ASSESSMENT OF MOSTLY CULTIVATED BORO RICE VARIETIES AGAINST RICE BLAST CAUSED BY *MAGNAPORTHE ORYZAE* AND ESTABLISHMENT OF KOCH'S POSTULATE THROUGH PATHOGENESIS

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CERTIFICATE

This is to certify that the thesis entitled '**ASSESSMENT OF MOSTLY CULTIVATED BORO RICE VARIETIES AGAINST RICE BLAST CAUSED BY *MAGNAPORTHE ORYZAE* AND ESTABLISHMENT OF KOCH'S POSTULATE THROUGH PATHOGENESIS'** *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 results of a piece of bona fide research work carried out by* ***SHAMMY AKTER,*** *Registration No.* ***13-05470*** *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: June, 2020
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DEDICATED

TO

MY BELOVED PARENTS,

MY SPOUSE

&

MY RESPECTED SUPERVISOR

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ABSTRACT

In Bangladesh last few years, Rice blast caused by fungus, *Magnaporthe oryzae* played vital role in reducing the yield. In the current study, a field, lab and cup tray experiment was carried out in the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 during the period October, 2018 to May, 2019. The field experiment was conducted in central farm of SAU for the evaluation of the mostly cultivated boro rice varieties viz. BRRI dhan 28, BRRI dhan 29, BRRI dhan 58, BINA dhan 8 and BINA dhan 10, two high yielding varieties viz. BRRI dhan 74 and BRRI dhan 81 and one wild variety against rice blast pathogen. The lab experiment was done in Molecular Biology and Plant Virology Lab for the etiology study of rice blast pathogen. Potato sucrose agar (PSA) media was used to isolate the blast pathogen and identification was done on the basis of cultural and morphological structures of *Magnaporthe oryzae*. A cup tray experiment was also carried out to establish the Koch's postulate through pathogenicity test. From the field experiment, it was found that the disease incidence and severity of rice blast was showed significant variants among the tested varieties and ranged from 0.00 to 21.48 and 0.00 to 60.48% respectively. The highest disease incidence and severity was recorded in BRRI dhan 58. There was no disease incidence (0.00%) and severity (0.00%) was found in selected wild variety throughout the growing season. Other varieties showed moderate disease incidence and severity that was statistically not significant each and other. From the study, it was noted that the disease severity of rice blast in tested varieties was showed moderately resistant to susceptible and the selected wild variety showed tolerance/highly resistance to blast pathogen. From the cultural and morphological study of the derived isolate from the field samples, it was evident that the disease was caused by *Magnaporthe oryzae*, as the isolate produced hyaline, pyriform three celled conidia from leaf sample and media respectively. The etiology of rice blast pathogen was also studied through establishment of the Koch's postulate. In the study, yield and yield contributing characters of selected boro rice varieties against blast disease was also studied, and it showed statistically non-significant. However from the study it may be concluded that wild cultivar can be alternative of BRRI dhan 28 and BRRI dhan 58, but it needs further more trial. This wild variety can also be used in resistance breeding program to develop the induced resistance system in rice plant.

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ABBREVIATIONS AND ACRONYMS

%	: percent
^0C	: Degree Celsius
μm	: micrometer
cm	: centimeter
g	: gram
ha	: hectare
i.e.,	: that is
l	: liter
μl	: microliter
mg	: milligram
ml	: milliliter
mm	: millimeter
MT	: Million Tones
CRD	: Completely Randomized Design
RBD	: Randomized Block Design
LSD	: Least Significant Difference
DS	: Disease severity
DI	: Disease incidence
DAT	: Days After Transplanting
DAS	: Days After Sowing
et. al.	: and others
No.	: Number
RH	: Relative Humidity
Viz.,	: Namely

BBS	: Bangladesh Bureau of Statistics
BER	: Bangladesh Economic Review
SRDI	: Soil Resource Development institute
IRRI	: International Rice Research Institute
BRRI	: Bangladesh Rice Research Institute
FAO	: Food and Agriculture Organization

INTRODUCTION

Rice (*Oryza sativa*), a monocot, is normally grown as an annual plant, although in tropical areas, it can survive as a perennial and can produce a ratoon crop. (IRRI, 2009). Rice is the seed of the family Poaceae or Gramineae. As a cereal grain, it is one of the most important cereals of the world and is consumed by almost 50% of the world population (Luo *et al*; 1998). It is the agricultural commodity with the third-highest worldwide production after sugarcane and maize. (FAOSTAT, 2018)

In the world, the major rice growing countries are China, India, Myanmar, Indonesia, Bangladesh, Vietnam, Thailand, Philippines, Brazil and Japan. Almost 90 percent of the population of Bangladesh, Myanmar, Sri Lanka, Vietnam and Kampuchea are rice eaters (FAOSTAT, 2018). In 2017, world production of paddy rice was 769.7 million tonnes, led by China and India with a combination 49% of this total. Other major producers were Indonesia, Bangladesh and Vietnam (FAOSTAT, 2018). More than 90% of rice is grown and consumed in Asian countries. In terms of food consumption, what distinguishes Asia from the rest of the world is its great dependency on rice. It is the basic staple food for the majority of the population, including the region's 560 million poor. Sub-Saharan Africa produces about 19 million tons and Latin America about 25 million tons. In Asia and sub-Saharan Africa, almost all rice is grown on small farms of 0.5−3 ha. (RICEPEDIA, 2018).

Asia is the leader in rice production that leads about 90 per cent of the world's production. Over 75 per cent of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. In Asian countries more than 80% people take rice in their daily diet as a staple food. The global annual demand for rice is expected to be around 800 million tons by 2025 in view the expected increase in population.

Bangladesh is an agro-based developing country and still striving hard for rapid development of its economy. In this country, rice is grown in three distinct seasons, namely Aus (April to August), Aman (August to December) and Boro (December to April) covering almost 11.0 million hectares of land (DAE, 2010). About 95 % of the total food requirements are fulfilled by producing rice in three different seasons, but there is still need to be increased production to feed the growing population which increases at the rate of 1.32 % per annum (BER, 2010). The average world yield of rice is 3.84 ton/ha (Ahmed *et al.,* 2013). Rice is grown on about 10.5 million hectares which has remained almost stable over the past three decades. The country is now producing about 25.0 million tons to feed her 160 million people (Hossain *et al.,* 2017). This indicates that the growth of rice production was much faster than the growth of population. This increased rice production has been possible largely due to the adoption of modern rice varieties on around 66% of the rice land which contributes to about 73% of the country's total rice production (DAE, 2010). Rice, however, continues to remain main sources of food supply for the people of all the sectors, producing nearly 95 percent of the total food requirements, but there is still a need to increase production to feed the growing population which increases at the rate of 1.32 percent per annum (BER, 2010). The International Rice Research Institute, Philippines, estimates that in order to feed the growing global population, rice production must be increased by another one-third by the year 2020.

Rice output is likely to rise 4 percent year-on-year to 5.36 core tonnes during the current calendar year. The overall production of the staple food would be higher than the five-year annual average of 5.17 core tonnes (equivalent of around 3.53 core tonnes of rice) between 2013 and 2017. The Bangladesh Bureau of Statistics estimated that boro rice production rose 8.67 percent year-on-year to 1.95 core tonnes in 2018 from that a year ago. Because of ample supply from record output, Bangladesh's rice imports may decline to 8.50 lakh tonnes in fiscal 2018-19. Total rice import hit a record at 38.92 lakh tonnes the previous year (FAO, 2018).

Rice is known to be attacked by many pests and diseases which cause huge losses annually worldwide. Rice crop is subjected to attack by fifty diseases include twenty one fungal, six bacterial, four nematodes, twelve viral and seven miscellaneous diseases and disorders (Jabeen *et al.,* 2012). Asia's hot and humid climate during the long and heavy monsoon season provide the most favourable agro ecological environment for rice cultivation as well as diseases development. So far in Bangladesh, about thirty one diseases are recorded to occur in rice including ten major diseases (Miah *et al.,* 1985, Shahjahan *et al.,* 1987). Among the major rice diseases that often causes great economic losses, the rice blast caused by *Magnaporthe oryzae*, is a vicious threat to the country's economy (Ganesh *et al.,* 2012). Rice blast is a serious fungal disease of rice that is threatening global food security. It has been extensively studied due to the importance of rice production and consumption and because of its vast distribution and destructiveness across the world (Aziiba *et al.,* 2019). Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world including Bangladesh. It is estimated that each of the three years enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al.,* 1994).

The disease initially appears as whitish or greyish specks along the leaf margins. Later on they turn into elliptical spots which were elongated and nearly diamond shaped with pointed ends. Those spots became necrotic in the centre with brown or reddish-brown margins. Those spots collapse each other and forms large lesions. On neck of the plants, fungus produced elongated, greyish to black color lesions which causes the plants to break at the neck point. Disease also appears on rice seeds as brown diamond shaped spot. Bangladesh has experienced several epidemic outbreaks of rice blast since 1980 (Ahmed *et al.,* 1985; Shahjahan *et al.,* 1994). During 2016, serious yield losses occurred due to epiphytotic of rice blast caused by *Magnaporthe oryzae* have been recorded in different regions in Bangladesh such as Dinajpur, Rangpur, Thakurgaon, Panchagarh, Kushtia, Jashore, Pabna, Barishal, Mymensingh, Munshigonj, Chuadanga etc. and the production of Boro rice and transplanted Aman in Bangladesh were greatly reduced due to severe outbreak of rice blast infestation.

Considering the above mentioned facts, the current study was carried out to assess the mostly cultivated boro rice varieties, including one wild variety in Bangladesh against rice blast pathogen. The etiology of rice blast pathogen, *Magnaporthe oryzae* and to establish the Koch's postulate of this pathogen through pathogenesis was also studied.

OBJECTIVES

The present study was conducted to achieve the following specific objectives-

- To assess the selected boro rice varieties against rice blast pathogen
- To study the etiology of rice blast pathogen on the basis of cultural and morphological characteristics of *Magnaporthe oryzae* and
- To establish the Koch's postulate through pathogenicity test

REVIEW OF LITERATURE

The available literature of work previously done on blast disease of rice and its management strategies have been reviewed in this section. The review of literature relating to this exposition is presented in the subsequent headings and sub-headings.

2.1. Significance of rice

Rice (*Oryza sativa* L.) is one of the most vital cereal crops of the world and is consumed by 50% of the world population (Luo *et al.*, 1998). There are two species cultivated *Oryza sativa* L. (Asian rice) and *Oryza glaberrima* S (African rice) (Silue and Notteghem, 1991).

Oryza glaberrima is by tradition found in diverse West African agro ecosystems but it is largely uncontrolled in favor of high yielding *Oryza sativa* cultivar that has higher agronomic performance (Seebold *et al.*, 2004). However, *Oryza sativa* cultivars are often not adequately adapted to various abiotic and biotic environments in Africa. *Oryza glaberrima* has been found to have several useful traits like being moderate to high in their level of resistance to blast (Silue and Notteghem, 1991), rice yellow mottle virus (Attere and Fatokun, 1983); (Jabeen *et al.*, 2012), rice gall midges, insects (Alam, 1988) and nematodes (Ram *et al.*, 2012). The variety has also been found to be tolerant to abiotic stresses such as acidity, iron toxicity, drought, and weed competition (Sano *et al.* 1984).

