EVALUATION OF SOME SELECTED RICE GENOTYPES AGAINST *MAGNAPORTHE ORYZAE*

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CERTIFICATE

This is to certify that thesis entitled, "Evaluation of some selected rice genotypes against Magnaporthe oryzae" 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 MRINMOY KUMAR ROY TANU bearing Registration No. 14-05816 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: 20/02/2022

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DEDICATED

TO

MY BELOVED PARENTS

AND

ELDER BROTHER

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ABSTRACT

Rice blast caused by *Magnaporthe oryzae* is one of the most important diseases in rice growing areas of the world. It is one of the most devastating rice diseases which can lead a complete failure of the crop under severe infection. Development of durable blast resistant variety is the best option to control the disease. Thirty rice germplasm, collected from Bangladesh Rice Research Institute (BRRI) Gene bank and various districts of Bangladesh including one susceptible check, US2 were evaluated to find out new sources of resistance and assess their diversity based on the reactions against *M. oryzae* in uniform blast nursery (UBN) of BRRI during June 2019 to December 2020. While five germplasm viz. Shakkhor Khora, Shompa Kathari (Indian Variety), Shail Dhan, BR 16, BRRI Dhan 30 were observed resistant and Kalijeera was found moderately resistant (disease score 2-3). The 30 genotypes were grouped in 3 clusters. The grouping of some genotypes in same cluster is based on their similar reaction against leaf blast. The results of this study can be useful for the development of blast-resistant rice varieties.

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is that the supply of subsistence for quite one third of human population. it's the most staple food at intervals the Asia and thus the Pacific region, providing nearly thirty-nine you look after calories (Yaduraju and Rao, 2013). it's one amongst the traditional cultivated crops (Islam and Catling, 2012; Qiu *et al*., 2019) belong to the family Gramineae (former Gramineae) (USDA-NRCS, 2019).

Bangladesh could be a little developing country with largely associate with agro-based economy. Agricultural sector plays a crucial role within the overall economic development and food security of this extremely inhabited country. Agriculture is that the mainstay of Bangladesh economy and it contributes concerning 32 % of the gross domestic product (GDP). or so, 6.20 % of GOP has been derived from crops whereas rice alone contributes 9.5 % to the agricultural gross domestic product (BBS, 2019). Most of the thirteen million farm families of the country grow rice. concerning seventy fifth of the complete cropped space and over eightieth of the complete irrigated space is planted to rice (BBS, 2019).

Bangladesh is that the fourth prime rice manufacturing countries round the world with three, 265,000 MT of rice production. Rice is that the staple food of concerning 166.5 million folks in Bangladesh (BBS, 2021). It provides nearly 46% of provincial work, around two-third of absolute calorie offer and around one-portion of the whole macromolecule admission of a traditional individual within the nation (Rahman, 2017). In Bangladesh, rice sector contributes to common fraction of the agricultural gross domestic product and sixth of the value (Elahi, 2017). most of the fifteen million farm families of the country grow rice (Ghosh *et al*., 2017). Rice is mature on concerning 114 million hectares that has remained nearly stable over the past 3 decades (BBS, 2021). Rice is planted on concerning seventy fifth of the whole cropped space and over eightieth of the whole irrigated space (BBS, 2017). Thus, rice plays a serious role within the resource of the folks of Bangladesh.

The low production of rice is attributed to abiotic and organic phenomenon stresses, poor grain quality and lack of recent improved and custom-made varieties. The abiotic stresses are drought, cold, salinity, acidity and iron toxicity whereas the organic phenomenon factors embody pests, weeds and diseases. Pests like rice dipterous insect cause nice losses within the field (Onyango,

2014). Weeds like striga species and false ragi contend with rice for nutrients, water, and lightweight so busy with its growth. They conjointly harbor pests and manufacture root exudates that have an effect on chemical action so reducing yield (Dzomeku *et al*., 2007). Johnson (1996) unconcealed that yield loss in fields vary between 20 - 100% betting on weeds management levels by farmers. Additionally, rice is attacked by many infectious agent, flora and microorganism diseases that harm numerous elements of the crop (Webster and Gunnell, 1992; Jabeen *et al*., 2012). Researchers have unconcealed that the main diseases are rice blast, brown spot, microorganism blight and leaf streak, sheath blight, sheath rot, wilt disease, stem rot, Tungro virus and smut (Sharma and Bambawale, 2008).

Globally, rice blast is that the most damaging sickness caused by plant life acknowledged *Pyricularia oryzae* (Koultroubas *et al*., 2009). it's been calculable to destroy rice leading to economic losses of over $70 billion (Scheuermann *et al*., 2012). The plant life happens in 85 countries worldwide (Scardaci *et al*., 2003). continent is among the regions most affected wherever nearly four-hundredth of the rice consumed is foreign (Seck *et al*., 2012). In Kenya, an important eruption of rice blast was rumored in 2007 and over the last six years there has been a discount of 26% in production.

The sickness has been managed through numerous ways like use of resistant varieties, cultural practices and treatment with fungicides (Ribot *et al*., 2008). Cultural practices are inexpensive measures and embody burning sickness straw, cacophonic N chemical and water management. However, wherever the setting is favorable for blast, these practices are seldom economical. Chemical management has been used effectively in India, Japan and Philippine to cut back rice blast incidence and severity (Kumbhar, 2005). In Kenya, the few registered fungicides to manage rice blast are terribly high-ticket, un-friendly to the setting and lead to the event of resistance to infectious agent. The foremost effective means for resource-poor farmers is growing of resistant cultivars (Sharma *et al*., 2012). However, the offered resistant varieties have undesirable traits for shopper satisfactoriness. Rice improvement through breeding offers a property resolution. Through breeding, desired traits like higher yield and sickness resistance are often introduced to the custom-made varieties. this can improve rice productivity, scale back rice import bills, give extra financial gain to the poor and result to property management of the sickness (Seck *et al*., 2012).

Rice is that the most genetically and morphologically wide-ranging crop. Bangladesh has affluent rice genetic diversity within the past. In 1982, a survey conducted by BRRI found that 12487

totally different rice varieties were still cultivated in numerous seasons (Biswas, 2012). Nowadays BRRI features an assortment of quite 8000 rice germplasms (BRRI, 2019). Among them around 6000 native germplasm that aren't however characterized against blast resistance. These native germplasms may need possessed novel genes for blast resistance thanks to its wide-ranging genetic characteristics. Screening of those germplasm each phenotypically and with tightly joined marker might determine some novel blast resistance genes which may utilize for study blast resistant selection.

The objective of the present study is to screen rice genotypes against *Magnaporthe oryzae*.

CHAPTER II

REVIEW OF LETERATURE

The available literature of work done on blast disease of rice has 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

FAO (2018) 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 Tones to 476 million tons), trade by 8% (from 38 million tons to 41 million tons) and consumption by 3% (from 469 million tons to 481 million tons) (Commodity profile for rice - January 2015).

Yaduraju and Rao (2013) studied that rice is the main staple food in the Asia and the Pacific region, providing almost 39% of calories.

Kumar *et al.,* (2012) stated that rice is one of the most important cereal crops of developing countries and the staple food of about 65% of the world's population. Rainfed lowland rice is the second most important rice ecosystem, representing about 25% of the total rice production area. FAO (2009) stated in their annual report that rice is grown on 161 million hectares worldwide, with an annual production of about 678.7 million tons of paddy. 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.

Von Braun (2007) stated the importance of rice as 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. Its production is concentrated in Asia (90%) in subsistence agriculture farms with the grain destined for local consumption and only 4% exported to international markets. Fifty percent of the production area is located in China and India.

Seebold *et al.,* (2004) studied that *Oryza glaberrima* is traditionally found in diverse West African agroecosystems but it is largely abandoned in favor of high yielding *Oryza sativa* cultivar that has higher agronomic performance.

Luo *et al.,* (1998) studied that rice (*Oryza sativa* L.) is one of the most important cereals of the world and is consumed by 50% of the world population.

Faure and Mazaud (1996) stated in their study that rice is the most economically important staple food 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. It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories. In several developed countries such as North America and European Union (EU) also, rice consumption has increased due to food diversification and immigration.

FAO (1995) reported that rice is the most economically important staple food 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.

Oryza sativa cultivars are often not sufficiently adapted to various abiotic and biotic conditions in Africa. *Oryza glaberrima* has been found to have several useful traits like being moderate to high in their level of resistance to blast, rice yellow mottle virus, rice gall midges, insects and nematodes (Reversat, 1995; Silue and Notteghem, 1991; Alam, 1988; Attere and Fatokun, 1983).

Jones *et al.,* (1993) and Sano *et al.,* (1984) studied that the rice variety *Oryza glaberrima* has also been found to be tolerant to abiotic stresses such as acidity, iron toxicity, drought, and weed competition.

Khush and Toenniessen (1991) studied that its production is concentrated in Asia (90%) in subsistence agriculture farms with the grain destined for local consumption and only 4% exported to international markets. Fifty percent of the production area is located in China and India.

Silue and Notteghem (1991) studied that there are two species cultivated *Oryza sativa* L. (Asian rice) and *Oryza glaberrima* Steud (African rice).

2.2. Significance of blast disease of rice

Shahriar *et al.,* (2020) studied that economically relevance with 60 percent of total population of world depending on rice as the main source of calories, may have destructive effects of the disease, rice blast however, this pathogen has developed into a pioneering model system for researching host-pathogen interactions. The disease outbreak depends on the weather and climatic conditions of the various regions. The disease's occurrence and symptoms vary from country to country.

Susceptible cultivars cause huge rice production loss in yield. The principal cause of resistance breakdown in rice against rice blast disease is pathogenic variability. During sexual hybridization, pathogenic changes may provide evidence of pathogenic variation found at the asexual stage of the fungus. The virulent pathotypes cause severe disease incidence. Only through pathogenicity research the pathotypes can be determined using a collection of different rice varieties that are usually different carrying various resistance genes.

Rahman and Jashim (2017) studied that the rice blast disease is caused by the fungi *Magnaporthe grisea* (renamed as *Magnaporthe 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. In Bangladesh the blast disease was relatively unimportant in late sixties and early seventies. The outbreak of blast disease was recorded on 1980 and 1990 in boro season in Bangladesh.

Suprapta and Khalimi (2012) recorded 21-37% in Bali Indonesia due to rice blast.

Sundaram *et al.,* (2011) reported that In India, blast epidemics were from sub-Himalayan regions of Jammu and Kashmir, Andhra Pradesh, Tamil Naidu and Coorg regions of Karnataka and North eastern region comprising the states of Arunachal Pradesh, Manipur, Mizoram, Meghalaya and Assam.

Nutsugah *et al.*, (2008) observed that In West Africa, the largest area of African production, this pathogen is the main constraint to production with yield losses ranging from 3-77%. 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.

