MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Rhizoctonia solani* CAUSING SHEATH BLIGHT AND ITS REACTION PATTERN TO RICE GERMPLASM

YASIN AFROJA

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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BY

YASIN AFROJA

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Approved by:

Dr. M. Salahuddin M. Chowdhury
Professor
Department of Plant Pathology Sher-e-Bangla Agricultural University, Dhaka
Research Supervisor

Dr. Tahmid Hossain Ansari
Principal Scientific Officer
Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur
Co-Supervisor

Prof. Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka-1207

Dr. M. Salahuddin M. Chowdhury
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Mobile: +880-1713382588
E-mail: smc_1968@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Rhizoctonia solani* CAUSING SHEATH BLIGHT AND ITS REACTION PATTERN TO RICE GERMPLASM"** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE*** in ***PLANT PATHOLOGY***, embodies the result of a piece of bona-fide research work carried out by **Registration No. 13-05444** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 30. 12. 2020
Dhaka, Bangladesh

Dr. M. Salahuddin M. Chowdhury
Research Supervisor
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207

Dedicated to

My

Beloved Parents

LIST OF ACRONYMS AND ABBREVIATIONS

ABBREVIATIONS	FULL FORMS
%	Percentage
et al.	And others
spp.	Species
J.	Journal
No.	Number
viz.	Namely
h	Hour(s)
&	And
etc.	Etcetera
°C	Degree Celsius
@	At the rate of
cm	Centimeter
cm^2	Centimeter square
mm	millimeter
ml	mililitre
µL	microlitre
kg	kilogram
g	gram
Avg.	Average
Var.	Variety
DAP	Day After Planting
CP	Constriction Point
MNR	Multinucleate *Rhizoctonia*
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
pH	Hydrogen ion concentration
i.e.	That is

T	Treatment
PDA	Potato Dextrose Agar
WA	Water Agar
PDB	Potato Dextrose Broth
Acc. no.	Accession number
CV%	Percentages of Co-efficient of Variance
ShB	Sheath Blight
BRRI	Bangladesh Rice Research Institute
BBS	Bangladesh Bureau of Statistics
QTLs	Quantitative Trait Locus
SES	Standard Evaluation System

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The Author

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Rhizoctonia solani* CAUSING SHEATH BLIGHT AND ITS REACTION PATTERN TO RICE GERMPLASM

ABSTRACT

The experiment was conducted for morphological characterization, molecular identification and to study pathogenic variation among *Rhizoctonia solani* isolates collected from different areas of Bangladesh. The experiment was performed in the laboratory of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur during the period of June 2019 to February 2020. Eight isolates were isolated from seven different areas in Bangladesh and all isolates showed wide morphological variation in terms of size of sclerotia (1.62 mm to 4.38 mm), colony colour and texture of sclerotia ranging from dark brown to light brown with rough to smooth in texture. Initiation of sclerotia formation time was varied from 4 to 6 days. Based on the colony pigmentation, all the isolates were assigned into 4 groups: dark brown, dirty white, milky white and yellowish brown. All the isolates had growth rates between 5.00-5.87cm^2 per day with an average of 5.33cm^2/day. All the isolates except RS-2 and RS-7 were confirmed as *Rhizoctonia solani* with the molecular identification using the primer pair ITS1/ITS4 and showed band around 700bp. No genetic variation was found among the isolates. A field trial with 35 rice germplasm was conducted during the Transplanted Aman season to screen the *Rhizoctonia solani* resistant germplasm in BRRI research field. BR11 was used as susceptible variety. Highest relative lesion height (97%) was recorded in susceptible check BR11. The lowest relative lesion height (15%) was recorded in genotype Var24. It was revealed that three genotypes (Var23, Var24 and Var29) were found resistant out of 35 genotypes tested. These germplasm could be used in future rice breeding programmes aimed at improving resistance to sheath blight.

INTRODUCTION

Rice belongs to the family Gramineae (Poaceae) tribe Oryzae. This tribe has 11 genera of which *Oryza* is the only one with cultivated species. Although it is predominant in Asia, this crop has also been cultivated in Europe since the 15th century, mainly in Mediterranean countries including Italy, Spain, Portugal, Greece, and France (FAO, 2016). About 77.12% of cropped area of the country is used for rice production. Bangladesh is ranked as fourth based on the annual milled rice production of 36.39 million metric tons during 2018-19 in the world (BBS, 2020). It is cultivating almost one fifth of the total land area covered under cereals. Rice sector contributes one-half of the agricultural GDP and one-sixth of the national income in Bangladesh. The population of Bangladesh is still growing by two million every year and may increase by another 30 millions over the next 20 years. Rice yield therefore, needs to be increased from the present 2.74 to 3.74 t/ha (BRKB, 2019).

Productivity of rice fluctuates significantly from region to region, season to season due to various biotic and abiotic stresses. There are so many constraints responsible for low yield of rice in Bangladesh. Among all the pathogenic organisms, fungal pathogens are the major limiting stresses for rice productivity. More than 60 diseases attack the rice crop every year among which sheath blight, bacterial leaf blight, stem rot and blast are considered important diseases at various parts of rice growing areas of the world (Latif *et al.,* 2011).

Sheath blight (ShB) is considered as the most damaging major epidemic disease of rice (Li *et al.,* 2012). It is one of the major constraints of rice production in almost all rice growing areas and in all season of Bangladesh. Incidence and development of ShB of rice depends on climate, host and soil factor (Damicone *et al*., 1993). Most of the cultivated high yielding rainfed lowland rice varieties are severely affected by this disease. It is a great threat to successful rice cultivation during rainy season especially in northern part of the country (Rahman *et al.*, 2016). Short duration and semi-dwarf cultivars are more

susceptible to ShB (Groth and Lee, 2002).The intensity of the disease is severe in Boro and Transplanted Aman seasons. Northern and central part of the country experiences frequent epidemic outbreak of the disease.In Rangpur, at T. Aman 2018 season, average sheath blight (incidence: 24.5% and severity 3.4), In cumilla, average disease incidence was 5-80%, where in Keshobpur, jashore, it was found higher (90%). In Boro 2018-19 season, on an average sheath blight disease was widespread in Rangpur. Sheath blight (incidence: 32.5%, severity: 5.4) was predominant compared to brown spot, Bacterial Blight and blast disease (BRRI, 2019).

The disease is caused by *Rhizoctonia solani* AG1-1A Kuhn (Teleomorph: *Thanatephorus cucumeris*) is one of the most ubiquitous and destructive soil borne pathogen. Miyake (1910) first reported the disease on rice from Japan and it has become a major constraint to rice production only during the last two decades (Hashiba and Kobayashi, 1997). The disease first reported in Bangladesh in 1973 (Miah *et al.*, 1995). It is also known as “kholpora rog” by local farmers of Bangladesh. Rice sheath blight (ShB), caused by *Rhizoctonia solani* Kuhn is a soil borne, semi-saprophytic fungus (Almasia *et al*., 2008). Due to the semi-saprophytic nature and uncharacterized pathogenicity mechanism, *R. solani* has a wide host range and infects at least 200 plant species including most of the important crops in the world such as rice, maize, wheat, potato, soybean, carrot, tomato, cotton etc (Lehtonen *et al*., 2008).

The yield reduction due to sheath blight in Bangladesh has estimated to be from 14 to 31% under experimental and farmers field condition (Shahjahan *et al.,* 1986) and 50% was reported when susceptible cultivars were planted (IRRI, 2017 and Ou, 1985). The disease can cause yield losses up to 50% in advanced stage and adversely affects quality of straw, limiting its value as fodder (Prakasam *et al*., 2013 and Ali *et al.,* 2004). In Bangladesh yield losses occurred 6-36% in Aus and 19% in transplanted Aman rice (Dey *et al*., 1996). In hot and high humid condition, yield loss can even reach as high as 50% (Meng *et al*., 2001). On infection by *Rhizoctonia solani*, semi-dwarf varieties show

more than twice the reduction in yield and milling quality. During rice ShB epidemics, severe lodging may occur (Wu *et al.*, 2012). Differences in yield loss between very susceptible and moderately resistant cultivars are substantial. The estimation of losses due to sheath blight of rice in India has been reported to be up to 54.3% (Chahal *et al*., 2003). In Eastern Asia, this disease causes a yield loss of 6 million tons of rice grain per year by affecting nearly 15-20 million ha of rice land under irrigated condition (Bernardes *et al*., 2009 and Groth, 2008). Studies at IRRI showed that sheath blight causes a yield loss of 6% in tropical Asia, 20% in Japan and 50% in USA.

Around 8000 native land races of rice have been preserved in Bangladesh Rice Research Institute (BRRI) gene bank. Very few of them are screened against rice sheath blight and the others are still remains unscreened which indicates there is a scope to find resistance in unevaluated germplasm of rice. Till date, no rice germplasm in the world has been found to be completely resistant to this fungus. This is mainly due to the lack of sources for resistance in cultivated and wild rice species possessing ShB resistance traits. (Lalita *et al.,* 2018 and Adhipathi *et al*., 2013). Fortunately, rice land races have proven to be highly adaptive to diverse environmental conditions and are believed to harbour a number of valuable genetic resources for crop improvement (Ganie *et al*., 2014; and Karmakar *et al*., 2012). Therefore, local or land races of rice need to be exploited for getting resistant or moderately resistant or even better tolerant sources for ShB disease. In this study, morphological traits that contribute to resistance of the genotype assessed in detail due to the complex inheritance nature of ShB resistance.

Plant breeding could not effectively overcome the sheath blight of rice because of the wide diversity of *R. solani* so that genetically resilient properties are hard to find. This research was important as a study on the characteristics of isolate *R. solani*. By understanding the characteristics and virulence of the isolate, it can be used as a reference in controlling the disease effectively and safely. Although disease management strategies using an integrated approach are required, these

are hindered by the current lack of knowledge of pathogen variability and lack of availability of methods to detect and diagnose the pathogens.

The conventional diagnostic methods of pathogens are multi-stage, time consuming and labor-intensive (Ball and Reeves, 1991). Biotechnology is able to discriminate reliably, among closely related organisms within a population (Dobinson *et al.,* 1998). Molecular approaches may overcome the limitations of morphological characterization (Reeves, 1995; Foster *et al*., 1993 and Ball and Reeves 1991). Thus, on the basis of above facts and views the present study has been aimed to achieve the following objectives:

OBJECTIVES:

i. To isolate and identify the causal agent of sheath blight disease of rice through cultural and molecular marker.

ii. To study the morphological and genetic variability of different isolates collected from different areas in Bangladesh.

iii. Screening of rice germplasm against sheath blight disease using virulent isolate of *Rhizoctonia solani.*

REVIEW OF LITERATURE

Nagaraj *et al*. (2019) worked with 65 isolates collected from rice and rice associated weed species from different agroclimatic zones of Karnataka, India. All isolates were characterized for their morphological, genetic and virulence diversity. All isolates showed wide morphological variation in terms of size of sclerotia (0.13 mm to 9.44 mm), colour and texture of sclerotia ranging from dark brown to light brown with rough to smooth in texture and abundance (0-1054). The genetic diversity of *R. solani* populations was dissected based on anastomosis grouping. Anastomosis group (AG) was identified by restriction fragment length polymorphism (RFLP) of internal transcribed spacer (ITS) sequences. They analyzed that the virulence of all 65 isolates of *R. solani* was based on relative lesion height on four different rice varieties such as TN1 (Susceptible), BPT 5204 (Susceptible), IR 64 (Moderately Susceptible) and Tetep (Moderately Resistance) by artificial inoculation. Out of 65 isolates, 11 isolates were categorized as highly virulent, 23 isolates as moderately virulent and 31 isolates were less in virulence. The relative lesion length on rice varieties varied with respect to isolate and cultivar combination. They also conducted DNA extraction and PCR amplification. The ITS region of all 65 isolates was amplified using Primers RS1 (5 -CCTGTGCACCTGTGAGACAG-3) and RS4 (5 - TGTCCAAGTCAATGGACTAT-3). Sixty-five isolates of *R. solani* were characterized by PCR- RFLP with the selected enzymes. RFLP of rDNA-ITS amplified product was used to determine the AGs belonging to subgroup IA, IB, or IC and others of *R. solani* isolates.

Parveen *et al*. (2018) worked with Fifty-seven rice germplasm collected from BRRI Genebank were screened against sheath blight (ShB) by artificial inoculation in field and laboratory conditions in T. Aman 2012. Significant differences on relation to lesion height (RLH) among the germplasm were observed, where the highest (83%) was recorded in susceptible check, BR11 and the lowest (8.33%) was in Orgoja. Severity score of ShB was recorded maximum

(9) in Dudhsail, Basi, Chaula mari, Holdemota, Calendamota, Semmua, Kotijira, Halisail, Horakani, Kalisura, Ashfuli, Huglapata and BR11 as highly susceptible to ShB, whereas it was minimum (1) in Orgoja. Gopal ghosh was observed as moderately tolerant with 27.33% RLH and severity score 3, while Kala binni, Khazur chari, Binni, Kalagora, Patjait and Dorkumur found moderately tolerant with severity score 5. Finally the local cultivar, Orgoja (acc. no. 5310) showed resistance to ShB in both hill inoculation in field and detached sheath inoculation in test tube, which could be used in resistance breeding for varietal improvement programme of rice.

Lalitha *et al*. (2018) screened 196 germplasm lines under natural conditions with the susceptible check (Pusa Basmati-1) and resistant check (Tetep) varieties after inoculation by the virulent isolate of *R.solani* (RS 49). None of the entries were found immune or resistant. Fifty seven entries were found moderately resistant, moderately susceptible and rest of the entries showed highly susceptible reaction.

Dey *et al.* (2016) evaluated a total of 1013 germplasm comprising of mutants, introgression lines from wild species, A, B, R lines, Tropical japonica accessions, land races from north eastern India, wild rice accessions of *O. nivara* and *O. rufipogon* and gall midge biotype differentials maintained at ICAR-IIRR with artificial inoculation for resistance to sheath blight disease during 2012 under field conditions. The germplasm identified as resistant/moderately resistant were further tested both under glass house and field conditions in the subsequent years (2013 and 2014) and seasons (Both Kharif and Rabi). Based on three years of testing, seven genotypes such as SM 801 (N 22 mutant), 10–3 (Introgression line), Ngnololasha, Wazuhophek, Gumdhan and Phougak (land races from north east) and RP 2068-18-3-5 (gall midge biotype differential) were identified as moderately resistant to sheath blight.

Yadav *et al.* (2015) evaluated forty rice germplasm lines including 8 wild, 4 land races, 26 cultivated, and 2 advanced breeding lines were evaluated for their reaction to sheath blight. A moderate level of resistance to this disease was identified in Tetep and ARC10531, a land race with the relative lesion height

percentage of 21-30%, while highly susceptibility was recorded in BPT 5204 with 80% relative lesion height. Tetep is well known source for sheath blight resistance and mapping of quantitative trait loci involving this as a resistance source has been already reported earlier and several QTLs have already been mapped by number of researchers. The land race ARC10531 identified in the present study was observed with equal levels of resistance as that of Tetep, could be used as an alternate source for ShB resistance, so in present study ARC10531 was selected as a contrasting moderate resistant parent with highly susceptible line BPT-5204 for parental polymorphic survey.

Chen *et al*. (2014) improved japonica rice resistance to Sheath blight by pyramiding qSB-*9TQ* and *qSB-7TQ* on chromosomes 9 and 7 respectively.

Dey (2014) utilized indigenous lines in future breeding programmes through the development of a databank with detailed agro-morphology, physio-biochemical and molecular screening with trait-linked markers and specific genes and suggested some of the landraces such as Buhjan, Banshpata, Bhasamanik, Nagra Sail, Raghu Sail which are tolerant to rice ShB.

Dubey *et al*. (2014) screened a set of 100 rice lines set of 100 rice genotypes including a susceptible check (Pusa Basmati-1) and a resistant check (Tetep) were used for phenotyping against sheath blight diseases. Four entries, viz., BPL7-12, BML27-1, BML 21-1 and Kajarahwa possess high degree of tolerance to sheath blight but none of the rice entries was completely resistant.

Shiobara *et al.* (2013) identified three landraces Jarjan, Nepal 555 and Nepal 8, resistant to sheath blight after three years of continuous field testing. They screened 33 rice accessions and proved that early- heading accession had a wider range of rate of tillers with lesion (~80%) than late heading accessions (~40%).

Willocquet *et al.* (2010) indicated that morphological traits were strongly associated with disease intensity and their effect was larger than that of genetic groups. Particularly, plant height was associated with low sheath blight intensity.

Zuo *et al*. (2009) mentioned that the resistance levels of Zhongbaiyou 1 and Teyou 338 are as high as YSBR1, a rice line that has been identified with high resistance to sheath blight.

Sharma *et al.* (2009) studied with a population of 279 F2:3 progeny rows derived from a cross between two tropical *japonica* U.S. rice cultivars, Rosemont (semi dwarf, SB susceptible) and Pecos (tall, SB resistant), was used to map SB resistance. Progeny families were evaluated for disease reactions, plant height (PH), and heading date (HD) in replicated field trials for 2 yr and genotyped with 149 simple sequence repeat markers. Correlation analysis between SB ratings with PH and HD showed that both agronomic traits were significantly correlated with SB resistance. Four significant (logarithm of odds ratio   3.6) quantitative trait loci (QTLs) were identified for SB resistance, with individual effects explaining 5.6 to 33.4% of the total phenotypic variation. Plant height appears to have a direct influence on SB resistance, with QTLs for these traits colocated on chromosome. Consistent results across years indicate the stability of the identified QTLs and their potential for improving rice SB resistance using marker assisted selection.

Groth (2008) stated that no strong genetic sources of resistance are reported against rice ShB disease. The rice ShB resistance among the cultivable varieties in the southern United States currently ranges only from very susceptible to moderately resistant. The yield losses were reported to be 8% in moderately resistant (cv. Jupiter) and up to 30% in very susceptible (cv. Trenasse) in rice fields with artificial inoculation

Jena and Mackill (2008) founded that Both wild species and landraces of the wild rice have been used successfully to develop resistance against many rice diseases which thought to possess under-exploited alleles that may have a strong potential for the improvement of Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* Steud).

