

**INCIDENCE AND SEVERITY OF BROWN SPOT (BS), BACTERIAL LEAF
BLIGHT (BLB) AND TUNGRO DISEASE IN MOSTLY CULTIVATED
HYBRID AND INBRED RICE VARIETIES IN BANGLADESH**

By

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A Thesis

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CERTIFICATE

This is to certify that the thesis entitled, “**INCIDENCE AND SEVERITY OF BROWN SPOT(BS), BACTERIAL LEAF BLIGHT (BLB) AND TUNGRO DISEASE IN MOSTLY CULTIVATED HYBRID AND INBREED RICE VARIETIES IN BANGLADESH**” submitted to the Department Of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY**, embodies the result of a piece of *bona fide* research work carried out by **SANIA AKTER ROZY Registration No. 13-05752** under my direct supervision and guidance. No part of this thesis has been submitted for any other degree in any other institutions.

I further certify that any help or sources of information received during the course of this investigation have been duly acknowledged.

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Dedicated To
My
Beloved Parents

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INCIDENCE AND SEVERITY OF BROWN SPOT (BS), BACTERIAL LEAF BLIGHT (BLB) AND TUNGRO DISEASE IN MOSTLY CULTIVATED HYBRID AND INBREED RICE VARIETIES IN BANGLADESH

ABSTRACT

Present studies had been conducted to evaluate the effect of different hybrid and inbreed varieties of rice against major field diseases during the period from December, 2013 to June, 2014. The experiment was conducted in the Agronomy Farm, Sher-e-Bangla Agricultural University, Dhaka-1207. Five Hybrid varieties namely BRRi Hybrid Dhan 2, BRRi Hybrid Dhan 3, Aloran, Jagoran, Shakti 2 and five Inbreed varieties namely BRRi Dhan 28, BRRi Dhan 29, BRRi Dhan 50, BINA 8 and BINA 10 were evaluated against incidence and severity of brown spot, bacterial leaf blight and tungro disease at three growth stages (40 DAT, 60 DAT and 80 DAT). The effect of diseases on yield and yield contributing characters were also observed. The incidence and severity of diseases varied significantly from one another. All the varieties were developed brown spot and bacterial leaf blight disease under field condition. At 80 DAT, among hybrid varieties the highest incidence and severity of brown spot was found in Aloran (incidence in hill, leaf, grain and severity in leaf 19.74, 6.62, 18.09 and 1.74% respectively) and BLB was recorded in Shakti 2 (incidence in hill, leaf and severity in leaf 16.67, 4.03 and 0.46 respectively). The lowest incidence and severity of both diseases viz. brown spot and BLB were observed in BRRi Hybrid Dhan 2. The highest yield was found in BRRi Hybrid Dhan 2 (10.46 t/ha), and lowest yield was recorded in Aloran (5.57t/ha) and Shakti 2 (7.43t/ha). Incase of inbreed varieties, the highest incidence and severity of brown spot was found in BRRi Dhan 29 (incidence in hill, leaf, grain and severity in leaf 16.66, 7.23, 19.29 and 1.41% respectively) and BLB was recorded in BRRi Dhan 28 (incidence in hill, leaf and severity in leaf 9.48, 3.89 and 0.46% respectively). The lowest incidence and severity of brown spot was observed in BRRi Dhan 50 and BLB was found in BINA 10. The highest yield (8.92 t/ha) was recorded in BRRi Dhan 50 and BINA 10 and lowest was found in BRRi Dhan 28 (4.74 t/ha) and BRRi Dhan 29 (5.43 t/ha). Incase of Tungro, rice green leaf hoppers (*Nephotettix virescens*) were inoculated on each varieties of rice except control plants to develop the disease. Among the hybrid varieties, BRRi Hybrid Dhan 3 and Aloran were found highly susceptible and Shakti 2 was recorded highly resistance to tungro disease followed by control plant. While in the inbreed varieties, BRRi Dhan 29 was found highly susceptible and BRRi Dhan 50 and BINA 8 were observed highly resistance to tungro followed by control plant. It was noted that, every infected plant of both types of varieties (Hybrid and Inbreed) initiated lower number of tiller and panicle than untreated plant and the tiller number with panicle number become decreases with the increase of disease incidence. In case of all diseases, it was observed that disease incidence and severity was gradually increased with the age of the plant and minimum incidence and severity gave the maximum yield.

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Chapter 01
INTRODUCTION

INTRODUCTION

Rice (*Oryza sativa*) is a cereal crop of the grass family Gramineae. It has been under intensive cultivation originating in Asia for over 4,000 years and has since spread across the world, where almost a third of the world population depends on rice for vital nutrition. As a cereal grain, Rice is the most widely consumed staple food for a large part of the world's human population. About 40 percent of the world's population derives most of their calories from rice. In the world, the major rice growing countries are China, India, Myanmar, Indonesia, Bangladesh, Vietnam, Thailand, Philippine, Brazil and Japan. Almost 90 percent of the population of Bangladesh, Myanmar, Sri Lanka, Vietnam and Kampuchea are rice eaters (FAOSTAT., 2010).

In Bangladesh, rice is grown in three distinct seasons, namely Aus (April to August), Aman (August to December) and Boro (January to June) 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). Total production of rice in Bangladesh; Aus, 23,26,000 metric tons (2.44 ton/ha), Aman 1,30,23,312 metric tons (2.59 ton/ha) and Boro 1,90,07,206 metric tons (4.37 ton/ha) respectively (BBS 2013-14). So, the average yield per hectare production of rice in Bangladesh is extremely low as compare to other rice growing countries of the world for example China, India, and Vietnam etc.

There are so many constraints to increase the production of rice in Bangladesh of which disease and pest play a major role (Fakir, 1982). Rice diseases, caused by different groups of microorganisms are grouped into fungi, bacteria, virus and nematodes. Thirty six fungal, six bacterial, twenty one viral and five nematode diseases are recorded in rice (Ou, 1985). Asia's hot and humid climate during the long and heavy monsoon season provide the most favorable agro ecological environment for rice cultivation as well as diseases development. So far in Bangladesh, about 31 diseases are recorded to occur in rice including 10 major

diseases (Miah *et al.*, 1985, Shahjahan *et al.*, 1987). Major diseases are seedling blight, blast, brown spot, sheath blight and rot, bakanae, false smut, bacterial leaf blight (BLB), bacterial leaf streak, ufra and tungro disease. Among these diseases, brown spot caused by fungus, bacterial leaf blight caused by bacterium and tungro disease caused by virus played vital roles in reducing yield of rice.

Brown spot (BS) of rice caused by *Bipolaris oryzae*, is an orphan disease of rice. It has been despite the fact that the disease chronically affects millions of hectares worldwide every year (Zanao Junior *et al.*, 2009). Brown spot has been associated with two major epidemics in India, the first in 1918–19, in the Krishna- Godavari delta and the second, during 1942 in today's India and Bangladesh. It was responsible for the Bengal famine (Chakrabarti 2001). It especially occurs in environments where water supply is scarce and it is often combined with imbalances in plant mineral nutrition, especially the lack of nitrogen. Brown spot disease is characterized by oval spots on the leaves, about the size and shape of the sesame seeds. Reported yield losses in relative terms vary widely from 4 to 52 % (Chakrabarti, 2001)

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae*, occurs at all the growth stages of rice and is manifested by leaf blight symptoms. The causal organism invades plants through water pores and wounds (Tabei and Mukoo, 1960). Since the water pores are located at the margins of upper parts of the leaf, the lesion usually starts from the leaf margin near its tip. As the disease progresses, the tiny water soaked lesion turns yellow, enlarges in size progressively and develops into an elongated irregular lesion with wavy margins. Bacterial ooze, which consists of small, yellowish, spherical masses, may sometimes be seen on the margins or veins of the freshly infected leaf under moist conditions. With the passage of time, the lesion may cover on the entire blade, which turns white and later grayish owing to saprophytic growth (Ou, 1985). The incidence of BLB was found to be aggravated by high dosages of nitrogen. There may be 50% reduction in yield in case of severe infection (Mew *et al.*, 1993) whereas 10-12% yield reduction has been recorded in case of mild infection (Ou, 1985). Losses ranging from 2.7% to 41.0% in grain yield were inflicted on the rice

crop by BLB. The yield loss caused by BLB in Bangladesh has been estimated as 10-30% (Ashrafuzzaman, 1992).

Tungro disease of rice is caused by *Rice tungro spherical virus (RTSV)* and *Rice tungro bacilliform virus (RTBV)*. In Bangladesh, tungro was first recorded in 1966, and reported as the most damaging viral disease. Stunting of the plant along with yellowish or orange discoloration and twisting of the leaves, reduced tillering, delayed flowering etc. are the distinguishing symptoms of this disease. The disease causing viruses is transmitted by Green leafhoppers (*Nephotettix virescens*, *N. nigropictus*, and *Recilia dorsalis*) and mostly by *Nephotettix virescens*. Due to the potential injury and unpredictability tungro outbreaks can cause yield losses up to 80% (Quazi *et al.*, 2009).

In view of the above facts the present study was conducted to investigate the incidence and severity of brown spot, bacterial leaf blight and tungro disease of rice in mostly cultivated hybrid and inbred varieties in Bangladesh and to know the most susceptible growth stage of rice due to these diseases. To achieve these goals, we selected five hybrids and five inbreds rice varieties which are mostly cultivated in Boro season in Bangladesh.

Objectives:

The present study was undertaken with the following specific objectives-

1. To estimate the incidence and severity of brown spot, bacterial leaf blight and tungro disease of rice.
2. To determine the effect of these disease on the yield and yield contributing characters of rice.
3. Screening of resistant varieties against these three major diseases of rice in Boro season.



Chapter 02
REVIEW OF LITERATURE

REVIEW OF LITERATURE

Rice is one of the major crops in the world. It suffers from many diseases. The literatures on incidence, severity, effect on the yield, yield contributing characters and screening of resistance varieties against brown spot, bacterial leaf blight and tungro are accumulated in this chapter.

2.1. Brown Spot

Baranwal *et al.*, (2013) reported that the disease especially occurs in environment where water supply is scarce combined with nutritional imbalance particularly lack of nitrogen.

Khalili *et al.*, (2012) reported that the disease has been reported to occur in all the rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philippines, Saudi Arabia, Australia, Malaya and Thailand.

Dallagnol *et al.*, (2011) reported that the environmental factors influence survival of fungus in seed and soil. The temperature and relative humidity at which seeds are stored influence the variability of the pathogen.

There is indication that BS is becoming more frequent and severe as drought is becoming more frequent (Savary *et al.*, 2005), perhaps due to increased variability in rainfall consider BS as a model pathosystem for a synergistic understanding on a range of diseases (Savary *et al.*, 2011).

Kamal and Mia (2009) observed that reduction in yield ranged from 18.75-22.50%. Glume blotch phase of the disease has been reported to cause more damage (Kulkarni *et al.*, 1986) and reduce seed germinability (Hiremath *et al.*, 1983).