Arshad *et al.,* (2008) recorded that In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and place like Gaggoo Mandi.

Chandrasekhara *et al.,* (2008) reported that rice blast caused by *Magnaporthe oryzae* is one of the devastating diseases of rice resulting in yield losses up to 65% in susceptible rice cultivars.

Munoz (2008) studied the effect of temperature and relative humidity on the airborne concentration of *Magnaporthe oryzae* spores and the development of rice blast in Southern Spain.

Puri *et al.,* (2006) studied the effect of blast disease on Rice plants over 45 regions of India and found the higher Percent Disease Index (PDI) at dough stage (30.45%) followed by booting stage (29.77%) and tillering stage (15.4%) in low land rice growing areas.

Agrios (2005) recorded that several rice blast epidemics have occurred in different parts of the world, resulting in yield losses ranging from 50 to 90 per cent of the expected crop.

Zhu *et al.,* (2005) identified neck blast as the most destructive phase of the disease and can occur without being preceded by severe leaf blast. 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.

Leong (2004) described from his experiment that the Rice blast 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 and adult plant stages on the leaves, nodes and panicles. It appears in irrigated low land or rainfed 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.

Oerke and Dehne (2004) studied that one of the main limitations in production of rice is rice blast disease caused by the fungus *Magnaporthe oryzae.* Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production.

Couch and Kolin (2002) observed that rice blast is one of the most important diseases of rice, caused by the fungus *Magnaporthe oryzae.*

Kato (2001) recorded that the disease causes yield losses from between 1- 100% in Japan and 70% in China due to rice blast disease.

Jia *et al.,* (2000) observed that blast disease caused by *Magnaporthe oryzae* Cavara [Synonym *Magnaporthe grisea* Sacc., the anamorph of *Magnaporthe grisea* (T.T Hebert) Yaegashi and Udagawa], upsets production statistics of rice in Pakistan.

Manandhar *et al.,* (1998) reported that *Magnaorthe oryzae* is one of the most important fungal pathogens of rice 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.

Baker *et al.,* (1997) and Scardaci *et al.,* (1997) recorded 30-50% in South America and Southeast Asia due to rice blast disease.

Chiba *et al.,* (1996) reported that rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated, in both paddy and upland conditions. Rice blast is present wherever rice is cultivated, but the disease occurs with highly variable intensities depending on climate and cropping system. Environments with frequent and prolonged dew periods and with cool temperature in daytime are more favorable to blast.

Bonman (1989); Tsai (1981); Litsinger *et al.* (1982); Padmanabhan (1965) and Goto (1965) studied that the disease often results in a significant loss of yield which has been estimated to be as high as 70-80% during an epidemic season.

Ou (1985) reported 40 percent loss in Nigeria due to rice blast.

IRR1 (1976) observed that lower temperature, dew formation and high humidity favor the blast disease development in upland rice growing environments

Chin (1975) observed that *Magnaporthe grisea* (Anamorph *Magnaporthe grisea* Sacc. synonym *Magnaporthe oryzae* Cav.) causes rice blast disease in rice cultivation areas worldwide.

Awoderu and Esuruoso (1975) reported 50 per cent losses in Philippines due to rice blast.

Ou (1985) studied that rice blast, caused by the fungus *Magnaporthe grisea.* (Hebert) Barr *[(Magnaporthe grisea*, Sacc) *Magnaporthe oryzae,* Cavara] is one of the most destructive diseases of cultivated rice. The disease is reported from 85 countries world-wide and remains a serious constraint to rice production in all irrigated and upland environments.

Manandhar *et al.,* (1996) reported that in Nepal, the disease caused 10-20 per cent yield reduction in susceptible varieties, but in severe cases yield reduction had gone up to 80 percent.

Hawksworth (1990) from Commonwealth mycological institute (CMl) provided the description of the culture of *Magnaporthe oryzae*: Cultures greyish, conidiophores single or in fascicles, simple,

rarely branched, showing sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodial and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, three septate rarely one or two septate, hyaline to pale olive, 19-23 x 7-9 pm, with a distinct protruding basal hilum. Chlamydospores often produced in culture, thick-walled, 5-12 pm diameter.

Prabhu and Morais (1986) reported that in Brazil, rice blast has been considered to be one of the major yield constraints in both irrigated and upland ecosystems.

Ou and Nuque (1985) observed that the disease often results in a significant yield loss, as high as 70-80% during an epidemic. On susceptible cultivars, lesions may initially appear greyish green and water soaked with a darker green boarder and they expand rapidly to several centimeters in length. Symptoms of infection of the collar node consist of general area necrosis at the union of the two tissues. Collar infections can kill the entire leaf and may extend a few millimeters into and around the sheath later the plant is broken at the collar region.

Padmanabhan (1965) reported losses of grains to the tune of 75 per cent has been reported in India due to rice blast.

Hashioka (1950) studied that blast can cause severe losses to susceptible cultivate grown in areas conducive to disease development rice is grown under diverse ecosystems, but blast is usually more prevalent in the upland cultivations. It has been postulated that blast outbreaks in the temperate and sub-temperate regions, are influenced by temperature and also by the relation of the age of the plant to resistance.

McRae (1922) first reported the blast disease in India and gave a yield loss estimate over 50% yield reduction was recorded at Rampur in Uttar Pradesh.

Hemmi and Yokogi (1927) reported that rice crop at all stages of its growth from seedling to about three weeks before harvest is susceptible to the attack of disease. The extent of losses varies with severity of the disease, stage of the crop and the affected plant part.

2.3. Symptoms

Blast symptoms develop on all the aerial organs of the rice plant, mainly on the coleoptiles, leaf sheaths and leaf blades, neck of panicles, stem nodes and spikelet. Oval or diamond-shaped spots (5-15 mm long and 3-5 mm wide) with dark borders occur on the leaves. Often, the spots have

yellow haloes. Spots develop quickly under moist conditions and produce large numbers of spores on both sides of the leaves. As they age, the spots become longer, the centers turn whitish grey and the borders become wider and red-brown. The spots join together and the leaves die. The foliar lesions reduce the leaf area available for photosynthesis and, when they are severe and occur in the early development stages, they are likely to destroy the whole tiller. Severely infected fields have a scorched appearance (Jackson, 2015).

2.3.1. Taxonomy and nomenclature

Teleomorph: Magnaporthe oryzae (Hebert) Barr

Kingdom: Fungi

Division: Ascomycota

Subdivision: Pezizomycotina

Class: Sordariomycetes

Subclass: Sordariomycetidae

Order: Magnaporthales,

Family: Magnaporthaceae

Genus: *Magnaporthe*

Species: *Oryzae*

Anamorph: *Pyricularia oryzae* (Couch and Kohn, 2002)

The blast causal agent is an ascomycete fungus described on many gramineous species and in its asexual form called *Pyricularia grisea* (Cke.) Sacc, whose perfect stage was known as *Magnaporthe grisea* (Hebert) Barr. However, Couch and Kohn (2002) distinguished through multilogues gene genealogy and host preference - two clades within *M. grisea*. One, associated with the grass genus *Digitaria*, is named *M. grisea*, while the other, associated with *Oryza sativa* and other cultivated grasses, was described as a new species, *M. oryzae*. Thus, the correct name of the blast pathogen is currently *Magnaporthe oryzae* B. Couch [anamorph: *Pyricularia oryzae* Cavara] (Couch and Kohn, 2002). The asexual stage is the most common form of the fungus. It

has been classified based on the anamorphic stage, *Pyricularia oryzae* (Deuteromycota: Hyphomycetes: Moniliales: Dematiaceae).

2.3.2. Occurrence and distribution

Rice blast disease has been recognized in 85 rice-growing countries (Wang *et al*., 2014). Blast is considered the most destructive rice disease as the environmental conditions favored for disease occurrence and distribution. Rice blast disease has been reported in all the rice growing countries of the world, firstly in China from 1637, Japan from 1704, America from 1876, India from 1913, and Australia from 2011 (Shafaullah *et al*., 2011). It was found as destructive disease in West Africa, Iran (Mousanejad *et al.*, 2010), Malaysia (Rahim, 2010) and Savanas of South America (Bonman *et al*., 1986). Rice blast was reported for the first time in Africa in 1930 (Feakin, 1974). Climatic changes accompanying with the global warming could prompt its spread in to other parts around the world (Kohli *et al.*, 2011). Rice varieties resistant to blast frequently lose their resistance within a few years because of shifts in strains of the fungal population (Huang, 2011).

In Myanmar, the occurrence of rice blast disease in Ayeyarwady (Central Agricultural Research Institute, 2000), leaf blast epidemic and neck blast around Yezin area on the variety IR50 in 2002-2003 cold and dry seasons (Naing, 2004), the occurrence of blast disease in 2013 early summer and 2014 rainy season in Nay Pyi Taw Union Territory (Aye *et al*., 2015), the leaf blast and neck blast occurrence in Aungban, Pindaya, Taunggyi, Kyaukme, Yezin, Lapputa and Bago during 2015 to 2018 rice growing season (Khaing *et al*., 2018) and leaf blast disease incidence in Aungban research farm every year (Department of Agricultural Research, 2018) has been reported.

2.3.3. Infection process and disease cycle

A spore (conidium) landing on the rice organ surface initiates the infection. Conidium is attached to the host plant until it can germinate (Koga and Nakayachi, 2004). Thereafter, the conidium of *M. oryzae* develops germ tubes and appressorium. The conidium attachment and germination, and differentiation of the appressorium belong to the passive stage of the host-pathogen relationship since they occur for both compatible and incompatible host-pathogen combinations (Arase *et al*., 1994).

In compatible interactions, the appressorium differentiates a peak, which penetrates the epidermal cells, allowing the pathogen to colonize the host tissues (Howard and Valent, 1996). At this stage, the active interaction between blast fungus and rice begins. Host plant resistance manifests itself

either by preventing the subsequent hyphal growth inside the host cell through hypersensitive reaction, or by reducing the damaged cell and therefore the size of the lesions and their sporulating abilities, slowing epidemic development and finally leading to a partial resistance of rice to the blast fungus.

The disease is particularly serious in areas of frequent and prolonged showers and temperatures in the range of 24-28° C. This is because the leaves need to be wet for 6-8 hours for spore germination. High humidity, close to 100 %, is needed for infection and spore formation. In upland areas, conditions are favorable to the disease because differences between day and night temperatures cause dew to form on the leaves and the overall temperatures are cooler. By contrast, in lowland tropical areas, leaf infection is less, but blast is still serious in seedling nurseries and on panicles (Jackson, 2015).

Spread occurs in irrigation water. Spores are spread short and long distances on air currents and wind. Survival between crops is in straw and stubble, in or on seed, volunteer rice plants, and alternative hosts, mostly grass species (Jackson, 2015).

