VARIATION IN *MAGNAPORTHE ORYZAE* AND SCREENING RICE GERMPLASMS AGAINST RICE BLAST

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VARIATION IN *MAGNAPORTHE ORYZAE* AND SCREENING RICE GERMPLASMS AGAINST RICE BLAST

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This is to certify that thesis entitled, **"VARIATION IN *MAGNAPORTHE ORYZAE* AND SCREENING RICE GERMPLASMS AGAINST RICE BLAST"** submitted to the Faculty of AGRICULTURE, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) in PLANT PATHOLOGY, embodies the result of a piece of bona fide research work carried out by **Azmira Arefin, Registration No. 19-10039** 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.

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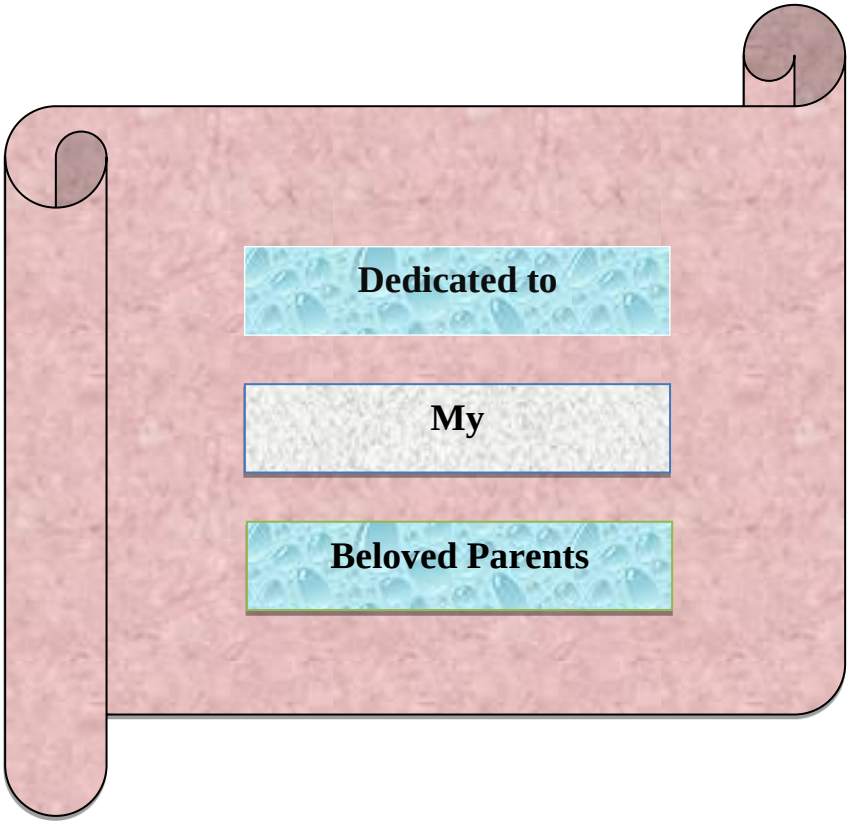
Dedicated to
My
Beloved Parents

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important crops and over half of the world's population consumes it as the staple energy source. Rice blast pathogen is widely distributed and highly destructive under favorable conditions caused by the fungus *Magnaporthe oryzae*, affecting global rice production. The blast pathogen affects different parts of a rice plant during pathogenesis. One of the serious forms of rice blast is neck blast but the leaf blast is well studied and the screening method for the same is precisely standardized. Blast resistance, tended to be unreliable with resistance often failing, or broken down, under field conditions, therefore there is always continuous search for resistant donors/lines. In Boro season 2020-2021, a survey on rice blast has been carried out in different locations of Cumilla and Brahmanbaria districts of Bangladesh. Blast incidence and severity ranged from 0 to 9 scale and 0% to 57%, respectively. A total of 6 isolates of *Magnaprothe oryzae* was isolated, identified and their cultural characterization was done. Radial mycelial growth of all isolates ranged from 7 mm to 72 mm in PDA, 10 mm to 66 mm in OMA and 10 mm to 90 mm in RfYA and 7.5 mm to 48.5 mm in PSA. A total of 18 rice germplasms were screened at UBN, BRRI, Gazipur in Robi season of 2020 to 2021 to determine the source of resistance in rice germplasm against *Magnaporthe oryzae*. Germplasms screening was done by following 0-9 Standard Evaluation Scale (SES) for leaf blast. Among 18 rice lines, only BRRI dhan32, BRRI dhan33 and BINA17 were shown resistance against rice blast under nursery condition upon artificial inoculation. These three varieties i.e. BRRI dhan32, BRRI dhan33 and BINA17 could be used for breeding purpose to develop high yielding blast resistant rice variety.

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CHAPTER-1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops and over half of the world's population consumes it as the staple energy source (Fones *et al*., 2020). It plays a vital role in the human diet and feeds more than 50% of the world's population (Rathna Priya *et al*., 2019). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Kole, 2006). Globally rice occupies an area of 163 m ha with a production of 719 MT of paddy (FAO, 2012). Rice is grown with the second highest worldwide production after maize (Boumas, 1985). Rice has a high social and economic importance, playing a major role in the world production of cereals, serving approximately three billion people around the world, with a total production of ca. 756.5 million tons of husked grains (Filippi *et al*., 2009, SOSBAI 2018) and global rice demand is estimated to reach8.52 × 108 t by 2035 (Khush 2013). By 2050, global demand for rice is projected to rise more than 40% to feed the rapidly growing world population (Milovanovic and Smutka 2017). Despite impressive global increases in production from 289 million tons in 1968 to 782 million tons in 2018, this quantum jump still has to keep pace with demand for rice from the rising population (FAOSTAT 2020). Rice grain contains on an average 7% protein, 62-65 % starch, 0.7% fat & 1.3% fiber and rice is a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) & B5 (pantothenic acid).

The challenge, however, is to continue maintaining the current surplus of rice in the coming decades in order to sustain rice security in the country. Before searching the way forward, it is needed to quantify the future rice demand and requirement, and address the resource utilization obligations. Rice is known to be attacked by many pests and diseases which cause huge annual losses worldwide. Among fungal diseases, rice blast caused by *Magnaporthe oryzae* is of significant economic importance. Out breaks of rice blast is a serious and recurrent problem in all rice growing regions of the world. It is estimated that 60 million people could feed each year by the produce of rice which is destroyed by rice blast (Zeigler *et al*., 1994).

Rice crop is subjected to attack by 50 diseases that include 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Hollier *et al*., 1993; Webster and Gunnell, 1992; Jabeen *et al*., 2012).

Rice blast pathogen is widely distributed and highly destructive under favorable conditions. Temperature, moisture, sunshine, humidity and wind speed play a major role in the infection and development of the disease. Blast disease is caused by the fungus *Magnaporthe oryzae* Chavara Teleomorph: *Magnaporthe oryzae* B. Couch] (Couch and Kohn, 2009). It causes disease in rice, wheat, rye, barley, pearl millet in which rice and wheat are economically more important. Blast is the most destructive fungal disease affecting global rice production. In temperate flooded and tropical upland rice ecosystems rice blast cause significant yield loses (Shahjahan, 1994). Incidence and severity of blast disease of rice was recorded in ten agro-ecological zones (AEZs) of Bangladesh during Boro (November to May; irrigated ecosystem) and Transplanted Aman (July to December; rain fed ecosystem) seasons. Disease incidence and severity was higher in irrigated ecosystem (Boro season) (21.19%) than in rain fed ecosystem (Transplanted Aman season) (11.98%) regardless of locations (AEZs). It was as high as 68.7% in Jhalak hybrid rice variety followed by high yielding rice cultivar BRRI dhan47 (58.2%), BRRI dhan29 (39.8%), BRRI dhan28 (20.3%) during Boro and in BRRI dhan34 (59.8%) during T. Aman season. Maximum yield loss was noted in AEZ9 for both the seasons. Most popularly adopted Boro rice was BRRI dhan28 (29.6%) followed by BRRI dhan29 (25.9%) and T. Aman rice was BRRI dhan34 (22.9%). The rice blast disease is caused by the fungi *Pyricularia oryzae* (renamed as *Magnaporthae oryzae*) and first documented in 1637 in China, then in Japan in 1704. In Italy, the USA and India the disease was also identified in 1828, 1876 and 1913, respectively.

The disease can be managed by the use of fungicides, resistant cultivars, agronomic practices and biotechnological methods (Ribot *et al*., 2008). The disease was under intensive investigation during the past four to five decades. Six international symposia organized by the International Rice Research Institute, LusBanos, the Philippines (Anonymous. I965. l979). Although fungicides can be used to control rice blast, they generate additional costs in rice production and chemical contamination of environment and foods.

Therefore, the use of resistant varieties (host plant resistance) is thought to be one of the most economically and environmentally efficient ways of crop protection.

However, the use of resistant cultivars is the most economical and environment friendly method for the management of rice blast (Castano *et al*., 1990; Saifullah *et al*., 1995; Khan *et al*., 2001; Haq *et al*., 2002) but the resistance is subject to break down due to appearance of new/more virulent races of the pathogen. The rapid genetic evolution of the fungus often overcomes the resistance conferred by major genes after a few years of intensive agricultural use. Therefore, screening germplasm requires continuous efforts of enriching the reservoir of resistance genes/alleles to effectively tackle the disease. The severity of biotic stresses in rice production is increasing at a startling pace of late because of rapid changes in climate (Jamaloddin *et al*., 2020). Changing climatic conditions are contributing to the emergence of new virulent races and the occurrence of diseases in new localities. Many diseases considered as minor thus far have become economically significant in many rice-cultivating areas and are exacerbating their impact (Anderson *et al*., 2004). Plant resistance genes (R genes) harbor tremendous allelic diversity, constituting a robust immune system effective against microbial pathogens. Nevertheless, few functional R genes have been identified for even the best- study pathology systems. There is thus a need to investigate and avail broad alternative spectrum, sustainable and environmentally friendly ways of managing the disease. The genetic nature of this fungus enables it to overcome the resistance offered by major R-genes (Roy Chowdhury *et al*., 2012; Vasudevan *et al*., 2014). The high existence of retro-transposons and repetitive segments make the fungus more virulent and change its pathogenicity very often (Dean *et al*., 2005).

As evidence to this, researchers have reported the breakdown of rice blast resistance and occurrence of high disease incidence with severe yield losses (Khush and Jena 2009; Sharma *et al*., 2012; Nalley *et al*., 2016),. This phenomenon is not for Bangladesh but for the other 85 countries where rice is grown. Rice has tremendous adaptation ability from Hokkaido to Honolulu (Biswas *et al*., 2017),. As a result, the pests of rice also sustain everywhere. Hence, rice blast is considered as a serious and recurrent problem in many of rice growing countries. The resurgence of rice blast in the form of neck blast is dominant this year.

Because there was rain during the flowering stage of BRRI dhan28, BRRI dhan50, BRRI dhan61 and BRRI dhan63 (the varieties are popular in those areas). None of these varieties is tolerant to the blast diseases.

Thus, disease pressure is in increasing trend and may be a devastating experience in the near future. Rice blast, caused by *Magnaporthe oryzae* with high pathogen plasticity and mutation rate considered as most damaging disease in rice.

Blast resistance, tended to be unreliable, with resistance often failing, or broken down, under field conditions. Therefore there is always continuous search for resistant donors/lines. The present study reports on the screening/evaluation of rice germplasm for sources of resistance against rice blast disease. The uniform blast nursery method, approved by International Rice Research Institute (IRRI) was used for screening of rice germplasm against *M. oryzae*. From the above-mentioned facts, this research work was under taken to fulfill the following objectives;

Objectives

1. To determine incidence and severity of rice blast in selected rice growing regions of Bangladesh.
2. To isolate, identify, pathogenicity study and morphological characterization of rice blast pathogen *Magnaporthe oryzae.*
3. To screen and rice genotypes against *Magnaporthe oryzae* causing rice blast disease.

CHAPTER-2

REVIEW OF LITERATURE

The available literature of work done on blast disease of rice and it's germplasm screening strategies have been reviewed in this chapter. The review of literature pertaining to this dissertation is presented in the following headings and sub-headings.

2.1. Importance of rice

Khan and Rashid (1986) reported that according to Food and Agricultural Organization rice is a staple food providing 2400 calories per day that is the least food safety required of a person per day. Unfortunately, this crop is more susceptible to several diseases, Rice is second only to wheat in terms of area harvested and is the most important grain in terms of calorie consumption.

According to FAO (1995) rice is the 1st economically important crop in India, China, East-Asia, South East Asia, Africa and Latin America catering to nutritional needs of 70% of the population in these countries.

Gowda *et al*., (2003) reported that rice (*Oryzae sativa*) belongs to the genus *oryzae* (Family: Poaceae) was domesticated probably in north-eastern India and southern China about 8000 years ago and is the staple food for more than 50% of the world's population.

Harriss-White, (2005) reported that rice is the primary staple in the diets of over 50% of the globe's population (Childs, 2012) with over two and a half billion mouths depending on the grain as their primary source of calories.

Asghar *et al.,* (2007) experimented that this yield is very low as compared to other developed countries of the world and this low production is attributed to several biotic and abiotic factors. Among the biotic factors disease is the most important factor which results in crop losses of $ 5 billion every year.

FAO (2009) reported that rice is found all over the world and in thousands of varieties; there are more than 8,000 varieties found in India alone where it all originated, and in one of the smaller rice-producing countries, the Philippines, there are about 3,500 varieties. Some of these well-known varieties include basmati (India), sushi rice (Japan) and jasmine rice (Thailand) each having a different consistency and flavor. Worldwide, rice is grown on 161 million hectares, with an annual production of about 678.7 million tons of paddy and about 90% of the world's rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia.

