

**SCREENING OF SELECTED RICE VARIETIES AGAINST
TUNGRO DISEASE CAUSED BY *Rice Tungro Viruses***

DANISH MAHMUD



**DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH**

June, 2021

**SCREENING OF SELECTED RICE VARIETIES AGAINST
TUNGRO DISEASE CAUSED BY *Rice Tungro Viruses***

**BY
DANISH MAHMUD
REGISTRATION NO. 14-06196**

*A Thesis
Submitted to the Department of Plant Pathology,
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of*

MASTER OF SCIENCE (MS)

IN

PLANT PATHOLOGY

SEMESTER: JANUARY-JUNE, 2021

APPROVED BY

.....
Prof. Dr. Md. Belal Hossain
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Supervisor

.....
Prof. Dr. Fatema Begum
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Co-Supervisor

.....
Prof. Dr. Fatema Begum
Chairman
Examination Committee



**DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207**


CERTIFICATE

*This is to certify that thesis entitled “**SCREENING OF SELECTED RICE VARIETIES AGAINST TUNGRO DISEASE CAUSED BY Rice Tungro Viruses**” submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), 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 **DANISH MAHMUD**, Registration No. **14-06196** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged and style of this thesis have been approved and recommended for submission.

Dated: June, 2021
Place: Dhaka, Bangladesh

Prof. Dr. Md. Belal Hossain
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Supervisor



**DEDICATED
TO
MY BELOVED
PARENTS**

ACKNOWLEDGEMENT

All the praises and gratitude are due to the omniscient, omnipresent and omnipotent Almighty Allah, who has kindly enabled the author to complete her research work and complete this thesis successfully for increasing knowledge and wisdom.

*The author really does not have adequate words to express his heartfelt sense of gratification, ever indebtedness and sincere appreciation to his benevolent teacher and research supervisor, **Prof. Dr. Md. Belal Hossain**, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for his constant help, scholastic guidance, planning experiment, valuable suggestions, timely and solitary instructive criticism for successful completion of the research work as well as preparation of this thesis.*

*It is a great pleasure for the author to express his sincere appreciation, profound sense, respect and immense indebtedness to his respected co-supervisor & Chairman **Prof. Dr. Fatema Begum**, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for providing necessary facilities and conducive atmosphere to accomplish the research work. Special thanks and indebtedness are also due to all the teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, sympathetic co-operation and inspiration throughout the period of the study.*

The author would like to express his deepest respect and boundless gratitude to all the respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 and Bangladesh Rice Research Institute for their sympathetic co-operation and inspiration throughout the course of this study and research work.

The author deeply acknowledges the profound dedication to his beloved parents and sisters for their moral support, steadfast encouragement and continuous prayer in all phases of academic pursuit from the beginning to the completion of study successfully.

Finally, the author is deeply indebted to his friends and well-wishers especially Shohag vai and Md. Ariful Islam for their kind help, constant inspiration, co- operation and moral support which can never be forgotten.