2.3.4. Disease development

Several environmental factors can influence the infection rate and spread of the disease, including temperature, nitrogen levels, intermittent rain showers or drizzle airflow, high relative humidity and drought conditions. Blast susceptibility is inversely related to soil moisture. Plants grown under upland conditions are more susceptible, while plants grown under lowland condition are more resistant. The pathogen requires free moisture for spore penetration. High relative humidity (90-92 %) is also reported to be essential for infection. Severe blast epidemics are usually associated with moist weather. Low solar radiation and cloudy skies are also good deeds to blast (Miah *et al*., 2017). Using 13-years data, Padmanabhan (1963) concluded that whenever the minimum temperature of 24 °C or below was associated with relative humidity of 90 % or above, the conditions were favorable to blast infection.

2.4. Source of inoculum

Infested seeds are a source of primary inoculum. Dead infested grains could serve as primary inoculum when placed on the field during seedling development (Long *et al*., 2001). Seed contamination and panicle symptoms are interrelated using naturally infested seeds as primary

inoculum in field conditions (Manandhar *et al*., 1998). They observed that sporulation of *M. oryzae* on infested seeds was favorably found at the embryonic end of germinating seeds. A seed lot with 21 % contamination led to <4 % seedlings with blast lesions. Tests employing different ways of covering seeds with soil and underwater seeding (no covering) pointed out that complete covering or seedings underwater induce a lower infection frequency (Manandhar *et al.*, 1998). Guerber and Tebeest (2006) conveyed similar experiments in the USA, but no disease was observed when infested seeds were germinated under water. When infested seeds were sown in the field, the fungus was recovered from different seedling parts, including roots. These results clearly indicated that the fungus can survive on the grains used for seeding and could serve as primary inoculum (Miah *et al.,* 2017).

2.5. Climatic conditions

Most severe blast disease occurs when more than a few days of continuous rains and average temperatures between 18-25 °C during the flowering stage of the crop followed by sunny, hot and humid days (Kohli *et. al*., 2011). Under controlled growth chamber conditions, the highest blast intensity was observed at 30 °C which increased with a longer wet period, and low at 25 °C with a wet period of less than 10 hr. (Cardoso *et al*. 2008). However, at 25° C and 40 hr. of wetting, blast intensity exceeded to 85 % (Miah *et al.,* 2017).

2.6. Isolation and identification of causal agent

Dutta (2017) stated in his study that serious yield losses due to epiphytotic of blast diseases have been recorded in different regions in India, such as Tanjore delta, Nellore, Hyderabad, Bombay, parts of Orissa, Kashmir and Kerala. *Magnaporthe* pathogen isolated in PDA appears white colored at first and then blackish fungal growth was observed in the media. The leaves started to produce symptoms from 4th day onwards; initially the spots were small, yellow, round to oval. At later stage the spots became enlarged and spindle shaped having ash colored center. The spot was similar in appearance with the rice blast spots found in the field.

Priya *et al.,* (2013) conducted an In-vitro experiment on rice blast disease where 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). 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. They also studied culturing of different isolates of *Magnaporthe oryzae* and reported that colonies of *M. oryzae* appeared as white on oat meal, rice polish and malt extract agar, grey 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.

Motlagh and Javadzadeh (2010) studied on blast affected leaves of rice cultivars, 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 25°C for 2-3 days. Later, Petri dishes were incubated at 25°C 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.

Silva *et al.,* (2009) worked on eight samples of rice leaves infected with blast collected from commercial fields of upland rice cultivars in the state of Goias, Brazil. 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°C.

Roumen *et al.,* (1997) obtained single conidiospore culture from the conidial stage of *Magnaporthe oryzae.* Growth and sporulation were induced by placing inoculated Petri dishes filled with rice polish agar at 27°C under fluorescent light for about eight days. Conidia were harvested by adding little water to Petri dishes with sporulating cultures. The spores were dislodged with a camel brush, the suspension was filtered, and the spore concentration measured using a hemocytometer. Spray inoculation was done spraying 30 ml of suspension containing 50,000 conidia ml^{1} and 0.5% gelatin to each tray of plants using a paint brush powered by compressed air. The plants were inoculated when the plants had 5 or 6 leaves on the main culm. Trays with plants were placed on a rotating table and inoculated by spraying the suspension in slow systemic moments. Immediately following inoculation, the plants were kept in the dark inside a phytotron at 25° C and 100% humidity for 16 hours.

Yaegashi and Udagawa (1978) finally proposed *Magnaporthe oryzae* as a perfect stage of *Magnaporthe oryzae* (cooke.) Sacc instead of *Ceratosphaeria oryzae.* The found in their study that fungal hyphae are hyaline and septate. 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. Conidium is three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown.

2.7. Media suitable for culturing *Magnaporthe oryzae*

Akhilesh *et al.,* (2017) studied on 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. Among all the solid media the highest mean mycelial growth of the fungus *Magnaporthe oryzae* (Cav.) was recorded on oat meal agar (77.6mm) followed by rice leaf extract (75.9mm) and least mean mycelial growth of the *M. oryzae* (Cav.) on Sabourad's media (44.7mm) followed by Takahashii's media (52.5mm).

Malviya (2014) used four culture media for the study of mycelial growth of *P. grisea* under *Invitro.* Among them PDA media supported maximum mycelial growth followed by Richard's Agar medium after 168 hr. of incubation. Then sporulation of *P. grisea* was observed in traces in Potato dextrose agar medium and Richard's Agar medium after 168 hrs. of incubation. However, Czapek Dox medium was not found effective for both vegetative growth and sporulation of the test pathogen.

Mahdieh (2013) reported that PDA culture medium could provide the best medium for *P. oryzae* vegetative growth, regardless of light condition. However, *M. oryzae* could sporulate when light was provided either continuously or at intervals. A combination of 16/8 hr. light/darkness intervals and adding rice materials to culture media could induce *P. oryzae* for abetter sporulation. Sun *et al.,* (1989) studied the effects of 17 media on 41 isolates of *P. oryzae.* They found that, com meal and rice straw agar media were most conducive for sporulation.

Ram *et al.,* (2012) estimated the linear growth of the colonies of the *Magnaporthe* isolated from rice was measured on standard medium agar, oat meal agar, French bean agar and decoction agar made out of the leaf material of rice. He also determined the weight of mycelial mat produced by the isolates in the standard medium, Richards's medium, Browns medium and decoctions of leaf material of rice. The isolates produced good growth on the decoctions of their host material. The dimensions of conidia produced by *Magnaporthe oryzae* ranged from 17.6 to 24.0 pm in length and 8.0 to 9.6 pm in width.

Metz and Chandra (2011) studied the effect of five media i. e. potato dextrose agar (PDA), Oat meal agar (OMA), V8 agar (V8A), prune juice agar (PJA) and St. Augustine grass agar (STAA) on the vegetative mycelial growth, pigmented mycelial production and conidial production in *P. oryzae.* After inoculation, different media were kept at 26° C for 11 days under diurnal (12hr) fluorescent light. He observed that vegetative mycelial growth was highest on V8A, PDA and OMA, while the culture media produced the highest number of pigmented mycelia on OMA, STAA and V8A. Highest conidial production was obtained on STAA.

Ram *et al.,* (2007) reported that 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. They also found that the 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 oryzae* 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 spoilation (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.

Meena (2006) described the colony color of all the rice blast (*Magnaporthe oryzae)* 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 color with smooth colony margin and good growth.

Mijan (2000) observed that among the non-synthetic media, potato dextrose agar supported linear growth of *P. grisea.* The fungus reached maximum growth on 14th day of incubation. Richards's medium was best synthetic medium to support maximum growth of the fungus.

Priya *et al.,* (2013) studied culturing of different isolates of *Magnaporthe oryzae* was studied by reported that colonies of *M. oryzae* appeared as white on oat meal, rice polish and malt extract agar, grey 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 *M. 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.

Kumar and Singh (1996) studied *Magnaporthe grisea (M. grisea)* from rice on different solid culture media. They found that, maximum colony diameter of rice isolate occurred on malt extract agar and Leonine agar.

Du *et al.,* (1995) reported that *Magnaporthe* isolates from hosts including rice and common weeds in paddy fields sporulated abundantly on sterilized barley and sorghum grains.

2.8. Blast resistance genes

Till date 99 blast R genes have been identified; in which 45% were found in japonica cultivars, 51% in indica cultivars, and the rest 4% in wild rice species (Wang *et al.*, 2014). More than 100 blast resistance loci or genes have been mapped to rice chromosomes (Fang *et al.*, 2016)

Two types of resistance genes are responsible for rice blast resistance: major resistance (R) genes that confer race-specific resistance and quantitative trait loci (QTLs) that control partial, non-race specific resistance (Skamnioti and Gurr, 2009).

Cloned R genes are distributed across all 12 chromosomes except chromosome 3 (Qin Zhong *et al.*, 2009). All of the cloned R genes except for *Pi-d2*, *pi21* and *Ptr* contain nucleotide-binding domain leucine-rich repeat (NLR) proteins (Zhao *et al.*, 2018).

R genes are highly specific to *M. oryzae* races, resistance of a single R gene is often rapidly overcome by the selection of compatible pathogen races (Hittalmani *et al.*, 2000). In response to

the rapid evolution of *M. oryzae*, the rice genome has evolved R gene polymorphism, which confers multiple forms of race-specific resistance (Hayashi *et al.*, 2004).

2.9. Genetic association study

Genetic association study has recently been used for assessing associations between genetic markers and blast resistance in rice (Li *et al.*, 2019). It was first used to identify genes underlying complex diseases in humans (Altshuler *et al.*, 2008). Genetic association modeling has a wider use (Liu *et al.*, 2016).

Genetic association has become a powerful approach for mapping agronomic traits of rice (Zhao *et al.*, 2018). Genetic association analysis was applied for QTL mapping using large germplasm collections (Huang *et al.*, 2010).

CHAPTER III
MATERIALS AND METHODS

The materials used and methods applied or followed for conducting the experiments under the present study are described in this chapter.

3.1. Location of the experiment

The experiments were conducted at Uniform Blast Nursery (UBN) of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur (23°59'26" N latitude 90°24'28" E longitudes) during June 2019 to December 2020.

3.2. Isolation, purification, identification and maintenance of *Magnaporthe oryzae*

Collected infected leaf and panicle samples were cut in 3-5 cm sections. These sections were surface sterilized by dipping in 10% Clorox for one minute and were washed by sterilized water for several times and then the cut sections were placed on moist filter paper (Whatman: 9.0 cm) in a sterile petri plate. Plates were incubated for 24 hours at room temperature (25^0 C) and the infected parts were examined under stereo microscope (Motic SMZ-168). Conidial masses were picked by using very fine tip needle and spread on 3% water agar plate. The plates were observed under stereo microscope and single conidia picked by using needle and transferred it to another agar plate. That plate was incubated at 25^0 C temperature for 2 or 3 days and finally the mycelium was transferred to potato sucrose medium plates (Hayashi *et al*. 2009). The sub culturing was done at an interval of 15 days and preserved at low temperature (5±1ºC) in refrigerator. 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 oat meal agar slants and allowed to grow. The pure culture slants were sealed with paraffin wax and stored in a refrigerator for further use. The pathogen isolated from the diseased specimen and established in pure form on OMA was identified on the basis of colony, morphological characters and pyriform shaped conidia.

