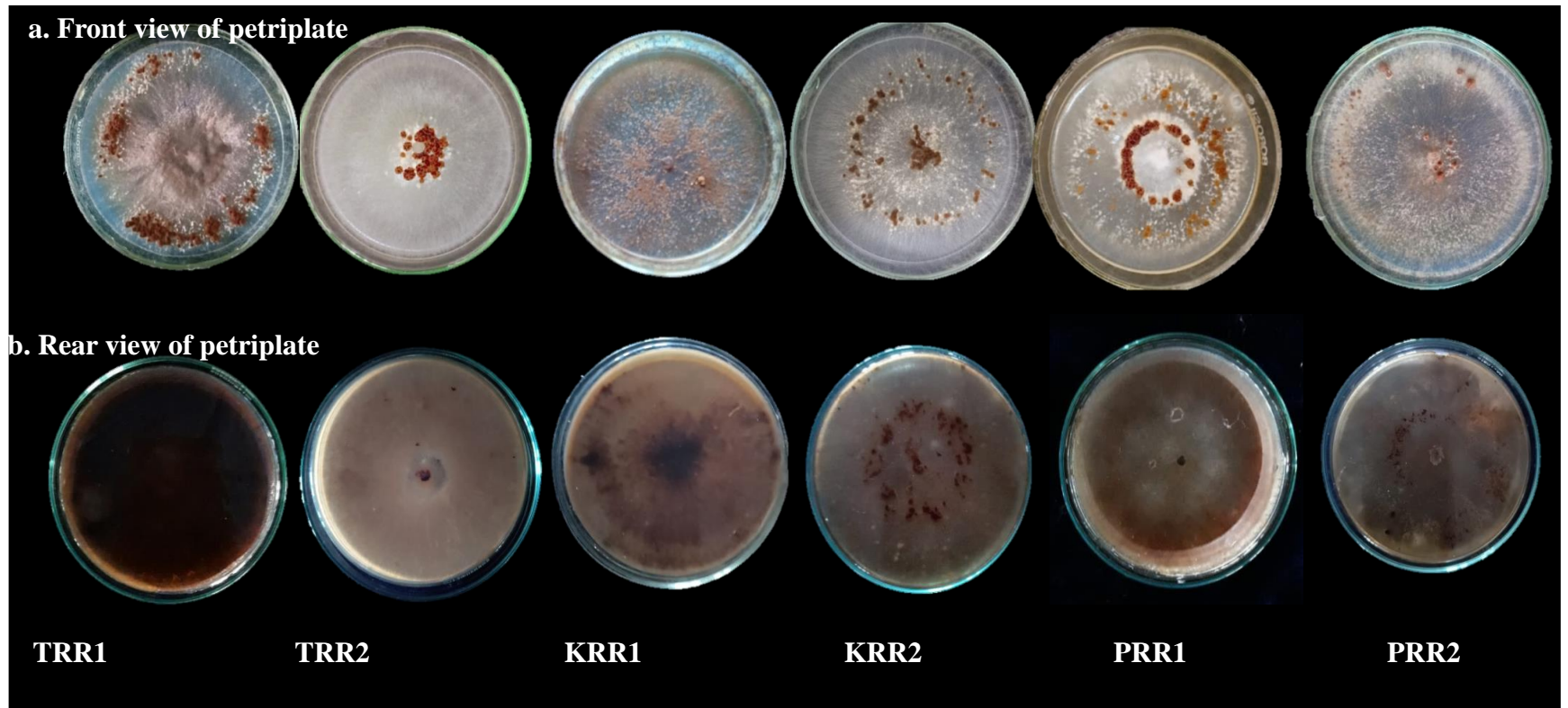
Plate 8. Variation in colony colour, zonation and orientation of sclerotial formation of *R. solani* rice isolates on PDA at 7 days after growth
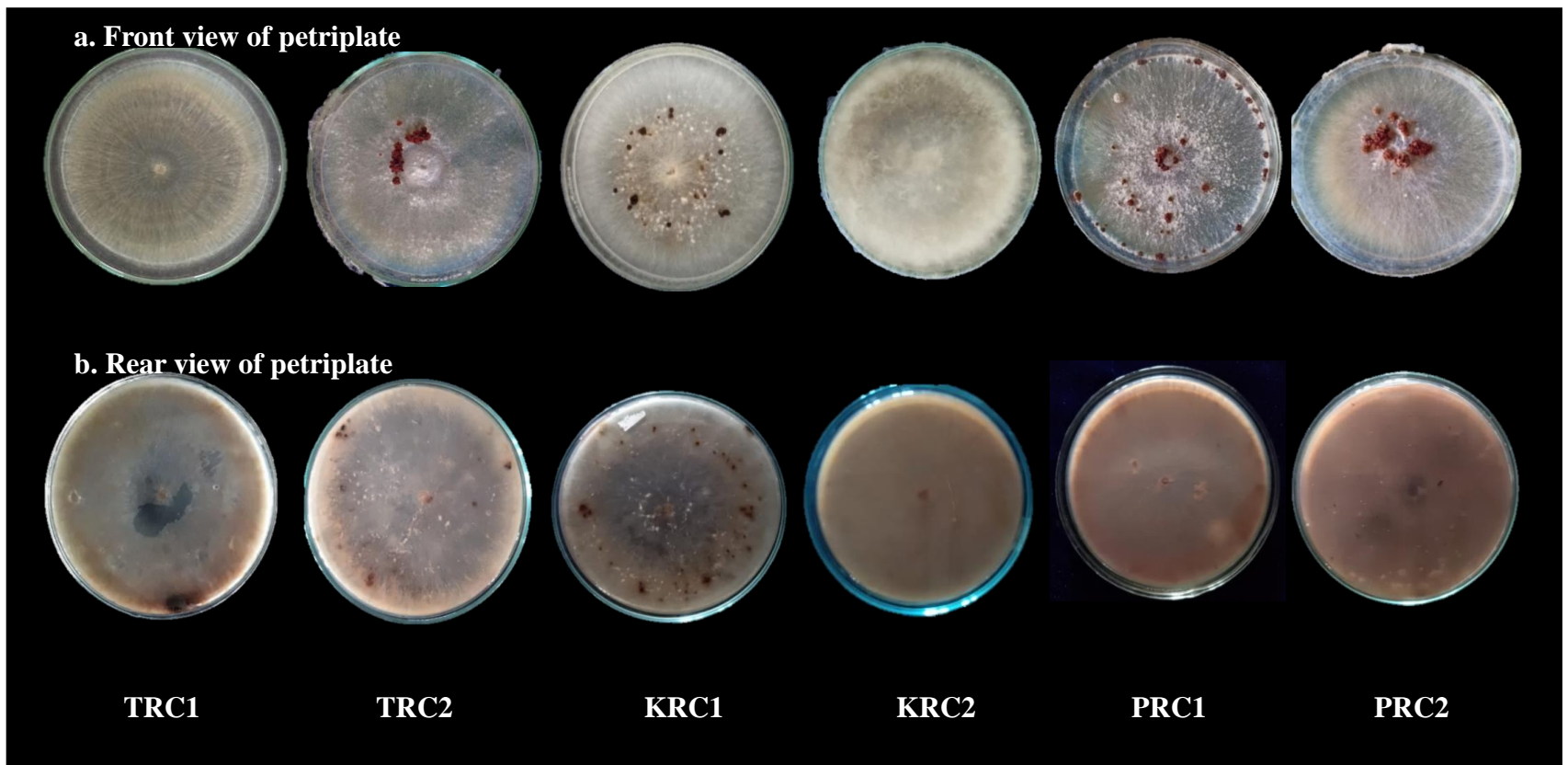
Plate 9. Variation in colour colour, zonation and orientation of sclerotial formation of *R. solani* vegetable cowpea isolates on PDA at 7 days after growth

a. Front view of petriplate

b. Rear view of petriplate

TRA1   TRA2   KRA1   KRA2   PRA1   PRA2
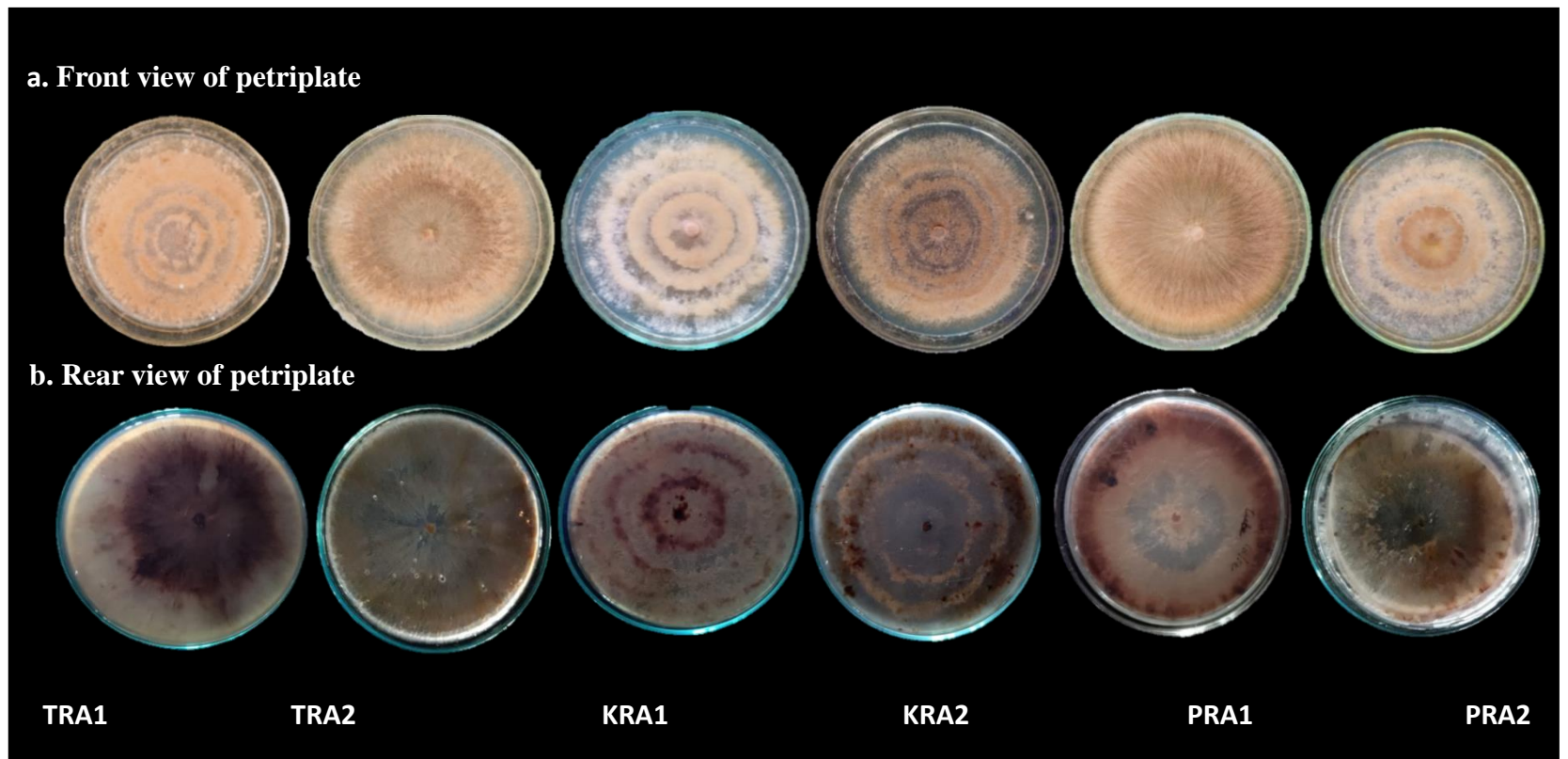
Plate 10. Variation in colour colour, zonation and orientation of sclerotial formation of *R. solani* of amaranth on PDA at 7 days after growth

Plate 11. Pigmention of *R. solani* isolates of rice, vegetable cowpea and amaranth on PDA at 2 weeks after growth
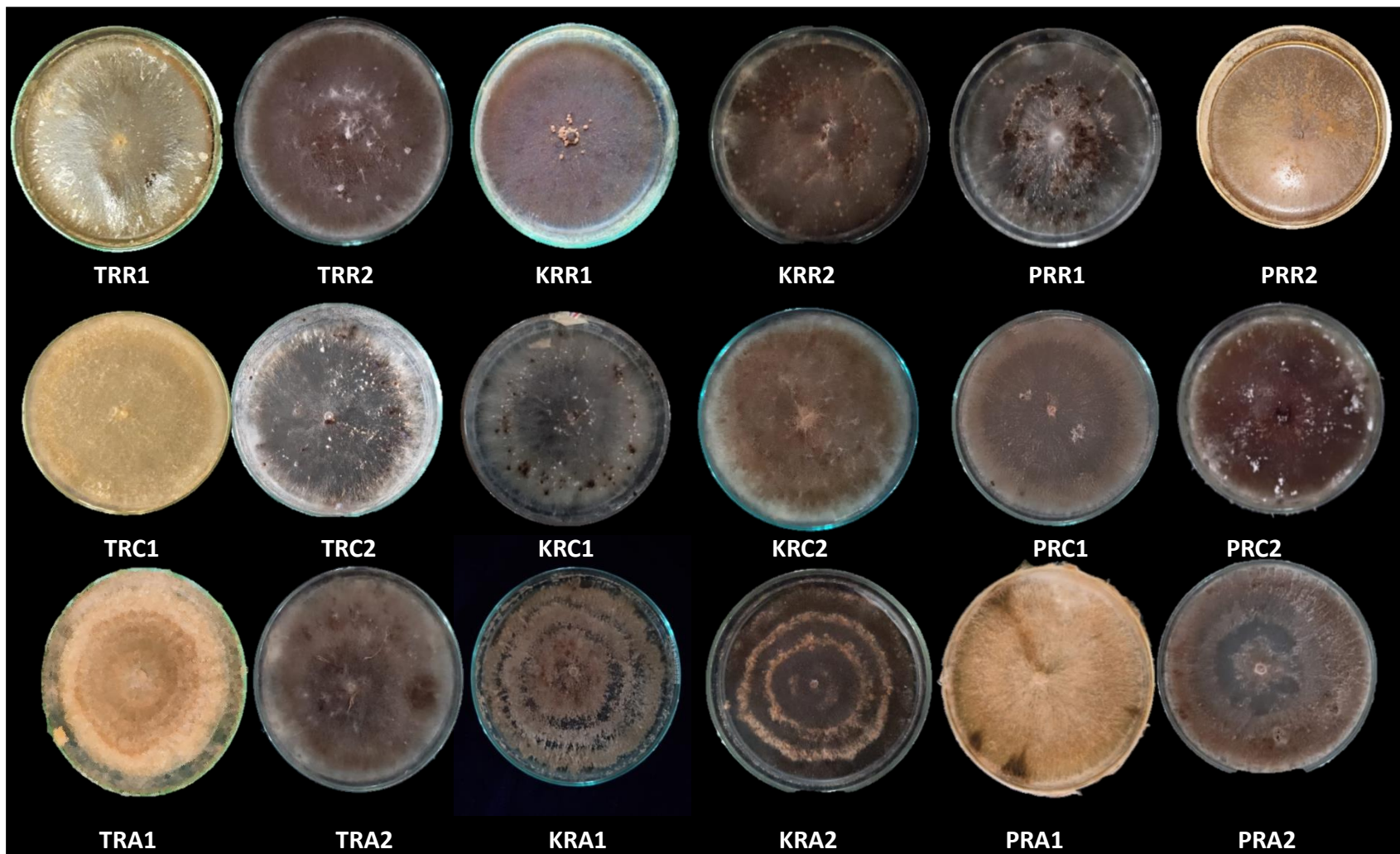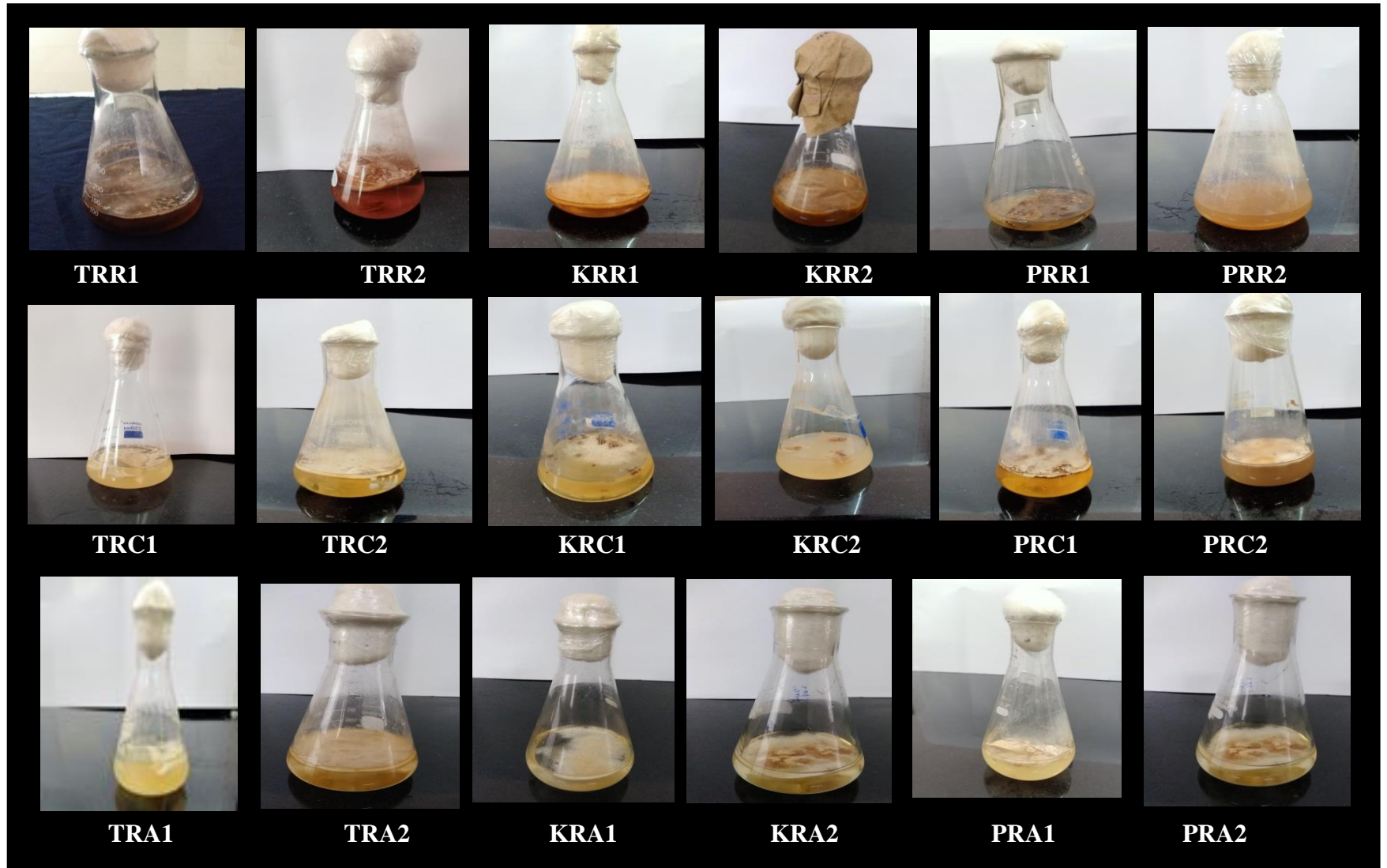
Plate 12. Growth and pigmentation of *R. solani* isolates of rice, vegetable cowpea and amaranth in PDB at 7 days after growth
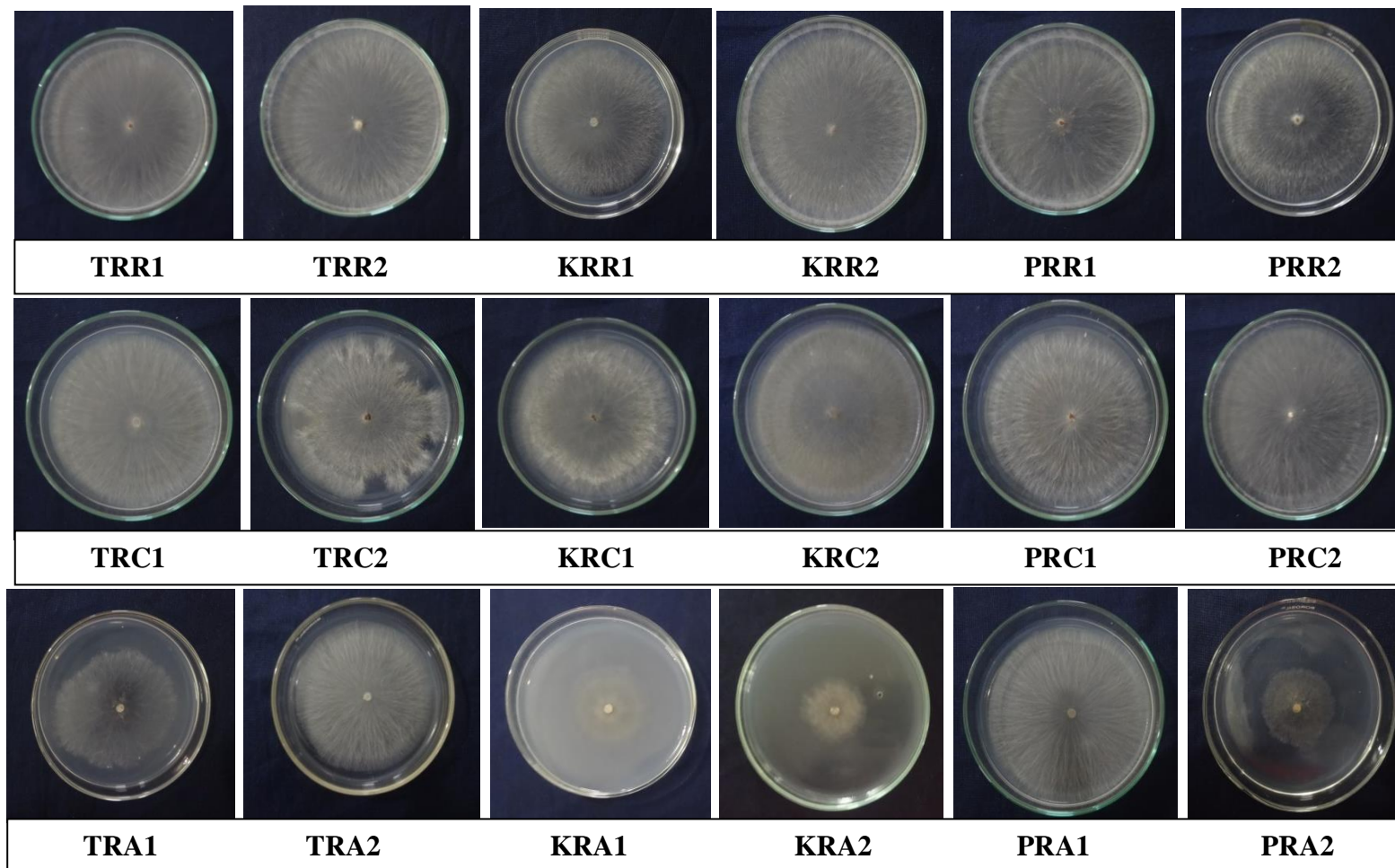
Plate 13. Colony diameter of eighteen *R. solani* isolates on PDA at 3 days after inoculation (DAI)