Park *et al*. (2008) analyzed detail study of sheath blight resistance at genetic, molecular, biochemical and functional genomic levels by using 3 different

inoculum types (agar block, liquid cultured mycelia ball, and mycelia suspension) and concluded that cultivars with late heading dates generally are more resistant to sheath blight than those with early heading dates.

Pinson *et al*. (2008) developed three rice germplasm lines TIL 455, TIL 514, TIL 642 containing introgressed sheath blight resistance alleles.

Groth and Bond (2007) conducted a separate study during 2003 through 2005, following artificial inoculations with ShB pathogen, a significant increase in disease incidence and severity was observed in moderately susceptible and very susceptible cultivars. Further, a yield loss of 4% was noticed in moderately susceptible cv. Francis and 21% was found in very susceptible cv. Cocodrie

Sharma *et al.* (2005) underlined the importance of land races in exploring valuable genes for fungal diseases.

Loan *et al*. (2004) reported that resistance is less affected by culm angle and highly affected by heading date and plant height and there is negative relationship between early maturity, short plant stature, and sheath blight disease rating must be considered when selecting plants for sheath blight resistance.

Peng *et al.* (2003) studied the relationship between morphological traits and sheath blight resistance by using a segregant population consisting of 240 inbred recombination lines, derived from an elite combination of Zhenshan 97X Minghui 63 in year 1999 and 2000. Results indicated that plant compactness was significantly correlated with resistance to sheath blight in both the years; eight traits such as plant height, heading date, and penultimate leaf angle were significantly correlated with sheath blight resistance in either of the two years and other morphological traits were not consistently associated with sheath blight resistance in the two years.

Singh and Borah (2000) also screened sixty local upland rice cultivars in Assam and reported that only one variety i.e. Chingdar was found to be resistant, seven moderately resistant and rest 52 were susceptible.

Rangaswami and Mahadevan (1999) reported that the pathogen causes spots or lesions mostly on the leaf sheath, extending to the leaf blades under favourable conditions. At first, the spots are greenish gray, ellipsoid or ovoid and about 1 cm long. They enlarge and may reach 2 or 3 cm in length and become grayish white with brown margins and somewhat irregular in outline. In the advanced stages brown sclerotia are formed which are easily detached from these spots. Under humid conditions, the fungal mycelium spreads to other leaf sheaths and blades. Eventually, the whole sheath rots and the affected leaf can easily be pulled of from the plant. In severe cases, all the leaves of a plant are blighted. resulting in death of the plant. Plants are usually attacked at the tillering stage. when the leaf sheaths become discolored at or above water level.

Matsumoto *et al*. (1997) used Restriction fragment length polymorphism (RFLP) analysis for DNA products amplified by the polymerase chain reaction (PCR) was for the direct detection of *Rhizoctonia solani* AG 1 IA and AG 2-2 IIIB, *R. oryzae, R. oryzae-sativae* and *R. fumigata* from the diseased rice sheaths using two restriction enzymes HhaI and MspI.

Reddy *et al*. (1997) tested 457 breeding lines for sheath blight resistance and found two lines (RNR 15336 and RNR 82096) as resistant.

Hashiba & Kobayashi (1996) cited the size of the sclerotia also varied from pinhead to 6 mm in diameter. They are generally superficial on PDA. On the host plant, sclerotia are dark brown to black 4-5 mm in diameter, spherical but flattened when pressed between leaf sheath and culm. Five phases of sclerotial morphogenesis have been recognised in *R. solani:* a) repeated hyphal branching resulting in short, thick, lateral monilioid hyphae; b) hyphal aggregation as clusters of thick-walled cells rich in nutrients and network formation (5 µm wide X 0.09 µm thick); c) formation of sclerotial initial; d) formation of white and immature sclerotium attaining maximum size in 30h; e) maturation and pigmentation by melanin incrustation.

Sharma and Teng (1996) cited that the development of sheath blight on two rice cultivars IR-42 and IR-72 inoculated at different growth stages with *Rhizoctonia solani*. They reported that disease development was highest at later growth stages. Disease progress was faster at flowering and booting stages than at tillering and panicle initiation stages in both cultivars. The percent productive tillers, grain weight per plants, filled grain number per panicle, 1000 grain weight, filled grain percent and total biomass per plant were usually higher at early stages of inoculation and lower at booting and flowering stages of inoculation. The disease development was also higher at later growth stages and consequently the yield parameters also declined.

Rao (1995) reported that the fungus produces sclerotia and occasionally basidiospores and overwinters in soil as sclerotia or as mycelia in the organic fraction of the soil. Ecologically, the fungus is diversified; it can survive with dead plant parts as a saprophyte, as a pathogen on many hosts and occasionally with seed when infection reaches panicles or basidiospores initiate infection.

Singh and Dodan (1995) reported cultivars KK2, Dodan, IR40 and Camor for sheath blight resistance sources.

Lakpale *et al.* (1994) reported that viability of sclerotia remained unchanged when sclerotia were submerged for periods up to 96 h. Followed by drying treatments of 24-168 h. However, viability decreased with an increase in submergence until it was completely lost after 168 h. Submergence also lowered the inoculum potential by reducing the production of sclerotia from those which had been under water.

Jiang *et al*. (1993) evaluated 1,188 rice varieties from Zhejiang province, no highly resistant materials were found and only 2.4% of accessions displayed resistance.

Leano (1993) noted that the infection efficiency in sheath blight significantly increased with prolonged wet and dry daily cycles, particularly with three days of intermittent wet and dry (12/12 h) periods. Sheath blight intensity and

yield loss were higher in plots where flooding was intermittent compared to continuously flooded ones.

Dasgupta (1992) reported that the fungus produces three types of hyphae on the host. These are: runner mycelium-straight, creeping, trophic, non infecting hyphae, sometimes thick and flattened; lobate mycelium- branching out from the runner hyphae as short, swollen much branched, single or multiple lobed appressoria and penetration pegs, which later become intracellular and grow through the host causing lesions. Such mycelium can withstand desiccation. Monilioid mycelium - forming the sclerotia but also formed on the culture plates. Young hyphae are hyaline. Pigmentation of hyphae appears to be restricted to various shades of brown or silvery, becoming brown with maturity. Hyphal diameters vary between isolates of *R. solani*, ranging from 5-17 µm, and cell length varies between 50 and 250 µm. the length to width ratio of vegetative hyphal cells is generally greater than 5: 1.

Dasgupta (1992) reported that when humidity exceeds 95 percent and temperatures are in the range of 25-27 "C, infection spreads rapidly by means of runner hyphae in three ways: a) inward spread- from outer to inner sheaths with bleached centre and irregular purple brown border; b) vertical spread- upward rapidly invading the lamina, loosening the sheath from the culm, even causing blight of boot, flag leaf, panicle and lodging. Such lesions are ovoid, smaller, 5-10mm X 5 mm. which coalesce rapidly, more so on lower leaf sheaths than on upper leaves. Rice grains becomes chaffy or partially filled particularly in the lower parts of the panicle. c)Horizontal spread- laterally, the disease spreads from tiller to tiller and hill to hill apparently by physical contact in a dense planting. When the conditions are less favourable the lesions remain restricted to lower leaf sheaths and appear as a typical cobra skin pattern.

Rush and Lee (1992) reported that the symptoms of sheath blight do not appear until plants are in the late tillering or early internode elongation growth stages. Initial symptoms consist of circular, oblong or ellipsoid.

green-gray, water-soaked spots about 1 cm long that occur on the leaf sheaths near the waterline. The lesions enlarge to approximately 2-3 cm in length and 1 cm in width and the centers of the lesions become pale green or white and are surrounded by an irregular purple-brown border. Under favourable conditions i.e., low sunlight, humidity near 95% and high temperature (28-32°C), infection spreads rapidly by means of runner hyphae to upper plant parts including leaf blades and to adjacent plants. Lesions on the upper portion of plants may coalesce to encompass entire leaf sheaths and stems. Sclerotia are produced superficially on or near infected tissue after symptoms first appear and are loosely attached and easily dislodged from the plant when they are mature. Heavily infected plants produce poorly filled grains particularly in the lower portion of the panicle. Additional losses in yield result from increased lodging or reduced ratoon production as a result of death of culm.

Suparyono and Nuryanto (1991) narrated that the severity of sheath blight and its effect on rice yield depended on the rice variety and the host growth stage at which plants were inoculated. Disease development was greatest when plants at maximum tillering and primordial stages were inoculated with *Rhizoctonia solani*. Disease severity of the early maturity varieties was less when plants were inoculated at the later stage, although disease severity was affected long duration rice varieties. Yields of both early and long duration varieties were significantly reduced by the disease when the plants were inoculated at the maximum tillering and primordial plant growth stages. Yield reduction decreased when plants of the early maturing varieties were inoculated at the later stage, although the reduction of the yield loss was smaller on the long duration varieties.

Ansari *et al.* (1989) observed some Local accessions BogII, Aduthurni, Chinese galendopuram, Arkavati, Saket-4, Neela, MTU-3, MTU-7, MTU-13, MTU-3642, BPT-6 to be promising genotypes for sheath blight resistance.

Singh *et al*. (1988) noted that the pathogen normally attacked the leaf sheath and leaf blade but the symptoms were also found on emerging panicles which chaffy, grayish brown and matted together by fungal mycelium. Numerous white and brown sclerotia were found on diseased panicles.

Ahn and Mew (1986) studied relation between rice sheath blight and yield. No significant yield reduction was found when RLH was less than 20%. If lesion reached the 3rd sheath from the sheath bearing the flag leaf, and if RLH was about 30% a significant yield loss was seen. A 46% yield loss is possible in milled rice if sheath blight lesion reaches 90% of RLH.

Miah *et al*. (1985) informed that the disease severity and yield losses were significantly higher when inoculated at booting stage than when inoculated at maximum tillering, panicle initiation and flowering stages.

Ou (1985) reported that the size and color of spots and the formation of sclerotia depend upon environmental conditions mycelium of the fungus may grow over the surface of the leaf sheaths and can spread to a considerable distance.

Kim and Min (1983) recorded that the percentage of infected hills/stems increased more rapidly from 11 July to 1st August than 1st September (i. e., Booting stage than flowering stage of crop growth).

Lee and Rush (1983) reported that short and medium grain type Japonica rice showed highest degree of resistance.

Yu and Fan (1981) conducted an experiment for estimation of sheath blight resistance in 226 lines for 1st crop and 214 lines for 2nd crop by using Yoshimura‟s formula. Results indicated that early maturing varieties were more susceptible to sheath blight than those of late maturing varieties, while tall varieties and varieties with few tillers were less susceptible to sheath blight disease.

Boyette and Lee (1979) reported that plant growth stages have significant effects on *R. solani* Kuhn and development of rice sheath blight in the field. In field

trials maximum yield losses occurred when plant becomes infected at the 1/2 in internode elongated growth stage.

Kannaiyan and Prasad (1979) informed that soil moisture was important factor determining the survival and persistence of the fungal pathogen in soil. The increase in moisture levels gradually decreased the seedling infection. Low moisture favored the survival of *R. solani*, whereas, higher moisture inhibited the survival and the multiplication of the propagules of *R. solani*. Higher moisture content also stimulated the bacterial activity which in turn might result in lysis of *R. solani* mycelium.

Manian and Rao (1979) identified the sources of ShB resistance by screening the land races from Assam viz., ARC 5925, ARC 5943, ARC 14529, ARC 10572 and ARC 10618 were moderately resistant for sheath blight. The earlier reports and the response of ARC 10531, a landrace from Assam, throws an indication that accessions from Assam has good potential to act as a prominent source for sheath blight resistance.

Rajan and Nair (1979) revealed certain rice varieties IR24, IR26, IR29, Jaya, Jaganath, Mashoori, Pankaj, Rajeshwari, Supriya, Sabari, TKM6 were resistance to sheath blight.

Bhaktavatsalam *et al*. (1978) reported that the land races ARC15762 and ARC 18119 as promising genotypes for sheath blight resistance.

Yuno *et al.* (1978) reported that there was no difference in growth between the isolates at 15ºC. With a daily average temperature of 26-28ºC (daily maximum 30-32ºC), the rate of upward development of the disease caused by higher temperature isolates was greater than that of these caused by the low temperature isolates. There was no significant difference in the growth of *R. solani* on rice leaf sheath held in the dark or light but lesions development more rapidly in the light. Mycelial growth increased with temperature 20-28ºC. From 25-26ºC after disease development, lesions of leaf sheaths were cleanly defined and the lesions diameter then remained constant.

Yu CM (1975) investigated that outer leaf sheaths are first affected, gradually extending towards the inner sheath. Sheath blight lesion spread more rapidly on the leaf sheath blade than the sheath, and spread is hastened by loosening. Lesion length increases faster at heading than at booting and maximum at tillaring stage. At booting the developments is more rapid on lower leaf sheaths than the upper, but reverse at heading off the rice plants.

Hashiba *et al.* (1974) isolated the pure cultures of *R. solani* isolates from high temperature regions grow well on PDA at 35ºC but tented to grow poorly at 12ºC, whereas those from low temperature regions at 35ºC and well at 12ºC.

Wu (1971) obtained Chin-kou-tsan, Zenith, CO.17, Dinominga, Puang Nahk 16, Baok, Toma-112, R.T.S.31, Kele Kala as promising genotypes for sheath blight resistance of rice in Southern Taiwan.

Das (1970) identified the sources of ShB resistance cultivars NC 678, Dudsor, Bhasamanik as promising genotypes.

MATERIALS AND METHODS

3.1. Experimental site

The experiments were conducted in the Laboratory of Plant Pathology Division and Research Field at Bangladesh Rice Research Institute (BRRI). This research was a part of the core research programme of Plant Pathology Division, BRRI, Joydebpur, Gazipur.

3.2. Experimental period

The experiments were conducted during the period on June, 2019 to February, 2020.

3.3. Cleaning and Sterilization of glassware

All the *in vitro* experiments were conducted in aseptic conditions. The equipment used for the experiments were glassware, petriplates (9 cm diameter) block cutter, needles, forceps etc. were sterilized by keeping them in cleaning solution. Media and glassware were sterilized by using autoclave (PETRRA-IRRI, C-Astell, England) and hot air oven, respectively. Corning or Borosil made Glasswares were used throughout the present investigation. At first, glassware washed thoroughly with a detergent and rinsed in running tap water. Then they were soaked in cleaning solution for 24h and finally rinsed with distilled water for 3-4 times and air dried and then sterilized in hot air oven (BINDER XMTA-1000, Yangming, China) at 160°C. Different media and water used in this study were sterilized at 15 PSI (121.6ºC) for 20 minutes in an autoclave. Work benches are sterilized using ethyl alcohol.. Sodium hypochlorite (NaOCl) 1.0 % was used for surface sterilization of plant materials and rectified spirit for other equipments like inoculation needles, forceps, scalpel inoculation chamber and hands. Cork borer were sterilized over flame.

Composition of cleaning solution:

Potassium dichromate ($K_2Cr_2O_7$)	:	60g Concentrated
Sulphuric acid (H_2SO_4)	:	60 ml
Distilled water	:	1000 ml

3.4. Equipments

Single pan electronic balance with a sensitivity of 0.001g was used for weighing chemicals. BOD incubator (Hettcube 400R, Hettich, Germany) were used for incubating cultures at desired temperature and were stored in a refrigerator at 4ºC (Siemens, Germany).

3.5. Media used for the different experiments

Two percent Water Agar and Potato Dextrose Agar were used for isolation, purification and maintenance of isolates. The media required for the experiment were prepared in steel containers and distributed equally into 250 ml. conical flasks @ 100 ml/flask and autoclaved at 121°C under 15 PSI pressure for 20 minutes. The following media were used for the different experiments.

3.5.1. Preparation of Potato Dextrose Agar media

Composition of ingredients for Potato Dextrose Agar (PDA) described by (Ricker and Ricker, 1936)

Potato - 200g

Dextrose - 20g

Agar powder - 20g

Distilled Water - 1000 ml

200g disease free and fresh potato was cleaned and washed thoroughly under running tap water and then rinsed with sterilized water. Cleaned potatoes were taken in sterilized vessels and peeled with sterilized and sharp knife. Peeled potato were sliced and boiled in 500ml distilled water for 20 minutes. It was fitered through cheesecloth, saving effluent which is potato infusion. Requisite

amounts of dextrose (20 g/l) was added and the extract was made up to 1 liter. Agar powder was weighted precisely and added gently through shaking, then oven heated for 30 seconds to dissolve properly. The media thus obtained was poured equally into several 250 ml. conical flasks. The flasks were closed by cotton and covered with aluminium foil and sterilized by autoclaving.

3.5.2. Preparation of Water Agar Media

Water Agar is recommended for enumeration, cultivation and observation of sporulation of some fungi. Composition of ingredients for media preparation (Lingappa and Lockwood, 1960).

Distilled water - 1L

Agar - 20g

20 grams of Agar powder was weighed precisely and was added in 1000 ml distilled water. Ingredients were mixed thoroughly by shaking. The mixture was oven heated to dissolve the medium completely. It was sterilized by autoclaving at 121°C under 15 PSI pressure for 15 minutes.

3.6. Collection of isolates

In the present study, diseased samples were collected from seven different rice growing areas representing different agroclimatic zones of Bangladesh such as Dhaka, Rangpur, Gopalgonj, Cumilla, Gazipur, Feni, Satkhira (Figure 1).