Date: June, 2021

The Author

SAU, Dhaka

SCREENING OF SELECTED RICE VARIETIES AGAINST TUNGRO DISEASE CAUSED BY *Rice Tungro Viruses*

ABSTRACT

Tungro disease of rice is one of the most destructive and devastating viral disease in South Asian countries including Bangladesh that causes yield loss up to 100%. The present study was carried out to assess the selected rice varieties against tungro disease through virus-vector relationship. The field experiment was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka-1207 and virus-vector relationship study was conducted in net house. In total, twelve aman rice varieties *viz.* BRRI dhan34, BRRI dhan71, BRRI dhan70, BRRI dhan87, BRRI dhan80, BR11, BRRI dhan75, BRRI dhan72, BRRI dhan51 BRRI dhan30, BRRI dhan49 and Binadhan-7 were evaluated. From the field experiment it was found that the incidence of rice tungro disease showed significant variants among the tested varieties. The highest (53.34%) disease incidence was found in BRRI dhan34 and in BR11 and the lowest in BRRI dhan51 (13.35%). There was no disease incidence found in BRRI dhan80, BRRI dhan87, BRRI dhan30, BRRI dhan49 and Binadhan-7. In control condition, tested varieties were artificially inoculated with viruliferous insect Green Leaf Hopper (GLH) at 10, 20, 25 days after inoculation (DAI) and it was noted that most of the varieties except BRRI dhan49 and Binadhan-7 were infected with tungro viruses and expressed the tungro disease symptoms. But the typical symptoms were appeared after 20 DAI. The effect of yield and yield contributing character were also studied. From the result it was revealed that among the tested varieties BRRI dhan34, BRRI dhan71 and BR11 was found susceptible, and BRRI dhan49 and Binadhan-7 was showed resistance reaction to tungro viruses. It was also found that 20 days was the optimum acquisition period for transmission of tungro viruses through GLH.

TABLE OF CONTENTS

| CHAPTER | TITLE | PAGE |
|--------------|---------------------------------------|------|
| | ACKNOWLEDGEMENT | I |
| | ABSTRACT | II |
| | TABLE OF CONTENTS | III |
| | LIST OF TABLES | IV |
| | LIST OF FIGURES | V |
| | LIST OF APPENDICES | VI |
| | LIST OF ABBREVIATIONS AND ACRONYMS | VII |
| CHAPTER I | INTRODUCTION | 01 |
| CHAPTER II | REVIEW OF LITERATURE | 04 |
| CHAPTER III | MATERIALS AND METHODS | 11 |
| CHAPTER IV | RESULTS | 22 |
| CHAPTER V | DISCUSSIONS | 31 |
| CHAPTER VI | SUMMARY AND CONCLUSION | 33 |
| CHAPTER VII | REFERENCES | 34 |
| CHAPTER VIII | APPENDICES | 44 |

LIST OF TABLES

| TABLE NO. | NAME OF THE TABLES | PAGE NO. |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------|----------|
| 1 | Disease reaction features of tested rice varieties against tungro disease in control condition at DAI (different day after inoculation) | 25 |
| 2 | Effect of tungro disease on plant height, tiller number and panicle length of tested varieties | 27 |
| 3 | Effect of tungro disease on grains per panicle of tested varieties | 28 |
| 4 | Effect of tungro disease on fresh and dry weight of tested varieties | 29 |

LIST OF FIGURES

| FIGURE NO. | TITLE | PAGE NO. |
|---------------|-----------------------------------------------------------------------------------------------------------------|-------------|
| 1 | Soaking of tested rice varieties seed | 13 |
| 2 | Sprouting of tested rice varieties seed | 13 |
| 3 | Collection of data of disease incidence from field | 15 |
| 4 | Data of dry weight from individual plot | 17 |
| 5 | Seedling transplantation in pot | 17 |
| 6 | collection of tungro infected rice plant | 18 |
| 7 | Collection of GLH and inoculated with tungro infected rice plant | 19 |
| 8 | Release of GLH in net house | 19 |
| 9 | Collection of data from net house | 20 |
| 10 | Performance of tested rice varieties on disease incidence against tungro disease in field condition | 23 |
| 11 | Performance of tested rice varieties on disease incidence against tungro disease in control condition at DAI | 24 |
| 12 | Performance of tested rice varieties on disease incidence against tungro disease in control condition At DAI | 30 |

LIST OF APPENDICES

| Appendix No. | Title | Page No |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| I | Map showing the experimental sites under study | 41 |
| II | Characteristics of soil of experimental field | 42 |
| III | Monthly record of air temperature, relative humidity and total rainfall of the experimental site during the period from June-November, 2019 | 43 |
| IV | Disease Rating Scale of MLND | 43 |
| V | Analysis of variance of the data on disease incidence at field condition (%) of tested rice varieties | 44 |
| VI | Analysis of variance of the data on Growth parameters of tested rice varieties | 44 |
| VII | Analysis of variance of the data on yield contributing and yield parameters of tested rice varieties | 44 |