Table 8. Variations in the cultural characters of *R. solani* isolates of rice, vegetable cowpea and amaranth on PDA medium

Isolate	Colony colour		Type of growth				Pigmen-tation in media	Colony diameter 3 DAI (mm)	Zona-tion	Days for full plate coverage (8 cm)	Dry weight of mycelium 7 DAI (g)	Days for sclerotial initiation
	Front view	Rear view	Margin	Texture	Growth rate	Growth rate (cm)						
1	2	3	4	5	6	7	8	9	10	11	12	13
TRR1	Olive brown	Dark olive brown	Regular	Appressed	Fast	5.2	+++	9	Absent	2	6.02	3
TRR2	Yellowish brown	Dark brown	Regular	Appressed	Fast	5.8	+++	90	Absent	2	6.50	3
KRR1	Dark yello-wish brown	Dark grey-ish brown	Regular	Appressed	Fast	5.9	+++	90	Absent	2	3.32	3
KRR2	Light olive brown	Dark olive brown	Regular	Appressed	Fast	4.8	+++	90	Absent	2	2.67	3
PRR1	Yellowish brown	Dark yello-wish brown	Regular	Appressed	Fast	5.0	++	90	Absent	2	7.10	3
PRR2	Light olive brown	Dark olive brown	Regular	Appressed	Fast	4.9	+++	90	Absent	2	2.80	3
TRC1	Light brown	Light olive brown	Regular	Appressed	Fast	3.4	++	90	Slight	3	5.63	No sclerotia
TRC2	Light yello-wish brown	Dark olive brown	Irregular	Appressed	Fast	4.2	++	90	Absent	3	6.19	3
KRC1	Light yello-wish brown	Dark olive brown	Irregular	Appressed	Fast	4.3	++	90	Absent	3	5.53	3
KRC2	Olive yellow	Light olive brown	Irregular	Fluffy	Fast	4.5	++	90	Absent	3	4.63	No sclerotia

Table 8. Variations in the cultural characters of *R. solani* isolates of rice, vegetable cowpea and amaranth on PDA medium (contd.)

PRC1	Olive brown	Dark reddish brown	Regular	Appressed	Fast	4.3	++	90	Absent	3	6.29	3
PRC2	Yellowish brown	Dark yellowish brown	Regular	Appressed	Fast	4.6	+++	90	Absent	2	6.63	4
TRA1	Light yellowish brown	Dark yellowish brown	Slightly Irregular	Appressed	Medium	2.2	++	85	Present	4	2.53	
TRA2	Dark brownish yellow	Dark brown	Regular	Appressed	Fast	3.3	+++	90	Slight	2	2.73	
KRA1	Light yellowish brown	Dark yellowish brown	Slightly irregular	Appressed	Slow	1.9	++	60	Present	7	2.10	No sclerotia
KRA2	Brownish yellow	Dark yellowish brown	Slightly irregular	Appressed	Slow	1.5	++	46	Present	7	4.13	
PRA1	Dark yellowish brown	Dark yellowish brown	Regular	Appressed	Fast	4	++	90	Absent	2	4.9	
PRA2	Yellowish brown	Dark yellowish brown	Slightly irregular	Appressed	Slow	1.7	++	54	Present	5	3.77	

+++ High   ++ Moderate   + Low

4.3.10. Days for Sclerotia Initiation

The days taken for sclerotia initiation in the culture was ranging from three to four days under room temperature (28$\pm$2 ^{0}C). *R. solani* isolates in rice took three days for initiation of sclerotial bodies under laboratory conditions. Similarly, the vegetable cowpea isolates also showed initiation of sclerotia within 3- 4 DAI except TRC1 (Vellayani) and KRC2 (Pooapally) where there was no sclerotia present. None of the amaranth isolates showed production of sclerotial bodies under artificial conditions.

4.4. STUDYING THE MORPHOLOGICAL VARIABILITY OF THE ISOLATES

Microscopic as well as macroscopic characters such as sclerotial characters and mycelial compatibility were studied. Microscopic characters showed variability in mean hyphal width and shape of moniliod cells (Table 9). There was considerable variation in size, texture, shape, weight, diameter, number, orientation, position, colour, clump formation and exudation in the culture of sclerotial bodies produced by the isolates (Table 10). Mycelial compatibility between cultures was tested with macroscopic vegetative reactions and microscopic hyphal anastomosis.

4.4.1. Microscopic Variability

Light microscopic studies revealed that all the isolates of *R. solani* obtained from rice, vegetable cowpea and amaranth showed mycelia branching at right angle in the distal end of the cell. Mycelium of most of the isolates showed branching at nearly 90°. In addition, all the isolates also showed a characteristic constriction at the point of branching. Septation was found at the point of branching or adjacent to branching (Plate. 14). Clamp connection was absent in all the mycelium of the isolates. Presence of moniliod cells was found in all the isolates and this act as an important taxonomic key in the identification of *R. solani*. The isolates showed significant difference in the microscopic characters such as width of hypha and shape of moniliod cells.

TRR1	TRR2	KRR1	KRR2	PRR1	PRR2
TRC1	TRC2	KRC1	KRC2	PRC1	PRC2
TRA1	TRA2	KRA1	KRA2	PRA1	PRA2
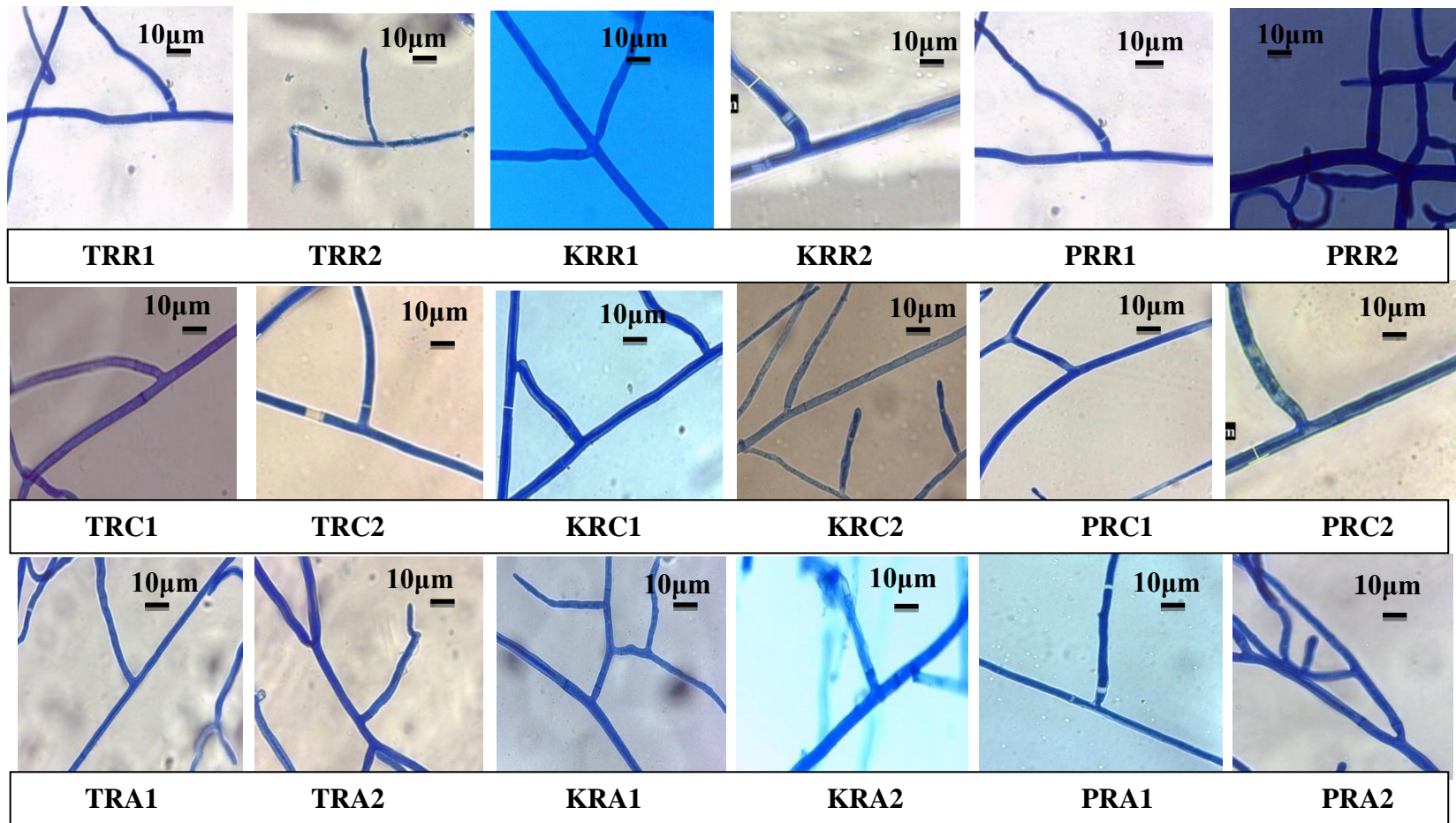
Plate 14. Hyphal characters of *R. solani* isolates from rice, vegetable cowpea and amaranth (400X)
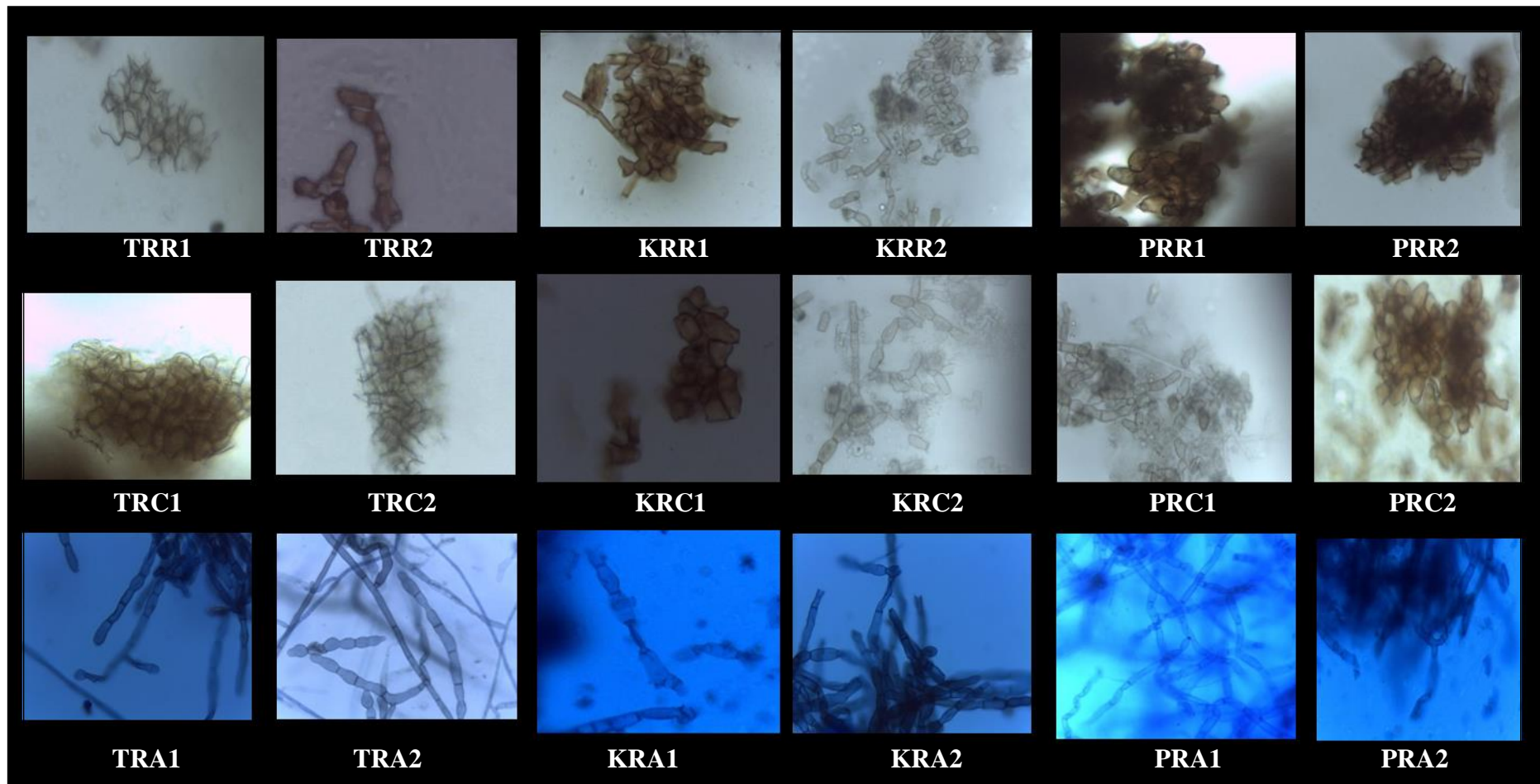
Plate 15. Moniliod cells of _R. solani_ isolates of rice, vegetable cowpea and amaranth (400X)

Table 9. Variations in the morphological characters of *R. solani* isolates from rice, vegetable cowpea and amaranth

Isolate	Mean hyphal width (µm)	Constriction at the point of branching	Shape of moniliod cells	Septation on hypha
TRR1	4.04	Present	Barrel	present
TRR2	3.81	Present	Irregular	present
KRR1	4.4	Present	Barrel	present
KRR2	4.42	Present	Barrel	present
PRR1	4.52	Present	Barrel	present
PRR2	6.95	Present	Barrel	Present
TRC1	4.32	Present	Irregular	present
TRC2	3.75	Present	Irregular	present
KRC1	5.03	Present	Irregular	present
KRC2	6.24	Present	Barrel	present
PRC1	6.04	Present	Barrel	present
PRC2	5.59	Present	Barrel	Present
TRA1	4.80	Present	Barrel	present
TRA2	4.20	Present	Barrel	present
KRA1	4.70	Present	Barrel	present
KRA2	5.90	Present	Barrel	present
PRA1	5.50	Present	Barrel	present
PRA2	5.70	Present	Barrel	present

4.4.1.1. Hyphal Width

Mean hyphal width of the culture was recorded and it ranged from 3.81 to 6.95 µm. Among the isolates, TRR2 (Chirayinkeezh) was showing the lowest hyphal width and PRR2 (Thiruvalla) was having the highest hyphal width.

4.4.1.2. Shape of Moniliod Cells

In addition to ordinary vegetative hyphae, *R. solani* produces simple or branched chains of short broad cells sometimes hyaline or brown, barrel shaped, pyriform, irregular or lobate known as monilioid cells in the cultures. Presence of moniliod cells were found in every isolate and shape of the moniliod cells were varied among the isolates. TRR2 (Chirayinkeezh), TRC1 (Vellayani), TRC2 (Chirayinkeezh) and KRC1 (Kottarakkara) isolates produced irregular moniliod cells in the culture and rest of the isolates produced barrel shaped moniliod cells (Plate 15).

4.4.2 Sclerotial Characters

4.4.2.1. Size of Sclerotia

There was a significant difference in the size of sclerotia among the isolates. TRR2, PRR1, TRC2, KRC1 and PRC2 produced bigger sclerotia whereas TRR1, KRR1, KRR2 and PRR2 produced smaller sclerotia. Diameter of single sclerotia was ranging from 1.0 to 3.2 mm. The isolate PRC2 produced the biggest sclerotia with a diameter of 3.2 mm and the smallest one was produced by KRR1 (1.0 mm). Most of the sclerotial bodies produced by the vegetable cowpea cultures were found bigger compared to rice isolates. Among the rice isolates, PRR1 recorded the highest sclerotial diameter of 2.8 mm. The isolate TRC2 was having lowest sclerotial diameter (2.0 mm) among vegetable cowpea isolates. No sclerotial bodies were produced by the isolates TRC1 and KRC1 under laboratory conditions.

4.4.2.2. Texture of Sclerotia

The texture of the sclerotial bodies showed great variation and it ranged from smooth to rough in culture. Isolates such as TRR1, KRR1 and KRR2 produced smooth

sclerotia whereas; TRR2, PRR1, PRR2, TRC2, KRC1, PRC1 and PRC2 produced rough sclerotia. Most of vegetable cowpea isolates produced rough bordered sclerotia. Rice isolates produced both smooth and rough bordered sclerotia in the cultures (Plate 16).

4.4.2.3 Shape of Sclerotia

Variation in the shape of sclerotial bodies was also found among the isolates. They were oval, round or irregular in shape. Rice isolate TRR1 (Vellayani) produced oval shaped sclerotia whereas round shaped sclerotia was produced by the cowpea isolate TRC2 (Chirayinkeezh). Rest isolates of *R. solani* such as TRR2, KRR1, KRR2, PRR1, PRR2, KRC1, PRC1 and PRC2 produced irregular shaped sclerotia.

4.4.2.4. Colour of Sclerotia

Colour of the sclerotia was determined according to the dominant spectral colour present in the Munsell's colour chart. Initially the sclerotia showed cream colour, later it changed to different shades of brown. Colour of mature sclerotia varied from dark olive brown (10 YR 3/3), dark yellowish brown (10 YR 4/4), dark reddish brown (5 YR 3/4) and dark brown (10YR 3/3). Most of the cultures were having dark olive brown sclerotia (KRR1, PRR1, KRC1 and PRC2) followed by dark brown (TRR1, TRR2 and TRC2), dark yellowish brown (KRR2 and PRC1) and dark reddish brown (PRR1).

4.4.2.5 Orientation of Sclerotia

Peripheral (TRR1), central (TRR2, TRC2 and PRC2), scattered (KRR1), central and peripheral (KRR2, PRR1, PRR2, KRC1 and PRC1) were the type of sclerotial arrangement seen in the culture. In peripheral arrangement, sclerotia were arranged at the periphery of the petri plate. The sclerotial bodies were conglomerated in the centre of the petri plate in central arrangement. In peripheral and central arrangement, sclerotia can be found in both these positions.