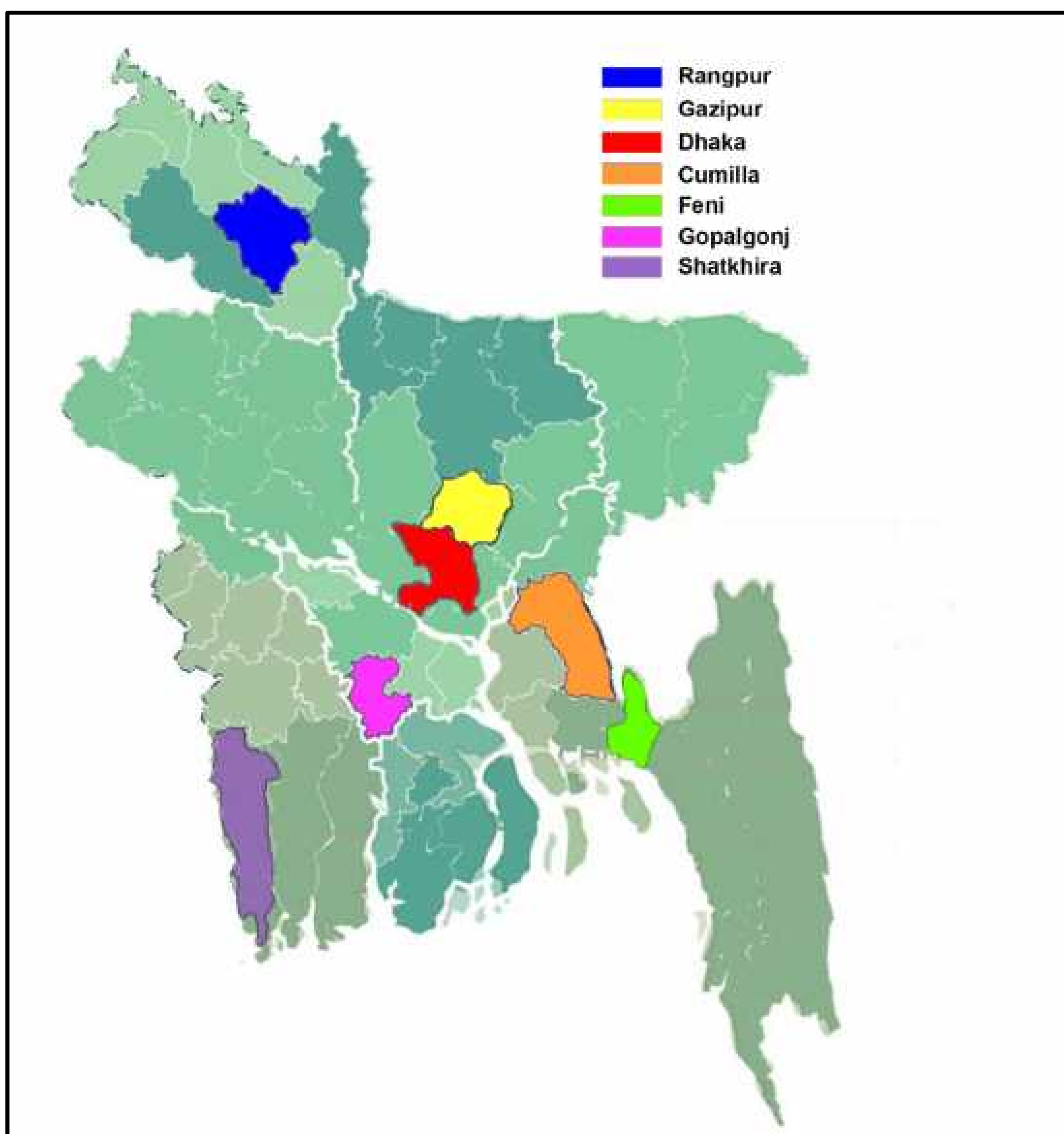


Figure 1: Map showing the location of the isolates sources.

3.7. Isolation of pathogen

The fungus was isolated from infected parts of the rice plants following tissue planting method (Yugander *et al.,* 2015; Bashar *et al*., 2010). The diseased plant parts were washed in running water followed by sterile distilled water to remove surface contaminants and any attached organic debris. Then the tissues were dissected into 0.5-1 cm size with a sterile scalpel from the infected portion along with some healthy portion. The cut pieces were surface sterilized by dipping it

into 2% Clorox solution for 1 min and rinsed three times with sterile distilled water. After surface drying by sterilized tissue paper, disease tissues were placed on water agar (2%) and incubated at 26±1°C for 24h. Mycelial tips with morphological characters typical of *R. solani*, growing outfrom the infected plant tissue was cut and transferred in fresh PDA medium.

3.8. Identification and purification of pathogen

Identification of pathogen causing sheath blight of rice was carried out by studying the cultural and morphological characters and compared with relevent literature. The isolates were purified following hyphal tip technique (Tuite, 1969). Hyphal tip of each isolate was subcultured on water agar for purification. The isolates were further purified by cutting 3x3 mm hyphae along with the medium with a sterile knife then subcultured on PDA medium in petri dish diameter 90 mm with three replications. On the 5th day, the macroscopic morphology was identified based on its appearance in PDA. After 2 to 3 days, Each isolate were examined under compound microscope (OLYMPUS CX43, Japan) for macroscopic morphology (hyphal characteristics) typical to *R. solani.*

3.9. Maintenance and preservation of culture

Pure cultures were maintained on potato dextrose agar plates by sub culturing those at 30 days intervals. The cultures were sealed by parafilm and preserve into incubator. The sclerotia of isolates were inoculated in PDA slant and maintained at 26 ± 2 °C. After 3 to 4 days when the mycelia had almost covered the full slant (Figure 2). Following sufficient growth and production of sclerotia, culture tubes were kept at 4 °C for short term storage. For long time preservation, they were kept in zip lock bag and preserved by slowly freezing process. Initially kept in 4°C refrigerator for 10 minutes and then finally in -40 °C refrigerator (VWR Scientific, USA).

Figure 2: Preservation of *Rhizoctonia solani* isolates on PDA slant media.

3.10. Morphological characterization

Isolates were grown on PDA at 26°C for 3 days until hyphae had almost reached the periphery of the plates, after which mycelial disks of 4 mm diameter were transferred aseptically by block cutter from the margin of growing colonies to at the center of new PDA petridishes (Figure 3). The fungal isolates were subcultured on PDA plates with two replications (Plate 1). Each plate was wrapped with Parafilm tape to protect from contamination and they were incubated at 26 ± 1°C upto 2 weeks for studying mycelial, hyphal and sclerotial characteristics. Morphological and cultural characters were investigated on the basis of colony and sclerial characters.

Figure 3: Cutting of mycelial disk by block cutter from the edge of growing colony.

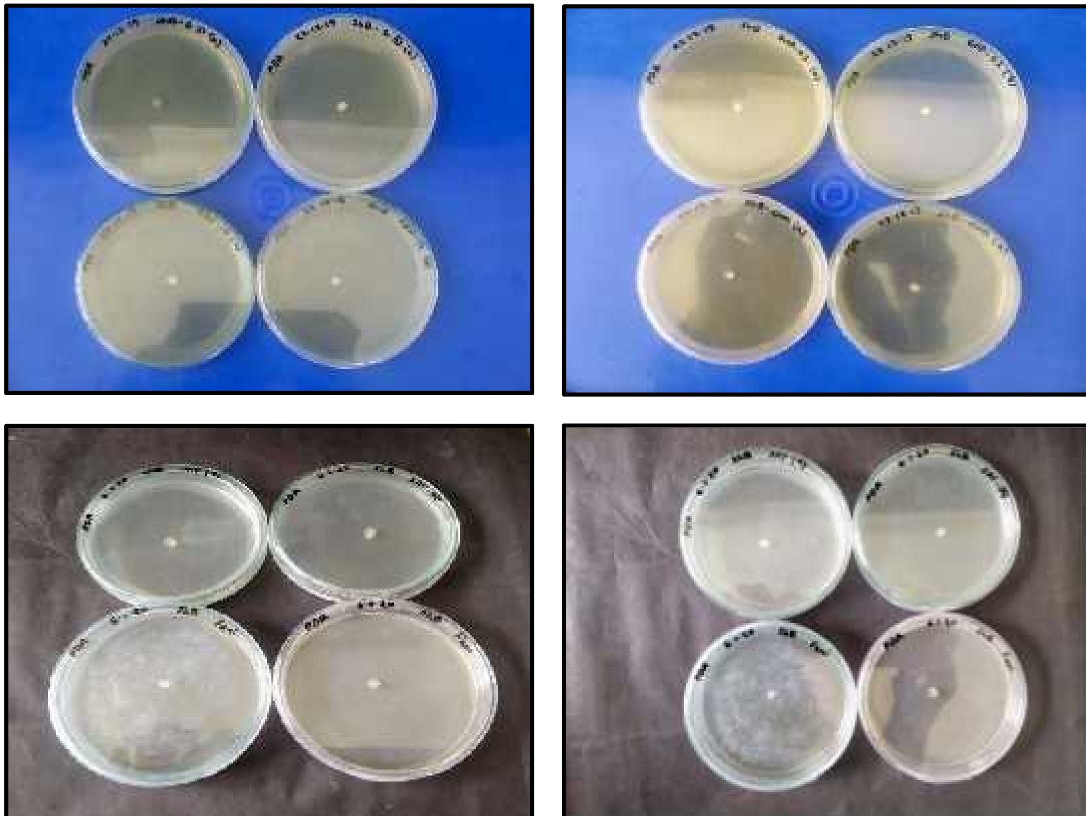


Plate 1: Subculture of eight fungal isolates on PDA plates for morphological characterization**.**

3.10.1. Colony characteristics

Growth pattern was recorded by visual observation according to the growth of hyphae. Observations were taken when the pathogen covered complete petriplate. The colony color, reverse colony pigmentation, aerial mycelium quantity and compactness were recorded after seventh day of incubation. The colour of the colony was observed from the both of upper and bottom side of petri dish.

3.10.1.1. Measurement of radial growth of colony

The bottom of the each Petri dish was marked in four directions with the help of linear scale. In case of irregular colonies measurements were recorded at the broadest and narrowest diameter and average of four different directions were taken as growth. Radial growth rate and growth pattern was measured for each isolate with three replications using meter scale at interval of 24, 48 and 96 h after incubation of the inoculated Petri dish at 26±2°C. White and black arrow indicates the edge of radial growth after 24 hours and 48 hours of incubation period respectively (Plate 2).

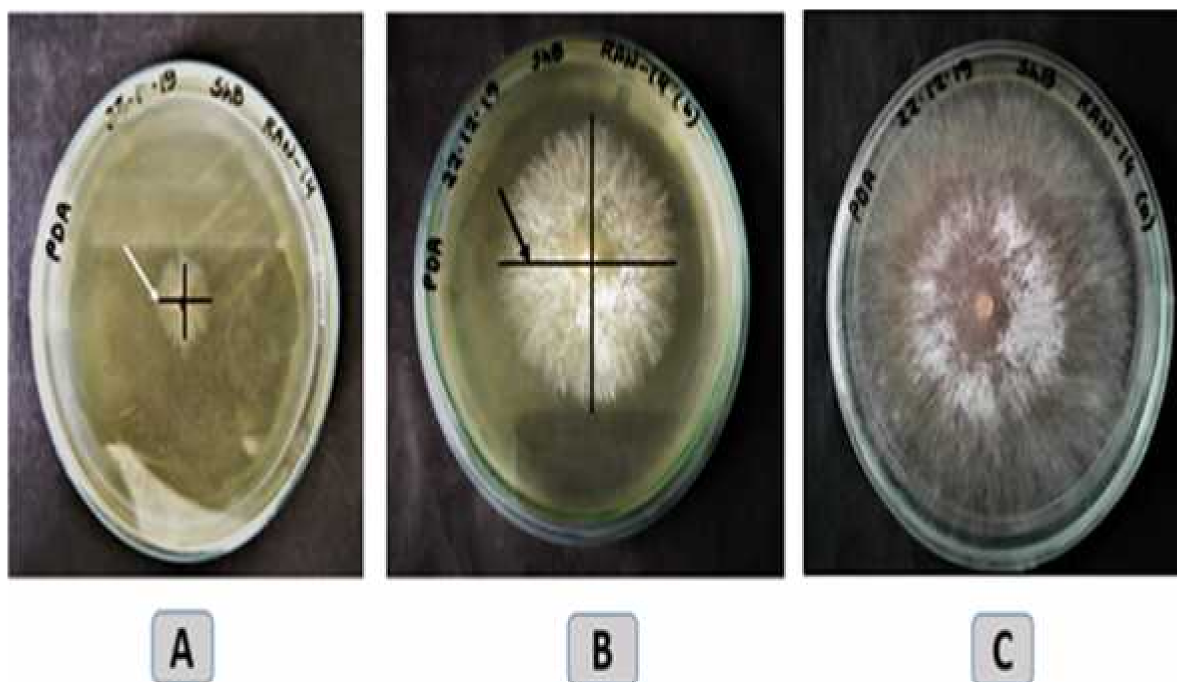


Plate 2: Measurement of radial mycelial growth diameter (cm). (A), (B) and (C) after 24 hours, 48 hours and 72 hours of incubation period respectively.

3.10.2. Sclerotia characters

Sclerotia characters such as formation of sclerotia, size of sclerotia, diameter and number of sclerotia The observation on sclerotial characteristics such as time taken for initiation of sclerotia, size (macro, micro) colour, number, topography, shape, distribution, arrangements and texture were recorded by visual observation. (Plate 3).

3.10.2.1. Size of the sclerotia

The bottom of the each petri dish was marked or drawn cross wise at random and in four directions with the help of linear scale. Diameter of the sclerotia was measured in respect of randomly selected sclerotia near the cross line of bottom of the plate with the help of Digital Vernier Calipers after 10 days of incubation (Mitutoyo Corp, Japan). 12 measurements per isolate were taken from two plates (Six from of each) (Plate 3).

Plate 3: (A) and (B) Measurement of the sclerotia diameter (mm) after 10 days of incubation with digital vernier calipers.

3.11. Analysis of data

The data were statistically analyzed in Statistix 10 software programme. Treatment means were compared by LSD at 0.05 level of significance. The data obtained in the present investigation for various parameters were carried out by Complete Randomized Design (CRD) with 3 replications for in vitro studies.

3.12. Molecular identification of fungal isolates

3.12.1. Reagent Preparation

3.12.1.1. 1M Tris-HCL Buffer Stock Solution (pH 8.0, 1L)

121.1 g Tris, UltraPure (Invitrogen, USA) was dissolved in 800 ml ddH_2O. pH was adjusted to 8.0 with the appropriate volume (~150 mL) of concentrated HCL, fuming 37% (Merck, Germany). Then finally the volume was brought to 1litre with deionized water.

3.12.1.2. 0.5M EDTA solution (pH 8.0, 1L)

Ethylene diamine tetra acetic acid (EDTA) solution, at 0.5 M pH 8.0, is a commonly used solution utilised as a ligand and chelating agent. The solutions are used to protect DNA and RNA from degradation.

For 1L solution preparation 186.12 g of EDTA (VWR International, USA) was added into 800 mL of H_2O. The solution was stirred vigorously using a magnetic stirrer. 18 to 20 gm of NaOH (Merck, Germany) solution was added to adjust the pH to 8.0. The EDTA will slowly go into solution as the pH nears 8.0. Finally the solution was diluted to 1 litre with distilled water.

3.12.1.3. 5M NaCl solution (1L)

292.2 gm NaCl (Duchefa Biochemie, Netherlands) was dissolved in 800 mL of H_2O. The volume was adjusted to 1litre with deionized water.

3.12.1.4. 2X Cetyl trimethyl ammonium bromide (CTAB)

The use of CTAB (cetyl trimethyl ammonium bromide), a cationic detergent, facilitates the separation of polysaccharides during purification while additives,

such as polyvinylpyrrolidone, can aid in removing polyphenols. CTAB based extraction buffers are widely used when purifying DNA from plant tissues.

For 1L solution preparation, 100 mL 1M Tris was Dissolved in about 700 ml of H_2O pH was brought down to 8.0 by adding about 50 ml concentrated HCl. 280 mL 5M NaCl was added and autoclaved the solution.

10 g Polyvinylpyrrolidone (PVP) (Bio Basic Inc., Canada) and 20g CTAB (Bio Basic Inc., Canada) was added to the solution and heated to dissolve. Then Total volume was brought to 1 L with ddH2O. Before use, the solution was preheated in 65 °C water bath.

3.12.1.5. TE buffer solution (1L)

TE buffer is used as a protective measure against DNA and RNA degredation, storing the two molecules and maintaining proper pH levels.

To prepare 1L of TE Buffer, 10 mM Tris (desired pH 8.0) and 2 mL 0.5M EDTA (pH 8) was measured. Microliter amounts of high molarity HCl was added to lower the pH to 8. Final volume of 1 L was filled with ddH_2O.

3.12.1.6. 10X and 1X TBE buffer (1L)

TBE buffer is commonly prepared as a 10X concentrated stock.

For 1L 10X TBE buffer preparation, 108 grams of Tris (MP Biomedicals, USA) and 55 grams of boric acid (Bio Basic Inc.,Canada) was dissolved into 900 milliliters of distilled deionized water. Then 9.3 g EDTA solution at a pH of 8.0 was added. Finally the volume was adjusted to 1 L with ddH_2O.

1X TBE buffer was prepared from 10X TBE following the formula

$$V_1S_1=V_2S_2$$

Where,

S_1 = Concentration of stock solution (10X)

S_2 = Desired concentration of solution (1X)

V_1 = Volume of stock solution (10X)

V_2 = Final volume of buffer with desired concentration

3.12.2. Fungal DNA extraction

Genomic DNA were extracted from fungal mycelia of each representative isolates following the CTAB method (Ferdous *et al.*, 2012) with minor modification.

