तमसो मा ज्योतिर्गमय

Isolation and characterization of *Rhizoctonia solani* causal agent of sheath blight of paddy

THESIS

SUBMITTED TO THE
ACHARYA NARENDRA DEVA UNIVERSITY OF AGRICULTURE & TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN

AGRICULTURAL BIOTECHNOLOGY

By

Minakshi
I.D. No. A-**10557/18**

DEPARTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
Dr. Ram Manohar Lohia Institute of Plant Biodiversity and Biotechnology
ACHARYA NARENDRA DEVA UNIVERSITY OF AGRICULTURE & TECHNOLOGY
NARENDRA NAGAR (KUMARGANJ), AYODHYA-224 229 (U.P.) INDIA

2020

Dr. Adesh Kumar
Assistant Professor

DEPARTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
A.N.D. University of Agri. & Technology,
Kumarganj, Ayodhya-224 229
(U. P.) India.

CERTIFICATE-I

This is to certify that the thesis entitled"**Isolation and characterization of *Rhizoctonia solani* causal agent of sheath blight of paddy.**" submitted for the degree of '**Master of Science'** in the subject of **Agricultural Biotechnology** to the Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj) Ayodhya, is a bonafide research work carried out by **Minakshi , Id. No. A- 10557/18** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation has been duly acknowledged.

Narendra Nagar
July 2020

(**Adesh Kumar)**
Major Advisor and Chairman

CERTIFICATE - II

This is to certify that the thesis entitled **"Isolation and characterization of *Rhizoctonia solani* causal agent of sheath blight of paddy."** submitted by **Minakshi , Id. No. A-10557/18** to the Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya, in partial fulfilment of the requirements for the degree of '**Master of Science**' in the subject of **Agriculture Biotechnology** has been approved by the student's Advisory Committee after an oral examination on the same in collaboration with an external examiner.

(23/09/2020)
External Examiner

ADVISORY COMMITTEE

Major Advisory & Chairman

(Adesh Kumar)
Assistant Professor
Deptt. of Plant Molecular Biology
& Genetic Engineering

Members (Major)

(D.K. Dwivedi)
Professor & Head
Deptt. of Plant Molecular Biology
& Genetic Engineering

Member (Minor)

(O.P Verma)
Assistant Professor
Department of Genetics &
Plant Breeding

Member (Related field)

(S.K. Singh)
Associate Professor
Department of Plant Pathology

Dean's Nominee

(Suresh Kumar)
Associate Professor
Department of Soil Science

Dean
College of Agriculture

Head
Deptt. of Plant Molecular
Biology & Genetic Engineering

Dedicated to My Beloved Grandfather

Late (Prof.) Dr. Kedarnath Tripathi

(Rastrapati Awarded)
Former President Kashi Vidvat Parishad, Vns.
Former Head of Department Philosophy
Banaras Hindu University, Varanasi

Minakshi.....

ACKNOWLEDGEMENT

First and for most, I owe my deep sense to almighty 'God' for giving me strength to overcome difficulties which came across my way in accomplishing of this endeavor and without whose blessing my present thesis would not have existed.

I express my indebtedness and inestimable gratitude to my esteemed major advisor and chairman Dr. Adesh Kumar, Assistant Professor, Deptt. Of P.M.B & G.E., N.D.U.A & T, Kumarganj, Faizabad for the invable assistance provided by him, I honesty thank him for his rhetoric and pearliness guidance, imperative and persistent encouragement facile counselling, constructive criticism and instant bearance in the current endeavor.

I am highly grateful to the member of advisory committee, Dr. D.K. Dwivedi, Professor & head Deptt. Of P.M.B & G.E, Dr. O.P Verma, Asst. Professor, Deptt. Of Genetics & Plant breeding, Dr. S.K Singh, Associate Professor, Deptt. Of Plant Pathology,Dr.S.uresh Kumar, Associate Professor , Deptt. Of Soil Science

I now express my gracious veneration and gratitude to Dr. Bijendra Singh, Vice Chancellor of the university, Dr. V.N Rai Dean College of Agriculture for providing necessary facilities to carry out this research work without any hurdle. I also lend my deep sense of gratitude to Dr. D.K Dwivedi, Dr.N.A Khan, Dr.Sambhu Prasad, including non-teaching staff Mr. S.P Singh, Mr. Keshav, Mr. Aditya,Mrs. Sita Pati, Mr. Ashok Kumar and Mr. Ram kumar. Other staff member of the Department of Plant Molecular Biology and Genetic Engineering for providing necessary facilities and cordial help when ever needed.

Words are inadequate to express the love and gratitude to my grandparents Late Prof. KedarNath Tripathi and Late Smt. Ganga Devi. I offer my regards to my loving parents, Dr. V.K Tripathi & Smt. Neeta Tripathi, and my In-laws Adv. Lal Ji Tripathi & Smt. Poonam Tripathi whose love, teachings and blessings brought me this far. I thank my father for his unconditional love and support throughout my educational career.I would never be able to pay back the love and affection showered upon by my parents. also, I express my thanks to my brother Ashutosh Kumar Tripathi, and brother in law Anurag Tripathi for their support and valuable prayers. Thanks

to my sister Shreeya Tripathi for her selfless love, care and dedicated efforts which contributed a lot for completion of my thesis. I would like to special thanks to my uncle Mr. I.D Singh for their strong support in my thesis work.

And most of all, I owe my deepest gratitude to my husband, Er. Anupam Tripathi for his affection, encouragement, understanding and patient. He supported me without any complaint or regret that enabled me to complete my M.Sc. Thesis.

I wish to extend my sincere thanks to my respected super senior Mrs. Shalini Srivastava and my senior Mrs. Komal and my nearest friend and roommate Shivani Chandra for her support and Supportive junior Mr. Niyaj.

My parents, husband and siblings form the backbone of my happiness and I dedicate my thesis to them. Finally, I thanks to my God for always being with me.

Narendra Nagar
Kumarganj, Ayodhya **(Minakshi)**
July 2020

CONTENTS

LIST OF TABLES

LIST OF PLATES

Plate No.	DISCRIPTION
4.1	Growth of fungal isolates on PDA plates after 10 days of incubation.
4.2	Growth of fungal isolates on PDA slants after 10 days of incubation.
4.3	Plant disease symptoms after inoculation it with different isolates of fungus.
4.4	PCR amplification with primer ITS1/ITS4 pair from total genomic DNA of *Rhizoctonia* isolate.
4.5	Phylogenetic tree of *Rhizoctonia solani* based on DNA bands.

Chapter I

INTRODUCTION

Rice (*Oryza sativa* L.*)* is India's predominant crop and one of the oldest cultivated crops for several thousand years. It is a staple food for the people of eastern, north eastern and southern part of country.It is the main food source for more than two-third of the world's population (Sasaki and Burr, 2000) especially in Southeast Asia (Nwugo and Huerta, 2011; Wang *et al.,* 2011). With the completion of the rice genome sequencing program, rice has become the model organism in molecular biological research of monocotyledons (Agarwal and Rakwal, 2011; Li *et al.,* 2011). The cultivated rice belongs to genus *Oryza* and there are about 24 species of rice distributed in tropical, sub-tropical and warm temperature regions of the world. Out of these, most commonly cultivated species are *Oryza sativa* and *Oryza glaberrima*. The *Oryza sativa* is divided into three sub-species, namely *indica , japonica* and *javonica.* It contains approximately 6-12% protein, 70-80% carbohydrate, 1.2-2.0% mineral matter and significant content of fats and vitamins. Rice supplies 23% of global human/capita energy and full fill its 16% of per capita protein requirements (USDA, 2010). Although India has the largest area under rice cultivation, the productivity is low which has been attributed to several biotic and abiotic stresses (Mohanty and Yamano, 2017). In-depth understanding of the pathogens involved is necessary, for the effective management of plant diseases. The most common and severe diseases of rice are blast, sheath blight and bacterial leaf blight (Woperies *et al.,*2009). *Rhizoctonia solani* causes Sheath blight disease in rice. The Sheath blight disease causes significant grain losses, yield losses of up to 50% have been reported under most conducive environment. Sheath blight of paddy occurs in areas with high

temperature (28-32 ^{0}C), high level of nitrogen fertilizer, and relative humidity of crop canopy from 85-100%. Symptoms are usually observed from tillering to milk stage in a rice crop initial symptoms are noticed on leaf sheaths near water levels. On the leaf sheath oval or elliptical or irregular greenish grey spots are formed and as the spots enlarge, the centre becomes greyish white with an irregular blackish brown or purple brown border and at severe stage it forms sclerotia. *Rhizoctonia solani* is a soil borne Basidiomycetes fungus and occurs worldwide (Lehtonen, 2009). According to Agrios (1997) soil borne pathogenic fungi can cause disease on roots and other underground plant parts *i.e.* stolon's, tubers and basal parts of the stems. Though Rhizoctonia *solani* is a soil borne pathogen it can attack stern and leaf of plants (Snehet *et al*., 1991). *Rhizoctonia solani* causes significant damage on crop quantity and quality of crop species annually. (Weinhold *et al*., 1982; Ban ville, 1989; Martin and Loper, 1999; Green and Jenson, 2000; Bottonal., 2006; Wagacha and Muthomi, 2007). The genus *Rhizoctonia spp.* is a species complex of highly heterogeneous group of filamentous fungi. These fungi have similarities in their sterile as well as anamorphic state. The fungus does not produce any sexual spores *i.e.* Conidia. Sexual spores *i.e.* basidiospores occur only rarely. The asexual stages of these fungi are known as anamorphic state and the sexual stages of this fungus are known as teleomorphic state. *Rhizoctonia solani* produces thread like hyphae. Colour of the hyphae is white to brown. Immature hyphae are white in colour, as the hyphae mature it turns into brown or dark brown in colour. Dolipore septum is present within the cross wall of the hyphae. Each cell is multinucleate, though binucleate *Rhizoctonia* is also present. Branches produce from the main hypha at right angles. Asexual spores are not formed by the mycelium. Small, oval cells are produced in branched chains or clusters. These

cells are called monilioid cells. Monilioid cells have slightly thicker walls than the mycelium. When these monilioid cells are aggregated large amounts, they form a resting structure called sclerotia. Sclerotia are brown coloured and 3 to 5 mm long.

Rhizoctonia solani can be divided into 13 anastomosis groups (AGs) based on hyphal anastomosis reaction (Parmeter *et al.*, 1969). Some AGs of *Rhizoctonia solani* have been further divided into subgroups based on cultural morphology, host range, virulence, and molecular techniques (Vilgalys *et al*., 2007). Isolates within the same AG, or within the same subgroup, may have similar characteristics, such as host preference and disease symptoms. Currently the AG groups of *Rhizoctonia solani* have been further divided into subgroups AG 1, AG 2, AG 3, AG 4, AG 5, AG 6, AG 7, AG 8, AG 9, AG 10, AG 11, AG 12, and AG 13 based on various features (Harikrishnan *et al.*, 2004, Meyer *et al.*, 2006). As of recent, it has become increasingly common to designate anastomosis groups through molecular approaches. Most available molecular techniques are based on the detection and typing of genomic polymorphisms at several levels. Sequencing of ribosomal DNA (rDNA) has been widely employed in recent years to reconstruct phylogenetic relationships between different organisms at the genus level (Susan *et al*., 2006, Peth *et al*., 2007). Techniques in molecular biology have contributed to determining genetic diversity and taxonomic classification within fungal species (Steinberg *et al.*, 2003,Tamura *et al.*, 2004). Currently, rDNA internal transcribed spacer region ((ITS) composed of ITS1, 5.8S, and ITS2 regions) sequence information offers the most accurate method of establishing the

taxonomic and phylogenetic relationships for *Rhizoctonia spp.* (Sharon *et al*., 2008, Reddy *et al.*, 2013). Sequence analysis of the genomic regions encoding the ITS-rDNA is convenient for AG determination and has become increasingly common with the accumulation of sequences from different isolates in databases (Woodhall *et al*., 2007, Lehtonen *et al*., 2008). Both within and between the various AGs, ITS-rDNA sequencing has been used to analyze the genetic diversity of *Rhizoctonia solani*. Keeping all these points in view, the present study was planned to isolate the sheath blight of rice causal agent *Rhizoctonia solani* from various rice growing regions of Uttar Pradesh and screening for their ability of pathogenicity and of their molecular characterization to establish the diversity with following objectives:

1. Isolation of fungus from sheath blight diseased sample from different regions.
2. Phenotypic, cultural and morphological characterization of isolates.
3. Pathogenicity test and molecular characterization.