3.2.1. Pathogenicity study

A pot culture technique was used to prove the pathogenicity of the test organism. The blast susceptible BRRI dhan28 seeds were sown in sterilized earthen pots containing sterilized soil + Farm Yard Manure (FYM) (1:1). The seedlings with vigorous growth were selected for artificial inoculation. Then the pathogen was inoculated by spraying over the seedlings @ $1x10^5$ conidia/ml suspension. After spraying the seedlings were covered with polyethene paper. The observations on

the development of symptoms were recorded daily for a period of 15 days from the day of inoculation.

3.3. Screening of rice germplasm

3.3.1. Plant materials

Thirty rice germplasm were collected from Gene bank, BRRI and different districts of Bangladesh (Table 1). One additional genotype, US2 was included with this set as blast susceptible check.

Table 1. Source of rice germplasm used in the experiment

SL. No.	Genotypes	Source
1	Jangli	BRRI
2	Nerica - 1	BRRI
3	Chinigura	Dinajpur
4	Chini Atop	Dinajpur
5	Shakkhor Khora	Rangpur
6	Badsha Vog	Dinajpur
7	BRRI Dhan 72 (Mota)	BRRI
8	Kalijeera	Thakurgaon
9	BRRI Dhan 37	BRRI
10	BRRI Hybrid 6	BRRI
11	Guti Dhan	Thakurgaon
12	Mali Sorno	Thakurgaon
13	Sumon Sorno	Thakurgaon
14	Shompa Kathari (Indian Variety)	Dinajpur
15	Jeera-34 (Aromatic)	Dinajpur
16	Philipine Kathari (Aromatic)	Dinajpur
17	Sonamukhi (Indian Variety)	Dinajpur
18	Balam	BRRI
19	Katarivog	Dinajpur
20	Shail Dhan	BRRI
21	Pajam	BRRI
22	BR 11	BRRI
23	BR 16	BRRI
24	BR 22	BRRI
25	BRRI Dhan 30	BRRI
26	BRRI Dhan 50	BRRI
27	BRRI Dhan 51	BRRI
28	BRRI Dhan 52	BRRI
29	Ranjit	Bogura
30	BRRI Dhan 34 (Atap)	Bogura

3.3.2. Phenotypic screening against leaf blast disease

Rice germplasm with susceptible check US2 were screened for their reaction to leaf blast at UBN, BRRI, Gazipur. The screening was done two times in June 2019 and December 2020 with four replications. Each entry was sown in rows in the UBN. Each row was 50 cm long and row to row distance was 10 cm. Susceptible check, US2 was sown after every ten entries as well as two rows in border around the rows. A virulent blast isolate collected from the pure culture of *Magnaporthe oryzae* (Figure 1), was inoculated at 21 days after seeding in both seasons. Disease reaction was recorded visually at 7 days after inoculation following the scoring system developed by Japan International Research Center for Agricultural Sciences (JIRCAS) (Hayashi *et al.*, 2009). Detailed scoring system is presented in Table 2. Infection type 0 to 1 was considered as resistance, 2 to 3 was considered as moderately resistant and infection type 4 to 5 was considered as susceptible. Disease scoring was done by considering the highest disease score among the replications. Additionally, the location severity index (LSI) was calculated to find the severity of blast disease using following formula (Wheeler, 1969):

$$\text{LSI} = \frac{\text{Sum of multiplication of entries and scales}}{\text{Total member of entries}}$$

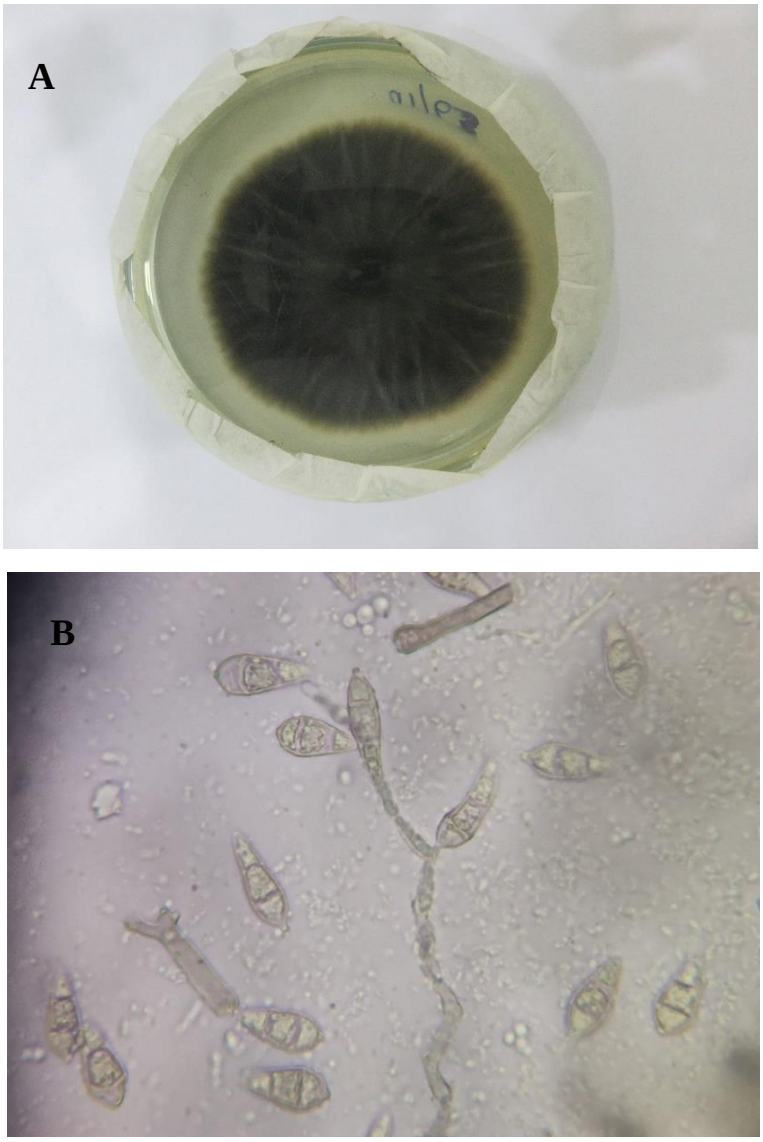

Figure 1. Pure culture of *Magnaporthe oryzae*. A. Culture on oat meal agar media. B. Conidia of *Magnaporthe oryzae* under compound microscope (40X).

Table 2. Leaf blast scoring system of JIRCAS

Disease score (0-5)	Leaf blast symptom
0	No lesions.
1	Uniform or scattered brown specks.
2	Small lesions with distinct tan centers surrounded by a darker brown margin approximately 1mm in diameter.
3	Small eye spot lesions less than one and a half times of the interval between thin veins or less than 1.5 mm in diameter surrounded by dark brown.
4	Intermediate size eyespot lesions less than twice the interval between thin veins or less than 2 mm in diameter.
5	Large eyespot lesions more than twice the interval between thin veins or more than 2 mm in diameter.

Figure 2. Rice genotypes used in this experiment. A. Collected rice genotypes for screening against *Magnaporthe oryzae*. B. Water-soaked rice genotypes before sowing.

3.4. Statistical Analysis

Cluster analysis classifies the genotypes into different group on basis of difference. These groups are called clusters. Different techniques are used to group the data in clusters and represented in graphical dendrogram. In this study, we used the complete linkage hierarchical cluster analysis and distances measured by Euclidean distance. It is calculated as follows:

Distance (x, y) = {Σ I (xi-yi) 2}1/2

This analysis was done by using the computer-based program STATISICA version 10.0 (Stat Soft, 2013) to find the relationship among the genotypes in response to rice leaf blast.

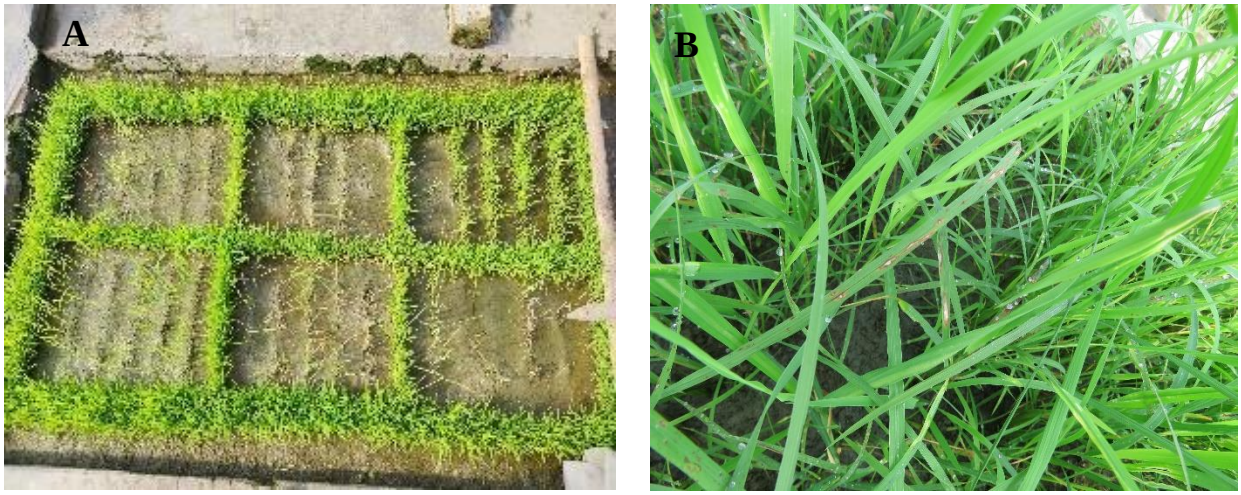

Figure 3. Activities at uniform blast nursery (UBN). A. uniform blast nursery (UBN) for rice genotypes evaluation. B. Spindle shaped on the inoculated seedlings.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Phenotyping for blast resistance

Based on the screening scores of different germplasms in UBN, five germplasms (R: 2.67%) exhibited resistant with the disease score 1. The resistant germplasms are Shakkhor Khora, Shompa Kathari (Indian Variety), Shail Dhan, BR 16 and BRRI Dhan 30. One germplasm (MR: 6.67%) was found (Kalijeera) moderately resistant (disease score 2-3) and twenty-four germplasm (S: 50.69%) were susceptible with the disease score >3 (Table 3). The location severity index (LSI) found 2.2, 2.5, 4 and 3.7 respectively over four observations which confirm the adequate disease pressure in the study location.