4.4.2.6. Position of Sclerotia

According to the position of the sclerotia in the culture, it was divided as aerial and embedded. In aerial type, sclerotia were visible in the superficial position of the

culture. In embedded type, sclerotia present mostly in between the mycelial mat. Aerial type of sclerotia was found in TRR1, TRR2, KRR2, PRR1, PRR2, TRC2, KRC1, PRC1 and PRC2. KRR1 produced embedded sclerotia in the culture. All vegetable cowpea isolates produce aerial sclerotia in the culture.

4.4.2.7. Number of Sclerotia

Sclerotial number in the culture was ranging from 15 to 230. Minimum number of sclerotia was produced by the isolate PRR2 (Thiruvalla) and maximum number was seen in KRR1 (Kottarakkara) among the rice isolates. PRC1 (Ranni) was having lowest number of sclerotia and PRC2 (Thiruvalla) was having highest number of sclerotia among vegetable cowpea cultures. They were grouped into good (TRR2, PRR2, TRC2, PRC1 and PRC2), very good (KRC1) and excellent (TRR1, KRR1 and PRR1) sclerotia forming cultures. Most of the rice cultures produced sclerotia significantly higher than vegetable cowpea isolates in the culture.

4.4.2.8. Clump Formation

Sclerotia usually occur as aggregations in the culture and such structures are called as clumps. Based on the aggregation pattern of sclerotia, the cultures were divided into high, moderate and low clump forming cultures. TRR1 and PRR1 sclerotia were showing high clump forming pattern. TRR2, KRR2, TRC2, KRC1, PRC and PRC2 produced moderate clump forming sclerotia whereas KRR1 and PRR2 were low clump forming.

4.4.2.9. Exudation on Sclerotia

On the top of the sclerotia, liquid secretion usually called as honey dew may be present. Presence of honey dew secretion was recorded after 15 days incubation. All sclerotia producing cultures of rice and vegetable cowpea exhibited honey dew secretion on it. However, vegetable cowpea isolates TRC1 and KRC1 do not secret honey dew in the culture. Honedew secretion was also absent in all amaranth isolates.
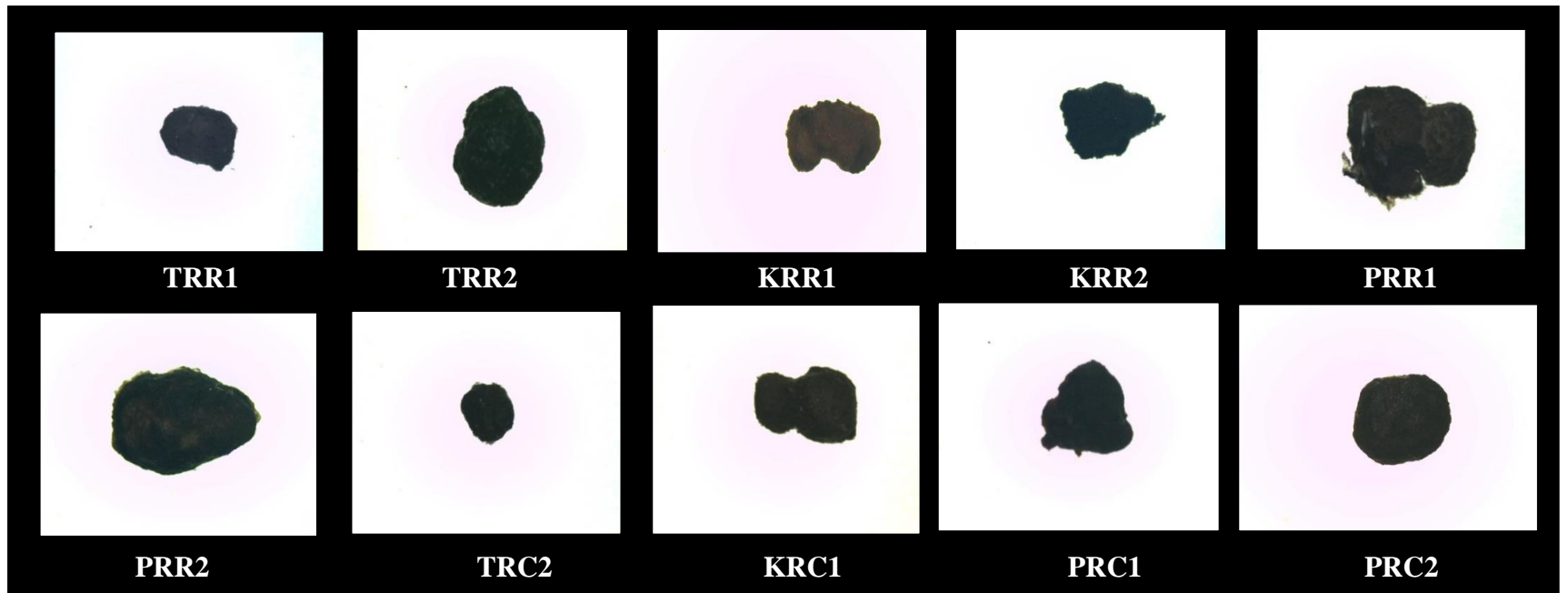
Plate 16.Variation in shape and texture of sclerotia forming *R. solani* isolates of rice and vegetable cowpea under stereo microscope (100X)

Table 10. Sclerotial characters of *R. solani* isolates of rice, vegetable cowpea and amaranth

Isolate	Size	Texture	Shape	Number / plate	Weight (mg)[*]	Orientation	Position	Colour of sclerotia	Clump formation
TRR1	Small	Smooth	Oval	91	4.0	Peripheral	Arial	Dark brown	More
TRR2	Large	Rough	Irregular	20	9.0	Central	Arial	Dark brown	Moderate
KRR1	Small	Smooth	Irregular	230	2.0	Scattered	Embbeded	Dark olive brown	Less
KRR2	Small	Smooth	Irregular	80	4.0	Central& peripheral	Arial	Dark yello-wish brown	Moderate
PRR1	Large	Rough	Irregular	62	6.0	Central& peripheral	Arial	Dark olive brown	More
PRR2	Small	Rough	Irregular	15	3.0	Central& peripheral	Arial	Dark reddish brown	Less
TRC2	Large	Rough	Round	4.0	23	Central	Arial	Dark brown	Moderate
KRC1	Large	Rough	Irregular	9.0	25	Central & peripheral	Arial	Dark olive brown	Moderate
PRC1	Large	Rough	Irregular	10.0	20	Central & periphery	Arial	Dark yellowish brown	Moderate
PRC2	Large	Rough	Irregular	9.0	35	Central	Arial	Dark olive brown	Moderate
Sclerotial formation was absent in rest isolates									

* Mean of 10 observations

4.4.2.10. Weight of Sclerotia

Weight of single sclerotia in the culture was ranging from 2 to 10 mg. Ranni vegetable cowpea isolate PRC1showed the maximum weight of 10 mg whereas, the Kottarakkara rice isolate KRR1 recorded the minimum weight of 2 mg.

4.4.3. Macroscopic Vegetative Compatibility between Isolates

Macroscopic vegetative compatibility was analyzed using dual culture experiment. The culture combinations showed type of compatibility like merge, merging line, tuft, barrage and barrage line. Out of the 171 macroscopic reactions, merge reactions were found most common followed by barrage line and merging line combination (Plate 17).

4.4.4. Microscopic Hyphal Anastomosis Reactions between Isolates

Microscopic hyphal anastomosis reactions were analyzed using cellophane technique and observed that reactions were categorised as C0, C1, C2 and C3. In C0, there was no observable reaction between the hyphae. Hyphal contact and apparent connection of walls is visible in C1. In C2 reaction, wall connection and a pore plus death of the anastomosing hyphae and adjacent cells is observed. In C3, there was a fusion of wall and membrane without the death of anastomosing hyphae.

Out of the 171 combinations analysed, C2 reactions were the most commonly found followed by C3 reactions. C3 reaction was vegetatively compatible; and C0, C1 and C2 reactions were vegetatively incompatible. There was less anastomosis reaction between amaranth and rice isolates; and amaranth and vegetable cowpea isolates (C0 and C1). More hyphal anastomosis reactions were present between rice and vegetable cowpea isolates and within the anastomosis reaction between same hosts *i.e,* rice and rice, vegetable cowpea and vegetable cowpea, and amaranth and amaranth (C2 and C3) (Plate 18).

| a. Merge | b. Merging line | c. Tuft | d. Barrage | e. Barrage line |
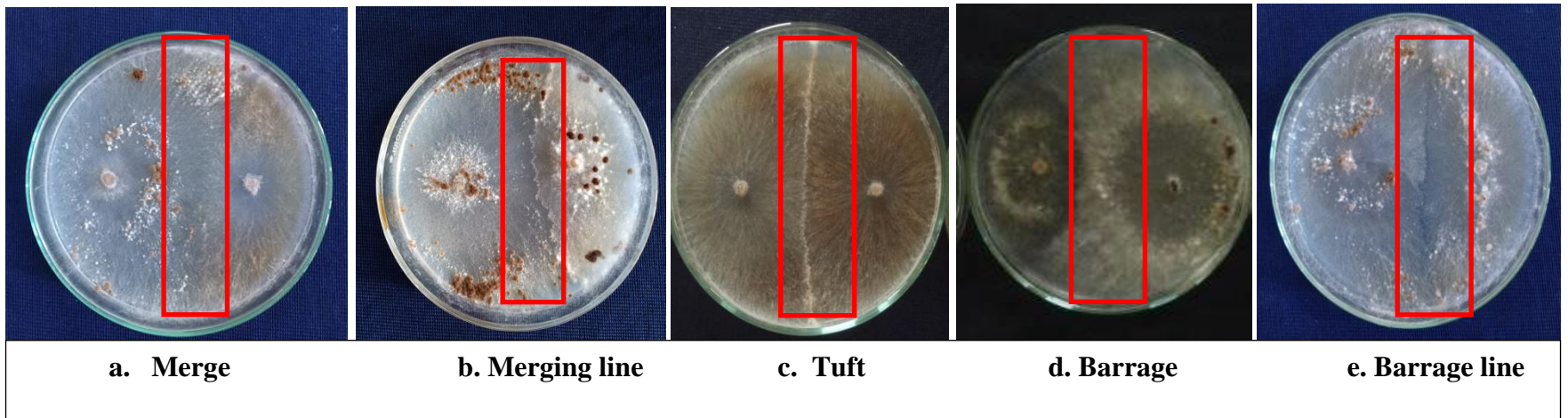
Plate 17 a – e. Macroscopic vegetative compatibility reaction between isolates of *R. solani* on 2% agar medium
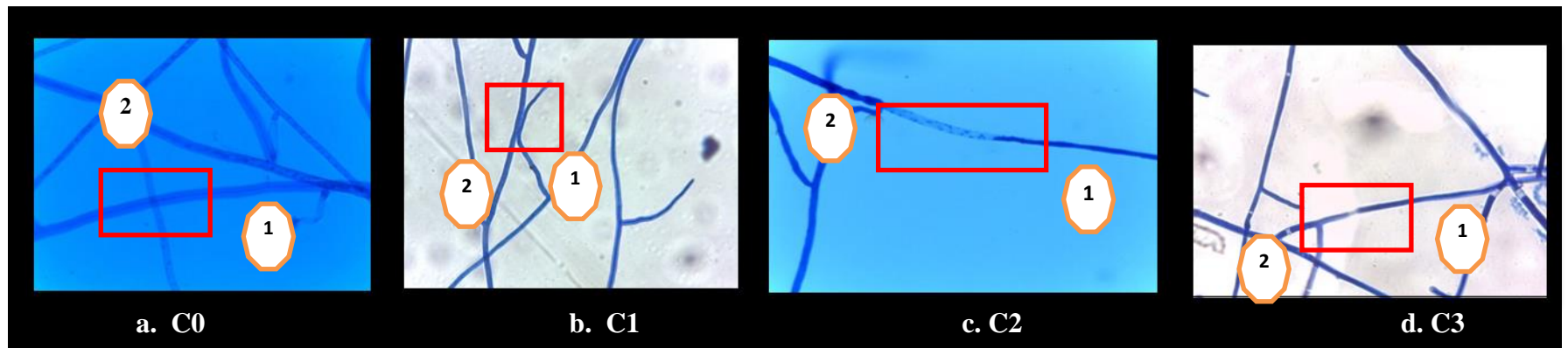
Plate 18 a-d. Microscopic hyphal anastmosis reaction between *R. solani* isolates a. C0-no reaction; b. C1-contact fusion; c. C2-imperfect fusion; d. C3-perfect fusion

4.5. STUDYING THE PATHOGENIC VARIABILITY OF THE ISOLATES

The ability of the isolates to cause infection in amaranth was studied by pot culture experiment. All isolates from rice, vegetable cowpea and amaranth were artificially inoculated on Arun variety of amaranth by laying out CRD experiment with 18 treatments and three replications (Plate 19).

R. solani from different hosts exhibited variation in their pathogenicity (Table 11). All isolates produced symptoms on amaranth plants within 2-4 days after inoculation. There was variation in the number of lesions, size of lesion and PDI. All the rice isolates produced leaf blight symptom within 2-4 days of inoculation. Similarly, vegetable cowpea isolates took only 2-3 days for producing symptom on amaranth. Amaranth isolates produced leaf blight symptom within 2-4 days after inoculation.

The lesion number produced on amaranth plant was ranging from 3 to 12. Rice isolates produced 3-8 lesions on amaranth plant and amaranth isolates produced 3-9 lesions on the leaves. Vegetable cowpea isolates were found to produce highest number of lesions (8-12) on amaranth leaves on artificial inoculation.

Lesion size produced by the isolates on amaranth plant was ranging from 0.2-5 x 0.2-4.2 cm. Among rice isolates, KRR1 (Kottarakkara) produced the largest lesions (5 x 4.2 cm). Among the vegetable cowpea isolates, PRC2 (Thiruvalla) produced the biggest lesion (4.3 x 2.7cm) whereas among the amaranth isolates, KRA1 produced bigger sized lesion (2.2 x 1.2 cm) (Plate 20). Leaf blight severity was ranging from 22.22 to 58.33 per cent. It was found that, PRA1 (Ranni) was more virulent amaranth isolates of *R. solani* on amaranth (55.55%). PRR2 (Thiruvalla) was found more virulent among rice *R. solani* isolates on amaranth (46.66%). PRC1 (Ranni) was more virulent among vegetable cowpea *R. solani* isolates on amaranth (58.33%). Among all isolates of *R. solani* artificially inoculated on amaranth plant, vegetable cowpea isolate PRC1 was most virulent, followed by PRA1 and KRC2. Pooyapally amaranth isolate KRA2 was having PDI of 22.22 per cent and produced leaf blight symptom on three DAI. KRA2 was found to be the least virulent on amaranth among all isolates. Among the vegetable cowpea

Plate 19. Layout of experiment to study pathogenic variability of eighteen *R. solani* isolates on amaranth
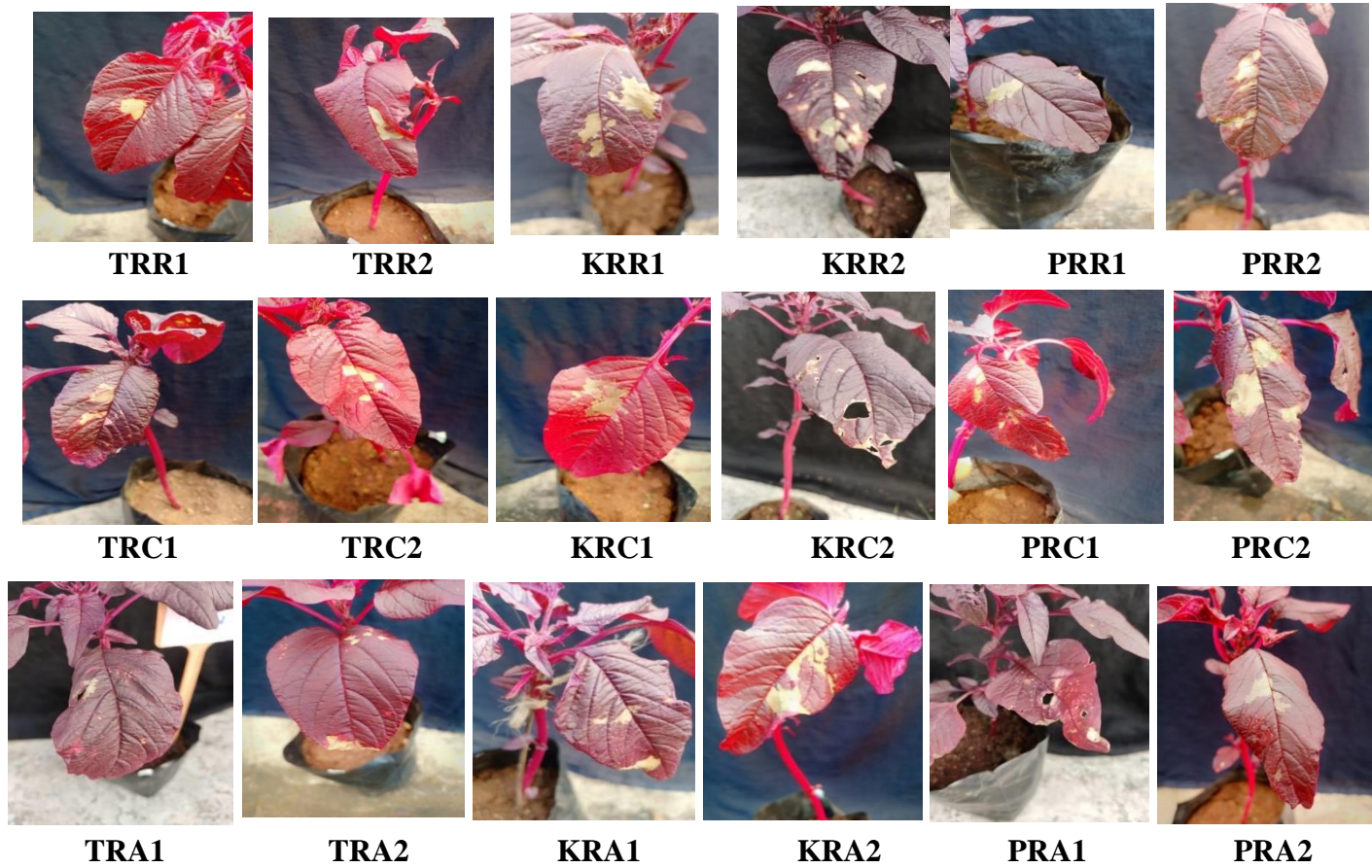
Plate 20. Symptom development on artificial inoculation by rice, vegetable cowpea and amaranth isolates of *R. solani* on amaranth

Table 11. Symptom development by *R. solani* isolates of rice, vegetable cowpea and amaranth isolates on amaranth

Isolate	Symptom on amaranth	Days for symptom development	No. of lesions	Size of lesion (lXb in cm)	PDI (%)
TRR1	Yes	2	4	0.3-2.1 X 0.3-1.3	34.28 (35.837)[g]
TRR2	Yes	3	6	0.3-3.4 X 0.2-2.5	30.86 (33.745)[h]
KRR1	Yes	2	6	0.7-5 .0X 0.6-4.2	44.44 (41.808)[e]
KRR2	Yes	3	8	0.7-3.1 X 0.6-0.8	31.11 (33.898)[h]
PRR1	Yes	4	3	0.3-1.4 X 0.2-0.7	33.33 (35.261)[g]
PRR2	Yes	2	7	1.2-2.5 X 0.65-1.2	46.66 (43.085)[d]
TRC1	Yes	2	8	0.7-3.5 X 0.5-2.2	43.20 (41.091)[e]
TRC2	Yes	2	10	0.4-2.5 X 0.5-2.2	50.00 (45.000)[c]
KRC1	Yes	3	8	0.4-2.5 X 0.3-1.7	37.77 (37.921)[f]
KRC2	Yes	3	11	0.4-1.3 X 0.3-0.8	55.55 (48.187)[b]
PRC1	Yes	2	9	0.8-2.6 X 0.7-0.8	58.33 (49.795)[a]
PRC2	Yes	3	12	0.9-4.3 X 0.4-2.7	47.22 (43.406)[d]
TRA1	Yes	4	3	0.3-1.5 X 0.2-1.2	33.33 (35.262)[g]
TRA2	Yes	3	5	0.3-2.4X 0.2-0.7	33.33 (35.260)[g]
KRA1	Yes	3	3	0.2-2.2X 0.2-1.2	33.33 (35.255)[g]
KRA2	Yes	3	7	0.4-2.5 X 0.3-2.3	22.22 (28.121)[j]
PRA1	Yes	3	9	0.6-1.5X 0.5-1.2	55.55 (47.995)[b]
PRA2	Yes	4	8	0.3-3.2 X 0.2-2.6	26.98 (31.293)[i]
C.D(p=0.05)					0.853
S.E (M)±					0.297
Values in parenthesis are arcsine transformed					

isolate, KRC2 was least virulent producing PDI of 37.77 per cent on 3rd day. TRR2. (Chirayinkeezh) was least virulent among rice isolates which produced PDI of 30.86 per cent on three days after inoculation

4.6. STUDYING THE MOLECULAR VARIABILITY OF THE ISOLATES BY RAPD ANALYSIS

4.6.1. DNA Isolation

Genomic DNA was isolated using QIAGEN DNeasy plant mini kit from one week old *R.solani* isolates which were grown in PD Broth.