Khandakar *et al*., (2013) found that in Bangladesh, rice is the most staple cereal crop and central to Bangladesh's economy, accounting for nearly 20 percent of gross domestic product (GDP) and providing about one-sixth of the national income of Bangladesh.

BBS (2014) reported that combined efforts of farmers, rice scientists, extension personnel and Government of Bangladesh have enabled the country with a surplus of about 2 MT of rice in 2014-15. In the last few years (2009-10 to 2013-14), rice production has increased by 0.34 MT per year.

Salam *et al.,* (2014) found that Bangladesh agriculture involves food production for 163.65 million people from merely 8.75 million hectares of agricultural land.

BER (2015) reported that this has transformed the country from so called "Bottomless Basket" to a "Full of Food Basket". In recent years, the country has not only earned self-sufficiency in rice production, but also gradually entering into the export regime.

Commodity profile for rice (January, 2015) reported that rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. During 2012-13 and 2013-14, the world production has increased by 1% (from 472 Million Tonnes to 476 Million Tonnes), trade by 8% (from 38 Million Tonnes to 41 Million Tonnes) and consumption by 3% (from 469 Million Tonnes to 481 Million Tonnes).

LaFranchi (2015) observed that Bangladesh has emerged as a global model for combating hunger and obtained great success in becoming a country of food surplus from a country lagged with chronic food shortages.

Brolley (2015) stated that the challenge, however, is to continue maintaining the current surplus of rice in the coming decades in order to sustain rice security in the country. Sufficient rice production is the key to ensure food security in Bangladesh. In fact, 'Rice security' is synonymous to 'Food security' in Bangladesh as in many other rice growing countries.

AIS (2016) reported that more food will be required in future because of increasing population. Since independence, there has been a three-fold increase in rice production in Bangladesh, which jumped from nearly 11 MT in 1971-72 to about 34.86 MT in 2014-15.

2.2. Significance of blast disease of rice

Awodera and Esuruoso (1975) observed that heavy yield losses have been reported in many rice growing countries such as 75, 50 and 40 percent grain loss may occur in India (Padmanabhan, 1965), Philippines (Ou, 1985) and Nigeria.

Bonman *et al*., (1989) found that the symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting.

Seebold *et al.,* (2004) found that the fungus *Magnaporthe oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes.

Bedendo and Prabhu (2005) reported that the first records of blast occurrence date from the year 1600 and were found in China and Japan, where it was first described as "rice fever".

Prabhu and Filippi (2006) found that in Brazil, the first diagnosis of rice blast occurred in 1912 in São Paulo and in Rio Grande do Sul in 1918.

Garrett *et al*., (2006), and Milus *et al.,* (2009) found that the efficacy of resistant genes might decrease in crops due to change in weather pattern, ultimately it could result in evolution of highly virulent strains.

Katsantonis *et al.,* (2007) found that the disease appears early as white to grey/brown leaf spots or lesions, followed by nodal rot and as neck blast, which can cause necrosis and frequently breakage of the host panicles.

Wang and Valent (2009) reported that as rice production expanded through Asia, Latin America and Africa, the disease followed the expansion, and now occurs in more than 85 countries.

Zeng *et al.,* (2009) found that under favorable conditions, rice blast can be the most important rice disease in China, Japan and the USA, causing severe damage to rice yields.

Devi and Sharma (2010) reported that it has been estimated that M. *oryzae* destroys rice grain each year that would feed 60 million people.

Anonymous (2011) reported that the BRRI dhan29 and BRRI dhan28 are the most popular and mega varieties recognized highly susceptible to blast disease in Bangladesh.

Dean *et al.,* (2012) reported that the pathogen is scientifically important because it has been developed as a model system for the study of the plant pathogen interactions.

Vasudevan *et al*., (2014) reported that disease pressure, weather conditions and genome stability of the pathogen determine the virulence.

Wang *et al.,* (2015) stated that rice blast, caused by the fungus *Magnaporthe oryzae* Cavara [synonym *P*. *oryzae* Sacc, teleomorph *Magnaporthe grisea* (Hebert) Barr] has been identified as one of the major rice cultivation constraints worldwide.

Richa *et al.,* (2016) reported that rice blast, caused by *Magnaporthe oryzae* with high pathogen plasticity and mutation rate considered as most damaging disease in rice.

Yan *et al*., (2017) found that rice blast is one of the most damaging diseases affecting rice production worldwide, is caused by the non-obligate filamentous Ascomycota *Magnaporthe oryzae* (cyn. *Magnaporthe grisea*), (*Anamorph = Pyricularia grisea*).

Biswas *et al.,* (2017) reported that at present 267 races of rice blast have already been identified in our environment.

Agbowuro *et al.,* (2020) stated that the disease has been found in virtually all regions where rice is grown on a commercial scale. Losses are variable even 40 to 100% depending on the cultivar and environmental conditions.

2.3. *Magnaporthe oryzae* the causal organism of rice blast

Saccardo (1880) reported that the causal agent of rice blast has been referred to by different names over the years and its asexual phase was named *Pyricularia grisea.*

Cavara (1892) Named it *Magnaporthe oryzae.*

Hebert (1971) found that the perfect stage of *Pyricularia grisea* was earlier named as *Ceratosphaeria g*risea.

Yaegashi and Nishihara (1976) Later suggested the genus *Magnaporthe*.

Yaegashi and Udagawa (1978) finally proposed M. Sacc instead of *Ceratosphaeria grisea* Rahnema (1979), Longer duration of susceptive condition such as relative humidity and darkness increased conidia germination and appressoria formation.

Bonman *et al*., (1989) found that the symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting.

Hawksworth, (1990) Contributed on Commonwealth mycological institute (CMI) description about blast. The Cultures are greyish in color, conidiophores single, either simple or rarely branched and show sympodial growth. Conidia formed singly at the tip of the conidiophore, pyriform and narrowed toward tip which is hyaline to pale olive in color.

Mijan Hossain (2000) found that the mycelium was first hyaline then to olive in color. It usually 0.5 – 5µm, septation occurs and branched hypha.

Seebold *et al*., (2004) reported that the fungus *Magnaporthe oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes.

You *et al*., (2012) reported that the mycelium consists of septate, uninucleate, branched hyphae. However, as the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot darker on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in color, produced acrogenously, one after another.
Conidia is three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Conidia is rarely two celled or four celled. Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown.

Moreira (2015) and Zhang. (2016) stated that the recent studies point to *Magnaporthe oryzae*, both for the asexual and for the sexual phase, as the correct way to name the causative agent of rice blast, due to its pathogenicity and ecological and evolutionary traits.

Moreira *et al.,* (2015) found that its sexual phase (teleomorphic) is not observed naturally but can be performed by pairing compatible individuals in vitro.

Zhang *et* al., (2016) recommended that the synonym mentioned in publications such as "*Magnaporthe oryzae*".

2.4. Growth of *Magnaporthe oryzae* on different culture media

Riker and Riker (1936) isolate blast affected rice leaves and investigate mycelial growth and sporulation of *Magnaporthe oryzae* in different media such as Potato dextrose agar, Oat meal agar, Malt extract agar, Rice polish agar, White rice agar at different temperature levels (10°C, 15°C, 20°C, 25°C, 30°C, 35°C), on different carbon sources (Glucose, Dextrose, Maltose, Sucrose, Fructose), on different nitrogen sources (Potassium nitrate, Sodium nitrate, Ammonium nitrate, Calcium nitrate) and at different Hydrogen ion concentration (pH) levels (pH-5, pH-6, pH-7, pH-8, pH-9, pH-10).

Padmanabhan *et al*., (1970) isolate *Magnaporthe oryzae* from samples of diseased leaves, necks and nodes of the infected rice plant on oat meal agar with traces of biotin and thiamine. Cultures were purified by dilution method and single spore isolates were grown and multiplied on OMA + Biotin and Thiamine at 25°c.

Xia *et al*., (1993) collected the panicles with the symptoms of neck blast, single germinating conidium was isolated and transferred to potato dextrose agar and get pure culture of *Magnaporthe oryzae*.

Greer and Webster (1997) identified *Magnaporthe oryzae* by isolating the fungus from panicle neck, nodes and leaf collars of rice on potato dextrose agar.

Choi *et al*., (1999) was used spore drop mathod to identify *Magnaporthe oryzae* on OMA media. Each single spore colony was then picked and transferred onto oatmeal agar (OMA; 15 g of instant oatmeal and 7.5 g agar stick/500mL).

Uddin *et al.,* (2003) maintained the cultures of *Magnaporthe oryzae* on PDA during their study on detection of *Magnaporthe oryzae* causing leaf spot of perennial rye grass by Rapid Immuno-Recognition Assay.

Silva *et al.,* (2009) isolated *Magnaporthe oryzae* from monoconidial isolates which were obtained by directly transferring one conidium per lesion on 5% water agar from two to three lesions per leaf and the collected isolates were conserved on sterilized filter paper discs in a freezer at -20 ± 10c.

Priya Vanaraj *et al.,* (2013) isolated *Magnaporthe oryzae* from blast lesions that surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28±20c). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance.

Kariaga, Wakhungu J. And Hassan K. (2016) identified *Magnaporthe oryzae* from blast affected leaves using steeped tissues in glass plates containing filter paper plate and subsequently transferring a small sector of the growing mycelia to plates containing potato dextrose agar.

Atitaya Chumpol *et al.,* (2018) identified *Magnaporthe oryzae* from infected rice leaves which are cultured on rice polish agar (rice bran 20 g, yeast extract 2 g, agar 20 g, and distilled water 1000 ml) and incubated for 10 days under dark conditions at 25 ± 2 _C.

Kalpana Kulkarni and Dr. Swati Peshwe (2019) isolated *Magnaporthe oryzae* from blast lesions that treated with 1% mercuric chloride as per the protocol prescribed (Patel,1989) and then surface sterilized lessions were placed on potato dextrose agar medium.

FEI Li-wang *e al*. (2021) isolating a single fungal spore from rice blast diseased leaves by oatmeal tomato agar (OTA) solid medium.

Nazifa (2021) identified Magnaporthe oryzae by isolating blast affected leaf in Oat Meal Agar media which show high mycelial growth of the pathogen.

2.5. Screening of rice germplasm for resistance against rice blast disease

Castano *et al*., (1990) Saifullah *et al*., (1995) Khan *et al*., (2001) and Haq *et al*., (2002) screen rice germplasms to get resistant cultivars which is the most economical and environment friendly method for the management of rice blast.

Mohanta (2003) in his screening trials at Bangladesh, screened twenty-eight restored line and four standard checks to obtain resistance against blast.

Amanzadeh (2004) screened rice germplasms to know the prevalence of blast susceptibility among local cultivar and improved cultivar in nursery condition.

Mohapatra (2008) screened rice germplasms to know disease severities in the susceptible germplasms and slow-blasting germplasms to find new resistant variety againt M. *oryzae*.

Garrett *et al*., (2006) and Milus *et al*., (2009) try to decrease the effectiveness of existing resistance genes in crop varieties by promoting more aggressive races of pathogens by screening germplasm against rice blast caused by the fungus *Magnaporthe oryzae* which is one of the most devastating rice pathogens because it can infect the plant during nearly all growth stages.

Ribot *et al*., (2008) and Bhat *et al*., (2013) suggested screening so that the disease can be managed by the use of resistant cultivars.

Ghazanfar (2009) establish a disease screening nursery of rice germplasm consisting of course and fine varieties which was established during the kharif 2007 to determine the source of resistance in rice germplasm against *Magnaporthe oryzae.*

Pandey (2016) screened twelve cultivars of rice which were investigated in upland agricultural conditions in rewa, madhya pradesh in India during year 2011 and 2012 for quantification of apparent infection rate of leaf blast.

Yan (2017) screened a set of 32 germplasm by artificial inoculation with M. *oryzae*.

Atitaya Chumpol (2018) screened 256 indigenous upland rice plants for blast resistance under greenhouse and field conditions for obtaining new sources of rice blast resistance.

CHAPTER 3

MATERIALS AND METHODS

The various aspect of present investigation on blast disease of rice (*Oryzae sativa* L.) incited by *Magnaporthe oryzae* were conducted in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during 2020-2021. The materials used and methods or techniques adopted during the course of present investigations are describe in this chapter.

3.1 Experimental site

Survey was conducted in different villages of Cumilla, and Brahmanbaria district namely Noapara, Cumilla Sadar, Majhi Gacha, North Durgapur, Burichang Sadar, Champak Nagar, Nabinagar, Nawabpur, Chandina & Nasir Nagar, Ibrahimpur.

Isolation of *Magnaporthe oryzae*, determination of morphological characteristics, pure culture preparation and germplasm screening against *Magnaporthe oryzae* for improve resistance against blast disease conducted in the Plant Pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka -1207 and Uniform Blast Nursery (UBN), Bangladesh Rice Research Institute (BRRI), Gazipur 1701.

3.2. Experimental period

The experiment was conducted during the period from August 2020 to June2021.

3.3. Survey on incidence and severity of rice blast and collection of diseased samples

The survey on rice blast disease was conducted in farmers' fields of Bangladesh namely Cumilla and Rangpur during Boro (November to May; irrigated ecosystem) season. In this season survey was conducted during pre-flowering stage of the rice crop to observe leaf and node blast. Soil type cropping pattern and cropping intensity are taken into consideration in order to select locations.