3.12.2.1. DNA extraction

For DNA extraction aerial mycelium was collected from the three day old culture. Mycelial mats were taken carefully by spatula. Mycelium for DNA extraction was grown by inoculating 50 ml potato dextrose broth (PDB) in 125 ml conical flasks with hyphal threads of *Rhizoctonia* spp. Cultures were incubated on shaker incubator 100 rev/min at room temperature (26°C) for seven days. Fungal mycelia were collected from the broth by filtering through muslin cloth. The mycelia were dried carefully by pressing them with 3 layer of filter paper to extract the liquid broth completely (Plate 4). The dried mycelia ball (around 20gm) were placed by a forcep into a separate 1.5 ml microcentrifuge tube (Axygen, Inc., USA) for each. A zirconium oxide ball (3mm) was put into each tube and 300 µL of 2X CTAB was added into the tube and the tubes were placed into Mixer Mill (MM400, Retsch, Germany). The mycelia were mashed at frequency 30 per second for 2 minutes. Then 300 µL CIP (Chloroform: Iso-amyl alcohol: Phenol) was added into the tubes and were vortexed (Whirlimixer, Fisher Scientific, England). After vortex, tubes were centrifuged (Microfuge 20R, Beckman Coulter, Inc., Germany) at 13000 rpm for 10 minutes. 200 µL supernatant was taken into a new 1.5 mL microcentrifuge tube. 200 µL cold isopropanol (Molecular Biology Grade, Fisher BioReagents, USA) was added with the supernatant. The tubes were inverted for few minutes to mix them properly and kept the solution at room temperature for 10 minutes. Again, the samples were centrifuged at 13000 rpm for 10 minutes and then the DNA pellet at the bottom of the tube was visualized. Finally, Supernatants were removed very carefully. After that, DNA pellets were washed with 700 µL 100% cold ethanol and centrifuged at 13000rpm for 3 minutes. After discarding the liquid carefully, finally the DNA pellets were air dried. After complete drying, the pellets were

resuspended with 100 µL sterilized distilled water). And that solutions were used as stock DNA solution and kept at -20°C. The DNA samples were later diluted with ultra-pure water to the concentration of 20 ng/µL for polymerase chain reaction (PCR).

3.12.3. Molecular markers for *R. solani* detection

Targeting Internal transcribed spacer (ITS) region of rDNA of *R. solani* for identification of the fungus (Table 1). The molecular markers were synthesized by Integrated DNA Technologies, Inc.,USA.

Table 1: Details of primer used to detect *R. solani* isolates.

Primer	Sequence	Direction	Expected Size (bp)	Reference
ITS1	TCC GTA GGT GAA CCT GCG G	Forward (5'-3')	~700	(Sandoval and Cumagun,2019)
ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse (5'-3')		

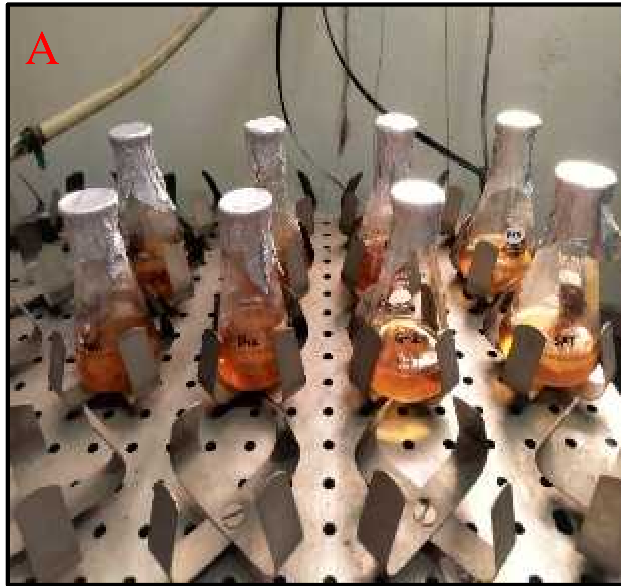


Plate 4: DNA harvesting from fungi isolates.

A. Cultures are incubated on shaker incubator for 7 days.

B. Mycelia are grown on potato dextrose broth (PDB).

C. Fungal mycelia are collected from the broth by filtering.

D. The mycelia are dried by pressing them with filter paper to extract the liquid broth.

3.12.4. PCR amplification

Each DNA sample was run into PCR Thermal Cycler (SimpliAmp, Applied Biosystems, Singapore) to amplify the targeted sequence for ITS region.

3.12.5. PCR reaction volume preparation

A 20 µL PCR reaction volume was prepared for each PCR sample. Each PCR sample containing 20 ng template DNA, 1.0 µL of each primer (0.4 µmol/L), 10 µL of Taq PCR Master Mix (Promega Corporation, USA) and nuclease-free water to final volume of 20 µL.

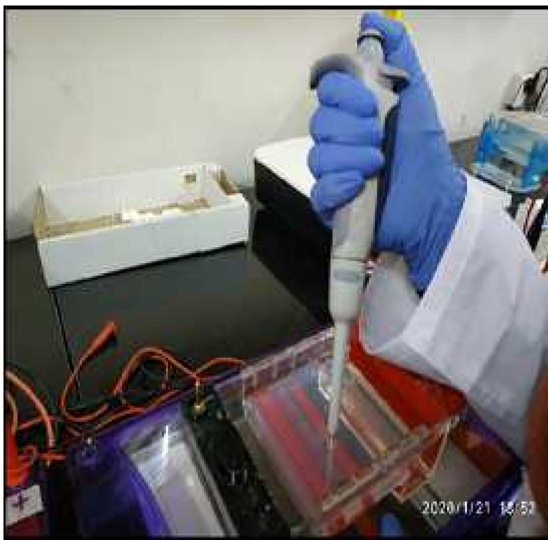
3.12.6. PCR condition

PCR program was set up as follows: initial denaturation at 94°C for 1 minutes followed by 30 cycles of denaturation at 94°C for 15 seconds, primers annealing at 58^0C for 15 seconds, and extension at 72°C for 15 seconds, followed by a final extension at 72°C for 7 minutes.

3.12.7. Gel electrophoresis and gel documentation

The amplified PCR products along with 100 bp ladder (Promega Corporation, USA) were separated using gel electrophoresis (multiSUB Mini, Cleaver Scientific Ltd., UK) in 2% UltraPure agarose (Invitrogen, USA) gel in 1X TBE buffer at 100V for 1 hours. After electrophoresis, the gel was stained with UltraPure ethidium bromide (0.5 µg/mL) (Invitrogen, USA) and visualized under ultra-violet (UV) illumination. The picture was taken in a gel documentation system (BioDocAnalyze, Biometra, Germany) (Plate 5).

All PCR reactions of each sample were repeated twice to confirm the results.



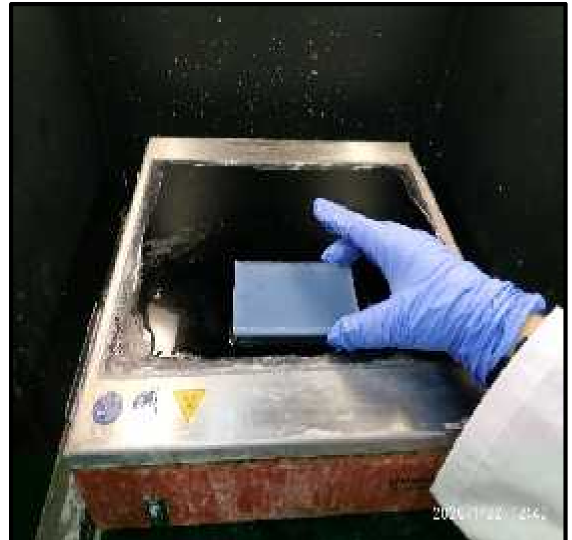


Plate 5: Part of research workings in molecular lab for PCR amplification and gel documentation.

3.13. Screening of rice germplasm against ShB of rice

3.13.1. Plant material

A total of 35 rain-fed low land rice germplasm were screened against ShB disease of rice and BR11 was used as susceptible check. These were tested during Transplanted Aman season (monsoon season, June-November). All the materials were evaluated with artificial hill inoculation for resistance to sheath blight disease under field conditions.

3.13.2. Collection of seedlings

Seedlings of rain-fed low land rice germplasms were collected from Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur.

3.13.3. Land preparation and fertilizer application

The land was prepared 15 days before transplanting/ seedling. The land was first plowed in late July. A levee was made surrounding plots to maintain standing water up to 5.0 cm inside. Four-plowings and cross plowings followed by 3 laddering were done by power tiller. The following doses of manures and fertilizers were applied in the fields as recommended by BARC.

Fertilizers Rate (Kg/ hectare)	Rate (kg/hectare)
Urea	150 kg
TSP	100 kg
MP	70 kg
Gypsum	60 Kg
Zinc Sulphate	10 Kg

TSP, MP, Gypsum, Zinc sulphate were applied at the time of final land preparation. Nitrogen in the form of urea was applied in three equal splits at growth stages 15, 30 and 50 days after transplanting (DAT). All fertilizers were applied in basal, except urea.

3.13.4. Transplanting and lay-out

The experiment was laid out in a Randomized Complete Block Design with three replications. Thirty day old seedlings were transplanted at the rate of one seedling per hill in three rows of two-meter length with plant to plant distance of 15 cm and row to row distance of 16 cm. Plot size was $2m^2$ ($2m \times 1m$).

3.13.5. Irrigation and weeding

Irrigations were given as per need and weeding was done manually whenever needed to keep the plots weed free as far as possible. For agronomic, weed management, irrigation and drainage and insect management current standard recommendations were followed (BRRI, 2007).

3.13.6. Preparation of inoculum

Forty PDA petridishes were prepared for fungal inoculum following the standard procedure. The fungus was incubated at 26±1°C for 7 days for growth and development of the pathogen (Plate 6 A).

3.13.7. Pathogen inoculation

Inoculations were done at maximum tillering stage viz, approximately 40 days after planting (Bhaktavatsalam *et al*., 1978). Total 4 tagged hill of the plot were inoculated with the pathogen culture medium. Inoculation was done by inserting a piece of culture medium (cutting the culture medium into eight pieces) at the middle of each hill in the morning (Plate 6 B). Rice plants was inoculated with a virulent *R. solani* isolate (RS-5) by placing fungal mycelia block (with sclerotia) between tillers in the central region of rice hills at 5–10 cm above the waterline (Plate 6 C). The field was maintained standing water onward of the crop growth to maintain high moisture below canopy level for disease development (Sharma and Teng, 1990).

3.13.8. Harvesting of crop and disease scoring

The crop was harvested on November 2019 at fully ripening stage from field in plot wise. The all bundled were carried to farm office for data collection. Data on plant height and lesion height was recorded at 21 days after inoculation (DAI) to calculate relative lesion height (RLH) (Plate 6 D). Lesion height (cm) was calculated for each leaf as the length of lesion from the collar down the leaf sheath and from the collar up the leaf blade.

RLH in each tiller was calculated by using the following formula and graded as per 0–9 Standard Evaluation System (SES) scale (IRRI 2002) (Table 2).

RLH (%) = [highest point of a lesion is seen (cm) x 100] / [plant height (cm)] and then making the average (Vidhyasekaran,1997).

3.13.9. Data Collection

Data were collected on following parameters:

- Plant height (cm)
- Number of tiller/hill
- Lesion height (cm)
- Relative lesion height (RLH) (%)

3.13.10. Data analysis

The field experiment for screening of germplasm was followed the RCBD design with three replications. Data were analyzed using Statistical Analysis software R (R Core Team, 2020).

Table 2. Standard Evaluation System (SES) (IRRI 2002) for sheath blight of rice Diseas score

Disease score	Disease Reaction	Description (based on relative lesion height-RLH %)
0	Immune	No infection
1	Resistant	Vertical spread of lesion up to 20% of plant height
3	Moderately Resistant	Vertical spread of lesion up to 21-30% of plant height
5	Moderately Susceptible	Vertical spread of lesion up to 31-45% of plant height
7	Susceptible	Vertical spread of lesion up to 46-65% of plant height
9	Highly Susceptible	Vertical spread of lesion up to 66-100% of plant height

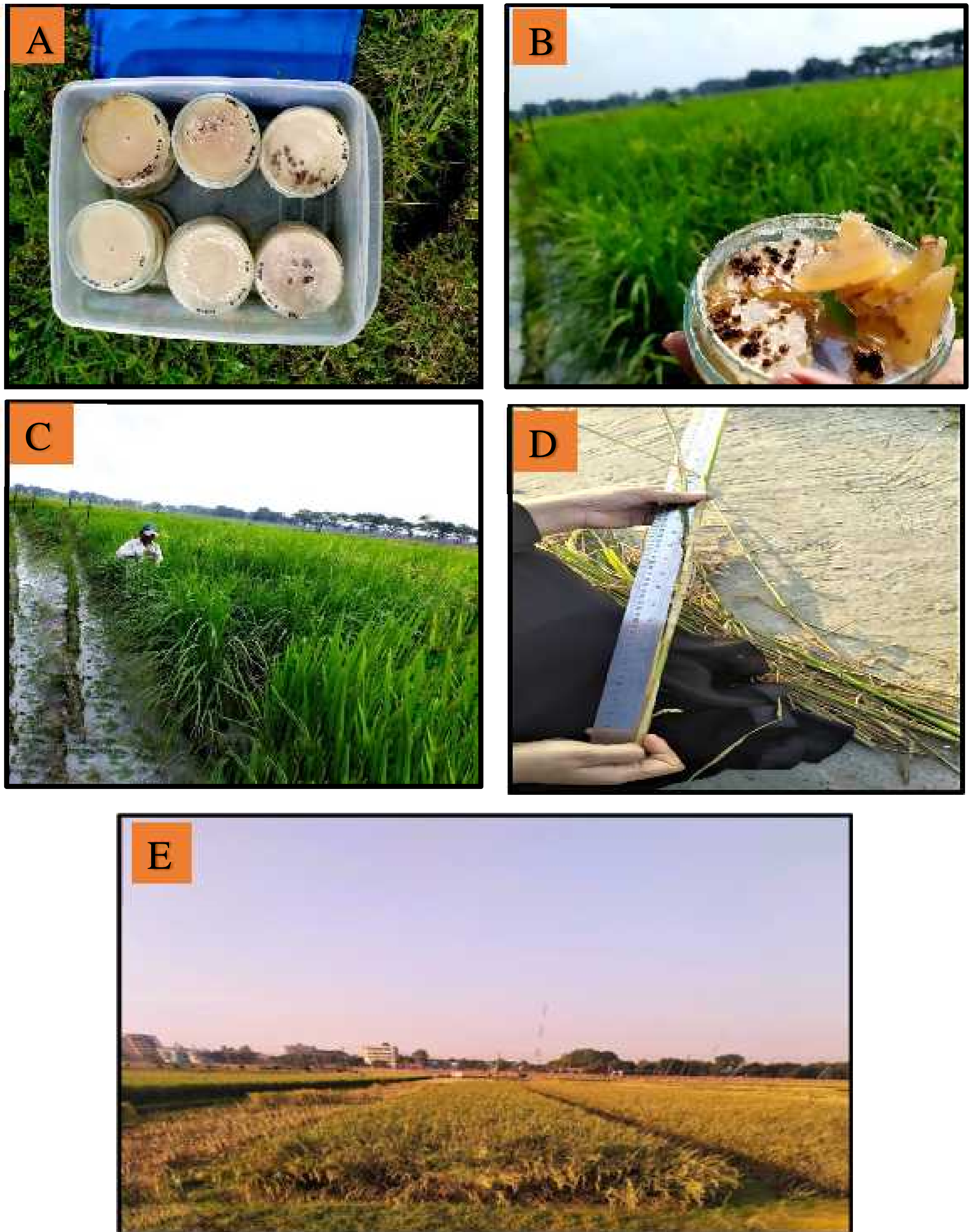


Plate 6: Artificial inoculation of pathogen under field condition.

- **A.** Fungal inoculum on PDA petridishes prepared for inoculation.
- **B.** Fungal mycelia block cutting (with sclerotia) before inoculation.
- **C.** Placing the fungal mycelia block in the central region of rice hills.
- **D.** Measurement of plant lesion height (cm).
- **E.** Field view of experimental plot.

RESULT AND DISCUSSION

4.1. Symptomatoloy

The symptoms of rice sheath blight were investigated and visually observed in the BRRI research field. Early symptoms developed on the leaf sheaths at or just above the water line as circular, oval or ellipsoid, water-soaked spots which are greenish-gray in color (Plate 7 A). As the disease progressed, they enlarged and tended to coalesce forming larger lesions with grayish white centers surrounded by tan to dark brown irregular borders or outlines. Infection was spreaded to leaf blades and caused irregular lesions with dark green, brown, or yellow-orange margins (Plate 7 B). The lesions was developed extensively and coalesced on partial or whole leaf blades, which may usually produce the characteristic rattlesnake skin like pattern (Plate 7 C). These damaged tissues had interrupted the normal flow of water and nutrients to the plant tissues above (leaves and panicles). As the plant had approached heading, the canopy became dense, created a humid microclimate that was favorable for the rapid development of the disease. The disease was moved up the plant and infected the flag leaves and panicles under severe conditions. The fungus was spreaded into the culms from early sheath infections and weaken the infected culms, resulted in the lodging and collapse of tillers (Plate 7 D). Rangaswami and Mahadevan (1999) as well as Lakshaman and Amaradasa (2016) also reported the same typical even irregularly elongated (3cm x 1cm) and discolored discrete lesions with pale greenish grey to grayish white centre with narrow blackish to dark brown margin lesion on sheath. The similar types of symptoms of sheath blight were also observed earlier by Singh *et al.,* 2012; Hollier *et al.*, 2009 and Sivalingam *et al.*, 2006 and on sheath and leaf of rice. Uppala and Zhou (2018) also described that the symptom almost looks like a cobra snake skin from distance.

Plate 7: Characteristics Sheath blight disease symptoms.

A) Irregular water soaked greenish gray lesion.

B) Grayish white center surrounded by dark brown irregular border.

C) Lesion coalesced on leaf blade produce cobra skin like pattern.

D) Collapse of culm and panicle under severe condition.