4.6.2. RAPD Analysis

Genetic relatedness *of R. solani* isolates of rice, vegetable cowpea and amaranth was assessed with Random Amplified Polymorphic DNA analysis. RAPD analysis was carried out by using 4 oligonucleotide random primers namely OPA 04 (AATCGGGCTG), OPA 19 (CAAACGTCGG), OPA 20 (GTTGCGATCC) and OPW 13 (CACAGCGAACA)(Table 4). All the primers yielded PCR products (Plate 21). A total of 75 polymorphic and scorable bands ranging approximately as low as 250 bp to as high as 3000 bp was generated with these 4 primers. These primers were very informative that they could well display the genetic polymorphism among the *R. solani* isolates.

OPA 04 produced 21 RAPD bands of size ranging from 250 to 1000 bp (0.25-1.0 kb). There was monomorphic band found in the amplified product which indicated cent per cent polymorphism with this primer. OPA 19 produced 18 RAPD bands of size ranging from 250-3000 bp (0.25-3.0 kb). As there were no monomorpic bands present, this primer also gave cent per cent polymorphism. OPA 20 produced 16 RAPD bands of size ranging from 250-1500 bp (0.25-1.5 kb). This primer also provided cent per cent polymorphism due to the absence of monomorphic bands. OPW 13 produced 20 RAPD bands of size ranging from 200-2000 bp (0.2-2.0 kb). This primer also exhibited cent per cent polymorphism as monomorphic bands are absent. All four primers were highly discriminative and provided higher number of bands. Polymorphism Information Content (PIC) value of the primers were ranging from 0.17 to 0.25. In these selected primer PIC

value was found to be higher in OPA 19. So primer OPA 19 was more informative with higher polymorphism content among all the seleted primers.

Out of the 57 RAPD bands scored, cent per cent polymorphism was seen as there was no monomorphic band present. There was no band found common among all isolates. The similarity values of RAPD profile ranged from 0.59 to 0.92 (Table 12).

4.6.3. Genetic Similarity and Cluster Analysis

Each amplified bands were considered as RAPD marker. Based on the presence or absence, bands were scored using binary digits '0' and' 1'and data were entered using a matrix. The bivariate data thus obtained were then analysed to generate Jaccard's similarity coefficient for pair wise comparison.   Statistical analysis was done for the results obtained with the primers using NT SYSpc 2.2 version.

A dendrogram was generated by Sequence Agglomerative Hierarchial Nested (SAHN) subroutine clustering using Jaccrad similarity coefficient. The estimated similarity of the isolates with the primer ranged from 59 to 92 per cent reflecting wide variability among the isolates of rice, vegetable cowpea and amaranth from different collection locations of Southern Kerala at their molecular level. The maximum similarity value (0.92) was obtained between the rice isolates TRR1 and KRR1 which belongs to Vellayani and Kottarakkara locations.

Based on the The Unweighted Pair Group Arithmetic Mean method (UPGMA) analysis, all the isolates were divided into two major clusters namely A and B at a similarity of 70 per cent. The first group contains 15 isolates, which is further constituted to two subgroups A1 and A2. Subgroup A1 contained 13 isolates namely TRR1, KRR1, PRR2, KRR2, KRA1, TRA1, TRC2, PRR1, TRR2, TRC1, PRA1, TRA2, KRA2 and PRA2 which again divided into several clusters. Isolate KRC1 formed a separate cluster as A2 under major cluster A. The second group B contained 3 isolates which is again divided into subcluters B1 and B2. KRC2. Vegetable cowpea isolate KRC2 formed a separate cluster as B1 under major cluster B. The subgroup B2 contained isolates namely PRC1 and PRC2. There was a difference in the clustering of isolates from the same hosts.

M-1kb ladder,
Lane 1-18: 18 isolates of *R. solani*

a

M-1kb ladder,
Lane 1-18: 18 isolates of *R. solani*

b

M-1kb ladder,
Lane 1-18: 18 isolates of *R. solani*

c

M-1kb ladder,
Lane 1-18: 18 isolates of *R. solani*
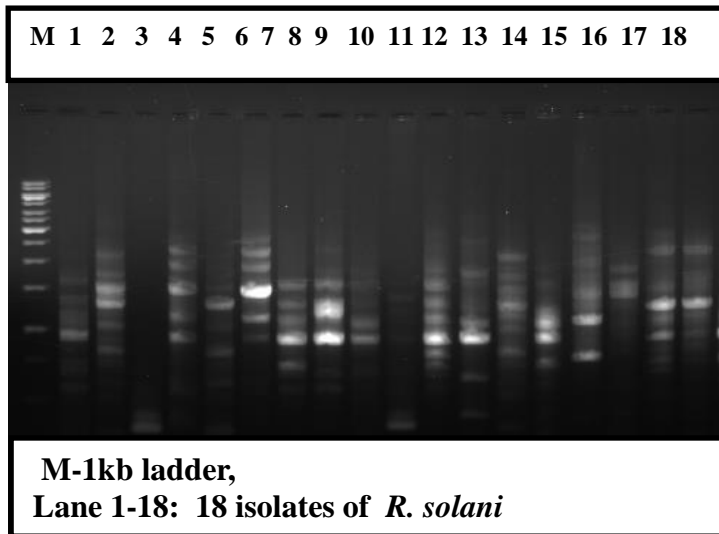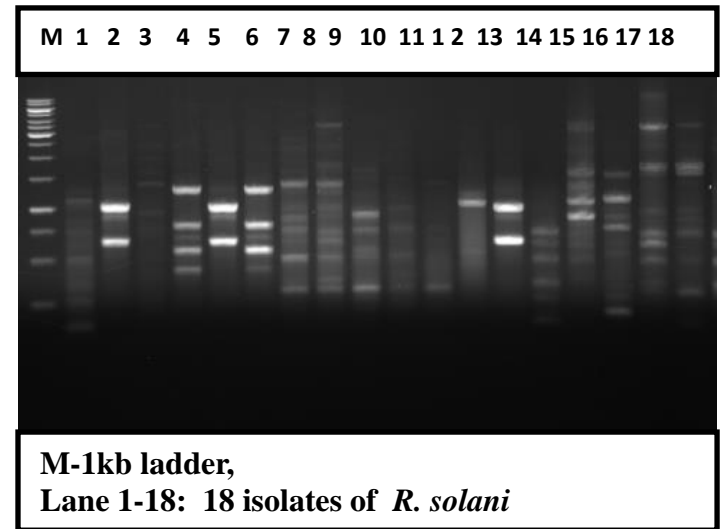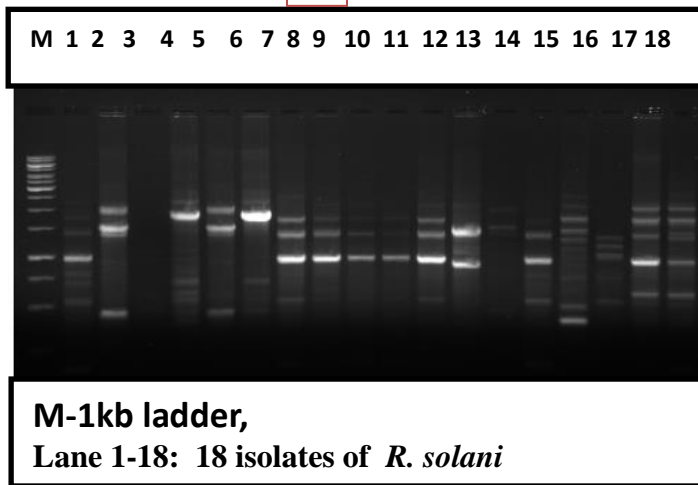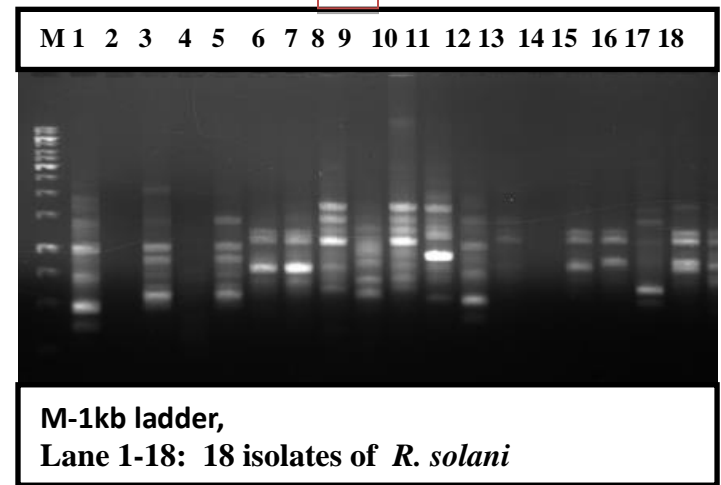
d

Plate 21a-d. RAPD banding pattern of 18 *R. solani* isolates from rice, vegetable cowpea and amaranth with the oligonucleotide primers. a) OPW 13; b) OPA 19; c) OPA 20; d) OPA 04

Table 12. Jaccard's similarity coefficient of 18 isolates of *R. solani* from rice, vegetable cowpea and amaranth based on DNA bands characters

	TRR1	TRR2	KRR1	KRR2	PRR1	PRR2	TRC1	TRC2	KRC1	KRC2	PRC1	PRC2	TRA1	TRA2	KRA1	KRA2	PRA1	PRA2
TRR1	1.00																	
TRR2	8.55	1.00																
KRR1	9.21	8.03	1.00															
KRR2	8.16	8.03	8.68	1.00														
PRR1	8.29	7.89	8.29	8.03	1.00													
PRR2	8.16	7.50	8.95	8.42	8.29	1.00												
TRC1	7.76	7.37	8.29	8.29	7.37	8.03	1.00											
TRC2	8.16	7.24	8.68	8.16	8.03	8.16	7.24	1.00										
KRC1	6.97	6.84	7.24	7.24	7.11	7.50	6.58	7.24	1.00									
KRC2	7.50	6.84	8.03	7.76	7.37	7.76	7.37	6.97	7.11	1.00								
PRC1	6.97	6.58	6.97	6.97	6.58	6.45	6.58	6.45	7.11	7.37	1.00							
PRC2	7.11	7.24	7.37	7.89	6.71	6.84	6.97	6.84	6.97	7.50	8.29	1.00						
TRA1	8.29	7.89	8.82	8.82	8.16	8.29	8.16	8.03	7.11	7.63	6.84	7.50	1.00					
TRA2	7.11	6.71	7.63	7.89	7.24	7.63	7.76	7.37	6.71	6.71	5.92	6.32	7.76	1.00				
KRA1	8.16	8.03	8.68	8.95	8.03	8.68	8.29	8.16	7.50	7.76	6.97	7.63	8.82	8.42	1.00			
KRA2	7.37	6.71	7.63	7.37	7.50	7.63	6.71	7.63	7.24	6.71	5.92	5.79	7.24	7.63	7.63	1.00		
PRA1	7.63	7.24	7.89	8.16	7.50	7.89	8.55	7.37	6.71	7.24	6.97	6.84	8.29	8.16	8.68	6.84	1.00	
PRA2	7.50	6.84	7.50	7.50	6.84	6.97	6.84	6.97	7.11	6.84	6.32	6.45	7.37	6.45	7.24	8.03	6.71	1.00

Discussion

5. DISCUSSION

Rhizoctonia solani Kuhn. (Teleomorph: Thanatephorus cucumeris (Frank) Donk) is an omnipresent pathogen having a wide host spectrum and distributed worldwide causing severe yield losses. This pathogen plays a major constraint in the cultivation of economically important crops in Kerala. Sheath blight, web blight and collar rot, and leaf blight are the diseases caused by *R. solani* in rice, vegetable cowpea and amaranth respectively. Although previous studies have addressed the management of the disease, a knowledge gap remains for understanding of variability among the Rhizoctonia isolates infecting the major crops in Kerala. Various results obtained in the present study entitled "Studies on the variability of *Rhizoctonia solani* Kuhn. infecting rice, cowpea and amaranth" are discussed below.

5.1. SYMPTOMATOLOGY OF THE DISEASE

Rice, vegetable cowpea and amaranth infected by *R. solani* were collected from Vellayani and Chirayinkeezh in Thiruvananthapuram district; Kottarakkara and Pooyapally in Kollam district; Ranni and Thiruvalla in Pathanamthitta district (Fig. 1-4) (Plate 1-3). The disease was prevalent in all the surveyed locations but the disease incidence and severity varied from location to location. Percent disease index and disease incidence was ranging from 11.11 to 99.99 per cent and 18.0 to 92.0 per cent in rice tracts, 33.33 to 77.77 per cent and 32.0 to 88.0 per cent in vegetable cowpea fields and 11.11 to 77.77 per cent and 30.0 to 98.0 per cent in amaranth fields respectively. Various factors like heavy rain, temperature, soil moisture, host genotype and virulence spectrum of the pathogen influenced the symptom development which resulted in variation in disease incidence and disease index in different survey locations. Taheri *et al.* (2007) stated that geographical area is responsible for the variation among rice *R. solani* isolates and not the host cultivar. Disease severity alteration at different locations due to the pathogenic variability has been reported in maize *R. solani* isolates (Akhtar *et al.*,

Fig. 1: Different districts selected for survey of *R. solani* infection from rice, vegetable cowpea and amaranth in Kerala

Chirayinkeezh (8.666165 N, 76.79173E)
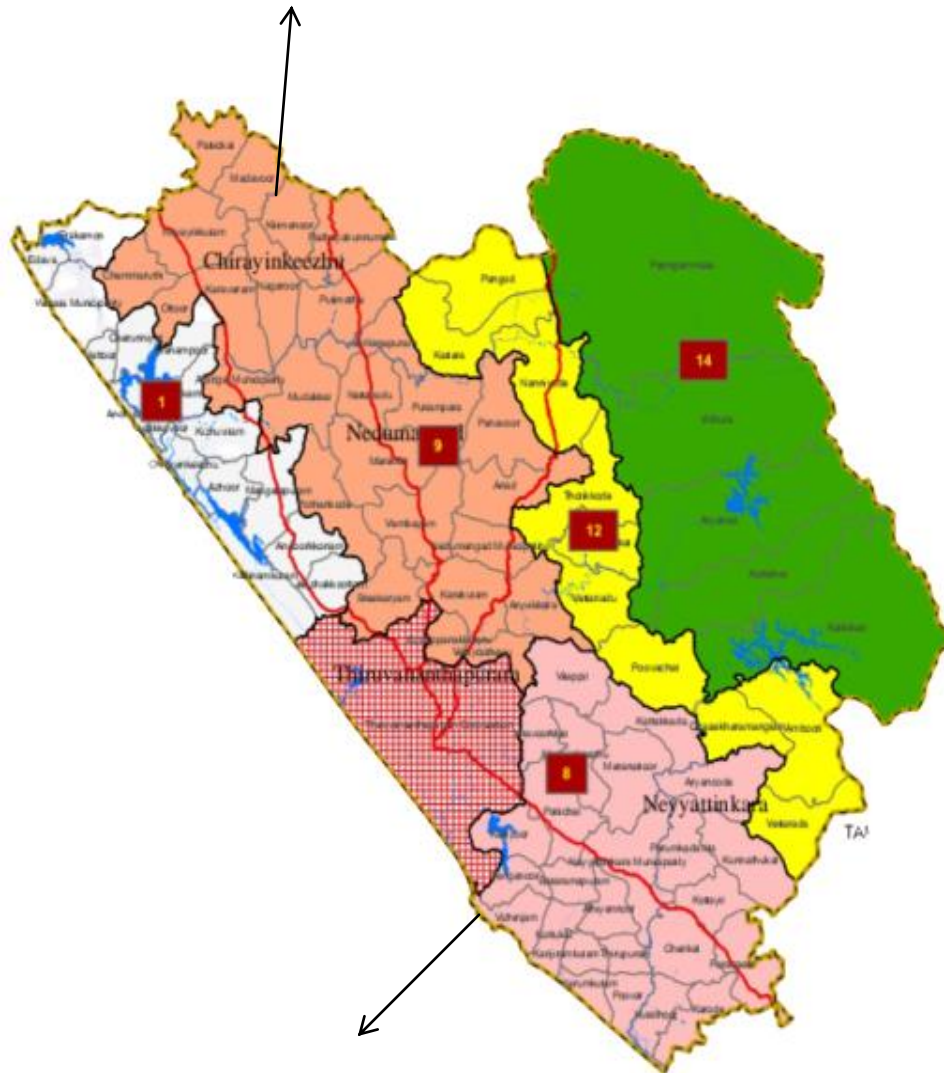
Vellayani (8.4294 N , 76.98736 E)

Fig. 2: Different survey locations for collection of diseased samples of rice, vegetable cowpea and amaranth plants infected with *R. solani* in Thiruvananthapuram district with GPS data

Kottarakkara (8.982463N, 76.8086083E)
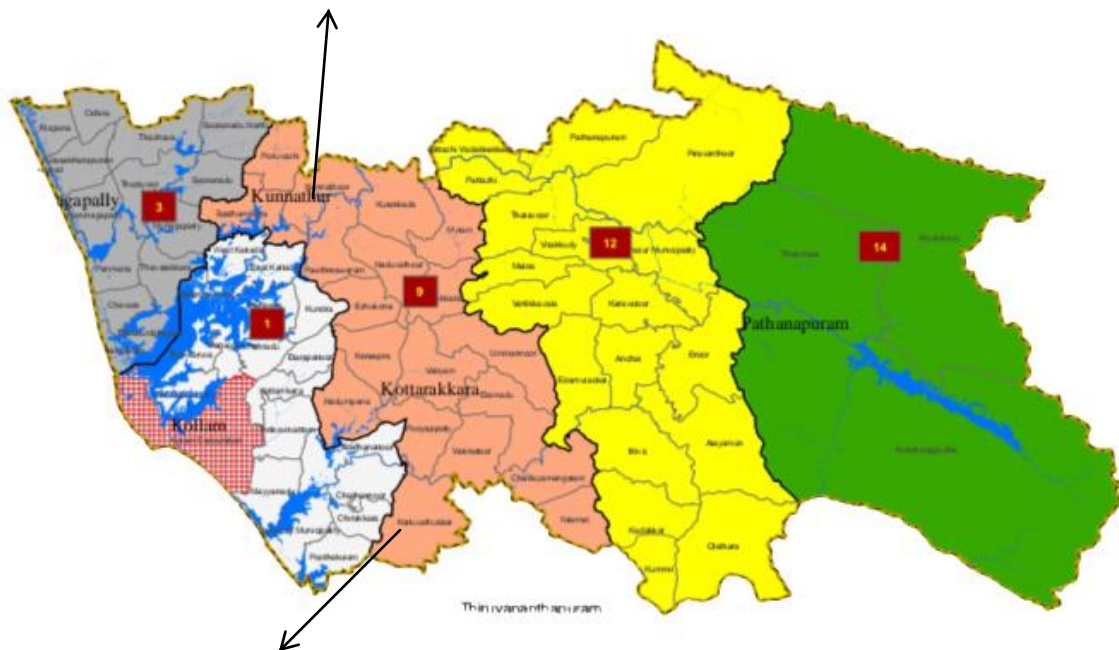
Pooyapally (8.9074N, 76.7627E)

Fig. 3: Different survey locations for collection of diseased samples of rice, vegetable cowpea and amaranth plants infected with *R. solani* in Kollam district with GPS data

Thiruvalla (9.383452 N, 76.578702 E)

Ranni (9.3856633N, 76.574059 E)

Fig. 4: Different survey locations for collection of diseased samples of rice, vegetable cowpea and amaranth plants infected with *R. solani* in Pathanamthitta district with GPS data

2009). High relative humidity, less temperature and water stagnation are the reasons for the disease spread; and cultivation of susceptible crop varieties continuously on the same field increased the chance of pathogen perpetuation (Parashuram *et al.*, 2017).

R. solani can infect different plant parts at different stages. It can cause infection in leaf, leaf sheath, collar region, shoot, pods and seeds. Various stages of the plants like active tillering stage, panicle initiation stage, late tillering stage, internodes elongation stage, booting and flag leaf emergence stage in rice, seedling stage, vegetative stage, flowering stage and pod development stage in vegetable cowpea and vegetative stage, flowering stage and maturation stage in amaranth were infected by this pathogen. Among these stages, the most susceptible stage is active tillering stage in rice, seedling stage in vegetable cowpea and vegetative stage in amaranth. Adhipathi *et al.* (2013) reported most severe stage of sheath blight in rice as late tillering, flag leaf emergence, booting and intermodal elongation. Vavilapalli *et al.* (2014) observed most severe stage of web blight in vegetable cowpea at vegetative stage and collar rot at seedling stage which is similar to the present study. According to Karthika (2017), active tillering stage is the most susceptible stage for the occurrence of infection in rice which is with the confirmation of the present study.