Two plots of each location were selected having a size of at least 1500 square meter. In each location intensive rice areas under irrigated conditions were selected. For the survey of blast disease, a zigzag sampling pattern were followed in this study at every 50-steps interval single hill (consists of several tillers/plants were selected and recorded for disease incidence and severity. Disease incidence and severity was recorded during survey of farmers' field. Disease incidence of blast disease across all selected locations was recorded following. Disease incidence was conducted using the following formula:

Disease Incidence (%DI) =Total no. of infected plants/Total no. of plants X100

Assessment of the disease severity in the field from each unit plot were randomly selected and tagged for grading the severity of diseases. Disease incidence was recorded on the basis of the percent diseased panicle present in the field. Disease severity was recorded as 0 to 9 scale (Table 1) developed by International Rice Research Institute (IRRI, 2014)

Table 1. Scale for panicle blast disease based on symptom

Scale	Symptom on panicle
0	No visible lesion or observed lesions on only a few pedicels
1	Lesions on several pedicels or secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
5	Lesion partially around the base (node) or the uppermost internode or the lower part of panicle axis near the base
7	Lesion completely around panicle base or uppermost internode or panicle axis near base with more than 30% of filled grains
9	Lesion completely

The diseased leaves and panicle of rice showing typical symptom of rice blast were collected from surveyed area. Samples were sun dried and put into brown paper envelop and were brought to the laboratory for further studies.

3.4. Preparation of culture media used in this study

The isolates of *Magnaporthe oryzae oryzae* (MoO) was grown on PDA for 10 days at room temperature. From the margin of actively growing fungus, 5-mm discs were cut out. Sterile Petridishes containing Water Agar (WA), Potato Dextrose Agar (PDA), Rice flour Yeast Agar (RfYA) and Oat Meal Agar (OMA) were inoculated each with a single 5mm disc of the fungus and incubated at room temperature for 7 days. Three replications were maintained for each medium. The fungal growth was measured at 7 DAI. Further, the colony characters of the 24 isolates were grown on PDA and their colony morphology was observed. Water Agar (WA), Potato Dextrose Agar (PDA), Rice flour Yeast Agar (RfYA) and Oat Meal Agar (OMA) medium was used for isolation, purification of the causal organism from the infected rice blast samples.

3.4.1. Water agar (WA) medium preparation

Thirty gram of agar was put it in a 2-liter capacity flask. 1000 ml distilled water was mixed with agar in the flask. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi. (Hayashi *et al*., 2009).

3.4.2. Potato Dextrose Agar (PDA) medium preparation

200 gram of potato was boiled in 500 ml distilled water for one hour. After boiling, potato juice was filtrated through cheese cloth or nylon mesh. 20 g Dextrose and 20 gm Agar was poured into the potato juice and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 15 min at 15 psi (Okunowo, 2010).

3.4.3. Rice flour Yeast Agar (RfYA) medium preparation

15-gram Rice flour put in a 1-liter capacity flask, sieved and rice extract was made. Then 4-gram yeast extract with 20-gram agar was mixed in the flask. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi.

3.4.4. Oat Meal Agar (OMA) medium preparation

60 gram oat meal was put it in a 2-liter capacity flask. 12.5gram agar was mixed with 1000 ml distilled water was added into this. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi.

3.4.5. Potato sucrose agar (PSA) medium preparation

200 gram of potato was boiled in 500 ml distilled water for one hour. After boiling, potato juice was filtrated through cheese cloth or nylon mesh. 20 g Sucrose and 20 gm Agar was poured into the potato juice and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 15 min at 15 psi (Okunowo, 2010).

Fig. 1. Culture media for isolation of *Magnaporthe oryzae Oryzae*

3.5. Isolation and identification of *Magnaporthe oryzae*

Rice neck tissue, leaves and panicles with symptoms of rice blast were sampled throughout rice production field of Noapara, champaknagar and B.Baria at Cumilla sadar. Air dried panicles were placed in paper bags, returned to the laboratory prior for isolation.

Symptomatic rice necks or panicles from the collected crops were trimmed to approximately 5-7 cm and surface sterilize with 1% Sodium hypochlorite for two minutes. These cut pieces were then washed with sterilized water and incubated in 100 % relative humidity overnight to induce sporulation. Single germinating conidia were isolated under the microscope. Conidia of *M. oryzae* were removed from panicle nodes and panicles with a sterile loop and placed on water ager medium Petri dishes aseptically.

These WA plates were incubated at 25° C for 7 days for the isolation of causal agent. Five seeds/pieces of diseased plant parts were placed in each Petri dish. These Petri dishes were incubated at 25° C for 7days to induce sporulation of the fungi. After 7 days, small pieces of mycelia were cut from the edge of the culture and then transferred to new potato dextrose agar (PDA) and oat meal agar (OMA) petri dish for pure culture. Different fungal colonies were appeared, which were purified and multiplied on PDA and OMA. The identification of the pathogen was made by studying the colony characteristics of the isolates on the PDA plates by following the method described in a technical bulletin on seed borne disease and seed health testing of rice (Agrawal *et al*., 1989). The pathogenicity of isolates was confirmed by following Koch's postulates.

3.6. Purification and maintenance of pure culture

When pure growth of the fungus was achieved, 5 mm culture discs of the fungal mycelium were cut with the help of sterilized cork borer and transferred aseptically in potato dextrose agar, potato sucrose agar, rice flour yeast agar AND oat meal agar slants and allowed to grow. The pure culture slants were sealed with paraffin wax and stored at 5° C in a refrigerator for further use.

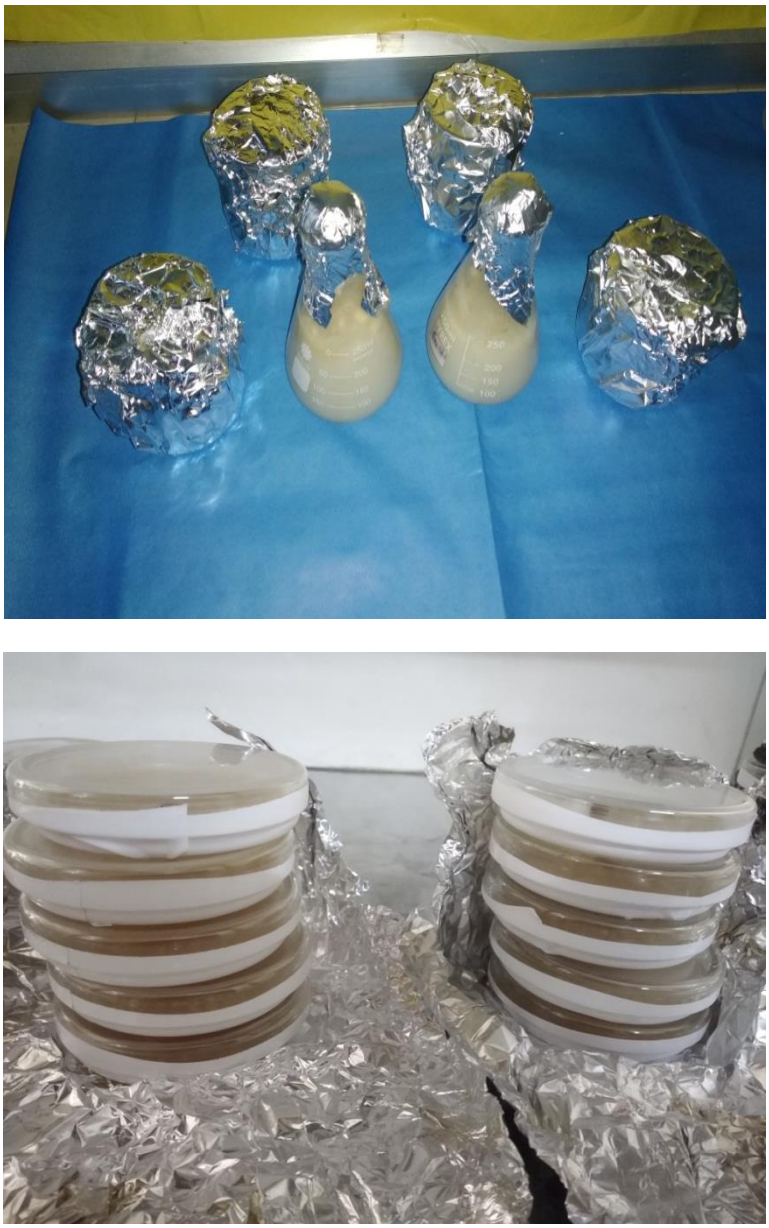

Fig. 2. Purification and maintenance of pure culture of MoO isolates

3.7. Identification of the causal organism

The pathogen isolated from the diseased specimen and established in pure form was identified on the basis of colony and morphological characters.

3.8. Sample seed collection

Thirty-two (32) rice germplasms consisting of traditional, hybrid and local rice lines or variety were collected from Rangpur and Chapainawabgonj were collected during the Robi season of 2020 to determine the source of resistance in rice germplasm against *Magnaporthe oryzae*, the cause of rice blast disease at UBN, BRRI, Gazipur (Fig. 3. and Table 2). The list of these rice lines/variety are given bellow:

Fig. 3. Rice seed samples (32 rice lines/variety) collected from different rice growing region of Bangladesh

Table 2. Different rice genotypes collected from Rangpur and Chapainawabgonj region of Bangladesh

SL No	Variety Name	Cultivated Area
1	Bashmoti (BD)	Tikrampur, Chapainawabganj
2	BINA-17	Kaunia, Rangpur
3	BINA-7	Shothibari, Rangpur
4	BR 11	Taragonj, Rangpur
5	BR 14	Rangpur Sadar
6	BR 16	Shothibari, Rangpur
7	BR 22	Taragonj, Rangpur
8	BRRI Dhan28	Taragonj, Rangpur
9	BRRI Dhan29	Taragonj, Rangpur
10	BRRI Dhan32	Pirgonj, Rangpur
11	BRRI Dhan33	Kaunia, Rangpur
12	BRRI Dhan34	Amnura, Chapainawabganj
13	BRRI Dhan36	Volahat, Chapainawabganj
14	BRRI Dhan47	Pirgonj, Rangpur
15	BRRI Dhan48	Rangpur Sadar
16	BRRI Dhan39	Kaunia, Rangpur
17	BRRI Dhan49	Shothibari, Rangpur
18	BRRI Dhan54	Volahat, Chapainawabganj
19	BRRI Dhan51	Mithapukur, Rangpur
20	BRRI Dhan52	Pirgonj, Rangpur
21	BRRI Dhan56	Chapainawabganj
22	BRRI Dhan57	Taragonj, Rangpur
23	BRRI Dhan58	Mithapukur, Rangpur
24	BRRI Dhan69	Mithapukur, Rangpur
25	BRRI Dhan81	Rangpur Sadar
26	BRRI Dhan82	Islampur, Chapainawabganj
27	Guti Sorna	Tanor, Chapainawabganj
28	Nerica	Taragonj, Rangpur
29	Summon Sorna	Dariyapur Chapainawabganj
30	Chini Atop	Bashmohol, Shibgonj, Chapainawabganj
31	Vadoi	Tiklichor, Chapainawabganj
32	Moajjemm	Tikrampur, Chapainawabganj

3.9. Inoculum preparation, inoculation and evaluation of tested line in diseased nursery (UBN)

Seeds of each of 32 test rice lines were placed on 32 petridish and water was sprayed over them with sprinkler. After two days sprouted seeds of each line were sown in a single row on a raised bed of the disease nursery (UBN) plot of BRRI. The length of each row sown was 100 cm and after every four test rows/lines there was a row of a susceptible spreader of US2. The rows were kept 10 cm apart. The disease nursery was also bordered around with two lines of susceptible US2. The Nursery was watered on 5th day of seed sowing and irrigation and weeding were done simultaneously. At four week stage, the test entries of the nursery were sprayed with already prepared inoculum of *M. oryzae* as described by Khan *et al*., (2001) by adjusting the spore suspension to 1×10^5 spores/ml. The nursery was continuously sprayed with tap water to maintain the humidity. Normal agronomic practices were followed and data of disease were recorded three weeks after spray inoculation by using disease rating scale of IRRI.,1996 (Table 3).

Table 3. 0-9 grade disease rating scale used for screening rice genotypes in blast nursery

Blast disease Grade	Disease severity	Host response
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions of 3mm or longer infecting 4-10% of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

3.10. Pathogenicity study of collected *M. oryzae* isolates

Pure culture of each isolates are grown on OMA for 30 days at 25°C under alternating 14 hour of fluorescent light and 10 hour dark cycle to induce sporulation. The conidial suspension was harvested, filtered and centrifuged at 5000rpm.The mass of spore sedimentation was collected, resuspended with sterilized distilled water and spore density was adjusted to a concentration of 1×10^5 spore/ml using hemocytometer. The conidial spore suspension was sprayed at 3-4 leaf stage on rice leaves collectively. Inoculated seedlings were kept in incubation chamber at 25°C. The sterile water was used instead of spore suspension served as control under controlled condition. Seedlings were evaluated on typical spindle shaped blast lesion after 10 days of inoculation.

3.11. Mycelial growth and morphological characterization

Mycelial growth of *Magnaporthe oryzae* on PDA, PSA, RfYA and OMA was recorded at 7 Days after Inoculation (DAI), 14 DAI and 21 DAI. Mycelia growth and growth rate were recorded and morphological characters like growth character, colony color, surface structure and colony shape were recorded.

3.12. Experimental design

Experiment was laid out in Completely Randomized Design (CRD) with four replications.

3.13. Statistical analysis of data

The data was analyzed by using the “R” Software (R Core Team, 2018). The mean value was compared according to LSD range test at 5% level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

Blast of rice, caused by *Magnaporthe oryzae* is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favorable conditions. Incidence and severity of blast disease is increasing especially in the Boro season. In recent years, in Bangladesh, frequency of blast occurrence has increased with invasion into new areas (north and northwest parts of the country). The most popular and mega varieties BRRI dhan29 and BRRI dhan28 are recognized highly susceptible to blast disease.

Occurrence the disease in both the seasons created interest to conduct this study. The present study was taken to initiate the work on survey, isolation, pure culture in screening germplasms resistant to rice blast caused by *Magnaporthe oryzae*. The results of the experiments conducted on these lines are presented in this chapter.