Table 3. Evaluation of various germplasm for leaf blast resistance

Sl. No.	Genotypes	Observation 1	Observation 2	Observation 3	Observation 4	LSI (%)	Highest score	Disease reaction
1	Jangli	1	2	5	5	43.33	5	S
2	Nerica - 1	5	5	5	5	66.67	5	S
3	Chinigura	0	2	5	5	40.00	5	S
4	Chini Atop	0	0	5	2	23.33	5	S
5	Shakkhor Khora	0	0	1	0	3.33	1	R
6	Badsha Vog	1	0	5	5	36.67	5	S
7	BRRI Dhan 72 (Mota)	3	5	5	5	60.00	5	S
8	Kalijeera	0	0	2	0	6.67	2	MR
9	BRRI Dhan 37	3	2	2	4	36.67	4	S
10	BRRI Hybrid 6	0	0	5	5	33.33	5	S
11	Guti Dhan	4	5	5	5	63.33	5	S
12	Mali Sorno	0	0	5	5	33.33	5	S
13	Sumon sorno	5	5	5	5	66.67	5	S
14	Shompa Kathari (Indian Variety)	1	1	0	0	6.67	1	R
15	Jeera - 34 (Aromatic)	4	3	5	5	56.67	5	S
16	Philipine Kathari (Aromatic)	1	1	5	5	40.00	5	S

17	Sonamukhi (Indian Variety)	0	0	5	0	16.67	5	S
18	Balam	5	4	5	5	63.33	5	S
19	Katarivog	1	2	5	5	43.33	5	S
20	Shail Dhan	0	1	0	0	3.33	1	R
21	Pajam	1	5	5	5	53.33	5	S
22	BR 11	5	5	5	5	66.67	5	S
23	BR 16	0	0	0	0	0.00	0	R
24	BR 22	5	5	5	5	66.67	5	S
25	BRRI Dhan 30	0	0	0	0	0.00	0	R
26	BRRI Dhan 50	5	5	5	5	66.67	5	S
27	BRRI Dhan 51	5	5	5	5	66.67	5	S
28	BRRI Dhan 52	1	4	5	5	50.00	5	S
29	Ranjit	4	5	5	5	63.33	5	S
30	BRRI Dhan 34 (Atap)	5	3	5	5	60.00	5	S
31	US2 (Sus. Ck.)	5	5	5	5	66.67	5	S
	LSI	2.2	2.5	4.0	3.7			

*R= Resistant, MR= Moderately Resistant and S= Susceptible, Sus. Ck. = Susceptible Check

4.2. Cluster Analysis

Improvement of crop varieties can only be successful with higher genetic variation and the heritability of desirable traits (Ravi *et al*., 2003). Therefore, for establishing relationship among different varieties, assessment of genetic variability has become very important (Kibria *et al.*, 2003; Sivaranjani *et al*., 2010). Cluster analysis of the present study classified the rice genotypes in three clusters depending upon the variation present for character under study. Cluster analysis gave dendrogram which distributed the thirty genotypes into three clusters shown in Table 4 and Figure 4.

Table 4. Distribution of rice genotypes to different clusters

Cluster no.	Disease score	Genotypes
1	0 - 1	Shakkhor Khora, Shompa Kathari (Indian Variety), Shail Dhan, BR 16, BRRI Dhan 30
2	2 - 3	Kalijeera
3	4 - 5	Jangli, Nerica - 1, Chinigura, Chini Atop, Badsha Vog, BRRI Dhan 72 (Mota), BRRI Dhan 37, BRRI Hybrid 6, Guti Dhan, Mali Sorno, Sumon sorno, Jeera - 34 (Aromatic), Philipine Kathari (Aromatic), Sonamukhi (Indian Variety), Balam, Katarivog, Pajam, BR 11, BR 22, BRRI Dhan 50, BRRI Dhan 51, BRRI Dhan 52, Ranjit, BRRI Dhan 34 (Atap)

Dendrogram constructed by cluster analysis revealed that the most diversified clusters were 1 and 3. This show for the selected character they had great genetic variation between each other. Most close cluster was 3 showed the presence of less variation. As we move from cluster 1 to 3, the similarity index between cluster increases indicates presence among the genotypes. Genotypes grouped in cluster 2 showed moderately resistant response against leaf blast with disease score of 2.

Cluster analysis for 30 Rice Genotypes against Blast

Complete linkage

Euclidean distance

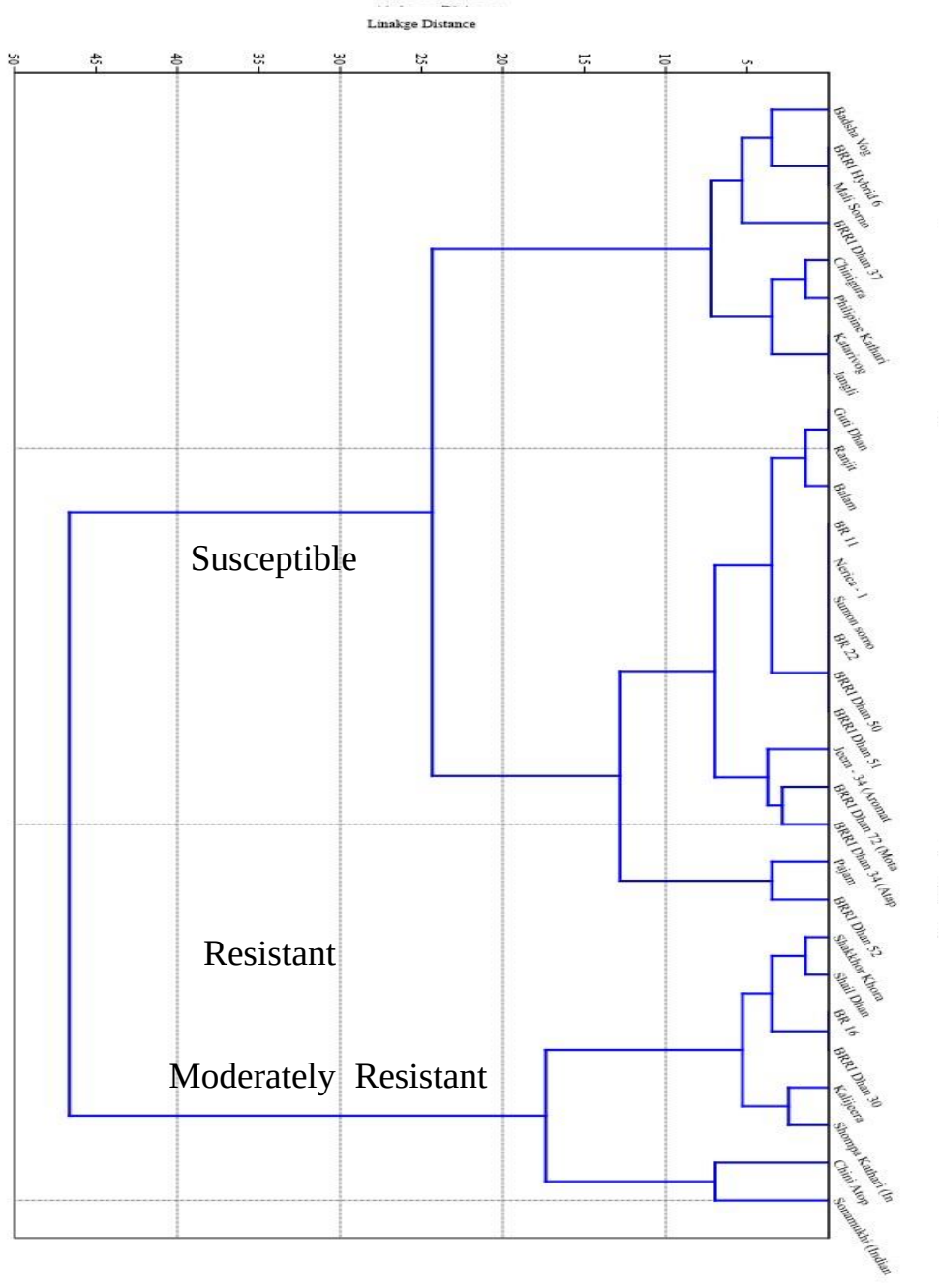

Figure 4. Dendrogram of rice genotypes response to leaf blast by Ward's method showing linkage and Euclidean distance.

Dendrogram constructed by cluster analysis revealed that the most diversified clusters were 1 and 3. This show for the selected character they had great genetic variation between each other. Most close cluster was 3 showed the presence of less variation. As we move from cluster 1 to 3, the similarity index between cluster increases indicates presence among the genotypes. Genotypes grouped in cluster 2 showed moderately resistant response against leaf blast with disease score of 2.

In cluster 1 included the genotypes showing resistant against rice blast among which BR 16 and BRRI Dhan 30 were superior to other genotypes within the cluster. Shakkhor Khora, Shompa Kathari (Indian Variety) and Shail Dhan were more similar to each other may be because of the similarity in their origin. Moderately resistant genotype of disease score of 2 was included in cluster 2. Cluster 3, most diverse cluster than cluster 1 and 2, included the genotypes with susceptible response to rice leaf blast disease with >3 score. The most diverse genotypes within the cluster were Nerica – 1, Sumon sorno, BR 11, BR 22, BRRI Dhan 50, BRRI Dhan 51. Some extent of distinctness could also be seen between other genotypes of this cluster, such as, Jangli, Chinigura, Chini Atop, Badsha Vog, BRRI Dhan 72 (Mota), BRRI Dhan 37, BRRI Hybrid 6, Guti Dhan, Mali Sorno, Jeera - 34 (Aromatic), Philipine Kathari (Aromatic), Sonamukhi (Indian Variety), Balam, Katarivog, Pajam, BRRI Dhan 52, Ranjit, BRRI Dhan 34 (Atap). Similar studies were carried out by Telebanco-Yanoria *et al*., (2008), in which 922 rice varieties collected from Asia were evaluated against rice blast. These varieties were grouped in six clusters (A-F) based on the reaction pattern to 20 standard differential blast isolates from the Philippines. Most susceptible varieties in clusters B and C were from Japan, while the varieties form East Asia and Southeast Asia were grouped in the clusters E and F with most resistance. Varieties from South Asia showed the widest variation, occurring in all clusters but less frequently in cluster B.