4.2. Pure culture of pathogen

A total of eight fungal isolates were cultured from seven different rice growing areas representing different agroclimatic zones of Bangladesh i.e. Dhaka (RS-1), Rangpur (RS-2), Gopalgonj (RS-3), Cumilla (RS-4), Gazipur (RS-5, RS-6), Feni (RS-7), Satkhira (RS-8). Young colonies of all isolates were identical, they were hyaline on water agar medium (Figure 4) and on PDA medium, mycelium usually very light brown or whitish; coarsely or finely radiate; occasionally moderately aerial, white patches scattered over the surface (Plate 8). The fungus *R. solani* produced silver colony at initial stage which later turned white in color and appeared smooth. The number of sclerotia was few to abundant with usually about 1.5 mm to 2 mm diameter on agar surface. Dasgupta (1992) reported that young hyphae are hyaline and pigmentation of hyphae appears to be restricted to various shades of brown or silvery, become brown with maturity. Similar findings were also reported by Gnanamanickam (2009) that *R. solani* isolates are known as fast growers that produce hyaline mycelia when young, which eventually turns yellow to brown with age.

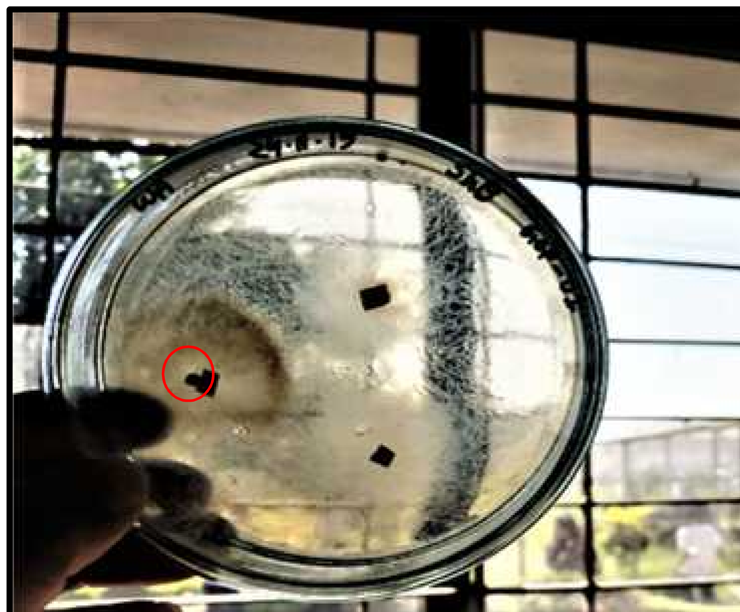


Figure 4: Cut pieces of samples on agar plate showing hyaline web like mycelial branching of the fungus with sclerotia.

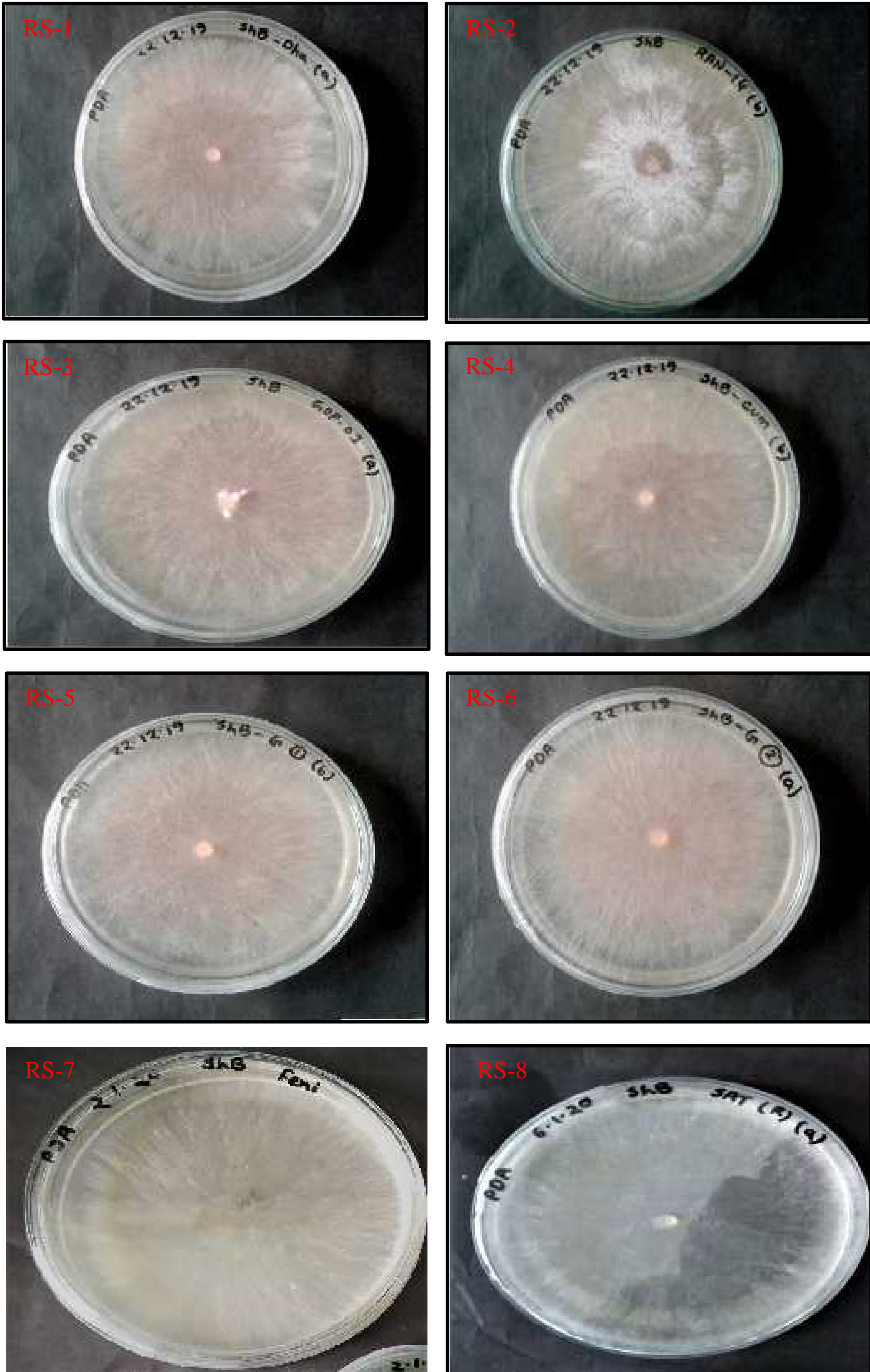


Plate 8: Pure cultures of *R. solani* isolates on PDA medium at 4 DAI.

4.3. Identification of pathogen: Pathogen was identified on PDA based on characteristic mycelial branching. Microphotographs were taken to show the typical microscopic characters of hyphae under compound microscope. Branching of the mycelium was found near the distal end of a cell in young and advanced hyphae. The young hyphae *R. solani* had branches forming 45° angles (Plate 9: RS-4, RS-5) and the more mature branches were perpendicular (90°) and same size (Plate 9: RS-1, RS-2, RS-3, RS-6, RS-7). Adult hyphae become stiff because of the thickened cell walls (Plate 9: RS-2, RS-6). In older hyphae, branching occurred at any place along the cell. Young hyphal branches are invariably somewhat constricted at the point of union with main hyphae. A septum is formed in the branch near the constriction (Plate 9). Constriction at the point of branching of the mycelium and presence of a septum near or at right angle of the branching junction have immense taxonomical importance. Microscopic studies revealed that all the eight isolates of *R. solani* in the present study characteristically had produced long multinucleate cells that grow approximately at right angles to the main hypha with a slight constriction at the junction of branching. The mycelial characteristics are in conformity as previously described by (Lal and Kandhari 2009; Vidhyasekaran *et. al.,* 1997; Parmeter *et al.,* 1970; Bracker C. K. and Butler 1963) on rice sheath and plant debris. The variations of hyphal branching was also noticed among the isolates. Butler and Bracker (1970) also described the concept of species of *R. solani* with young hyphae branching at an acute to right angle, with slight constriction at the point of branching, and septum formed near the constriction point.

These three characteristics were observed in all the isolates; thus, they were initially identified as *Rhizoctonia* spp.

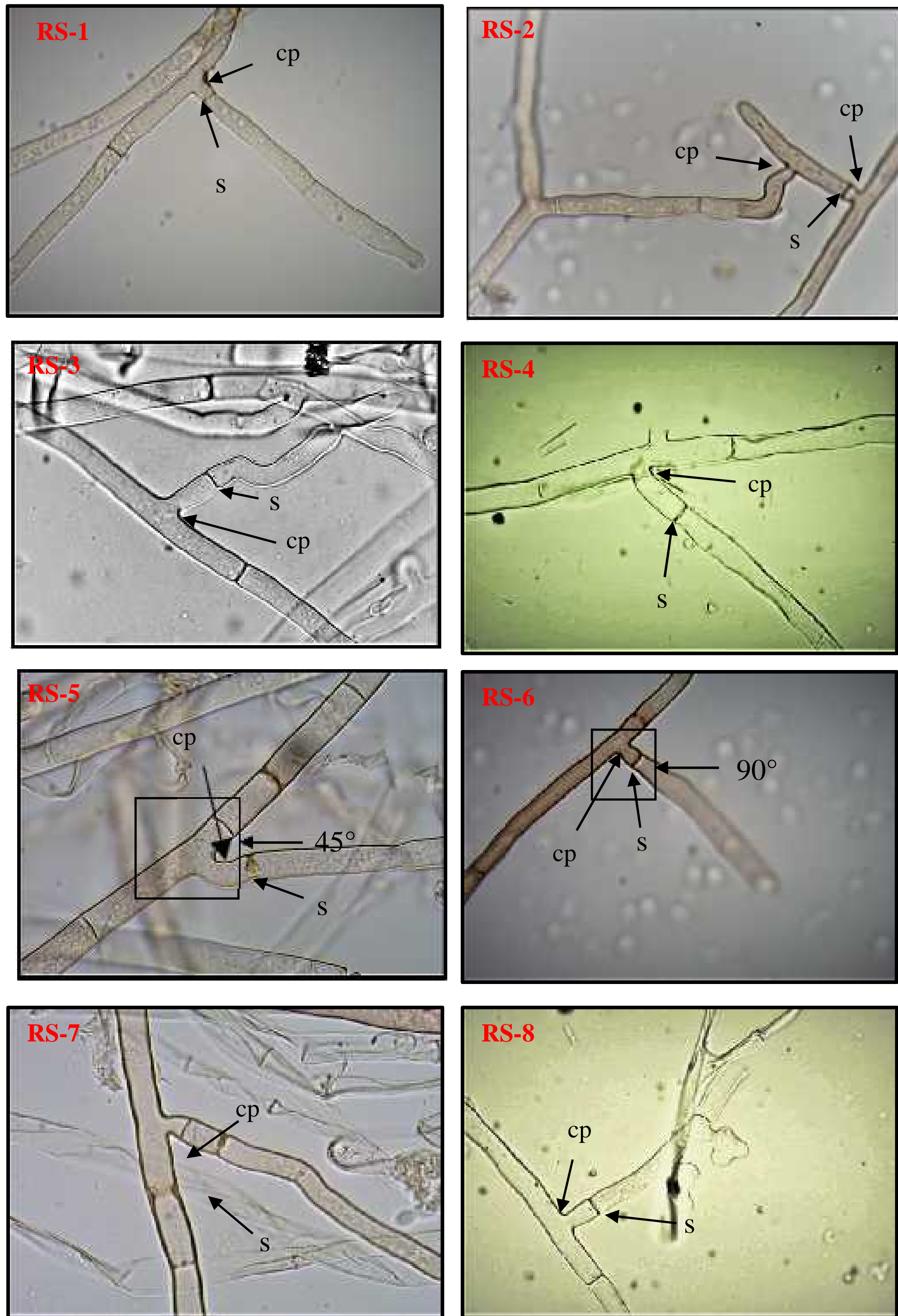


Plate 9: Microscopic view (40X magnification) of the 8 isolates showing the characteristics mycelia branching at an acute (45°) or 90° angle with slight constriction at the point (cp) of branching and septum (s) formed near the branching point.

4.4. Morphological study of *Rhizoctonia solani* isolates

The morphological characteristics of the eight *R. solani* isolates were observed at different intervals of time after inoculation on PDA. Young colonies of all isolates were identical, they were hyaline on water agar and white on PDA. When the culture plates were incubated for two weeks, isolates showed a high degree of variation in visual appearance. The diversity of macroscopic characters was seen in each replication, although it subcultured from the same isolate. Morphological characterization of *R. solani* isolates were done on the basis of mycelial colour, size and position of sclerotia in petri-plate on the PDA medium which is also reported by (Banniza *et al.,* 1996; Vilgalys and Cubeta 1994; Vijayan *et al.,* 1985 and Sherwood *et al*., 1969). Cultural and morphological characteristics in any living organism are governed and controlled by genetic and environmental factors. These characteristics also determine the virulence and aggressiveness of the pathogen. Singh *et al*. (2002) reported that *Rhizoctonia Solani* has been thoroughly studied for variation in its different aspects and has shown great variability in its cultural, morphological charectaristics. Zhou *et al.* (2016) explained that this inconsistency in macroscopic character of isolate *R. solani* might be due to the nature of the cell nucleus in the fungus. The earlier studies suggest that sheath blight pathogen *R. solani* was found homogenous in nature reported by (Kuninaga and Yokosawa, 1982) but recent investigations of (Susheela *et al.,* 2004; Yu *et al*., 2003; Neeraja *et al.,* 2002 and Singh *et al.,* 2002) revealed that the pathogen is more diverse than previously assumed. The existence of variability at mycelial, sclerotial and genetic level in *R. solani* causing sheath blight of rice was reported by many workers (Singh *et al.,* 2018; Singh *et al*., 2015; Susheela *et al.,* 2013; Guleria *et al*., 2007; Sunder *et al.,* 2003 and Singh *et al*., 1999) in India.

A. RS-1 (Dhaka)

B. RS-2 (Rangpur)

C. RS-3 (Gopalgonj)

D. RS-4 (Cumilla)

E. RS-5 (Gajipur)

F. RS-5 (Gajipur)

G. RS-6 (Feni)

H. RS-6 (Satkhira)

Plate 10. Pure cultures of *R. solani* isolates after two weeks of incubation on PDA media.

4.4.1. Mycelial characters

Mycelial characters including colony colour (upper side and reverse side), growth pattern, growth rate showed significant variation among the isolates which are listed in Table 3.

Table 3. Colony characteristics of different isolates on PDA Culture media

Isolate name	Location	Colony characters				
		Mycelia color		Growth pattern	Aerial mycelium quantity	Compactness
		Upper side	Reverse side			
RS-1	Dhaka	Milky white	Yellowish brown	Abundant	Air space in dish half filled	Compact
RS-2	Rangpur	Yellowish brown	Yellowish brown	Abundant	Almost all air space filled	Slightly loose
RS-3	Gopalgonj	Milky white	Dirty white	Moderate	Air space in dish half filled	Loose
RS-4	Cumilla	Dark brown	Yellowish brown	Moderate	All hyphae close to surface of plate	Slightly loose
RS-5	Gazipur	Yellowish brown	Milky White	Moderate	All hyphae close to surface of plate	Loose
RS-6	Gazipur	Yellowish brown	Milky White	Moderate	All hyphae close to surface of plate	Loose
RS-7	Feni	Dirty White	Dirty White	Scarce	Absent	Loose
RS-8	Satkhira	Dirty White	Dirty White	Scarce	Absent	Loose

4.4.1.1. Colony color

Based on the colony pigmentation, all the isolates were assigned into 4 groups: dark brown, dirty white, milky white and yellowish brown. Two isolates (RS-5, RS-6) were found yellowish brown on upper side and milky white on reverse

side on PDA media. Two isolates (RS-7, RS-8) produced dirty white and one isolate RS-2 produced yellowish brown color on both side of the plate. Isolate RS-1 produced mycelia color with milky white upper side and yellowish brown reverse side. Isolate RS-3 also produced milky white color mycelia on upper side and dirty white on reverse side and remaining one isolate RS-4 produced mycelia color with dark brown upper side and milky white reverse side (Plate 10). Raja *et al.* (2016) collected six isolates from (Gujarat, Karnataka, Pantnagar, Uttar Pradesh, Haryana and Punjab) of India of which two isolates of Haryana and Punjab were found yellowish brown, two isolates of Pant Nagar, Uttar Pradesh were found dirty white, one isolate of Gujarat was found milky white and one isolate of Karnataka was found dark brown. Sing *et al.* (2015) found more variation in colony color. Out of twenty-five isolates, five isolates of *Rhizoctonia Solani* were of whitish brown color, seven isolates were of light brown color, six isolates were of yellowish brown color, four isolates were of dark brown color, two isolates were of pale brown color, and one isolate was of milky brown color. Lal *et al.* (2014) while studying variability of *R. solani* isolate found six isolates as light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown. Sunder *et al*. (2003) had also reported that colony colour ranged from brown, light brown, dark brown and yellowish brown. The results are also supported by Hoa (1994), reported that colony colour ranged from brown, light/dark brown, black brown, chocolate brown, salmon and dark salmon. The discolorations of the growth media are mainly attributed to the production of pigments by the pathogen. The difference in the intensity of the colour may also correspond to the amount of pigments released by respective isolate in the media.