4.1. Survey on rice blast disease

In Boro season 2020-2021, a survey was done in different villages of Cumilla and Brahmanbaria district of Bangladesh (Fig. 6). The blast disease incidence and severity were recorded during the survey is shown in Table 4.

From the survey, it is exposed that the highest incidence of blast disease was recorded from Noapara (57%) with a severity score of 9. The lowest incidence of blast disease was recorded from Burichang and Champaknagar (10% and 9%, respectively) with a severity score 1 for each location. No disease was recorded on BRRI dhan89 in Uttor Durgapur. In Chandina BRRI dhan28 and BRRI dhan29 showed 50% and 40% blast incidence with severity score 7 for both varieties. Nasir Nagar and Ibrahimpur showed incidence 30% and 40% with severity score 5 and 7, respectively.

Table 4. Survey on blast disease in different villages of Cumilla and Brahmanbaria district of Bangladesh in Boro season 2020-2021

Name of district	Name of villages	Varieties	Blast disease	
			Incidence (%)	Severity Score
Cumilla	Noapara	BRRI dhan81	57%	9
	Cumilla Sadar	BRRI dhan29	51%	7
	Majhi Gacha	BRRI dhan50	40%	3
	North Durgapur	BRRI dhan89	0%	0
	Burichang Sadar	BRRI dhan74	10%	1
	Champak Nagar	BRRI dhan69	9%	1
	Nabinagar	BRRI dhan17	20%	5
	Nawabpur Chandina	BRRI dhan28	50%	7
Brahmanbaria	Nasir Nagar	BRRI dhan88	30%	5
	Ibrahimpur	BRRI dhan84	50%	7

Fig. 4. Field view of blast infected rice field in Chandina and Noapara (A and B); Field view of infected leaf and neck seen in Burichung and Ibrahimpur, Cumilla & Brahmanbaria region (C and D).

A significant variation in degree of disease susceptibilities were observed among different rice cultivars when scoring against M. *oryzae* infection scale. The prevalence of disease was more in highly susceptible local cultivars, than improved cultivars. These results are in agreement with Amanzadeh *et al*., (2004) who reported that local cultivars are susceptible, breeding cultivars were resistant and some cultivars were semi susceptible to leaf blast in nursery.

The disease incidence varied among collection field sites and varieties and the variation could be in weather condition, temperature, humidity, soil condition, soil management techniques etc. The fields management practices may also account for variation in disease incidence. A survey on rice blast was conducted in 5 districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla and among those Muktagachha, Mymensingh was found as the highest rice blast disease infected area and Bakerganj, Barishal was found as the lowest in Boro season 2017-2018 reported by Rayhanul *et al*., (2018). The report exposed that the highest incidence of blast disease was recorded from Muktagachha (60%) and severity score was 5. The highest severity score of blast disease was observed in Hossainpur, Kishoreganj (7) but percent incident was observed only 20%. From the survey of Nazifa *et al*., (2021), in Boro 2018-19 and Aman 2019, a survey was done in three northern districts of Bangladesh namely Gaibandha (Gobindogonj and Mohimagonj), Dinajpur (Birampur) and Bogura (Dupchanchia). The highest incidence of blast was recorded from Gobindogonj (84.26%) where severity score was 7. The highest severity score of blast was recorded in Mohimagonj that was 9 with 65% severity but the percent incidence was only 29.12%.

4.2. Isolation and pure culture of *Magnaporthe oryzae*

Collected infected leaf and panicle samples were cut in 5-7cm sections. These sections were surface sterilized by dipping in 1% Sodium hypochlorite for two minutes and were washed by sterilized water for several times and then the cut sections were placed on moist filter paper (Whatman: 9.0cm) in a sterile petridish (Figure 8). Plates were incubated for 24 hours at room temperature (250C). After 24 hours the infected parts were examined under stereo microscope (Motic SMZ-168). Conidial masses (Figure 6) were picked by using very fine tip needle and spread on 3% water agar plate for 3 days to induce sporulation of the fungi. After 3 days, a small pieces of mycelia were cut from the edge of the culture and then transferred to new potato dextrose agar (PDA) and oat meal agar (OMA) petridish for pure culture. Different fungal colonies were appeared, which were purified and multiplied on PDA and OMA.

Fig. 5 Infected neck segment of panicle in moist chamber

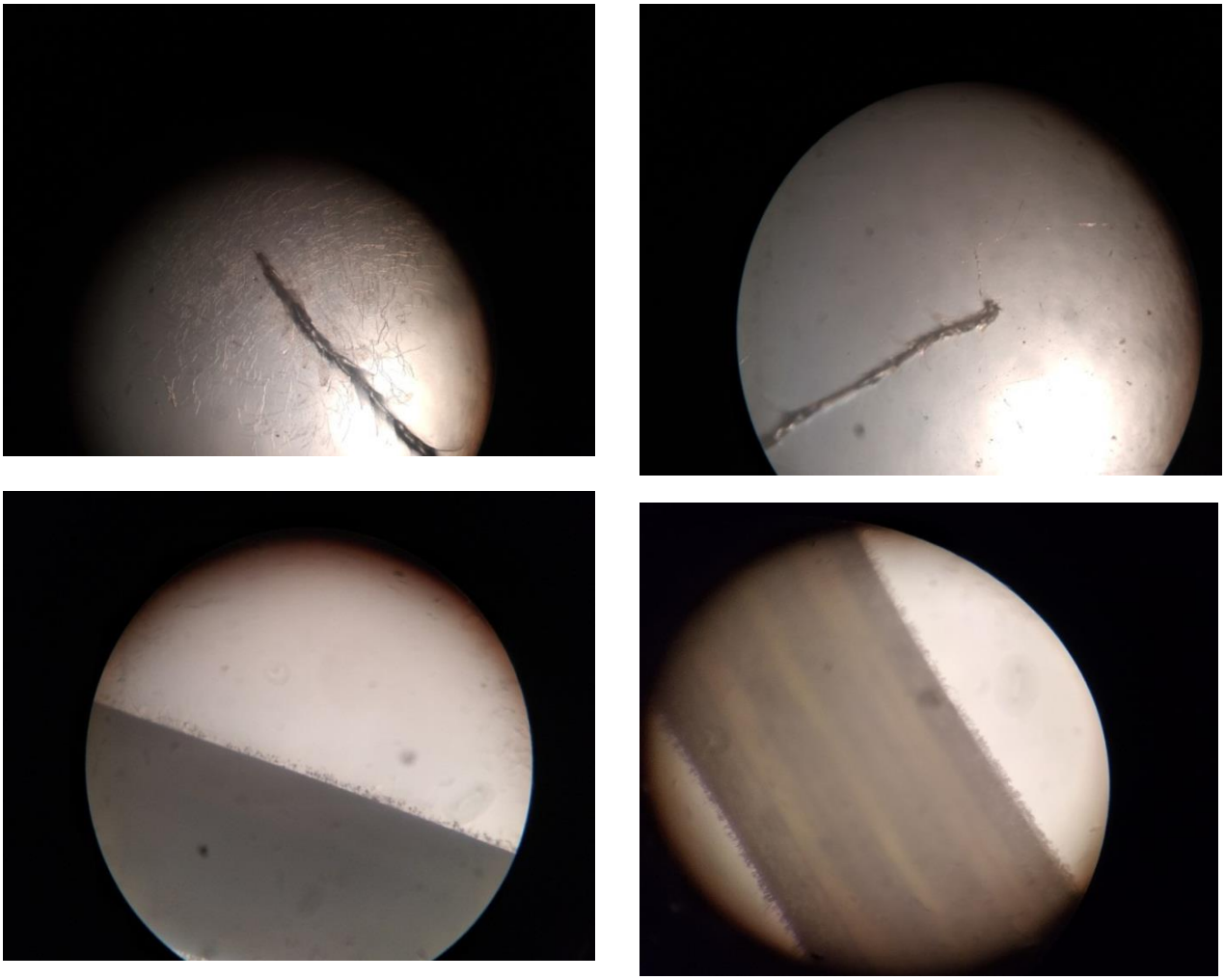

Fig. 6 Conidial mass seen under stereomicroscope (×50)

4.3. Confirmation of *Magnaporthe oryzae*

Conidial masses were picked by using very fine tip needle and placed in glass slide. Then the slide was observed under compound microscope with cover slip. Typical pyriform conidia was found. The conidia were found to show variations in septation, ranging from one to three septations and the majority of the conidia had three septations. Different solid media viz., potato dextrose agar,potato carrot agar, Kirchoff‟s, medium, Richard‟s medium, Sabourad‟s medium, Takahashii‟s medium, rice leaf extract agar and oat meal agar and liquid media viz., potato dextrose broth, potato carrot broth, Kirchoff‟s broth, Richard‟s broth, Sabourad‟s dextrose broth, Takahashii‟s broth and rice leaf extract broth was also used to culture rice blast pathogen (Akhilesh,2017). Among all the solid media the highest mean mycelial growth of the fungus *Magnaporthe oryzae* (Cav.) was recorded on oat meal agar (77.6 mm) followed by rice leaf extract (75.9 mm) and least mean mycelial growth of the *M. oryzae* (Cav.) on Sabourad‟s media (44.7 mm) followed by Takahashii‟s media (52.5 mm). They found the highest mean mycelia growth of the fungus *Magnaporthe oryzae* (Cav.) was recorded on oat meal agar.But in our study highest mycelial growth was found in RfYA media. The shape, color and compactness of the fungal colonies varied with the media and isolates.

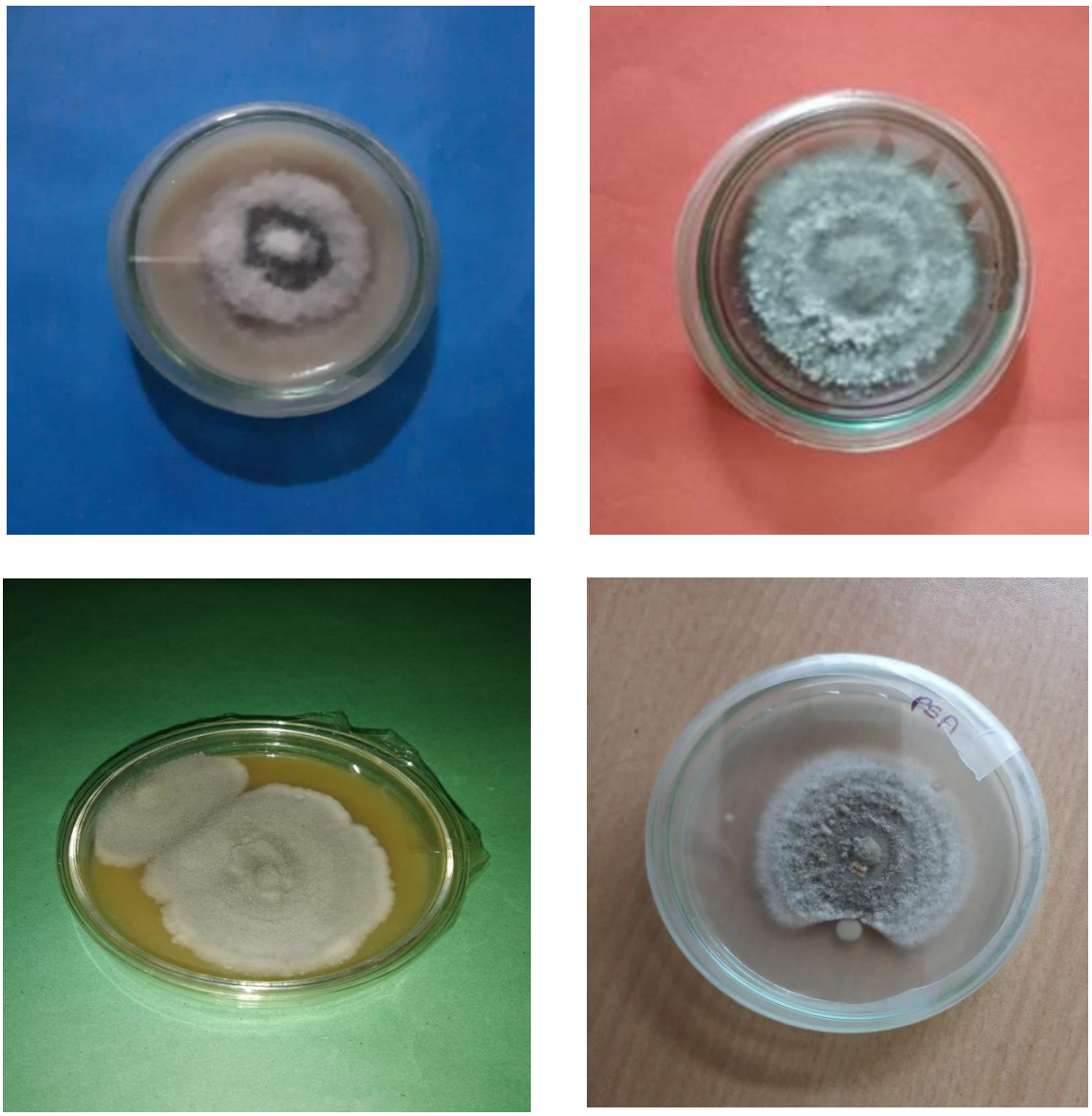

Fig. 7 Mycelial growth of four isolates of *Magnaporthe oryzae* on OMA, PDA, RfYA and PSA media.