Chapter-II

REVIEW OF LITERATURE

Review of literature pertaining to the topic "Isolation and characterization of *Rhizoctonia solani*causal agent of sheath blight of paddy". Was collected in this chapter to present brief account of research carried out in India and Abroad. Whenever the literature on particular aspect of *Rhizoctonia solani*on Paddy was scanty, it was amply supplemented with and supported by other pathogens and crops. The literature relevant to present investigation was collected under the following heads:

2.1. Isolation of fungus from sheath blight diseased sample from different regions.

2.2. Phenotypic, cultural, and morphological characterization of isolates.

2.3. Pathogenicity test and Molecular characterization.

2.1. Isolation of Fungus from sheath blight diseased sample from different regions.

***Surya et al.,* (2017)** found six isolates of *Rhizoctonia solani*,*i.e.,* two isolates collected from infected rice plants and four isolates from laboratory collection studied by using morphological characters and molecular analysis.

Hossain *et al.,* (2014) isolated, identified and validated the sheath blight resistance in rice (*Oryzasativa L.*) cultivars against *Rhizoctonia solani*Six moderately resistant cultivars, namely 'Teqing', 'Jasmine85', 'Tetep', 'Pecos', 'Azucena' and 'Taducan', one susceptible local cultivar, 'MR 219', and two new

advanced breeding lines, 'UKMRC 2' and 'UKMRC 9', were screened using micro-chamber and mist-chamber methods.

Webb *et al.*, (2011) worked with the long-term preservation of *Rhizoctonia solani*isolates and from their experimental result they reported that cryogenic methods (storage in liquid nitrogen) are suitable for the preservation or storage of *Rhizoctonia solani*cultures. They also reported that efficiency may vary in different isolates.

Sharma *et al.*, (2005) collected twenty- four isolates of *Rhizoctonia solani*(teleomorph: *Thanatephoruscucumeris*) from soil, root and collar rot or foliage blight- infected plants from several locations of north India were used for the analysis of variability by using morphological and molecular markers.

Meisong*et al.*, (1994) studied fifteen isolates of *Rhizoctonia solani*obtained from rice plant affected by sheath blight from five provinces of Thailand were studied for cultural characteristics. The isolates were identified as AG 1-1A.

2.2. Phenotypic, Cultural and Morphological characterization ofIsolates.

Joseph *et al.*, (2019) observed forty-two *Rhizoctonia* isolates which were collected from rice, mung bean, and grasses from Laguna, Philippines. Sixteen isolates were binucleate *Rhizoctonia* (BNR), while 26 were multinucleate *Rhizoctonia* (MNR). BNR isolates produced white to brown, small sclerotia (<1.0 mm) except for mung bean isolates. Twenty MNR isolates produced big (>1.0 mm), light to dark brown sclerotia, three produced salmon-colored masses in the medium, and three did not produce sclerotia. Twenty-three MNR isolates were identified as *Rhizoctonia solani*AG1-IA using specific primers. Deduced Internal Transcribed Spacer (ITS) sequences of BNR isolates D1FL, NVL, and ScNL

shared 100, 97, and 100% identity with *R. oryzae-sativae*, respectively, while MNR isolates BMgL, IbMgL, and MaSL that produced salmon-coloured masses shared 100, 90, and 100% identity with *Rhizoctonia oryzae*, respectively. Preliminary analysis of the DNA fingerprint patterns generated by repetitive-element PCR (rep-PCR) clustered the 42 isolates into three: *Rhizoctonia solani*, *Rhizoctoniaoryzae-sativae, and Rhizoctoniaoryzae*, together with*Ceratobasidium sp., Rhizoctonia solani*isolates were pathogenic on rice (TN1), barnyard grass, mungbean (Pagasa 3), and tomato (Athena), while *Rhizoctonia oryzae* and *Rhizoctonia oryzae-sativae* isolates were only pathogenic on rice, *Echinochloa crus-galli*, and tomato. *Rhizoctonia solaniand Rhizoctonia oryzae* were found to be more virulent than Rhizoctonia oryzae-sativae.

Dilip *et al.*, (2018)studied morphological and molecular variability among Indian isolates of *Rhizoctonia solani*causing banded leaf and sheath blight in maize. All the tested isolates caused symptoms of BLSB on maize and were also cross infective on rice and sugarcane hosts,but showed significant variability in hyphal diameter,mean hyphal cell size, weight, size and distribution of scleorotia, culture pigmentation, incubation period, pathogenicity and expression of symptoms. Neighbour joining cluster analysis placed the 62 isolates of *Rhizoctonia solani*into four major groups, A, B, C and D. Pathogenicity testingof*Rhizoctonia solani*isolates on maize genotype (CM 501) re-vealed highly variable virulence pattern of the pathogen population suggesting its high evolutionary potential, and hence adaptability to diverse geographical regions. of new genotypes into the region.

Surya *et al.*, (2017) studied by using morphological characters and molecular analysis of *Rhizoctonia solani*. Unweighted pair group method with arithmetic

mean dendrogram constructed based on cluster analysis showed that these isolates were grouped into three clusters at the 0.77 similarity coefficient. Cluster I consisted of BA, BNJ, and NBR isolates with 100% similarity and indicated that those were from AG 1 IA sub group, cluster II consisted of BND, and cluster III consisted of SL1 and SL2. Mycelium was very light brown or whitish with few and moderate sclerotia except SL1 and SL2.

Pankaj (2011) isolated *Rhizoctonia solani*from rice, maize and green gram were studied for their variability with respect to cultural, morphological and pathogenicity characterstics.Isolates of*Rhizoctonia solani*from rice, maize and green gram were studied for their variability with respect to cultural, morphological, pathogenicity and genetical characteristics.

Sharma and Tripathi (2001) reported that thirty-three plant species would be attacked by *Rhizoctonia solani*experimentally. The plants were of families like *Leguminosae* (11 plants), *Gramineae* (5 plants), *Solanaceae* (11 plants), *Brassicaceae* (5 plants),*Malvaceae*{3 plants), Cyperaceae (2 plants) one each of *Cucurbitaceae, Commelinaceae and Chenopodiaceae.* Host range of*Rhizoctonia solani*is very broad and several diseases have been reported by several scientists till date. Characteristics of *Rhizoctonia solani*as a pathogen *Rhizoctonia solani*is not an obligate parasite and it can stay in the soil as saprophyte for long period. Due to the lack of conidia and the scarcity of sexual spores *Rhizoctonia solani*remain as vegetative hyphae or in sclerotia! form. Sclerotium is an encapsulated, tightly compact hyphal clump that gives the fungus protection from environmental stress. The fungus *Rhizoctonia solani*generally spread through sclerotia, plant materials contaminated with this fungus, soil spread by wind, water ordering agricultural activities such as tillage and seed transportation.

Prahlad (2018) studied morphological and pathological characters of *Rhizoctonia solani*. They were fast growing in culture medium with differences in sclerotia formation and exhibited varying degree of virulence on the same cultivar BPT5204, a variety susceptible to sheath blight. Becomes tedious because of similarity in Symptoms.

Vimal *et al.*, (2014) identified cultural and morphological variabilityexamined in 15 isolates of *Rhizoctonia solani*from trans-gangetic plains covering parts of Haryana, Punjab and Delhi.The isolates were collected from maize and rice host origin showed a considerable variability in the parameters studied . The maize isolates formed a yellowish brown to off white colony on PDA.

Ritchie *et al.*, (2009) observed the mycelium of *Rhizoctonia solani*isolates from potato (AGs-2-1 and AG-3) grew best between 20°C and 25°C on all media (Potato dextrose agar, Malt yeast extract agar, water agar, soil extract agar) tested. Mycelial growth of all isolates occurred between pH4 pH9 with an optimum pH 5.6. In AG-3 isolates sclerotia formation occurred between pH4 -pH8 and in case of AG2-1 isolates the sclerotia formed between pH 5- pH6. Sclerotia germination took place in between 20-30℃and pH 5-6 in case of AG3 on all media tested. AG 2-1 isolates grew significantly slower compared to AG-3 in soil Mycelia grew best in soil between 20-25°C . Germination of sclerotia of AG3 isolates in soil took place between 10°Cand 30°C.

Kliejunas and Ko (1975) observed *Rhizoctonia solani*grew continuously at a steady growth rate on agar media but its' growth ceased on liquid media after a certain time. Like agar media the growth of *Rhizoctonia solani*also occurred continuously in autoclaved soil media. for AG 2-1.

Gottlie (1971) observed that mycelial growth of all the fungi was not indefinite. Experiment indicated that an age dependent, growth regulating mechanisn1 exist in at least some fungi and is responsible for restricted growth. The growth rate of *Rhizoctonia solani*increased to maximum at 4-5 days then decreased until growth ceased at 8-9 days. The respiration of the peripheral hyphae of mycelia that have ceased growth is greater than that of the older parts of the thallus.

Elarosi (1957)stated that the optimum pH value for*Rhizoctonia solani*growth is approximately 5.9. He also observed that the growth of *Rhizoctonia solani*is poor in pectin media when used as a sole source of carbon. But in pectin-agar medium the growth rate of *Rhizoctonia solani*increased.

Singh *et al.*, (2007) observed many white and mustard seed like brown sclerotia, from on the diseased panicles. A series of characteristic coppery band also appear across the lesions; hence, the disease was named banded blight. Sclerotia appeared among loose fine silvery threads of mycelium after 6 days on or near the lesions on or between leaf sheath and culm within lumen of culm and within large cells of sheath. They are easily detached at maturity.

Basu and Gupta (1992) found positive correlation between the size of sclerotia and pathogenicity. Less than 50micrometre propagules are generally non-pathogenic to rice seedlings cut sclerotia into 0.2,4,8and 16 pieces were able to infect the plant, but larger pieces produced large lesions.

Singh *et al.*, (2002) reported that the size of sclerotia affected not only the incubation period but also the number and size of lesions after inoculation, incubation period decreased with increases in mass of sclerotia, while the number and size of lesions increases.

Aggarwal *et al.*, (2007) observed morphological and pathological variability in rice isolates of *Rhizoctonia solani*collected from different rice varieties grown in various regions of Punjab were studied for their morphological and pathological characterization. Majority of the isolates were fast growing with raised and fluffy colonies and hyphal width of 9.6μm while four exhibited moderate growth rates. Colony colour in all except two isolates was light yellowish brown. While sclerotia number per 5.0 mm culture disc of the test isolates ranged between 2.1 and 11.2 mm, their size varied between 1.31 and 2.08 mm. Sclerotial colour in all except two isolates was dark brown and most of these were found scattered in the colony. There was no relationship between morphologically similar isolates and their pathogenic behaviour. Majority of the isolates produced lesion length between 45.6 and 58.2 mm on detached rice leaves.

Singh *et al.*, (2002) observed genetic diversity of *Rhizoctonia solani*isolates from rice plant differentiation by morphological characteristics.

Lal *et al.*, (2014) stated diversity analysis of *Rhizoctonia solani*causing sheath blight of rice in India. Twenty-five isolates were collected from different parts of India. Morphological and cultural characters were investigated, on the basis of colony colour, growth pattern, hyphal width, size sclerotia.

2.3. Pathogenicity test and Molecular characterization.

Sobhita*et al.*, (2019) studied morphological and molecular Variability of *Rhizoctonia solani*Caused Sheath Blight of Rice and In-vitro Management. in the experiment Molecular variability studies were carried out by using RAPD and ISSR primers. The results of RAPD analysis revealed that isolates were clustered to two main clusters at 49% degree of similarity, where cluster I consists of 4 isolates and cluster II consists of 5 isolates. Similarly, ISSR analysis revealed that

isolates were clustered to two main clusters at 39% degree of similarity, where cluster I consists of 2 isolates and cluster II consists of 7 isolates. Among fungicides tested Propiconazole showed highest percent (92.59%) of inhibition at a concentration of 120ppm. Propiconazole and Difenconazole combination and Metalaxyl and Cholrothalonil combination among the combination of fungicides viz., had revealed cent (100%) percent of inhibition at 180ppm concentration. Among the herbicide and insecticide, Glyphosate and Chlorpyriphos showed highest (100%) percent of inhibition at 0.2% of concentration each respectively. Among the plants extracts *Parthenium spp*. revealed highest (76.29%) percent of inhibition. Hypocrealixii (KX0113223) resulted showed highest growth inhibition (75.5%) among the six tested bioagents.

Aqeel *et al.*, (2019) observed molecular characterization of *Rhizoctonia solani*isolated from cucumber (*Cucumis sativus L.)* and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. In the experiment results of polymerase chain reaction (PCR) amplification and nucleotide sequence analysis using BLAST demonstrated that *Rhizoctonia solani*isolate was genetically different from the *Rhizoctonia solani*isolates in the National Centre for Biotechnology Information (NCBI). Therefore, it was recorded in GenBank under the accession number MK105921.