Rice is the most economically important staple food crop in Bangladesh, India, China, East-Asia, South East Asia, Africa and Latin America catering to nutritional needs of 70% of the population in these countries. It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories (Yaduraju, 2013).

In several developed countries such as North America and European Union (EU) also, rice consumption has increased due to food diversification and immigration (Dubey S.C., 1995).

Worldwide, rice is grown on 161 million hectares, with an annual production of about 678.7 million tons of paddy (FAO, 2017). 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 (FAO, 2017).

Five Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. During 201213 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) (Commodity profile for rice - January 2015). Rice is one of the three most important food crops of the world and the main staple food for nearly a half of the world's population (Von Braun, 2007).

2.2. Significance of blast disease of rice

Magnaporthe grisea (*Anamorph Pyricularia grisea Sacc. synonym Pyricularia oryzae Cav*.) causes rice blast disease in rice cultivation areas worldwide (BBS, 2017; Kato, 2001). The disease causes yield losses from between 1- 100% in Japan (Kato, 2001), 70% in China, 21-37% in Bali, Indonesia, 30-100% in Bangladesh (Singh and Prasad, 2007) and 30-50% in South America and Southeast Asia (Baker *et al.*, 1997).

Rice blast is one of the most important diseases of rice, caused by the fungus *Pyricularia oryzae* B. C. Couch (Couch and Kohn 2002). One of the main limitations in production is rice blast disease caused by the fungus *Pyricularia oryzae*. Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production (Oerke and Dehne, 2004).

In West Africa, the largest area of African production, this pathogen is the main restraint to production with yield losses ranging from 4-77%. The fungus is able to infect plants at all stages of growth and development in both upland and lowland rice production systems. Lowland rice produced in temperate and subtropical climates of Asia are highly susceptible to the pathogen, while tropical upland areas are susceptible only under irrigation (Nutsugah *et al.* 2008).

Blast disease caused by *Pyricularia oryzae* Cavara [Synonym *Pyricularia grisea Sacc*. the anamorph of *Magnaporthe grisea*, upsets production statistics of rice in Pakistan (Tirmali and Patil, 2000). In Pakistan during the last two decades, rice blast is 6 mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and place like GaggooMandi (Ali *et al.,* 2009).

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

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

Heavy yield losses have been reported in many rice growing countries. For example 95, 50 and 40 percent grain loss may occur in Bangladesh and India (Padmanabhan, 1970), Philippines (Ou and Nuque, 1985) and Nigeria (Arshad *et al.,* 2008).

The most usual approaches for the management of rice blast disease include planting of resistant cultivars, application of fungicides and manipulation of planting times, fertilizers and irrigations (Georgopoulos and Ziogas, 1992).

Blast is known to attack nearly all above ground parts as well as during all growth stages of plant. Recent reports have shown that the fungus has the capacity to infect plant roots also (Shafaullah *et al.,* 2011).

The infection of rice blast occur when fungal spores land and attach themselves to leaves using a special adhesive released from the tip of each spore (Hamer *et al.,* 1988).

The germinating spore develops an aspersorium, a specialized infection cell which generates enormous turgor pressure (up to 8MPa) that ruptures the leaf cuticle, allowing invasion of the underlying leaf tissue (Dean, 1997; Hamer et al., 1988).

2.3. Nature and disease symptoms

The pathogen may infect all the above ground parts of a rice plant at different growth stages: leaf, collar, node, internode, base, or neck, and other parts of the panicle, and sometimes the leaf sheath (Peterson, 1994). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting (Bonman *et al.*, 1989).

Magnaporthe oryzae infects and produces lesions on the following parts of the rice plant: leaf (leaf blast), leaf collar (collar blast), culm (culm nodes), panicle neck node (neck rot) and panicle (panicle blast). In leaf blast initial lesions/spots are white to grey-green with darker borders. Older lesions are white-grey, surrounded with a red-brown margin and are diamond shaped (wide centre and pointed toward either end). Lesion size is commonly 1-1.5 cm long and 0.3-0.5 cm wide. Under favourable conditions, lesions can coalesce and kill the entire leaf. In case of collar rot, lesions are located at the junction of the leaf blade and leaf sheath and can kill the entire leaf (Padmanabhan, 1974).

Infection to the neck node produces triangular purplish lesions, followed by lesion elongation to both sides of the neck node, symptoms which are very serious for grain development. When young neck nodes are invaded, the panicles become white in colour the so called 'white head' that is sometimes misinterpreted as insect damage. Infected panicles appear white and are partly or completely unfilled. The whitehead symptoms can easily be confused with a stem borer attack which also results in a white and dead panicle. Panicle branches and glumes may also be infected. Spikelet attacked by the fungus change to white in colour from the top and produce many conidia, which become the inoculum source after heading. Panicle blast symptoms include the panicle appearing 11 brown or black. Node infection includes infected nodes appearing black-brown and dry and often occur in a banded pattern. This kind of infection often causes the culm to break, resulting in the death of the rice plant. The pathogen is most common on leaves, causing leaf blast during the vegetative stage of growth, or on neck nodes and panicle branches during the reproductive stage, causing neck blast (Bonman, 1992).

Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu *et al.,* 2005). The neck blast infects the panicle causing failure of the seeds to fill or causing the entire panicle to fall over as it is rotted. Infection of the necks can be very destructive and directly reduces the economic value of the produce. The lesions are often greyish brown discoloration of the branches of the panicle and over time, the branches may break at the lesion. Out of three symptoms, neck blast is more destructive (Sreenivasa *et al.,* 2011).

The most important fungal pathogen of rice is *Magnaporthe oryzae,* because of its widespread occurrence and destructive nature. The fungus can attack any aerial part of the rice plant, including seeds. They also suggested systemic transmission of the fungus from seeds to seedlings. The fungus *Magnaporthe oryzae* was able to infect and produce lesions on all organs of the rice plant and when the fungus attacks young leaves, purple spots could be observed changing into spindle shape which has a grey centre and purple to brown border. Brown spots appeared only on older leaves or leaves of resistant cultivars. In young or susceptible leaves, lesions coalesce and cause withering of the leaves, especially at seedling and tillering stage. Infection to the neck results formation of triangular purplish lesions followed by elongation on both sides of neck. When young necks are infected, the panicles become white in colour and later infection caused incomplete grain filling and poor grain quality (Hajimo, 2001).

Leaf blast fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. When the last node is attacked, it causes partial to complete sterility.

Rice blast pathogen infect all the above ground parts of rice plants at different growth stages, i.e., leaf, collar, nodes, internodes, base or neck and other parts like panicle and leaf sheath. It starts a typical blast lesion on rice leaf as grey at the centre with a dark border and is spindle shaped (Ram *et al.*, 2007). The environment with frequent and prolonged dew periods and with cool temperature in day time is most favourable for the spread of the disease (Castilla *et al.*, 2009).

2.4. Occurrence and distribution

Rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated, in both low land and upland conditions. Rice blast exists wherever rice is cultivated, but the disease occurs with highly variable strengths depending on climate and cropping system. Environments with frequent and prolonged dew periods and with cool temperature in daytime are more favourable to blast (Luo *et al.,* 1998).

In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Vehari and places like Gaggoo Mandi (Arshad *et al.,* 2008). Rice blast has been recorded in the Northern Territory, Brazil (Prabhu and Morais,1986), Queensland, Australia (Perrot and Chakraborty 1999), Sri Lanka, Colombia, Philippines, Japan, South Korea (Ou, 1985).

Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *Magnaporthe oryzae* (Ravindramalviya, 2014). It is the most destructive pathogen of rice worldwide. Around 50% of production may be lost in a field moderately affected by infection (Supriya and Sharma, 2010). It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al.,* 1994).

It was first reported as rice fever disease in China by Soon ying-shin in 1637 (Ou, 1985), in Japan it was reported as Imochi-byo by Tsuchiya in 1704. In Italy it was reported as brusone by Astolifi (1828) and in India it was first reported in

Thanjavur delta of Tamil Nadu in 1913 (Padmanabhan, 1965). It is a disease of immense importance in temperate, tropical, subtropical Asia, Latin America and Africa and found in approximately 85 countries throughout the world (Kapoor and Abhishek, 2014).

The disease is also a major problem in Penna river belts and Godavari in Andhra Pradesh. The blast fungus can attack more than fifty other species of grasses. It causes disease at seedling to adult plant stages on the leaves, nodes and panicles. It appears in irrigated low land or rain fed upland rice as well as in submerged or deep water rice. Rice blast is the most serious disease found in the extensive rice areas of Latin America, Africa, and Southeast Asia and is a worldwide problem in rice production. Rice blast disease is a significant constraint to global food security and agricultural trade (Leong, 2004).

2.5. About *Magnaporthe oryzae*

The fungus *Magnaporthe oryzae* (Anamorph: *Pyricularia oryzae* (cooke.) Sacc. is the causal agent of rice blast disease. The perfect stage of *Pyricularia oryzae* was earlier named as *Ceratosphaeria oryzae* (Habert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus *Magnaporthe*.

Finally, *M. oryzae proposedas* a perfect stage of *Pyricularia grisea* (cooke.) Sacc instead of *Ceratosphaeria grisea.* 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 more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in colour, 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. (Luo *et al.*, 1998).

Commonwealth mycological institute CMI (Luo *et al.*, 1998) description of the culture: Cultures are greyish in colour, conidiophores single or in fascicles, simple or rarely branched, show sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodially and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, three celled rarely one or two celled, hyaline to pale olive, 19-23 × 7-9 µm, with a distinct protruding basal hilum. Chlamydospores often produced in culture, thick-walled, 5-12 µm diameter.

Fungus produce sexual fruiting bodies called perithecia within 21 days. Perithecia are flask-shaped that carry asci containing ascospores, the products of meiosis. Ascospores are arranged as unordered octads or as larger populations of randomly selected ascospores (Nicholas J. Talbot, 2003).

SYSTEMATIC POSITION

Kingdom: Fungi

Division: Eumycota

Subdivision: Deuteromycotina

Class: Hyphomycetes

Order: Moniliales

Family: Moniliaceae

Genus: *Magnaporthe*

Species: *Magnaporthe oryzae*

2.6. Isolation of *Magnaporthe oryzae*

Padmanabhan *et al.* (1970) isolated *Magnaporthe oryzae* from samples of diseased leaves, necks, and nodes of the infected rice plant on oat meal agar (OMA) with traces of biotin and thiamine (B and T). Cultures were purified by dilution method, and single spore isolates were grown and multiplied on OMA + B & T at 250C.

The panicles were collected with the symptoms of neck blast, washed once with sterile distilled water, and placed on moist filter paper in Petri dishes at room temperature to induce sporulation. Conidia from the lesion surface were spread onto 3% water agar with a sterile loop and incubated overnight. Single germinating conidium was isolated and transferred to potato dextrose agar.