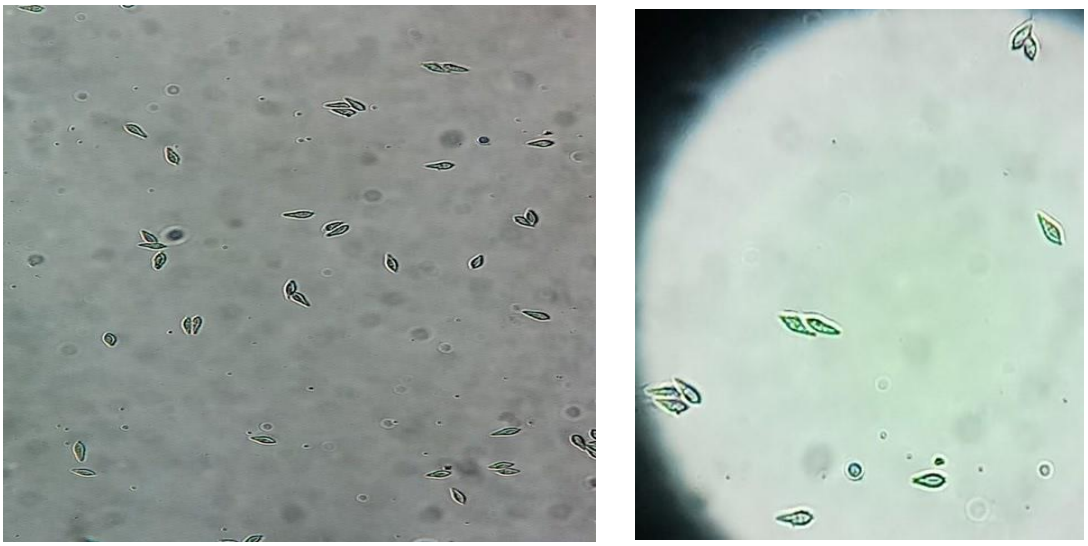

Fig. 8. Conidia of *Magnaporthe oryzae* on potato dextrose agar observed under compound microscope (X200)

Table 5. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 3 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 3 DAI	Colony Character	Surface Texture	Shape
MoO1	24	Blackish white	Rough cottony	Irregular
MoO2	11.5	White	Rough cottony	Irregular
MoO3	7	White	Rough cottony	Irregular
MoO4	20	White	Rough cottony	Irregular
MoO5	13.5	Grayish white	Smooth cottony	Regular
MoO6	8.5	Grayish white	Smooth velvety	Irregular

Table 6. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 7 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 7 DAI	Colony Character	Surface Texture	Shape
MoO1	36	Blackish white	Rough velvety	Regular
MoO2	27.5	White	Rough velvety	Irregular
MoO3	40.5	Blackish white	Rough cottony	Irregular
MoO4	29	White	Rough cottony	Irregular
MoO5	35	Grayish white	Rough velvety	Irregular
MoO6	52	Grayish white	Rough velvety	Irregular

Table 7. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	41	Blackish white	Rough velvety	Irregular
MoO2	72	Blackish white	Rough velvety	Irregular
MoO3	53	Blackish white	Rough velvety	Irregular
MoO4	48.5	Grayish white	Rough cottony	Regular
MoO5	66	Grayish white	Rough velvety	Irregular
MoO6	47	Blackish gray	Rough cottony	Irregular

In vitro mycelia growth at different treatment found significantly different. Six isolates of MoO were cultured on PDA and their mycelia growth and growth rate were recorded and morphological characters like growth character, color, surface structure and shape were observed. The highest growth was observed in MoO1 that was 24 mm at 3 DAI growth with white colony color and rough cottony, irregular surface structure. The lowest growth was observed in MoO3 that was 7 mm with grayish white colony color and smooth velvety, irregular surface structure.

At 7 DAI, the highest growth was observed in MoO6 that was 52 mm with Grayish white colony color and rough velvety, irregular surface structure. The lowest growth was observed in MoO2 that was 27.5mm with white colony color and rough velvety, irregular surface structure.

At 14 DAI, the highest growth was observed in MoO2 that was 72 mm with blackish white colony color and rough velvety, irregular surface structure. The lowest growth was observed in MoO1 that was 41 mm with blackish white colony color and rough velvety, irregular surface structure.

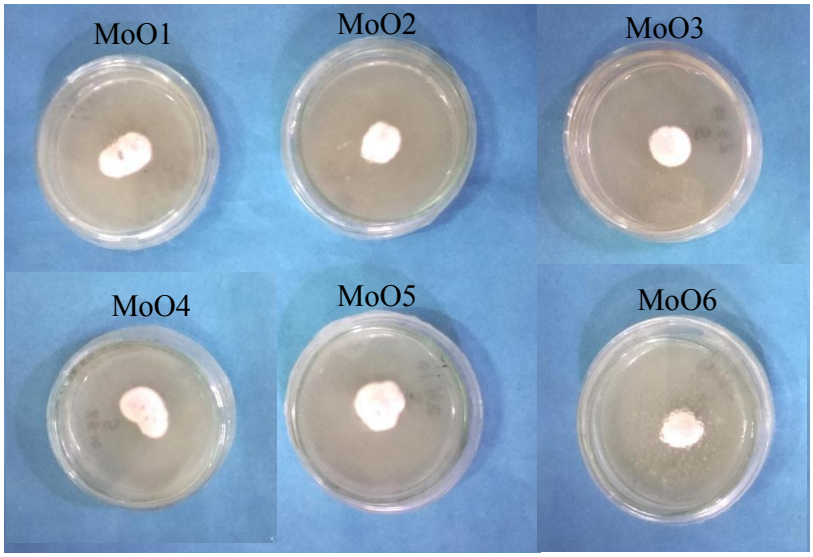

Fig. 9. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 3 DAI.

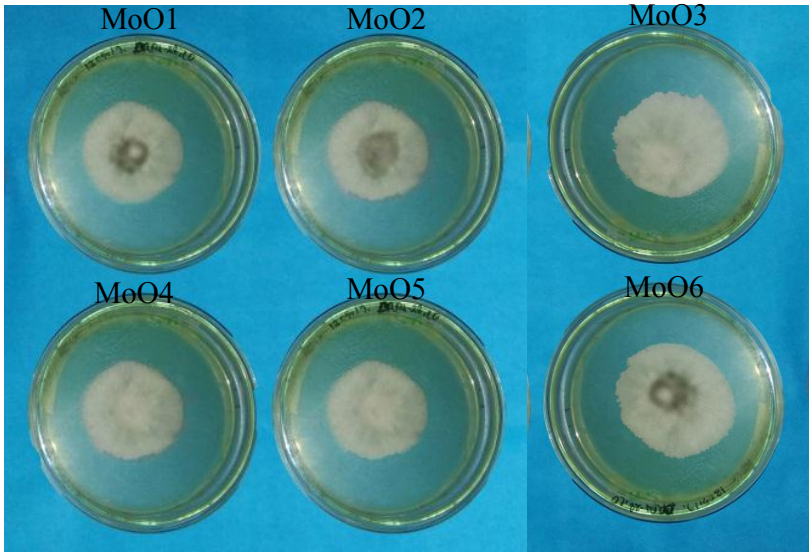

Fig. 10. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 7 DAI.

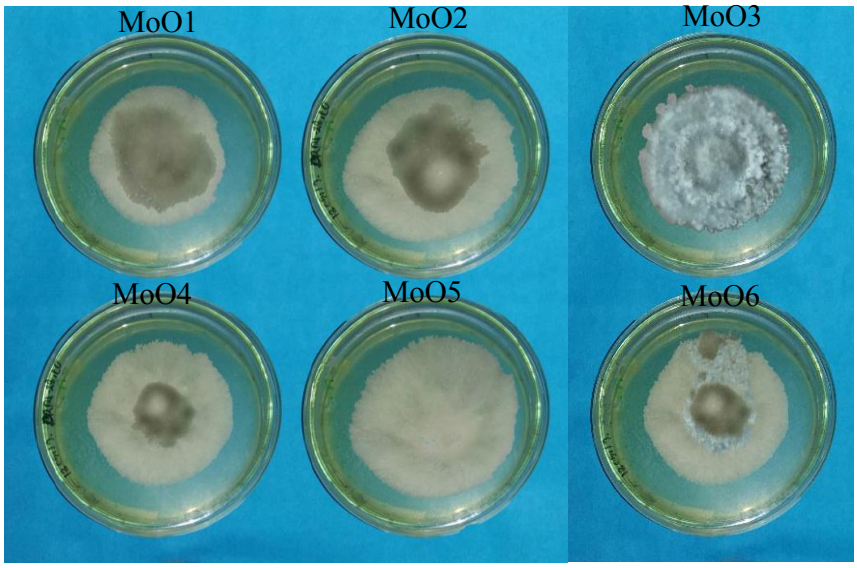

Fig. 11. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 14 DAI.

Table 8. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 3 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 3 DAI	Colony Character	Surface Texture	Shape
MoO1	22	White	Rough velvety	Regular
MoO2	10	Pinkish white	Rough velvety	Regular
MoO3	26	White	Rough velvety	Regular
MoO4	15.5	Blackish white	Rough velvety	Regular
MoO5	18	Grayish white	Rough cottony	Irregular
MoO6	30.5	White	Rough velvety	Regular

Table 9. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 7 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 7 DAI	Colony Character	Surface Texture	Shape
MoO1	60	Grayish white	Rough velvety	Regular
MoO2	41	Pinkish white	Rough velvety	Regular
MoO3	46.5	Grayish white	Rough velvety	Regular
MoO4	33	Grayish black	Rough velvety	Regular
MoO5	31.5	Grayish white	Rough cottony	Irregular
MoO6	48	Pinkish white	Rough velvety	Regular

Table 10. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	32.5	Grayish white	Rough velvety	Regular
MoO2	40	Blackish white	Rough velvety	Regular
MoO3	51.5	Grayish white	Rough velvety	Irregular
MoO4	44	Grayish black	Rough velvety	Regular
MoO5	66	Grayish black	Rough cottony	Regular
MoO6	60	Whitish Gray	Rough velvety	Regular

In vitro mycelia growth at different treatment found significantly different. Another six isolates of MoO were cultured on OMA and their mycelia growth and growth rate were recorded and morphological characters like growth character, color, surface structure and shape were observed. The highest growth was observed in MoO6 that was 30.5 mm at 3 DAI growth with white colony color and rough velvety, regular surface structure. The lowest growth was observed in MoO2 that was 10 mm with grayish white colony color and rough cottony, irregular surface structure.

At 7 DAI, the highest growth was observed in MoO1 that was 60 mm with grayish white colony color and rough velvety, regular surface structure. The lowest growth was observed in MoO5 that was 31.5 mm with grayish white colony color and rough cottony, irregular surface structure.

At 14 DAI, the highest growth was observed in MoO5 that was 66 mm with grayish white colony color and rough velvety, regular surface structure. The lowest growth was observed in MoO1 that was 32.5mm with grayish black colony color and rough velvety, regular surface structure.

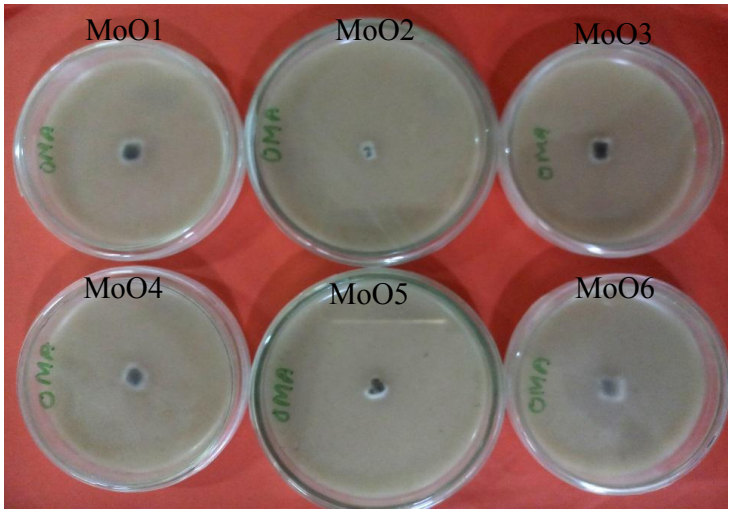

Fig. 12. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 3 DAI.

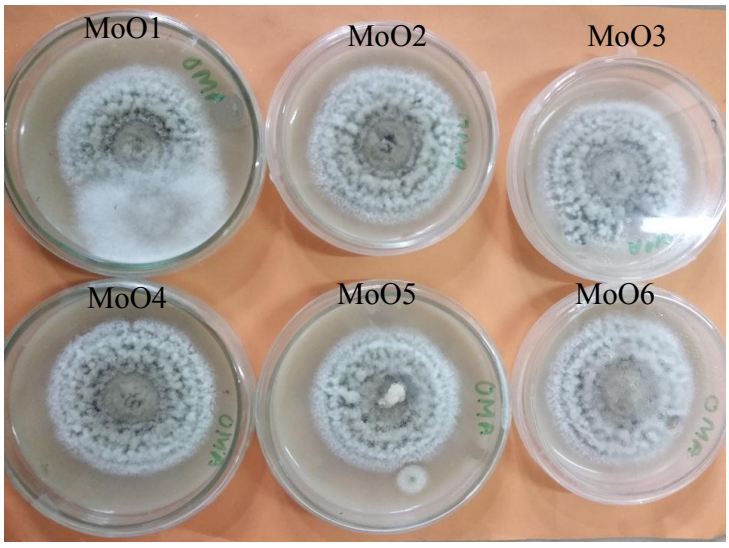

Fig. 13. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 7 DAI.