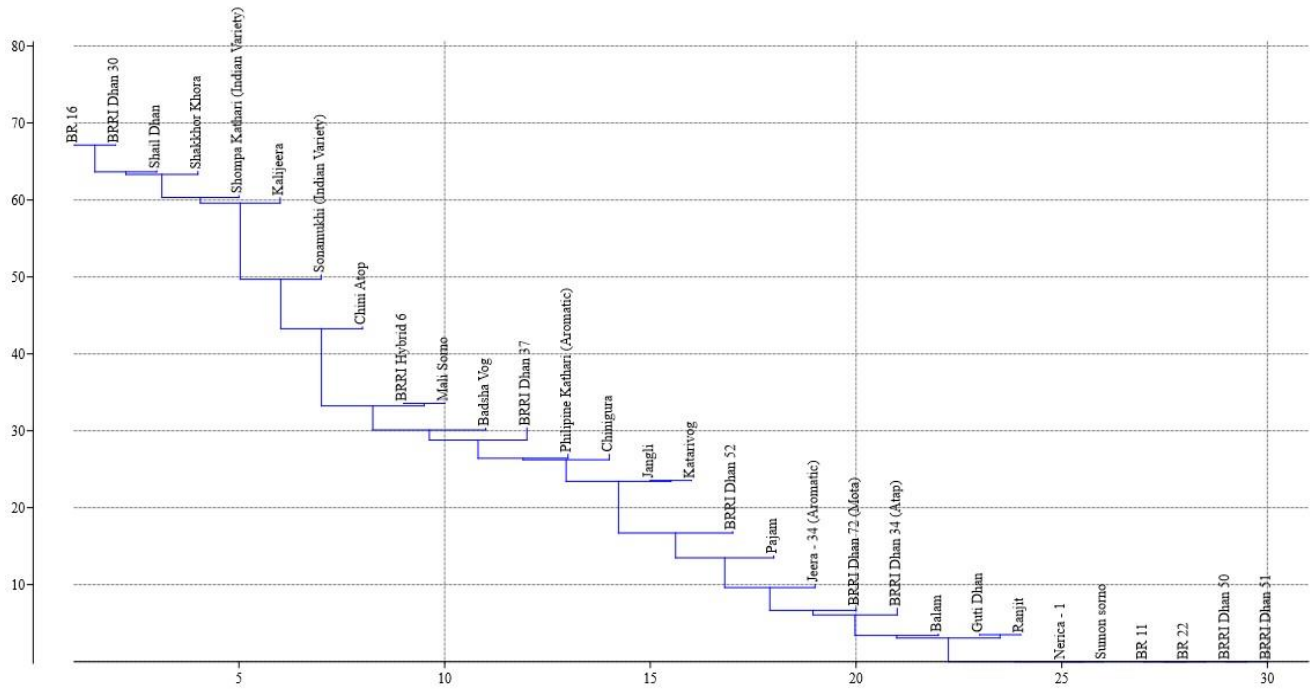

Figure 5. Dendrogram of rice genotypes response to leaf blast showing neighbor joining cluster and Euclidean distance.

Cluster analysis is used in many studies regarding resistance of rice genotypes against rice blast. Mukherjee *et al*., (2013), obtained three clusters of rice genotypes according to their response in respect of the various components of resistance. Two clusters had fast-blasting genotypes and one had slow-blasting genotypes. Thirteen genotypes were grouped in slow blasting cluster. It means these genotypes have characters of rate-reducing resistance and such resistance has the effects of slowing down the development of an epidemic. Jaihom and Parinthawong, (2014) evaluated different blast pathogen isolates in Thailand for their virulence and tested them on 25 rice varieties. The fugal isolates infecting the rice varieties at similar rate were grouped in same cluster. Some isolates caused severe infection and some showed lowest virulence on the tested varieties,

indicating that extent of plant resistance may also depends on the virulence level of the pathogen Isolate.

Qudsia *et al*., (2017), evaluated fifty-two rice genotypes including one susceptible check, Basmati C-622, were evaluated to find out new sources of resistance and assess their diversity based on the reactions against *P. oryzae*. The test genotypes were evaluated against leaf blast after three weeks of inoculation by following the standard evaluation system for rice introduced by the International Rice Research Institute, Philippines. Diversity of the 52 genotypes was also assessed based on blast symptoms. Moderately resistant reactions were observed with genotypes KSK-470, KSK463, KSK- 460, PK 8685-5-1-1-1, KSK-462, KSK-474, PK 3810-30-1, KSK-471 and KSK-472. The 52 genotypes were grouped in 4 clusters. The grouping of some genotypes in same cluster is based on their similar reaction against leaf blast.

CHAPTER V
SUMMARY AND CONCLUSION

Blast disease caused by *Magnaporthe oryzae* is considered as a major limiting factor in the worldwide rice production because of its wide distribution and destructiveness and it's been inflicting significant yield loss in all rice growing areas of Bangladesh. Host resistance is that the most fascinating suggests that of managing blast, especially in developing countries. Think about the importance of this disease a screening experiment was conducted to evaluate resistant rice genotypes against *Magnaporthe oryzae*.

Phenotypic screening of thirty germplasm against rice blast disease to identify the potential germplasm for blast resistance. The present study gives an overview of genetic diversity of blast resistance among the collected germplasm of rice. These rice germplasms were collected from BRRI gene bank and various districts of Bangladesh and were screened against blast disease at UBN, BRRI, Gazipur during June 2019 to December 2020.

Among the germplasms five resistant germplasms, one moderately resistant germplasm and twenty-four susceptible germplasms were found respectively. Three cluster groups were formed to differ clusters from other susceptible and resistant groups. Kalijeera, Shakkhor Khora, Shompa Kathari (Indian Variety), Shail Dhan, BR 16 and BRRI Dhan 30 could be used for the development of durable blast resistant variety. However, further studies are needed for identification of more blast resistance rice genotypes.

CHAPTER VI
REFERENCES

Agrios, G.N. (2005). Plant pathology (5th ed.). Elsevier Academic Press, London, UK. 922.

Akhilesh, K.K., Neha, S., Mukesh, K., Roshan, K., Kushram, T. and Sanath, V.B. (2017). Growth of rice blast fungus *Pyricularia oryzae* (Cav.) on different solid and liquid media. *International Journal of Current Microbiology and Applied Sciences*. ISSN: **6**(6):1154-1160.

Alam, M.S. (1998). Evaluation of rice cultivars for resistance to *Diopsis longicornis* (Diptera: Diopsidae). *Journal of Economic Entomology*. **81**(3): 934-936.

Altshuler, D., Daly, M.J. and Lander, E.S. (2008). Genetic Mapping in Human Disease. *Science*, **322**(5903): 881–888.

Arase, S., Miyahara, K., Honda, Y. and Nozu, M. (1994). Preinfectional interactions between *Magnaporthe grisea* spores and rice plants. *Bulletin Faculty of Agriculture Shimane University*. **28**: 45-51.

Arshad, H.M., Khan, I.J.A. and Jamil, F.F. (2008). Screening of rice germplasm against blast and brown spot disease. *Pakistan Journal of Phytopathology*. **20**: 52-57.

Attere, A.F. and Fatokun, C.A. (1983). Reaction of *Oryzae glaberrima* accessions to rice yellow mottle virus. *Plant Disease*. **67**: 420-421.

Awoderu, V.A. and Esuruoso, O.F. (1975). Reduction in grain yield of two rice varieties infected by rice blast disease in Nigeria. *Nigerian Agricultural Journal*. **11**:170- 173.

Aye, K.S., Win, N., Hnin, H.W., Aung, M.Z., Sai, W.M., Maung, T.T. and Naing, T.A.A. (2015). Occurrence of rice diseases in Nay Pyi Taw Council Area. *Journal of Agricultural Research*. **2**(1):46-56.

Dzomeku, B.M., Bam, R.K., Adu-Kwarteng, E., Darkey, S.K. and Ankomah, A.A. (2007). Agronomic and Physio-Chemical Evaluation of FHIA-21 in Ghana. *International Journal of Agricultural Research*. **2**: 92-96.

Baker, B., Zambryski, P., Staskawicz, B. and Dinesh, K.S.P. (1997). Signaling in plant microbe interactions. *Science.* **276**(5313):726-733.

BBS. (2017). Yearbook of Agricultural Statistics-2017. Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning, Government of the People's Republic of Bangladesh. P. 603.

BBS. (2019). Yearbook of Agricultural Statistics-2019. Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning, Government of the People's Republic of Bangladesh. P. 577.

BBS. (2021). Yearbook of Agricultural Statistics-2021. Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning, Government of the People's Republic of Bangladesh. P. 580.

Biswas, J.K. (2012). Dhan Gobeshonar Shata Barsher Kichu Katha. Technology Today, Dhaka, Bangladesh. p.163.

Bonman, J.M., Bandong, J.M., Lee, E.J. and Valent, B. (1986). Race specific partial resistance to blast in temperate Japonica rice cultivars. *Plant Disease*. **73**:496-499.

Bonman, J.M., Estrada, B.A. and Bandong, J.M. (1989). Leaf and neck blast resistance in tropical lowland rice cultivars. *Plant Disease.* **73**:388-390.

BRRI. (2019). Genetic Resource and Seed Division (GRS). Retrieved November 25, 2019, from Bangladesh Rice Research Institute website: http://www.brri.gov.bd/site/page/bd9bd45caf5e-423d-b370-21ec38997e 14/

Cardoso, C.A.A., Reis, E.M. and Moreira, E.N. (2008). Development of a warning system for wheat blast caused by *Pyricularia grisea*. *Summa Phytopathologica*. **34**(3):216-221.

Central Agricultural Research Institute. (2000). Research findings of CARI. Myanmar Agricultural Services. P. 109.

Chandrasekhara, M.V., Gururaj, S., Naik, M.K. and Nagaraju, P. (2008). Screening of rice genotypes against rice blast caused by *Pyriclaria oryzae* Cavara. Karnatak *J. Agril. Sci*. **21**(2):305.

Chiba, K., Tominaga, T. and Urakawa, F. (1996). Occurrence and control of rice blast disease in northern region of Iwate Prefecture in 1995. *Annual Report of the Society of Plant Protection of North Japan*. **47**:8-10.

Chin, K.M. (1975). Fungicidal control of the rice blast disease. *Malaysian Agricultural Journal.* **50**(2):221-228.

Chin, K.M. (1975). Fungicidal control of the rice blast disease. *Malaysian Agricultural Journal.* **50**(2):221-228.

Couch, B.C. and Kohn, L.M. (2002). A multilogues gene alloy concordant with host preference indicates segregation of new species, *Magnaporthe oryzae* from *M. grisea*. *Mycology*. **94**(4), 683-693.

Department of Agricultural Research. (2018). Research highlights during 2011-2012 to 20152016. Department of Agricultural Research, Yezin, Nay Pyi Taw, Myanmar. Organized by Asian Food and Agricultural Cooperation Initiative (AFACI-ATIN).

Du, X., Sun, S., Zheng, Z. and Tao, R.X. (1995). Evaluation of culture techniques for stimulating sporulation of *Pyricularia*. *Acta Agricultural Zhejian gensis*. **7**: 408-472.