Various symptoms were expressed by *R. solani* isolates under natural conditions. In each host there was variation in nature of symptoms. In rice, sheath blight symptom appeared as oval or irregular shaped, discoloured mostly as pale greenish-grey to greenish white centered water soaked lesions with light brown to dark brown margin as explained by Dasgupta (1992). Later leads to the girdling of leaf sheath, culm, boot and flag leaf and the tiller is succumbed to death. In vegetable cowpea, the collar rot symptom appeared as brownish black lesions in the collar region leading to girdling of stem followed by yellowing of leaves. Web blight appeared as small circular greyish brown spots on the foliage surrounded by water soaked areas. The spots enlarged to form lesions with oblong to irregular shape and webbing of leaves. Later the lesions on the leaves resulted in severe foliar blighting (Viswanathan and Viswambharan, 1979). In addition to this, web blight symptom can also cause seedling mortality, pre and post

emergence mortality, seed decay. The mycelium on the infected leaves shows spider web pattern (Shaibala and Tripati, 2007).

Leaf blight symptom on amaranth appeared as greenish, grey or cream coloured spots or lesions with irregular shape which gradually coalesces under humid condition. Symptoms of leaf blight on amaranth was studied by Gokulapalan *et al.* (2000) and observed similar type of irregular greenish, grey or cream coloured spots or lesions. In severe cases, shot hole symptoms appear on the leaves (Gireesh, 2016).

Generally the necrotrophic pathogen *R. solani* diverts the cells of host to undergo programmed cell death (Mondal *et al.*, 2012). During the establishment in the host cells, necrotic spots and lesions are formed due to loss of chlorophyll and programmed cell death. As an initial source of nutrients, pathogen utilizes these necrotic lesions (Basu *et al.*, 2016). This rapid breakdown of tissues is due to the production of cell wall degrading enzymes (CWDEs) or toxins. Presence of non-host specific toxins was reported by Jain and Thaplial (1980) in *R. solani*. Zhou *et al.* (2016) analyzed the virulence of Rhizotonia isolates on maize plants. They observed a positive correlation between both CWDEs and phytotoxins to the virulence of the Rhizoctonia isolates. Invaded cortical tissues subsequently collapsed to form lesions.

5.2. ISOLATION, PURIFICATION OF PATHOGEN, PATHOGENICITY TESTING AND VIRULENCE RATING

In the current study 18 isolates of *R. solani* were collected from the infected samples of rice, vegetable cowpea and amaranth. The pathogenicity and virulence of the isolates were assessed by artificial inoculation on respective hosts. The nature of symptom, days for symptom development, number of lesions and lesion size differ in the respective hosts according to the virulence spectrum of the pathogen. All isolates on respective host produced symptoms within two to three days (Plate 5-7).

By assessing the lesion number, days taken for symptom development, lesion size and PDI, virulence of the isolate was evaluated in respective hosts. PDI of *R. solani*

isolates on artificial inoculation ranged from 10.0 to 30.0 per cent in rice, 22.22 to 44.44 per cent in vegetable cowpea and 11.11 to 55.55 per cent in amaranth. TRR1, KRC1 and TRA2 were more virulent among rice, vegetable cowpea and amaranth isolates respectively. TRR1 was having PDI of 30 per cent and it produced symptom on second day after inoculation. PDI of KRC1 was 44.44 per cent and produced symptom on two days after inoculation. TRA2 was more virulent among *R. solani* amaranth isolate with PDI of 55.55 per cent and it produced symptom on two days after inoculation (Table 7). Variation in the degree of pathogenicity was observed among isolates. Isolates with higher virulence produced more number of lesions and bigger lesions early on artificial inoculation under in vitro condition. Uppala (2007) noticed slight variation in symptom expression by *R. solani* from red and green amaranth on artificial inoculation. Green amaranth is tolerant to leaf blight compared to red amaranth. While in the case of other hosts only tolerant varieties are available. Similar kind of pathogenicity experiments were also done by Guleria *et al.* (2007) on rice, Takegami *et al.* (2004) on cowpea and Gireesh (2016) on amaranth and observed that more virulent cultures can cause severe infection.

High genetic variability may be present among the isolates prevalent in different geographical area which may leads to the variations in the pathogenic reactions. Nuclear status like multinucleate, binucleate and uninucleate conditions of isolates are the reason for the significant difference in virulence among isolates (Zhou *et al.*, 2016).

5.3. STUDYING THE CULTURAL VARIABILITY OF THE ISOLATES

In the present study, there was significant variation in the cultural characters of *R. solani* isolates(Table 8). Permanent white mycelia or other than brown pigmented mycelia were not considered as *R. solani* isolates (Palo, 1926). In this study, colony colour was initially white then turns to different shades of brown. It was observed as yellowish brown, olive yellow, dark yellowish brown, brownish yellow, light brown and light olive brown. Reverse colour of the cultures varied as dark yellowish brown, dark olive brown, light olive brown, dark brown, dark greyish brown and dark reddish brown. Lal *et al.* (2014) also noted colony colour like light brown, yellowish brown, whitish

brown, dark brown and very pale brown in the *R. solani* rice isolates. Pigment production in the culture leads to colour change in the medium. Based on the pigmentation, culture were divided as low, medium and high pigmented. Release of other secondary metabolites (toxins) in the culture may also serve as the reason for the pigmentation in the medium (Gopireddy *et al*., 2017). In general, polynucleate Rhizoctonia are found to be more pigmented than binucleated isolates (Helmy *et al*., 2015). Heterokaryosis and parasexual cycle resulted in these variations (Parmeter *et al.,* 1969).

The cultures were showing regular, slightly irregular and irregular margins; and texture of growth was appressed and fluffy in nature. Abundant aerial cottony mycelial growth was observed in isolates due to their inherent nature. In early stages of growth, the pathogen revealed the quick and profuse growth of mycelium before setting the sclerotia in the medium (Gopireddy *et al.,* 2017)

In the present study, based on the colony diameter and growth rate of the isolates on medium, the isolates of *R. solani* are divided into fast, medium and slow. While studying the morphological characters. Lal *et al.* (2014) noted variation in growth rate as fast, medium and slow. All rice and vegetable cowpea isolates of *R. solani* were fast in growth. TRA2 and PRA1 isolates of amaranth were fast growers. Amaranth isolates KRA1 and KRA2 were the slow growing isolates among all isolates and they completed the full growth in petriplate on seven days after inoculation. Generally all the fast growing isolates completed their growth in petriplate within two to three days after inoculation.

Growth of the isolates can be connected with virulence of the pathogen. Fast growing isolates were more pathogenic than slow growing isolates. Similarly, Meena *et al.* (2001) also found a correlation between growth rate and virulence of the pathogen. Fast growing maize isolates were more aggressive on susceptible maize cultivars. Contradictory to this, Basu *et al*. (2004) stated that there is no such relations exist between mycelial growth and virulence in the rice isolates of *R. solani.* According to Helmy *et al*. (2015), polynucleate Rhizoctonia possesses higher growth rate. The

diversities between polynucleate Rhizoctonia were very strong compared binucleate isolates.

Zonation was present in cultures of all amaranth isolates of *R. solani* except PRA1 (Ranni isolate). Zonation was absent in all rice and vegetable cowpea isolates except TRC1 where a slight zonation was present. Among the different isolates, variation was also observed in the dry weight of mycelium and was ranging from 2.10 (KRA1) to 7.10 g (PRR1).

In the present study, days for sclerotia initiation in the culture extended from three to four days. Lal *et al.* (2014) noted sclerotial initiation ranging from three to five days in rice sheath blight isolates. But Meena *et al.* (2001) noted days for sclerotial initiation as 3-11 days in rice isolates. Noticeable variation can be observed in the cultural characters between isolates from different hosts in the PDA medium. All these cultural variability observations were in consonance with Singh *et al.* (2014) where he observed variation in cultural characters like colony colour and growth rate in banded leaf and sheath blight isolates from Transgangatic plains of India. *R. solani* being a complex pathogen, there can be existence of many races in it. The occurrence of different races is the reason for the cultural variation in the media as they respond differently to the culture conditions.

5.4. STUDYING THE MORPHOLOGICAL VARIABILITY OF THE ISOLATES

Light microscopic studies revealed that all the isolates of *R. solani* from rice, vegetable cowpea and amaranth were showing characteristic right angle branching in the distal end of the cell along with a characteristic constriction at the point of branching and a septa can be seen at the point of branching or adjacent to branching in every isolates (Plate 14). There was no clamp connection detected in the mycelium. Similar observation were also noted by Gopireddy *et al.* (2017) where he failed to observe clamp connection in the isolates of *R. solani* f. sp. *sasaki* collected from Andhra Pradesh. Moniliod cells were present in every culture. These characters play immense taxonomical importance. In this study, there were some variations detected in the microscopic characters like width of hypha and shape of moniliod cells (Plate 15).

R. solani isolates exhibited a great diversity in morphological characters (Table 9). TRR2 showed lowest hyphal width (3.81 μm) and PRR2 had the highest (6.95 μm). In addition to ordinary vegetative hyphae, R. solani produces simple or branched chains of short broad cells sometimes hyaline or brown, barrel shaped, pyriform, irregular, or lobate cells known as moniliod cells in the cultures. Doliform cells, short cells, barrel shaped cells, sclerotial cells and chlamydospores are various names of moniliod cells (Madhavi, 2012). These are actually the histological change of the cells. Presence of moniliod cells were detected in every isolate and shape of the moniliod cells were varied in the culture. Gopireddy *et al*. (2017) observed moniliod cells with barrel and irregular shape in the *R. solani* f. sp. *sasaki* isolates collected from Andhra Pradesh which was correlating with the present study. TRR2, TRC1, TRC2 and KRC1 isolate produced irregular shaped moniliod cells in the culture and rest produced barrel shaped moniliod cells. However, wide variations in the measurements of monilod cells were observed by Matsumoto (1921) and emphasized such variation were not sufficient to distinguish species.

Sclerotia are the compact mass of hardened structure present in the culture of *R. solani*. Due to the increased branching of hyphae, melanin encrusted multicellular structures are formed (Divya *et al.*, 2018). Sclerotial characters show variability in case of size, texture, shape, weight, diameter, number, orientation, position, colour, clump formation, exudation in the culture etc (Table 10). Lal *et al.* (2014) also conducted similar study and found variation in sclerotial size, number, formation and diameter of the sheath blight isolates. Based on the size of sclerotia, they are divided into big and small. Most of the sclerotia present in the vegetable cowpea cultures were bigger compared to rice culture. Jayaprakashavel and Mathivanan (2012) observed macrosclerotia and microsclerotia among rice *R. solani* isolates in South India.

In this present study, there was no correlation found between size of sclerotia and virulence. Vegetable cowpea and rice cultures were highly virulent though they were having difference in size of sclerotia. The vegetable cowpea isolates like TRC1 and KRC2, were fast growing and virulent even without the presence of sclerotia. In the case

71

of amaranth isolates also, even without the production of sclerotia, cultures like TRA2 and PRA1 were having higher growth rate. On contrary, Dath (1984) reported that sheath blight isolates having larger sclerotia were more virulent than other isolates. Among rice, maize and green gram isolates, *R. solani* having bigger size sclerotia produce higher disease incidence irrespective of host (Mishra *et al.*, 2014).

Basu and Gupta (1992) collected *R. solani* rice isolates from West Bengal. They noticed smaller sclerotia forming culture or cultures without sclerotia produced significantly less polygalacturonase and cellulase compared to large sclerotia forming cultures and reported a positive correlation between the size of sclerotia and pathogenicity. According to Singh *et al*. (2000) the isolates with macro sclerotia were fast growers and highly virulent, while micro sclerotia formers were considered as slow growers and less virulent. Basu *et al*. (2004) stated that no correlation exists between the mycelial growth and virulence of the pathogen; but it is the abundance and size of the sclerotia determines the virulence of the isolates.

Cultures were divided into those having smooth bordered and rough bordered sclerotia. Most of vegetable cowpea isolates produced rough bordered sclerotia. Rice isolates produced both smooth and rough bordered sclerotia in the cultures. Oval, round and irregular were the shape of the sclerotia present in the culture. Colour of the sclerotia will help to distinguish it easily (Anderson, 1982). Initially the sclerotia were cream in colour, and then changes to different shades of brown. Most of the cultures were having dark olive brown sclerotia followed by dark brown, dark yellowish brown and dark reddish brown. Colour difference was predominant even in the isolates from same crop.

Sclerotia were arranged in the culture as peripheral, central scattered, central and peripheral. In peripheral arrangement, sclerotia were seen at the periphery of the culture near the side of petriplate. In central arrangement, the sclerotia are conglomerated in the central position in the culture. In peripheral and central arrangement, sclerotia were observed in both these positions. Mishra *et al.* (2014) observed sclerotial arrangements like central, sub-central, peripheral, scattered, irregular and scattered in *R. solani* isolates

from rice, maize and green gram. Based on the position of the sclerotia in the culture, it was divided as aerial and embedded. KRR1 produced embedded type of sclerotia in the culture. All vegetable cowpea isolates produce aerial type of sclerotia in the culture. Most of cultures except KRR1 produced aerial type sclerotia. Sclerotial number in the culture was ranging from 15 to 230. They were grouped into good, very good and excellent sclerotia formers. Most of the rice cultures produced sclerotia significantly higher than vegetable cowpea isolates in the culture. Weight of single sclerotia in the culture ranged from 2 to10 mg.

On the sclerotia, liquid secretion usually called as honey dew may be present. All sclerotia producing cultures of rice and vegetable cowpea exhibited honey dew secretion on it. Honey dew was not secreted in the culture of amaranth isolates and vegetable cowpea isolates namely TRC1 and KRC1. These observations are consonant with Manjunatha (2016) that he observed honey dew secretions in the isolates of rice *R. solani.* Sclerotia usually form aggregations in the culture called clumps. According to the aggregation of sclerotia, Gopireddy *et al.* (2017) classified isolates of banded leaf and sheath blight into more, moderate and less clump formed cultures which is similar as the present study. This type of sclerotial aggregation will increase the survival rate of the pathogen (Gopireddy *et al.*, 2017). Faster growth of the pathogen and higher sclerotia production increase the survival of the pathogen to the next season (Surulirajan, 2003). Aggregation of sclerotia may hinder the quick dispersal but increases the chances of germination as the surface area get enhanced (Madhavi, 2012). Sclerotial formation was absent in amaranth isolates and the vegetable cowpea isolates TRC1 and KRC2. According to Meyer (1965), absence of sclerotia in the culture does not exclude them from *R. solani* as in certain condition sclerotia may be absent.

Even there was inconsistency in the sclerotial characters during each subculturing. This may be due to nuclear condition of the isolates (Desvani *et al.,* 2018). Morphological diversities were not depended on the geographical origin (Ganeshmoorthi and Dubey, 2015; Divya et al., 2018) which was found similar to our study. There is no specific pattern followed in these characters according to locations. Due to the presence

73

of different races in the isolates, there can be changes in the macroscopic characters and these results point towards the genetic variability.

Macroscopic vegetative compatibility of the isolates was tested using dual culture experiment in all combination. Out of the 171 combinations, merge reactions were the most common followed by barrage line and merging line combination (Fig. 5)(Plate 17). Merge reaction indicates that the perfect fusion can occur between the cultures (compatible) or there is no reaction present between the cultures, the hyphae will overgrow each other (incompatible). Other reactions types like merge line, tuft, barrage and barrage line were also incompatible. Isolates exhibiting barrage reaction at the colony junction were considered as different somatic compatibility group (SCGs) and failed to exhibit barrage at the junction were same SCGs (Jahan *et al*., 2018). Use of the easily recognizable macroscopic vegetative reactions can be utilized to clarify the interpretation of the microscopic examinations (MacNish *et al.,* 1997). Tuft reaction formed in the macroscopic reactions due to the flush of growth occurred from either or both isolates. In unsuccessful anastomosis, reallocation of cytoplasmic matter from the dead cells occurs leading to tuft formation (MacNish *et al.,* 1997).

Microscopic compatibility of the isolates was tested using hyphal anastomosis reactions. Anastomosis is the genetic mechanism of somatic cell fusion between different strains of R. solani and allows gene exchange and generates variability (Menzies, 1970; Anderson, 1982). C0, C1, C2 and C3 are the different type of reactions (Plate 18). In C0 reaction the hyphae from the cultures does not anastomose each other. They will overgrow each other without showing any specific reactions. In C1contact fusion type, hyphae of one culture get contact with the cell wall of other hyphae without producing pore and death of the cells. In C2 imperfect fusion type, hyphae of the one isolate get attached to hyphae of another isolate and pore plus death of the cells occurs (killing reaction). C2 reaction tries to prevent genetic exchange possible between like populations or otherwise limit the invasion of a niche occupied by a different strain of *R. solani* (MacNish *et al.,* 1997). Different genotype interactions either prevent the hyphal anastomosis or lead to cell death

	TRA1	TRA2	KRA1	KRA2	PRA1	PRA2	TRR1	TRR2	KRR1	KRR2	PRR1	PRR2	TRC1	TRC2	KRC1	KRC2	PRC1	PRC2
TRA1	M	ML	M	M	M	M	ML	M	M	M	M	M	ML	M	B	M	ML	M
TRA2		M	M	M	T	M	ML	ML	M	ML	ML	ML	T	ML	ML	M	BL	BL
KRA1			M	M	M	B	M	M	M	B	M	M	M	M	M	M	M	M
KRA2				M	M	M	M	M	B	M	M	M	B	M	B	M	M	M
PRA1					M	BL	M	M	M	M	ML	M	T	M	ML	BL	M	ML
PRA2						M	M	M	M	M	M	M	ML	M	B	M	ML	M
TRR1							M	B	B	M	B	M	BL	B	B	M	B	B
TRR2								M	M	BL	BL	BL	BL	ML	ML	M	BL	M
KRR1									M	BL	BL	ML	M	BL	BL	M	BL	BL
KRR2										M	BL	BL	BL	ML	BL	ML	BL	BL
PRR1											M	ML	BL	BL	M	BL	BL	BL
PRR2												M	M	M	M	ML	BL	ML
TRC1													M	ML	M	ML	BL	ML
TRC2														M	BL	ML	M	M
KRC1															M	M	ML	ML
KRC2																M	M	ML
PRC1																	M	BL
PRC2																		M

M	Merge	**ML**	Merge line	**B**	Barage	**B**	Barage line	**T**	Tuft

Fig. 5: **Macroscopic vegetative reactions between rice, vegetable cowpea and amaranth isolates of *R. solani***

	TRA1	TRA2	KRA1	KRA2	PRA1	PRA 2	TRR1	TRR2	KRR1	KRR2	PRR1	PRR2	TRC1	TRC2	KRC1	KRC2	PRC1	PRC2
TRA1	C3	C2	C3	C3	C3	C3	C1	C0	C0	C0	C0	C0	C1	C0	C2	C0	C0	C0
TRA2		C3	C3	C3	C2	C3	C1	C2	C3	C1	C2	C2	C2	C1	C1	C0	C2	C2
KRA1			C3	C3	C3	C2	C0	C0	C0	C0	C1	C0	C0	C0	C0	C0	C0	C0
KRA2				C3	C3	C3	C0	C0	C0	C1	C0	C0	C2	C0	C2	C0	C0	C0
PRA1					C3	C2	C0	C0	C0	C0	C0	C2	C2	C0	C1	C0	C2	C1
PRA2						C3	C0	C0	C0	C0	C0	C0	C1	C0	C0	C0	C0	C0
TRR1							C3	C2	C2	C3	C2	C3	C2	C2	C2	C2	C2	C2
TRR2								C3	C3	C2	C3	C3	C2	C3	C2	C3	C2	C3
KRR1									C3	C2	C3	C2	C2	C2	C2	C2	C2	C2
KRR2										C3	C2	C2	C2	C2	C2	C2	C2	C2
PRR1											C3	C2	C2	C2	C2	C2	C2	C2
PRR2												C3	C3	C3	C3	C2	C2	C2
TRC1													C3	C2	C3	C2	C2	C2
TRC2														C3	C2	C2	C3	C3
KRC1															C3	C3	C2	C2
KRC2																C3	C2	C2
PRC1																	C3	C2
PRC2																		C3

C0	No reaction	C1	Contact fusion	C2	Imperfect fusion	C3	Perfect fusion

Fig. 6: Macroscopic vegetative reactions between rice, vegetable cowpea and amaranth isolates of _R. solani_

(Leach and Yoder, 1983). In C3 reaction type, perfect fusion of the hyphal components occurs without the death of the fusing cells. This is similar to the description given by Sneh *et al.* (1996) where he describes perfect fusion as one of the hyphal anastmois reaction which do not lead to the cell death of fusing hyphae.