Rice leaves infected with blast were collected by Bonman *et al.,* (1989) and isolated by placing each lesion in a moist Petri dish and incubated at 250C until sporulation. Conidia from the lesion surface were spread on to water agar and the germinating conidium was isolated and transferred to agar slants. Leaves were collected and panicles infected with rice blast from rice cultivars obtained from germ plasm bank at the Centro Internacional de Agricultura Tropical (CIAT) and the International Rice Research Institute (IRRI) by Correa *et al.,* (1993). They derived cultures from either mass or single conidial isolates obtained from single lesions. Cultures were maintained on V8 juice agar and multiplied for inoculations on rice-polish agar at 28^{o}C under continuous light. They stated that *Magnaporthe oryzae* expressed its virulence spectrum irrespective of geographical location.

Eight samples of rice leaves infected with blast were collected from commercial fields of upland rice cultivars in the state of Goias, Brazil (Silue and Notteghem, 2009). Monoconidial isolates were obtained by directly transferring one conidium per lesion on 5% water agar from two to three lesions per leaf. The isolates from panicles in the majority of the cases were obtained from one conidium per panicle. The collected isolates were conserved on sterilized filter paper discs in a freezer at -20 ± 1^{o}C.

Blast affected leaves of rice cultivars were collected from rice fields in Guilan province of Iran. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed with sterile distilled water and placed on potato dextrose agar in Petri dishes at 25ºC for 2–3 days. Later, Petri dishes were incubated at 25ºC in the dark or artificial fluorescent light on a 12 h light/dark photoperiod for 15–25 days. Monoconidial isolates of the recovered fungi were maintained on half-strength potato dextrose agar slants in test tubes as stock cultures (Motlagh and Javadzadeh, 2010).

Blast lesions were 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 ± 2°C (Priya *et al.*, 2013). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance. The causal organism was identified as *Magnaporthe oryzae* based on the spore morphology.

2.7. Sporulation of the pathogen

Culturing of different isolates of *Magnaporthe oryzae* was studied by Priya *et al.*, (2013) and reported that colonies of *Magnaporthe oryzae* appeared as white on oat meal, rice polish and malt extract agar, pinkish white on potato dextrose agar and whitish grey on rice agar. Spore induction was hastened on maize stem pieces than on rice and *Panicum repens*. When spores of 11 isolates of *Magnaporthe oryzae* were compared, conidia of the isolate from *Pennisetum purpureum* were significantly bigger than the other isolates. The spores of rice isolates from Erode and Gopichettipalayam were significantly smaller in length and width.

Blast fungal isolates produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff colour, greyish black to black colour (Sreevastava *et al.*, 2014).

The colony diameters of different groups ranged from 67.40 to 82.50 mm and the conidial shape of the different groups was pyriform (pear-shaped) with rounded base and narrowed towards the tip which is pointed or blunt. On oat meal agar, colony colour of all the isolates was usually grey with good growth. All the isolates showed raised mycelial growth with smooth colony margin (Gashaw *et al.*, 2014).

Colony colour of all the rice blast (*Magnaporthe grisea*) isolates was usually buff with good growth on Oat meal agar, greyish black with medium growth on host seed extract + 2% sucrose agar, the raised mycelial growth with smooth colony margin on potato dextrose agar and raised mycelium with concentric ring pattern on Richard's agar medium. On host seed extract + 2% sucrose agar all the blast pathogenic isolates showed black to greyish black colour with smooth colony margin and good growth (Meena, 2005).

Mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm × 4 – 7 µm (Average, 17.4 µm × 5.2 µm) (Mijan, 2000).

Ram *et al.,* (2012) found isolates of the fungus from different hosts differed in their response in media for mycelial growth and sporulation. Radial mycelial growth and days of sporulation of *Magnaporthe grisea* were studied by culturing three fungal isolates from rice, finger millet and *Panicum sp*. on six different media: prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA) and finger millet meal agar. The highest RMG was found in the isolates from finger millet and the lowest in the isolates from rice. The shortest days of sporulation (1 week) was found in the isolate from rice and the longest (>2 weeks) in the isolate from finger millet. Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet. The shape, color and compactness of the fungal colonies varied with the media and isolates used. Cross inoculation studies showed that the fungus isolates from rice were able to infect all the plant species while isolates from finger millet were only able to infect three plant species (*E. coracana, Setaria sp. and E. indica*).

MATERIALS AND METHODS

The present study entitled "Assessment of Mostly Cultivated Boro Rice Varieties against Blast Disease of Rice Caused by *Magnzporthe oryzae* and Establishment of Koch's Postulate through Pathogenesis" was carried out in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The details of the materials used and methodology adopted during the study are described below with some headings and sub-headings.

3.1. Experimental Site and Duration

The experiment was conducted in two steps. The field experiment was conducted at agronomy farm of Sher-e-Bangla Agricultural University, Dhaka. The experimental field area was situated at 23°46' N latitude and 90°22' E longitude at an altitude of 8.6 meter above the sea level (Anon. 2011). The lab experiment was done in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The experiment was carried out during the period from October, 2018 to May, 2019. (Appendix-I).

3.2. Soil Characteristics

The soil of the experimental site was a medium high land which belongs to the Madhupur tract, Agro Ecological Zone no. 28. The soil texture was silt loam, High level of nutrients, non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment soil samples of the experimental plots were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU) at a depth of a 0 to 30 cm and analysed in the Soil Resources Development Institute (SRDI), Farmgate, Dhaka. (Appendix-II).

3.3. Climate

The climate of the Madhupur tract varies slightly from north to south, the northern part reaches being much cooler in winter. Average temperatures vary from 28^0C to 32^0C in summer, reducing to 20^0C in winter, with extreme lows of 10^0C. Rainfall ranges between 1,000 mm and 1,500 mm annually, heavy rainfall in kharif season (May-September) and scanty in rabi season (October-March). Severe storms are unusual but tornadoes have struck the southern areas sometimes. During the month of December, January and February there was no rainfall. During the period of study the average maximum temperature was 32^0C and average minimum temperature was 20^0C. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka. (Appendix-III).

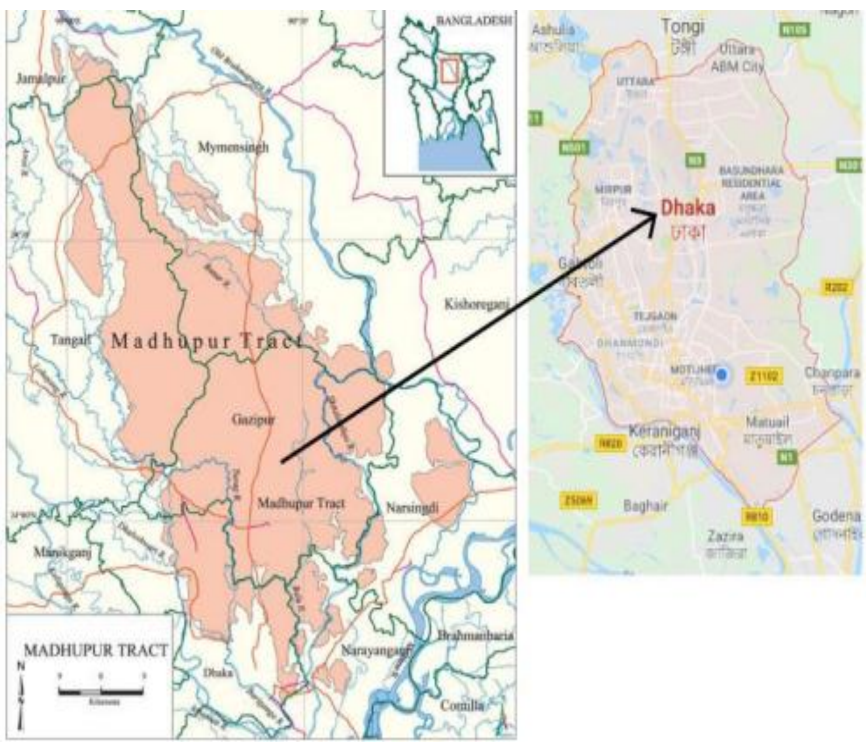

Figure 1. Madhupur tract, AEZ No-28.

3.4. Variety selection and Planting material

In total eight rice varieties were selected which are mostly cultivated in Boro season in Bangladesh. Among the selected varieties, five varieties were inbred viz.

BRRI dhan 28, BRRI dhan 29, BRRI dhan 58, BINA dhan 8 and BINA dhan 10, two high yielding varieties viz. BRRI dhan 74 and BRRI dhan 81 and one wild variety. Seeds of all the selected varieties of rice were used as planting material.

3.5. Seed collection

Seeds were collected from Bangladesh Rice Research Institute (BRRI), Bangladesh Institute of Nuclear Agriculture (BINA) and Bangladesh Agricultural Development Corporation (BADC).

3.6. Sprouting of seeds

Seeds were soaked in eight different plastic pots separately with tap water for 24 hrs. Before sowing in seed bed and earthen pot, seeds were taken out from water followed by put in eight different gunny bags and kept at room temperature for 72 hours for sprouting.

Figure 2. Sprouted seeds

3.7. Seed bed preparation and sowing of sprouted seeds

Seed bed was prepared by paddling the soil with the help of power tiller and harrow in Agronomy farm, Sher-e-Bangla Agricultural University, Dhaka. Proper manuring was done in the seed bed. Sprouted seeds were sown in wet seed bed on 11 November, 2018. Seedlings were properly taken care of. Weeds were removed and irrigation was given in the seed bed as and when needed.

Figure 3. Sprouted seeds in seed bed

3.8. Field Experiment

3.8.1. Land preparation

The land was prepared with the help of power tiller and harrow. The land was first opened on 2nd January 2019 and ploughed. The final ploughing was performed with the help of power tiller followed by laddering in order to level the soil surface. Weeds and stubbles were removed from the land.

Figure 4. Final land preparation for field experiment

3.8.2. Fertilizer application

Fertilizers were applied as per recommendation of Bangladesh Agricultural Research Council, 2012 (Fertilizer Recommendation Guide). The following doses of fertilizers were applied to the experimental plots:

Fertilizer	Dose (kg/ha)
Urea	100
TSP	16
MP	66
Gypsum	12
Zinc sulphate	1.3

All fertilizers except 2/3 urea were incorporated with soil during final land preparation. Rest of the urea was applied in equal two installments at 30 and 45 days after transplanting.

3.8.3. Experimental Design

The experiment was laid out in randomized complete block design (RCBD) with three replications. Blocks were representing the replication. Each block comprised eight unit plots and total number of plots were 24 (8 X 3=24). Size of each unit plot was 3.0 X 2.28 = 6.84 m^2. The distances between unit plot was 1.0 m and block 1.5 m.

3.8.4. Seedling transplantation

Seedlings were uprooted from the bed very carefully, and then transplantation was done on 9th January, 2019 in the main field. Row to row spacing was maintained as 1m and hill to hill 15 cm. Three seedlings were transplanted together in an individual hill.

Figure 5. Seedlings transplanted in the main field

3.8.5. Intercultural operation

The actions undertaken during the whole period of crop life in the field condition, are known as intercultural operations. Different types of intercultural operations performed in the field, are described in the following headings and sub-headings.