Dutta, S. (2017). Study on blast disease of Rice and its management strategies. M.Sc. Thesis. Department of Plant Pathology. Faculty of Agriculture. Uttar Bangla Krishi Viswavidyalaya. Pundibari, Cooch Behar, West Bengal-736165.

Famoso, A., Edwards, J.D., Wamishe, Y., Valent, B., Wang, G.L. and Yang, Y. (2018). The rice blast resistance gene Ptr encodes an atypical protein required for broad-spectrum disease resistance. *Nature Communications*. **9**(1): 1–12.

Fang, N., Wang, R., He, W., Yin, C., Guan, C., Chen, H., Huang, J., Wang, J., Bao, Y. and Zhang, H. (2016). QTL mapping of panicle blast resistance in japonica landrace heikezijing and its application in rice breeding. *Molecular Breeding*. **36** (12): 171.

FAO. (1995). World Rice Information, issue No.1. FAO, Rome, Italy.

FAO. (2009). FAOSTAT Database. Retrieved June 30, 2019, from Food and Agricultural Organization website: http://www.fao.org/faostat/en/#home.

FAO. (2018). Rice Market Monitor (RMM) (No. XXI (1); p. 35). Retrieved June 30, 2019, from FAO website: http://www.fao.org/economic/est/ publications/rice-publications/ricemarket-monitor-rmm/vn/.

Faure, J. and Mazaud, F. (1996). Rice quality criteria and the European market. In: Proceedings of the 18th Session of the International Rice Commission, 5-9 September 1996, Rome, Italy. pp. 121-131.

Feakin, S.D. (1974). Pest Control in Rice. PANS Manual No 3. Centre for Overseas Pest Research, London.

Ghosh, T.K., Aparicio-Sánchez, J.J., Buxton, S., Ketley, A., Mohamed, T., Rutland, C.S., Loughna, S. and David, B.J. (2017) Acetylation of TBX5 by KAT2B and KAT2A regulates heart and limb development. *Journal of Molecular and Cellular Cardiology*. **114**:185-198.

Goto, K. (1965). Estimating losses from rice blast in Japan. In: *The Rice Blast Disease*, Johns Hopkins Oress, Baltimore and Maryland, USA. 195-202.

Guerber, C. and TeBeest, D. O. (2006). Infection of rice seed grown in Arkansas by *Pyricularia grisea* and transmission by seedlings in the field. *Plant Disease*. **90** (2):170-176.

Halima, Q., Awais, R. and Muhammad, A. (2017). Evaluation of rice germplasm for resistance against *Pyricularia oryzae* the cause of rice leaf blast. *Asian Research Journal of Agriculture.* **4**(3): 1-6.

Hashioka, Y. (1950). Studies on the mechanism of prevalence of rice blast disease in tropics. *Technical bulletin. Taiwan Agricultural Research Institute.* **8**:237.

Hawksworth, D.L. (1990). CMI Descriptions of Fungi and Bacteria. *Mycopathologia,* 111(2): 109.

Hayashi, K., Hashimoto, N., Daigen, M. and Ashikawa, I. (2004). Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. *Theoretical and Applied Genetics*. **108**(7): 1212–1220.

Hayashi, N., Kobayashi, N., Cruz, C.M.V. and Fukuta, Y. (2009). Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. **In:** Development and Characterization of Blast Resistance Using Differential Varieties in Rice (JIRCAS Working Report No. 63. pp. 17–33.

Hemmi, T. and Yokogi, K. (1927). Studies on *Sclerotium* diseases on rice plant. *I. Agriculture and Horticulture, Tokyo.* **2**:955-1094. *Review of Applied Mycology.* **7**:266.

Hittalmani, S., Parco, A., Mew, T.V., Zeigler, R.S. and Huang, N. (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theoretical and Applied Genetics*. **100**(7): 1121–1128.

Hosseyni, M. and Soltani, J. (2013). An Investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae*. *Progress in Biological Sciences*. **3**:135-143.

Huang, H. (2011). Molecular mapping of the new blast resistance genes Pi47 and Pi48 in the durably resistant local rice cultivar Xiangzi 3150. *Phytopathology*. **101**(5):620-621.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y. and Han, B. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*. **42**(11): 961–967.

IRRI. (1976). Manual on genetic conservation of rice germplasm for evaluation and utilization. 81.

Islam, Z. and Catling, D. (2012). Rice pests of Bangladesh: Their ecology and management. The University Press Limited, Dhaka. P. 422.

Rodenburg, J. and Johnson, D. E. (2009). Weed Management in Rice-Based Cropping Systems in Africa. *Advances in Agronomy*. **103**: 149-218.

Jabeen, R. (2012). Medicinal plants - a potent antibacterial source against bacterial leaf blight (blb) of rice. *Pakistan Journal of Botany*. **43**: 111-118.

Jackson, G. (2015). *Magnaporthe grisea* (rice blast disease) Crop Protection Compendium. Pacific Pests and Pathogens - Fact Sheets, 252.

Jaihom, N. and Parinthawing, N. (2014). Disease assessment and cluster analysis of rice blast fungus collected in Thailand. Conf. Kasetsart University, Thailand.

Jia, Y., Adams, S.A.M., Bryan, G.T., Hershay, H.P. and Valent, B. (2000). Direct interaction of resistance genes products confers rice blast resistance. *EMBO Journal*, **19**:4004- 4014.

Jones, M.P., Dingkuhn, M., Aluko, G.K. and Semon, M. (1997). Interspecific *Oryza sativa* L. x *Oryza glaberrima* Steud. progenies in upland rice improvement. *Euphytica*. **92**:237-246.

Kato, H. (2001). Rice blast disease. *Pesticide Outlook February*. **pp**: 23-25.

Khaing, T.Y., Win, N.K.K., Win, K.K. and Naing, T.A.A. (2018). Race study of *Pyricularia oryzae* cav. causing rice blast from different rice growing areas of Myanmar. Proceeding of the 11th Agricultural Research Conference, 17-18 January, Yezin Agricultural University, Nay Pyi Taw. **5**:57-68.

Khaing, T.Y., Win, N.K.K., Win, K.K., Htun, T.M., Thein, M.S. and Naing, T.A.A. (2018). Pathogenicity analysis of *Pyricularia oryzae*, causing rice blast and varietal resistance of cultivated rice in Myanmar. *Journal of General Plant Pathology***. 15**(6): 48-56.

Khush, G.S. and Toenniessen, G.H. (1991). Prospects for the future. Rice Biotechnology (No 6). CAB International, UK in association with *Rice Research Institute*.

Kibria, K., Nur, F., Begum, S.N., Islam, M.M., Paul, S.K., Rahman, K.S. and Azam, S.M.M. (2009). Molecular marker based genetic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. *Int. J. Sustain. Crop Prod*. **4**:23-34.

Koga, H. and Nakayachi, O. (2004). Morphological studies on attachment of *Magnaporthe grisea* to leaf surface of rice. *Journal of General Plant Pathology*. **70**: 11-15.

Kohli, M.M., Mehta, Y.R., Guzman, E., Deviedma, L. and Cubilla, L.E. (2011). *Pyricularia* blast a threat to wheat cultivation. *Czech Journal of Genetics and Plant Breeding*. **47:** 130-134.

Kumar, A. and Singh, R.A. (1996). *In-vitro* evaluation of fungicide against *Magnaporthe oryzae* isolated from rice, finger millet and peal millet. *Indian Phytopathology*. **48(**2):187-188.

Kumar, S., Rajamanickam, E., Singh, S., Mall, A., Sangle, U., Sundaram, P. and Singh, A. (2012). Evaluation of rice genotypes for resistance to blast disease under rainfed lowland ecosystem. *J. Plant Disease Sci*. **8**:175-178.

Kumbhar, R. (2005). Spectator based faceted depth classification's application in thesaurus construction. *Annals of Library and Information Studies*. **52**:15-24.

Lee, F.N. and Gunnell, P.S. (1992). False Smut. In**:** Webster, P.K. and Gunnell, P.S., Eds., Compendium of Rice Diseases. *American Phyto pathological Society Press*. St. Paul. P. 28.

Leong, H. and Shi, Z. (2004). Genetic Regulation of Sporulation in the Rice Blast Fungus. In**:** Rice blast disease. CAB International, Wallingford, Oxon, U.K. pp. 35–50.

Li, C., Wang, D., Peng, S., Chen, Y., Su, P., Chen, J., Zheng, L., Tan, X., Liu, J., Xiao, Y., Kang, H., Zhang, D., Wang, G.L. and Liu, Y. (2019). Genome wide association mapping of resistance against rice blast strains in South China and identification of a new *Pik* allele. *Rice Science*. **12**(1): 47.

Litsinger, J.A., Cruz, C.D., Raymundo, F., Barrion, A.T., Lumaban, M.D., Bandong, J.P., Venugopal, M.S., Paragna, F. and Balete, E. (1982). Insect pests and insecticide response of dry-seeded bunded rice. *Cropping systems research in Asia*. **11**: 179-194.

Liu, X., Huang, M., Fan, B., Buckler, E.S. and Zhang, Z. (2016). Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome Wide Association Studies. *PLOS Genetics*. **12**(2): e1005767.

Long, D., Correll, Lee, J.F., and TeBeest, D. (2001). Rice blast epidemics initiated by infested rice grain on the soil surface. *Plant Disease*. **85**(6): 612-616.

Malviya, R. (2014). Studies on integrated approaches for the management of leaf blast of rice caused by *Pyricularia grisea* (Cooke) Sacc. M.Sc. Thesis, Department of Plant Pathology, College of Agriculture, Rewa (M.P.) JNKVV, Jabalpur, Madhya Pradesh.

Manandhar, H.K. (1996). Rice blast disease: Seed transmission and induced resistance. Ph.D. thesis. The Royal Veterinary and Agricultural University.

Manandhar, H.K., Jorgensen, H.J.L., Mathur, S.B. and Petersen, V.S. (1998). Seed borne infection of rice by *Pyricularia oryzae* and its transmission to seedlings. *Plant Disease.* **82**(10): 1094-1099.

McRae, W. (1922). Report of the Imperial Mycologist. *Scientific report, Pusa Agricultural Research Institute.* 44-50.

Meena, B.S. (2005). Morphological and molecular variability of rice blast pathogen *Pyricularia grisea* (Cooke) Sacc. M.Sc. Thesis. University of agricultural sciences, Dharwad. pp. 45 – 46.

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A. and Latif, M.A. (2017). Marker assisted introgression of broad-spectrum blast resistance genes into the cultivated MR219 rice variety. *Journal of the Science of Food and Agriculture*. **97**:2810-2818.

Mijan, H.M.D. (2000). Studies on Blast disease of rice caused by *Pyricularia grisea* (Cooke) Sacc. in upland areas. M.Sc. Thesis, University of Agricultural Sciences, Dharwad. **pp**. 52 – 53.