Out of the 171 combinations analysed, C2 reactions were most common followed by C3 reactions (Fig. 6). C3 hyphal anastomosis reaction indicates a much closer level of relationship than C1 or C2 (Carling, 1996). C3 reaction was vegetatively compatible and C0, C1 and C2 reactions were vegetatively incompatible. These results of hyphal anastomosis are in consonant with Divya *et al.* (2018) where they studied anastomosis behaviour of different isolates from different crops such as rice, maize, moongbean, groundnut and soyabean. Pairing between rice and maize with moongbean and groundnut, moongbean, soyabean and groundnut resulted in C2 reaction. Pairing between soybean, rice and maize isolates resulted in C1 reaction.

The frequency of anastomosis reactions was less in amaranth isolates with rice isolates and vegetable cowpea isolates. Hyphal anastomosis was observed between rice and vegetable cowpea isolates, and between isolates of same hosts i.e, rice and rice, cowpea and cowpea and amaranth and amaranth. This is due to the difference in anastomosis group of the isolates. Hyphal fusion occurs at a high frequency within the same AG (Hyakumachi and Ui, 1988) and between the isolates of different AGs, anastomosis occurs at a low frequency or sometimes there is no anastomosis reaction at all. Thus it is clear that for characterizing variability of *R. solani* isolates, anastomosis grouping based on hyphal fusion can also be used.

C3 reactions were observed in isolates coming under same AG. C2 reactions were coming under the same AG but only within same vegetative compatible population. C1 reactions were found under same AG or different AG. C0 reactions are shown by isolates coming under different AG.

In the present study, there is no strict correlation exist between microscopic C2 and C3 reaction and macroscopic tuft and merge reactions respectively. MacNish *et al.*

(1997) noticed C2 and C3 microscopic reactions were corresponding to tuft and merge macroscopic reaction in AG-3, AG-7, AG-8 and AG-10. But there is no such correlation exists in *R. solani* isolates coming under other AGs.

Thus macroscopic and microscopic vegetative compatibility also differentiate the races present in the R. solani. Within AG group, tremendous amounts of variation exist in terms of genetic makeup, pathogenicity and aggressiveness of the isolates. AG can be correlated to some extent in terms of pathogenicity and host specialization.

5.5. STUDYING THE PATHOGENIC VARIABILITY OF THE ISOLATES

R. solani possesses wide host range as it infects many plant species coming under different families. Pathogenic variability of *R. solani* isolates from rice, vegetable cowpea and amaranth were evaluated by artificially inoculating the isolates on amaranth variety Arun. All isolates produced symptoms on amaranth plants within two to four days after inoculation. *R. solani* can easily cross infect plants and generally not very host specific (Jayaprakashavel and Mathivanan, 2012). There was variation in the number of lesions, size of lesion and PDI (Table 11) (Plate 20). The lesion number produced on amaranth plant was ranging from 3 to 12. Lesion size produced by the isolates on amaranth plant was ranging from 0.2-5.0 x 0.2-4.2 cm. Leaf blight severity was ranging from 22.22 to 58.33 per cent. Among all isolates of *R. solani,* vegetable cowpea isolate PRC1 was most virulent on amaranth, followed by PRA1 and KRC2 (Fig. 7). Mishra *et al.* (2014) conducted similar cross infectivity test on varieties such as Pusa 1121 (rice), IPM 99-125 (green gram) and Vivek Hybrid 9 (maize) with the *R. solani* isolates of rice, maize and green gram collected from different parts of India. All isolates infected the hosts with varied pathogenicity. More aggressive isolates can be utilized in the resistant breeding programmes.

It was noted that vegetable cowpea isolates produced more leaf blight incidence on amaranth plants compared to amaranth and rice isolates. Percent disease index was higher for vegetable cowpea isolates than most of the amaranth and rice isolates. Sometime virulent *R. solani* isolate obtained from a host might be virulent or less virulent
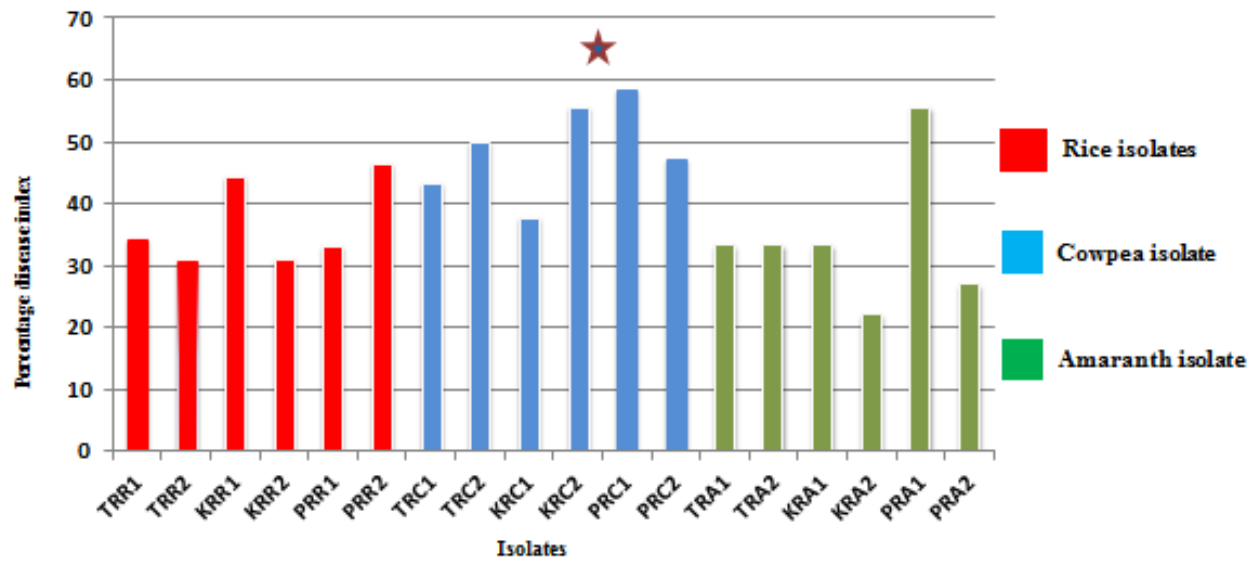
76

Fig. 7: Percentage disease index of rice, vegetable cowpea and amaranth isolates of *R. solani* on amaranth

or avirulent on other hosts (Kang and King 1986; Ogoshi *et al.*, 1990; Nelson *et al.,* 1996; Yang *et al.,* 1996). Variability noticed in the nature of symptom production, days taken for disease development, lesion size, lesion number, host range of R. solani isolates suggest that there exists several strains within the species (Madhavi, 2012).

There was no significant correlation exists between geographic origin and virulence of isolates. Difference in virulence of the pathogen is because of the presence of different races generated due to hyphal anastomosis. Multinucleate *R. solani* isolates usually produce heterogeneous characteristics (Zhou *et al.*, 2016). Mahmoud *et al.* (2007) stated that there is no relation between geographical location, pathogenicity and host plant which was similar as the present finding. Cultural, morphological and pathological variability visible among the isolates of *R. solani* from rice, vegetable cowpea and amaranth but there is no specific relationship exists between the culturally and morphologically similar isolates with their pathological behavior in the present study. Guleria *et al.* (2007) in the variability studies of *R. solani* also opined that there was no specific relationship between pathogenic behavior and morphological traits.

Dash *et al.* (2000) reported pathological differences of rice *R. solani* isolates belonging to different AGs. All isolates produced symptoms on the amaranth irrespective of the anastomosis group. Even within the anastomosis group also there was remarkable variation in pathogenicity. Environmental condition may also influence the pathogenic variability of the isolates. Pathogenicity of different isolates varied in different hosts and even within the same host, the pathogenic potential was found variable in plant parts like stem and leaf (Khandaker *et al*., 2008).

Environmental condition also influences the pathogenic variability of the isolates (Singh *et al*., 2002). Behaviour and virulence of the isolates may show changes due to mutation and gene shift, which make the isolates more adaptive to various hosts irrespective of the anastomosis group (Mishra *et al.,* 2014). Pathogenic variation were also observed by Ganeshmoorthi and Dubey (2015) where they classified *R. solani*

77

isolates from chickpea, mungbean and urdbean into less, moderate and highly virulent isolates.

5.6. STUDYING THE MOLECULAR VARIABILITY OF THE ISOLATES BY RAPD ANALYSIS

. Genetic relatedness of *R. solani* isolates of rice, vegetable cowpea and amaranth was assessed with Random Amplified Polymorphic DNA as this technique has great potential in analyzing the population biology. RAPD analysis was carried out by using 4 oligonucleotide random primers namely OPA 04, OPA 19, OPA 20 and OPW 13(Plate 21). A total of 75 polymorphic and scorable bands ranging approximately as low as 250 bp to as high as 3000 bp was generated with these 4 primers. These primers were very informative that they could well display the genetic polymorphism among the *R. solani* isolates (Singh *et al.*, 2015).

All the four primers were highly discriminative and provided higher number of bands. Polymorphism Information Content (PIC) value of the primers were ranging from 0.17 to 0.25. In these selected primers, OPA 19 was more informative with higher polymorphism content. Although the primers used in the study were less in number, they could effectively establish the genetic variation among the isolates from different host. Out of the 75 RAPD bands scored, there was cent per cent polymorphism with different electrophoretic mobility of fragments can be seen. The similarity values of RAPD profile in the present study ranged from 0.59 to 0.92. Similarly, on analyzing 22 isolates of sheath blight fungus, Banerjee *et al.* (2012) reported similarity value ranging from 0.41 to 0.94.

A dendrogram was generated by Sequence Agglomerative Hierarchial Nested (SAHN) subroutine clustering using Jaccrad similarity coefficient. The estimated similarity of the isolates with the primers ranged from 59 to 92 per cent polymorphism, reflecting wide variability among the isolates of rice, vegetable cowpea and amaranth from different locations of Southern Kerala at their genetic level. The maximum similarity value (0.92) was obtained between the rice isolates TRR1 and KRR1which
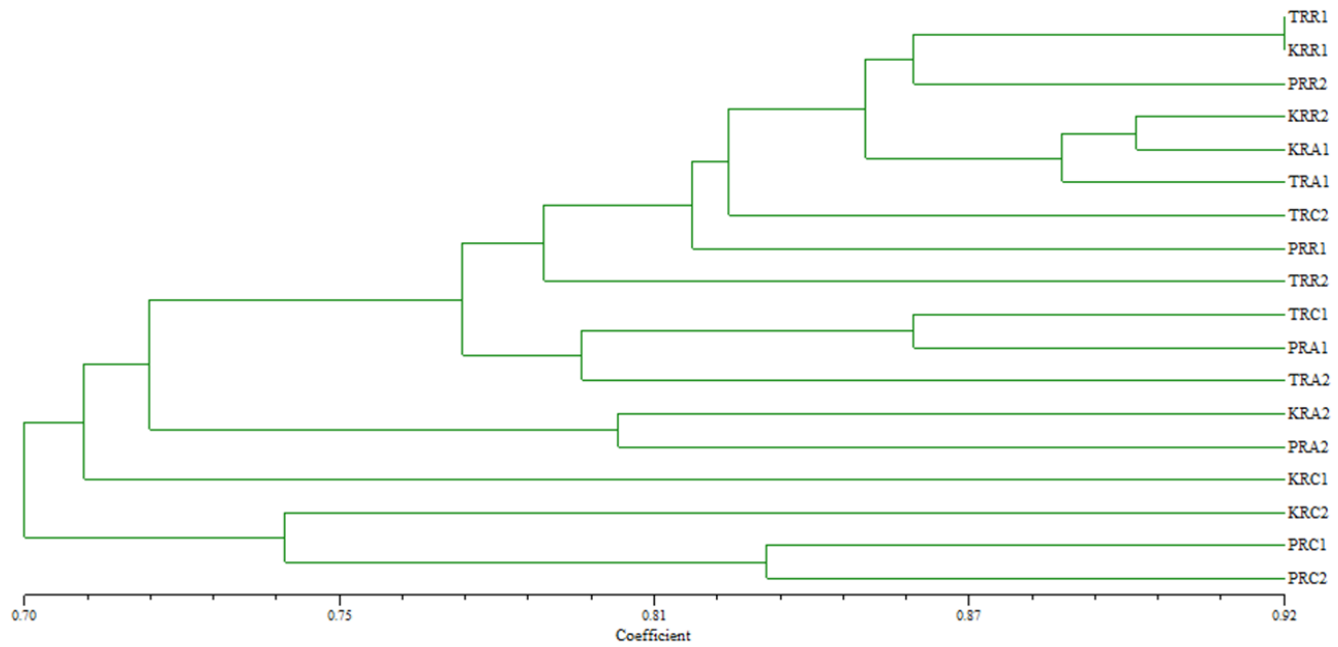
Fig. 8 : RAPD based dendrogram of rice, vegetable cowpea and amaranth isolates of *R. solani* constructed using SAHN of NTSYS v 2.0 and computed according to Jaccard similarity matrix.

belongs to Vellayani and Kottarakkara locations (Fig. 8). This is may be due to presence of the shared clones irrespective of geographical locations.

Different host isolates from same area didn't clustered together even though they are from same geographical area. This result was contradictory to Banerjee *et al*. (2012) that they observed most of the rice isolates from similar geographical location in West Bengal clustered together. Even isolates from different host collected from different locations clustered together. This was supported by Mishra *et al.* (2014) where they observed isolates from rice, green gram and maize clustered together. This is due to co-evolution and recombination of the isolates. Intraspecific variability of *R. solani* isolates collected from rice, vegetable cowpea and amaranth from same and different geographical regions was expressed through RAPD analysis. This is in accordance with Singh *et al.* (2015) where the observed intraspecific variability rice *R. solani* isolates from different parts of India. This method is suitable for screening large number of samples. There was no strict correlation observed between isolates based on morphological characters, cultural characters, anastomosis interaction, pathogenicity, geographical locations and RAPD fingerprinting. In contrast, Sharma e*t al*. (2005) reported some relationship between sclerotial characteristics and molecular banding pattern. Virulent isolate may not contain similar genetic makeup (Lal *et al*., 2014).

The rice isolates of Vellayani and Kottarakkara locations was found similar irrespective of geographical locations. This suggests the evolutionary potential of the isolates (Singh *et al.,* 2018). These isolates may be evolved from a common ancestor. Other than this, there may be chances of migration of pathogen via human activity, soil or natural mechanism (Madhavi, 2012). Genetic variation even among closely related isolates addressed through RAPD. The electrophoretic banding pattern within isolates belonging to same anastomosis group was found variable indicates the presence of different races of the pathogen. Thus RAPD markers provided a basis for identifying patterns, colonization and dispersal of the pathogen and also suitable for race and biotype characterization. This suggests effective management and appropriate resistant breeding programmes (Banerjee *et al.,* 2012).

Summary

SUMMARY

Rhizoctonia solani is a ubiquitous destructive pathogen causing severe damage in economically important crops like rice; and several vegetables like vegetable cowpea and amaranth. In this context, the research work entitled "Studies on the variability of *Rhizoctonia solani* Kuhn. infecting rice, cowpea and amaranth." was undertaken in the Department of Plant Pathology, College of Agriculture, Vellayani during the year 2018-2020, with the objective to study the cultural, morphological, pathogenic and molecular variability of *R. solani* infecting rice, vegetable cowpea and amaranth in Southern Kerala.

R. solani infected samples of rice, vegetable cowpea and amaranth were collected from two locations each from the three southern districts of Kerala *viz.*, Vellayani and Chirayinkeezhu in Thiruvananthapuram; Kottarakkara and Pooyappally in Kollam; Thiruvalla and Ranni in Pathanamthitta. Eighteen isolates of *R. solani* were obtained for the study. In these surveyed locations, Percent Disease Index and Disease Incidence was ranging from 11.11 to 99.99 per cent and 18 to 92 per cent in rice tracts, 33.33 to 77.77 per cent and 32 to 88 per cent in vegetable cowpea fields and 11.11 to 77.77 per cent and 30 to 98 per cent in amaranth fields respectively were recorded.

The expression of symptoms of *R. solani* isolates was found variable in each host. In rice, sheath blight symptom appeared as oval or irregular shaped, discoloured mostly greyish water soaked lesions with light brown, dark brown and purple coloured margin. In vegetable cowpea plants, the collar rot symptom appeared as brownish black lesions in the collar region leading to girdling of stem. Web blight symptom on vegetable cowpea plants appeared as small circular greyish brown spots on the foliage surrounded by water soaked areas. The spots enlarged to lesions with oblong to irregular shape and webbing of leaves also occurred. Leaf blight symptoms on amaranth were visible as greenish, grey or cream coloured irregular spots or lesions gradually coalesce and enlarged under humidity. In severe cases, shot hole symptoms also appeared on the leaves.

The pathogenicity and virulence of the isolates obtained from rice, vegetable cowpea and amaranth were assessed by artificial inoculation on respective hosts. Nature

80

of symptom, days for symptom development, number of lesions and lesion size were found variable in the respective hosts according to the virulence spectrum of the pathogen. PDI of *R. solani* isolates on artificial inoculation was showing a range from 10 to 30 per cent in rice, 22.22 to 44.44 per cent in vegetable cowpea and 11.11-55.55 per cent in amaranth. TRR1 (Vellayani, PDI - 30%) was more virulent among rice isolates. Among vegetable cowpea isolates, KRC1 (Kottarakkara, PDI - 44.44%) was more virulent. TRA2 (Chirayinkeezh, PD1 - 55.55%) was more virulent among amaranth *R. solani* isolates. There were variations in the degree of pathogenicity.

Various cultural characters like colony colour, pigmentation, rate of growth, texture of growth, days for sclerotial initiation, zonation, growth margin and dry weight of mycelium were studied and variations were observed among isolates. Colony colour of the isolates was initially white then turns to different shades of brown colour. The cultures were low, medium and high pigmented and showing regular, slightly irregular and irregular margins. Texture of growth was appressed and fluffy.

All isolates from rice and vegetable owpea and TRA2, PRA1 from amaranth were fast growers. Rate of growth was high in KRR1 (Kottarakkara), PRC2 (Thiruvalla) and PRA1 (Ranni) collected from rice, vegetable cowpea and amaranth respectively in PDA medium compared to other isolates. The isolates took 2 to 7 days for full petri plate coverage. Fast growing isolates were more pathogenic than slow growing isolates. Dry weight of mycelium was ranging from 2.10 - 7.10 g among the isolates. Zonation was absent in all rice and vegetable cowpea isolates except TRC1 (Vellayani) where a slight zonation was present. Days for initiation of sclerotial production ranged from 3 to 4 days.

Light microscopic studies revealed that all the isolates of *R. solani* were showing characteristic right angle branching along with a characteristic constriction at the point of branching and a septa can be seen at the point of branching or adjacent to branching. Mean hyphal width varies from 3.81 μm (TRR2) to 6.95 μm (PRR2). Isolates show variation in the shape of moniliod cells. TRR2, TRC1, TRC2 and KRC1 isolate produced

81

irregular shaped moniliod cells in the culture and rest produced barrel shaped moniliod cells.

A noticeable variation could be seen in sclerotial characters like colour, size, texture, shape, number, weight, orientation, position and clump formation. There was no correlation found between size of sclerotia and virulence. Sclerotia were grouped into smooth bordered and rough bordered. Sclerotia were arranged in the culture as peripheral, central scattered, central and peripheral; and position of the sclerotia was aerial and embedded. Sclerotial number in the culture was ranging from 15 to 230. Weight of single sclerotia in the culture was ranging from 2-10 mg. All sclerotia producing cultures of rice and vegetable cowpea exhibited honey dew secretion on it. According aggregation of sclerotia, cultures were divided into more, moderate and less clump formed culture. Sclerotia formation was absent in all amaranth cultures and vegetable cowpea culture such as TRC1 and KRC2.

Macroscopic vegetative compatibility reaction between isolates of *R. solani* are merge, merging line, tuft, barrage, barrage line and microscopic hyphal anastmosis reaction are C0, C1, C2 and C3.In the macroscopic vegetative reactions, 93 were merge, 31 were merging line, 3 were tuft,14 were barrage and 30 were barrage line. In the microscopic anastomosis reactions, 46 were C3, 67 were C2,11 were C1 and 47 were C0.