Savary *et al.*, (2006) reported that a number of damage mechanisms lead to BS yield losses, in addition to leaf area index (LAI) reduction and presumably self-shading of lesions on underlying canopy.

Vu-Van and Sangchote (2006) found the highest incidence of *Bipolaris oryzae* in rachilla followed by sterile lemma, palea, embryo and endosperm of rice kernels with typical brown spot.

Brown spot is still widely reported across India and more generally in the South and South-East Asian countries (Savary *et al.*, 2000). It causes yield losses that, on average, are in the range of 10 % of the attainable yield wherever it occurs (Savary *et al.*, 2000, 2006) in the lowlands of tropical and subtropical Asia.

Pannu *et al.*, (2005) reported that within a 5-year period (2000– 2004) two rainy season crops (2001 and 2003) in India led to terminal severities of 9.2 to 8.8 %, corresponding to accumulated rainfalls of 410.5 to 502.0 mm, respectively. However, in 2002 lower rainfall corresponded with higher severity.

Igawa *et al.*, (2005) Timmusk and Wagner (1999) reported that, BS might be strongly associated with the combined plant physiological disorders, disease development processes, and disease resistance mechanisms. This association between exposure to physiological stresses and disease development brings about generic and important questions pertaining to interacting metabolic pathways, their genetic bases and the interaction amongst genes or clusters of genes.

Brown spot is generally not observed in years with regular rainfall (Singh *et al.*, 2005) whereas seasons with limited rainfall but heavy dew are conducive to stronger epidemics (Sherf *et al.*, 1947).

Jha *et al.*, (2004) observed a higher disease severity in direct sown plants than in transplanted crop.

Holanda *et al.*, (2002) reported that this disease occurs more frequently in soils with high pH, low organic matter and low levels of N, K, Mn, Si and free Fe and low CEC.

Minnatullah and Sattar (2002) reported that temperature and humidity, in the form of leaf wetness, interact on infection efficiency. This may explain why decreasing daily minimum temperatures (9.3 to 7.5 °C) lead to more severe epidemics.

Jha (2001) reported that plants are more susceptible during dough and mature stages.

Singh *et al.*, (2000) and Jha (2001) observed that the disease severity was more in late sown crop as compared to early planted crop

2.2. Bacterial leaf blight

Akhtar *et al.*, (2011) reported that the disease reduces grain yield to varying levels depending on the stage of the crop, degree of cultivar susceptibility and a great extent to the conduciveness of the environment in which it occurs.

Basso *et al.*, (2011) reported that recent studies in West African countries such as Burkina Faso, Niger and Mali revealed the occurrence of BLB causing significant crop damages.

Kadai (2010) reported that occurrence of bacterial leaf blight in most of rice-growing ecozones of Togo with high incidence and severity, and the virulence of the pathogen was determined.

Waheed *et al.*, (2009) reported that Bacterial leaf blight has the potential to become a destructive bacterial disease of rice in Pakistan and can cause huge losses mainly because of the lack of information regarding the pathogen and its effective measure of control.

Dai *et al.*, (2007) reported that Bacterial Leaf Blight Disease (BLB), one of the major diseases of rice, known to occur in epidemic proportion in many part of the world, can reduce production by more than 50%.

Sere *et al.*, (2005) reported that BLB was observed to occur in fields with high incidence of 70 to 80% in several West African countries.

Bacterial leaf blight of rice (BLB), caused by *Xanthomonas oryzae pv. oryzae* (Swings *et al.*, 1990), is one of the most widespread and destructive diseases of rice in several countries in tropical rice-growing areas of Asia, Australia, United States, Latin America and Africa (Mew, 1987, 1989; Mew *et al.*, 1993; Sere *et al.*, 2005).

Ou (1985) and Sere *et al.*, (2005) reported that Yield losses due to BLB ranging from 50 to 90%.

Ezuka and Kaku (2000) reported that BLB occurrence reported from Australia, Bangladesh, Cambodia, Indonesia, India, Korea, Mainland China, Malaysia, Sri-lanka, Thailand, Philippines, USA, West Africa and Vietnam.

Veena *et al.*, (2000) reported that in India, the yield loss due to this disease is up to 81.3%.

2.3. Tungro

Dai and Beachy (2009) reported that Rice tungro disease (RTD); caused by the co-infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus is one of the most destructive rice diseases in South and Southeast Asia with outbreaks affecting thousands of hectares.

Rice tungro disease, the most important viral disease of rice, is widespread in South and Southeast Asia and is believed to be responsible for annual losses nearing 10⁹ US dollars worldwide (Herdt, 1991). More recent estimates reveal that the disease causes on an average about 2% losses in rice production in India, although at the regional level, losses can be more significant (Muralidharan *et al.*, 2003).

Cabunagan *et al.*, (2001) reported that the vector population, extensive cultivation of susceptible varieties and asynchrony of planting were identified as potential factors influencing the disease epidemics.

Azzam *et al.*, (1999) reported that in India in 1998, an outbreak of tungro-like yellow stunt syndrome occurred in the Punjab and damaged 40,000 ha of the 490,000 ha planted to rice in the area. Yield losses on the affected land were estimated at 30-100%.

Marmey *et al.*, (1999) and Hibino (1996) reported that Rice tungro disease occurs in south and southeastern Asia and in southern China with estimated annual crop loss of \$680 million. Its significance has become increasingly since mid-1960s, as

a consequence of planting susceptible but high yielding cultivars and double cropping of rice become common in irrigated areas in Asian tropics.

Hibino (1996) made the point that intensification of rice cultivation, and in particular, practices such as double-cropping, has significantly increased the incidence of virus disease. It is also noted that RTD was endemic in double-cropped rice areas.

In Mindanao (the Philippines) RTD was listed as the most destructive disease on rice (Sanchez and Obien, 1995) also in the Philippines, Cabauatan *et al.*, (1995) described a variety of different strains of both viruses with differing pathogenicity.

Medina *et al.*, (1994) reported that Leafhopper transmission of the disease complex is dependent on the presence of RTSV however; both agents contribute to symptoms and severity. The dependent transmission of RTBV can be explained by the association of both viruses with an inclusion body matrix in infected cells.

The disease is confined to Asia and there are reports from India (Nagarajan, 1993) through Southeast Asia to China (Zhou *et al.*, 1992).

Dasgupta *et al.*, (1991) reported that the most conspicuous symptoms of tungro are the stunting of plants and yellow-orange discoloration of leaves, both of which are believed to be caused by RTBV, as observed in symptomatic plants subjected to *Agrobacterium*-mediated inoculation of the virus.

Rice tungro is caused by the joint infection of two unrelated viruses *Rice tungro bacilliform virus* (RTBV), a double-stranded DNA-containing virus, belonging to the genus tungro virus and rice tungro spherical virus (RTSV), a single-stranded RNA virus belonging to the genus

Waikavirus (Jones *et al.*, 1991). RTBV and RTSV, also known as the “Tungro virus complex”, are transmitted exclusively by the Green leafhopper, GLH, *Nephotettix virescens* (Hibino and Cabauatan, 1987).



Chapter 03
MATERIALS AND METHODS

MATERIALS AND METHODS

In this chapter the details of different materials used and methodology followed during the experimental period are described.

3.1. Experimental site

The experiment was conducted at agronomy farm of Sher-e-Bangla Agricultural University, Dhaka -1207, during the period from December, 2013 to June, 2014.

3.2. Variety selection

We were selected five hybrids and five inbreed varieties of rice which are mostly cultivated in Boro season in Bangladesh. Varieties were as follows-

Hybrid varieties	Inbreed varieties
BRRRI Hybrid Dhan 2	BRRRI Dhan 28
BRRRI Hybrid Dhan 3	BRRRI Dhan 29
Aloran	BRRRI Dhan 50
Jagoran	BINA 8
Shakti-2	BINA 10

3.3. Seed collection

Seeds were collected from Bangladesh Rice Research Institute (BRRRI), Joydebpur, Gazipur, Bangladesh Institute of Nuclear Agriculture (BINA), Bangladesh Agricultural University, Mymensingh and BRAC center, Gazipur.

3.4. Sprouting of seeds

Seeds were soaked in ten 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 ten different gunny bags and kept at room temperature for 72 hours for sprouting.

3.5. 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. As the land was rich in organic matters, therefore no manuring was done. Sprouted seeds were sown in wet seed bed on 1st December, 2013. Seedlings were properly taken care of. Weeds were removed and irrigation was given in the seed bed as and when necessary.

3.6. Field Experiment

3.6.1. Land preparation

The land was prepared with the help of power tiller and harrow. The land was first opened on 25 December 2013 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.

3.6.2. Fertilizer application

Fertilizers were applied as per recommendation of BRRI, 2004 (Adhunic Dhaner Chash). The following doses of fertilizers were applied to the plots:

Fertilizers	Dose (kg/ha)
Urea	53.00
TSP	20.56
MP	23.89
Gypsum	13.61
Zinc Sulphate	2

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.

3.6.3. Design and layout

The experiment was laid out in randomized complete block design (RCBD) with three replications. Blocks were representing the replication. Each block comprised 10 unit plot and total number of plots were 30 (10 X 3=30). Size of each unit plot was 2.5x2.0 = 5.0 m². The distances between unit plot was 0.70 m and block 1m. (Appendix I)

3.6.4. Seedling transplantation

Seedlings were uprooted from the seed bed very carefully, and then transplanted on 2nd January, 2014 in the main field. Row to row spacing was maintained as 25 cm and hill to hill 15 cm. Four seedlings were transplanted together in individual hill.



Figure 1. Seedlings of different varieties of rice in seedbed

3.6.5. Intercultural operation

Weeding and irrigation was done in the field as and when necessary.

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

Each of the plots was investigated for recording the incidence and severity of brown spot (BS) and BLB diseases. 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 (40 DAT, 60 DAT, 80 DAT). Data was recorded on; hill/plot, infected hill/plot, total leaf/infected hill, infected leaf/infected hill, % disease infection/leaf. To estimate the incidence of brown spot in grain five hill of each unit plot were

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.