LIST OF ABBREVIATIONS AND ACRONYMS

| Abbreviation | Full meaning |
|---------------------|---------------------------------------|
| % | Percent |
| @ | At the rate |
| ^o C | Degree Centigrade |
| Agril. | Agricultural |
| BAU | Bangladesh Agricultural University |
| BBS | Bangladesh Bureau of Statistics |
| BCR | Benefit Cost Ratio |
| BE | Biological Efficiency |
| Cm | Centi-meter |
| CV | Coefficient of variation |
| d.f. | Degrees of freedom |
| DAS | Days After Stimulation |
| e.g. | For example |
| et al. | And others |
| FAO | Food and Agriculture Organization |
| G | Gram |
| J. | Journal |
| Kg | Kilogram |
| LSD | Least Significant Difference |
| Mg | Milligram |
| m ² | Meter Squares |
| SAU | Sher-e-Bangla Agricultural University |

CHAPTER I

INTRODUCTION

Rice, a monocotyledonous angiosperm belonging to the Poaceae family, genus *Oryza*, is the world's most significant staple food crop, serving as the primary source of calories consumed by humans and occupying over one-fifth of the total land area covered by cereals. Early rice farming has been documented in several cultures, including China, India, and Southeast Asian civilizations. However, the earliest archaeological evidence is from 7000–5000 BC and comes from central and eastern China. More than 90% of the world's rice is produced in Asia, primarily in China, India, Indonesia, and Bangladesh, with smaller amounts produced in Japan, Pakistan, and other Southeast Asian countries. Rice is also grown in portions of Europe, North and South America, and Australia as well.

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). According to BBS (2019) the total area of rice cultivation of Bangladesh was 11,717,903 ha. According to BBS (2019) the production of rice was 37,607,756 Metric ton. Unfortunately, the yield of rice in this country is low (average 3.4 t/ha) compared to other rice growing countries like South Korea and Japan where the average yield is 6.00 and 5.6 t/ha, respectively (FAO, 2003). 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 2 diseases (Miah *et al.*, 1985). It is the most serious and wide spread disease occurring in the rice growing countries like Bangladesh (Miah, 1973), India (John, 1968), Malaysia (Ou *et al.*, 1965) and the Philippines (Rivera and Ou, 1965). Among those disease, Tungro is one of the most damaging and destructive diseases of rice in South and Southeast Asia. In Bangladesh, tungro was identified in 1969 (Nuque and Miah 1969). Since then it has been one of the major constraints in rice production particularly in the Aus and T. Aman seasons (Miah, 1984, Miah *et al.*, 1985). It is the severe and most widespread disease being in 1990 when both Aus and T. Aman crops were affected in almost all over the country (Shahjahan *et al.* 1992). Tungro is caused by tungro virus (RTV) which is a complex of two bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino *et al.*, 1978). The viruses are transmitted by green leafhopper, *Nephotettix virescens* (Ling, 1967).

The disease can attack rice plants at all growth stages, but the symptoms and yield losses are severe when infection takes place at seedling or early growth stages (Miah, 1973; Nahar *et al.*, 1985). For a susceptible variety without any recovery ability RTV may cause 100% infection and resulting in total yield loss under favorable condition (BRRI. 1983).

There is no efficient method to cure tungro infected plants in the field. To control the vector serves as the indirect measure to control the disease. The use of insecticides provides good control measures for the vector but the vector may have already transmitted the virus before being killed. The use of resistant variety is one of the most effective ways to control rice disease. This concept is applicable to tungro disease (Rivera and Ou, 1965; Ling, 1967) because tungro disease controlled by killing the vector with insecticides is often not effective. Resistance of a variety to tungro may be due to its resistance to the insect vector, or to the virus or to both (Karim, 1978; Ling, 1979). Besides, acquisition period of insect vector is responsible for disease.