Out of the 171 culture combinations, merge reactions and C2 hyphal anastomosis were the most commonly found. C3 reaction is vegetatively compatible and C0, C1 and C2 reactions are vegetatively incompatible reactions. The frequency of anastomosis reactions were less between amaranth and rice isolates and amaranth and vegetable cowpea isolates. Frequency of hyphal anastomosis was more between rice and vegetable cowpea isolates, and within the anastomosis reaction between same hosts *i.e,* rice and rice, cowpea and cowpea, and amaranth and amaranth.

Pathogenic variability among the isolates was analysed on Arun variety of amaranth. All *R. solani* isolates from rice, vegetable cowpea and amaranth were found

infecting amaranth whereas variations in degree of pathogenicity were observed among isolates. PRR2 (Thiruvalla, PDI - 46.66%), PRC1 (Ranni, PDI - 58.33%) and PRA1 (Ranni, PDI - 55.55%), were found most virulent among rice, vegetable cowpea and amaranth isolates respectively. Among all isolates, PRC1 (Ranni) was the most virulent on amaranth. Microscopic, microscopic characters and virulence were not affected in geographical origin. All isolates produced symptoms on the amaranth irrespective of the anastomosis group. Even within the anastomosis group also, there is remarkable variation in pathogenicity.

Genetic variability was analyzed through RAPD using four random primers such as OPW-13, OPA-04, OPA-19, OPA-20. The primers were found very informative in generating high level of polymorphism in the RAPD electrophoretic banding pattern. A total of 75 polymorphic and scorable bands ranging approximately as low as 250 bp to as high as 3000 bp was generated with these 4 primers. Polymorphism Information Content (PIC) value of the primers were ranging from 0.17 to 0.25. Primer OPA 19 generated higher PIC.

A dendrogram was generated by Sequence Agglomerative Hierarchial Nested (SAHN) subroutine clustering using Jaccard similarity coefficient. The estimated similarity of the isolates with the primers ranged from 59 to 92 per cent. The maximum similarity value (0.92) was obtained between the rice isolates TRR1 (Vellayani) and KRR1 (Kottarakkara). Intraspecific variability of *R. solani* isolates was expressed among the population from different as well as from same geographical regions. The electrophoretic banding pattern within isolates belonging to same anastomosis group was found variable indicates the presence races of the pathogen

References

7. REFERENCES

Acharya, S. and Sengupta, P. K. 1998. Collateral hosts of rice sheath blight fungus *Rhizoctonia solani. Oryza.* 35 (1): 89-90.

Adhipathi, P., Singh, V., and Meena, S. C. 2013. Virulence diversity of *Rhizoctonia solani* causing sheath blight disease in rice and its host pathogen interaction. *The Bioscan* 8 (3): 949-952.

Ahuja, S. C. and Payak, M. M. 1985. Comparative biology, pathology and karyology of rice and maize isolates of *R. solani f. sp. sasakii. Int. Rice Res. Newsl*. 10: 5-6.

Akhtar, J., Kumar, J. V., Kumar, A., and Lal, H. C. 2009. Occurence of banded leaf and sheath blight of maize in Jharkhand with reference to diversity in *Rhizoctonia solani. Asian J. Agric. Sci*. 1: 32-35.

Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani. Ann. Rev. Phytopathol*. 20: 329-347.

Banerjee, S., Dutta, S., Mondal, A., Mandal, N., and Bhattacharya, S. 2012. Characterization of molecular variability in *Rhizoctonia solani* isolates from different agro-ecological zones by random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol*. 11 (40): 9543-9548.

Banniza, S., Rutherford, M. A., Bridge, P. D., Holderness, M., and Mordue, J. E. 1996. Biological characterization of *Rhizoctonia solani* in rice-based cropping systems. Proceedings of Brighton Crop Protection Conference. *Pests Dis.* 1: 399-404.

Basu, A., Chowdhury, S., Chaudhuri, R. T., and Kundu, S. 2016. Differential behavior of sheath blight pathogen *Rhizoctonia solani* in tolerant and susceptible rice varieties before and during infection. *Plant Pathol*. 65 (8): 1333-1346

Basu, A. and Gupta, P. K. S. 1992. Cultural and pathogenic variation in rice isolates of *Rhizoctonia solani* Kuhn. *Beitrage zur Tropischen Landwirtschaft Veterinarmedizin* 30 (3): 291-297.

.Basu, A., Podder, M., Prasanta, K., and Sengupta. 2004. Variability and anastomosis among the rice isolates of *Rhizoctonia solani.Indian Phytopathol*. 57 (1): 70-72.

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A. P. K., and John, V. T. 1978. Evaluation of sheath blight resistance in rice. *Int. Rice Res. Newsl.* 3: 9-10.

Biswas, B., Dhaliwal, L. K., Chahal, S. K., and Pannu, P. P. S. 2011. Effect of meteorological factors on rice sheath blight and exploratory development of a predictive model. *Indian J. agric. Sci*. 81: 256-260.

Bourn, W. S. and Jenkins, B. 1928. *Rhizoctonia* disease on certain aquatic plants. *Bot. Gaz.* 85: 413-425.

Burpee, L. and Martin, B. 1992. Biology of *Rhizoctonia* sp. associated with Turf grasses. *Plant Dis. Reptr*. 76: 112-118.

Burpee, L. L., Sanders, H. C., Sanders, J., and Sherwood, R. T. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* (Bourd) Rogers and related fungi. *Mycologia*. 72: 689-701.

Carling, D. E. 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis. In: Sneh, B., Jabaji- Hare, S., Neate, S., and Dijst, G. (eds.), *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology, and Disease Control. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 37-47.

Carling, D. E., Baird, R. E, Gitaitis, R. D., Brainard, K. A., and Kuninaga, S. 2002. Characterization of AG-3, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathol.* 92: 893-899.

Carling, D. E., Kuninaga, S., and Leiner, R. H. 1988. Relatedness within and among intraspecific groups of *Rhizoctonia solani*: a comparison of grouping by anastomosis and by DNA hybridization. *Phytoparasitica* 16: 209-210.

Carling, D. E., Leiner, R. H., and Kelber, K. M. 1987. Characterization of a new anastomosis group (AG-9) of *Rhizoctonia solani*. *Indian Phytopathol*. 77: 1609-1612.

Chand, T. and Logan, C. 1983. Cultural And Pathogenic Variation in Potato isolates of *Rhizoctonia solani* in Northern Ireland. *Trans. Br. Mycol. Soc.* 81 (3): 585-589.

Chiocchetti, A., Ghignone, S., Minuto, A., Gullino, M. L., Garibaldi, A., and Migheli, Q. 1999. Identification of *Fusarium oxysporum f. sp. basilici* isolated from soil, basil seed and plants by RAPD analysis. *Plant Dis*. 83 (6): 576-581.

da Cunha, F. S., Costa, A. E. D., Galvao, K, M, D., Capucho, A, S., and Ishikawa, F. H. 2018. Characterization, pathogenicity and anastomosis groups of *Rhizoctonia solani* from watermelon. *Communicata Scientiae*. 9 (4): 710-717.

Dasgupta, M. K., 1992. Rice sheath blight: The challenges continue. In: Singh, U. S., Mukhopadhy, A. N., Kumar, J., and Chaulke, H. S. (eds), *Plant Diseases of International Importance: Diseases of Cereals and Pulses,* Vol. I, Prentice hall Eaglewood cliffs, New Jersey, pp. 130-150.

Dash, A. B., Lodha, S. B., and Gangopadhyay, S. 2000. Selection of differentials against AG groups of rice sheath blight organism, *Rhizoctonia solani*. *Indian Phytopath* 53: 197–201.

Dath, P. A. 1984. Effect of some cultural factors on the aggressiveness of *Rhizoctonia solani* on rice. *Indian Phytopathol.* 37: 469-472.

Desvani, S. D., Lestari, I. B., Wibowo, Supyani, H. R., Poromarto, S. H., and Hadiwiyono. 2018. Morphological characteristics and virulence of *Rhizoctonia solani* isolates collected from some rice production areas in some districts of Central Java. In: *AIP Conference Proceedings,* 020068, 2014, AIP Publishing, Melville. https://doi.org/10.1063/1.5054472

Divya, R., Dantre, R. K., and Kotasthane, A. S. 2018. Studies on variability of *Rhizoctonia solani* isolated from different hosts and its virulence on rice crop. *Int. J. Chem. Stud*. 6 (2): 1798-1801.

Dodge, B. B. and Stevens, N. E. 1924. The *Rhizoctonia* brown rot and other rots of strawberry. *J. Agric. Res.* 28: 643-648.

Duggar, B. M. 1915. *Rhizoctonia crocorrum* (Pers.) DC and *Rhizoctonia solani* Kuhn *(Corticium vagum* B & C) with notes on other species. *Annu. Missouri Bot. Garden*. 2: 403-458.

Elewa, I. S., Abd-Allah, S. M., Mostafa, M. H., and Mhuanna, N. A. 2000. Variability of pathogenesis and fungal characteristics of *Rhizoctonia solani* Kuhn. I. Morphological and cultural characteristics. *An. Agric. Sci.* (*Cairo)* 4: 1373-1387.

Ganeshamoorthi, P. and Dubey, S. C. 2015. Morphological and pathogenic variability of *R. solani* isolates associated with wet root rot of chickpea in India. *Legume res*. 38 (3): 389-395.

Gangopadyay, S. and Chakrabarti, N. K. 1982. Sheath blight on rice. *Rev. Plant Pathol*. 61: 451-460.

Ghosh, S., Kanwar, P., and Jha, G. 2017. Identification of candidate pathogenicity determinants of *Rhizoctonia solani* AG1-IA, which causes sheath blight disease in rice. *Curr. Genet.* 64 (3): 729-740.

Gireesh. 2016. Integrated mamangement of *Rhizoctonia* leaf blight of amaranth (*Amaranthus tricolor* L.). M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 108p.

Gokulapalan, C., Nayar, K., and Umamaheshwaran, K. 2000. Foliar blight of amaranthus caused by *Rhizoctonia solani* Kuhn. *J. Mycol. Plant Pathol.* 30: 131-132.

Gopireddy, B. M., Devi, G. U., Kumar, K. V., Babu, T. R., and Naidu, T. C. M. 2017. Cultural and morphological characterization of *Rhizoctonia solani f. sp. sasakii*

isolates collected from different districts of Andhra Pradesh. *Int. J. Curr. Microbiol. App. Sci.* 6 (11): 3457-3469.

Goswami, B. K., Bhuiyan, K. A., and Mian, I. H. 2010. Morphological and pathogenic variations in the isolates of *Rhizoctonia solani* in Bangladesh. *Bangladesh J. Agril. Res.* 35 (3): 375-380.

Guleria, S., Aggarwal, R., Thind, T. S., and Sharma, T. R. 2007. Morphological and pathological variability in rice isolates of *Rhizoctonia solani* and molecular analysis of their genetic variability. *J. Phytopathol*. 155: 654-661.

Haware, M. P. and Nene, Y. L. 1980. Sources of resistance to wilt and root rots of chickpea. *Int. Chickpea Newsl*. 3: 11–12.

Helmy, M. M., Emad, G., Samir, E., and Mostafa, M. H. 2015. Phenotypic diversity and molecular identification of the most prevalent anastomosis group of R*hizoctonia solani* isolated from diseased faba bean plants. *Am. J. Life Sci.* 3 (1): 47-55.

Hyakumachi, M., Mushika, Y., Ogosi, T., Toda, K., Kageyama., and Tsuge, T. 1988. Characterization of new cultural type of *R. solani* AG-2-2 isolated from warm season turf grass and its genetic differentiation from other cultural type. *Plant Pathol*. 47: 1-9.

Hyakumachi, M. and Ui, T. 1988. Development of the teleomorph of non-self-anastomosing isolates of *Rhizoctonia solani* by a buried-slide method. *Plant pathol.* 37 (3): 438-440.

IRRI. 2002. Standard evaluation system for rice. 5th Edition, Nov, 2002, INGER, Genetic Resources Centre, IRRI, Manila, Philippines. 56p.

Jain, R. K. and Thapliyal, P. N. 1980. Toxic metabolites from *Rhizoctonia solani* Kuhn : Production and possible role in pathogenesis. *Indian J. Expt. Biol*. 18: 316-318.

Jayaprakashvel, M. and Mathivanan, N. 2012. Morphological and pathological variations of rice sheath blight inciting south Indian *Rhizoctonia solani* isolates. *Arch. Phytopathol. Plant Protect.* 45 (4): 455-467.

88

Jahan, S. B., Ali, M. A., Alam, M. S., Moni, Z. R., and Latif, M. A. 2018. Morphological Aand molecular characterization of *Rhizoctonia oryzae sativae* in Bangladesh. *SAARC J. Agri.* 16(2): 119-128.

Jones, R. K. and Belmar, S. B. 1989. Characterization and pathogenicity of *Rhizoctonia* spp. isolated from rice, soybean and other crops grown in rotation with rice in Texas. *Plant Dis.* 73: 1004-1010.

Karthika, S. R. 2017. Organic strategy for the management of sheath blight disease of rice. M. Sc. thesis. Kerala Agricultural University, Thrissur, 101p.

Kang, S. W. and King, H. K. 1986. Incidence and control of bottom rot of Chinese cabbage caused by *Rhizoctonia solani* Kuhn. *Korean J. Plant Pathol.* 2 (3): 193-198.

Kataria, H. R., Hugelshofer, U., and Gisi, U. 1991. Sensitivity of *Rhizoctonia* species to different fungicides. *Plant Pathol.* 40 (2): 203-211.

Khandaker, M. M., Khair, A., and Bhuiyan, M. K. A. 2008. Disease reaction of different crops against virulent potato isolates of *Rhizoctonia solani* Kühn. *Bangladesh J. Bot.* 37: 75-80.

Khodayari, M., Safaie, N., and Shamsbakhsh, M. 2009. Genetic diversity of Iranian AG1-IA isolates of *Rhizoctonia solani,* the cause of rice sheath blight, using morphological and molecular markers. *J. Phytopathol.* 157 (11-12): 708-714.

Kotasthane, A. S., Agrawal, T., Shalini., and Saluja, M. 2004. A specialized approach for managing on unspecialized fungus causing sheath blight disease in rice. In: *International Symposium on Rainfed Rice Ecosystems: Perspective and Potential,* Indira Ghandi Agri Univ, Raipur, Chhattisgarh, India, p. 40.

Kuiry, S. P., Mondal, Banerjee, C., and Duttaa, S. 2013. Morphological variability in *Rhizoctonia solani* isolates from different agro-ecological zones of West Bengal. *Arch. Phytopathol Plant Prot.* 12: 1224-1226.

Kumar, M., Singh, V., Singh, Prashant., and Vikram, K. N. 2008. Morphological and virulence characterization of *Rhizoctonia solani* causing sheath blight of rice. *Environ. Ecol*. 26 (3): 1158-1166.

Kumari, S. P. and Niza, R. T. J. 2005. Propiconazole - a new fungicide for sheath blight of paddy, Karnataka. *J. Agric. Sci*. 18 (3): 833-835.

Lakshmanan, P., Nair, M. C., and Menon, M. R. 1979. Collar rot and web blight of cowpea caused by *Rhizoctonia solani. Plant Dis. Reptr*. 63: 410-413.

Lal, M. and Kandhari, J. 2009. Cultural and morphological variability in *Rhizoctonia solani* isolates causing sheath blight of rice. *J. Mycol. Plant Pathol.* 39 (1): 77-81.

Lal, M., Singh, V., Kandhari, J., and Sharma, P. 2014. Diversity analysis of *Rhizoctonia solani* causing sheath blight of rice in India. *Afr. J. Biotechnol*. 13 (51): 4594-4605.

Leach, S. S. and Clapham, W. M. 1992. *Rhizoctonia solani* on white lupine. *Plant Dis*. 76 (4): 417-419.

Leach, J. and Yoder, O. C. 1983. Heterokaryon incompatibility in the plant pathogenic fungus *Cochliobolus heterostrophus*. *J. Heredity* 74: 149–52.

Lore, J. S., Thind, T. S., Hunjan, M. S., and Goel, R. K. 2007. Performance of different fungicides against multiple diseases of rice. *Indian Phytopathol*. 60 (3): 296-301.

MacNish, G. C., Carling, D. E., and Brainard, K. A. 1993.Characterization of *Rhizoctonia solani* AG-8 from bare patches by pectic isozyme (zymogram) and anastomosis techniques. *Phytopathol*. 83: 922-927

MacNish, G. C., Carling, D. E., and Brainard, K. A. 1997. Relationship of microscopic and macroscopic vegetative reactions in *Rhizoctonia solani* and the occurrence of vegetatively compatible populations (VCPs) in AG-8. *Mycol. Res*. 101 (1): 61 - 68.

Madhavi, M. 2012. Variability in *Rhizoctonia solani* f. sp. *sasakii* (Kuhn) Exner the incitant of banded leaf and sheath blight of maize (*Zea mays* l.). Ph. D thesis, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad, 272p.

Maier, C. R. and Stafferdt, E. E. 1960. Cultural variability of selected isolates of *Rhizoctonia solani* and *Thielaviopois basicola* and the variability in their pathogenicity to acala and pima cotton, respectively. *Plant Dis. Reptr*. 44: 956-961.

Manjunatha, O. 2016. Studies on variability of sheath blight of rice caused by *Rhizoctonia solani* Kuhn. and its management. M.Sc.(Ag) thesis. College of Agriculture Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, 102p.

Mathew, K. A. and Gupta, S. K. 1996. Studies on variability on web blight of French bean caused by *Rhizoctonia solani* and its management. *J. Mycol. Plant Pathol.* 26: 171-177.

Matsumoto, T. 1921. Studies on the physiology of the fungi XII. Physiological specializeation in *Rhizoctonia solani* Kuhn. Annu. *Missouri Bot. Garden*. 8: 1-62.

Matsumoto, T. and Yamoto, W. 1935. Hypochnus sasakii Shirai in comparision with *Corticium stevensii* Burt. and *Corticium coleroga* (cook) V. Hohn. *Trans. Nat. Hist. Sic. (Formosa)* 25: 161-175.

Matsumoto, T., Yamamoto, W., and Hirane, S. 1932. Physiology and parasitology of the fungi generally referred to as *Hypochnus sasakii* Shirai. I. Differentiation of the strains by means of hyphal fusion and culture in differential media. *J. Society Trop. Agric.* 4: 370-388.

Mayee, C. D. and Datar, V. V. 1986. *Phytopathometry.* Technical bulletin-1, Marathwada Agricultural University, Parabhani, p. 95.

Mccabe, P. M., Gallagher., M. P., and Deacon, J. W. 1999. Microscopic observation of perfect hyphal fungi *Rhizoctonia solani. Mycological Res*. 103 (4): 487-490.

Meena, B., Ramamoorthy, V., and Muthuswamy, M. 2001. Morphological and pathological variation in isolates of *Rhizoctonia solani* causing sheath blight of rice. *Plant Dis. Res.* 16: 166-172.

Menzies, J. D. 1970. The first century of *Rhizoctonia solani*. In: Parmeter Jr, J. R. (ed.), *Rhizoctonia solani: Biology and Pathology*, University of California Press, Berkeley, U.S.A., pp. 3-5.

Meyer, R. W. 1965. Heterokaryosis and Nuclear Phenomenon in Rhizoctonia. Ph. D. Thesis, University of California, Berkeley, 118 p.

Mishra, P. K., Gogoi, R., Singh, P. K., Rai, S. N., Singode, A., Kumar, A., and Manjunatha, C. 2014. Morpho-cultural and pathogenic variability in *Rhizoctonia solani* isolates from rice, maize and green gram. *Indian Phytopathol*. 67 (2): 147-154.

Mondal, A., Dutta, S., Nandi, S., Das, S., and Chaudhuri, S. 2012. Changes in defence-related enzymes in rice responding to challenges by *Rhizoctonia solani*. *Arch. Phytopathol. Plant Prot*. 45: 1840–1851.

Monga, D. and Raj, S. 1994. Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root tor of cotton. *Indian Phytopathol*. 47: 403-408.

Moni, Z. R., Ali, M. A., Alam, M. S., Rahman, M. A., Bhuiyan, M. R., Mian, M. S., Iftekharuddaula, K. M., Latif, M. A., and Khan, M. A. I. 2016. Morphological and genetical variability among *Rhizoctonia solani* isolates causing sheath blight disease of rice. *Rice Sci*. 23 (1): 42-50.

Mosaddeque, H. Q. M., Talukder, M. I., Islam, M. M., Khusrul Amin, A. K. M., and Alam, M. A. 2008. Screening of some restorer and maintainer hybrid rice lines against sheath blight *(Rhizoctonia solani). J .Soil. Nat*. 1: 23-29.