Incidence: % disease incidence was estimated by using the following formula (Rajput and Bartaria, 1995)

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

Severity: The severities of the diseases were recorded by following IRRI recommended grading scale (0-9 scale of Standard Evaluation System for Rice, 1980). The grades of each disease are given below:

Brown Spot

- 0 = No incidence
- 1 = Less than 1% leaf area affected
- 2 = 1-3% leaf areas affected
- 3 = 4-5 % leaf areas affected
- 4 = 6-10 % leaf areas affected
- 5 = 11-15 % leaf areas affected
- 6 = 16-25 % leaf areas affected
- 7 = 26-50 % leaf areas affected
- 8 = 51-75 % leaf areas affected
- 9 = 76-100 % leaf areas affected

Bacterial leaf blight (BLB)

- 0 = no lesion
- 1 = 1-5% lesion area
- 3 = 6-12% lesion area
- 5 = 13-25% lesion area
- 7 = 26-50% lesion area
- 9 = 51-100% lesion area

3.6.7. Isolation of *Bipolaris oryzae*

Isolation from leaf:

Bipolaris oryzae was isolated from infected leaves by using tissue planting method (Agrios, 2006). Diseased rice leaves were collected from the field and cut into small pieces along with healthy portion. Cut pieces were sterilized by the surface disinfectants, 0.1% mercuric chloride for 30 seconds. After sterilization, the cut pieces were washed three times with sterile water and then placed on sterile blotter paper to remove excess water. After removal of excess water, the cut pieces were placed on the sterile PDA media plate. The plates were labeled and placed in the incubation chamber at 25⁰C for 7 days. After 7 days of incubation, the fungus grown on culture media. A portion of culture was taken on slide and observed under compound microscope and identified the pathogenic structures i.e. *Bipolaris oryzae* with the help of relevant literature (Mew and Gonzales, 2002). A portion of culture was taken by inoculating needle on another sterile PDA plate. A small portion from the sub-culture was inoculated to another PDA plate for pure culture. The fungus, thus purified, was kept in refrigerator for future use. All these operations were done aseptically in the laminar air flow chamber. The pure culture was examined at 10x and 40x magnification to identify the fungus.

Isolation from seed:

The experiment was conducted at Seed Health Testing Laboratory, Department of Plant Pathology, SAU. Some diseased rice seeds were collected from the field. These seeds assessed for the detection of seed borne fungi *Bipolaris oryzae* through blotter paper and potato dextrose agar method followed by ISTA (2003). These seed were surface sterilized with the 0.5 % HgCl₂. After sterilization, seeds were washed three times with sterile water and then placed on sterile blotter paper to remove excess water. After removal of excess water, the diseased seeds were placed on the sterile PDA media plate. The plates were labeled and placed in the lab for period of seven days at 24°C ± 1 and sufficient light 12 hours alternating cycles of NUV (near ultra violet) light and darkness. After 7 days of incubation, the fungus grown on culture media. A portion of culture was taken on slide and observed under compound microscope and identified the pathogenic structures i.e. *Bipolaris oryzae* with the help of relevant literature (Mew and Gonzales, 2002). A

portion of culture was taken by inoculating needle on another sterile PDA plate. A small portion from the sub-culture was inoculated to another PDA plate for pure culture. The fungus, thus purified, was kept in refrigerator for future use. All these operations were done aseptically in the laminar air flow chamber. The pure culture was examined at 10x and 40x magnification to identify the fungus.



Figure 2. Brown spot infected leaf used for pure culture of *Bipolaris oryzae*



Figure 3. Brown spot infected seed with colony used for pure culture of *Bipolaris oryzae* (40x)



Figure 4. Pure culture of *Bipolaris oryzae*

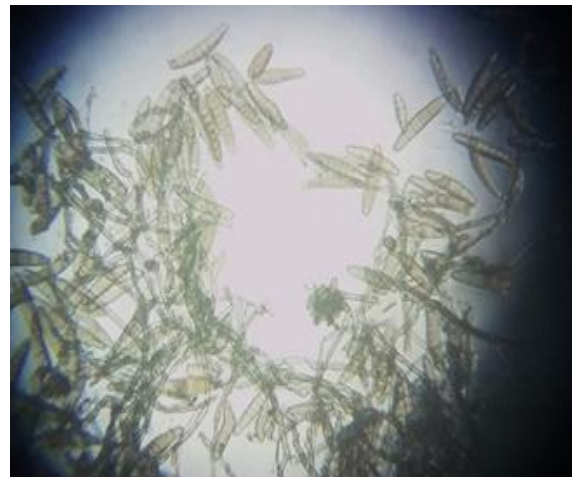


Figure 5. Microscopic view of pathogenic structure of *Bipolaris oryzae* (40x)

3.6.8. Isolation of *Xanthomonas oryzae* pv. *Oryzae*

The diseased leaves were washed under running water. Then the young lesions with green healthy portion of diseased leaves were cut into small pieces. Surface sterilize were done by soaking them in 5 % sodium hypochlorite solution for 2-3 minutes and then washed them three times with sterile water. After surface sterilization, the cut pieces were kept in a test tube containing 3-4 ml of sterile water and kept for 30 minutes for bacterial streaming and getting stock. One ml of this stock solution was transferred with the help of sterile pipette into the second test tube containing 9 ml sterile water and shaken thoroughly resulting 10^{-1} dilution. Similarly final dilution was made up to 10^{-4} . Drops of leaf extract are streaked onto the NA media plates and incubated at 27 ± 2 °C for 3 days. After incubation period, single colonies were grown over the NA plate. Then, it was kept in refrigerator at 4°C for future use. Identification of the pathogen causing bacterial leaf blight (BLB) disease of rice was determined by gram's staining.

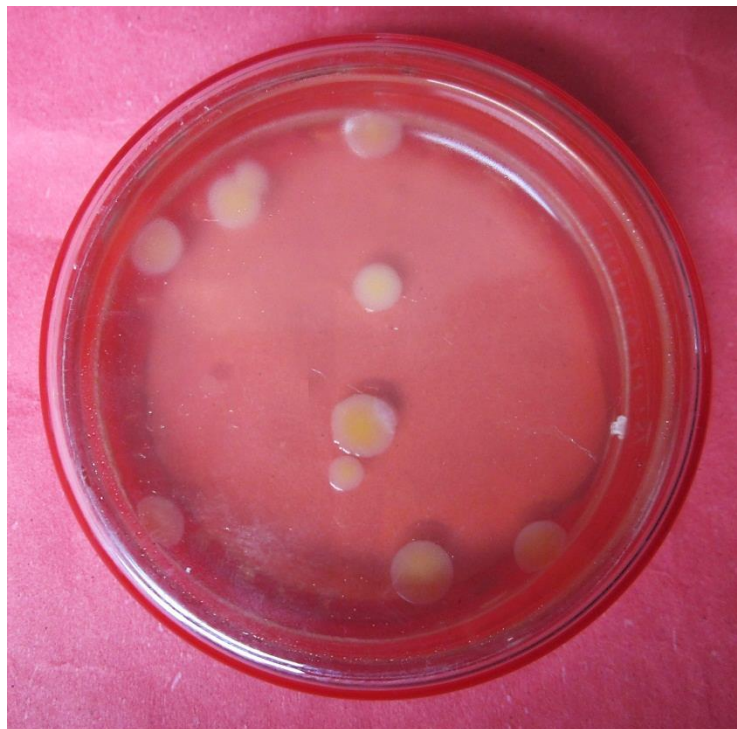


Figure 6. Pure culture of *Xanthomonas oryzae* pv. *oryzae*

Gram's staining: A small drop of sterile water was placed on a clean microscope slide. Part of a young yellow colony (18-24 hours old) was removed with a cold, sterile loop from the nutrient agar medium and the bacteria were smeared onto the slide that was very thin. The thinly spread bacterial film was air dried. Underside of the glass slide was heated by passing it four times through the film of a spirit lamp for fixing the bacteria on it. Then the slide was flooded with crystal violet solution for one minute. It was rinsed under running tap water for a few seconds and excess water was removed by air. Then it was flooded with lugol's iodine solution for 1 minute. Then it was decolonized with 95 % ethanol for 30 seconds and again rinsed with running tap water and air dried. Then it was counter stained with 0.5% safranin for 10 seconds. It was rinsed under running tap water for a few seconds and excess water was removed by air. Then the glass slide was examined at 40x and 100x magnification using oil immersion.

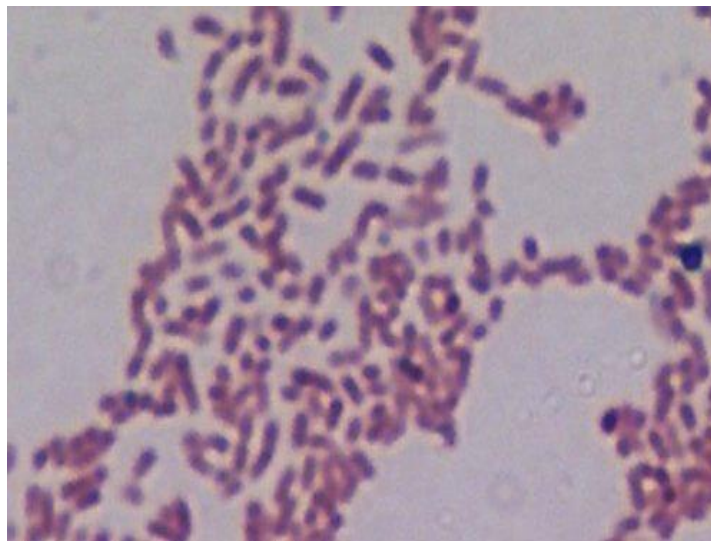


Figure 7. Microscopic view of pathogenic structure of *Xanthomonas oryzae pv. oryzae* after gram's staining (100x)

3.6.9. Harvesting and collection of data on yield and yield contributing parameters

Each variety of rice was harvested at full ripening stage. Moreover five hill of each unit plot were harvested randomly and separately. Then fifteen hill (As each variety consists three replication) from each variety were mix together and select 30 panicle randomly from that mixture. The data on the following yield

contributing parameters were recorded: number of panicle/15 plant, total grain/panicle, infected grain/panicle, total yield/variety.

3.7. Pot experiment:

3.7.1. Pot preparation

Pots were prepared manually by hand. Soil was collected from the same field where the field experiment was conducted.

3.7.2. Design and layout

The experiment was laid out in Completely Randomized Design (CRD) with three replication. Each variety comprises three replication (three pots) and one control (one pot). So, the total number of pot was 40 ($10 \times 3 = 30$ and $10 \times 1 = 10$).

3.7.3. Seedling transplantation

Seedlings were uprooted from the seedbed very carefully and then transplanted on 2 January, 2014 in the pot. Three seedlings were transplanted together in individual pot.

3.7.4. Intercultural operation:

Weeding and irrigation was done in the pot as and when necessary.

3.7.5. Inoculation with green leaf hopper



Figure 8. Green leaf hopper in Infected rice plant (at green house)



Figure 9. Green leaf hopper associated with rice plant (after inoculation)

At first, pots (except control pots) were covered by net (1 mm pores) during tillering stage (Appendix II). A significant number (7-10) of green leaf hoppers (*Nephotettix virescens*) was collected from BRRI and reared to feed on covered rice plants to transmit the disease.

Rearing procedure of green leaf hopper in BRRI:

The culture is usually started by collecting GLH from the field. A greenhouse and rearing cages are the major items required for rearing GLH. The green house should be well lighted and should provide a suitable environment for growing plants throughout the year. The greenhouse is equipped with pan trays, rearing cages and earthen pots. The size of the trays depends on the size of the greenhouse and on the volume of the materials to be used. The trays must be deep enough to cover the basal parts of the potted plants. For rearing cages use mesh. A bottom less cage placed in a water pan tray and the plants (food plants of 10 days) with earthen pot sit in water. Potted plants sit in about 8 cm of water inside the cage to provide optimum humidity. Then inoculate the collected GLH in food plant inside the cage. Hoppers are usually collected through aspirator and mylar also used to transfer the hopper from old plant to new one.