4.4.1.2. Growth pattern

Growth pattern was recorded by visual observation according to the growth of hyphae: abundant= aerial mycelium obscures surface mycelium and touches the lid of the petri dish (RS-2) (Plate 10: B); moderate= aerial mycelium obscures

surface mycelium but does not touch the lid of petri dish (RS-1, RS-3) (Plate 10: A, C) and scarce= aerial mycelium does not obscure surface mycelium of the inoculated petri dish at 26±2°C (RS-4, RS-5, RS-6, RS-7, RS-8) (Plate 10: D, E, F, G, H). The mycelial growth was considered rapid if on the third day of mycelia has grown reach on petri dish diameter (90 mm) and slow if on the third day growth was less than 90 mm. The growth was rapid in the majority of isolates. Among the eight fungal isolates, seven had covered the entire petri plate surface of 90 mm diameter after three days of incubation; indicating their fast growing nature, while one isolate (RS-6) had slow mycelial growth (Figure 5). Similar growth pattern was also described by Desvani *et al.* (2018). Raja *et al.* (2016) also found two isolates (Gujarat and Karnataka) of India which covered the whole plates (90 mm or 89.07) in 72 hrs. The similar types of cultural characters of *R. solani* were observed earlier by (Singh *et al.*, 2002 and Dasgupta, 1992). Sanjay *et al.* (2019) described different attributes for growth pattern. They found 31 (27.6%) isolates as aggregate growth pattern, 38 (33.9%) isolates were moderate dispersion of mycelia, while 43 (38.3%) isolates were spatial growth pattern out of 112 isolates. Similar type of observation was made by Susheela and Reddy, 2013; Singh and Singh, 1994 who also noticed aggregate, moderate and spatial type of dispersion in *R. solani* isolates. Adhipathi *et al.* (2016) described the criteria for fast growing isolates, which was fixed >45 mm, for moderate growers 35-45 mm and for slow growers <35 mm on the basis of mean colony diameter after 48h of inoculation on PDA medium at 28 ± 2ºC. On the basis of growth pattern Lal *et al.* (2014) categorized the isolates into three groups- abundant, moderate and slight. Eight isolates showed abundant growth, four isolates were moderate and 13 isolates only scarce growth pattern and on the basis of mean of three readings, they classified the isolates into three groups: fast, medium and slow growing. 12 isolates were included into fast growing (>65 mm) group. 11 isolates were categorized into medium growing (60-65 mm) group and two isolates were categorized into slow growing (40-59 mm) group. Burpee *et al.* (1980) had also grouped the growth into same three pattern- fast, medium and slow. Lal and Kandhari (2009) recorded diameter of mycelial growth after 24,

48 and 72 h. Ten isolates covered the whole plate (90mm) in 48h and one isolate did not cover the plate even after 72 h. They also informed that fast and medium growing isolates were more pathogenic than the slow growing isolates. Basu *et al.* (2004) found that there was no correlation between the mycelial growth of an isolates and its virulence on the host. But Shahjahan *et al.* (1987) reported that growth rate and the virulence of the fungus were related to each other.

The frequency distribution of growth pattern reveals that all the isolates follow a symmetrical increase of growth upto three days of incubation period (Figure 5).

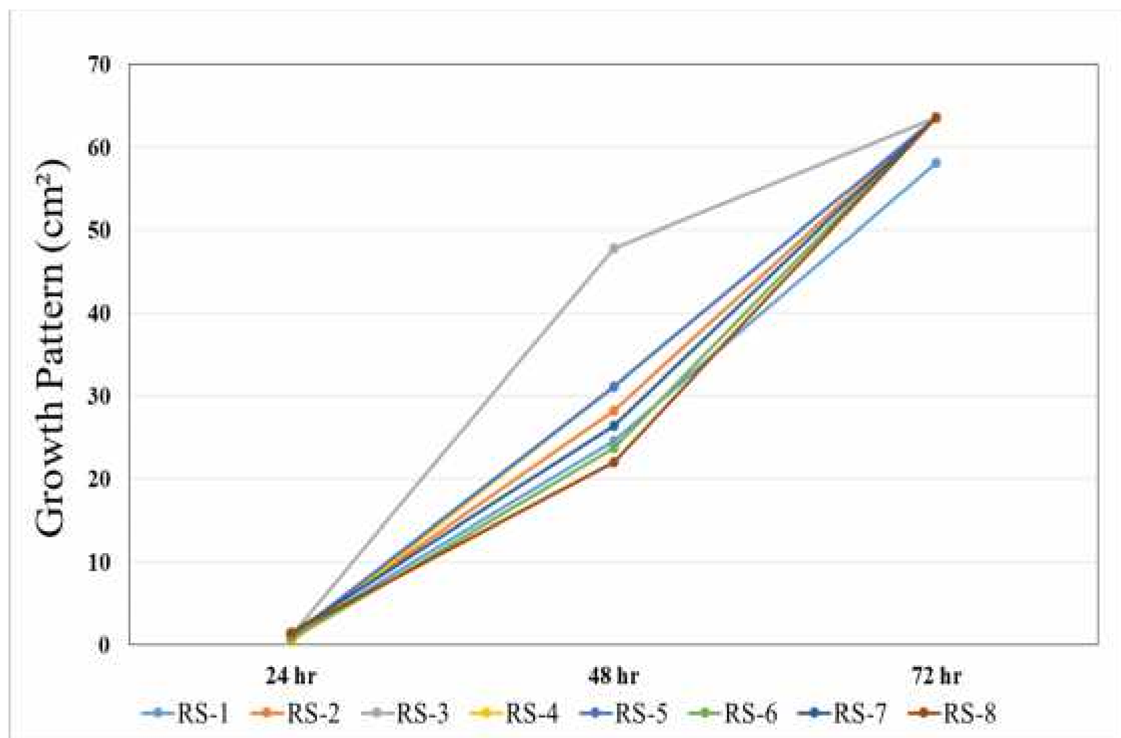
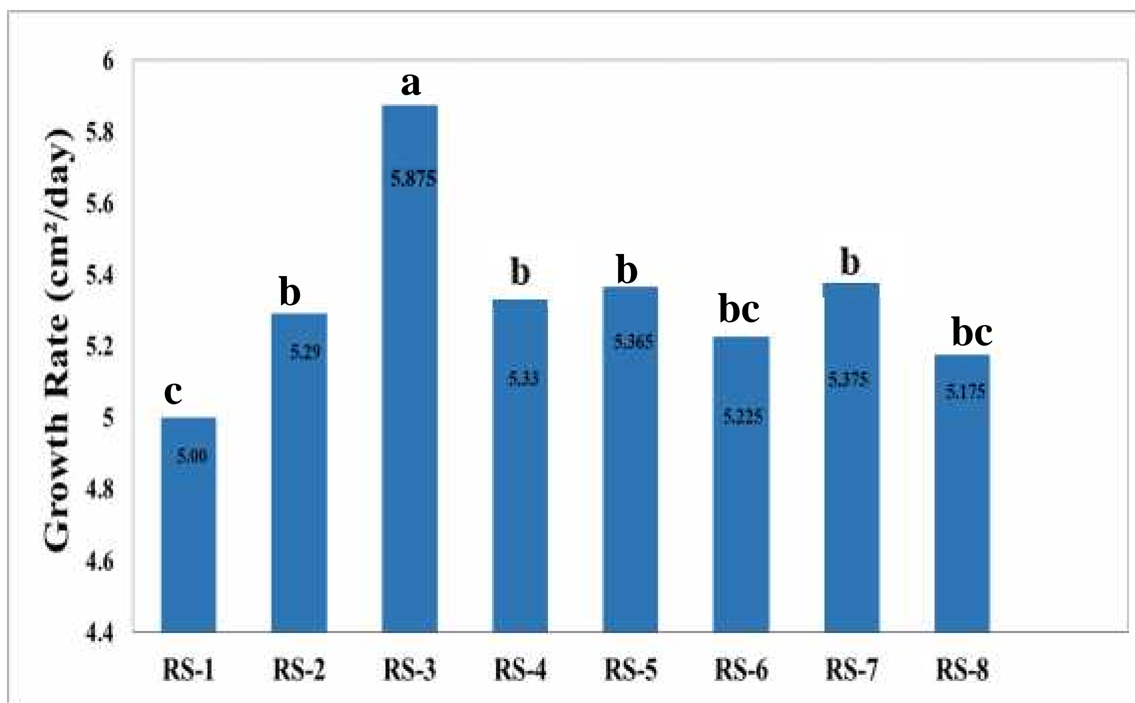


Figure 5: Growth Pattern of different isolates of *R. solani* on PDA media.

4.4.1.3. Growth Rate

Based on the colony growth rate, the isolates were divided into three groups like fast (> 5.37 cm^2/day), moderate (5.29–5.37 cm^2/day) and slow (< 5.29 cm^2/day). All isolates had growth rates between 5.00-5.87 cm^2 per day

with an average of 5.33 cm^2/day. RS-1 had low growth rate (5.00 cm^2/day) followed by RS-8 (5.17 cm^2/day) and RS-6 (5.22 cm^2/day). Hence, this three isolates belong to slow growth rate. Four isolates (RS-2, RS-4, RS-5, RS-7) exhibited moderate growth rate that had growth rate 5.29 cm^2/day, 5.33 cm^2/day, 5.36 cm^2/day and 5.37 cm^2/day respectively. Isolate RS-3 had high mycelial growth rate of 5.87 cm^2 per day and were categorized as fast colony growth rate (Figure 6). The frequency distribution plot reveals the isolates group into several distinct peaks. This result is in confirmity with Ali (2002), who recorded the linear mycelial growth rates in mm of the 72 isolates. All isolates had growth rates between 22.3-25.7mm per day with an average of 24.1 mm. The median or mid value was slightly higher than the mean at 24.2mm per day. Low growth rate was (22.3-mm per day) for 16 isolates and (25.7-mm per day) was higher for 17 isolates. Sanjay *et al.* (2019) recorded colony diameter after 24, 48, 72, 96 and 120 h of inoculation at 26 ± 2 °C. The colony growth rate was divided into three groups like fast (> 26.0 mm day−1), moderate (25.0–26.0 mm day−1) and slow (< 25.0 mm day−1). They observed maximum growth rate was (28.3 mm day−1), while the minimum growth rate was (18.7 mm day−1). A total of 45, 35 and 32 isolates were categorized as fast, moderate and slow grower respectively. Guleria *et al.* (2007) observed colony growth rate of 21.1 mm day−1 in 13 isolate out of 19 studied isolates. Four isolates exhibited moderate growth rate that ranged between 16.1 and 19.2 mm day−1. Slowest growth rate of 9.8 mm day−1 was observed in one isolate. Susheela and Reddy (2013) have also reported the variability in growth rate of AG1IA isolates including 16 fast growing, 13 moderate and 6 slow growing.



CV (%)- 2.27

Figure 6: Colony growth rate of different isolates of *R. solani* on PDA media.

4.4.1.4. Aerial mycelium quantity

All hyphae was close to the surface of plate for RS-1, RS-2, RS-4, RS-5 and RS-6 isolates. For the isolate RS-3 air space in dish was half filled (Plate 10: A, B, D, E, F, C). Aerial mycelium was absent for the isolate RS-7 and RS-8. (Plate 10: G, H). Ali (2002) and Moni *et al*. (2016) described the same aerial mycelial attributes as 0, absent; 1, all hyphae close to surface of agar; 2, air space in dish half filled; 3, almost all air space filled.

4.4.1.5. Compactness

Isolate RS-1 was more compact, while slightly loose texture was appeared in RS-2 and RS-4. Rest of the isolates RS-3, RS-5, RS-6, RS-7 and RS-8 showed loose compactness (Plate 10: A, B,C, D, E, F, G, H). Sifat and Monjil (2017) studied four different isolates in which isolate 1 produced regular shaped compact mycelia, isolate 2 produced slightly loose mycelia, at the same time isolate 3 and isolate 4 produced regular shaped loose mycelia.

4.5. Sclerotial characters

In our study considerable variation was found in sclerotial morphology of *R. solani* isolates. The observation on sclerotial characteristics such as number, size, shape, colour, texture, sclerotia initiation time and clump formation and number of clump were recorded. Main sclerotial characteristics of different isolates on PDA culture media are listed in Table 4.

Table 4. Scerotial characteristics of different isolates on PDA culture media

Isolate name	Location	Sclerotial characters						
		Distribution	Shape	*Quality of sclerotia number per petridish	Topography	Color	Sclerotia initiation time (day)	**Clump formation & Number (10DAI)
RS-1	Dhaka	Circular concentrated circle(CCC)	Irregularly globose with crust	Excellent	Immersed	Olive Brown	5	Circular at center (More)
RS-2	Rangpur	Wide sparsely(WS)	Flattened bottom and rounded top	Excellent	Superficial	Yello wish Brown	5	Periphery (Moderate)
RS-3	Gopalgo nj	Circular concentrated sparse(CCS)	Irregularly globose with pitted surface	Very good	Superficial	Olive Brown	4	Periphery (Less)
RS-4	Cumilla	Concentrated at the edges and lots(CEL)	Irregularly globose with crust	Excellent	Superficial	Olive Brown	5	Scattered (Moderate)
RS-5	Gazipur	Large concentric rings (LCR)	Irregularly globose with pitted surface	Excellent	Immersed and superficial	Dark brown	5	Circular at center (Moderate)
RS-6	Gazipur	Large concentric rings (LCR)	Irregularly globose with pitted surface	Excellent	Immersed and superficial	Dark brown	5	Circular at center (Moderate)
RS-7	Feni	Concentrated at the edges and rare(CER)	Spherical	Poor	Superficial	Black	6	Absent
RS-8	Satkhira	Concentrated at the edges and rare(CER)	Spherical	Moderate	Superficial	Black	6	Absent

*Scale for number of sclerotia: No sclerotia forming isolates/ (0)= poor, 1-10= fair, 11-20= Moderate, 21-40= Good, 41-60= very good, >60= excellent. (Lal *et al.,* 2014).

**Scale for number of clump: > 16= More, 11-16= Moderate, 1-10=Less, (0)= Absent (Sanjay *et al*., 2019).

4.5.1. Distribution of sclerotia

The distribution pattern of sclerotia was diverse. On PDA, smooth sclerotial bodies were formed in the aerial parts as well as surface of mycelium having central, peripheral and scattered position. However, distribution of the sclerotia was recorded more precisely as Circular Concentrated Circle (CCC) for RS-1 (Plate 10: A), Wide Sparsely (WS) for RS-2 (Plate 10: B), Circular Concentrated Sparse (CCS) for RS-3 (Plate 10: C), Concentrated at the edges and lots (CEL) for RS-4 isolate (Plate 10: D), Same distribution type viz; Large Concentric Rings (LCR) was noticed for both RS-5 and RS-6; (Plate 10: E, F), they are collected from Gazipur. Similarly, For RS-7 and RS-8 Concentrated at the edges and rare (CER) type distribution was found (Plate 10: G, H). The distribution pattern of sclerotia under the present study was found in line with the characters reported earlier by Desvani *et al*. (2018). They found Rare concentric rings (RCR), Large concentric rings (LCR), Wide sparsely (WS), Spread very rare (SVR), Concentrated at the edges and lots (CEL), Concentrated at the edges and rare (CER), Circular concentrated circle (CCC), Circular concentrated sparse (CCS) for a total of 8, 13, 28, 28, 12, 16, 9, and 19 isolates. Regina *et al.* (2019) also found in their study that the sclerotial bodies were formed in central, peripheral, scattered, or ring patterns. Twenty eight isolates had sclerotia scattered all over the plates. Five had sclerotia formed at the center. The peripheral formation pattern was observed for three isolates and two isolates had a ring pattern. Raja *et al.* (2016) classified the formation of sclerotia into three groups: peripheral, scattered and ring at periphery. One isolate was found in ring, two isolates were found scattered and three were found peripheral. Singh *et al.* (1990) also reported the sclerotial formation in the same manner i.e. central,

peripheral or scattered. Lal and Kandhari (2009) found sclerotia formation in aerial mycelium for nine isolates and on the surface of the mycelium for all isolates except one. In nine isolates sclerotia were formed on both aerial and on surface of the mycelium. Not a single isolate showed embedded sclerotia.

4.5.2. Shape and texture of the sclerotia

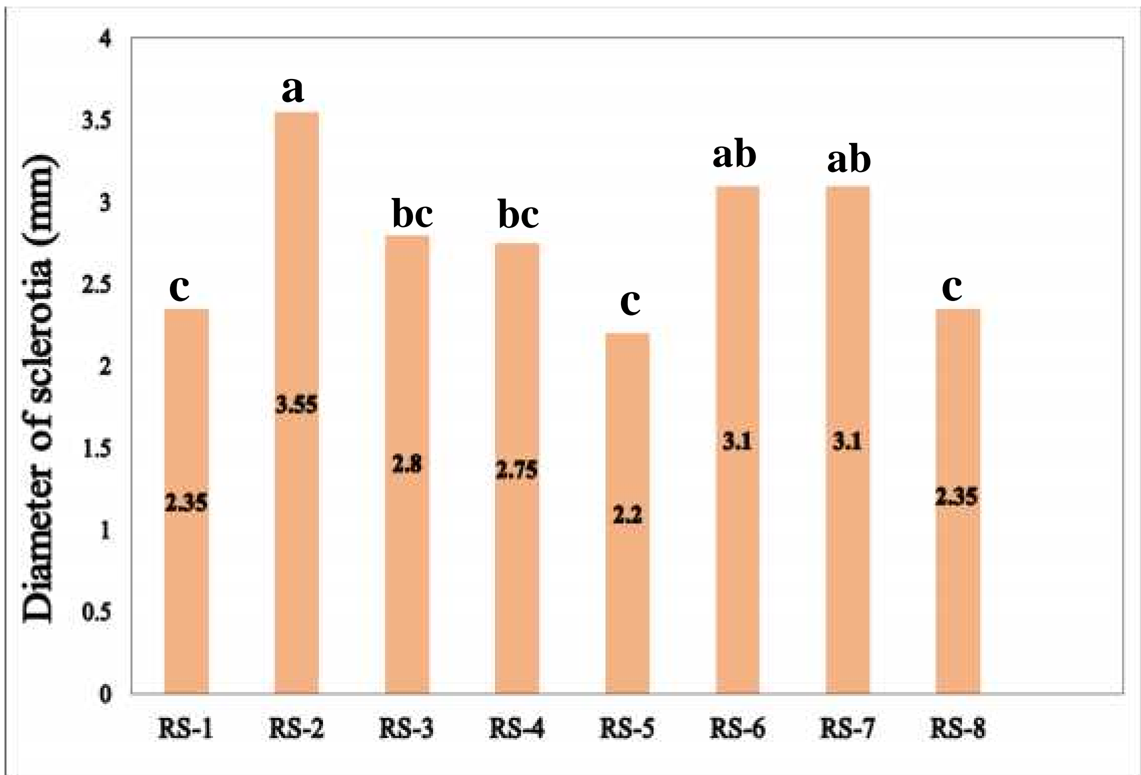
Shape of the sclerotia was variable ranging from irregular with a smooth surface to globose with an irregular surface or as slightly raised areas radiating from the centre of a culture. Isolate RS-7 and RS-8 formed spherical shape sclerotia while RS-2 formed flattened bottom and rounded top sclerotia (Plate 10: G, H, B). Irregularly globose with crust type sclerotia was formed for RS-1 and RS-4 (Plate 10: A, D). Isolate RS-3, RS-5 and RS-6 showed irregularly globose with pitted surface sclerotia (Plate 10: C, E, F). Superficial sclerotia produced exudate droplets on sclerotial body surface which was found in almost all isolates excluding RS-7 and RS-8. Nagaraj *et al.* (2019) described the texture of the sclerotia ranging from rough to smooth in texture. Sifat and Monjil (2017) characterized sclerotial shape as small round and bigger round. Moni *et al.* (2016) found that sclerotia shape flattened bottom and round to oval shape in most of the isolates. Based on texture of sclerotia, Singh *et al*. (2015) classified the isolates into two groups *i.e.* smooth and rough. Of all twenty five, ten isolates were having smooth category of sclerotium and remaining 14 isolates were having rough category of sclerotium. Matz (1921) reported that sclerotia were irregular and flat, tending to elongation. Valdez (1955) observed them in the form of a crust on PDA and Weber (1939) described them as subglobose. Ryker (1939) described them as flattened on the bottom and rounded on the top.