3.8.5.1. Weeding

Weeds are mainly perennial plants that compete with the crop for space, water, air and nourishment. It is one of the limiting factors for successful rice production. Rice plant in boro season also suffers from weed problem. Different kind of weeds such as, *Fimbristylis miliacea*, *Paspalum scrobiculaturm*, *Cyperus rotundus*, *Monochoria vaginalis*, *Ischaemum rogusum*, *Scirpus supinus*, *Cyperus difformis*, *Ipomoea aquatica and Marsilea minuta* etc. are some common weeds in the rice field in boro season. Whenever the weed community increased in the field, hand weeding was successfully done to control.

3.8.5.2. Irrigation

Rice is a water loving plant. For proper growth and development of rice and production of grains, proper and regular watering to the field is a must. Rice in boro season needs more frequent irrigation than other seasons as, it hardly rains throughout the season. Flood irrigation was given to the field crop at a regular interval.

3.8.5.3. Drainage

Though rice is a water loving crop, the constant stagnant water in the field hampers the growth of the plant. It restricts the plant's tillering and also affects the roots. The constant standing water in the field also increases the nuisance of many water scavengers and larvae of mosquito. That is why proper drainage in the field was done.

3.8.5.4. Application of fertilizer

Two splits foliar application of urea was given to the rice field during the entire crop life, one is at tillering stage and another is at the time of panicle initiation.

3.8.5.5. Application of insecticide

Insecticide was applied in the field when any insects were observed at a considerable number. Rodents are a great problem in the rice field. That is why, rodenticide was applied when it was necessary.

3.8.5.6. Netting

After panicle initiation, the entire field was surrounded with net, so that the insects and birds cannot attack and destroy the panicles.

3.8.6. Treatments (varieties) of the experiment

The selected most commonly and widely cultivated boro rice varieties were considered as treatments. So, the treatments (varieties) were as follows-

V_1 = BRRI dhan 28

V_2 = BRRI dhan 29

V_3 = BRRI dhan 58

V_4 = BINA dhan 8

V_5 = BINA dhan 10

V_6 = BRRI dhan 74

V_7 = BRRI dhan 81

V_8 = A wild variety

3.8.7. Parameters assessed

Different parameters were considered for the field experiment. Data was collected regarding the following parameters:

(i) Disease Incidence (%)
(ii) Disease Severity (%)
(iii) Tiller number
(iv) Plant height
(v) Panicle number
(vi) Grain/panicle
(vii) Fresh and dry weight of grains
(viii) Yield/plot (kg/plot)

3.8.7.1. Assessment of the disease incidence and severity in the field

Each of the plots was investigated for recording the incidence and severity of Blast disease. Data were recorded visually by observing the typical symptoms. Affected plants from each unit plot were selected for assessing the incidence and severity. Data were recorded three times at an interval of 20 days (45 DAT, 65 DAT and 85 DAT). Data were recorded on; hill/plot, infected hill/plot, total leaf/infected hill, infected leaf/infected hill, % disease infection/leaf. To estimate the incidence of Blast disease in grain five hill of each unit plot were 12 harvested randomly and separately during ripening stage. Then fifteen hill (As each variety consists three replication) from each variety were mixed together and select 30 panicle randomly from that mixture. Data were recorded on grain/panicle and infected grain/panicle.

Disease Incidence: % disease incidence was estimated by using the following formula,

$$\%\ \text{disease incidence} = \frac{\text{No. of infected hill or hill parts}}{\text{No. of inspected hill or hill parts}} \times 100$$

Disease Severity: The severity of the disease was recorded by following IRRI recommended grading scale (0-5 scale of Standard Evaluation System for Rice, 1980) first used by American Scientist Dr. Horsfall and Barrett (1945). The grades of the disease scale are given below:

%Leaf Area Disease (%LAD)	Rating/Grading
0	0
0.1-5.0	1
5.1-12.0	2
12.1-25.0	3
25.1-50.0	4
>50.0	5

3.8.7.2. Counting of tiller number

Tillering in rice generally starts at 45 to 55 days after transplanting. Five random plants of each replication of a distinct variety were selected, from which the data was recorded. Data was collected from total 15 plants of a variety and the average value was recorded. The first data on tiller number was recorded at 45 days after transplanting. It was done by counting the total tiller number per hill at the base of the selected plants. The second data was recorded at 65 days after transplanting and the third one was recorded at 85 days after transplanting when the plants reach at maximum tiller number.

3.8.7.3. Measurement of Plant height

The plant height was measured with a measuring scale. It was done by placing the scale at the ground level of selected 15 plants and the length of the plant from its base towards the flag leaf was recorded and average value was considered as plant height. Plant height was also recorded three times, at 45, 65 and 85 days after transplanting.

3.8.7.4. Counting of Panicle number

The data of panicle number was recorded after harvesting. The number of panicle per hill was counted of the selected plants and the average value was considered.

3.8.7.5. Counting of Grain/panicle

This data was also recorded after harvesting. The grains per panicle per plant were counted with the help of hands. The average value was considered as grain number per panicle.

3.8.7.6. Measurement of fresh and dry weight of grains

The assessment of fresh and dry weight of grains was done by a digital balance after harvesting. The fresh weight was recorded immediately after harvesting when the moisture level was high inside the grains. Whereas dry weight was taken after two weeks of sun drying, when the moisture level in the grains was reduced to a certain level. Average data was considered.

3.8.7.7. Yield/plot (kg/plot)

The yield/plot data was recorded by making average of the yields of the plots containing three replications of each treatment or variety. From this data, yield/ha was estimated.

3.8.8. Statistical analysis

The data was analysed by using the "Statistix 10" Software. The mean value was compared according to LSD range test at 5% level of significance. Tables, bar diagram, linear graphs and photographs were used to present the data as and when necessary

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3.9. Lab experiment:

3.9.1. Sample collection from the experimental field

The pathogen attacks at all stages of rice plants. It can also infect all the plant parts like leaves, stem, nodes, panicles etc. So, diseased samples like leaves, necks, nodes etc. were collected from the experimental field to obtain the pure culture of *Magnaporthe oryzae* for further lab experiment and establishment of Koch's postulate.

3.9.2. Experimental site and design of the experiment

The experiment was carried out in Molecular Biology and Plant Virology Lab under the Department of Plant Pathology, SAU. Experiment was run in blotter paper method and artificial culture media under control conditions with Complete Randomized Design (CRD).

3.9.3. Preparation of Potato Sucrose Agar (PSA) Media

The composition of PSA media is given below:

Ingredient	Quantities
Potato	200g
Sucrose	20g
Agar	20g
Water	1 liter

Cleaned potatoes were taken in sterilized vessels and peeled with sterilized and sharp knife. Peeled potatoes were sliced and then boiled in 500 ml distilled water for half an hour. It was later filtered using a markin cloth. Sucrose was added and the extract was made up to one liter. 20g Agar powder was weighted and then added gently, stirred well using a glass rod to get uniform distribution. The media thus obtained was dispensed equally into 250 ml. @100 ml conical flask and sterilized. Then 1 ml lacto phenol was added after autoclaving in laminar airflow cabinet.

3.9.4. Isolation of *Magnaporthe oryzae*

Fungi was isolated by tissue planting method. Diseased rice leaves and neck portions of the infected stems which were collected from the field, were cut into small pieces of 5mm size along with healthy portion. Cut pieces were sterilized by the surface disinfectant Sodium hypochlorite (1%) for 3 minutes (Ellis, 2001). After sterilization the cut pieces were washed three times with sterilized water. The cut pieces were then placed on sterile paper towels to remove excess water. The cut pieces were then placed on the petri dish under sterile condition. The plates were labeled with necessary information and placed in the incubation chamber for 7 days at 25 ± 1^0C. During incubation they were exposed to NUV light for 12 hours per day. After 7 days of incubation, the fungi was grown on culture media. A portion of culture was taken on slide and observed under compound microscope and identified the pathogenic fungi that was *Magnaporthe oryzae* with the help of pertinent literature. The growing mycelia on petri plates were then placed in PSA media and then were incubated at 25 to 26°C for 7 days. After 7 days the fungi grown on the culture media. A portion of culture was taken on slide and observed under compound microscope and identified the pathogenic fungi that is *Magnaporthe oryzae* with the help of pertinent literature. The growing mycelia on Potato sucrose agar plates were incubated at 25^0 to 26°C for 48 to 72 hours to induce sporulation of the fungus. For cultural characterization the plates were further incubated for fifteen days. A piece of the young sporulated fungus was picked using a needle, under binocular microscope. The sector of growing mycelia was placed on a slide with a drop of glycerin, the mycelia was covered by a cover slip and observed under the microscope for classical characterization. Single spores were carefully picked and transferred to PSA in Petri dishes for incubation under 25 to 26°C for a maximum of 48 hours. Replicates of the same were stored as spore aseptically on slides and kept under sealed polythene bags wrapping with aluminum foil. The whole process was gone through sterile condition. In the next step a small portion of mycelia from the pure culture was collected by a needle and placed it on PSA media and then incubated for 3 days under 25 to 26°C.The best growth and sporulation was observed in this media. PSA media is the best media for the sporulation of blast pathogen.

Figure 6. Isolation of *Magnaporthe oryzae*

3.9.5. Cultural, Morphological and Sporulation study of *Magnaporthe oryzae*

Magnaporthe was identified based on their morphological growth pattern on PSA medium. Morphological variation was examined and characterized according to their conidial structure (size, shape, color and septation of conidia). Mycelial growth, conidiophore and conidia was observed under light microscope, at first low power and then with high power objective lens. Sporulation study was done by using hemaecytometer.

3.9.6. Storage of pure culture

After obtaining the pure culture of blast pathogen, *Magnaporthe oryzae*, the isolate was stored for further study. It was stored at -20°C for keeping the pure culture contamination free and viable**.**

3.9.7. Establishment of Koch's Postulate

Pathogenicity test was done in the net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. For this test, pathogen free viable seedlings were collected from the seedbeds. The 21 days old seedlings were transplanted in a "cup tray". A "cup tray" is a tray containing 50 small cup sized pot in a plastic tray. Its measurement was 30 inches × 18 inches. Each cup's diameter was 2 inches.

Figure 7. Plastic cup tray

Soil mixed with fertilizers was taken from the land and water was added for muddy consistency, and then the mud was placed in cup tray. One seedling per cup was transplanted in each cup and the tray contained total 50 seedlings. Everyday water was provided on the tray for two times to keep soil always moist. After 7 days of transplanting when the seedlings were at three to four leaf stage than inoculation of fungal inoculum in the plant was done. Inoculation was done by spraying spore suspension. Each plant was inoculated with the test pathogen isolate. Spore suspensions of $1x10^5$ spore/ml sprayed on leaves by using hand sprayer with 2.5 ml of 20% Tween 20 to 1000 ml spore suspension (Han *et al*., 2003). For maintaining environment that is needed for successful infection, a large plastic box was used. It was internally wrapped with wet paper to seal moisture. Then the cup tray containing inoculated plants was placed in the box. Nearly 95% humidity for 24 hours give better inoculation (Sreenivasaprasad *et al*., 2005). The box provided nearly 98% humidity. The tray was there for 24 hours in darkness for quick penetration. This box method is one of the successful and cheap method for quick penetration process. After that the tray was placed in natural condition. Then waited for development of blast symptoms on the leaves. The temperature in the net house during the study period were $24±5^0$ C in late January. After that the plants were taken care for 45 days after transplantation for necessary study.