Figure 10. Green leaf hoppers were reared on potted plants in the cage.



Figure 11. Green leaf hoppers are on potted plants with mylar.

3.7.6. Collection of data on yield and yield contributing parameters

Disease incidence and severity of tungro disease are same. Three weeks after inoculation, the plants were scored based on visual observation of the symptoms.

The data on the following parameters were recorded; symptom on leaf blade leaf or sheath,% disease incidence and severity, number of tiller/hill, time of panicle initiation, panicle/infected hill, panicle/control hill, % plant height reduction and reaction level to rice tungro virus.

3.7. Weather report

The data on average temperature, relative humidity and rainfall were recorded at different date from mini weather station, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka 1207 (Appendix III).

3.8. Data Analysis

The data on different characters were subjected to statistical analysis using analysis of variance to find out the variation resulting from experimental treatments. The analysis was done using MSTAT computer software. Mean differences among the treatments were compared by Duncan's Multiple Range Test.



Chapter 04
RESULTS

RESULTS

The main objective of this research was to screening of resistant varieties against brown spot, bacterial leaf blight and tungro disease of rice. To achieve this goal, the disease incidence and severity was estimated at different DAT. In case of brown spot and bacterial leaf blight, the incidence was estimated on the basis of hills and leaves infection while the severity on the basis of leaf areas infection. The incidence of brown spot of rice in grain was also estimated on the basis of number of grains infection. Among the selected varieties, the incidence and severity of tungro disease of rice was checked by artificial inoculation with viruliferous insect vector, rice green leaf hopper. In this study, we also determined the effect of these diseases on the yield and yield contributing characters of rice.

4.1. Effect of different varieties on incidence of brown spot of rice in hill at field condition

The effect of different varieties on incidence of brown spot of rice in hill was recorded at 40, 60 and 80 days after transplanting (DAT). The incidence of brown spot was showed significant variants among the varieties at 40 DAT and ranged from 1.78-15.64%. Among the hybrid varieties, the highest incidence was found in Aloran (15.64%) which was significantly different from other varieties and the lowest incidence was recorded in BRR I Hybrid Dhan 3 (1.78%) which was statistically similar to all other hybrid varieties. In case of inbreed varieties, the highest incidence was recorded in BRR I Dhan 29 (14.35%) that was significantly different from other varieties and the lowest was found in BRR I Dhan 50 (1.78%) which was statistically similar to BINA 8 and BINA 10. After 60 DAT, in case of hybrid varieties the highest incidence was found in Aloran (17.69%) that was statistically different from all other varieties and the lowest incidence (4.35%) was recorded in BRR I Hybrid Dhan 2 and Shakti 2 which was statistically similar to rest two varieties. Among the inbreed varieties, the highest incidence was recorded in BRR I Dhan 29 (15.38%) that was significantly different from other varieties and lowest incidence (3.32%) was observed in BRR I Dhan 50 and BINA 8 which was statistically similar to two other varieties. At 80 DAT, among the hybrid varieties the highest incidence was also found in Aloran (19.74%) which was

statistically different from other varieties and the lowest incidence (5.89%) was also recorded in BRRH Hybrid Dhan 2 which was statistically similar to all other varieties. In case of inbred varieties, the highest incidence was recorded in BRRH Dhan 29 (16.66%) that was statistically different from all other varieties and lowest incidence was (5.12%) in BRRH Dhan 50 and BINA 8 which was statistically similar to two other varieties. It was noted that the disease incidence was gradually increased with the increase of the age of plant. These results are presented in table 1.

Table 1. Effect of different varieties on incidence of brown spot of rice in hill at different days after transplanting

Variety	% Incidence in hill		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	2.30 c	4.35 b	5.89 b
BRRH Hybrid Dhan 3	1.78 c	6.15 b	6.66 b
Aloran	15.64 a	17.69 a	19.74 a
Jagoran	2.04 c	6.15 b	8.45 b
Shakti 2	2.04 c	4.35 b	7.17 b
BRRH Dhan 28	7.43 b	7.69 b	9.48 b
BRRH Dhan 29	14.35 a	15.38 a	16.66 a
BRRH Dhan 50	1.78 c	3.32 b	5.12 b
BINA 8	3.07 c	3.32 b	5.12 b
BINA 10	2.55 c	4.09 b	6.92 b
LSD _{0.05}	4.05	5.21	5.52
CV (%)	44.60	41.96	35.29

4.2. Effect of different varieties on incidence of brown spot of rice in leaf at field condition.

The effect of different varieties on incidence of brown spot of rice in leaf was recorded at 40, 60 and 80 days after transplanting (DAT). The incidence of brown spot was showed significant variants among the varieties at 40 DAT and ranged from 2.12 to 6.52%. In case of hybrid varieties, the highest incidence was found in Aloran (3.76%) that was statistically similar with Shakti 2 and the lowest incidence (2.55%) was recorded in BRRH Hybrid Dhan2. Among the inbred varieties, the highest incidence was recorded in BRRH Dhan29 (6.52%) which was statistically different from other varieties and the lowest incidence was found in BINA 10 (2.12%). The disease incidence varied significantly at 60 DAT where, in case of hybrid varieties the highest incidence was found in Aloran (4.82%) which was statistically different from other varieties and the lowest incidence was recorded in BRRH Hybrid Dhan 2 (3.20%). Among the inbred varieties, the highest incidence was recorded in BRRH Dhan 29 (7.16%) that was statistically different from other varieties and the lowest incidence was found in BRRH Dhan 50 (2.97%). At 80 DAT, among the hybrid varieties the highest incidence was found in Aloran (6.62%) and lowest incidence was recorded in BRRH Hybrid Dhan2 (5.18%). In case of inbred varieties, the highest incidence was found in BRRH Dhan 29 (7.23%) which was statistically different from other varieties and the lowest incidence was in BRRH Dhan 50 (5.41%). It was noted that the disease incidence was gradually increased with the increase of the age of plant. These results are presented in Table 2.

Table 2. Effect of different varieties on incidence of brown spot of rice in leaf at different days after transplanting

Variety	% Incidence in leaf		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	2.55 c	3.20 c	5.18 b
BRRH Hybrid Dhan 3	2.69 c	4.46 bc	5.98 ab
Aloran	3.76 bc	4.82 b	6.62 ab
Jagoran	2.64 c	4.32 bc	5.47 b
Shakti 2	3.66 bc	4.05 bc	5.87 ab
BRRH Dhan 28	3.94 bc	4.25 bc	6.39 ab
BRRH Dhan 29	6.52 a	7.16 a	7.23 a
BRRH Dhan 50	2.21 c	2.97 c	5.41 b
BINA 8	2.59 c	4.45 bc	5.68 b
BINA 10	2.12 c	2.99 c	6.31 ab
LSD _{0.05}	1.94	1.34	1.36
CV (%)	28.34	18.94	13.32

4.3. Effect of different varieties on severity of brown spot of rice in leaf at field condition.

The effect of different varieties on severity of brown spot of rice in leaf was recorded at 40 DAT, 60 DAT and 80 DAT. The severity of brown spot was showed significant variants among the varieties at 40 DAT and ranged from 0.016 to 1.04%. In case of hybrid varieties, the highest severity was found in Aloran (1.04%) that was statistically different from other varieties and the lowest severity was recorded in BRRH Hybrid Dhan 2 (0.01%). Among the inbreed varieties, the highest severity was recorded in BRRH Dhan 29 (0.43%) which was statistically similar with BRRH Dhan 28 and BINA 8 and the lowest severity was found in BRRH Dhan 50 (0.03%). The disease severity varied significantly at 60 DAT where, among the hybrid varieties the highest severity was found in Aloran (1.72%) which was statistically different from other varieties and the lowest severity was recorded in BRRH Hybrid Dhan 2 (0.05%). In case of inbreed varieties, the highest severity was recorded in BRRH Dhan 29 (1.33%) that was statistically different from other varieties and the lowest was in BRRH Dhan 50 (0.04%). After 80 DAT, in case of hybrid varieties, the highest severity was found in Aloran (1.74%) which was also statistically different from other varieties and the lowest severity was recorded in BRRH Hybrid Dhan 2 (0.08%). Among the inbreed varieties, the highest severity was found in BRRH Dhan 29 (1.41%) that was statistically different from other varieties and lowest was recorded in BRRH Dhan 50 (0.15%). It was noted that the disease severity was gradually increased with the increase of the age of plant. These results are presented in Table 3.

Table 3. Effect of different varieties on severity of brown spot of rice in leaf at different days after transplanting

Variety	% Severity in leaf		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	0.01 d	0.05 d	0.08 c
BRRH Hybrid Dhan 3	0.02 d	0.06 d	0.09 c
Aloran	1.04 a	1.72 a	1.74 a
Jagoran	0.16 cd	0.18 cd	0.23 bc
Shakti 2	0.16 cd	0.17 cd	0.22 bc
BRRH Dhan 28	0.15 cd	0.17 cd	0.25 bc
BRRH Dhan 29	0.43 bc	1.33 a	1.41 a
BRRH Dhan 50	0.03 d	0.04 d	0.15 c
BINA 8	0.11 cd	0.22 bcd	0.23 bc
BINA 10	0.03 d	0.17 cd	0.22 bc
LSD _{0.05}	0.33	0.21	0.21
CV (%)	57.60	49.90	45.14

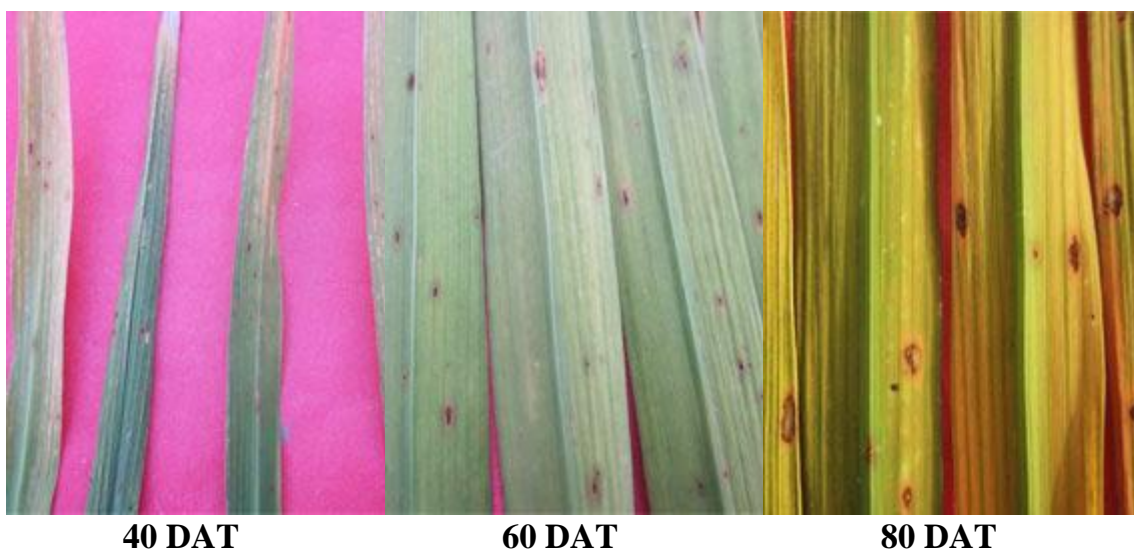


Figure 12. Brown spot infected leaf of hybrid variety Aloran at (40 DAT, 60 DAT and 80 DAT) different days after transplanting