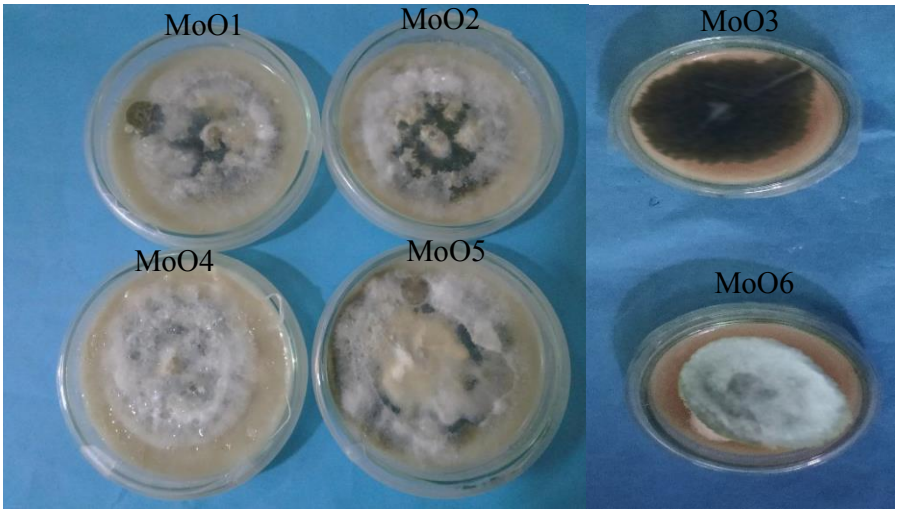

Fig. 14. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 14 DAI.

Table 11. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 3 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 3 DAI	Colony Character	Surface Texture	Shape
MoO1	24	White	Rough cottony	Irregular
MoO2	10	Whitish black	Rough cottony	Irregular
MoO3	26	Grayish white	Rough cottony	Regular
MoO4	20	Blackish white	Rough cottony	Irregular
MoO5	31.5	Blackish gray	Rough velvety	Irregular
MoO6	18.5	Grayish black	Rough velvety	Irregular

Table 12. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 7 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 7 DAI	Colony Character	Surface Texture	Shape
MoO1	60	Blackish white	Rough cottony	Irregular
MoO2	30	White	Rough cottony	Irregular
MoO3	52	Blackish white	Rough cottony	Irregular
MoO4	57	White	Rough cottony	Regular
MoO5	39.5	White	Rough cottony	Regular
MoO6	53	Grayish white	Rough velvety	Regular

Table 13. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	40	Blackish white	Rough cottony	Regular
MoO2	90	White	Rough velvety	Regular
MoO3	68.5	Blackish white	Rough cottony	Irregular
MoO4	49.5	Grayish white	Rough cottony	Irregular
MoO5	51	Blackish Gray	Rough cottony	Regular
MoO6	65.5	Blackish Gray	Rough cottony	Irregular

In vitro mycelia growth at different treatment found significantly different. Another six isolates of MoO were cultured on RfYA and their mycelia growth and growth rate were recorded and morphological characters like growth character, color, surface structure and shape were observed. The highest growth was observed in MoO5 that was 31.5 mm at 3 DAI growth with blackish gray colony color and rough velvety, irregular surface structure. The lowest growth was observed in MoO2 that was 10 mm with grayish black colony color and rough velvety, irregular surface structure.

At 7 DAI, the highest growth was observed in MoO1 that was 60 mm with blackish white colony color and rough cottony, irregular surface structure. The lowest growth was observed in MoO2 that was 30 mm with white colony color and rough cottony, regular surface structure.

At 14 DAI, the highest growth was observed in MoO2 that was 90 mm with grayish white colony color and rough cottony, irregular surface structure. The lowest growth was observed in MoO1 that was 40 mm with blackish white colony color and rough cottony, regular surface structure.

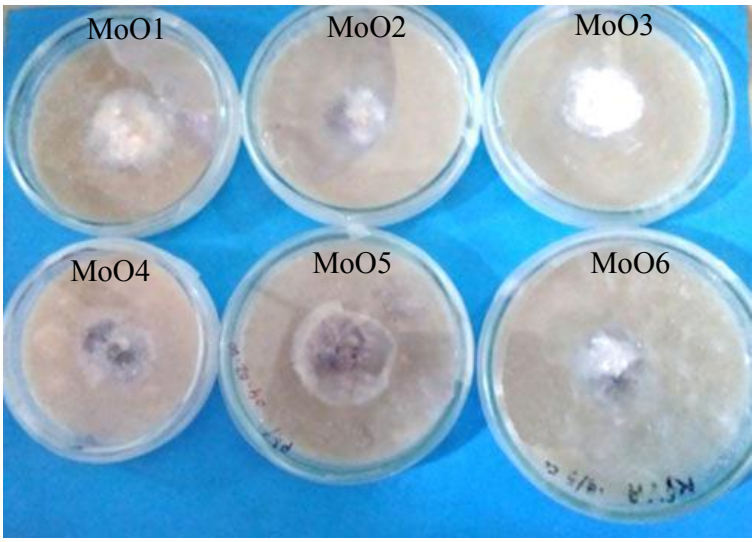

Fig. 15. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 3 DAI

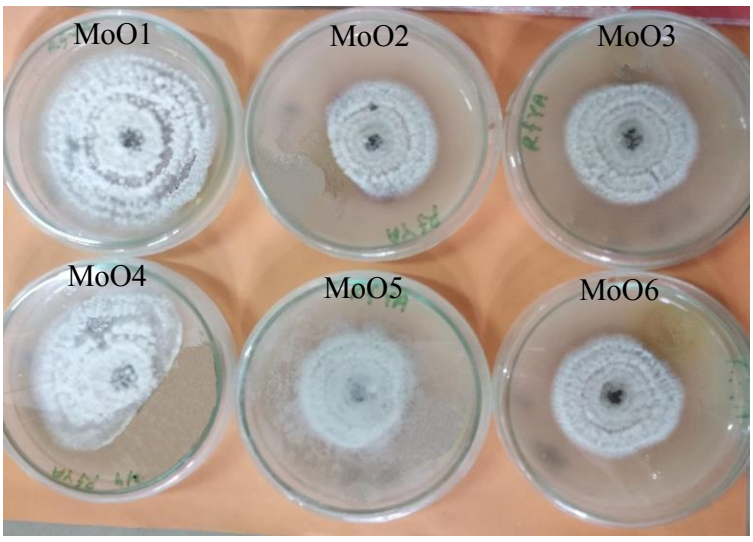

Fig. 16. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 7 DAI

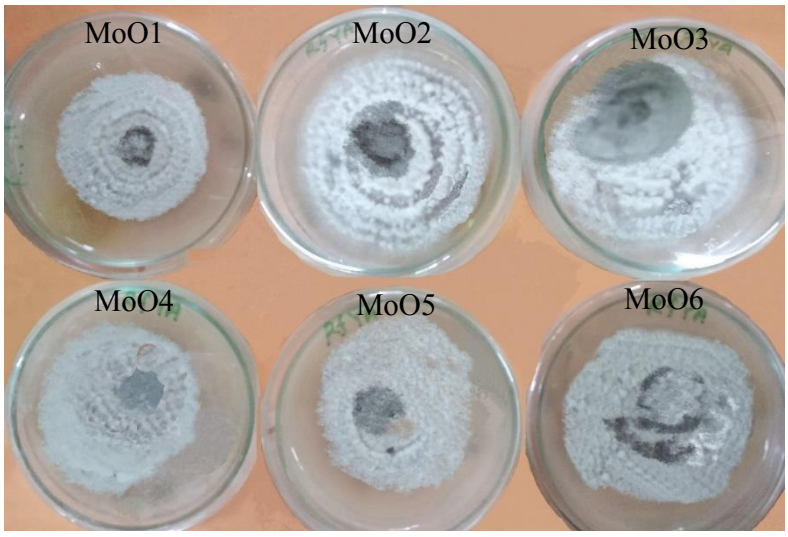

Fig. 17. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RfYA at 14 DAI.

Table 14. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 3 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 3 DAI	Colony Character	Surface Texture	Shape
MoO1	14	Blackish white	Rough cottony	Irregular
MoO2	9.5	White	Rough cottony	Irregular
MoO3	18	White	Rough cottony	Irregular
MoO4	7.5	White	Rough cottony	Irregular
MoO5	13.5	Grayish white	Smooth cottony	Regular
MoO6	10	Grayish white	Smooth velvety	Irregular

Table 15. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 7 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 7 DAI	Colony Character	Surface Texture	Shape
MoO1	19	Pinkish white	Smooth cottony	Irregular
MoO2	24.5	Grayish white	Rough velvety	Regular
MoO3	41	Pinkish gray	Rough cottony	Regular
MoO4	20.5	Grayish white	Rough velvety	Regular
MoO5	25	Grayish white	Smooth cottony	Irregular
MoO6	22	Pinkish white	Smooth cottony	Regular

Table 16. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	48.5	Blackish white	Rough velvety	Irregular
MoO2	37.5	Pinkish white	Rough cottony	Regular
MoO3	46.5	Whitish	Rough cottony	Irregular
MoO4	28.5	Grayish white	Smooth velvety	Regular
MoO5	43	Blackish white	Smooth velvety	Irregular
MoO6	23.5	Pinkish white	Smooth velvety	Irregular

In vitro mycelia growth at different treatment found significantly different. Another six isolates of MoO were cultured on PSA and their mycelia growth and growth rate were recorded and morphological characters like growth character, color, surface structure and shape were observed. The highest growth was observed in MoO3 that was 18 mm at 7 DAI growth with grayish white colony color and smooth cottony, regular surface structure. The lowest growth was observed in MoO4 that was 7.5 mm with white colony color and rough cottony, irregular surface structure.

At 7 DAI, the highest growth was observed in MoO2 that was 41 mm with Grayish white colony color and rough velvety, Regular surface structure. The lowest growth was observed in MoO1 that was 19 mm with grayish white colony color and rough velvety, regular surface structure.

At 14 DAI, the highest growth was observed in MoO1 that was 48.5 mm with blackish white colony color and rough velvety, irregular surface structure. The lowest growth was observed in MoO6 that was 23.5 mm with pinkish white colony color and smooth velvety, irregular surface structure.

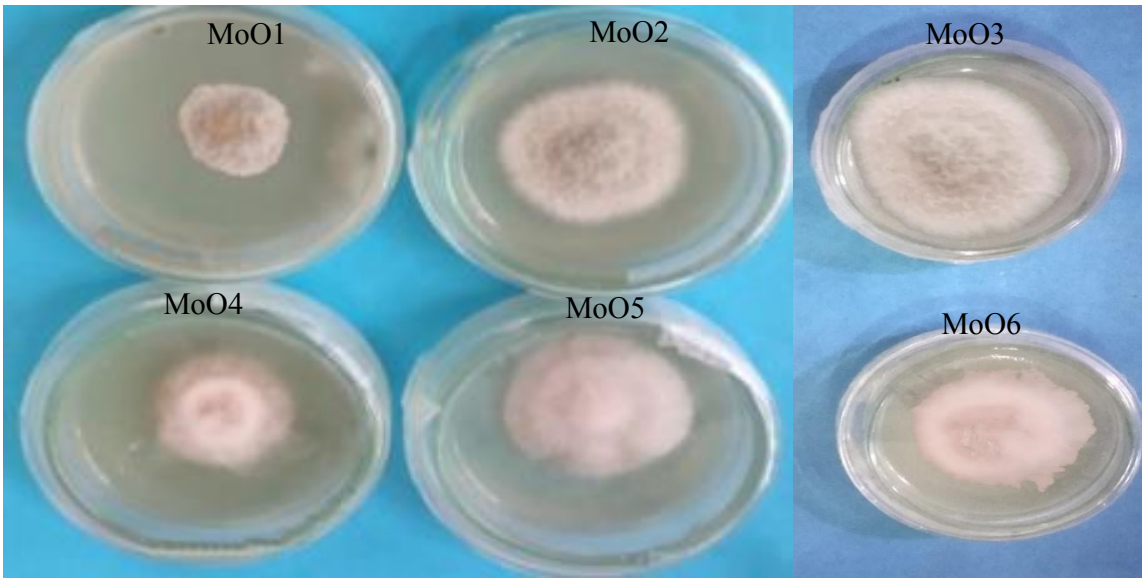

Fig. 18. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 7 DAI.

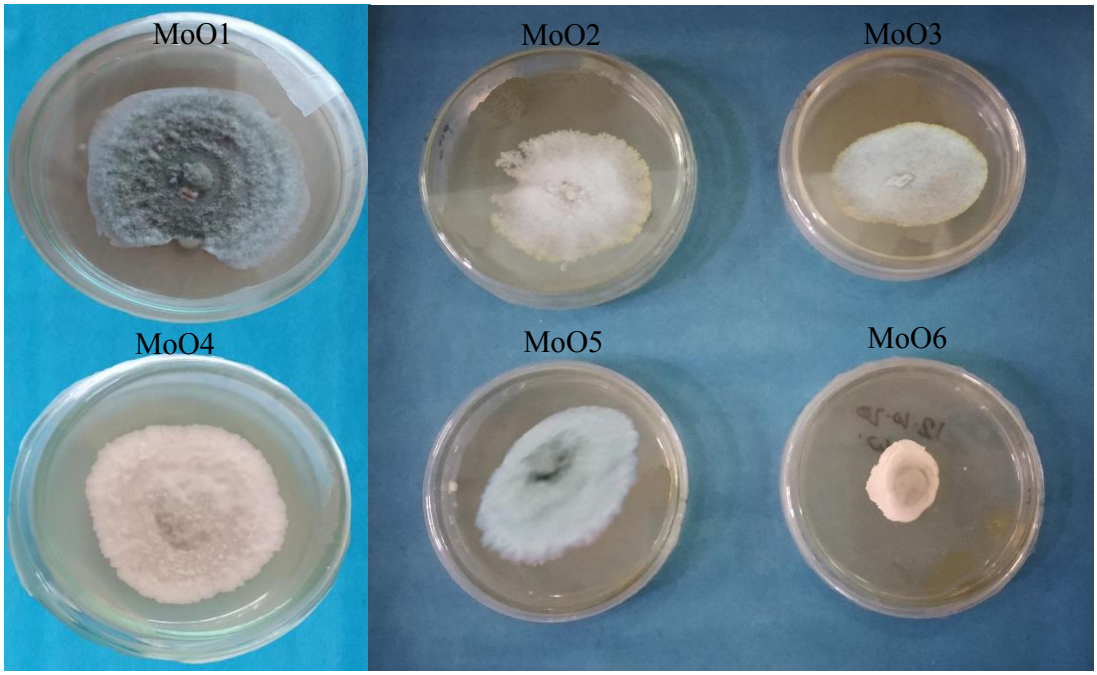

Fig. 19. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 14 DAI.