(i). Cup tray filled with mud

(ii). Seedlings transplanted to cup tray

(iii). Diseased leaf collected from field

(iv). Pure culture of *Magnaporthe oryzae*

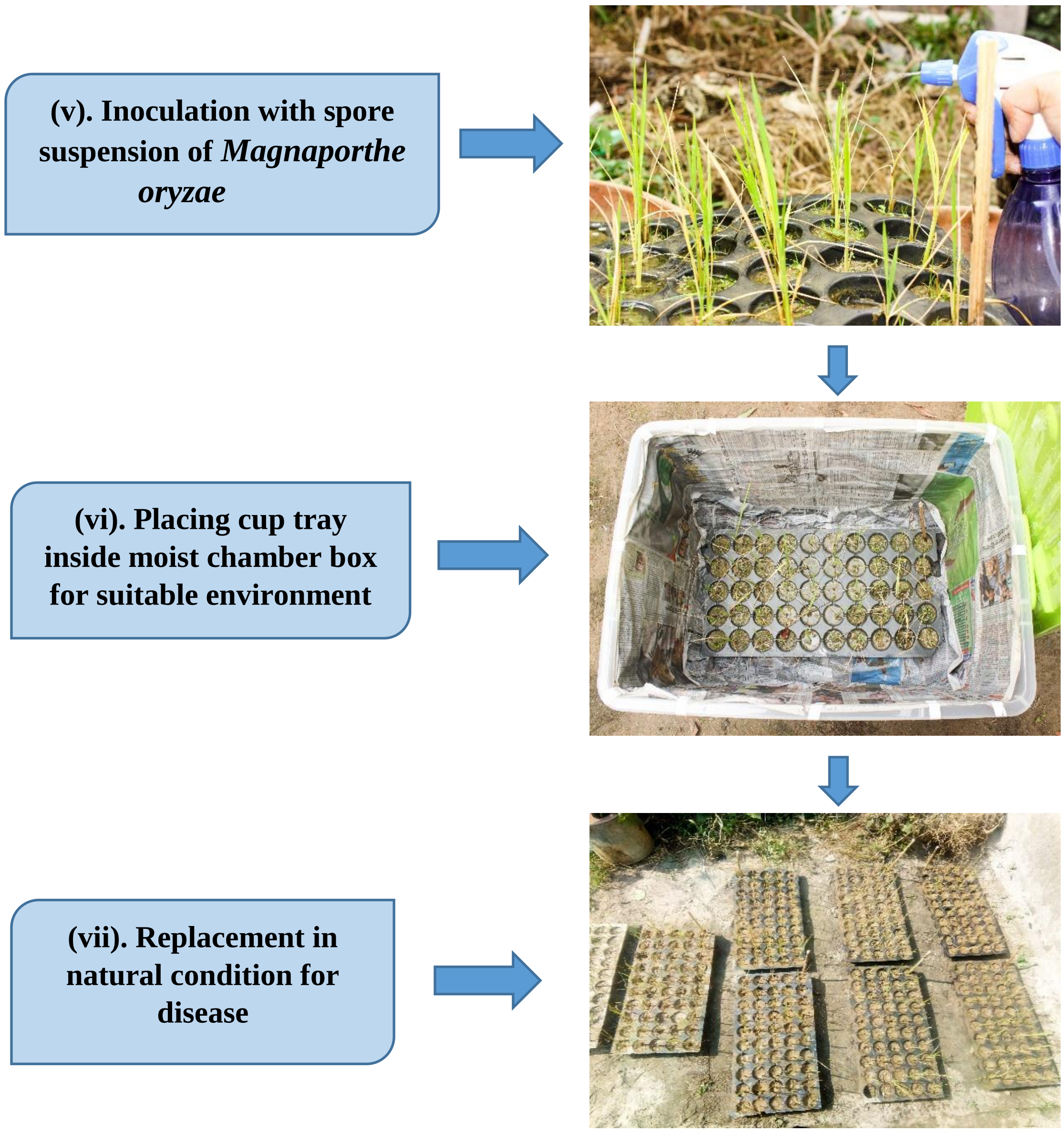


Figure 8. Steps involved in establishment of Koch's Postulate

Results

This chapter comprises the description and demonstration of the results obtained from the field experiment and the lab experiments on rice blast pathogen *Magnaporthe oryzae*. The result is being described in the following headings and sub-headings,

4.1. Field experiment

4.1.1. Effect of selected boro rice varieties on blast disease

4.1.1.1. Effect of selected boro rice varieties on the Disease incidence of rice blast

The effect of selected boro rice varieties on disease incidence of leaf blast was recorded at 65 and 85 days after transplanting (DAT). The incidence of blast was showed significant variants among the varieties at 65 DAT and ranged from 0.00 to 3.89%. In case of both selected high yielding varieties, the highest disease incidence was found in BRRI dhan 81 (3.33%) that was statistically similar with BRRI dhan 74 (2.78%). Among the inbred varieties, the highest disease incidence was recorded in BINA dhan 8 (3.89%) followed by BRRI dhan 58 (3.88%), BRRI dhan 28 (3.52%), BRRI dhan 29 and BINA dhan 10 (2.96%) which was statistically similar each and other. No disease incidence was found in wild variety (0.00%). The disease incidence varied significantly at 85 DAT and ranged from 0.00 to 21.48%. In case of both selected high yielding varieties, the highest disease incidence was found in BRRI dhan 81 (8.89%) that was statistically similar with BRRI dhan 74 (8.15%). Among the inbred varieties, the highest disease incidence was recorded in BRRI dhan 58 (21.48%) that was statistically different from other inbreed varieties and the lowest incidence was found in BINA dhan 10 (6.11%) that was statistically identical with BINA dhan 8 (7.04%). BRRI dhan 28 and BRRI dhan 29 showed moderate disease incidence at 85 DAT, both were statistically identical each and other. It was noted that the disease incidence of leaf blast was gradually increased with the increase of the age of plant. Again no disease incidence was found in wild variety. Results are presented in table 1.

Table 1. Effect of selected boro rice varieties on the Disease incidence of rice blast

Varieties	Disease Incidence (%)	
	@65 DAT	@85DAT
BRRI Dhan 28	3.52 a	11.67 bc
BRRI Dhan 29	2.96 a	12.22 b
BRRI Dhan 58	3.89 a	21.48 a
BINA Dhan 8	3.89 a	7.03 bc
BINA Dhan 10	2.96 a	6.11c
BRRI Dhan 74	2.78 a	8.89 bc
BRRI Dhan 81	3.33 a	8.15 bc
Wild variety	0.00 b	0.00 d
LSD Value	4.988	4.988
CV%	17.07	20.96

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

Figure 09. The highest DI showed in BRRI dhan 58

Figure 10. DI in BRRI dhan 74

Figure 11. No DI in Wild variety

4.1.1.2. Effect of selected boro rice varieties on the Disease severity of rice blast

The effect of selected boro rice varieties on disease severity of leaf blast was recorded at 85 DAT. The severity of blast was showed significant variants among the varieties and ranged from 0.00 to 60.48%. In case of both selected high yielding varieties, the highest disease severity was found in BRRI dhan 74 (26.52%). that was statistically similar with BRRI dhan 81 (26.13%). Among the inbred varieties, the highest disease severity was recorded in BRRI dhan 58 (60.48 %) that was statistically different from other inbred varieties and the lowest disease severity was found in BINA dhan 10 (23.03 %) that was statistically similar with BINA dhan 8 (25.82 %). BRRI dhan 28 and 29 was showed moderate disease severity at 85 DAT that was 38.18% and 37.58% respectively, both was statistically similar each and other. It was noted that the disease severity of blast in both hybrid and inbreed selected varieties was moderately resistant to susceptible. No disease severity was recorded in wild variety. Results of disease severity are presented in table 2.

Table 2. Effect of selected boro rice varieties on the Disease severity of blast of rice

Varieties	Disease severity (%)
BRRI dhan 28	38.18 b
BRRI dhan 29	37.58 b
BRRI dhan 58	60.48 a
BINA dhan 8	25.82 c
BINA dhan 10	23.03 c
BRRI dhan 74	26.52 c
BRRI dhan 81	26.13 c
Wild variety	0.00 d
LSD Value	4.988
CV%	6.58

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

Figure 12. The highest Disease Severity showed in BRRI dhan 58

4.1.1.3. Effect of selected boro rice varieties on tiller number and plant height (cm)

The tiller number showed significant variants among the varieties and ranged from 12.80 to 20.73 at 85 days after transplanting. In case of both selected high yielding varieties, the highest tiller number was found in BRRI dhan 81 (14.00) that was statistically different from BRRI dhan 74 (13.93). Among the inbred varieties, the highest tiller number was observed in the wild variety (20.73), that was statistically different from other inbred varieties. The lowest tiller number was observed in BINA dhan 8 (12.80), preceded by BINA dhan 10 (13.53), both varieties were statistically identical with each other. BRRI dhan 28 (15.53), BRRI dhan 29 (17.40) and BRRI dhan 58 (18.27) showed moderate tiller number, and were statistically identical with each other.

The height of the selected plants of the varieties was measured at 85 days after transplanting (DAT). The plant height was showed significant variants among the varieties and ranged from 93.07 to 106.07cm. In case of both selected high yielding varieties, the maximum plant height was found in BRRI dhan 81 (94.67 cm) that was statistically similar to BRRI dhan 74 (93.07 cm). Among the inbred varieties, the maximum plant height was observed in BINA dhan 10 (106.07 cm) that was statistically different from the other inbred varieties. The lowest plant height was recorded in BRRI dhan 29 (95.60 cm), that was also statistically different from the other inbred varieties. BRRI dhan 58 (101.80 cm), BRRI dhan 28 (101.40 cm) and the wild variety (98.78 cm) showed moderate plant height Results are presented in table 3.

Table 3. Effect of selected boro rice varieties on tiller number and plant height

Varieties	Tiller number	Plant height (cm)
BRRI dhan 28	15.53 ab	101.40 ab
BRRI dhan 29	17.40 ab	95.60 b
BRRI dhan 58	18.27 ab	101.80 ab
BINA dhan 8	12.80 b	99.47 ab
BINA dhan 10	13.53 b	106.07 a
BRRI dhan 74	13.93 b	93.07 b
BRRI dhan 81	14.00 ab	94.67 b
Wild variety	20.73 a	98.78 ab
LSD Value	4.988	4.988
CV%	14.83	3.12

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

4.1.1.4. Effect of selected boro rice varieties on panicle number and panicle length (cm)

The data of panicle number and panicle length of all the selected varieties was taken after harvesting. From the above table it can be easily observed that the panicle number showed significant variants among the varieties and ranged from 12.47 to 18.40. In case of both selected high yielding varieties, the highest panicle number was found in BRRI dhan 74 (63.80 cm) that was statistically different from BRRI dhan 81 (12.53). In case of inbred varieties, the highest panicle number was recorded in the variety BRRI dhan 28 (18.40), followed by wild variety (17.50), both varieties were statistically identical. Whereas the lowest was found in BINA dhan 8 (12.47), preceded by BINA dhan 10 (12.67), that were statistically similar with each other. BRRI dhan 58 (16.00) and BRRI dhan 29 (14.47) showed moderate panicle number that were statistically identical with each other.