Dilip *et al.*, (2018) studied molecular variabilty among indian isolates of *Rhizoctonia solani*causing banded leaf and sheathblight in maize. Neighbour joining cluster analysis placed the 62 isolates of *Rhizoctonia solani*into four major groups, A, B, C and D. Sequence comparisons of the internal tran-scribed sequence-ribosomal DNA region of 62 isolatesdid not reveal much diversity among the isolates. Majority of the isolates (n= 61) clustered together with anasto-

mosis group (AG) AG1-IA used as reference strain in the phylogram, distinct from AG1-IB, AG2–2IIIB and Waiteacircinata used as reference strains. BLSB isolates representing distinct geographical locations shared identical sequences indicating long-distance dispers-all of the pathogen. The study confirms that the ge-netic flexibility of the pathogen allows for its adap-tation to variable ecological niches and long-distance introduction.

Prahlad (2018) studied three fungal isolates for morphological and pathological characters. They were exhibited varying degree of virulence on the same cultivar BPT5204, a variety susceptible to sheath blight. Precise identification of cause of disease based on morphological characters and symptoms induced by *Rhizoctonia* sp. Becomes tedious because of similarity in symptoms. The identification of isolates at genus and species level using molecular markers for genetic differentiation would be an ideal approach. The isolate RS4 showed 99 % homology with *Rhizoctonia solani*AG1-IA based on nucleotide sequence data for ITS.

Liu*et al.*, (2017) observed the antifungal properties and mechanism of three types of chitosan against the rice sheath blight pathogen, *Rhizoctoniasolani,* were evaluated. Each chitosan had strong antifungal activity against *Rhizoctonia solani*and protected rice seedlings from sheath blight, in particular, two types of acid-soluble chitosan caused a 60–91 % inhibition in mycelial growth, 31–84 % inhibition of disease incidence, and 66–91 % inhibition in lesion length. The mechanism of chitosan in protection of rice from *Rhizoctoniasolani*pathogen was attributed to direct destruction of the mycelium, evidenced by scanning and

transmission electron microscopic observations and pathogenicity testing.

Surya *et al.*, (2017) collected six isolates of *Rhizoctoniasolani*, from infected rice plants and four isolates from laboratory collection were studied by using morphological characters and molecular analysis. Unweighted pair group method with arithmetic mean dendrogram constructed based on cluster analysis showed that these isolates were grouped into three clusters at the 0.77 similarity coefficient. Cluster I consisted of BA, BNJ, and NBR isolates with 100% similarity and indicated that those were from AG 1 IA subgroup cluster II consisted of BND, and cluster III consisted of SL1 and SL2. Mycelium was very light brown or whitish with few and moderate sclerotia except SL1 and SL2. Molecular characterization showed that BA, BNJ, and NBR were amplified at 140 bp using Rs1F/Rs2R specific primer for R. solani AG1 IA. All isolates were amplified between 350−400 bp using Rhsp1 primer, meanwhile SL1 and SL2 were not amplified using AG2sp and AG22sp2 primers. Based on Maximum Likelihood tree analysis showed that SL1 and SL2 had high similarity based on ITS sequence data.

Khodayari *et al.*, (2017)studiedgenetic diversity of Iranian AG1- IA isolates of *Rhizoctonia solani*, the cause of rice sheath blight, using morphological and molecularmarkers. 25 out of 47 isolates of *R. solani* collected from rice paddies in northern (mostly Guilan province) Iran were examined for anastomosis grouping, colony morphology, growth rate and genetic diversity. Genetic diversity was estimated among 25 isolates using inter simple sequence repeats (ISSR) and enterobacterial repetitive consensus (ERIC) analysis. DNA bands of four ISSR and a pair of ERIC primers ranged from 0.25 to 3Kb in all isolates.

Taheri *et al.*, (2017)observed characterization, geneticstructure, and pathogenicity of Rhizoctonia spp. Associated with Rice Sheath Diseases in India. Isolates of *Rhizoctonia spp*. were obtained from rice in India during 2000-2003. Characterization by conventional techniques and polymerase chain reaction showed that from 110 isolates, 99 were *Rhizoctonia solani*and 11 were *Rhizoctoniaoryzae-sativae*. Of 99 isolates identified as *Rhizoctonia solani*, 96 were AG1-IA, 1 was AG1-IB, and 2 were AG1-IC. Amplified fragment length polymorphism (AFLP) analyses were used to determine genetic relationships in*Rhizoctonia* pathogen populations collected from different geographic regions. Cluster analysis based on the AFLP data separated isolates belonging to the three different intraspecific groups of *Rhizoctonia solani*AG1 and differentiated *Rhizoctonia solani*from*Rhizoctoni*oryzae-sativae. Analysis of molecular variance (AMOVA) revealed that geographic region was the dominant factor determining population structure of*Rhizoctonia solani*AG1-1A; host cultivar had no significant effect. Pathogenicity tests on Oryza sativa cv. Zenith revealed that isolates of *Rhizoctonia solani*AG1-1A and AG1-1B were more virulent than *Rhizoctonia solani*AG1-IC and R. oryzae-sativae isolates.

Nadarajah *et al*.,(2014) studied Molecular Characterization and Screening for Sheath Blight Resistance Using Malaysian Isolates of *Rhizoctonia solan*i. In his experiment two field isolates of *Rhizoctonia solani*were isolated from infected paddy plants in Malaysia. These isolates were verified via ITS-rDNA analysis that yielded ~720 bp products of the ITS1-5.8S-ITS4 region, respectively. The sequenced products showed insertion and substitution incidences which may result in strain diversity and possible variation in disease severity. These strains showed some regional and host-specific relatedness via Maximum Likelihood and further

phylogenetic analysis via Maximum Parsimony showed that these strains were closely related to *R. solani* AG1-1A (with 99-100% identity). Subsequent to strain verification and analysis, these isolates were used in the screening of twenty rice varieties for tolerance or resistance to sheath blight via mycelial plug method where both isolates (1801 and 1802) showed resistance or moderate resistance to Teqing, TETEP, and Jasmine 85. Isolate 1802 was more virulent based on the disease severity index values. This study also showed that the mycelial plug techniques were efficient in providing uniform inoculum and humidity for screening.

Pankaj (2011) has done molecular characterization of *Rhizoctonia solan* iinfecting different hosts. The Isolates of*Rhizoctonia solani*from rice, maize and green gram were studied for their variability with respect to cultural, morphological, pathogenicity and genetical characteristics.The isolates were further characterized using three molecular markers viz., random amplified polymorphic DNA (RAPD), universal rice primer (URP) and internal transcribed spacer region (ITS). The RAPD markers were suitable for resolving the genetic polymorphism of the isolates. It resulted into two major and distinct clusters for maize and rice isolates. The two green gram isolates, PRS21 and PRS22 were clustered with the maize isolates. URP markers could not differentiate the isolates properly. UPGMA analysis of URP data revealed that the green gram and maize isolates belonged to the same cluster. Further analysis of ITS region 1 and 2 using ITS1 and ITS4 universal primer resolved an amplicon of 700 bp in all the *Rhizoctonia solani*isolates.

Ou (1985) observed initial symptoms appeared as lesions on sheath of lower leaves when plants are in late tillering or early internode elongation stage of

growth. The symptoms of disease appeared on almost all the aerial plant parts such or lower leaf sheath, flag leaf and panicles.

Chapter-III

MATERIALS AND METHODS

The present investigation on **"Isolation and characterization of *Rhizoctonia solani* causal agent of sheath blight of paddy." w**as carried out in the department of plant molecular biology and genetic engineering, Narendra Deva University of Agriculture and Technology Kumar Ganj, Ayodhya (UP). The detail of material used, experimental procedures followed, and techniques adopted during the course of investigation have been described in this chapter.

3.1. Experimental Materials

3.1.1 Instruments used

1. Autoclave
2. BOD incubator
3. Hot air oven
4. Laminar air flow
5. Electronic balance
6. Forceps
7. Gas burner for flaming
8. Water bath
9. Centrifuge
10. Gel electrophoresis (bp)
11. Micropipette
12. PCR
13. Deep freezer

14. Shaker
15. Fridze
16. pH meter
17. Microwave oven
18. Spectrometer
19. Microscope
20. Scanner/ Gel documentation
21. Ice machine

3.1.2 Cultures:

Native strains of *Rhizoctonia solani* were isolated from infected plant part of paddy showing sheath blight disease symptom from different regions of Uttar Pradesh **(Taheri *et al.*, 2007).** These cultures were maintained on PDA slants at 4°C.

3.1.3 Media Used:

Media components used were from Hi-media and all other chemical and reagents were of analytical grade from Sigma. PDA media was used for isolation, cultivation and maintenance of *Rhizoctonia solani*

PDA media contained following constituents in g/litre

Potatoes (sliced washed unpeeled): 200g

Dextrose : 20g

Agar Powder : 20g

Distilled water : 1.0 litre

pH : 6.0

3.1.4 Sampling

Sheath blight symptoms showing diseased samples of *Rhizoctonia solani*were collected from different division of eastern Uttar Pradesh (Gorakhpur Division, Varanasi division, Mirzapur division, Mau division ,Ayodhya division). The detailed information of crops and location is shown in Table 3.1.

3.1.5 Chemical used for identification and Molecular characterization of *Rhizoctonia solani*

3.1.5.1 Material required for DNA isolation

(1) DNA extraction buffer	**100ml**
Tris Buffer (50mM)	0.605
EDTA (10mM)	3.12g
NaCl (150 mM)	0.87g
(2) 70 % Ethanol	**100ml**
Absolute ethyl alcohol	70ml
Distilled water	30ml
(3) 5 M Nacl solution	**20ml**
Nacl (5M)	5.8g
Distilled water	20ml
(4) 10% SDS	**100ml**
SDS	10g
Distilled water	80ml

(5)	**10% CTAB**	**20ml**
	CTAB	2g
	Nacl	0.82g
(6)	**Choloform: Isomyl alcohol (24:1)**	**100ml**
	Choloform	96ml
	Isomyl alcohol	4ml
(7)	**3M sodium acetate**	**100ml**
	Sodium acetate	40.81g
	Distilled water	100ml
(8)	**TE Buffer**	**100 ml**
	Tris Buffer(10mM)	0.121g
	EDTA 1mM,pH 8.0)	0.372g
(9)	**TAE Buffer (50 X)**	**1000ml**
	Tris base	242g
	Glacial acetic acid	57.0ml
	0.5 M EDTA (pH 8.0)	100ml
(10)	**Ethidium Bromide**	**10mg/ml**
Ethidium Bromide		0.1 g
	Distilled water	10 ml
(11)	**Loading Dye (6x)**	**10ml**
Sucrose		4 g
	Bromophenol-blue	2.5 mg
	10 mM Tris-HCl, pH 8.0	6 ml

1 mM EDTA (TE buffer)

(12) RNase Solution

RNase 10 mg
10 mM Tris-HCl (pH 8.0)
+ 1 ml
15mM NaCl

Preparation of PCR reaction mixture:

Following cocktail mixture was prepared in sterile PCR tubes-

Heated the tube in boiling Water bath at 100 degree Celsius for 15 minutes. Cooled slowly at room temperature.

Compounds (conc.)	**Concentration for single PCR tubes(µl)**
DNA template (50mg/µl)	1.0
dNTP mixture (10mM)	1.5
Taq DNA Polymerase (3U/µl)	0.5
PCR Reaction buffer (10X)	2.5
Magnesium Chloride	1.0
Double distilled water	17.5
Primer	1.0
Total	**25.0**

Primer details:

No.	**Oligo Name**	**Sequence (5' a' 3')**
1	18s Forward Primer	CCATGCATGTCTAAGTWTAA
2	16s Reverse Primer	ANCCATTCAATCGGTANT

3.2 Experimental methods

3.2.1. Cleaning and sterilization of metal and glassware

For cleaning of required glassware, the glasswares were kept in the solution containing 60-gram potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated Sulphuric acid (H_2SO_4) in one litre of water for a day, Then, they were cleaned by washing with several times in tap water. All glasswares solid and liquid media were subjected to sterilization by autoclaving at 15 psi (121.6℃) for 20 minutes or hot air even. The tip of inoculated needle and forceps were sterilized under flame. Dry glassware's were sterilized at 180 ℃ for 20 hours in a hot air oven.

3.2.2 Isolation of fungus from sheath blight diseased sample

Samples infected by *Rhizoctonia solani* exhibited Sheath blight symptoms on rice were collected from different regions of Uttar Pradesh then from the diseased sample isolation of fungus *Rhizoctonia soalni* was done. The samples of paddy sheath were thoroughly washed with 0.5% Mercuric Chloride solution for 1 minute and rinsed three times in sterile distilled water. Sample bits containing advancing margin of infection were cut in such a way that half healthy and half infected part is present. The samples were dried on sterilized filter paper , transferred to Potato dextrose Agar plates and incubated at 28℃. After 2 to 3 days cultures were examined for the mycelium of fungus and purified on PDA slants. The pure cultures were maintained on PDA slants at 4 ℃.