Mughal, M. N., Bashir, S., Bhat, N. A., and Bhat, K. A. 2017. Cultural and morphological variability and identification of anastomosis group of *Rhizoctonia*

solani (*Thanatephorus cucumeris*) causing sheath blight of rice in Kashmir. *Int. J. Curr. Microbiol. App. Sci.* 6 (11): 3787-3794.

Munsell Color Company. 1954. Munsell's Soil Color Chart. Munsell Color Co. Inc., Baltimore, Maryland, USA.

Nayak, P. and Sridhar, R. 1979. Host range of *Rhizoctonia solani*, the causal organism of sheath blight of rice. *Indian Phytopathol*. 32: 604-605.

Nayar, K., Gokulapalan, C., and Nair, C. 1996. A new foliar blight of amaranthus caused by *Rhizoctonia solani*. *Indian Phytopathol*. 49 (4): 407.

Neeraja, C. N., Vijayabhanu, N., Shenoy, V. V., Reddy, C. S., and Sarma, N. P. 2002. RAPD analysis of Indian isolates of rice sheath blight fungus *Rhizoctonia solani*. *J. Plant Biochem. Biotechnol*. 11 (1): 43-48.

Nelson, B., Helms, T., Christianson, T., and Kural, I. 1996. Characterization and pathogenicity of *Rhizoctonia* from soybean. *Plant Dis.* 80 (1): 74-80.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annu. Rev. Phytopathol*. 25: 125-143.

Ogoshi, A., Cook, R. J., and Bassett, E. N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathol*. 80 (9): 785-788.

Ou, S. H. 1985. Rice diseases. CAB International, Leutralsales, Fanham Royals Slough, UK, p. 380.

Palo, M. A. 1926. Rhizoctonia diseases of rice I. A. study of the disease and of the influence of certain conditions upon viability of the sclerotial bodies of the causal fungus. *Philippine Agric. 1*5: 361-376.

Paracer, C. S. and Chahal, D. S. 1963, Sheath blight of rice caused by *Rhizoctonia solani* Kuhn. A new record in India. *Curr. Sci.* 32: 328-329.

Parashuram, R., Yadav, S. C., Awadhiya, G. K., Prasad, M, S., and Prakasam, V. 2017. Survey and occurrence of sheath blight of rice in major rice growing areas of Chhattisgarh. *Int. J. Pure App. Biosci.* 5 (4): 838-845.

Parmeter, J. R. 1970. *Rhizoctonia solani*, Biology and Pathogenicity. Univ. California Press, Berkeley, USA, 255p.

Parmeter, J. R. J., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris. Phytopathol*. 59: 1270-1278.

Peltier, C. I. 1916. Parasitic *Rhizoctonia* in America Illinois. *Agr. Expt. Sta. Bull.* 189: 281-390.

Pillai, P. 1990. Laboratory screening of rice cultivars for sheath blight resistance. Extended summary, International Symposium Rice Research - New Frontiers, November 15-18, Directorate of Rice Research, Hyderabad, pp. 337-339.

Pollastro, S., Abbatecola, A., Dongiovanni, C., and Faretra, F. 2000. Usage of molecular markers (PCR-RAPD) for studying genetic variability in *Phellinus (Fomitiporia) species. Phytopathologia Mediterranea.* 39: 107-111.

Prasad, J., Gaur, V. K., Sharma, R. A., Mehta., and Sangeeta. 2014. Assessment of pathogenic and molecular variability in isolates of *Rhizoctonia solani* infecting chickpea. *J. Food Legumes* 27 (1): 42-45.

Reddy, C. S. and Susheela, K. 2005. Morphological characterization and pathogenicity of *Rhizoctonia solani* in rice. *J. Mycol. Pl. Path*. 35 (2): 403-409.

Reissig, W. H., Heinrichs, E. A., Litsinger, J. A., Moody, K., Fielder, L., Mew, T. W., and Barrion, A. T. 1986. Illustrated guide to integrated pest management in rice in Tropical Asia. International Rice Research Institute, Los Banos, Laguna, Philippines, 420p.

Richter, H. and Schneider, R. 1953. Studies on the morphological and biological differentiation of *Rhizoctonia solani. J. Curr. Res*. 2: 17-34.

Shahjahan, A. K. M., Duve, T., and Bonman, J. M. 1987. Climate and rice diseases.weather and rice. IRRI, Los Banos, Laquna, Philippines, pp. 125-128.

Shailbala and Tripathi, H. S. 2007. Current status of research on web blight disease of urd bean. *Agric. Rev*. 28 (1): 1-9.

Sharma, M., Gupta., S. K., and Sharma, T. R. 2005. Characterisation of variability in *Rhizoctonia solani* by using morphological and molecular markers. *J. Phytopathol*. 153 (7-8): 449-456.

Sharma, N. R., Teng, P. S., and Olivares, F. M. 1990. Comparison of assessment methods for rice sheath blight disease. *Philippines Phytopathol*. 26: 20-24.

Sharma, T. R., Aggarwal, R., Thind, T. S., and Guleria, S. 2007. Morphological and pathological variability in rice isolates of *Rhizoctonia solani* and molecular analysis of their genetic variability. *J. Phytopathol*. 155 (11-12): 654-661.

Singh, R., Murti, S., Mehilal, Tomer, A., and Prasad, D. 2015. Virulence diversity in *Rhizoctonia solani* causing sheath blight in rice pathogenicitya. *J. Plant Pathol. Microb*. 6: 8.

Singh, A., Singh, U. S., Singh, V., Zeigler, R. S., Hill, J. E., Singh, V. P., Duveiller, E. P., and Holderness. 2000. *Rhizoctonia solani* in Rice- Wheat System. *J. Mycol. Plant Pathol.*30 (3): 343-349.

Singh, A., Singh, U. S., Willocquet, L., and Savary, S. 1999. Relationship among cultural/ morphological characteristics, anastomosis behaviour and pathogenicity of *Rhizoctonia solani* Kuhn on rice. *J. Mycol. Plant Pathol.* 29 (3): 306-316.

Singh, H. K. and Singh, U. D. 2012. Evaluation of vertical and horizontal spread of sheath blight in rice varieties for resistance against *Rhizoctonia solani. Int. J. Agric. Env. Biotech*. 4: 367-372.

Singh, O. 2016. Variation among rice and maize isolates of *Rhizoctonia solani* Kuhn and management of sheath blight in rice. M.Sc.(Ag) thesis, G. B. PANT University of Agriculture & Technology, Pantnagar (U.S. Nagar), Uttarakhand, India, 87p.

Singh, V. and Singh, M. 2003. Fingerprinting the rice isolates of *Rhizoctonia solani* Kuhn using RAPD markers. *Int. Rice Res. Newsl*. 28 (2): 28-30.

Singh, V., Amaradasa, B. S., Karjagi, C. G., Lakshman, D. K., Hooda, K. S., Kumar, A. 2018. Morphological and molecular variability among Indian isolates of *Rhizoctonia solani* causing banded leaf and sheath blight in maize. *Eur. J. Plant Pathol.* https://doi.org/10.1007/s10658-018-1447-2.

Singh, V., Kumar, S., Lal, M., and Hooda, K. S. 2014. Cultural and morphological variability among *Rhizoctonia solani* isolates from trans-gangetic plains of India. *Res. Crops.* 15 (3): 644-650.

Singh, V., Singh, U. S., Singh, K. P., Singh, M., and Kumar, A. 2002. Genetic diversity of *Rhizoctonia solani* isolates from rice: differentiation by morphological characteristics, pathogenicity, anastomosis behaviour and RAPD fingerprinting. *J. Mycol. Plant Pathol*. 32: 332-344.

Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, Minnesota, USA.

Sneh, B., Jabaji- Hare, S., Neate., and Dijst, S. 1996. Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Springer Netherlands, Noordwijkerhout, Netherlands. 578p.

Srinivas, P. 2002. Studies on genetic and pathogenic variation in *Rhizoctonia solani f. sp. sasakii* incitant of banded leaf and sheath blight of maize. Ph. D. (Ag) thesis, Indian Agricultural Research Institute, New Delhi, 198p.

Srinivas, P., Ratan, V., Narayan, R. P., and Bindu, M. G. 2014. In vitro evaluation of fungicides, bio control agents and plant extracts against rice sheath blight pathogen. *Rhizoctonia solani*. *Int. J. Appl. Biol. Pharma. Technol*. 5:121-126.

Sunder, S., Kataria, H. R., and Sheoran, S. O. P. 2003. Characterization of *Rhizoctonia solani* associated with root/collar rots and blights. *Indian Phytopathol.* 56 (1): 27-33.

Surulirajan, M. 2003. Integrated management of sheath blight of rice *(Oryza Sativa* L.) caused By *Rhizoctonia Solani* Kuhn. Ph. D. thesis, Indian Agricultural Research Institute, New Delhi, 151p.

Susheela, K. and Reddy, C. S. 2013. Variability in Rhizoctonia solani (AG-1 IA) isolates causing sheath blight of rice. *Indian Phytopathol*. 66 (4): 341-350.

Swain, N. C., Chhotray, A. K., and Mahapatra, S. S. 2005. Pathogenic variability of *Rhizoctonia solani* causing sheath blight of rice and its management. *J. Plant Prot. Environ*. 2 (1): 96-99.

Syminis, T., Bandy, B. P., and Tavantzis, S. M. 1987. Vegetative incompatibility in *Rhizoctonia solani*. *Abstracts in Phytopathol.* 77: 1669-1670.

Taheri, P., Gnanamanickam, S. S., and Hofte, M. 2007. Characterization, genetic structure, and pathogenicity of *Rhizoctonia* spp. associated with rice sheath diseases in India. *Phytopathol.* 97: 373–383.

Takegami, J. C., Beaver, J. S., Lutz, G. G., Echavez-Badel, R., and Steadman, J. R. 2004. Inheritance of web blight resistance in common bean. *J. Agric. Univ. P. R*. 88 (1-2): 45-54.

Thakur, R. S., Sugha, S. K., and Sharma, B. M. 1992. Morphological grouping of different isolates of *Rhizoctonia solani* Kuhn. *Plant Dis. Res.* 7(1): 58-60.

Thind, T. S. and Aggarwal, R. 2008. Characterization and pathogenic relationships of *Rhizoctonia solani* isolates in a potato-rice system and their sensitivity to fungicides. *J. Phytopathol.* 156 (10): 615-621.

Thirumalaisamy, P. P., Singh, D. V., Aggarwal, R., and Srivastava, K. 2006. Pathogenic variability in *Tilletia indica* the causal agent of karnal bunt of wheat. *Indian Phytopathol.* 59 (1): 22-26.

Thomas, K. S. 1925. Onderzockinger over Rhizoctonia Electr.Drukkerij De Industries J. Van Drutten Utrecht. 98p.

Tiwari, A. and Khare, M. N. 1998. Variability among isolates of *Rhizoctonia solani* infecting mung bean. *Indian Phytopathol*. 51 (4): 334-337.

Toda, T., Hyakumachi, M., and Arora, D. K. 1999. Genetic relatedness among and within different *Rhizoctonia solani* anastomosis groups as assessed by RAPD, ERIC and REP-PCR. *Microbiol. Res*. 154 (3): 247-258.

Tu, J. C. 1967. Strain of *Pelicularia sasakii* isolated from rice in Taiwan. *Plant Dis. Reptr.* 51: 682-684.

Upadhyay, B. K., Dubey, S. C., Singh, R., and Tripathi, A. 2013. Morpho-molecular characterization of Indian isolates of *Rhizoctonia solani* infecting mungbean. *Res. J. Biotechnol.* 8 (11): 92-99.

Uppala, S. S. 2007. Potentiality of endophytic micro organisms in the management of leaf blight disease of amaranth. M.Sc. (Ag) thesis. Kerala Agricultural University, Thrissur, Kerala. 165p.

Vavilapalli, S., Celine, V. A., and Girija, V. K. 2014. Collar rot and web blight caused by *Rhizoctonia solani* Kuhn in vegetable cowpea (Vigna unguiculata (L) Walp.) and its organic management. *Agritechnol.* 2: 244p.

Vijayan, M. and Nair, C. M. 1985. Anastomosis grouping of isolates of *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk) causing sheath blight of rice. *Curr. Sci.* 54: 289-291.

Viswanathan, T. V. and Viswambharan, K. 1979. Screening germplasm collection of cowpea for tolerance or resistance to collar rot caused by *Rhizoctonia solani*. *Agric. Res. J. Kerala* 17: 272-274.

Wei, C. T., 1934. *Rhizoctonia* sheath blight of rice. Nanking College Agric. Forestry Bull., 15: 21p.

Welsh, J. H. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acid Res*. 18: 7213-7218.

Wheeler, B. E. J. 1969. An Introduction to Plant Diseases. John Wiley and Sons Ltd., London.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Kefalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.* 18: 6531–6535.

Xiao, Y., Liu, M., Li, G., Zhou, E., Wang, L, Tang, J., Tan, F., Zheng, A., and Li, P. 2008. Genetic diversity and pathogenicity variation in *Rhizoctonia solani* isolates from rice in Sichuan Province, China. *Rice Sci.*15: 137-144.

Yaduman, R., Singh, S., and Lal, A. A. 2019. Morphological and pathological variability of different isolates of *Rhizoctonia solani* Kuhn causing sheath blight disease of rice. *Plant cell biotechnol. mol. biol.* 20 (1&2): 73-80.

Yang, H. A., Sivasithamparam, K., and O' Brien, P. A. 1991. An improved technique for fluorescence staining of fungal nuclei. *Aust. Plant Pathol*. 20: 119-121.

Yang, H. A., Sivasthamparam, K., and O' Brien, P. A. 1993. Mycelial interactions and the potential use of tuft formation in characterizing *Rhizoctonia solani* isolates infecting cereals. *Australian J. Bot.* 41: 253-262.

Yang, J., Kharbanda, P. D., Wang, H., and McAndrew, D. W. 1996. Characterization, virulence and genetic variation of *Rhizoctonia solani*. *Plant Dis*. 80 (5): 513-518.

Yang, X. B., Snow, J. P., and Berggren, G. T. 1989. Morphogenesis of microsclerotia and sasakii-type sclerotia in *Rhizoctonia solani*, anastomosis group 1, intraspecific groups I A and I B. *J. Mycol. Res*. 93: 429-434.

Yokoyama, K. and Ogoshi, A. 1984. Studies on hyphal anastomosis of *R. solani* IV: Observation of imperfect fusion. *Ann. Phytopathol. Soc. Jpn*. 50: 398-401.

Yugander, A., Ladhalakshmi, D., Prakasham, V., Satendra, K., Mangrauthia, Prasad, M. S., Krishnaveni, D., Madhav, M. S., Sundaram, R. M., and Laha, G. S. 2015. Pathogenic and Genetic Variation among the isolates of Rhizoctonia solani (AG1-IA), the rice sheath blight pathogen. *J. Phytopathol.* 163: 465-474.

Zhou, S., Yang, L., Mei, Z., Li, B., Chen, X., and Liang, W. 2016. Comparison of the virulence and cognate virulence factors of multinucleate, binucleate, and uninicleate *Rhizoctonia* isolates, causing sheath blight on maize plants. *Eur. J. Plant. Pathol*. 145: 501-506.

Appendices

APPENDIX-1

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar (PDA)

Potatoes (Sliced)	-	200.00 g
Agar-agar	-	20.00 g
Dextrose	-	20.00 g
Distilled H_2O	-	1000 ml

APPENDIX – II
COMPOSITION OF STAIN USED

1. Lactophenol- Cotton blue

Anhydrous lactophenol	-	67.00 ml
Distilled water	-	20.00 ml
Cotton blue	-	0.1 g

Anhydrous lactophenol was prepared by dissolving 20g phenol in 16 ml lactid acid in 3ml glycerol.

APPENDIX - III
COMPOSITION OF BROTH USED

1. Potato Dextrose Broth (PDB)

Potato sliced)   - 200g

Dextrose   :- 20g

Distilled water   - 1000 ml

APPENDIX - IV

BUFFERS FOR PCR PRODUCTS AND GEL ELECTROPHORESIS

50 X TAE buffer

Tris Base   - 242 g

Glacial Acetic acid   - 57.1 ml

0.5 M EDTA (pH 8.0)   - 100 ml

Make up the volume to 1L using distilled water

1.2 % agarose is prepared for casting and examination of PCR product

STUDIES ON THE VARIABILITY OF *RHIZOCTONIA SOLANI* KUHN. INFECTING RICE, COWPEA AND AMARANTH

By

HARITHA J. KUMAR

2018-11-145

Abstract of the thesis
Submitted in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA

2020

ABSTRACT

Rhizoctonia solani is a ubiquitous destructive pathogen causing severe damage in economically important crops like rice and cowpea; and several vegetables like amaranth. In this context, the research work entitled "Studies on the variability of *Rhizoctonia solani* Kuhn. infecting rice, cowpea and amaranth." was undertaken in the Department of Plant Pathology, College of Agriculture, Vellayani during the year 2018-2020, with the objective to study the cultural, morphological, pathogenic and molecular variability of *R. solani* infecting rice, cowpea and amaranth in Southern Kerala.

R. solani infected samples of rice, cowpea and amaranth were collected from two locations each from the three southern districts of Kerala *viz.*, Vellayani and Chirayinkeezhu from Thiruvananthapuram; Kottarakkara and Pooyappally from Kollam; and Thiruvalla and Ranni from Pathanamthitta. Eighteen isolates of *R. solani* were obtained for the study. TRR1 (Vellayani) was more virulent among rice isolates. Among cowpea isolates, KRC1 (Kottarakkara) was more virulent. TRA2 (Isolate from Chirayinkeezh) was more virulent among amaranth *R. solani* isolates.

Various cultural characters like colony colour, pigmentation, rate of growth, texture of growth, days for sclerotial initiation, zonation, growth margin, dry weight of mycelium were studied for the different isolates and variations were observed among isolates. All isolates from rice and cowpea; and TRA2 (Chirayinkeezh), PRA1(Ranni) from amaranth were fast growers. Rate of growth was high in KRR1 (Kottarakkara), PRC2 (Thiruvalla) and PRA1 (Ranni) collected from rice, cowpea and amaranth respectively in PDA medium compared to other isolates. All *R. solani* isolates of amaranth and two cowpea isolates -TRC1 (Vellayani) and KRC2 (Pooyapally) didn't produce sclerotia in the culture. Zonation was absent in all rice and cowpea isolates except TRC1 (Vellayani) where a slight zonation was present. Days for initiation of sclerotial production ranged from 3 to 4 days.

Pathogen was highly variable in microscopic and sclerotial characters. Mean hyphal width varies from 3.81 μm (TRR2) to 6.95 μm (PRR2). Isolates show variation in the shape of moniliod cells which is the histological change of hyphae formed in the culture. A noticeable variation could be seen in sclerotial characters like colour, size, texture, shape, number, weight, orientation, position and clump formation. Macroscopic vegetative compatibility reaction between isolates of *R. solani* are merge, merging line, tuft, barrage, barrage line and microscopic hyphal anastmosis reaction are C0, C1, C2 and C3. Out of the 171 culture combinations, merge reactions and C2 hyphal anastomosis were most commonly found. There was less hyphal anastomosis reaction between amaranth and rice *R. solani* isolates and between amaranth and cowpea *R. solani* isolates(C0). More hyphal anastomosis reactions are present between rice and cowpea isolates and also within the same hosts (C2 and C3). C3 reaction is vegetatively compatible and C0, C1 and C2 reactions are vegetatively incompatible reactions.

Pathogenic variability among the isolates was analysed on Arun variety of amaranth. All *R. solani* isolates from rice, cowpea and amaranth were found infecting amaranth whereas variations in degree of pathogenicity were observed among isolates. PRR2 (Thiruvalla, PDI -46.66%), PRC1 (Ranni, PDI - 58.33%) and PRA1 (Ranni, PDI - 55.55%), were found most virulent among rice, cowpea and amaranth isolates respectively. Among all isolates PRC1 (Ranni) was the most virulent on amaranth.

Genetic variability was analyzed through Random Amplified Polymorphic DNA (RAPD) using four random primers such as OPW-13, OPA-04, OPA-19, OPA-20. The primers were found very informative in generating high level of polymorphism in the RAPD electrophoretic banding pattern. Intraspecific variability of *R. solani* isolates was expressed among the population from different as well as from same geographical regions.

R. solani isolated from amaranth plant does not produced sclerotia in the culture. All *R. solani* isolates produced symptoms on amaranth plant. Microscopic, microscopic characters and virulence were not affected in geographical origin. No correlation found

between virulence and cultural and morphologic characters. Intra specific variability among the population from the same and different geographical regions expressed through RAPD analysis. The study reveals that the isolates are cross infective between crops. The variation is observed at morphological, cultural and molecular level. This will help in developing suitable management practices. The specific gene responsible for the variation in   *R. solani* should be identified. Avirulent strain can be developed as biocontrol agent for the future disease management.