The panicle length showed significant variants among the varieties and ranged from 20.90 cm to 24.90 cm. In case of both selected high yielding varieties, the highest panicle length was found in BRRI dhan 74 (21.89 cm) that was statistically different from BRRI dhan 81 (20.90 cm). In case of inbred varieties, the highest panicle length was recorded in the wild variety (24.90 cm), followed by BINA dhan 10 (24.58 cm), both varieties were statistically identical with each other. The lowest panicle length was recorded in BRRI dhan 58 (21.70 cm) that was statistically different from other varieties. BINA dhan 8 (24.15 cm) and BRRI dhan 29 (24.11 cm) showed moderate panicle length, that were statistically identical. Results are presented in table 4 and figure 13.

Table 4. Effect of selected boro rice varieties on panicle number and length (cm)

Variety	Panicle no.	Panicle length (cm)
BRRI dhan 28	18.40 a	21.75 bc
BRRI dhan 29	14.47 ab	24.11 ab
BRRI dhan 58	16.00 ab	21.70 bc
BINA dhan 8	12.47 b	24.15 ab
BINA dhan 10	12.67 b	24.58 a
BRRI dhan 74	15.20 ab	21.89 bc
BRRI dhan 81	12.53 b	20.90 c
Wild variety	17.50 a	24.90 a
LSD Value	4.988	4.988
CV%	10.70	3.87

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

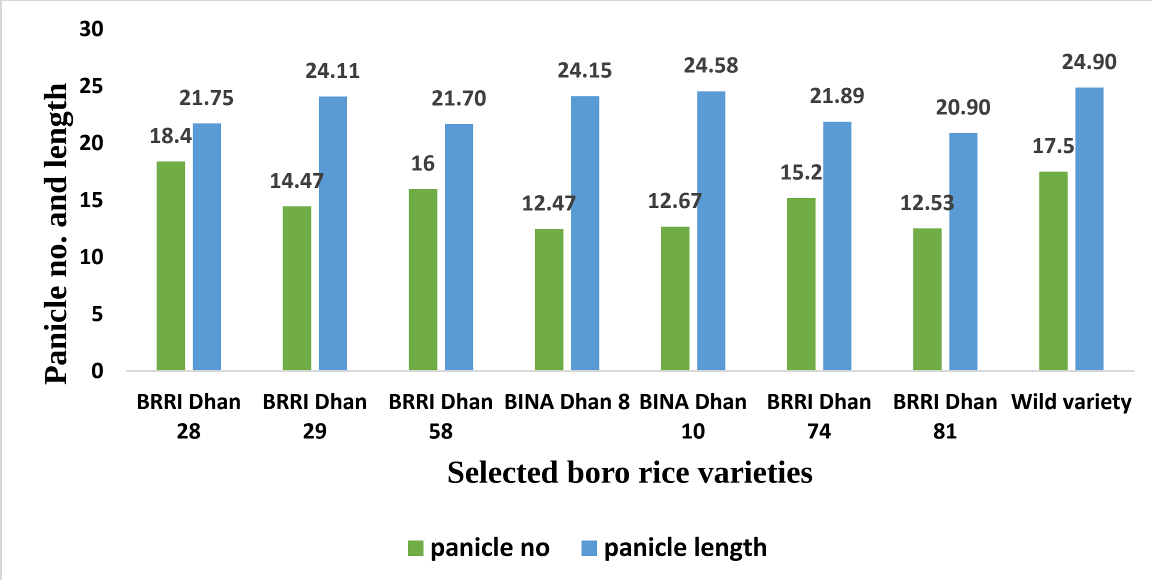


Figure 13. Effect of selected boro rice varieties on panicle number and panicle length (cm)

4.1.1.5. Effect of selected boro rice varieties on fresh weight (g) and dry weight (g)

Here, it can be seen that the fresh weight showed significant variants among the varieties and ranged from 47.33 g to 62.30 g. In case of both selected high yielding varieties, the maximum fresh weight was found in BRRI dhan 74 (50.03 g) that was statistically identical with BRRI dhan 81 (47.90 g). In case of inbred varieties the maximum fresh weight was observed in the wild variety (62.30 g) that was statistically different from the other varieties. The minimum fresh weight was recorded in BINA dhan 10 (47.33 g), preceded by BINA dhan 8 (48.66 g) and BRRI dhan 29 (51.69 g) that were statistically similar to each other. BRRI dhan 58 (55.27 g) and BRRI dhan 28 (53.57 g) showed moderate fresh weight, that were statistically identical with each other.

When considering dry weight, it showed significant variants among the varieties and ranged from 37.97 g to 51.70 g. In case of both selected high yielding varieties, the maximum dry weight was found in BRRI dhan 74 (40.95 g) that was statistically identical with BRRI dhan 81 (37.97 g). In case of inbred varieties the maximum dry weight was observed in the wild variety (51.70 g) that was statistically different from the other varieties. The minimum dry weight was recorded in BINA dhan 10 (38.34 g), preceded by BINA dhan 8 (39.65 g) and BRRI dhan 29 (41.81 g) that were statistically similar to each other. BRRI dhan 58 (43.59 g) and BRRI dhan 28 (44.24 g) showed moderate dry weight, that were statistically identical with each other. Results are presented in table 5.

Table 5. Effect of selected boro rice varieties on fresh and dry weight

Variety	Fresh wt. (g)	Dry wt. (g)
BRRI dhan 28	53.57 ab	44.24 ab
BRRI dhan 29	51.69 b	41.81 b
BRRI dhan 58	55.27 ab	43.59 ab
BINA dhan 8	48.66 b	39.65 b
BINA dhan 10	47.33 b	38.34 b
BRRI dhan 74	50.03 b	40.95 b
BRRI dhan 81	47.90 b	37.97 b
Wild variety	62.30 a	51.70 a
LSD Value	4.988	4.988
CV%	6.92	7.80

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

4.1.1.6. Effect of selected boro rice varieties on yield

The data of yield per plot showed no significant variants among the varieties and ranged from 4.77 to 6.63 kg/plot. In case of both selected high yielding varieties, the highest yield per plot was found in BRRI dhan 81 (5.43 kg/plot) that was statistically identical with BRRI dhan 74 (5.27 kg/plot). In case of inbred varieties, the highest yield per plot was observed in BINA Dhan 10 (6.63 kg/plot), followed by BRRI dhan 29 (6.53 kg/plot), BINA dhan 8 (6.27 kg/plot), BRRI dhan 58 (6.00 kg/plot), BRRI dhan 28 (5.96 kg/plot) and the wild variety (4.77 kg/plot), all the varieties were statistically similar to each other.

The data of yield per hectare showed no significant variants among the varieties and ranged from 6.95 to 9.67 ton/ha. In case of both selected high yielding varieties, the highest yield per hectare was found in BRRI dhan 81 (7.92 ton/ha) that was statistically identical with BRRI dhan 74 (7.68 ton/ha). In case of inbred varieties, the highest yield per hectare was observed in BINA dhan 10 (7.68 ton/ha), followed by BRRI dhan 29 (9.52 ton/ha), BINA dhan 8 (9.13 ton/ha), BRRI dhan 58 (8.75 ton/ha), BRRI dhan 28 (8.68 ton/ha) and the wild variety (6.95 ton/ha), all the varieties were statistically similar to each other. Results are presented in table 6.

Table 6. Effect of selected boro rice varieties on yield

Variety	**Yield (kg/plot)**	**Yield (ton/ha)**
BRRI dhan 28	5.96 a	8.68 a
BRRI dhan 29	6.53 a	9.52 a
BRRI dhan 58	6.00 a	8.75 a
BINA dhan 8	6.27 a	9.13 a
BINA dhan 10	6.63 a	9.67 a
BRRI dhan 74	5.27 a	7.68 a
BRRI dhan 81	5.43 a	7.92 a
Wild variety	4.77 a	6.95 a
LSD Value	4.988	4.988
CV%	11.75	11.76

Values followed by different letters within a column are significantly different at 5% level of significance by DMRT.

4.2. Lab experiment

4.2.1. Isolation of *Magnaporthe oryzae*

At first the pathogen, *Magnaporthe oryzae* was isolated in Potato Sucrose Agar (PSA) media and purified. At initial stage, the pathogen produced white mycelial growth and later on it turned into black in color. Sporulation was observed after 25-35 days of culture maintenance. Almost same media was also used for isolation of *Pyricularia grisea* by Motlagh and Javadzadeh, 2010), Priya Vanaraj *et al.* (2013) and Suman dutta *et al.* (2017), and they also observed the almost same colored mycelial growth in PSA media but no observed any sporulation even after 2-3 months of culture maintenance in proper temperature and moist conditions.

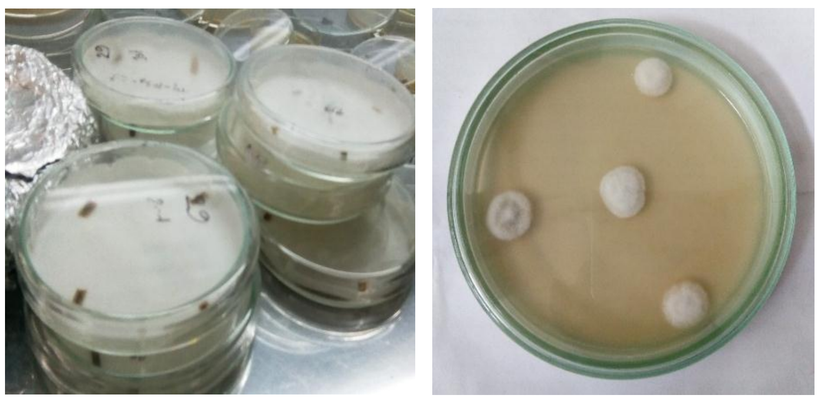


Figure 14. Diseased samples placed on blotter for the Isolation of *Magnaporthe oryzae*

4.2.2. Cultural characterization of *Magnaporthe oryzae*

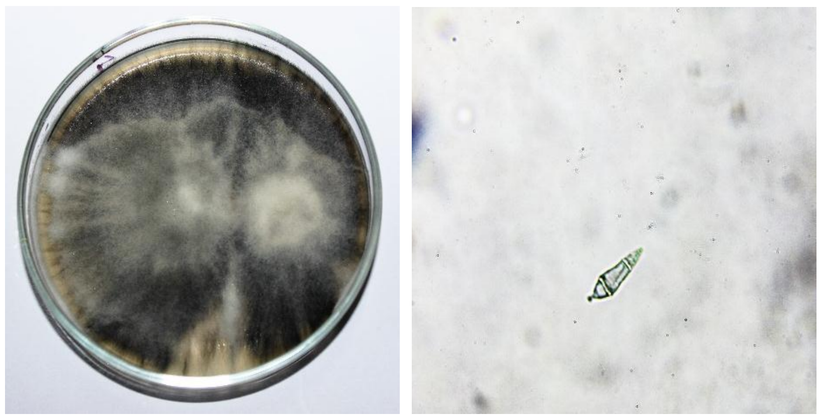
The pathogen was grown in Potato Sucrose Agar (PSA) media. The mycelial growth was recorded at 7, 14 and 21 days after inoculation. From the study it was found that the highest growth was recorded in Potato Sucrose Agar (PSA) at 21 days after inoculation (figure 15).