Motlagh, M. and Javadzadeh, A. (2010). Study of Alternaria pellucida as a promising mycoherbicide for controlling Arrowhead (*Sagitaria trifolia*) in paddy fields. *Plant Omics***.** **3**: 172-176.

Mousanejad, S., Alizadeh, A. and Safaie, N. (2010). Assessment of yield loss due to rice blast disease in Iran. *Journal of Agricultural Science and Technology*. **12**: 357-364.

Mukherjee A.K, Mohapatra N.K, Nayak, P. (2013). Identification of slow-blasting rice genotypes through multivariate analysis of components of resistance. *Journal of Applied Biological Science*. **8**:125- 138.

Muñoz, M. (2008). The effect of temperature and relative humidity on the airborne concentration of *Pyricularia oryzae* spores and the development of rice blast in southern Spain. *Spanish journal of Agricultural Research*. **6**(1). 61-69.

Nutsugah, S.K., Twumasi, J.K., Chipili, J., Sere, D. and Sree Nivasa prasad, H. (2008). Diversity of the blast pathogen populations in Ghana and strategies for resistance management. *Plant Pathology Journal.* **7**(1): 109-113.

Oerke, E.C. and Dehne, H.W. (2004). Safeguarding production losses in major crops and the role of crop protection. *Crop Protection*. **23**(4): 275-285.

Onyango, O.A. (2014) Analysis of Meteorological Drought in North Eastern Province of Kenya. *Journal of Earth Science Climate Change*. Article No. 219.

Ou, S.H. (1985). Rice Disease. 2nd Ed. Kew Survey: Commonwealth Mycological Institute. 380.

Ou, S.H. and Nuque, F.L. (1985). Rice Diseases, second ed. Commonwealth Mycological Institute, Kew and Surrey, UK.

Padmanabhan, S. (1963). The role of therapeutic treatments in plant disease control with special reference to rice diseases. *Indian Phyto pathological Society*. **1**:79-84.

Padmanabhan, S.Y. (1965). Estimating losses from rice blast in India. In: The rice blast disease. Johns Hopkins Press, Baltimore, Maryland. **50**:203-221.

Partridge-Metz, S. and Chandra, A. (2011). Culture media influence on vegetative growth and in-vitro conidia production of *Magnaporthe Oryzae*. *Biology.* **10**:6-9.

Prabhu, A.S. and Morais, O.P. (1986). Rice blast management in upland rice in Brazil. Progress in upland rice research. Manila. *Euphytica.* **63**:115–123.

Priya, G., Saravanan, K. and Renuka, C. (2012). Medicinal plants with potential antifertility activity - A review of sixteen years of herbal medicine research (1994-2010). *Int. J. PharmTech Res*. **4**(1): 481-494.

Priya, V., Kandasamy, S., Ambalavanan, S., Ramalingam, R. and Sabariyappan, R. (2013). Variability in *Pyricularia oryzae* from different rice growing regions of Tamil Nadu, India. *African J. Micro. Res*. **7**(26): 3379-3388.

Puri, K.D., Shrestha, S.M., Joshi, K.D. and Chhetri, G.K.B. (2006). Reaction of different rice lines against leaf and neck blast under field condition of Chaitwan Valley. *Journal of Institutional Agricultural and Animal Sciences*. **27**:37-44.

Qin, Z.Y., Fei, L., Shu, J.F., Ling, W. and Qing, H.P. (2009). Recent progress on molecular mapping and cloning of blast resistance genes in rice (*Oryza sativa* L.). *Scientia Agricultura Sinica*. **42**(5): 1601–1615.

Qiu, Z., Jiang, L., Wang, C., Hill, D.V. and Wu, Y. (2019). New evidence for rice cultivation from the Early Neolithic Hehuashan site. *Archaeological and Anthropological Sciences*. **11**(4): 1259–1272.

Rahim, H. (2010). Genetic studies on blast disease (*Magnaporthe grisea*) resistance in Malaysian rice. University Kebangsaan, Selangor.

Rahman, M.H. and Jashim, M.U. (2017). Blast: A threat to cereal crops in Bangladesh. *Barisal University Journal*. Part 1. **4**:237-260.

Ram, G., Thiruvengadam, V. and Kurungara, V. (2007). Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *Journal of Applied Genetics*. **48**:337-45.

Ram, H., Saini, K., Kler, D. and Timsina, J. (2013). Tillage and planting methods effects on yield, water use efficiency and profitability of soybean-wheat system on a loamy sand soil. *Experimental Agriculture*. **10**:1017.

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M. (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*. **133**:243-252.

Reversat, G. (1995). Resistance to *Heterodera sacchari* in rice. *Nematologica.* **41**: 333-334.

Ribot, C., Hirsch, J., Balzergue, S., Tharreau, D., Nottéghem, J.L., Lebrun, M. and Morel, J.B. (2008). Susceptibility of rice to the blast fungus, *Magnaporthe grisea*. *Journal of Plant Physiology*. **165:** 114-24.

Roumen, E., Levy, M. and Notteghem, J.L. (1997). Characterization of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. *European Journal Plant Pathology*. **103**: 363-371.

Koutroubas, S.D., Katsantonis, D., Ntanos, D.A. and Lupotto, E. (2009). The effect of blast disease on rice growth, yield and quality in the field. *Turkish Journal of Agriculture and Forestry*. **33**: 487-494.

Sano, Y., Sano, R. and Morishima, H. (1984). Neighbour effects between two occurring rice species, *Oryza sativa* and *O. glaberrima*. *Journal of Applied Ecology*. **21**: 245-254.

Scardaci, S.C., Webster, R.K., Greer, C.A., Hill, J.E., Williams, J.F., Mutters, R.G., Brandon, D.M., McKenzie, K.S. and Oster, J.J. (1997). Rice blast: A new disease in California. Agronomy Fact Sheet Series 1997-2. Davis: Department of Agronomy and Range Science, University of California. **1**: 2-5.

Scheuermann, K.K., Raimondi, J.V., Marschalek, R., and De, A.A. and Wickert, E. (2012). *Magnaporthe oryzae* genetic diversity and its outcomes on the search for durable resistance. *Life Sciences.* **6:** 10-13.

Seck, P.A., Diagne, A. and Mohanty, S. (2012). Crops that feed the world 7: Rice. *Food Sec*. **4**:7–24.

Seebold, K.W., Datnoff, L.E., Correa-Victoria, F.J., Kucharek, T.A. and Snyder, G.H. (2004). Effects of Silicon and Fungicides on the Control of Leaf and Neck Blast in Upland Rice. *Plant Disease*. **88**(3): 253–258.

Shafaullah, M.A.K., Khan, N.A. and Mahmood, Y. (2011). Effect of epidemiological factors on the incidence of paddy blast (*Pyricularia oryzae*) disease. *Pakistan Journal of Phytopathology*. **23**: 108-111.

Shahriar, S.A., Imtiaz, A., Hossain, B., Husna, A. and Nurjahan, M.E.K. (2020). Review: Rice Blast Disease. *Annual Research and Review in Biology*. **35**(1): 5-64.

Sharma, O.P., Bhosle, B.B., More, D.G., Bhede, B.V., Patange, T. and Bambawale, O.M. (2008). Pest records on transgenic cotton in the Marathwada region. *Indian Journal of Plant Protection*. **36**(2): 186-191.

Sharma, T.R., Rai, A.K., Gupta, S.K., (2012). Rice blast management through host-plant resistance: Retrospect and prospects. *Agricultural Research.* **1**:37–52.

Silue, D. and Notteghem, J. (1991). Resistance of *Oryza glaberrima* varieties to blast. *Int. Rice Res. News.***16**:13-14.

Silva, L. da., Barbosa, J.M. (2009). Seaweed meal as a protein source for the white shrimp (*Litopenaeus vannamei). J. Appl. Phycol*. **21**(2): 193–197.

Sivaranjani, A.K.P., Pandey, M.K., Sudharshan, I., Kumar, G.R., Madhav, M.S., Sundaram, R.M., Varaprasad, G.S. and Rani, N.S. (2010). Assessment of genetic diversity among Basmati and non-Basmati aromatic rice of India using SSR markers. *Current Science*. **99**(2): 221-226.

Skamnioti, P. and Gurr, S.J. (2009). Against the grain: Safeguarding rice from rice blast disease. *Trends in Biotechnology*. **27**(3): 141–150.

StatSoft, Inc. Electronic Statistics Textbook (Electronic Version). StatSoft, Inc. Tulsa. 2013. http://www.statsoft.com/textbook/ Cluster-Analysis.

Sundaram, R.M., Laha, G.S., Viraktamath, B.C., Sujatha, K., Yadla, H., Reddy, C.S., Rani, N.S. and Sonit. R.V. (2011). Marker Assisted Breeding for Development of Bacterial Blight Resistant Rice. *Genomics and Crop Improvement: Relevance and Reservations*. **5**:154-182.

Suprapta, D.N. (2012). Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. *Journal of the International Society for Southeast Asian Agricultural Sciences*. **18**: 1–8.

Telebanco-Yanoria M.J., Ohsawa, R., Senoo, S., Kobayashi, N. and Fukuta, Y. (2008). Diversity analysis for resistance of rice (*Oryza sativa* L.) to blast disease [*Magnaporthe grisea* (Hebert) Barr.] using differential isolates from the Philippines. *Plant Breeding*. **127**:355-363.

Tsai, W.H., Yang, Y.Z., Leo, S.C. and Chien, C.C. (1981). Low volume chemical control of rice blast. *J. Agric. Res. China.* **30**(2): 187-191.

USDA-NRCS. (2019). Plant Profile: *Oryza* L. [Database]. Retrieved June 21, 2019, from Plants Database website: https://plants.usda.gov/java/ClassificationServlet?source=Display&classid=ORYZA.

Von, B.J. (2007). World Food Situation: New Driving Forces and Required Actions. International Food Policy Research Institute, Washington DC.

Wang, J.M., Bianco, T. and Jia. Y. (2014). Current advances on genetic resistance to rice blast disease. *Rice: Germplasm, Genetics and Improvement*. **10**:195-217.

Wheeler, B.E.J. (1969). An introduction to plant diseases. UK. p. 374.

Yaduraju, N.T. and Rao, A.N. (2013). Implications of weeds and weed management on food security and safety in the Asia-Pacific region. Presented at the Proceedings of the 24th Asian-Pacific Weed Science Society Conference on 22-25 October, 2013. Bandung, Indonesia. **p**. 13–30.

Yaegashi, H. and Nishihara, N. (1978). Production of the perfect stage in *Pyricularia* from cereals and grasses. *Japanese Journal of Phytopathology*. **42**:511-515.

Zhu, Y.Y., Fang, H., Wang, Y.Y., Fan, J.X., Yang, S.S., Mew, T.W. and Mundt, C.C. (2005). Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology*. **95**(1): 433-436.