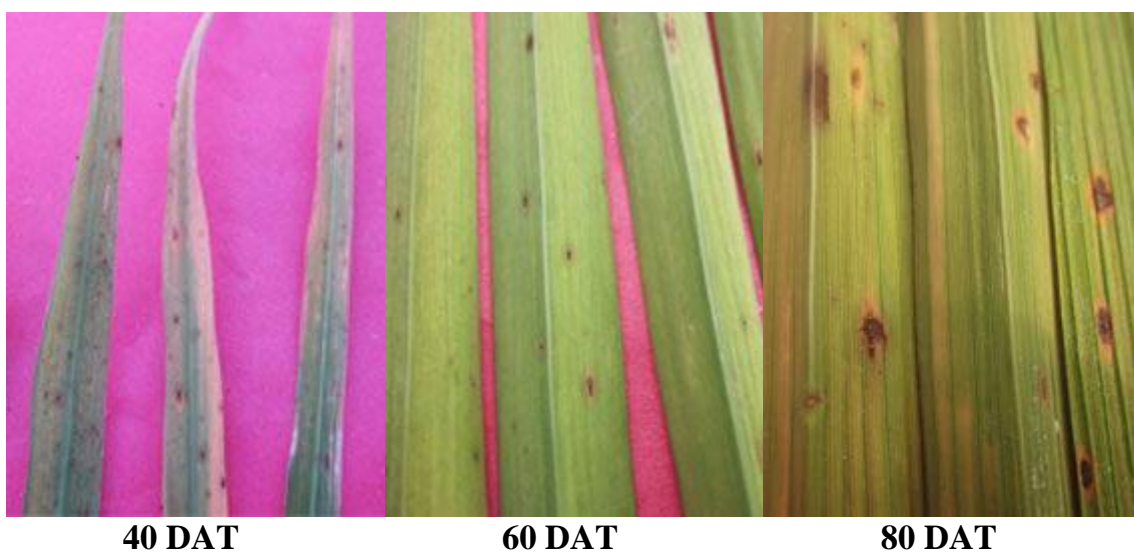


Figure 13. Brown spot infected leaf of inbred variety BRR1 Dhan 29 at (40 DAT, 60 DAT and 80 DAT) different days after transplanting

4.4. Effect of different varieties on incidence of brown spot of rice in grain at field condition.

The effect of different varieties on incidence of brown spot of rice in grain was recorded after harvesting of rice. The incidence of brown spot was showed significant variants among the varieties and ranged from 1.17 to 19.29%. In case of hybrid varieties, the highest incidence was found in Aloran (18.09%) which was statistically different from other varieties and the lowest incidence was recorded in BRRI Hybrid Dhan 2 (1.17%) that was statistically different from all other varieties. Among the inbreed varieties, the highest incidence was recorded in BRRI Dhan 29 (19.29%) that was statistically different from other varieties and lowest was recorded in BRRI Dhan 50 (4.26%) which was also statistically different from other varieties. These results are presented in Table 4.

Table 4. Effect of different varieties on incidence of brown spot of rice in grain

Variety	% Incidence in grain
BRRH Hybrid Dhan 2	1.17 h
BRRH Hybrid Dhan 3	2.46 f
Aloran	18.09 b
Jagoran	1.75 g
Shakti 2	2.64 f
BRRH Dhan 28	18.09 b
BRRH Dhan 29	19.29 a
BRRH Dhan 50	4.26 e
BINA 8	7.58 d
BINA 10	12.33 c
LSD _{0.05}	0.47
CV (%)	3.72



Figure 14. Brown spot infected grain of hybrid variety Aloran



Figure 15. Brown spot infected grain of inbred variety BRR1 Dhan 29

4.5. Effect of different varieties on incidence of Bacterial Leaf Blight of rice in hill at field condition

The effect of different varieties on incidence of Bacterial Leaf Blight of rice in hill was recorded at 40 DAT, 60 DAT and 80 DAT. The incidence of bacterial leaf blight was showed significant variants among the varieties at 40 DAT and ranged from 0.76 to 3.59%. In case of hybrid varieties, the highest incidence was found in Shakti 2 (3.59%) that was statistically similar with BRRH Hybrid Dhan 3 and the lowest incidence was recorded in Aloran (1.02%). Among the inbred varieties the highest incidence was recorded in BRRH Dhan 28 (1.53%) and the lowest incidence was in BINA 10 (0.76%). The disease incidence varied significantly at 60 DAT where, among the hybrid varieties, the highest incidence was recorded in Shakti 2 which was statistically similar with BRRH Hybrid Dhan 3 (11.79%) and the lowest incidence was recorded in BRRH Hybrid Dhan 2 (2.56%). In case of inbred varieties, the highest incidence was recorded in BRRH 28 (6.66%) and lowest was in BINA 10 (1.53%). After 80 DAT, in case of hybrid varieties the highest incidence was found in Shakti 2 which was statistically different from all other varieties (16.67%) and the lowest incidence (4.61%) was recorded in BRRH Hybrid Dhan 2 and Aloran. Among the inbred varieties, the highest incidence was found in BRRH Dhan 28 (9.48%) that was statistically different from all other varieties and lowest was recorded in BINA 10 (3.07%). It was noted that the disease incidence was gradually increased with the increase of the age of plant. These results are presented in Table 5.

Table 5. Effect of different varieties on incidence of bacterial leaf blight of rice in hill at different days after transplanting

Variety	% Incidence in hill		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	1.53 b	2.56 c	4.61 d
BRRH Hybrid Dhan 3	3.07 a	10.51 a	12.31 b
Aloran	1.02 b	2.82 c	4.61 d
Jagoran	1.28 b	6.41 b	8.46 c
Shakti 2	3.59 a	11.79 a	16.67 a
BRRH Dhan 28	1.53 b	6.66 b	9.48 c
BRRH Dhan 29	1.02 b	3.33bc	5.12 d
BRRH Dhan 50	1.02 b	2.05 c	3.59 d
BINA 8	1.28 b	4.10 bc	5.12 d
BINA 10	0.76 b	1.53 c	3.07 d
LSD _{0.05}	1.43	3.17	2.68
CV (%)	51.68	35.69	21.45

4.6. Effect of different varieties on incidence of Bacterial Leaf Blight of rice in leaf at field condition

The effect of different varieties on incidence of Bacterial Leaf Blight of rice in leaf was recorded at 40 DAT, 60 DAT and 80 DAT. The incidence of bacterial leaf blight was showed significant variants among the varieties at 40 DAT and ranged from 1.40 to 4.00%. Among the hybrid varieties, the highest incidence was found in Shakti 2 (4.00%) that was statistically different from other varieties and the lowest incidence was recorded in BRRH Hybrid Dhan 2 (1.61%) In case of inbreed varieties, the highest incidence was recorded in BRRH Dhan 28 (2.12%) which was statistically different from other varieties and the lowest incidence was found in BINA 10 (1.40%). The disease incidence varied significantly at 60 DAT where, Among the hybrid varieties the highest incidence was found in Shakti 2 (4.01%) which was statistically different from other varieties and the lowest incidence was recorded in BRRH Hybrid Dhan 2 (1.74%). In case of inbreed varieties, the highest incidence was found in BRRH DHAN 28 (2.21%) that was statistically different from other varieties and the lowest incidence was recorded in in BINA 10 (1.52%). After 80 DAT, in case of hybrid varieties the highest incidence was found in Shakti 2 (4.03%) which was statistically similar with BRRH Hybrid Dhan 3 and the lowest incidence was recorded in BRRH Hybrid Dhan 2 (3.63%). Among the inbreed varieties, the highest incidence was recorded in BRRH Dhan 28 (3.89%) that was statistically different from other varieties and the lowest incidence was recorded in BINA 10 (3.11%). It was noted that the disease incidence was gradually increased with the increase of the age of plant. These results are presented in Table 6.

Table 6. Effect of different varieties on incidence of bacterial leaf blight of rice in leaf at different days after transplanting

Variety	% Incidence in leaf		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	1.61 c	1.74 c	3.63 b
BRRH Hybrid Dhan 3	3.71 ab	3.77 ab	3.89 a
Aloran	2.56 abc	2.65 abc	3.75 ab
Jagoran	2.55 abc	2.56 abc	3.81 ab
Shakti 2	4.00 a	4.01 a	4.03 a
BRRH Dhan 28	2.12 bc	2.21 bc	3.89 a
BRRH Dhan 29	1.74 c	1.76 c	3.71 ab
BRRH Dhan 50	1.47 c	1.60 c	3.83 ab
BINA 8	1.60 c	1.66 c	3.26 c
BINA 10	1.40 c	1.52 c	3.11 c
LSD _{0.05}	1.55	1.67	0.23
CV (%)	39.39	28.73	11.89

4.7. Effect of different varieties on Severity of Bacterial Leaf Blight of rice in leaf at field condition

The effect of different varieties on severity of Bacterial Leaf Blight of rice in leaf was recorded at 40 DAT, 60 DAT and 80 DAT. The severity of bacterial leaf blight was significant among the varieties at 40 DAT and ranged from 0.01 to 0.35%. In case of hybrid varieties, the highest severity was found in Shakti 2 (0.35%) that was statistically different from other varieties and the lowest severity was recorded in BRRH Hybrid Dhan 2 (0.02%). Among the inbred varieties, the highest severity was recorded in BRRH Dhan 28 (0.31%) which was statistically different from other varieties and lowest severity was in BINA 10 (0.01 %). The disease severity varied significantly at 60 DAT where, among hybrid varieties the highest severity was found in Shakti 2 (0.38%) which was statistically similar with BRRH Hybrid Dhan 3 and Jagoran and the lowest severity was recorded in BRRH hybrid Dhan 2 (0.06%). In case of inbred varieties, the highest severity was recorded in BRRH DHAN 28 (0.37%) that was statistically different from other varieties and the lowest severity was in BINA 10 (0.03%). After 80 DAT, in case of hybrid varieties the highest severity was found in Shakti 2 (0.46%) was statistically similar with BRRH Hybrid Dhan 3 and Jagoran and the lowest severity was recorded in BRRH Hybrid Dhan 2 (0.18%). Among inbred varieties, the highest severity was found in BRRH Dhan 28 (0.46%) that was statistically different from other varieties and lowest was in BINA 10 (0.15%). It was noted that the disease incidence was gradually increased with the increase of the age of plant. The results are presented in Table 7.