4.5.3. Size of sclerotia

The size of sclerotia is classified into macro sclerotia and micro sclerotia. Isolates that produce sclerotia with an average diameter of > 3 mm were grouped as macro slerotia and those that produce sclerotia with an average

diameter of 3 mm were considered as micro sclerotia producers. In the present study, sclerotial diameter ranged from 1.62 mm to 4.38 mm where the minimum sclerotial diameter (1.62 mm) was observed in isolate RS-5 with minimum average diameter (2.2 mm) and maximum sclerotial diameter (4.38mm) was recorded in RS-2 isolate with maximum average diameter (3.55 mm) (Figure 7). In the present study, among the eight isolates, isolate RS-2, RS-6 and RS-7 formed macro sclerotia. Isolate RS-1, RS-3, RS-4, RS-5, RS-8 formed micro sclerotia and varied their number of sclerotia among the isolates. Pralhad *et al*. (2019) also categorized the sclerotial size into two group- macro and micro sclerotia. Sanjay *et al.* (2019) recorded diameter of sclerotia size at 10 DAI by using binocular microscope and divided those into two categories i.e. L isolates: large or macro sclerotia producers (> 1.25 mm) and S isolates: small or micro sclerotia producer (125 mm). Among all the 128 isolates, 56.2% isolates were categorized as S type isolates, while 41.9% isolates were designated as L-type or macro isolates. Kumar *et al.* (2008) and Goswami *et al.* (2017) had reported that isolates with macro sized sclerotia are highly virulent as compared to isolates with micro sized sclerotia. Nagaraj *et al.* (2019) found considerable variation in sclerotial size of *R. solani* isolates even within the agroclimatic zones. They recorded sclerotial diameter ranged from 0.13 mm to 9.44 mm. Eleven isolates produced more than 4 mm sclerotial diameter. Thirteen isolates produced 3-4 mm sclerotial diameter. The remaining isolates produced sclerotial diameter of less than 3 mm. Sing *et al.* (2015) categorized the isolates into two groups. Group -1 had diameter range from 1.21-1.75 mm included with seven isolates and group -2 had diameter range from 1.75-2.94 mm included with seventeen isolates. Lal *et al.* (2014) also categorized the isolates into 2 groups. Group 1 had diameter range from 1.13-1.5 mm and Group 2 from 1.5-2.03 mm. Nine isolates were categorized into group 1, while 15 isolates were categorized into group 2. Basu *et al.* (2004) also reported that sclerotial diameter ranged from 0.23 to 1.91 mm and found that the abundance and size of sclerotia determine the virulence of an isolate. Banniza *et al.* (2011) also informed that *R. solani* isolates specifically those belonging to subgroup AG1-

IA, produced brown to dark-brown sclerotia that were relatively spherical and large (1–3 mm). The size of the sclerotia also varied from pinhead to 6 mm in diameter reported by (Hashiba and Kobayashi, 1996). Dath (1985) and IRRI (1986) also reported that diameter of *R. solani* sclerotia ranged from 1 to 3 mm.



CV(%)- 10.51

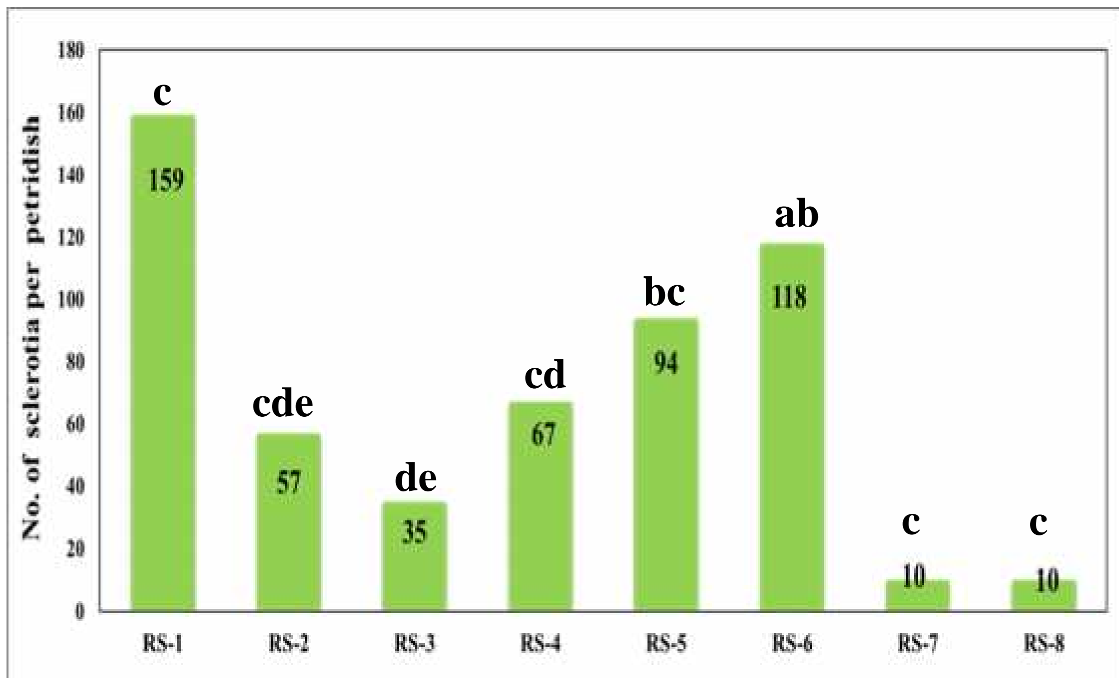
Figure 7: Average sclerotial diameter (mm) of the different isolates at 10 DAI.

4.5.4. Number of sclerotia

The number of sclerotia per petridish was counted for all the isolates after 7 day after inoculation. According to the scale for number of sclerotia, (No sclerotia forming isolates/ (0)= poor, 1-10= fair, 11-20= Moderate, 21-40= Good, 41-60= very good, >60= excellent) the quality of sclerotia number for isolates RS-1, RS-2, RS-4, RS-5 and RS-6 was found excellent (group 6) (Plate 10: A, B, D, E, F). Very good (group 5) amount of sclerotia was observed for only RS-3 isolate (Plate 10: C). Moderate (group 3) and poor (group 1) amount sclerotia was

noticed for RS-7 and RS-8 isolate respectively (Plate 10: H, G) (Figure 8). All isolates produced varied number of sclerotia on the lid at 8 DAI except RS-7 and RS-8. Abundant scelotial growth on lid was found in RS-1 and RS-3, Moderate to few growth was found in RS-2, RS-4, RS-5 and RS-6. No growth was found in RS-7 and RS-8 (Plate 10: A, C, B, D, E, F, G, H). According to the scale for number of sclerotia, Lal *et al.* (2014) found the sclerotia number ranged from 0 to >60. One isolate from New Delhi had no sclerotia at all and was categorized into group 1 (poor). None of the isolate was categorized in group 2 (fair) and group 3 (moderate). Group 4 (good) included six isolates. Group 5 (very good) included seven isolates and group 6 (excellent) included 11 isolates. Nagaraj *et al*. (2019) was found highest number of sclerotia among 65 isolates and number varied from 0-1504 per petri plate. Two isolates produced 1504 and 1308 sclerotia respectively. Seven isolates produced more than 500 sclerotia Petri plate-1, while five isolates produced very less sclerotia (less than 10 sclerotia petri plate-1). Two isolates did not produce any sclerotia. Sing *et al.* (2015) also categorized the isolates using the same scale for number of sclerotia. Raja *et al.* (2016) recorded the maximum number of sclerotia in isolate Gujarat (125.33) followed by Karnataka (75.67) and the minimum no. of sclerotia was observed in Pant Nagar (6.33) in India. Sanjay *et al.* (2019) calculated the sclerotial number by counting number of sclerotia per five mm culture disc under binocular microscope 7 DAI. They used three colony discs to count sclerotial number and divided into more (< 9.0), moderate (5.1–9.0), less (5.0) and absent categories. The number of sclerotia produced was in the range of 3.7–10.7 per 5 mm mycelium disc. According to the scale for number of sclerotia, 27.6% isolates were clustered in group-IV. Thirty four and 45 isolates were clustered in group-III and II respectively. Two isolates were unable to form sclerotia on PDA media were included in group-I. Five isolates produced maximum number (10.7) of sclerotia. They also recorded the sclerotia formation on under the surface of lid for their presence or absence at 10 DAI and found forty-seven (41.9%) isolates showed sclerotia formation on under surface of lid, while it was absent in 65 isolates (58.9%). These findings are also in consonance

with the observations of Mishra *et al.,* (2014). According to Meyer (1965), sclerotia may be absent in some *R. solani* isolates under certain cultural conditions therefore the absence of sclerotia does not mean that it is not a mycelium from *R. solani.*



CV (%)- 15

Figure 8: Number of sclerotia of different isolates of *R. solani* at 7 DAI.

4.5.5. Topography

The location of sclerotia was observed on the basis where actually the sclerotia were formed in the fungal colony. Based on the location of sclerotia formation, these isolates were categorized into three groups- immersed, superficial, superficial and immersed. Majority of isolates formed superficial sclerotia (RS-2, RS-3, RS-4, RS-7, RS-8), isolate RS-1 formed immersed sclerotia, isolates (RS-5, RS-6) formed both superficial and immersed sclerotia on the PDA medium ((Plate 10: B, C, D, G, H, A, E, F). Similar attributes of sclerotial topography were also described by Moni *et al.* (2016) and Ali (2002) as 0, immersed; 1, superficial; 2, superficial/ immersed. Based on the location of

sclerotia formation, Singh *et al*. (2015) categorized the isolates into three groups. First and second group included sclerotium formation within the aerial mycelium and at the surface of the mycelium respectively. Third group had sclerotia embedded in the fungal mycelium itself. Singh *et al*. (2002) reported the location of sclerotia as aerial, surface and embedded. Moni *et al.* (2016) found all the isolates was produced superficial sclerotia on potato dextrose agar plates and Hashiba and Kobayashi (1996) noticed that surface sclerotia were fast growers than embedded sclerotia on the PDA medium.

4.5.6. Color of sclerotia

All eight isolates of *Rhizoctonia Solani* showed great variation in the colour of sclerotium. Dark brown colored sclerotia has been observed in RS-5 and RS-6 isolates while isolate RS-2, formed yellowish brown (Plate 10: E, F, B). Isolates RS-1, RS-3, RS-4 produced olive brown or pale brown color sclerotia and remaining isolates RS-7 and RS-8 formed black color sclerotia (Plate 10: A, C, D, G, H). Lakshaman and Amaradasa (2016) and Raja *et al*. (2016) grouped sclerotial colour into two: dark brown and light brown. Moni *et al*. (2016) described the sclerotial colour dark brown in most of the isolates. Based on the pigmentation of the sclerotium, Sing *et al.* (2015) also assigned isolates into four groups *i.e.* dark brown color which included eight isolates, dark yellowish brown and olive brown color both included six isolates and light brown color which included three isolates. One isolate did not bear any sclerotia. Hoa (1994) observed the sclerotial colour ranged from brown, light or dark brown, black brown, chocolate brown, salmon and dark salmon. Kim *et al.* (2001) informed that the dark brown colored sclerotia forming isolates were found more virulent compared to yellowish or olive brown colored sclerotia forming isolates, i.e. melanin production also determines the pathogenicity factors of *R. solani* which indicated that melanin producing cultures i.e. brown coloured (M+ type) are more virulent than non melanin producing cultures (M-type). The only character that clearly distinguished the sclerotia is the colour as reported by (Anderson, 1982).

4.5.7. Time for first sclerotium formation

Time of initiation of sclerotia formation was varied between 4 to 6 days. Based on the time taken for the initiation of sclerotial formation, the isolates were divided in 3 groups i.e. (I) fast (4 days), (II) moderately fast (5–6 days) and (III) slow (> 6 days) grower. In the present study, RS-3 initiated sclerotia in PDA media at 4 days hence categorized as fast grower (group I). Isolates RS-1, RS-2, RS-4, RS-5, RS-6 took 5 days while solates RS-7, RS-8 took 6 days for sclerotia initiation and so considered as moderately fast grower (group II). Sanjay *et al.* (2019) recorded average time for first sclerotium formation varied between 4 to 6 days for most of the isolates at 26 ± 2 °C. Based on the time taken for the initiation of sclerotial formation, they also divided 128 isolates in same 3 groups i.e. (I) fast (< 4 days), (II) moderately fast (4–5 days) and (III) slow (> 5 days). Twenty-six (23.2%), 48 (42.8%) and 36 (32.0%) isolates were clustered in group-I, II and III respectively. Pralhad *et al.* (2019) found 4 days and 8 days for initiation of sclerotia in two test isolates RS3 and RS4 respectively. Singh *et al*. (2015) found the time ranged from 3 to 6 days. Lal *et al.* (2014) had mentioned in their result that it ranged from 3 to 5 days and reported that four isolates took 3 day, 17 isolates took 4 day and 3 isolates took 5 day for initiation of sclerotia formation. Meena *et al.* (2001) found a great diversity for time taken for initiation of sclerotia formation. They recorded the time ranged from 3-11 day. These results are also in agreement with (Debbarma and Dutta, 2015; Jayaprakashvel and Mathivanan, 2012). Susheela and Reddy (2013) informed that this variation in number of sclerotia formation by the *R. solani* may be due to more intra specific variability among the sub groups.

4.5.8. Clump formation

After 10 day of inoculation, sclerotial clump formation was noticed in distinct variation, viz; For isolate RS-1, RS-5 and RS-6 clump was formed in the circular at centre. Clump was formed in peripheral region for isolate RS-2, RS-3 and scattered clump formation was seen for RS-4 isolate. No clump was formed in isolate RS-7 and RS-8 (Plate 10: A, E, F, B, C, D, G, H). According

to the scale for number of clump, clump formation was divided into four different groups- (I) more (>16), (II) moderate (11–16), (III) less (1–10) and (IV) absent (0) clump groups. The number of clumps varied in the range of 9–34 among the eight isolates. More clump was formed for isolate RS-1. Maximum number of sclerotial clumps were recorded in RS-1 (34) followed by RS-5 (15) and RS-6 (13). Minimum number of clump (9) was in isolate RS-3. Isolate RS-2, RS-4, RS-5 and RS-6 had moderate amount of clump. Isolate RS-3 was fall in group III which showed less number of clump formation. Clump formation was absent for isolate RS-7 and RS-8 and belong to category IV. Sanjay *et al.* (2019) found the number of clumps varied in the range of 7.9–21.7 among all the 112 isolates. According to the scale for number of sclerotia, a total of 29 isolates did not show any sclerotial clump, less amount of clump was observed for nine isolates. Thirty six isolates were clustered in group-I (more). Similarly, in group-II, 36 isolates showed moderate level of sclerotial clump formation.

4.6. Molecular confirmation of isolates

The current study was aimed at studying morphological and pathological variations in fungus associated with sheath blight complex in rice, and identifying them through amplification of ITS 5.8S-rDNA region. Molecular confirmation was conducted by amplifying rDNA-ITS region by using ITS1/ITS4 pair. This primer pair was first used by White *et al.* (1990) for amplification of ITS region of rDNA of the fungal isolates. Nuclear ribosomal internal transcribed spacer (ITS) is a universal DNA barcode marker for fungi. ITS region of nuclear DNA (rDNA) has the most sequenced region to identify fungal taxonomy at species level and even within species. It has a specific ITS region for distinctive fungi. Several studies were done earlier on rice sheath blight using molecular markers such as RAPD (Singh *et al*., 2015; Lal *et al*., 2014; Guleria *et al*., 2007;), RFLP (Linde *et al*., 2005), AFLP (Taheri *et al*., 2007) and ISSR (Goswami *et al*., 2017; Yugander *et al*., 2015 and Guleria *et al*., 2007) along with morphological markers. Recently, rDNA-ITS sequencing has been used for identifying variations of *Rhizoctonia solani* by (Ramos-Molina and Chavarro-Mesa , 2016; Bintang *et al.,* 2017 and Singh *et al.,* 2018).