3.2.3 Preparation of PDA plates:

PDA medium consisting following composition was prepared and sterilized using method described by Waller *et al.*, (2001) was used for present study.

Peeled potato : 200.00g

Dextrose : 20.00g

Agar : 20.00g

Distilled water : 1000.00ml

The peeled potatoes were cut in 12 mm cubes. Two hundred gram of potato cubes were rinsed in water and boiled for 20 minutes in 500 ml water. Potato broth was filled through cheese cloth and kept in measuring cylinder. Agar was melted in 500 ml of water by heating and added to potato broth. Dextrose was added in it. The pH was adjusted to 7.0. The PDA was poured in test tube for preparation of PDA slants and also Petri dish. Then there were sterilized at 15 psi for 20 minutes in an autoclave.

3.2.4 Pure culture of pathogen

The pure culture of pathogen (*Rhizoctonia solani)* was obtained by using hyphal tip method. Water suspension of hyphal tip (1.0 ml) was poured aseptically over a molten but still warm plain agar (2%) Petri dishes to from a very thin layer. The growth of fungus was allowed on plain agar for 24-48 hours and critically observed under microscope. The areas having hyphal tips along with medium was scooped out and transferred to slants to obtain a single hyphal tip. The regular sub-culturing was done at 15 days interval to check contamination . These PDA slants having *Rhizoctonia solani* were kept in refrigerator at 6 to 8-degree Celsius temperature for further studies.

3.2.4.1 Maintenance of Rhizoctonia solani isolates

Isolates of*Rhizoctonia solani*was maintained on potato dextrose agar (PDA) at −80°C for long-term storage and for short-term cultures for immediate use was maintained at 4°C.

3.2.5 Soil preparation

Clayey loam soil from local paddy field was collected and autoclaved 3 times (1h, 121℃) at 12 hrs-intervals mixed with autoclaved manure. One Kg sterilized soil was filled per pot.

3.2.6 Identification of the fungal isolates:

The *Rhizoctonia solani* was identified on the basis of their cultural and morphological characters as given below:

3.2.6.1. Colony and growth characters:

The cultural and morphological characters of the fungus were recorded on PDA medium after 5-6 days of incubation. Colour and type of mycelium were observed with the help of microscope.

3.2.6.2. Mycelial characters:

The colour, septation and branching pattern of mycelium were microscopically recorded.

3.2.6.3. Sclerotia characters:

The colour shape and size of sclerotia were observed at 5-6 days of incubation.

3.2.7. Pathogenicity test:

Pathogenicity done during this investigation revealed that the pathogen could reproduce the similar symptoms of the disease after 5-6 days of inoculation. The experiment was conducted in pots of 12-15 cm diameter. The pots were filled up with compost, gently compacted, soaked with water and left overnight for drain out excess water. Three holes (1-2 cm depth) were made approximately at equal distance from each other on the compost surface of the pots and 4-6 sprouted seeds of BPT 5204 placed in each hole and covered up gently by compost. Seedlings were allowed to grow 5-10cm long and healthy seedlings of approximately same age were maintained as control and the seedlings growing together from a point were considered a hill. Inoculation were made with growing mycelial plug (3-mm dia.) at the base of seedlings and centre for each hill and the pots were covered up immediately (to prevent dry up on inoculum plug) with a polyethylene case or equivalent. The pots were left to prevent high temperature (the temperature inside plastic does not exceed 32 degree Celsius) The disease progress was monitored starting 7 days after incubation. Final disease measurement conducted as soon as disease (lesions) caused by any isolate cover. Twenty-five days old seedlings were transplanted. The plants sprayed with sterilized water, served as check could not produce any symptoms. Re-isolation of the pathogen from artificially inoculated diseased plant resulted in the growth of *Rhizoctonia solani* again. The morphology and other cultural behaviour of all isolates *i.e*., isolate from naturally infected and artificially inoculated rice plants were similar. Thus, Koch's postulate was proved.

3.2.7.1 Pathogenicity / Koch's postulate:

Pathogenicity test was done by following incubation method. For this purpose, at least three rice plants per pot (5kg capacity) were maintained in three replications. The white milky sclerotial stage of milky sclerotia was placed inside the sheath or rice plants wrapped with moist absorbent cotton to provide continuous moisture to the culture as well as plants. The process was done in the culture as well as plants.

The process was done in the month of August and if there was no rain, the cotton wrapping was regularly wetted to provide constant moisture at the place of inoculation. After four days, typical sheath blight symptoms appeared. The re-isolation at *Rhizoctonia solani* from inoculation rice plants confirmed *Rhizoctonia solani* as per Koch's postulates.

3.2.7.2 Inoculation technique:

Pathogenicity of *Rhizoctonia solani*is closely related with the inoculation technique used on the plant for disease development. Gangopadhyay (1982) described a simple inoculation procedure. In this method, a five mm mycelial disc containing milky sclerotia was placed inside the sheath of rice plant and wrapped with moist absorbent cotton to provide continuous moisture to the culture (inoculation) as well as the plant. After 4 days typical sheath blight symptoms appeared.

3.2.7.3 Disease incidence:

Disease incidence is the number or proportion of plants units that are diseased (*i.e.*, plant, flower, leaves, fruits *etc*,.) in relation to the total number of the units examined.

$$\text{Diseases incidence (\%)} = \frac{\text{Number of Plants infected}}{\text{Number of Plants observed}} \quad X \quad 100$$

Or

Number of infected units multiply by 100

Disease severity is equal to number of units multiplied by disease grade divided by total leaves observed in a set multiplied by maximum grade overall multiplied by 100.

3.2.7.4 Gradings:

1-10= Disease not easily visible, very few units/plants found diseased after carefully search,

11-25= Disease visible easily in each direction, but most (75%) ofthe unit look healthy

26-50= Both disease and healthy units are equally observed

51-75= Disease seen very easily, with some healthy units

75-100=All most all units are diseased with few healthy units seen on carefully search

3.2.8. Molecular characterization:

Some Sclerotium species are related to the genus *Rhizoctonia*, which form sclerotia and sterile mycelia with hyphae branching at right angles (*Tredway and Burpee*, 2001; *Xu et al*., 2010). Thus, the identification of disease based on morphological markers and symptoms induced by these fungi becomes tedious. Molecular diagnostic tools including PCR primers with enhanced specificity to the three *Rhizoctonia spp.* PCR protocols and DNA extraction methodologies for rapid and reliable analysis of fungal and plant specimens and pathogen/disease diagnosis have been designed, testedand developed. Utilisation of these tools in combination with intensive disease surveys confirmed *Rhizoctonia solani* causing sheath blight as dominant pathogen (more than 80%).

3.2.8.1 Procedure of DNA isolation

1. 0.5 g mycelium ground with Liquid nitrogen using mortar and pestle.
2. Powdered mycelium was suspended in DNA extraction buffer and shaken properly.
3. 1 ml 10% SDS was added and shaken gently then incubated in shaker incubator at 37℃ for 1 hr at 250 rpm.
4. 5M NaCl was added and mixed it with gently, after that added 1.25ml 10% CTAB was added and were mixed properly.
5. Mixture obtained in step 4 was incubated at 65℃ for 20minutes in water bath.

6. DNA was extracted by adding an equal volume of Chloroform isoamyl alcohol and mixed thoroughly then spined at 1000 rpm for 12 minutes at 4℃.
7. Aqueous, viscous supernatant was removed to a fresh tube and precipitate with 0.6 volume of cold isopropanol and 0.1 volume of sodium acetate and then left it inside the freeze for overnight at 4℃.
8. This mixture was centrifuged at 1000 rpm for 10 minutes at 4℃, supernatant was removed and pellet was washed with 70% ethanol and then dried it at room temperature.
9. Pellet was dissolved into the 100µl TE buffer.

3.2.8.2 Purification of DNA

Following are the step for purification of the DNA:

1. RNase solution (10mg/ml) @ 50 µg/µl was added to DNA sample and incubated at 37℃ for one hours.
2. Equal volume of phenol: Chloroform isoamyl alcohol (25:24:1) was added and mixed gently.
3. Mixture obtained in step 2 was spinned at 10000 rpm for 2 minutes at room temperature, aqueous phase was taken out and transferred to afresh microfuge tube. Extracted twice with equal volume of Chloroform: isoamyl alcohol (24:1), centrifuged and taken out the aqueous phase.

4. 0.1 volume of 3M sodium acetate (pH 4.8) was added to above mixture and mixed properly. 2.5 times absolute alcohol was added, mixed by quick gentle inversion to precipitate the DNA.
5. Mixture obtained in step 4 was centrifuged at 9000 rpm for 5 minutes in a microfuge to obtain the pellet. Removed the supernatant was removed carefully, pellet was washed with 70% cold ethanol; pellet was dried in air and dissolved pellet (DNA) in 100 micro-litre TE buffer.

3.2.8.3 Quantification of DNA:

For quantification 2-5 micro-litre of DNA was added to 1 ml TE buffer in a cuvette. The spectrophotometer was calibrated at 260 nm as well as at 280 nm wavelength. Optical density of DNA was estimated employing following

formula

$$\text{Amount of DNA (mg/µl)} = \frac{(\text{OD})260 \times 50 \times \text{dilution factor}}{100}$$

For the PCR amplification the DNA quantity must be 50-60 mg/ul

3.2.8.4 PCR Amplification:

DiagnosticPCR tests for each isolates of causal agent of sheath blight diseases were optimised by the primers based on sequence data available in the databases in general and characterised isolates. Further, thermal cycling parameters particularly higher annealing temperature and reduced annealing time were followed to enhance specificity. Th crudely extracted DNA in PCR analysis. DNA from each sample was tested with universal ITS1 forward and ITS4 primers

to confirm that the DNA samples was amenable to PCR amplification. *Rhizoctonia solani* genomic DNA obtained from fungal cultures. The reaction mix contained taq polymerase, dNTPs and buffer. The specific primers were used along with ITS1 and ITS4 conserved primers. PCR was conducted in a Phoenix thermal cycler (Helena Biosciences), at the following thermal cycling profile:

Cycle	Denaturation		Annealing		Extension	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94℃	5 minutes	-	-	-	-
10 cycle minutes	94℃	2 minutes	40℃	1 minutes	72℃	2
3-0 cycle minutes	94℃	1 minutes	45℃	50 seconds	72℃	2
Last cycle	-	-	-	-	72℃	3

Ready mix red taq from sigma was used in 20 ul reactions and an aliquote tested on agarose gel. To generate molecular profiles for genetic characterisation of various *Rhizoctonia solani*isolates simple sequence repeat (SSR) and amplification fragments length polymorphism (AFLP) PCR based markers was used up to Two primers. Aliquots of the reactions were run on agarose gel and photographic images recorded for analysing the profiles.

3.2.8.5 Agarose gel Electrophoresis:

Agarose gel (0.8 %) was casted in 1X TAE (Tris Acetate EDTA) buffer and loaded 3 µl of DNA sample mixed with 1 µl loading dye (6 X). Gel was run at constant voltage (50 V) for one hour. Stained the gel with ethidium bromide

(ErBr) solution (0.5 µg/ml) for 10 min., washed with distilled water and visualized under UV light Gel was then visualized on Gel documentation system (Alpha imager).

3.2.8.6 Data Analysis:

All the independently bands were scored, manually, band presence was indicated by 1 and its absence was indicated by 0. All monomorphic bands were also scored and included in analysis. Presence and absence of unique shared polymorphic as well as monomorphic product were used to generate similarity coefficient. The similarity coefficient was then used to construct a dendrogram UPGMA (unweighted pair group method with arithmetical average) using a computer programmed NTSYS_PC Version 2.1 (Rohlf,2000).

3.2.8.7 Alignment of 18S rRNA sequence data with known 18S rRNA sequences

Once the nucleotide sequence of the PCR product had been derived, it was BLAST using the NCBI's (National Centre for Biological Information) Basic Local Alignment Search Tool (BLAST) to compare the sequence data with known sequences submitted on the NCBI database. Phylogenetic trees were constructed using the Neighbour Joining (NJ) methods by Mega 7 software**.**

Chapter-IV

EXPERIMENTAL FINDING

The present chapter is devoted to the results of investigation entitled "Isolation and characterization of *Rhizoctonia solani* causal agent of Sheath blight of paddy." In this chapter phenotypic,cultural,morphological features, pathogenicity and molecular characterization of *Rhizoctonia solani* isolateswere determined. The results are described below:

4.1 Isolation of fungus *Rhizoctonia solani* from sheath blight diseased from differentregions.

Samples such as Sclerotia, plant leafs, tillers infected by *Rhizoctonia solani* exhibited sheath blight of Paddy from different regions of Uttar Pradesh (Table 4.1) were collected and used for isolation of *Rhizoctonia solani. F*or isolation, of fungus PDA media was used. *Rhizoctonia solani* was purified on PDA plates and slants at a particular favourable temperature and humidity. A total of seven isolates were picked up and purified (Plate 4.1 and plate4.2).