Table 7. Effect of different varieties on severity of bacterial leaf blight of rice in leaf at different days after transplanting

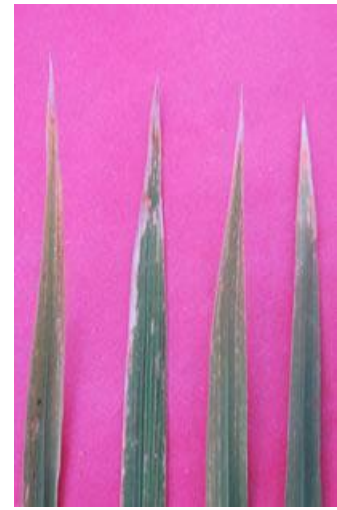
Variety	% Severity in leaf		
	40 DAT	60 DAT	80 DAT
BRRH Hybrid Dhan 2	0.02 b	0.06 b	0.18 b
BRRH Hybrid Dhan 3	0.02 b	0.25 a	0.38 a
Aloran	0.02 b	0.08 b	0.19 b
Jagoran	0.02 b	0.28 a	0.37 a
Shakti 2	0.35 a	0.38 a	0.46 a
BRRH Dhan 28	0.31 a	0.37 a	0.46 a
BRRH Dhan 29	0.02 b	0.06 b	0.18 b
BRRH Dhan 50	0.02 b	0.06 b	0.16 b
BINA 8	0.02 b	0.05 b	0.17 b
BINA 10	0.01 b	0.03 b	0.15 b
LSD _{0.05}	0.18	0.13	0.15
CV (%)	64.15	46.95	32.48



40 DAT



60 DAT



80 DAT

Figure 16. Bacterial Leaf Blight infected leaf of hybrid variety Shakti 2 at (40 DAT, 60 DAT and 80 DAT) different days after transplanting



40 DAT



60 DAT



80 DAT

Figure 17. Bacterial Leaf Blight infected leaf of inbred variety BRRI Dhan 28 at (40 DAT, 60 DAT and 80 DAT) different days after transplanting

4.8. Yield performance of different varieties of rice against BLB and brown spot at field condition

The yield performance of different varieties was recorded after harvesting. Among the hybrid varieties, the highest yield performance was recorded in BRRRI Hybrid Dhan 2 (10.46 t/ha) which was statistically similar with Jagoran and lowest yield was found in Aloran (5.57 t/ha) and Shakti 2 (7.43 t/ha) where both of them were statistically different from other varieties. In case of inbred varieties the highest yield performance (8.92 t/ha) was recorded in BRRRI Dhan 50 and BINA 10 that was statistically different from other varieties and lowest was recorded in BRRRI Dhan 28 (4.74 t/ha) which was statistically similar with BRRRI Dhan 29. These results are presented in table 8.

Table 8. Yield performance of different varieties of rice against BLB and brown spot diseases

Variety	Yield/plot (kg/5m ²)	Yield(t/ha)
BRRH Hybrid Dhan 2	5.23 a	10.46
BRRH Hybrid Dhan 3	4.96 a	9.92
Aloran	2.78 d	5.57
Jagoran	5.08 a	10.16
Shakti 2	3.71 c	7.43
BRRH Dhan 28	2.37 d	4.74
BRRH Dhan 29	2.71 d	5.43
BRRH Dhan 50	4.46 b	8.92
BINA 8	4.02 c	8.04
BINA 10	4.46 b	8.92
LSD _{0.05}	0.42	-
CV (%)	4.59	-

4.9. Screening of hybrid and inbred rice varieties against tungro virus inoculated with viruliferous green leaf hopper

The effect of different varieties on incidence of rice tungro disease was observed after the artificial inoculation with viruliferous insect vector rice green leaf hopper (GLH). In case of hybrid varieties, the highest incidence (66.67%) was found in BRRH Hybrid Dhan 3 and Aloran while no disease incidence was recorded in Shakti 2 and untreated/control plants. This results presented in Table 9 and also very much clear in figure 18. Among the inbred varieties, the highest incidence (66.67%) was recorded in BRRH Dhan 29 while no incidence was found in BRRH Dhan 50, BINA 8 and untreated/control plant. It was observed that, every infected plant of both types of varieties (Hybrid and Inbred) initiated lower number of tiller and panicle than untreated plant. It was also noted that, the tiller and panicle number become decreases with the increases of disease incidence. In case of resistance to rice green leaf hopper, among the five hybrid varieties only one variety, Shakti 2 was showed the highest resistance while Jagoran and BRRH Hybrid Dhan 2 showed moderate resistance. Aloran and BRRH Hybrid Dhan 3 were found susceptible. Among the five inbred varieties BINA 8 and BRRH Dhan 50 showed the highest resistance while BRRH Dhan 28 and BINA 10 showed moderate resistance. BRRH Hybrid 29 was found highly susceptible. These results are presented in table 9.

Table 9. Reaction of different rice varieties against rice tungro virus

Variety	Disease incidence in hill	Number of tiller/hill		Number of panicle		Disease reaction
		Inoculated plant	Control plant	Inoculated plant	Control plant	
BRRI Hybrid Dhan 2	33.33	12	18	10	16	MR
BRRI Hybrid Dhan 3	66.67	7	17	6	15	HS
Aloran	66.67	7	20	7	17	HS
Jagoran	33.33	7	18	5	18	MR
Shakti 2	0.00	20	21	20	20	HR
BRRI Dhan 28	33.33	9	13	7	13	MR
BRRI Dhan 29	66.67	5	13	5	12	HS
BRRI Dhan 50	0.00	12	11	11	11	HR
BINA 8	0.00	11	12	10	10	HR
BINA 10	33.33	8.33	12	6	11	MR



(A) (B)

**Control plant A and treated plant B
(BRR Hybrid Dhan 3)**



(A) (B)

**Control plant A and treated plant B
(Aloran)**

Figure 18. Comparison between control plant and tungro infected plant of hybrid variety BRR Hybrid Dhan 3 and Aloran respectively



(A)

(B)

Figure 19. Comparison between control plant (A) and tungro infected plant (B) of inbred variety BRR Dhan 29



Chapter 05
DISCUSSION

DISCUSSION

The present investigation had been conducted to evaluate the effect of incidence and severity of brown spot, bacterial leaf blight and tungro disease on different varieties of rice. The experiment was conducted under field condition in Boro season during the period November 2013 to May 2014. For this study, five hybrids viz BRRI Hybrid Dhan 2, BRRI Hybrid Dhan 3, Aloran, Jagoran, Shakti 2 and five inbreeds viz BRRI Dhan 28, BRRI Dhan 29, BRRI Dhan 50, BINA 8 and BINA 10 varieties were selected.

From the results it was observed that, all the selected varieties were infected with brown spot disease and developed typical symptoms under field condition. In case of hybrid varieties, the highest incidence and severity of brown spot was found in Aloran in hill, leaf and grain at different days after transplanting (40 DAT, 60 DAT and 80 DAT). The lowest incidence in hill was recorded in BRRI Hybrid Dhan 2 at 60 DAT and 80 DAT, BRRI Hybrid Dhan 3 at 40 DAT and Shakti 2 at 60 DAT. But, the lowest incidence and severity in leaf was recorded only in BRRI Hybrid Dhan 2 at different days after transplanting (40 DAT, 60 DAT and 80 DAT). The disease incidence in grain of brown spot was also found lowest in BRRI Hybrid Dhan 2 when investigated after harvesting. Among the inbreed varieties, the highest incidence and severity of brown spot was found in BRRI Dhan 29 at different days after transplanting (40 DAT, 60 DAT and 80 DAT). The lowest incidence of brown spot in hill was found in BRRI Dhan 50 at 40 DAT, 60 DAT, 80 DAT and BINA 8 at 60 DAT and 80 DAT. The lowest incidence of brown spot in leaf was found in BINA 10 at 40 DAT, BRRI Dhan 50 at 60 DAT and 80 DAT. The lowest severity of brown spot in leaf was recorded in BRRI Dhan 50 at different days after transplanting and the lowest incidence in grain was also found in BRRI Dhan 50 while observed after harvesting. The present findings were supported by Faruq *et al.*, (2015) who reported that in all growth stages, the highest incidence and severity was recorded in Aloran whereas the lowest incidence and severity was observed in Hira-2. Rashed (2001) reported that the incidence and severity of brown spot were observed 30.75 to 62.75% and 25.25 to 47.50%, respectively at 50 days after transplanting in the hybrids line 321H. The

incidence and severity were 40.50 to 80% and 45 to 77% respectively at 70 days after transplanting in the hybrids line 321H. Saifulla (1994) reported that mean brown spot severity ranged from 23.0 to 36.5% in IR9924-14 and IR9924-14.

From the result it has been found that, all the varieties were developed bacterial leaf blight disease (BLB) under natural condition. Among the hybrid varieties, the highest incidence and severity of BLB was found in Shakti 2 at different days after transplanting (40 DAT, 60 DAT and 80 DAT). The lowest incidence of BLB was found in Aloran (40 DAT and 80 DAT) and BRRI Hybrid Dhan 2 (60 DAT and 80 DAT). The lowest Incidence and severity in leaf of BLB was recorded in BRRI Hybrid Dhan 2 at all of the investigation (40 DAT, 60 DAT and 80 DAT). In case of inbreed varieties, the highest incidence and severity of BLB in both cases of hill and leaf, was recorded in BRRI Dhan 28 and lowest in BINA 10 at all of the investigation (40 DAT, 60 DAT and 80 DAT). Rahman *et al.*, (2013) reported that out of 15 tested hybrid rice varieties, Taj-1 and Krishan-2 showed the highest incidence and severity of bacterial leaf blight (BLB) at all the growth stages. The lowest disease incidence and severity of BLB was recorded from Hira-1 followed by BRRI hybrid dhan-2, Hira-2 and ACI-1. Latif *et al.*, (2011) reported that in the irrigated rice during 1999 to 2000, most of the tested hybrid (13) and inbred (35) varieties were found moderately susceptible, while hybrid variety Aalock6201, IR69690H, IR68877H and inbreed variety Anamika and BRRI dhan28 were found susceptible against BLB.

The yield of these varieties varied significantly due to brown spot and BLB disease. Among the hybrid varieties, the highest yield was recorded in BRRI Hybrid Dhan 2 and the lowest yield was recorded in Aloran and Shakti 2. In inbreed varieties, the highest yield was found in BRRI Dhan 50 and BINA 10 while the lowest was found in BRRI Dhan 28 and BRRI Dhan 29. It should be noted that the highly infected varieties gives the lowest yield and the lowest infected varieties gives the higher yield. Faruq *et al.* (2015) reported that Aloron, BRRI dhan-29, Modhumoti-2 and BRRI dhan-28 varieties were severely affected by brown spot disease and gives lower yield whereas Hira-2, ACI-1 and krishan-2 varieties give good performance against the disease. Rahman *et al.* (2013) reported

that Hira-1 followed by BRRi hybrid dhan-2, Hira-2 and ACI-1 produced the higher panicle length, number of filled grains/panicle, number of rachis/panicle, 1000-seed weight, grain yield and showed lowest disease incidence and severity of BLB.