In the present study, Among the test isolates, six isolates (RS-1, RS-3, RS-4, RS-5, RS-6 and RS-8) were showed consequently a single band of approximately 700bp size, while two isolates RS-2 and RS-7 did not show any band. As the tested six isolates amplified the expected region (around 700 or 700-800bp) of ITS, so we confirmed the isolates as *Rhizoctonia solani*. Remaining two isolates did not show any band so we assumed that it may be for the absence or poor concentration of DNA during fungal DNA extraction. Though this two isolates were identified as *R. solani* by morphological and pathological characters, so further study is required to confirm this two isolates as *Rhizoctonia solani* or other *Rhizoctonia* species.

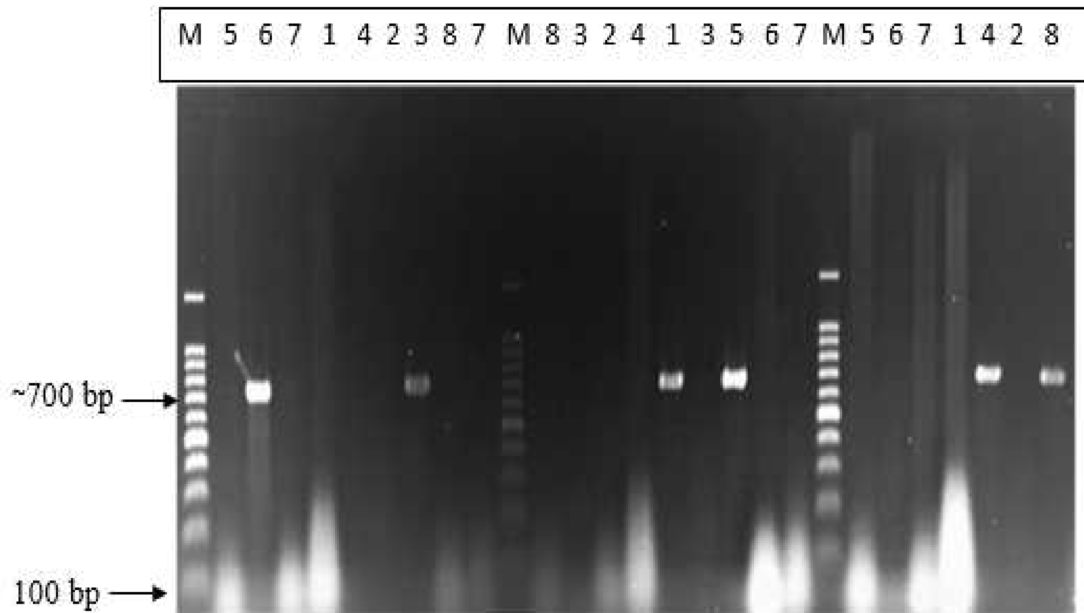


Figure 9: Gel picture of PCR amplified fungal DNA from *Rhizoctonia solani* isolates using the primer pairs ITS1/ITS4. (Lane: M= 100bp DNA ladder, 1= RS-1, 2= RS-2, 3= RS-3, 4= RS-4, 5= RS-5, 6= RS-6, 7= RS-7, 8= RS-8).

Agarose gel picture showing PCR amplified products 700bp (Figure 9). The band was found in the same size of around 700 bp in the ITS region. The approach did not reliably differentiate the variation among the stated six isolates. Apparently, no variation was found in molecularly among the isolates. The present findings have the similarity with Suryawanshi *et al.* (2019). They found almost the same band size of the present study. The amplification of rDNA-ITS region showed band of approximately 700bp for two isolates viz, RS3 (Accession number MK213723) showed band at 723 bp where RS4 (Accession number MK213724) showed band at 713 bp. Isolate RS3 and RS4 have the similarity with *Rhizoctonia solani* and *Rhizoctonia solani* AG1-IA repectively. The isolate RS4 showed 99% homology with *R. solani* AG1-IA based on nucleotide sequence data for ITS 5.8S-rDNA region. Nagaraj *et al.* (2019) characterized sixty-five isolates of *R. solani* by PCR- RFLP with the selected enzymes. RFLP of rDNA-ITS amplified product was used to determine the AGs belonging to subgroup IA. The rDNA-ITS region for 65 isolates was amplified

using primers ITS1 and ITS4. The amplicon length ranged from 475-550 bp among the isolates. Most of the isolates belong to AG1-IA and 40 isolates contributed to 61 per cent of the total isolates characterized. However, no genetic difference in the restriction pattern of ITS region was observed among AG1-IA isolates even though they have been collected from rice and weeds. Sandoval and Cumagun (2019) detected twenty-three multinucleate *Rhizoctonia* (MNR) isolates as *R. solani* AG1-IA using specific primers. They found a region of the 28S ribosomal DNA unit which was amplified for *R. solani* subgroup AG1-IA and produced amplicons of approximately 265 bp. Matsumoto (2002) conducted a trial of direct detection and identification of *Rhizoctonia solani* AG1 subgroups using specifically primed PCR analysis by primers ITS5 and ITS4. PCR analysis showed that AG1-IA primers sets were able to detect from the same AG isolates and could escape detection from different AG isolates at a high level of frequency and band size for AG1-IA, was~265bp. Mahendra *et. al*. (2016) also identified thirteen isolates by using ITS primers and these were able to differentiate AG1-IA from IB. Based on amplification with specific primers and band size the isolates were grouped under AG1-IA (~265bp and ~140bp) and AG1-IB (~300bp). Based on the band size (~265bp), among thirteen isolates total nine isolates KMR6, KMR7, KMR9, KMM3, KMM4, KMM6, KMM7, KMM8 and KMM9 were grouped under AG1-IA.

4.7. Screening of Germplasm against sheath blight

To identify the prominent sources of resistance to sheath blight, thirty five entries along with one susceptible check BR11 were screened against sheath blight of rice under artificial inoculation. The results presented in Table 5 which revealed that among all the tested genotypes none were found immune. However, Three genotypes var23, var24 and var29 were found resistant and other three var2, var20 and var25 was found moderately resistant. Three genotypes viz., var1, var19, and var28 were found moderately susceptible. Whereas, seven genotypes *viz.*, var3, var4, var7, var11, var16, var17, var35 were found susceptible and rest 19 genotypes *viz.*, (var5, var6 var8, var9, var10, var12, var13, var14, var15, var18, var21, var22, var26, var27, var30, var31, var32, var33, var34) were found highly susceptible to *R. solani*. The relative lesion height (RLH) recorded on different germlasm by *R. solani* is presented in Table 6. The intensity of the disease varied depending on the germplasm (Appendix-1). Among thirty five screened genotypes, highest relative lesion height (97%) was recorded in susceptible check (BR-11), which was followed by local genotypes var15 (95.33%) and var27 (85%). Whereas, the lowest relative lesion height (15%) was recorded in genotype var24 which was followed by genotype var29 (19.67%). Screening of paddy genotypes against sheath blight was also carried out earlier by different co-workers Dubey *et al.*, 2014 and Shiobara *et al.,* 2013 by artificial inoculation of sclerotial bodies in leaf sheath.

Table. 5 Screening of Germplasm for sheath blight under artificial inoculation condition

Sl. No.	Material Code No.	Relative Lesion Height (%)	Disese Index (0-9 Scale)	Disease Reaction
1	var1	34.33 no	5	MS
2	var2	29.67 op	3	MR
3	var3	48.33 m	7	S
4	var4	50.00 m	7	S
5	var5	70.00 hij	9	HS
6	var6	85.00 cd	9	HS
7	var7	48.33 m	7	S
8	var8	88.67 bc	9	HS
9	var9	82.67 cdef	9	HS
10	var10	66.67 ijk	9	HS
11	var11	61.33 kl	7	S
12	var12	97.00 a	9	HS
13	var13	71.67 hi	9	HS
14	var14	82.33 cdef	9	HS
15	var15	95.33 ab	9	HS
16	var16	62.67 jkl	7	S
17	var17	46.67 m	7	S
18	var18	66.67 ijk	9	HS
19	var19	38.33 n	5	MS
20	var20	22.33 pq	3	MR
21	var21	83.33 cde	9	HS
22	var22	76.67 efgh	9	HS

Table 5 (cont'd)

Sl.No.	Material Code No.	Relative Lesion Height (%)	Disease Index (0-9 Scale)	Disease Reaction
23	var23	20.33 q	1	R
24	var24	15.00 q	1	R
25	var25	29.33 op	3	MR
26	var26	73.00 ghi	9	HS
27	var27	85.00 cd	9	HS
28	var28	34.67 no	5	MS
29	Var29	19.67 q	1	R
30	var30	70.00 hij	9	HS
31	var31	71.67 hi	9	HS
32	var32	75.00 fgh	9	HS
33	var33	80.00 defg	9	HS
34	var34	83.33 cde	9	HS
35	var35	58.33 l	7	S
BR11 (Susceptible check)		93.33 ab	9	HS
CV(%)		8.2		

*MR= Moderately Resistant, MS=Moderately Susceptible, S= Susceptible, HS=Highly Susceptible.

Disease reaction scale followed the Standard Evaluation System of IRRI (2002)

Table 6. Grouping of Germplasm based on their reactions against sheath blight under field condition

Scale	Relative Lesion Height	Disease Reaction	Germplasms code	No. of Germplm percei
0	0	Immune	Nil	0
1	1-20	Resistant	var23, var24, var29	3
3	21-30	Moderately Resistant	var2, var20, var25	3
5	31-45	Moderately Susceptible	var1, var19, , var28	3
7	46-65	Susceptible	var3, var4, var7, var11, var16, var17, var35	7
9	66-100	Highly Susceptible	var5, var6 var8, var9, var10, var12, var13,var14,var15, var18, var21, var22, var26, var27,var30, var31, var32, var33, var34	19

Scale: SES, IRRI 2002.

In the present study, 35 germplasm lines were screened under field conditions after inoculation with virulent isolate of *R. solani* (RS-5). None of the entries were found immune. Three entries were found resistant, one was moderately resistant, five were moderately susceptible, seven were susceptible and rest of the entries showed highly susceptible reaction. The present result also support the earlier report of Lalita *et al*. (2018), Adhipathi *et al*. (2013), Jia *et al*. (2012) and Zhang *et al.* (2006), in which there is no immune cultivars had screened against sheath blight till date and informed none of the high yielding varieties is resistant to ShB disease neither in Bangladesh nor elsewhere in the world. However, a number of genotypes with varying levels of resistance have been reported. However, the rice variety Tetep shows moderate resistance which is

reported several times by (Yugander *et al*., 2015; Jia *et al*., 2012 and Zhang *et al.,* 2006). Parveen *et al.* (2018) also revealed local cultivar, Orgoja (acc. no. 5310) as resistant and Gopal ghosh as moderately tolerant to ShB disease with the lowest RLH (8.33%) and (27.33%) respectively. Moreover, Kala binni, Khazur chari, Binni, Kalagora, Patjait and Dorkumur were found moderately tolerant to ShB. The information generated in the present study is in accordance with different co-workers that some varieties with relatively high levels of resistance to this pathogen have been identified, viz Teqing (Pinson *et al.,* 2005) Jasmine 85 (Zou *et al.,* 2000), Minghui63 (Han *et al*.,2002), Xiangzaoxian 19 (Che *et al.,* 2003), WSS2 (Sato *et al.,* 2004), Pecos (Sharma *et al.,* 2009) and Tetep (Sha and Zhu 1989 and Channamallikarjuna *et al.,* 2010). Again, Dey (2014) informed of some of the land races such as Buhjan, Banshpata, Bhasamanik, Nagra Sail, Raghu Sail are tolerant to rice ShB. Reddy *et al*., (1997) also found two lines (RNR 15336 and RNR 82096) as resistant. Singh and Borah (2000) also screened sixty local upland rice cultivars in Assam and only one variety i.e. Chingdar was found to be resistant, seven moderately resistant and rest 52 were susceptible. Zuo *et al.* (2009) mentioned that the resistance levels of Zhongbaiyou1 and Teyou 338 are as high as YSBR1, a rice line that has been identified with high resistance to sheath blight. However, genotype or entries screened here are new entries, so no specific information was available related to these genotypes against sheath blight of rice. The information generated in the present study is valuable in future rice breeding programmes aimed at improving resistance to sheath blight.

SUMMARY AND CONCLUSION

Rice sheath blight has emerged in higher incidence in North-Western region of Bangladesh. In Boro 2018-19 season, sheath blight (incidence: 32.5%, severity: 5.4) was predominant compared to brown spot, bacterial blight and blast disease in the northern and central part of the country. Considering the seriousness of the disease the experiment was conducted for morphological characterization, molecular identification through sequencing of ITS rDNA region and to study pathogenic variation among *Rhizoctonia solani* isolates.

Eight isolates were isolated from seven different areas of Bangladesh. Pathogen was identified on PDA media based on characteristic mycelial branching. Microphotographs were taken to show the typical microscopic characters of hyphae. All the eight isolates showed the characteristics mycelia branching at an acute or 90° angle with slight constriction at the point of branching and septum formed near the branching point.

All the isolates showed wide morphological variation in terms of colony characters, size of sclerotia, colour and texture of sclerotia. Among the 8 isolates, seven had fast mycelial development while one (RS-6) had slow mycelial growth. All the isolates had growth rates between 5.00-5.87cm² per day with an average of 5.33cm²/day. Isolate RS-1 had low growth rate (5.00 cm²/day) and RS-3 had high mycelial growth rate of 5.87 cm² per day. Based on the colony pigmentation, all the isolates were assigned into 4 groups: dark brown, dirty white, milky white and yellowish brown. The number of sclerotia per petridish was counted for all the isolates after 7 day after inoculation. According to the scale for number of sclerotia, the quality of sclerotia number for isolates RS-1, RS-2, RS-4, RS-5 and RS-6 was found excellent. Moderate and poor amount sclerotia was noticed for RS-8 and RS-7 isolate respectively. Distribution of the sclerotia was recorded as Circular Concentrated Circle (CCC) for RS-1, Wide Sparsely (WS) for RS-2, Circular Concentrated Sparse (CCS) for RS-3, Concentrated at the edges and lots (CEL) for RS-4 isolate, Same distribution type viz; Large Concentric Rings (LCR) was noticed for both RS-5 and RS-6; they are collected from Gazipur. Similarly, For RS-7 and RS-8 Concentrated at the

edges and rare (CER) type distribution was found. Time of initiation of sclerotia formation was varied between 4 to 6 days. In the present study, isolates RS-1, RS-2, RS-4, RS-5, RS-6 took 5 days and RS-3 took 4 days for sclerotia initiation while RS-7 and RS-8 initiated sclerotia in PDA media at 6 days. Dark brown colored sclerotia has been observed in RS-5 and RS-6 isolates and RS-2 formed yellowish brown. Isolates RS-1, RS-3, RS-4 produced olive brown or pale brown color sclerotia and remaining isolates RS-7 and RS-8 formed black color sclerotia. Sclerotial diameter ranged from 1.62 mm to 4.38 mm. On PDA, smooth sclerotial bodies were formed in the aerial parts as well as surface of mycelium having central, peripheral and scattered position. Shape of the sclerotia is variable ranging from irregular with a smooth surface to globose with an irregular surface or as slightly raised areas radiating from the centre of a culture.

All isolates except RS-2 and RS-7 were confirmed as *Rhizoctonia solani* with the molecular identification using the primer pair ITS1/ITS4 and no variation was found among the isolates.

Thirty-five rice germplasm were collected from BRRI Genebank and screened using a virulent isolate against sheath blight (ShB) by artificial inoculation in field and in T. Aman 2019. Significant differences on relation to lesion height (RLH) among the germplasm were observed. It was revealed that three genotypes (var23, var24 and var29) were found resistant out of tested 35 genotypes.

The study gives us the basic idea about the existing variability of such organism and helps us in their grouping based on morphological variation. Further study with more isolates is required for better understanding of this fungal behavior. Due to the huge diversity and high morphological and biological similarities within the fungal kingdom, the taxonomic identification becomes cumbersome. Furthermore, molecular technique could be disclose their various morphological complexity. Apparently no genetic variation was found in molecularly but in morphologically we found many variation among the isolates.

This is the first time study of the genetic variability of the pathogen with ITS marker in Bangladesh. There will be required more ITS sequencing to clear understanding the genetic variability with the marker. Moreover, DNA extraction protocol have to be modified or improved by further research and trial to avoid having poor DNA concentration trouble in PCR programme. For isolate RS-2 and RS-7, more study and trials are required to get proper sequencing. As the local cultivars are believed to harbour a number of valuable genetic resources for crop improvement. These germplasm need to be exploited for getting resistant or moderately resistant or even better tolerant sources for ShB disease. These three resistant genotypes can be used in allelic analysis for confirming Quantitative Trait Locus (QTLs) or finding additional loci of shB resistance using recombinant inbred lines. The findings would also help pathologists for designing suitable management strategies such as pre-disease diagnosis and protective spray, deployment of resistance varieties, evaluation of chemical fungicides and biocontrol agents etc.

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Appendices

Appendix I

Germplasm Screening

ANOVA TABLE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
block	2	352	175.95	6.8908	0.001
Treatment	35	60845	1738.43	68.0812	0.000
Residuals	70	1787	25.53		

Appendix-2: Sample layout of germplasm screening in BRRI research field.

2m ↔ (plot width), 1m ↕ (plot length)

Replication		
R1	**R2**	**R3**
var7	var26	var15
var32	var17	var1
var13	var18	var2
var14	var9	var30
var35	var25	var34
var26	var11	var3
var17	var12	var33
var18	var19	var7
var9	var24	var32
var25	var5	var13
var11	var16	var14
var12	var20	var35
var19	var8	var26
var24	var31	var17
var5	var10	var18
var16	var28	var9
var20	var22	var25
var8	var23	var11
var31	var4	var12
var10	var6	var19
var28	var27	var24
var22	var21	var5
var23	var29	var16
var4	var15	var20
var6	var1	var8
var27	var2	var31
var21	var30	var10
var29	var34	var28
var15	var3	var22
var1	var33	var23
var2	var7	var4
var30	var32	var6
var34	var13	var27
var3	var14	var21
var33	var35	var29