4.2 Phenotypic, Cultural and Morphological characterisation ofisolates.

In present study, seven fungal isolates were studied for phenotypic, cultural and morphological characters. The observation of mycelia growth and sclerotia were recorded from plates with fungal cultures upto 2 weeks (Table 4.2& 4.3). All the seven fungal isolates covered the entire petri plate surface of 90mm diameter after 4 days of incubation; indicating their fast-growing nature. The isolates RS1 had fluffy colony texture. RS 3 formed round smaller

brown to dark brown sclerotia of size 1 mm scattered within the PDA plates after 7 days of incubation. Isolates RS2, RS4, RS5 and RS6 showed formation of few to many dark brown types of sclerotia of size 2-4 mm after 10 days of incubation. Isolate RS2, RS3, RS4,RS6 and RS7 showed rapid and fast growth

4.2.1 Growth of *Rhizoctonia solani* on PDAmedium:

Macro sized sclerotia were observed in RS1, RS3, RS5 and RS7 while other isolates such as RS2, RS4 and RS6 produced micro-sclerotia. In another observation, found that macro sized sclerotia forming isolates were fast grower i.e. RS2, RS3, RS4, RS6 and RS7. Rest two *Rhizoctonia* isolates *i.e..*, RS1 and RS5 are moderate and slow grower. (Table4.2)

4.2.2 Colour of sclerotia on PDAmedium

Sclerotia formation in most of the isolates was found dark brown colour (like RS1, RS4 and RS5) after 6-7 days of inoculation period. In RS2, RS3 and RS6 isolates light brown colour sclerotia were formed and RS7 showed medium brown colour sclerotia. Initially sclerotia were white in colour and later on turned in brown in case of allisolates.

4.2.3 Distribution pattern of sclerotia on PDAmedium

Following three types of distribution pattern recorded on the PDA medium (Table 4.3)

1. Near inoculation point: The sclerotia concentrated near centre of the

colony showed by the isolateRS4.

2. Throughout the plate: Sclerotia were scattered over colony of surface as recorded in RS1, Rs2, RS3, RS5 andRS7.
3. Aggregate at centre: Macro sclerotia which were mostly aggregated and near the point of inoculation like RS6.

4.3 Pathogenicity and molecularcharacterization.

4.3.1 Pathogenicity test of *Rhizoctonia solani*isolates

Pathogenicity test was done during this investigation revealed that the pathogen could reproduce the similar symptom of the disease after 5-6 daysof inoculation. The use of polythene covers helped in maintaining high humidity, which allowed high fungal establishment. The early sheath blight symptoms (Water soaked spot) were observed in BPT5204 after 5 days of pathogen inoculation. All seven isolates exhibited varying degree of virulence. (Plate 4.3, Table 4.4) on BPT5204, a sheath susceptible variety. Re-isolation of the pathogen from artificially inoculated diseased plant resulted in the growth of *Rhizoctonia solani* again.The morphology and other cultural behaviour both the culture *i.e,*. isolates from naturally infected and artificially inoculated rice plant were similar, thus Koch's postlates was proved. The isolates RS6 and RS7 were found to be highlyvirulentwith 78% of disease incidence, RS1 and RS2 were moderately virulent while RS3, RS4 were least virulent.

4.3.2 Molecular characterization of *Rhizoctonia solani*isolates

The identification of disease based on morphological markers and symptoms induced by these fungi become tedious. The identification of isolates at genus and species level using molecular markers for genetic differentiation would be an ideal approach. The amplification of rDNA-ITS region by ITS4 primer gave a single product approximately 700bp for all seven isolates (Plate 4.4). The nucleotide sequence data (ITS 18s-rDNA region) for isolates were deposited in NCBI database; accession number are given in Table 4.5. The isolate RS1and RS5 were 99.7% homologous to CsKa, while RS2 and RS7 were 99% homologous to Rh 28, RS 3 was found 99.74% homologous to 331-7 at molecular level in ourstudy.

4.3.3 Cluster analysis of *Rhizoctonia solani* based on DNA bands and construction of phylogenetictree.

The cluster dendogram analysis of seven *Rhizoctonia solani* isolates showed four clusters that were demarcated at a cutoff similarity coefficient level of 0.01, below which the similarity values narrowed conspicuously. Cluster I, cluster II, And cluster IV was included each of 2 fungal isolates, while cluster III included only 1 *Rhizoctonia solani* isolates (Plate 4.5). The input matrix for rDNA analysis among the seven *Rhizoctonia solani* isolates were prepared from the scoring of main DNA band of different bp obtained. A dendogram was generated by NTSYS version 2.1 to depict the DNA band

relationships among the seven *Rhizoctonia solani* isolates under this study. The cluster analysis showed the significant molecular variation among the fungal isolates. Clusters I contain 2 *Rhizoctonia solani* isolates namely isolate RS1 and RS5. Clusters II contain two fungal isolates namely isolate RS2 and RS7. Cluster IV contain two fungal isolates RS4 and RS6, and cluster III contain only one *Rhizoctonia solani* isolateRS3.

Table 4.1. Collection of *Rhizoctonia solani*isolates from different location

Isolate ID	Sample (Rice variety)	Location
RS 1	Narendra Dhan 359	ND university instructional Farm
RS 2	Moti Gold	Shardanagar (Ayodhya)
RS 3	Sambha Mansuri	Banaras Hindu University instructional farm
RS 4	Moti Gold	DamodarPathara (Mirzapur)
RS 5	Sampurna	Farmer's field Barahaj, Deoria
RS 6	Prasanna	Farmer's field Lohta, Varanasi
RS 7	Moti Super	Farmer's field, Mau

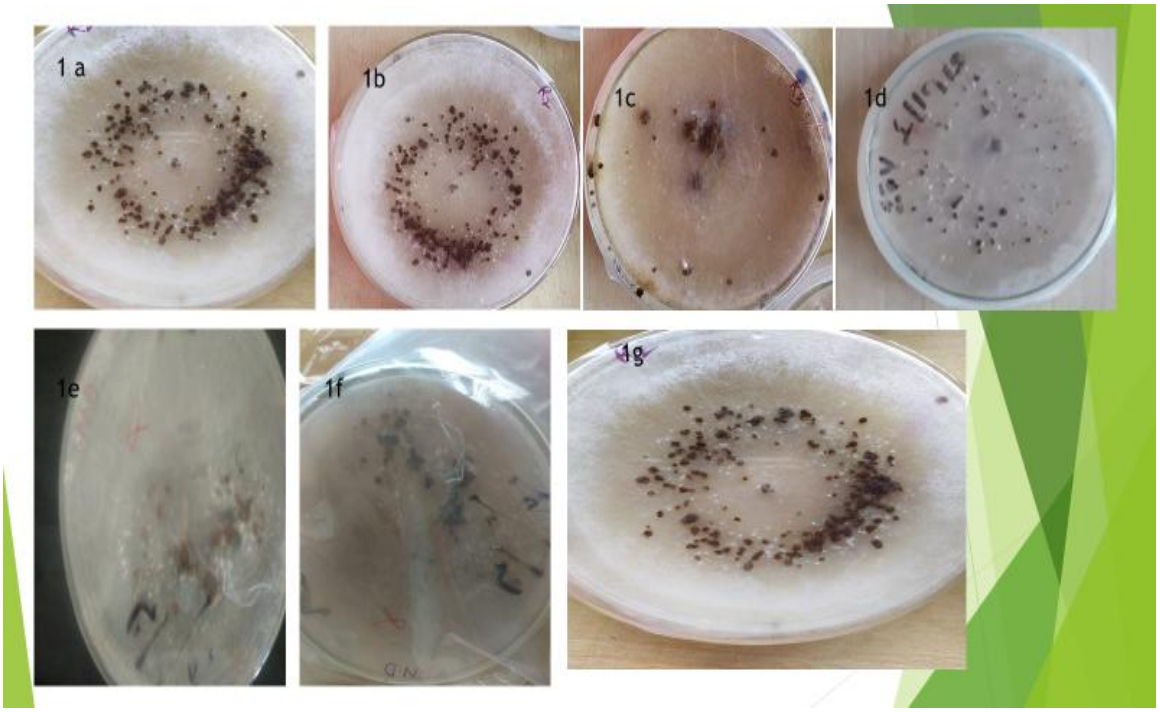

Plate. 4.1: Purified *Rhizoctonia solani*isolates on PDA medium plate after 10 days of incubation

Legend: 1a:RS1, 1b:RS2, 1c: RS3, 1d: RS4, 1e:RS5, 1f:RS6, 1g:RS7

Plate. 4.2 Preserved *Rhizoctonia solani*isolates on PDA slants
Legend: 1a:RS1, 1b:RS2, 1c: RS3, 1d: RS4, 1e:RS5, 1f:RS6, 1g:RS7

Table-4.2: Growth of *Rhizoctonia solani* on PDA medium.

Growth pattern	***Rhizoctonia solani*Isolates**
Fast	RS2,RS3,RS4,RS6 and RS7
Moderate	RS5
Slow	RS1

Fast grower= (>40 mm) mean colony diameter after 72 hr inoculation on PDA medium

Moderate= (35-40 mm) colony diameter after 72 hr of inoculation on PDA medium

Slow=(30-35 mm) colony diameter after 72 hr of inoculation on PDA medium

Table 4.3Variability in Sclerotia of *Rhizoctonia solani*on PDA medium at 28±3℃

S. No.	Isolates	Colour of sclerotia	Size	Pattern on plate	Position
1	RS 1	Dark brown	Micro	Throughout the plate	On surface of media
2	RS 2	Light brown	Macro	Throughout the plate	On surface of media
3	RS 3	Light brown	Macro	Throughout the plate	On surface of media
4	RS 4	Dark brown	Macro	Near inoculation point	On surface of media
5	RS 5	Dark brown	Micro	Aggregate at centre	On surface of media
6	RS 6	Light brown	Macro	Throughout the plate	On surface of media
7	RS 7	Medium brown	Macro	Throughout the plate	On surface of media

Table 4.4: Pathogenicity test of *Rhizoctonia solani* isolates on rice crop.

Isolates	Disease index	No. of Lesion/leaf	Plant part affected	Virulence nature
RS 1	1.61	2.50	Sheath	Moderate virulent
RS 2	1.80	2.83	Sheath, stem	Moderate virulent
RS 3	0.63	1.21	Sheath, leaf, stem	Least virulent
RS 4	0.21	0.41	Leaf, sheath	Least virulent
RS 5	4.97	4.67	Leaf, stem	Highly virulent
RS 6	5.09	3.50	Stem, sheath	Highlyvirulent
RS 7	5.07	4.67	Sheath	Highly virulent

Plate. 4.3 Rice plant showing disease symptoms after inoculation with *Rhizoctonia solani*isolates.