From the present study it has been observed that among hybrid varieties, BRRi Hybrid Dhan 3 and Aloran were showed highly susceptible to tungro disease and gave lower number of tillers and panicles than the untreated/control plants. On the other hand, Shakti 2 showed highly resistance to tungro disease and initiated higher/equal number of panicles like untreated/control plants. In case of inbred varieties, BRRi Dhan 29 was found highly susceptible to tungro disease and gave lower number of tillers and panicles than the untreated/control plants. On the other hand, BINA 8 and BRRi Dhan 50 were found highly resistance to tungro disease and initiated higher/equal number of panicles like untreated/control plants. Latif *et al.*, (2011) reported that among the inbreeds (35), BR10 and BR11 were susceptible to tungro. In contrast, BR5, BR22, BR23, BRRi dhan27 and BRRi dhan31, BRRi dhan32, BRRi dhan37 and BRRi dhan38 were moderately resistant to tungro. Among the thirteen (13) hybrid varieties, IR69690H was susceptible to tungro, while IR67161H and sonar bangla1 were moderately susceptible to tungro. Only the hybrid, IR68877H was moderately resistant to tungro. Islam *et al.*, (2001) reported that inbred varieties- BR 25, BRRi Dhan 28, BRRi Dhan 31 and BRRi Dhan 32 are moderately resistance to tungro while BR 11, BR 22, BRRi Dhan 34, BRRi Dhan 39 and BINA sail were severely infected by tungro. IR68877H and IR67161H hybrid lines were moderately resistant to tungro. However, IR69690H and Sonar Bangla 1 were moderately susceptible to tungro.

From the present investigation it has been found that most cultivated hybrid and inbred rice varieties got infection by brown spot, BLB and also by tungro disease. So, different measures should be taken to control the diseases and to increase the yield.



Chapter 06
SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The studies were conducted to evaluate the effect of different varieties of rice on incidence and severity of brown spot, bacterial leaf blight and tungro disease which cause serious damages in rice cultivation in worldwide. Different hybrid and inbred varieties were grown in the field of Sher-e-Bangla Agricultural University, Dhaka during the period from December 2013 to June 2014 with normal agronomic practices. The different varieties were BRRI Hybrid Dhan 2, BRRI Hybrid Dhan 3, Aloran, Jagoran, Shakti 2, BRRI Dhan28, BRRI Dhan 29, BRRI Dhan 50, BINA 8 and BINA 10.

From present study it has been observed that, all the varieties were developed brown spot and bacterial leaf blight disease under field condition. Among hybrid varieties the highest incidence and severity of brown spot was found in Aloran and BLB was found in Shakti 2. The lowest incidence and severity of brown spot and BLB was recorded in BRRI Hybrid Dhan 2. The highest yield was found in BRRI Hybrid Dhan 2 and lowest was recorded in Aloran and Shakti 2. In case of inbred varieties, the highest incidence and severity of brown spot was found in BRRI Dhan 29 and BLB was found in BRRI Dhan 28. The lowest incidence and severity of brown spot was recorded in BRRI Dhan 50 and BLB was observed in BINA 10. The highest yield was recorded in BRRI Dhan 50 and BINA 10 and lowest was found in BRRI Dhan 28 and BRRI Dhan 29.

From present study it has been found that, among hybrid varieties BRRI Hybrid Dhan 3 and Aloran were highly susceptible to tungro disease and Shakti 2 was highly resistance to tungro disease. In case of inbred varieties, BRRI Dhan 29 was found highly susceptible to tungro disease and BRRI Dhan 50 and BINA 8 were found highly resistance to tungro disease. It was noted that, every infected plant of both types of varieties (Hybrid and Inbred) initiated lower number of tiller and panicle than untreated plant and the tiller number with panicle number become decreases with the increase of disease incidence.

More studies are required to find out the effect of different varieties on incidence and severity of brown spot, BLB and tungro disease under different agro-ecological zones in the country



REFERENCES

REFERENCES

- Agrios, G. N. (2006). Plant Pathology. Fifth Edition, Elsevier Academic Press, pp. 398-400.
- Ahmed, M., Hossain, M., Hasan, K. and Dash, C. K. (2013). Efficiency of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. *sci.* **1**(3):66-73.
- Akhtar, M. A., Abbasi, F. M., Ahmad, H., Shahzad, M., Shah M. A. and Shah, A. H. (2011). Evaluation of rice germplasm against *Xanthomonas oryzae* causing bacterial leaf blight, *Pak. J. Bot.* **43**(6): 3021-3023.
- Ashrafuzzaman, H. (1992). Shasyer Rog (disease of crop). Forth reprint, published by Bangla Academy, Dhaka, Bangladesh. pp. 203 -207.
- Azzam, O. and Chancellor, T. C. B. (1999). The biology, epidemiology and management of tungro disease in Asia. Plant Dis. Research highlights, Directorate of Rice Research, Hyderabad, India, pp. 56-64.
- Bangladesh Economic Review (BER), 2010. Department of Finance, Ministry of Finance, Government of the People's Republic of Bangladesh, Dhaka.
- Baranwal, M.K., Kotasthane, A., Magculia, N., Mukherjee, P.K., Savary, S., Sharma, A.K., Singh, H.B., Singh, U.S., Sparks, A.H., Variar, M. and Zaidi, N. (2013). A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. *Eur. J. Plant Pathol.* **136**: 443-457.
- Basso, A., Onasanya, A., Issaka S., Sido, A.Y. and Haougui, A. (2011). Bacterial leaf blight of rice in Niger: Pathological diversity of isolates collected on irrigated lands. *J. Appl. Biosci.*, **38**: 2551-2563.
- BBS, (2013-2014). Statistical Pocketbook of Bangladesh. Agriculture Wing. Government of the People's Republic of Bangladesh. pp. 2.
- BRRI, (2004). Adhunic Dhaner Chas (Bengali Bulletin), Bangladesh Rice Research Institute, Gazipur. pp. 26.
- Cabauatan, P.Q., Cabunagan, R.C. and Koganezawa, H. (1995). Biological variants of rice tungro viruses in the Philippines. *Phytopathology* **85** (1): 77-81.
- Cabunagan, R.C., Castilla, N., Coloquio, E.L., Tiongco, E.R., Fernandez, J. Du. M.J., Zaragosa, B., Hozak, R.R., Savary, S. and Azzam, O. (2001). Synchrony of planting and proportions of susceptible varieties affect rice tungro disease epidemics in the Philippines. *Crop Protection* **20**: 499-510.

- Chakrabarti, N. K. (2001). Epidemiology and disease management of brown Spot of rice In India. In: Major Fungal Disease of Rice: Recent Advances. Kluwer Academic Publishers. pp. 293–306.
- Dai, L.Y., Liu, X.L., Xiao, Y.H. and Wang, G.L. (2007) Recent advances in cloning and characterization of disease resistance genes in rice. *J. Integr. Plant Biol.* **49**: 112-119.
- Dai, S. and Beachy, R.N. (2009). Genetic engineering of rice to resist rice tungro disease. *In Vitro Cell Dev. Biol. Plant.*, **45**: 517-524.
- Dallagnol, L.J., Rodrigues, F.A. and Da Matta, F.M. (2011). Brown spot of rice as affected by phyton irradiance and temperature. *J. Phytopathol.* **159**: 630-634.
- Dasgupta, I., Hull, R., Eastop, S., Poggi-pollini, C., Blakebrough, M., Boulton, M.I. and Davies, J.W. (1991) Rice tungro bacilliform virus DNA independently infects rice after Agrobacterium-mediated transfer. *J Gen Virol* **72**:1215–1221.
- Department of Agricultural Extension DAE, (2010). Government of the People's Republic of Bangladesh, Dhaka.
- Ezuka, A. and Kaku, H. (2000). A Historical Review of Bacterial Blight of rice. National Institute of Agrobiological Resources Bulletin, Japan. pp. 207.
- Fakir, G. A. (1982). An annotated list of seed borne diseases in Bangladesh Agricultural Information Service. Dhaka, Bangladesh. pp. 15-22.
- FAOSTAT. (2010). Worldwide rice area harvest and production. FAO Statistical Yearbook 2011. Finance, Government of the People's Republic of Bangladesh, Dhaka.
- Faruq, A. N., Rahman, M. M., Akhtar, N., Islam, M. T., Uddin M. M. and Ora N. (2015). Evaluation of Imported Hybrid Rice Varieties against Three Field Diseases under Natural Epiphytic Conditions of Bangladesh, *Adv. Agric. Biol.* **4** (1): 1-7.
- Herdt, R.W. (1991). Research priorities for biotechnology. In: Khush GS, Toennissen GH (eds) Rice biotechnology. CAB International, Wallingford, UK. pp. 19–54.
- Hibino, H. (1996). Biology and epidemiology of rice viruses. *Annual Review of Phytopathology* **34**: 249-274.

- Hibino, H. and Cabauatan, P.Q. (1987). Infectivity neutralization of rice tungro-associated viruses acquired by vector leafhoppers. *Phytopathology* **77**:473–476.
- Hiremath, P.C., Hegde, R.K. and Kulkarni, B.G. (1983). Effect of seed infection by brown leaf spot on rice seed germination. *Curr. Res.* **12**: 74-76.
- Holanda, F.S.R., Mendonca, M. da C., Melo, A.S., Cassetari, N.D., Carvalho, J.G. de and Bertoni, J.C. (2002). Influence of the availability of nutrients and irrigation water on the severity of brown spot and leaf scald in rice crops. *Revista Ensaios-e-Ciencia;-Serie Ciencias Biologicas, Agrarias- e-da-Saude* **6**: 85-96.
- Igawa, T., Tokal, T., Kudo, T., Yamagushi, I. and Kimura, M. (2005). A wheat xylanase inhibitor gene, Xip-I, but not Taxi-I, is significantly induced by biotic and abiotic signals that trigger plant defense. *Bioscience, Biotechnology, and Biochemistry* **69**: 1058 – 1063.
- IRRI, (1980). Standard Evaluation System for Rice. International Rice Testing Program. Losbanos, Philippines. pp. 7-20.
- Islam, M. J., Hassan, M. S., Islam M. R. and Badshah M. A. (2001). Post Flood Rehabilitation and Adaptive Research Support Project. BRRI, Comilla. pp.30-31.
- ISTA, (2003). International rules for seed testing, rules 2003 (Draper, S.R., Eds.) Zurich, Switzerland, ISTA. Seed mycoflora studies in rice. *Pak. J. Phytopathol.* **14**(2):132-134.
- Jha, A.C. (2001). Development and management brown spot of rice caused by *Drechslera oryzae* (Breda de Haan) Subramanian and Jain. Ph.D. Thesis, RAU Bihar, Pusa (Samastipur).
- Jha, A.C., Rai, B. and Jha, M.M. (2004). Response of direct seeded and transplanted rice varieties on the development of brown spot disease. *Annals Biol.* **20**: 195-197.
- Jones, M.C., Gough, K., Dasgupta, I., Rao, B.L., Cliffe, J., Qu, R., Shen, P., Kaniewska, M., Blakebrough, M., Davies, J.W., Beachy, R.N. and Hull, R. (1991). Rice tungro disease is caused by an RNA and a DNA virus. *J Gen Virol.* **72**: 757–761.
- Kadai, (2010). Diversity and pathogenicity of the rice brown spot pathogen, *Bipolaris oryzae* (Breda de Haan) shoem. in Bangladesh assessed by genetic fingerprint analysis. *Bangladesh. J. Bot.* **38** : 195-200.