In this study, PDA, PSA, OMA and RfYA media were used for obtaining cultural growth of 6 *Magnaporthe oryzae* isolates, in which highest mycelial growth was found in RfYA media that was 90 mm and lowest mycelial growth was found in PDA media that 7 mm. The shape, color and compactness of the fungal colonies varied with the media and isolates. Similarly, Mijan Hossain (2000) observed that among the non-synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm).

Varsha Gayatonde (2016) used spore of 26 isolates of *M. Oryzae* in Rice Straw Agar, Potato Dextrose agar and Oat Meal Agar media where colonies of *M. oryzae* appeared white on oat meal, whitish grey on rice agar and grey on potato dextrose agar. Highest radial growth of mycelia on sixteenth day was shown on three medias from three rice varietal isolates Viz, on OMA (83.23 mm), on PDA (70.42 mm), on RSA (75.3 mm). Rayhanul *et al.,* (2019) used PDA media for culturing 4 isolates of *M. oryzae* where the highest mycelial growth (79.50 mm) was observed. In another study Manjunatha (2019) characterized the growth of *M. oryzae* isolates on different solid media *viz*., Host Extract Agar (HEA), Oat Meal Agar (OMA), Potato dextrose agar, Richard's Agar medium, among all the solid media the highest mean mycelial growth of the fungus was recorded on Host Extract Agar (4.08 cm) followed by Oat meal agar (3.83 cm) and least mean mycelial growth of the *M. oryzae* on Richard's agar media (3.21cm). Kalpana Kulkarni (2019) used 15 different natural and synthetic media to understand the nutritional requirements of the blast pathogen–naming few Oat meal agar, rice media, Soyabean caesin Dextrose medium, starch agar medium, YSS agar, rice and soyabean extract media and KKSP formulated medium etc which was composed for growth and sporulation of *M. oryzae* , in which excellent mycelial growth was found in OMA media. The present study corroborates with the study of Nazifa *et al.,* (2021) where they used five different media including Water Agar, Potato Dextrose Agar, Potato Sucrose Agar, Rice flour Yeast Agar and Oat Meal Agar (OMA) to culture 28 MoO in which highest mycelial growth was observed in Oat Meal Agar (20 mm) and lowest in Water Agar (10mm).

Table 17. Significance of mycelial growth on culture media (3 DAI)

Isolates	Radial Mycelial Growth			
	PDA	OMA	RfYA	PSA
MoO1	24 a	22 a-c	24 ab	14 ab
MoO2	11.5 bc	10 c	10 c	9.5 ab
MoO3	7 c	26 ab	26 ab	18 a
MoO4	20 ab	15.5 bc	20 a-c	7.5 b
MoO5	13.5 bc	18 a-c	31.5 a	13.5 ab
MoO6	8.5 c	30.5 a	18.5 bc	10 ab
Critical Value	10.220	14.813	12.153	9.9647

*LSD (0.05) = 2.179

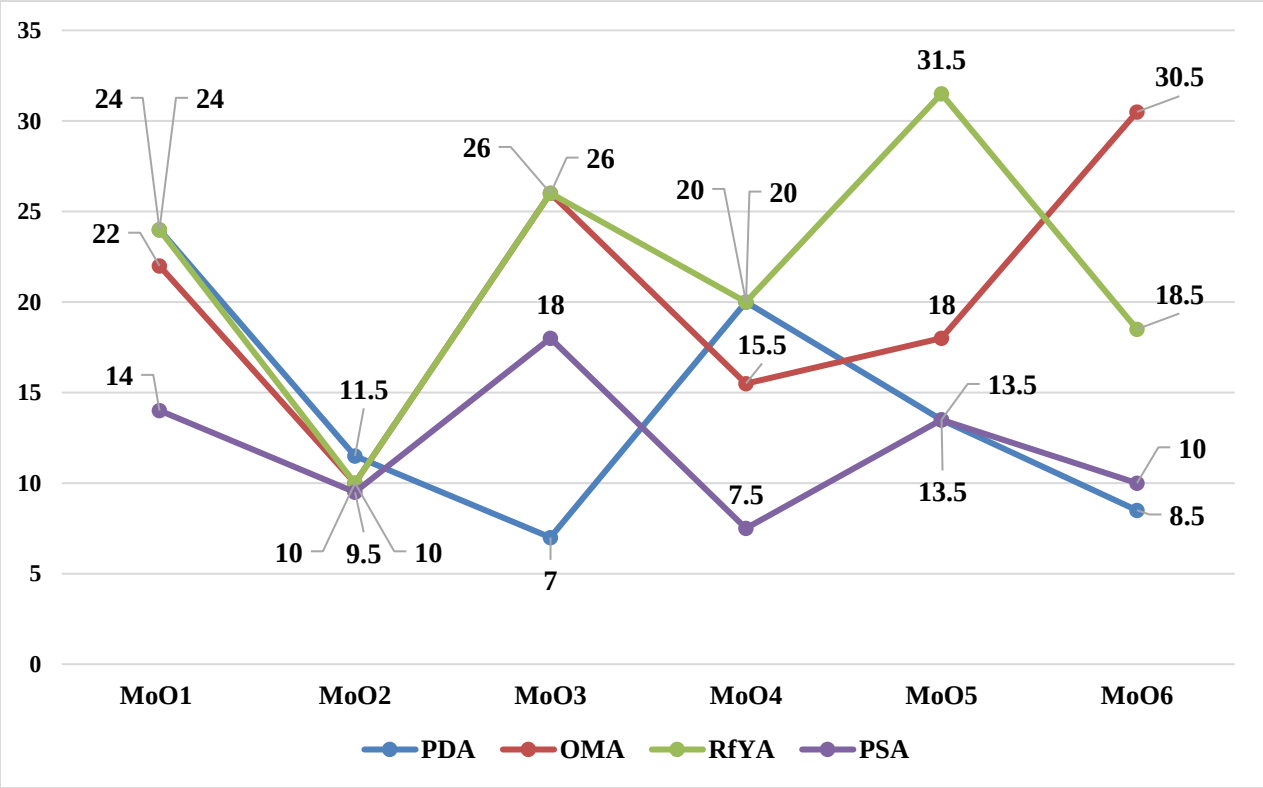

Fig. 20. mycelial growth on culture media (3 DAI)

Table 18. Significance of mycelial growth on culture media (7 DAI)

Isolates	Radial Mycelial Growth			
	PDA	OMA	RfYA	PSA
MoO1	36 ab	60 a	60 a	19 b
MoO2	27.5 b	41 ab	30 b	24.5 ab
MoO3	40.5 ab	46.5 ab	52 ab	41 a
MoO4	29 ab	33 b	57 ab	20.5 b
MoO5	35 ab	31.5 b	39.5 ab	25 ab
MoO6	52 a	48 ab	53 ab	22 b
Critical Value	23.879	24.821	29.205	17.431

*LSD (0.05) = 2.179

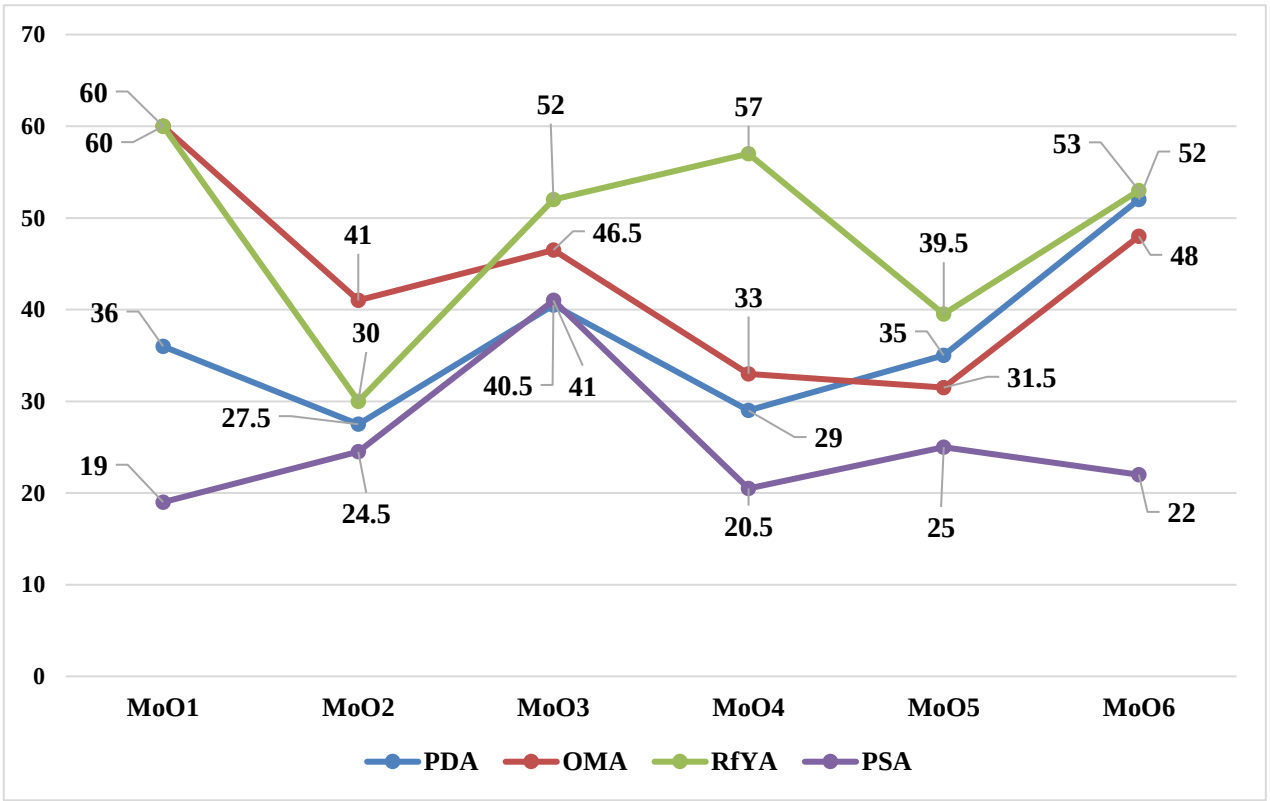

Fig. 21. mycelial growth on culture media (7 DAI)

Table 19. Significance of mycelial growth on culture media (14 DAI)

Isolates	Radial Mycelial Growth			
	PDA	OMA	RfYA	PSA
MoO1	41 b	32.5 b	40 b	48.5 a
MoO2	72 a	40 ab	90 a	37.5 ab
MoO3	53 ab	51.5 ab	68.5 ab	46.5 a
MoO4	48.5 ab	44 ab	49.5 ab	28.5 ab
MoO5	66 ab	66 a	51 ab	43 ab
MoO6	47 ab	60 ab	65.5 ab	23.5 b
Critical Value	29.160	28.649	48.725	21.606

*LSD (0.05) = 2.179

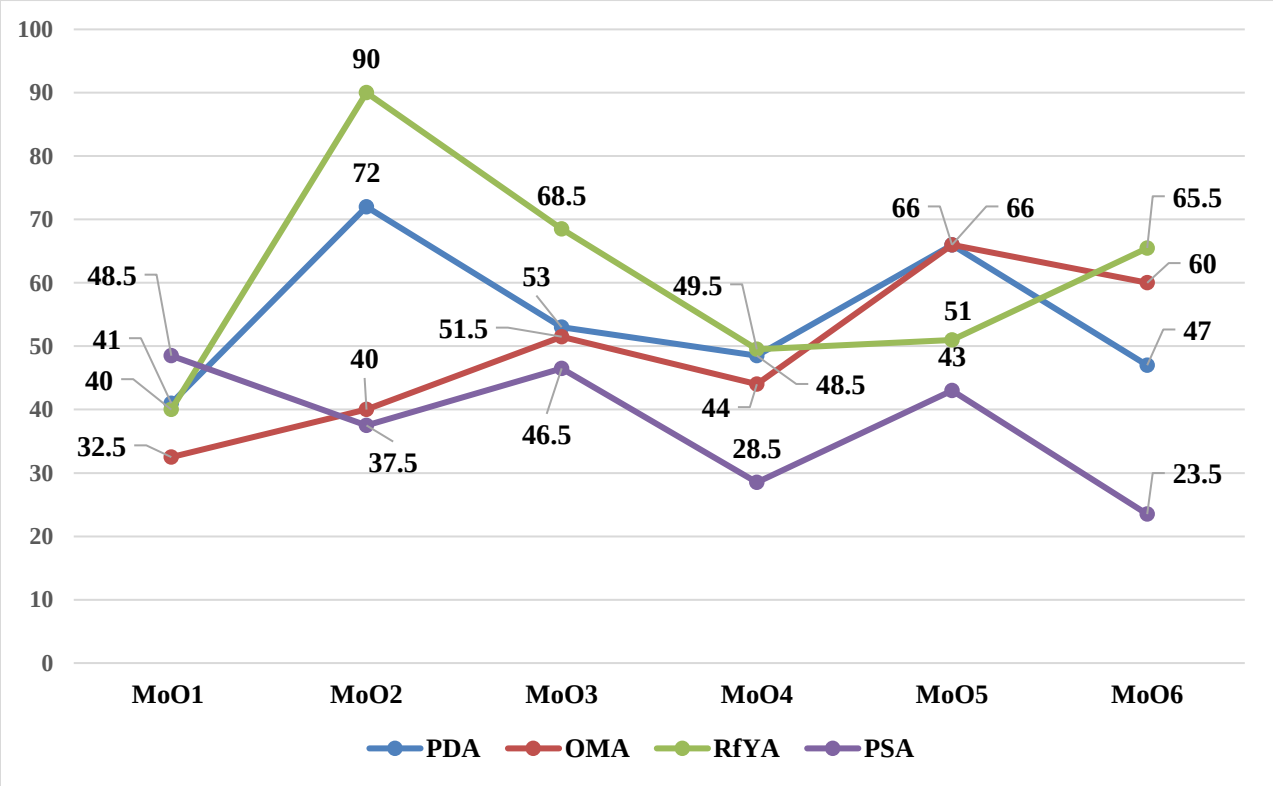

Fig. 22. mycelial growth on culture media (14 DAI)