Table 4.5Molecular identification of *Rhizoctonia solani* isolates by rDNA analysis

S. No.	Isolate ID	Similarity with	Accession number	Match (%)
1	RS1	CsKa	D 85629.1	99.87%
2	RS2	Rh 28	D 85631.1	99.87%
3	RS3	1156	D 85641.1	99.74%
4	RS4	001-7	D 85630.1	99.87%
5	RS5	CsKa	D 85629.1	99.87%
6	RS6	001-7	D 85630.1	99.87%
7	RS7	Rh 28	D 85631.1	99.87%

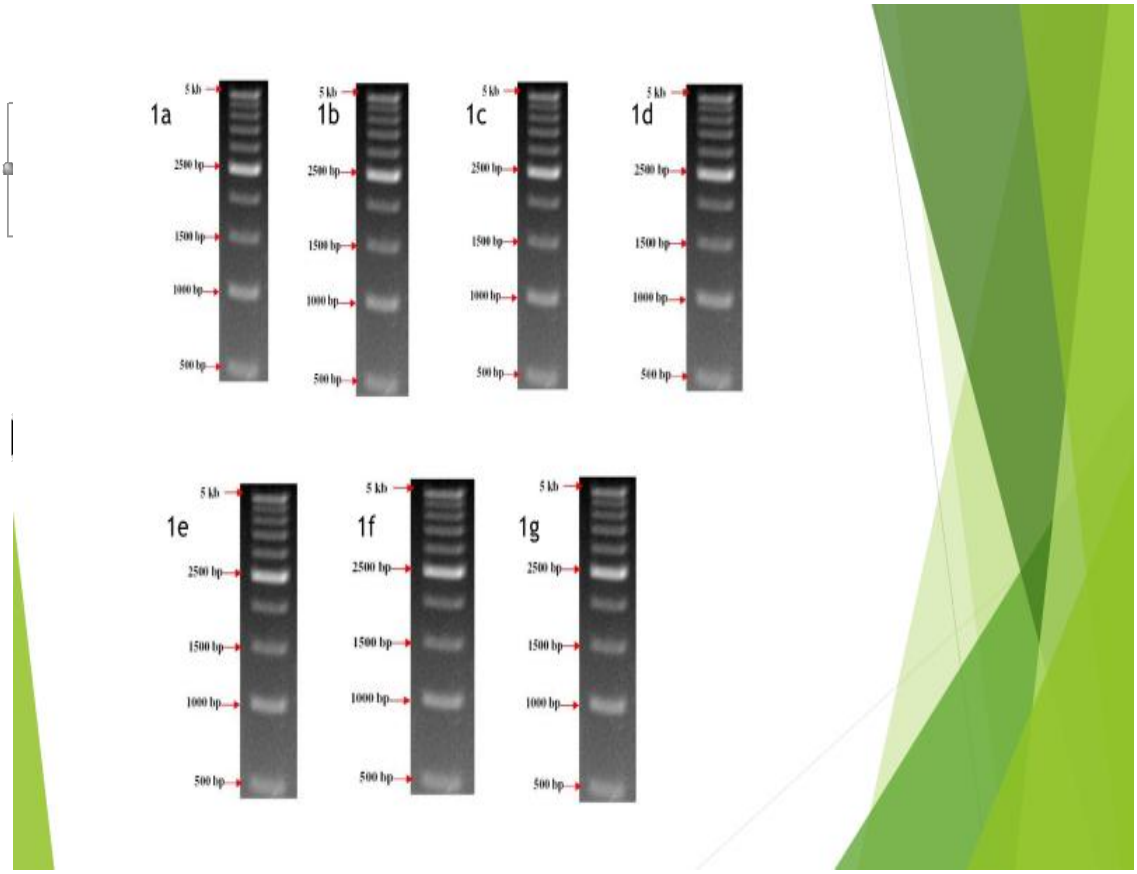

Plate 4.4 PCR amplification with primer ITS1/ITS4 pair from total genomic DNA of Rhizoctonia isolate

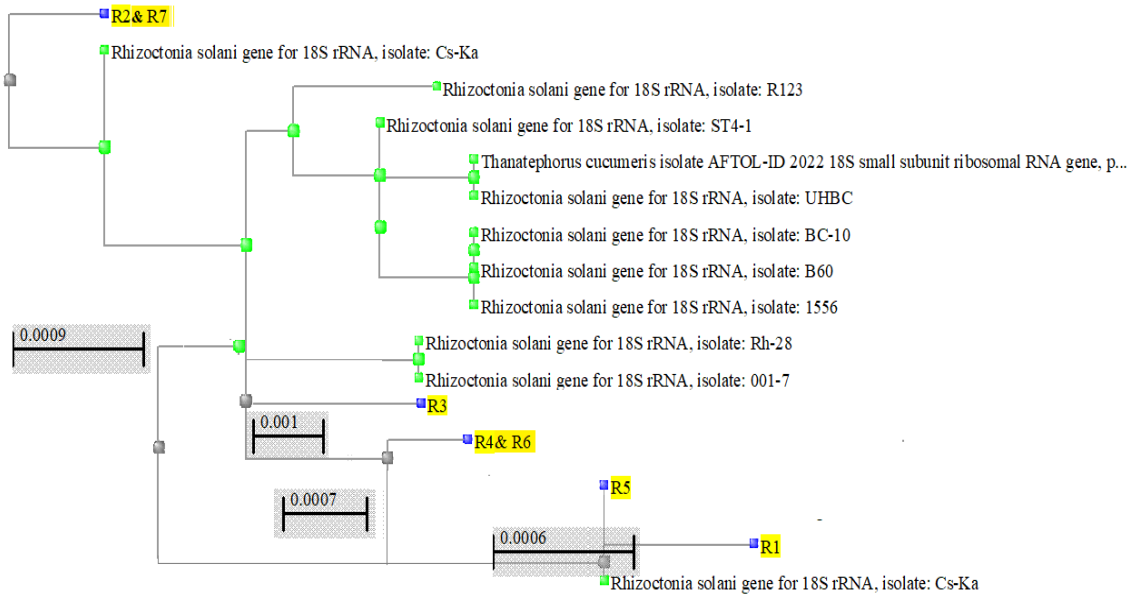

Plate 4.5 Phylogenetic tree of *Rhizoctonia solani*based on DNA bands.

Chapter-V

DISCUSSION

Investigation was carried out on *Rhizoctonia solani* isolated from rice crop grown in various regions of Uttar Pradesh. The isolates were characterized based on Morphological feature of colonies, Pathogenicity test and molecular characterization. Our objective here was to study the morphological and molecular characterization of *Rhizoctonia solani* causing sheath blight disease of rice and virulence determination. It is well known fact that high diversity exist within *Rhizoctoniasolani* isolates with respect to their morphological, virulence and molecular characters. Seven isolates of *Rhizoctonia solani* was isolated from different regions of eastern Uttar Pradesh. It is widely recognized that *Rhizoctonia solani* consists of many races, forms, or groups of various isolates diff ering in pathogenicity, morphology, and ecology.

5.1 Isolation and Identification of *Rhizoctonia solani*from rice

Isolation of different isolates of *Rhizoctonia solani*from rice grown in different geographical location of Uttar Pradesh was done to get variables. Seven isolates were collected from diseased plants of rice. Isolates were named as RS1, RS 2, RS3, RS4, RS5, RS6 and RS7. The detail of location of the collection with the name of isolates is given in table 4.1. Many researcher isolated *Rhizoctonia solani*from different crop plant parts for their study. Aisyay Surya *et al.,* (2017) were found six isolates of *Rhizoctonia solani, i.e.* two isolates collected from infected rice plants. Similarly, isolated four *Rhizoctonia solani*isolates were from different regions of India.Webb *et al.*, 2011 studies sho

that the great variabilty in *Rhizoctonia solani*causing blight in many cultivated crops, lying in the nature which is very important to be deciphered.

5.2 Phenotypic, cultural and morphological characterisation of isolates.

In present study, seven *Rhizoctonia solani* fungal isolates wereisolated from rice plants and studied for phenotypic, cultural and morphological characters. The observation of mycelia growth and sclerotia were recorded from plates with fungal cultures upto 2 weeks (Table 4.1 and plate 4.1). Morphological characterization of *Rhizoctonia solani* isolates was observed on the basis of their hypha, formation of sclerotia, sclerotia colour and position on petriplate on PDA medium were done by Banniza *et al.*, 1996. Similarly, Guleria *et al*., (2007) used cultural characters for differentiating the *Rhizoctonia solani* isolates from riceAll the seven fungal isolates covered the entire petri plate surface of 90 mm diameter after 4 days of incubation: indicating their fast-growing nature. The isolate RS1 had fluffy colony texture. RS3 formed round smaller brown to dark brown sclerotia of size 1 mm scattered within the PDA plates after 7 days of incubation. Isolates RS2, RS4, RS5 and RS6 showed formation of few to many dark brown types of sclerotia of size 2-4 mm after 10 days of incubation. Isolate RS 7 showed rapid growth. Similar findings were also observed by Meena *et al.,* (2001) as they recorded time taken for sclerotia formation ranged from 3-11 days.

5.3.1 Growth of *Rhizoctonia solani* on PDA medium:

Macro sized sclerotia were observed in RS1, RS3, RS5 and RS7 while other isolates such as RS2, RS4 and RS6 produced micros sclerotia. In another observation, found that macro sized sclerotia forming isolates were fast grower ie RS2, RS3, RS4, RS6 and RS7. Rest two *Rhizoctonia solani* isolates RS1 and RS5 were moderate and slow grower respectively. (Table 4.2 and plate 4.2). Guleria*et al*., (2007), Thind and Aggarwal (2008) and Khodaryari*et al*., (2009) stated that the *Rhizoctonia solani* isolates from rice were fast growing with >20 mm mycelia growth rate per day indicating their fastgrowing nature. Rapid growth rate among *Rhizoctonia solani*isolates have also been reported by Peltier (1916), Matz (1921), Matsumoto (1934) and Parmeter and Whitney (1970). the findings are corroborative with the earlier works.

5.2.2 Colour of sclerotia on PDA medium

Sclerotia formation in most of the isolates was found dark brown colour RS1, RS4 and RS5 after 6-7 days of inoculation period. In isolates namely RS2, RS3 and RS6, light brown colour sclerotia were formed and RS7 showed medium brown color sclerotia. Formation of sclerotia were observed in same pattern initially sclerotia were white in colour and later on turned in brown. The only character that clearly distinguished the sclerotia was the colour as reported by Anderson (1982). Sinha and Ghufran (1988) reported that more variations in the type and colour of mycelium and size, colour, number and type of sclerotia among the isolates of *Rhizoctonia solani*.Hoa (1994) also reported that sclerotial

colour ranged from brown, light/dark brown, black brown, chocolate brown, salmon and dark salmon.

5.3.3 Distribution pattern of sclerotia on PDA medium

Among seven *Rhizoctonia* isolates three types of distribution pattern recorded on the PDA medium. RS4 isolates showed sclerotia concentrated near centre of the colony, while RS6 aggregate at centre and RS1, RS2, RS3, RS5 and RS7 sclerotia were scsttered (Table4.2 and Plate 4.2). Similarly, Singh *et al* (1990) and Singh *et al* (2002) reported the sclerotial formation in the same manner *i.e.* central, peripheral or scattered. Not a single isolate showed embedded sclerotia. Location of sclerotia as aerial, surface and embedded was also reported by Singh *et al.*, (2002).

5.4 Pathogenicity and molecular characterization.

5.4.1 Pathogenicity test of *Rhizoctonia* isolates

Singh *et al.,* 2001 observed virulence pattern of *Rhizoctoniasolani* was tested after artificial inoculation of seven isolates on rice variety BPT5204. All of the isolates depicted typical sheath blight symptoms under greenhouse condition.

Inoculation of different isolates of *Rhizoctonia solani* on the rice variety BPT5204 resulted in the development of symptom of sheath blight of rice within 5 days after inoculation. Based on the minimum time required for initiation of symptoms and lesion size. In our investigation the seven isolates exhibited Varying degree of virulence (Plate 4.3 and table 4.3) on the BPT5204,

a Sheath blight susceptible variety. The isolate RS5, RS6, and RS7 was found highly virulent with 78% disease incidence, while RS3 and RS4 found least virulent on the variety BPT 5204 (Table 3). Many workers have reported morphological as well as pathological variation in fungal isolates associated with Sheath blight disease of rice. (Parmeter*et al.,* 1969, Mekwatanakarn*et al.*, 1999, Sivalingan*et al.,* 2006, Zhou *et al.,* 2007, Singh *et al.,* 2002, Guleria*et al.,* 2007; Singh *et al*., 2018). Macro-sized sclerotia forming isolates RS1, RS3 and RS5 were observed to be more virulent than isolate RS4, RS2, RS7 and RS6 sclerotia forming isolate was least virulent. Kumar *et al.,* (2008) and Goswami *et al.,* (2007) have also reported that isolates with macro- sized sclerotia are highly virulent as compared to isolates with micro-sized sclerotia. Non sclerotia producing isolates showing poor symptom expression in pathogenicity test was mentioned by Singh *et al.*, (2018). Similar resultes were observed in our study, where RS1, RS3 and RS5 was more virulent than RS2, RS4, RS6 and RS7 (Plate 4.3, Table 4.3).

5.4.2 Molecular characterization of *Rhizoctonia solani*isolates

The identification of disease based on morphological markers and symptoms induced by these fungi become tedious. The identification of isolates at genus and species level using molecular markers for genetic differentiation would be an ideal approach. The amplification of rDNA-ITS region by ITS4 primer gave a single product approximately 700 bp for all seven isolates (Plate 4.4). The

nucleotide sequence data (ITS 18s-rDNA region) for isolates were deposited in NCBI database; accession number are given in Table 4.4. The isolate RS1and RS5 were 99.7% homologous to CsKa, while RS2 and RS7 were 99% homologous to Rh 28, RS 3 was found 99.74% homologous to 331-7 at molecular level in our study Similarly many researcher's found the same result in molecular characterization of *Rhizoctonia solani* (Duncan *et al.,* (1993), Singh *et al.,* (2000), and (2002) Sharma *et al*., (2005), Khodayari *et al.,* (2009). Prasad *(*2017) as they showed PCR amplification with primer ITs1/ITS4 pair from total genomic DNA of *Rhizoctonia* isolates showed 99% similarity with *Rhizoctonia solani*. But, in our study more variability is seen in *Rhizoctonia solani* as four cluster are evident in only seven isolates.