- Kamal, M.M. and Mia, M.A.T. (2009). Diversity and pathogenicity of the rice brown spot pathogen, *Bipolaris oryzae* (Bredade Haan) shoem. in Bangladesh assessed by genetic fingerprint analysis. *Bangladesh J. Bot.* **38**: 195-200.
- Khalili, E., Sadravi, M., Naeimi, S. and Khosravi, V. (2012). Biological control of rice brown spot with native isolates of three *Trichoderma* species. *Braz. J. Microbiol.* **43** : 297-305.
- Kulkarni, S., Ramakrishnan, K. and Hegde, R.K. (1986). Demonstration on supervisory control of brown leaf spot of rice caused by *Drechslera oryzae* (Breda de Haan) Subram. and Jain ex. M.B. Ellis. *Pl. Pathol. Newsl.* **4** : 22.
- Latif, M. A., Badsha, M. A., Tajul, M. I., Kabir, M. S., Rafii, M. Y. and Mia, M. A. T. (2011). Identification of genotypes resistant to blast, bacterial leaf blight, sheath blight and tungro and efficacy of seed treating fungicides against blast disease of rice. *Scientific Research and Essays.* **6**(13): Pp. 2804-2811.
- Marmey, P., Brian, B., Emmanuel, J., De Kochko, A., Ching, A.O., Piere, Y., Gary, S., Roger, N. B. and Claude, M.F. (1999). RTBV open reading frame 3 encodes a single 37-Kda coat protein. *Virology* **253**: 319-326.
- Medina, V., Venkitesh, R. and Markham, P.G. (1994). Immunoelectron microscopy of viral complex causing rice tungro disease (RTD). pp. 343-354.
- Mew, T.W., Alvarez, A.M., Leach, J.E. and Swings, J. (1993). Focus on bacterial blight of rice. *Plant Dis.*, **77**:5-12.
- Mew, T.W. and Gonazales, P. (2002). A Handbook of Rice Seed borne Fungi, International Rice Research Institute, Los Banos, Philippines. pp. 20.
- Mew, T.W.(1987). Current status and future prospects of research on bacterial blight of rice. *Ann. Rev.Phytopathol.*, **25**: 359-382.
- Mew, T.W. (1989). An overview of the world bacterial blight situation. In: Bacterial Blight of Rice, Ogawa T, Khush GS (eds)., International Rice Research Institute, Manila, Philippines, pp. 7-12.
- Miah, S.A., Shahjahan, A.K.M., Hossain, M.A. and Sharma, N.R. (1985).survey of rice disease in Bangladesh. *Trop. Pest management* **31**(3): 208-213.
- Nagarajan, S. (1993). Plant diseases in India and their control. *Ciba Found Symp.* **177**, 208-223.

- Minnatullah, M. D. and Sattar, A. (2002). Brown spot development in Boro rice as influenced by weather condition. *Journal of Applied Biology*. **12**: 71–73.
- Muralidharan, K., Krishnaveni, D., Rajarajeshwari, N.V.L. and Prasad A.S.R. (2003) Tungro epidemic and yield losses in paddy fields in India. *Curr Sci* **85**:1143–1147.
- Ou, S.H. (1985). Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 61-96.
- Pannu, P. P. S., Chahal, S. S., Kaur, M. and Sidhu, S. S. (2005). Influence of weather variable on the development of brown leaf spot caused by *Helminthosporium oryzae* in rice. *Indian Phytopathology*. **58** : 489 – 492.
- Quazi, S.A.J., Rahman M.M., Akter, S., Tuhina-Khatun, M. and Monsur, M.A. (2009). Assessment of Yield Loss due to tungro in Bangladesh. *Bangladesh Journal of Plant Pathology*. **25**(1): 37-40.
- Rahman, M.M., Islam, M.T., Faruq, A.N., Akhtar, N., Ora, N. and Uddin M.M.(2013). Evaluation of Some Cultivated Hybrid Boro Rice Varieties Against BLB, ShB and ALS Diseases Under Natural Epiphytic Conditions. *Middle-East Journal of Scientific Research*. **15** (1) :146-151.
- Rajput, R.L. and Bartaria, A.M. (1995). Reaction of rice cultivars to brown spot. *Agricultural Science Digest Journal*. **15** (4): 205-206.
- Rashed, R. (2001). Effect of brown spot on the yield and yield contributing characters of different hybrids and varieties of rice grown in boro season, M.S. Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Saifulla, M. (1994). Field screening of rice genotypes of brown spot and leaf scald diseases. *Agricultural Science Digest Journal*. **4**(1): 68-70.
- Sanchez, L.M. and Obien, S.R. (1995). Profile of insect pests and diseases in Mindanao. Proceedings 8th National Rice Rand review and Planning Workshop, Philippine Rice Research Institute, Los Banos. pp. 21-36.
- Savary, S., Castilla, N. P., Elazegui, F. A. and Teng, P. S. (2005). Multiple effects of two drivers of agricultural change, labour shortage and water scarcity, on rice pest profiles in tropical Asia. *Field Crops Research*. **91**: 263– 271.
- Savary, S., Nelson, A., Sparks, A. H., Willocquet, L., Duveiller, E., Mahuku, G., et al. (2011). International agricultural research tackling the effects of global and climate changes on plant diseases in the developing world. *Plant Disease*, **48**:1– 40.

- Savary, S., Willocquet, L., Elazegui, F. A., Teng, P. S., Du, P. V. and Zhu, D., et al. (2000). Rice pest constraints in tropical Asia: characterization of injury profiles in relation to production situations. *Plant Disease*. **84**: 341 – 356.
- Savary, S., Teng, P.S., Willocquet, L. and Nutter, F.W. (2006). Quantification and modeling of crop losses: a review of purposes. *Annual Review of Phytopathology*. **44**:89 –112.
- Sere, Y., Onasanya, A., Verdier, V., Akator, K., Ouedraogo, L.S., Segda, Z., Mbare, M.M., Sido, A.Y. and Basso, A. (2005). Rice bacterial leaf blight in West Africa: Preliminary studies on disease in farmers' fields and screening released varieties for resistance to the bacteria. *Asian J. Plant Sci.* **4**: 577-579.
- Shahjahan, A.K.M., Duve, T. and Bonman, J.M. (1987). Climate and rice diseases. In weather and rice. IRRI, Los Banos, Laquna, Philippines. pp.125-128.
- Sherf, A. F., Page, R. M., Tullis, E. C. and Morgan, T. L. (1947). Studies on factors affecting the infectivity of *Helminthosporium oryzae*. *Phytopathology*. **37**: 281– 290.
- Singh, R. K., Singh, C. V. and Shukla, V. D. (2005). Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. *International Rice Research Notes*. **30** (2): 31–32.
- Singh, R.S., Singh, S.N. and Yadav, B.P. (2000). Management of brown spot disease of rice caused by *Drechslera oryzae*. *Madras Agric. J.* **87** : 372-375.
- Swings, J., Mooter, M.V., Vauterin, L., Hoste, B., Gillis, M., Mew, T.W. and Kersters, K (1990). Reclassification of the causal agents of bacterial blight of rice (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae*. *Int. J. Syst. Bacteriol.* **40**: 309-311.
- Tabei, H. and Mukoo, H. (1960). Anatomical studies of rice plant leaves affected with bacterial leaf blight, in particular reference to the structure of water exudation system. *Bulletin National Institute of Agricultural Science* **11**: 37-43.
- Timmusk, S. and Wagner, E. G. H. (1999). The plant-growth- promoting rhizobacterium *Paenibacillus polymyxa* induces changes in Arabidopsis thaliana gene expression: A possible connection between biotic and abiotic stress responses. *Molecular Plant-Microbe Interactions*. **12**:951–959.
- Veena, M.S., Shetty, H.S., Mortensen, C.N. and Mathur, S.B. (2000). Bacterial leaf blight of rice. Technical Bulletin, Department of Studies in Botany,

University of Misore, India and Danish Govt. Institute of Seed Pathology for Developing Countries, Denmark, pp.16.

- Vu-Van, B.A. and Sangchote, S. (2006). Seed borne and transmission of *Bipolaris oryzae*, the causal pathogen of brown spot of rice. *Kasetsart J.-Natural Sci.* **40**: 353-360.
- Waheed, M. A., Ahmad, H., Sirajuddin, H. A., Khan, A.Q. and Khan, A.(2009). Evaluation of rice genotypes for resistance against bacterial leaf blight. *Pak. J. Bot.* **41**(1): 329 – 335.
- Zanao Junior, L. A., Rodrigues, F. Á., Fontes, R. L. F., Korndörfer, G. H. and Neves, J. C.L. (2009). Rice resistance to brown spot mediated by silicon and its interaction with manganese. *Journal of Phytopathology.* **157**: 73–78.
- Zhou, Z.J., Lin, Q.Y. and Xie, L.J. (1992). Occurrence of rice tungro bacilliform virus in China. *Acta Phytopathologica Sinica.* **22**(1):15-18.



APPENDICES

APPENDICES

Appendix I. Field View of Experimental Plot



Appendix II. Green leaf hopper (*Nephotettix virescens*) inoculated pots covered by net



Appendix III. Average temperature, relative humidity and total rainfall at different date of the experimental site during the period from January to May 2014

Date	Air temperature (° C)		RH (%)	Total rainfall (mm)
	Maximum	Minimum		
02/01/14	22.40	15.60	63.15	0.00
25/02/14	27.32	21.20	47.65	0.00
13/03/14	30.65	23.40	54.17	0.00
24/03/14	30.55	24.40	58.85	0.00
04/04/14	27.82	20.20	60.12	12.50
10/04/14	32.45	21.00	49.17	0.00
03/05/14	33.25	22.50	53.35	0.00
07/05/14	33.57	22.60	68.60	4.92
17/05/14	34.75	24.30	57.10	0.00

Source: Mini weather station, SAU, Dhaka.