Figure 15. Conidium of *Magnaporthe oryzae*

4.2.3. Pathogenicity test

Pathogenicity test must be performed in order to prove the pathogenic nature and structure. The test showed that the typical disease symptoms were developed on the leaves 5 to 6 days after inoculation and increased rapidly. The rice blast fungus, usually caused infection at all growth stages of the rice plants. The disease initially appeared as whitish or greyish pin point spots along the leaf margins between 7 to 12 days after inoculation. Later on they turned into elliptical spots which were elongated and nearly spindle shaped with pointed ends. Those spots became necrotic in the centre with brown or reddish-brown margins. Those spots collapse each other and forms large lesions within 20-25 days after inoculation. The diseased leaves were collected, sterilized and placed on PSA media aseptically and re-isolation was done. The observation of the characteristic elliptical spindle shaped lesions with grey centres on the leaves and the re-isolation of the pathogen from infected rice seedlings showed that *Magnaporthe oryzae* incited the leaf and neck blast disease of rice in this study. The colony characteristics and conidial attributes derived from the culture of re-isolated fungi were compared with that of the isolates mentioned before. The hyaline pyriform conidia derived from the re-isolated cultures was a confirmation for the hypothesis that the disease causing pathogen was *Magnaporthe oryzae*.

a. Disease developed in tray plant for pathogenicity test



b. Pure culture and conidium of *Magnaporthe oryzae*

Figure 16. Pathogenicity test

DISCUSSION

Rice (*Oryza sativa* L.) is one of the most important cereals of the world second in rank next to wheat in terms of consumption rate (Yaduraju, 2013). In most of the Asian countries including Bangladesh, it is consumed as a staple food and is consumed by 50% of the world population (Luo *et al*, 1998). Among the reported rice diseases in the world, Rice blast caused by *Magnaporthe oryzae* is a serious fungal disease of rice that is threatening global food security. It has been extensively studied due to the importance of rice production and consumption, and because of its vast distribution and destructiveness across the world. In Bangladesh, the main constrains of rice production is also different kinds of diseases. Rice Blast disease has been identified as a devastating disease in all over Bangladesh since last few years. Both boro and aman rice was infected by blast pathogen, but in boro rice it was appeared as a severe outbreak and caused great yield losses. Rice blast is a great threat in rice production as well as threat to our existence and national economic growth (Jabeen *et al.*, 2012). Although rice production has improved substantially over time, it is inadequate to cope with the increasing global demand (Sasaki and Burr 2000).

Rice blast, caused by *Magnaporthe oryzae*, can infect aboveground tissues of rice plants at any growth stage and can cause total crop failure. The pathogen produces lesions on leaves (leaf blast), leaf collars (collar blast), culms, culm nodes, panicle neck nodes (neck rot), and panicles (panicle blast), which vary in color and shape depending on varietal resistance, environmental conditions and rice plant age. There is no resistant variety of rice still identified against blast disease and no specific treatment to control this disease (Ganesh *et al.*, 2012). To ensure our food security, necessary steps must be taken to manage this disease. Some popular varieties that are mostly cultivated in Bangladesh, become highly susceptible to rice blast pathogen. Considering the above mentioned facts, the current study was conducted to assess the mostly cultivated boro rice varieties in Bangladesh against rice blast pathogen, and to establish Koch’s postulate through pathogenicity test. The whole study hereby conferred with some headings and subheadings.

4.3. Symptomology of rice blast disease

Blast symptoms appear on leaves and panicles. Characteristic blast symptoms were showed in the leaves such as; brown colored spindle shaped spots with greyish centre was found on leaves. In severely affected leaves spots were gradually coalesced together and blighted the whole leaf area. Dark brown to black colored lesion was found on infected panicle represented the characteristic panicle blast symptoms. Out of two type’s symptoms, neck blast was more destructive. Neck blast was appeared the most destructive phase of the disease and can occur without being preceded by severe leaf blast. Symptomology recorded in the current study is almost similar to some recent studies (Zhu *et al.,* 2005, Sreenivasa *et al.,* 2011).

4.4. Disease incidence and severity of rice blast

Disease incidence was recorded three times at 45, 65 and 85 DAT. The disease incidence of rice blast was showed significant variants among the varieties and ranged from 0.00 to 3.89% and 0.00 to 21.48 at 65 and 85 DAT respectively. It was observed that there was no disease incidence found at 45 DT in all the selected varieties including selected wild variety. The highest disease incidence was recorded in BRRI dhan 58 at 65 and 85 DAT. Other varieties showed moderate disease incidence that was statistically not significant each and other. It was observed that there was no disease incidence found in selected wild variety throughout the growing season. The effect of selected boro rice varieties on disease severity of leaf blast was recorded only one time at 85 DAT. The severity of rice blast was showed significant variants among the varieties and ranged from 0.00 to 60.48%. In case of both selected high yielding varieties, the highest disease severity was found in BRRI dhan 74 that was statistically similar with BRRI dhan 81. Among the inbred varieties, the highest disease severity was recorded in BRRI dhan 58 that was statistically different from other inbred varieties and the lowest disease severity was found in BINA dhan 10 that was statistically similar with BINA dhan 8. BRRI dhan 28 and 29 was showed moderate disease severity at 85 DAT that was 38.18% and 37.58% respectively, both was statistically similar each and other. It was noted that the disease severity of blast in both high yielding and inbred selected varieties was moderately resistant to susceptible. No disease severity was recorded in wild variety i.e., according to severity scale used in the study (IRRI recommended grading scale by Dr. Horsfall and Barrett 1945), the selected wild variety was showed tolerance/highly resistance to blast pathogen. Results from the study are supported by previous reports. During 2016, serious yield losses occurred due to epiphytotic of rice blast caused by *Magnaporthe oryzae* have been recorded in different regions in Bangladesh such as Dinajpur, Rangpur, Thakurgaon, Panchagarh, Kushtia, Jashore, Pabna, Barishal, Mymensingh, Munshigonj, Chuadanga etc. and the production of boro rice and transplanted aman in Bangladesh were greatly reduced due to blast infestation with 21.19% and 11.98% disease severity. Most popularly affected boro rice was BRRI dhan 28 (29.6% disease severity) followed by BRRI dhan 29 (25.9% disease severity) BRRI dhan 61 (21.9% disease severity) and T. Aman rice was BRRI dhan 34 (22.9% disease severity) (Hossain and Ali, 2017). Rice blast can infect above ground tissues of rice plants at any growth stage and cause total crop failure.

4.5. Performance in yield and yield contributing characters of selected boro rice varieties against blast disease in the field condition

The tiller number of selected varieties was recorded and showed significant variation (12.80-20.73). The highest number of tillers were recorded in wild variety (20.73) followed by BRRI dhan 58, BRRI dhan 29, BRRI dhan 28 and BRRI dhan 81, all are statistically identical each and others. The lowest number of tillers were recorded in BINA dhan 8 (12.80) preceded by BINA dhan 10 and BRRI dhan 74 which are statistically similar each and other. The plant height of selected varieties was recorded after panicle initiation. The highest plant height was recorded in

BINA dhan 10 (106.07 cm) followed by BRRI dhan 58, BRRI dhan 29, BINA dhan 10 and selected wild variety, all are statistically identical each and others. The lowest plant height was recorded in BRRI dhan 74 (93.07 cm) preceded by BRRI dhan 81 and BRRI dhan 29, all are statistically similar each and others. Maximum number of panicles per hill were obtained in BRRI dhan 28 (18.40) and wild variety (17.50), both are statistically similar. Minimum number of panicles per hill were found in BINA dhan 8 and 10 (12.47 and 12.67 respectively), both are statistically similar. The highest panicle length was recorded in wild variety (24.90 cm) followed by BINA dhan 10, BINA dhan 8 and BRRI dhan 29, which are statistically identical. The lowest panicle length was recorded in BRRI dhan 81 (20.90 cm) preceded by BRRI dhan 28, BRRI dhan 58 and BRRI dhan 74, which are statistically identical. Maximum fresh and dry weight was recorded in the wild variety, BRRI dhan 28 and BRRI dhan 58, while minimum fresh and dry weight was found in BINA dhan 10, BRRI dhan 81 and BINA dhan 8. The yield performance of different varieties was recorded after harvesting. In case of both hybrid varieties, yield was almost same (7.92 and 7.68 ton/ha). Among the inbred varieties, almost same yield was obtained in BINA dhan 10, BRRI dhan 29 and BINA dhan 8 (9.67, 9.53 and 9.14 ton/ha respectively) and from BRRI dhan 28 and BRRI dhan 58 obtained the same yield that was 8.68 and 8.75 ton/ha respectively. From the selected wild variety yield was recorded 6.95 ton/ha. Regarding the yield performance of tested boro rice varieties, it was showed statistically non-significant.

4.6. Isolation of rice blast pathogen

At first the rice blast pathogen, *Magnaporthe oryzae* was isolated in Potato Sucrose Agar (PSA) media and purified. At initial stage, the pathogen produced white mycelial growth and later on it turned into black in colour. Sporulation was observed after 25-35 days of culture maintenance. Almost same media was also used for isolation of *Magnaporthe oryzae* by Motlagh and Javadzadeh, 2010), Priya Vanaraj *et al.* (2013) and Suman dutta *et al.* (2017), and they also observed the almost same colored mycelial growth in PDA media but did not observe any sporulation even after 2-3 months of culture maintenance in proper temperature and moist conditions.

4.7. Morphological and cultural characters of the *Magnaporthe oryzae*

In the study, the pathogen was re-isolated and grown in PSA media for sporulation purpose. Conidiophores were either single or in fascicles, simple, rarely branched, showing sympodial growth. Conidia were pyriform (pear-shaped with pointed tip), narrowed toward tip, rounded at the base, 2 septate, hyaline in color with a distinct basal hilum. The size of conidia was measured using ocular-micrometer and found about 22.13 to 28.47 µm × 9.13 to 11.72 µm (average 25.30 × 10.43 µm). Bonman *et al.,* (1992) conducted a research on *Magnaporthe oryzae*. For isolation of *Magnaporthe oryzae,* rice blast infected leaves were collected and each lesion was placed in a moist petri dish and incubated at 25^{o}C for isolation purpose. PDA was used for isolation of *Magnaporthe grisea* by Motlagh and Javadzadeh (2010) and Priya Vanaraj *et al*., (2013). Leong (2004), Mukund Variar *et al*., (2006) and Mebratu *et al.*, (2015) observed *Magnaporthe* culture black to olive gray color. Sun *et al*., (1989) reported that rice straw agar media is most supporting medium for sporulation of *M. oryzae* and observe that the spore measurements were 15 to 22 µm × 4 to 7 µm (Average, 17.4 µm × 5.2 µm). Mijan Hossain (2000) also observed *Magnaporthe* cultures and reported that, mycelia were first hyaline in color, then changed into olivaceous. It was observed that mostly 2 celled conidia were found from rice grain media and 3 celled conidia were found in infected leaf sample.