5.4.3 Cluster analysis

The cluster dendogram analysis of seven *Rhizoctonia solani*isolates showed four clusters that were demarcated at a cutoff similarity coefficient level of 0.01, below which the similarity values narrowed conspicuously. Cluster I, cluster II, And cluster IV was included each of 2 fungal isolates, while cluster III included only 1 *Rhizoctonia solani*isolates (Fig 4.5). The input matrix for rDNA analysis among the seven *Rhizoctonia solani*isolates were prepared from the scoring of main DNA band of different bp obtained. A dendogram was generated by NTSYS version 2.1 to depict the DNA band relationships among the seven *Rhizoctonia solani* isolates under this study. The cluster analysis

showed the significant molecular variation among the fungal isolates. Clusters I contain 2 *Rhizoctonia solani*isolates namely isolate RS1 and RS5. Clusters II contain two fungal isolates namely isolate RS2 and RS7. Cluster IV contain two fungal isolates RS4 and RS6, and cluster III contain only one *Rhizoctonia solani*isolate RS3. Similar results were found my many researcher Aggarwal *et al.,* 2007 in they observed fiveclusters with 49–89% genetic similarity. Most of the isolates showed grouping specific to the host variety. Out of these two types of DNA markers, RAPD markers were able to detect more genetic variability when compared to ISSR markers. According to Sharma *et al.,* 2005 four different cluster group are found showing 89% similarity.

Chapter-VI

SUMMARY AND CONCLUSION

The present investigation was done undertaken during the period of 2018-20 in the department of Plant Molecular biology and Genetic Engineering, Narendra Deva University of Agriculture and Technology, Ayodhya Uttar Pradesh. The sheath blight infected plant samples were collected from rice field of different regions of UP. The specific objectives were to isolates *Rhizoctonia solani* from sheath blight diseased sample, pathogenicity test, cultural, morphological characterization of isolates and molecular characterization by 18s rRNA reverse and forward primers in PCR. The salient features of the research findings are as follows:

1 The plant samples such as leaflets, shoot *etc.*, were collected from the different regions of Uttar Pradesh *viz.* Faizabad, Varanasi, Shardanagar (Ayodhya), Deoria and Mau.

2 A total of seven fungal strains were isolated from the collected samples of diseased plant showing symptom of sheath blight of paddy from different regions of Uttar Pradesh.

3 All the seven isolates were maintained and purified on Potato dextrose Agar medium on petriplates and slants and stored at 4^0C. Isolates were further purified on the Potato Dextrose Agar medium slants at favourable temperature and pH

4 The isolates were characterised on the basis of Morphological features such as colour and shape of hyphae, size, shape,colour and growth pattern of sclerotia.

5 Further pathogenicity test were conducted by Koch's postulates rule, inoculation of purified fungi on the rice plant variety BPT514 was done and observed incubation period, diseases emergence, lesion size and disease severity. All seven isolates followed the rule of Koch postulates

6 Identification and molecular characterization of all seven isolates was done for by 18srRNA reverse and forward primers. On the basis of obtained DNA sequence, all seven isolates were identified as *Rhizoctoniasolani* showed diversity in their DNA sequences and banding. The nucleotide sequence of the PCR product derived, it was BLAST using the NCBI's (National Centre for Biological Information) Basic Local Alignment Search Tool (BLAST) to compare the sequence data with known sequences submitted on the NCBI database. Based on dendogram, among identified *Rhizoctonia solani* isolates, RS1, RS2, RS6 and RS7 showed maximum percentage of similarity with *Rhizoctonia solani*.

7 The genetic diversity in rice sheath blight fungus *Rhizoctonia solani* using PCR amplification collected from the different regions of Uttar Pradesh was studied. Together with morphological characters, these

molecular characters revealed diversity between isolates different regions. These results suggested the presence of different races within the same geographic regions. Our finding may be helpful for the phylogenetic classification of this complex species and may provide knowledge about the spread of *Rhizoctonia solani* races in Uttar Pradesh. The information may also provide a new insight into the nature of variation in this pathogen. For better understanding of the pathogen population of this important pathogen and the existence of its races, further study is needed with more number of isolates covering all the agro-ecological regions of India.

BIBLIOGRAPHY

Aderson, N.A. (1982).Genetic and pathology of *Rhizoctonia solani*. *Annual review of phytopathology,***20**(1);329-347.

Agrawal, G.K., and Rakwal, R. (2011). Rice proteomics, A move toward expanded proteome coverage to comparative and functional proteomics uncovers the mysteries of rice and plant biology. *Proteomics.,***11**:1630-1649.

Agrios, G.N. (1969). Plant Pathology study of different fungus. *Academic Press, New York*.**10**:520-536.

Aisyah, S., Wilbowo A., Priyatmojo, A. and Subandiyah S. (2017). Morphological characters and molecular analysis of *Rhizoctoniasolani*.*JurnalPerlinduganTanamnIndone*. **21**(2):72.

Aisyah, S., Wilbowo, A., Priyatmojo A. andSubandiyah S.(2017).Morphological characters and molecular analysis of *Rhizoctoniasolani*. *JurnalPerlinduganTanamnIndone*. **21**(2):72.

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 CropProtection Conference. Pests and Disease*s. **1**: 399-404.

Banniza, S., Rutherford, M. A., Bridge, P. D., Holderness, M. and Mordue, J. E. (2001). Biological characterization of *Rhizoctonia solani* in rice-based

cropping systems. *Proceedings of Brighton Crop Protection Conference. Pests and Diseases*. **1**: 399-404.

Banville, G.J. (1989). Yield losses and damage to potato plants caused by *Rhizoctonia solani* Kuhn. *Amer. Potato J,*. 66, 821-834.

Basu, P.K. and Gupta, S. (1992). Cultural and pathogenic variation in rice isolates of *R. Solani*. *Beitrage-zur-tropischen-hand writschaft and veterinarmedizin*. **30**(3):291-297.

Bin, L., Bao-ping, L. and Rong, Yu. (2011). Isolation and molecular characterization of Sheath blight of paddy causal agent. *World Journal of Microbiology and Biotechnology*., **27**(10):2305-2313.

Bottonal G. E., Shaw M. W., Colyer A., Pietravalle S., Boonham N. (2009). Molecular tools to investigate *Rhizoctonia solani* distribution in soil. *Plant Pathol*. **58** 1071–1080.

Cécile, G., Véronique, E., Pierre, C., Claude, A., Marc, R.M. and Steinberg, C. (2003). Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. *Canadian Journal of Microbiology*. **49** (9): 556-568.

Cubeta, M.A., Echandi, E., Abernethy, T., and Vilgalys, R. (2007). Characterization of anastomsis groups of binucleated *Rhizoctonia spp.* Using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology,* **81**: 1395-1400.

Dilip, K., Susan, LF., Meyer, I., Zasada, A., Daniel, P., Roberts, B., Vinyard,T., Lakshman, J. and David, J. (2006). Plantago lanceolata and

plantagorugelii extracts are toxic to Meloidogyne incognita but not to certain microbes. *Journal of nematology*. **38** (3): 333.

Duncan, S., Barton, J.E. and O'Brien, P.A. (1993). Analysis of variation in isolates of *Rhizoctonia solani* by random amplified polymorphic DNA assay. *Mycol. Res.***97**: 1075-1082.

Elarosi, Hussein.(1957). Fungal associatio. Cultural studies on *Rhizoctonia solani*Kuhn. *Ann. Bot. N.S*, **21**:555-567.

Fadhal, A., Al-Fadhal, Aqeel, N., Abedy, A., and Duaa, A., and Alkhafije. (2019). Plantago lanceolata and Plantago rugelii extracts are toxic to Meloidogyne incognita but not to certain microbes. *Egyptian Journal of Biological Pest Control*. **47**:520-560.

Gangopadhyay, P. (1982). Simple inoculation procedure. *Indian Vet. J,*. **56**: 979-980.

Goswami, S.K., Singh, V. and Kashyap P. (2017). Population genetic structure of *Rhizoctonia solani* AG1IA from rice field in North India. *Phytoparasite*,**45**.299-316.

Gottlieb, D. (1971). Limited growth of fungi. Mycologia. *Taylor and francis Journal*.**63**: 619–629.

Green, H., and Jensen D.F. (2000). Disease progression by active mycellial growth and biocontrol of pypthiumultimum var. ultimum studied using a rhizobox system. *Phytopathology*, **90**:1049-1055

Grosch, R., Schneider, J.H.M., Peth, A., Waschke, P., Franken, P., Kofoet, A., and Jabaji, S.H. (2007). Development of a specific PCR assay for the detection of *Rhizoctonia solani* AG 1- IB using SCAR primers. *Journal of applied Microbiology***102** :(3), 806-819

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. *Journal of Phytopathology*. **155**: 654-661

Guleria, S., Aggarwal, R., Thund, T.S and Sharma, T.R. (2007). Morphological and pathological variabilty in rice isolates of *Rhizoctonia solani*and molecular analysis of their genetic variabilty. *Journal of phytopthology*.**155**:654-661.

Guleria, S., Aggarwal, R., Thund, T.S and Sharma, T.R. (2007). Morphological and pathological variability in rice isolates of *Rhizoctonia solani*and molecular analysis of their genetic variability. *Journal of phytopthology*.**155**:654-661.

Guleria, S., Aggarwal, R., Thund, T.S and Sharma, T.R. (2007). Morphological and pathological variability in rice isolates of *Rhizoctonia solani and molecular analysis of their genetic variability. Journal of phytopthology*.**155**:654-661.

Harikrishnan, R., and Yang, X. (2004). Recovery of anastomosis groups of *Rhizoctonia solani*from different latitudinal positions and influence of temperatures on their growth and survival. *Journal of Plant Disease*. **88**: 817–823.

Hoa TTC. (1994). Characterization and pathogenicity of *Rhizoctonia solani* Kuhn isolates from different rice zone and management of sheath blight of rice. Ph. D Thesis, Indian Agricultural Research Institute, New Delhi-12, 122p.

Hossain, Md., Kamal, T., Ong, S., Nadarajah, K. and Jena, K. (2014). isolated, Identified and validated the sheath blight resistance in rice (*Oryza sativa* L.) cultivars against *Rhizoctonia solani.Canadian Journal of plant pathology*.**36**(4):482-490.

James, W., Woodhall, P., Adams, J.C., Peters, Glyn Harper and Boonham, N. (2007). A new quantitative real-time PCR assay for *Rhizoctonia solani* AG3-PT and the detection of AGs of *Rhizoctonia solani* associated with potato in soil and tuber samples in Great Britain. *European Journal of Plant Pathology*, **136**: (2), 273-280.

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. *Journalof Phyto-Pathology*. **157**(11/12):708-714.

Kliejunas, J.T., Ko, W.H. andThompson, D. (1992). Inhibition of Mycelial Growth of Mycotoxigenic Fungi by Phenolic Antioxidants. *Mycologia***84**:(5) 791-793.

Kumar, B. and Dinesh, P. (2009). First record of banded sheath blight disease of barnyard millet caused by *Rhizoctonia solani.J Mycol Pl Pathol***39**(2):352-354.

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

Lehtonen, M.J. (2009). *Rhizoctonia solani* as a potato pathogen – variation of isolates in Finland and host response. *Journal of Molecular Evolution***69** (2), 150-163, 2009

Lehtonen, M.J., Somervuo, P. and Valkonen, J. (2008). Infection with *Rhizoctonia solani* induces defense genes and systemic resistance in potato sprouts grown without light. *Phytopathology Journal*. **98**:(11), 1190-1198.

liu, H., Wenxiao, T., Bin, L., Guoxing, W., Muhammad I., Zhongyun, T., Yangli W., Guanlin X., Hongye L. and GuochangS.(2017). Antifungal effect and mechanism of chitson against the rice sheath blight disease pathogen. *BiotechnolLett*..34(12):2291-8.

Martin, J. P.(1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Science,Philadelphia,*.**69** (3): 215-232.

Matz., Matsumoto, T. (1921). Some remarks on the taxonomy of the fungus Hypochnussasakii Shirai. *Sapparo Nat. Hist. Soc.***13**: 115–120.

Meena, B., Ramamoorthy. V. and Muthoswami, M., (2001). Morphological and pathological variabilty in isolates of *Rhizoctonia solani*., *University of California Journal.*, **55**:526-539.

Meisong, J., Jin. (1994). Characterization and variation of isolates of R. solani from rice in thailand. *Acta-Agriculture Zhejiangensis*. **6**(3): 150-155.