4.4. Screening rice genotypes against *Magnaporthe oryzae* in blast nursery

The screening of 32 traditional and local lines of rice germplasms against rice blast disease revealed that, 18 lines germinated and 14 lines failed to germinate on UBN of BRRI and none of the variety was immune, only BRRI Dhan32, BRRI Dhan33 and BINA Dhan17 were found to be resistant against blast and BRRI Dhan57 was found moderately resistant and the other lines are susceptible to blast. These results are considered after four observations of disease score on blast affecting leaf following 0-9 standard evaluation scale for rice blast (SES IRRI, 1996). Out of 18 rice lines BRRI Dhan32, BRRI Dhan33 and BINA Dhan17 shown phenotypic score of 1 & 0 which considered as resistant, BRRI Dhan57 shown phenotypic score of 2 which are moderately resistant and the other lines shown phenotypic score of 5 which subsidized as susceptible to blast. Among them BRRI Dhan28 shows highly susceptibility to blast pathogen *M. oryzae.* These sources of resistance identified from rice germplasm, can be explored in breeding programs for the development of disease resistant cultivars.

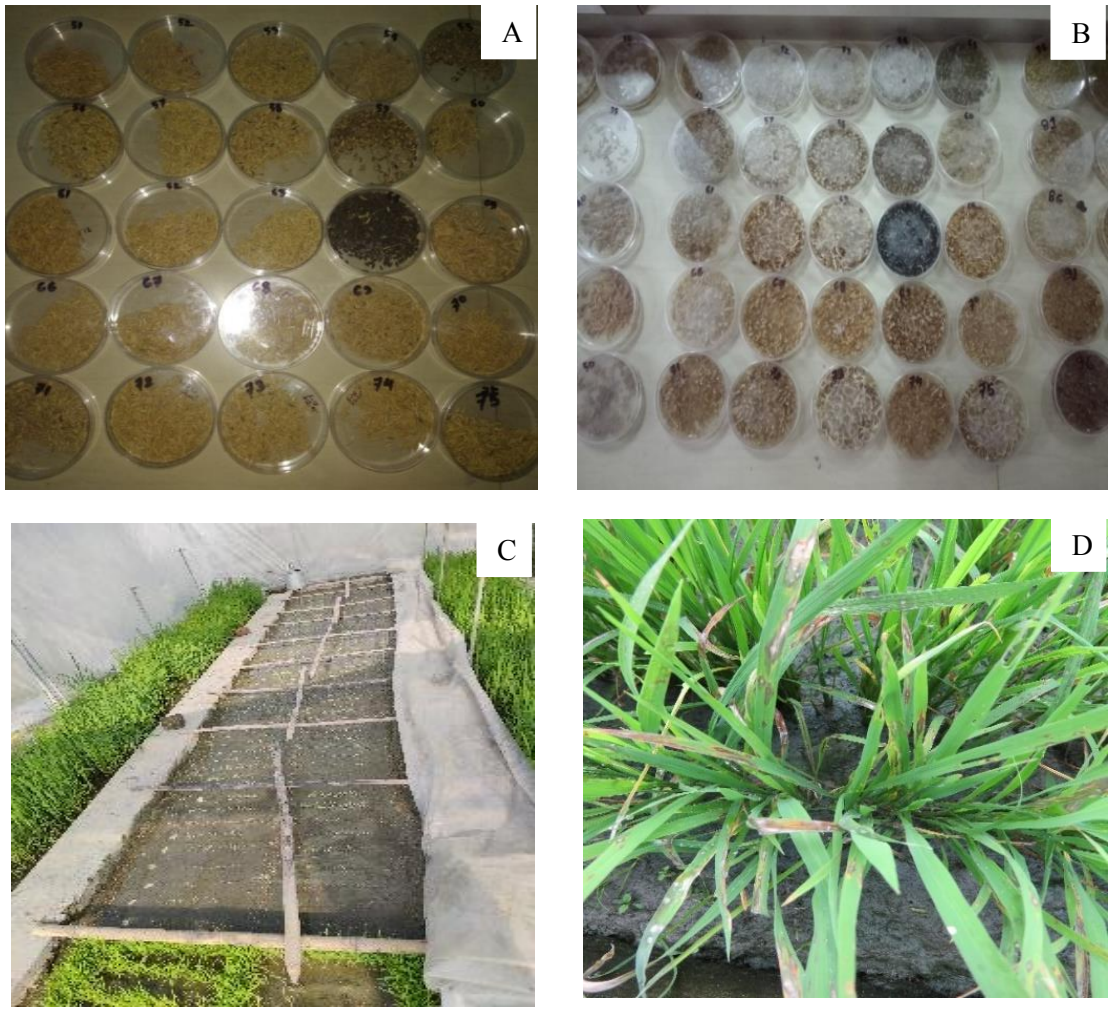

Fig. 23. Seed germination (A and B), seed sowing (C) and typical spindle shaped lesion (D) developed on inoculated seedlings in nursery bed.

Table 20. Reactions of rice germplasms against rice blast caused by *Magnaporthe oryzae*

Disease Grade/ Score							
SL	**Traditional Rice Variety**	**Observation 1**	**Observation 2**	**Observation 3**	**Observation 4**	**Highest Score**	**Disease Reaction**
1	BR11	1	5	5	5	5	S
2	BR14	5	5	5	5	5	S
3	BR22	2	3	5	5	5	S
4	BRRI DHAN28	5	5	5	5	5	S
5	BRRI DHAN29	2	2	5	5	5	S
6	BRRI DHAN32	1	1	0	0	1	R
7	BRRI DHAN33	0	0	0	0	0	R
8	BRRI DHAN39	0	0	0	0	5	S
9	BRRI DHAN47	0	2	0	4	4	S
10	BRRI DHAN48	0	0	3	5	5	S
11	BRRI DHAN49	5	5	5	5	5	S
12	BRRI DHAN51	5	5	5	5	5	S
13	BRRI DHAN52	0	0	5	5	5	S
14	BRRI DHAN57	2	0	0	0	2	MR
15	BRRI DHAN81	0	0	5	4	5	S
16	BINA DHAN7	0	0	5	5	5	S
17	BINA DHAN17	0	0	0	0	0	R
18	NERICA	5	5	5	5	5	S

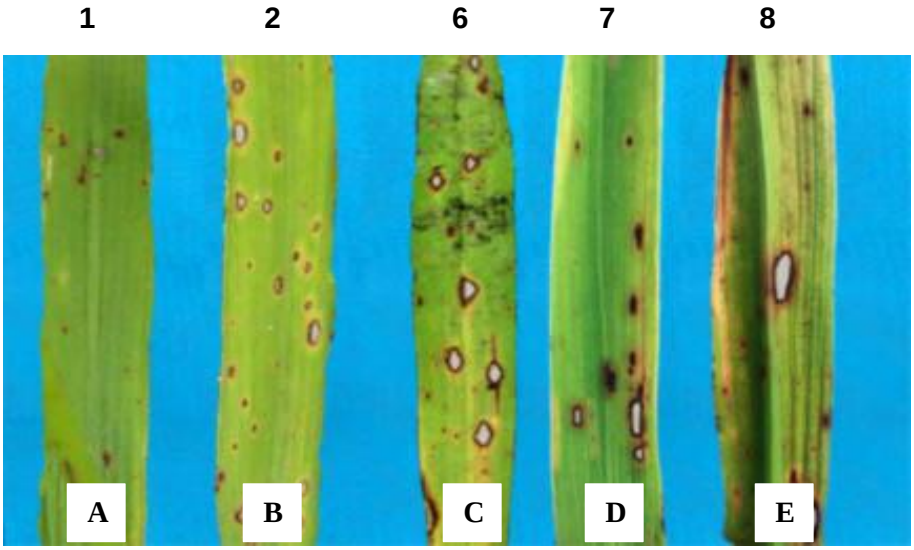

Fig. 24. Scoring of leaf blast using standard evaluation scale (SES IRRI, 1996)

Rice blast caused by the pathogen *Magnaporthe oryzae* is one of the most damaging disease affecting rice production worldwide. The use of resistant varieties is thought to be one of the most economically and environmentally efficient ways of crop protection. However, the use of resistant cultivars is the most economical and environment friendly method for the management of rice blast (Castano *et al*., 1990; Saifullah *et al*., 1995; Khan *et al*., 2001; Haq *et al*., 2002).

Many reports available on the screening of rice germplasms against the blast disease. Yan *et al*., (2017) screened a set of 32 germplasm by artificial inoculation with *M. oryzae* under UBN (Uniform Blast Nursery) at Linan, Zhejiang Province, China in 2012–2014. Similarly, Sowmya *et al*., (2014) screened different landraces for blast resistance and observed that HR 12 shows the highly susceptible reaction against blast which is similar with our results. They found some landraces (Beesginsali, Siddasala and Casebatta) which shown resistant reaction against blast. Mohanta *et al*., (2003) also conducted a screening trial at Bangladesh reported that among twenty-eight restored line and four standard checks, three were highly resistant, 12 resistant, 16 moderately susceptible.

Similar results were also reported by Haq *et al*., (2002) where they screened twenty-five rice germplasm lines and found that two lines KSK-282 and IRRI-6 were resistant. Field screening of 40 entries/varieties during 2005-2006 against the blast disease revealed that only one entry 99513 of PARC, one entry of KSK-10 from Rice Research Institute, Kala Shah Kaku and DM-2-25-9-02 from NIAB, showed resistant response (Arshad *et al*., 2008). Khan *et al*., (2001) screened 39 (course) and 40 (fine) entries/varieties for three years from Rice Research Institute Kala Shah Kaku and NIAB, Faisalabad. The screening revealed that amongst the course entries/varieties like IR-6 and KS-282 were found highly resistant in 1998 and resistant in 1999 and 2000 while on over all basis lR-8, DR-82 and DM- 15-1-95 were found resistant in the entire test. In another study Saifu1Iah (1995) screened 23 genotypes during 1990 and 1991 that 19 genotypes were highly resistance and 3 resistant to leaf and neck blast caused by *Magnaporthe oryzae*. Castano *et al*., (1990) also developed methods for screening of 437 upland germplasm from Indonesia (IAT), Colombia and IRRI (Philippines) for resistance to *M*. *oryzae* six times within two years and found that 176 germplasms were highly resistant while other had low to high susceptibility to rice blast disease. The blast pathogen affects different parts of a rice plant during pathogenesis. One of the serious forms of rice blast is neck blast. However, due to very complex nature of *M. oryzae*, the epidemiology of pathogen is not completely understood and the screening technique for neck blast is not standardized. The leaf blast is well studied and the screening method for the same is precisely standardized.

CHAPTER 5

SUMMARY AND CONCLUSION

In Boro season 2020-21, in different villages of Cumilla and Brahmanbaria district of Bangladesh a survey on blast disease of rice has been carried out. The highest incidence of blast was recorded from Noapara, *Cumilla* in BRRI dhan81 where blast incidence was 57% and severity score was 9. From this collected sample blast pathogen *Magnaporthe oryzae* has been isolated and pure culture of *M. oryzae* was prepared on different culture media. Colony characters like growth character, color, surface structure and shape of 6 isolates were observed in Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Rice flour Yeast Ager (RfYA), Oat Meal Agar (OMA) media. Maximum mycelial growth was measured on RfYA media and minimum growth was recorded on PDA media.

Thirty-two (32) rice genotypes were collected from Rangpur and Chapainawabgonj. Seeds of collected germplasms were allowed to germinate in moistened petridish and then sown in the nursery (UBN), BRRI during the Robi season of 2020 to 2021 to determine the source of resistance in rice germplasm against *Magnaporthe oryzae*. These test lines were sown in a single row of 50 cm long with row to row spacing of 10 cm. After every five test entries a local susceptible check BRRI dhan28 and highly susceptible check US2 was planted, respectively in the uniform blast nursery, BRRI, Gazipur. Here 18 test entries were germinated and shown blast reaction among 32 rice lines. Data was taken after four observations of the inoculated seedling leaf showing reaction of blast disease.

Among all the 18 rice genotypes along with susceptible checks BRRI dhan32, BRRI dhan33 and BINA dhan17 were shown to be resistant with phenotypic score 1 and 0. BRRI dhan57 shown to be moderately resistant with phenotypic score 2 and the rest of the genotypes namely BRRI dhan49, BRRI dhan51, BR14 and NERICA were susceptible to blast disease with phenotypic score of 5. The highest susceptibility with phenotypic score of 5 was recorded by five rice varieties along susceptible check variety BRRI dhan28.

In present study BRRI dhan32, BRRI dhan33 and BINA17 should shown resistant reaction against rice blast under nursery condition upon artificial inoculation. These varieties could be used as donors of blast resistant gene during breeding program to develop blast resistant rice varieties.

CHAPTER 6

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