4.8. Pathogenicity test for establishment of Koch's Postulate

Pathogenicity test showed that the typical disease symptoms were developed on the leaves after 7th days of inoculation and increased up to 30 days. The rice blast fungus caused infection at all growth stages of the rice plants. The disease initially appeared as whitish or greyish specks along the leaf margins between 7 to 12 days after inoculation. Later on they turned into elliptical spots which were elongated and nearly diamond shaped with pointed ends. Those spots became necrotic in the centre with brown or reddish-brown margins. Those spots collapse each other and forms large lesions within 20-25 days after inoculation. The fungi were re-isolated from the infected leaves of the plants. The observation of the characteristic elliptical spindle shaped lesions with grey centres on the leaves and the re-isolation of the pathogen from infected rice seedlings showed that *Magnaporthe oryzae* incited the leaf blast disease in this study. The colony characteristics and conidial attributes derived from the culture of re-isolated fungi were compared with that of the isolates from naturally infected leaf samples. The hyaline pyriform conidia derived from the re-isolated cultures was a confirmation for the hypothesis that the disease causing pathogen was *Magnaporthe oryzae*. Pathogenicity test was conducted for *Magnaporthe oryzae* by Perello *et al.*, (2015). Artificially inoculated Plants were incubated in a moist chamber at 22°C and 100% RH for 48 hours. Inoculated plants gave symptoms which were similar to those observed in the field. Dutta (2017) conducted Koch's postulate for *Magnaporthe* for the conformity of the Pathogen and found the exact fungus and symptoms from artificially inoculated plants that was found in natural condition.

Summary and Conclusion

Rice is the most important cereal crop and staple food of Bangladesh. It is central to economy, accounting for nearly 20 percent of gross domestic product (GDP) and providing about one-sixth of the national income of Bangladesh. This disease is distributed in 85 countries in all continents where, there rice is cultivated, in both paddy and upland conditions. The incidence and severity of blast disease is very common in Bangladesh as in that period the water scarcity is universal compared to aman season. In Bangladesh the frequency of blast occurrence has increased with invasion into new areas (north and north-west parts of the country) in recent years. The BRRI dhan 28 and BRRI dhan 29 are the most popular and mega varieties recognized highly susceptible to blast disease. The incidence of rice blast was recorded in boro season (November to May: irrigated ecosystem) and Transplanted aman (July to December: rain fed ecosystem) in all over Bangladesh. The government of Bangladesh is taking initiative to the extension of the aus coverage. It means rice is grown with intensive care throughout the year. Thus the popular varieties are getting susceptible to some pest and diseases. Even the pests are getting the opportunities to change their races for better adaptation. At present 267 races of rice blast have already been identified in our environment. Thus disease pressure is in increasing trend and may be turn into a devastating experience in the near future. Hence, rice blast is considered as a serious and recurrent problem in many of the rice growing countries. The blast is a trans-boundary disease, travels through the air from one region to the other, one country to another country. Infected seed also act as a carrier too. The favorable weather is low night temperature (conversely higher day temperature), unusually windy and foggy weather in the morning, and dew on the leaf, drizzle etc. The drizzle brings down the spores (seeds of fungi able to grow as new fungi) on the leaf, node or on panicles. No sooner a spore drops on the rice plant it starts growing to produce conidiophore (a fungal hypha) and conidium (asexually produced spore) within 3-

4 days. Within 7-10 days it starts producing millions of spores if favorable conditions prevail to cause a devastating impact on the rice crop.

The primary goal of the current study was to find out the resistant/tolerant boro rice varieties from mostly cultivated boro rice varieties against blast disease caused by *Megnaporthe oryzae*. The prime goal of the study was to assess the selected boro rice varieties against blast pathogen in natural condition as well as to study the etiology of *Magnaporthe oryzae* and to establish the Koch's postulate through pathogenesis. From the study, it was revealed that the incidence of rice blast showed significant variants among the selected varieties and ranged from 0.00 to 21.48%. In case of both selected high yielding varieties, the highest disease incidence was found in BRRI dhan 81 (8.89%) that was statistically similar with BRRI dhan 74 (8.15%). Among the inbred varieties, the highest disease incidence was recorded in BRRI dhan 58 (21.48%) that was statistically different from other inbred varieties and the lowest incidence was found in BINA dhan 10 (6.11%) that was statistically identical with BINA dhan 8 (7.04%). BRRI dhan 28 and BRRI dhan 29 showed moderate disease incidence, both was statistically identical each and other. It was noted that the disease incidence of leaf blast gradually increased with the increase of the plant age. The severity of rice blast was also showed significant variants among the selected varieties and ranged from 0.00 to 60.48%. In case of both selected high yielding varieties, the highest disease severity was found in BRRI dhan 74 (26.52%). that was statistically similar with BRRI dhan 81 (26.12%). Among the inbred varieties, the highest disease severity was recorded in BRRI dhan 58 (60.48%) that was statistically different from other inbred varieties and the lowest disease severity was found in BINA dhan 10 (23.03%) that was statistically similar with BINA dhan 8 (25.82%). BRRI dhan 28 and BRRI dhan 29 showed moderate disease severity that was 38.18% and 37.58% respectively, both were statistically similar each and other. It was noted that the disease severity of rice blast in both high yielding and inbred boro rice varieties showed susceptibility to moderate resistance. No disease incidence and severity was found in wild variety i e., selected wild variety showed tolerance/resistance to rice blast pathogen.

The rice blast pathogen, *Magnaporthe oryzae* was isolated in Potato Sucrose Agar (PSA) media and purified. At initial stage, the pathogen produced white mycelial growth and later on it turned into black in color. Sporulation was observed after 25-35 days of culture maintenance. From the morphological study of the blast pathogen, it was observed that conidiophores remain either single or in fascicles, simple, rarely branched, showing sympodial growth. Conidia were pyriform (pear-shaped with pointed tip), narrowed towards tip, rounded at the base, 2 septate, hyaline in color with a distinct basal hilum. Pathogenicity test showed that the typical disease symptoms were developed on the leaves after 7th days of inoculation and increased up to 30 days. The observation of the characteristic elliptical spindle shaped lesions with grey center on the leaves and the re-isolation of the pathogen from infected rice seedlings showed that *Magnaporthe oryzae* incited the leaf blast disease in this study. The colony characteristics and conidial attributes derived from the culture of re-isolated fungi were compared with that of the isolates from naturally infected leaf samples. The hyaline pyriform conidia derived from the re-isolated cultures was a confirmation for the hypothesis that the disease causing pathogen was *Magnaporthe oryzae*. However, from the study, it was found that the selected wild rice variety showed tolerance/resistance, and four selected mostly cultivated boro rice varieties showed moderate resistance to blast pathogen *Magnaporthe oryzae*. But BRRI dhan 28 and BRRI dhan 58 showed highly susceptibility to blast pathogen. The etiology and pathogenesis study also confirmed the hypothesis that the rice blast disease causing pathogen was *Magnaporthe oryzae*.

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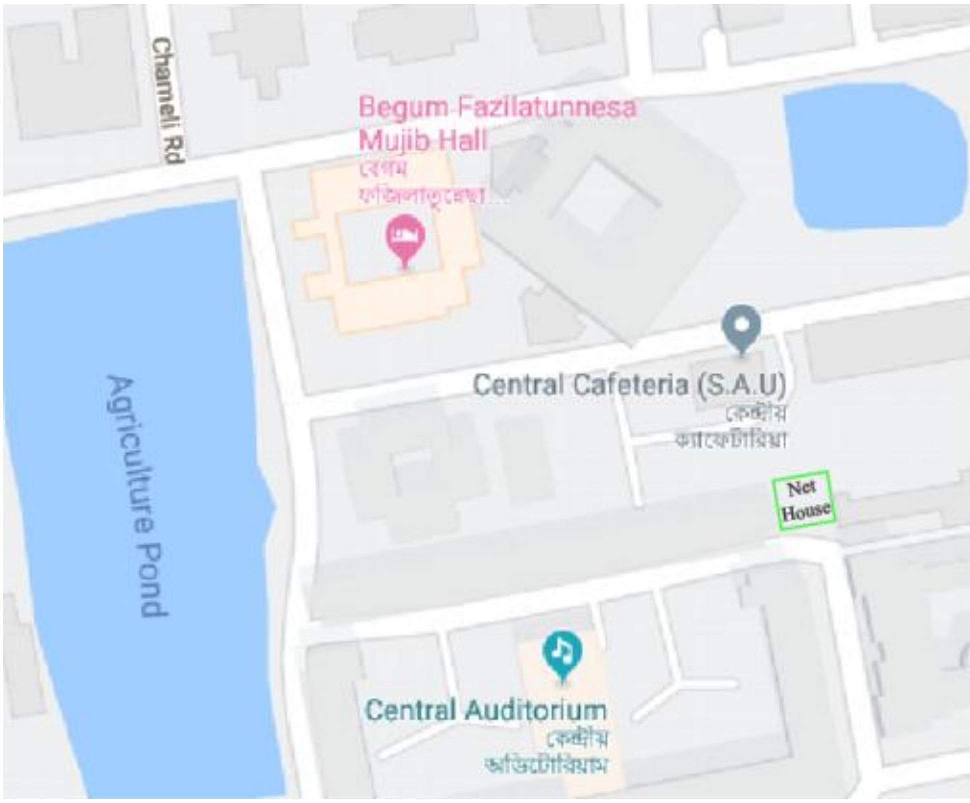
APPENDICES

Appendix-I. Map showing the experimental site under study



Appendix-II. Physiochemical properties of soil of the experimental field

Characteristics	Value
Sand (%)	25.67
Silt (%)	53.86
Clay (%)	20.48
Texture	Silty loam
pH	5.7-7.1
Organic carbon (%)	0.30
Organic matter (%)	0.55
Total N (%)	0.028
Phosphorus(µg/g soil)	23.59
Exchangeable K(milliequivalent/100 g soil)	0.61
Sulfur (µg/g soil)	28.45
Zinc (µg/g soil)	2.32

Source: Soil Resources Development Institute (SRDI), Dhaka-1207

Appendix-III. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour of the experimental period (October 2017- March 2018)

Month	Average RH (%)	Average Temperature (ºC)		Total Rainfall (mm)	Average Sunshine hours
		Min.	Max.		
October	79	25	32	175	6
November	65	21	30	35	8
December	74	15	29	15	9
January	68	13	24	7	9
February	57	18	30	25	8
March	57	20	33	65	7

Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka-1207.

Appendix-IV. The experimental field

Appendix-V. Sprouted rice seeds

Appendix-VI. Seedlings in plastic cup trays

Appendix-VII. Diseased leaves collected from the field and isolates of *Magnaporthe oryzae*