Mekwatanakarn. P, Kositratana. W, Phromraksa. T. and Zeigler. R.S. (1999). Sexually fertile Magnaporthe grisea rice pathogen in Thailand. *Scopus Google Scholar*.**83**(10).939-943.

Mohanty, S., and Yamano, T. (2017). Rice food security in India emerging challenges and opportunities In: The Future rice strategy for India (Eds.)., *Academic Press Cambridge*., pp 1-13.

Nadarajah, K.,Omar, N.S., Rosli, M.M. and Tze, O.S.(2014). Molecular Characterization and Screening for Sheath Blight Resistance Using Malaysian Isolates of *Rhizoctonia solani*. *Biomed Res. Intern*. **20**: 434-457.

Nwugo, CC., and Huerta, A.J. (2011). The effect of silicon on the leaf proteome of rice (*Oryza sativa* L.) plants under cadmiumstress. *J Proteome Res.*, **10**:518-528.

Ogoshi, A. (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. *Annual Review of Phytopathology***25**: 125-143.

Ou, A. (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. *Annual Review of Phytopathology*, **25**: 125-143.

Pankaj, K., Kumar, A. and Singh, R. (2019). Integrated management of *Rhizoctonia solani* causing sheath blight of rice (*Oryza sativa*). Haryana. *Indian Journal of Agricultural Sciences*. **89** (12): 2079–2084.

Pankaj, K., Kumar, A. and Singh, R. (2019). Integrated management of *Rhizoctonia solani* causing sheath blight of rice (*Oryza sativa*). Haryana. *Indian Journal of Agricultural Sciences*. **89** (12): 2079–2084.

Parmeter, J.R., and Whitney, H.S. (1970). Taxonomy and nomenclature of the imperfect state- *Rhizoctonia solani*. In:(Ed. J.R. Parmeter). Biology and Pathology. *University of California Press, Berkley University of California Press Berkelay, Los Angeles and London.***57**:1500-1560.

Parmeter, J.R.J., Sherwood, R.T. and Platt, W.D. (1969). Anastomosis grouping among isolates of *Thanatephoruscucumeris*. *Phytopathology* 59:1270–1278

Peltier GL. (1916). Parasitic Rhizoctonia in America. *Illinois AgrExpt Sta Bull***189**: 281-390.

Prahlad, S.,Krishnaraj, P. andPrashanti S.K.(2018).Morphological and pathological characters of *Rhizoctoniasolani*.*International Journal of Current Microbiolgy and Applied Science*.**8**(01):1714-1721.

Prasad, S., Meena, M., Hossain, M. and Sharma. R.S. (2017). Morphological and Genetical Study on Rhizoctonia Sheath Disease Complex of Rice in Bangladesh. *Universal Journal of Agricultural Research*,**5**(6): 344-349.

Ritchie, F., Bain, R.A., and McQuilken M.P. (2009). Mycelium of *Rhizoctonia*solani isolates from potato.*Journal of Plant Pathology*.**91** (3):589-596

Rohlf, F. J., and Corti, M. (2000). The use of two-block partial least-squares to study covariation in shape. *Systematic Biology*, **49**(4), 740–753.

Sasaki, T., and Burr, B. (2000). International rice genome sequencing project: The effort to completely sequence the rice genome. C*urrOpin Plant Biol*., **3**:138-141.

Satoru, M., Joseph G., Dubouzet, Y., Kondou, Yusuke J., Shigemi S., Kenji O., Minami M. and Hirohiko H. (2019). Molecular characterization of *Rhizoctonia solani*.*Masaki Mori Scientific reports*. **9** (1): 1-14.

Sharma M, Gupta SK, Sharma TR. (2005). Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. *Journal of Phytopathology*. **153**:449–456.

Sharma, J., and Tripathi, H. S, (2001). Influence of environmental factors on web blight development in Urdbean (Vignamumgo (L.0.hepper). *F.Mycol.Pl*. *Pathol*..**31**: 54-58

Sharma, M., Gupta, S.K. and Sharma, T.R. (2005). Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. *Journal of Phytopathology*.**153:** 449–456 .

Sharon, M., Freeman, S., Kuninaga, S. and Sneh, B.(2008). Genetic diversity, anastomosis groups, and pathogenicity of Rhizoctonia spp. isolates from Strawberry. *Eur J Plant Pathol*, **117**:247–265

Shivlingam, P.N., Vishwakarma, S.N. and Singh, U.S. (2006). Role of seed-borne inoculum of *Rhizoctonia solan*i in sheath blight of rice. *Indian Phytopathol*.**59**:445-452.

Singh, S.K., Satyanarayan, K., and Reddy, A.P.K. (1990). Studies on morphology growth habit, hyphal anastomosis and virulence pattern of five isolates of sheath blight pathogen of rice. *Indian Phytopth***43**: 368-371.

Singh, A., Rohila, R., Singh, U. S., Savary, S., Willocquel, L. and Duvoillor, E. (2001). An improved inoculation technique for sheath blight of rice caused by *Rhizoctonia solani*. *Canadian J. Plant Pathology*.**24**: 65-68.

Singh, A., Rohila, R., Singh, U. S., Savary, S., Willocquel, L. and Duvoillor, E. (2002). An improved inoculation technique for sheathblight of rice caused by *Rhizoctonia solani*. *Canadian J. Plant Pathology*.**24**: 65-68.

Singh, A., Singh, U. S., Singh, V., Zeigler, R. S., Hill, J. E., Singh, V. P., Duveiller, E. P. and Holderness (2007). *Rhizoctonia solani* in RiceWheat System. *J. Mycology and Plant Pathology*. **30**(3): 343-349.

Singh, V,, Singh, U.S., Singh, K.P., Singh, M. and Kumar, A. (2002). Genetic diversity of *R. solani* isolates from rice: Differentiation by morphological characteristics, pathogencity, anastomosis behavior and RAPD finger printing. *J Mycol Pl Pathol***32**: 332-344.

Singh, V., Bimal S., Amaradasa, G., Karjagi, Dilip K., Lakshman, K., and Aundy, K. (2002). Molecular characterization of *Rhizoctonia solani*. *Eur J. Plant Pathol*.152: 45-60.

Singh, V., Singh, U. S., Major, S. and Kumar, A. (2002). Genetic diversity of Rhizoctonia solani isolates from rice: Differentiation by morphological characteristics, pathogenicity, Anastomosis behaviour and RAPD finger printing. *J. Mycology and Plant Pathology*. **32**(3):332-344.

Singh, V., Singh, U.S., Singh, K.P., Singh, M. and Kumar, A. (2002). Genetic diversity of *R. solani* isolates from rice: Differentiation by morphological characteristics, pathogencity, anastomosis behavior and RAPD finger printing. *J Mycol Pl Pathol***32**: 332-344.

Sinha, B.P., and Ghufran SM. (1988). Physiopathological studies on five isolates of sheath blight of rice caused by *R. solani* Kuhn. *Journal of Reasearch- Rajendra Agricultural University*. **6**: 61-67.

Sneh, B., Burpee, L. and Ogoshi, A. (1991). Identification of Rhizoctonia Species. St. Paul. The American Phytopathological Society, APS Press, p. 134.

Surya, A., Wilbowo, A, Priyatmojo, A. and Subandiyah S. (2017). morphological characters and molecular analysis of *Rhizoctoniasolani*.*JurnalPerlinduganTanamnIndone*.**22**(02).72.

Susan, L.F., Meyer, A., Zasada, D., Roberts, P., Bryan, T., Vinyard, Dilip, K., Lakshman, J., David J.C., and Lynn, K. (2006). Lantago lanceolata and Plantago rugelii extracts are toxic to Meloidogyne incognita but not to certain microbes. *Journal of Nematology***38** (3):333

Susan, L.F., Meyer, I.A., Zasada, D., Roberts, B.T., Vinyard, Dilip K., Lakshman, J., David J. and Lynn, K.C.(2006). Pathogenicity and characterization of *Rhizoctonia solani. Journal of Nematology***38** (3), 333.

Susheela, K., and Reddy, C.S. (2013). Varibilty in *Rhizoctonia solani*isolates causing sheath blight of rice in India. *Indian phytopathology*,**66**: 341-350.

Sobita, P., Vignesh, L.N.K., Singh, Bireswar, Sinha, Devi, K., Sarda D., Bhuvaneswari, D. and Dinesh, K. (2019). In-vitro evaluation of Trichoderma spp. for the management of *Rhizoctoniaoryzae*-sativae causing aggregate sheath spot of rice in Manipur. *IJCS***7** (1), 2498-2501.

Taheri, P., Gnanamanickam, S. and Höfte M. (2017). Characterization, genetic structure, and pathogenicity of Rhizoctonia spp. associated with rice sheath diseases in India. *PopulBiol*.;**97**:373–383.

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

Tamura., Stecher, K., Peterson, G., Filipski, D. and Kumar, A.S. (2013). molecular evolutionary genetics analysis version 6.0. *Mol BiolEvol***30**:2725–2729.

Vimal., Singh, V., , S., Karjagi, G., Dilip, K. and Kumar, A. (2018). Morphological as well as pathological variation in fungal isolates

associated with Sheath blight disease of rice. *European journal of plant pathology*, **152**: 45-60.

Wagacha, J.M. and Muthomi, J.W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, 124: 1-12.

Waller, R. A., Sale, P.W.G., Saul, G. R. and Kearney, G. A. (2001). Tactical versus continuous stocking in perennial ryegrass-subterranean clover pastures grazed by sheep in south-western Victoria. 1. Stocking rates and herbage production. *Aust. J. Exp. Agric.*,**41** (8): 1099-1108

Wang, Y., Liu, H., Tian, W., Li,B., Guoxing Wu, Muhammad I. and Zhongyun T. (2011). Characterization of sheath blight of paddy. *Biotechnology Letters*,**34** (12), 2291-2298.

Wang, Z.T., Wei, Z. and Zhou, Z.K. (2007). Analyses of the temporal development and yield losses due to sheath blight of rice. *Agricultural Science in China***.6**(9):1074-1081.

Webb, K.M., Hill, A.L., Laufman, J., Hanson, L. E. and Panella, L. (2011). Long- term preservation of a collection of *Rhizoctonia solani* using cryogenic storage. *Annals of Applied Biology* **158**, 297–304 .

Webb, K.M., Hill, A.L., Laufman, J., Hanson, L.E., and Panella, L. (2011) Long- term preservation of a collection of *Rhizoctonia solani* using cryogenic storage. *Annals of Applied Biology*. **158**, 297–304 .

Weinhold, A. R.(1977). Population of *Rhizoctonia solani* in agricultural soils determined by a screening procedure. Phytopathology, Saint Paul,. v. 67, n. 4, p. 566-569.

Woperies, M.C.S., Defoer, T., Idinoba, P., Diack, S., and Dugue, M.J. (2009). Curriculum for participatory learning and action research (PLAR) for integrated rice management (IRM) in inland valleys of Sub-Saharan Africa., Technical Manual.,**10**: 105-109.

Xu, Z., Harrington, T.C., Gleason, M.L. and Batzer, J.C. (2010). Phylogenetic placement of plant pathogenic Sclerotium species among teleomorphgenera. *Journal of Mycologia*. **102**: 337-346.

Department of Plant Molecular Biology And Genetic Engineering

Narendra Deva University of Agriculture And Technology

Narendra Nagar (Kumarganj) Ayodhya-224 229 (U.P.)

Dr. Adesh Kumar
Assistant Professor
Department of PMB & GE

Name of Student: Minakshi
Id No.: A10557/18
M.Sc. (Ag. Biotech.)

Title: Isolation and characterization of *Rhizoctonia solani* Causal agent of Sheath blight of paddy.

ABSTRACT

Sheath blight of paddy is an economically significant rice disease worldwide. The disease cause significant grain losses, yield losses of up to 50%. In the present study ,seven strains of *Rhizoctonia spp* isolated from the infected paddy crop grown in different locations. Pathogenicity test of all seven *Rhizoctonia solani* isolates was done on rice crop under greenhouse condition. All tested isolate were able to infect rice plants causing Sheath blight of paddy with some different degree of severity Isolate RS1,RS2and RS3 showed significantly highest sheath blight severity while isolate RS5 gave the lowest percentage of sheath blight severity. The DNA markers obtained from all isolates showed genetically similarity among different isolates obtained from different geographical regions. Precise identification of cause of disease based on morphological characters and symptom induced by *Rhizoctonia spp.* Become tedious because of similarity in symptoms. The identification of isolates at genus and species level Molecular markers for genetic differentiation would be ideal approach. The isolate RS1and RS5 were 99.7% homologous to CsKa, RS2 and RS7 were 99% homologous to Rh 28, RS 3 is 99.74% homologous to 331-7 at molecular level as well as was found in our study.

(Adesh Kumar) **(Minakshi)**