In view of the above facts the present study was conducted to investigate the incidence of tungro disease of rice in selected genotype and to know the virus-vector relationship. To

achieve these goals, twelve inbreeds rice varieties were selected which are mostly cultivated in Amon season in Bangladesh.

Objectives:

The present study was undertaken with the following specific objectives-

- To estimate the incidence of tungro disease of rice in field condition,
- To determine the effect of tungro on the yield and yield contributing character of rice,
- To assess the virus-vector relationship of the rice-tungro viruses in selected rice varieties in control condition.

CHAPTER II

REVIEW OF LITERATURE

Rice is one of the world's most important crops. It is afflicted with a variety of ailments. This chapter compiles the literature on rice, incidence, effect on yield, yield contributing features, and screening of resistance cultivars against tungro.

2.1. Importance of Rice

Burlando & Cornara (2014) said that Rice (*Oryza sativa*) is the most staple and cereal component that sustains the two-third of the world population. The chief livelihood of human beings abundantly relies on rice due to their sovereignty nutritive property and energy value.

Burlando & Cornara, 2014; Goufo & Trindade, 2014; Juliano, 1993 reported that Rice is an important source of fiber, energy, minerals, proteins, vitamins, antioxidants and other biomolecules which may act in synergy and exerted an advantageous effect on health .

Burlando & Cornara, 2014; Goufo & Trindade, 2014 reported that Rice is also an important source of diverse antioxidant molecules that play a key role in health promotion.

Sen & Chakraborty, (2017) reported that Health benefits of antioxidant rich food, antioxidant molecules include prevention/treatment of cardiovascular diseases, metabolic diseases, neurodegenerative diseases, gastrointestinal problems, disease related to inflammation and oxidative stress.

Burlando & Cornara, (2014) reported that Nutritional value of different rice varieties and the presence of diverse bioactive compounds in rice were reported around the globe. The nutritional profile of rice includes 80% carbohydrates, 7–8% protein, 3% fat, 3% fiber and 7-6% is minerals and many other bioactive constituents.

Burlando & Cornara, (2014) reported that Starch, a polymer mixture of two α -glucans (i.e. amylose and amylopectin) is considered as main rice carbohydrate. Rice is a good

source of crude protein, soluble protein, albumin, globulin, prolamine, reducing sugar, non reducing sugar, glutelin and minerals like iron, calcium, phosphorus.

T.H. Fairhurst and Dobermann (2002) reported that since it was cultivated between 8,000 and 10,000 years ago, rice (*Oryza sativa L.*) has supported a bigger number of people for a longer period of time than any other crop (Greenland, 1997). Rice is now the staple food of more people than wheat, with Asia accounting for 90% of total rice production and consumption (Evans, 1998). Unlike maize and wheat, rice trades for less than 5% of total production, primarily within Asia and from Asia to Africa and Europe. As a result, self-sufficiency is emphasized in all rice economies. Rice self-sufficiency and political stability are intertwined in many Asian countries.

Peng and Yang (2003) reported that to assure food security in the rice-consuming countries of the world, farmers must produce more rice of better quality to meet the demands of consumers in coming years.

Bethell and Huang (2004) of Ventria Bioscience reported that genetically modified rice to express lactoferrin and human lysozyme which are proteins usually found in breast milk and have antiviral, antibacterial, and antifungal properties

2.2. Tungro disease of rice:

2.2.1. Occurrence and distribution

Tungro is a serious virus disease in South and Southeast Asia since 1960. Devastating outbreaks of the disease caused tremendous yield losses in the Philippines and elsewhere during 1960-1971 (Bergonia, 1978; Ling, 1979; Siwi *et al.*, 1987). In Indonesia alone, rice crops worth more than US \$ 89 million were damaged by tungro during 1967-1972 (Tantera, 1986) The disease has been epidemic rather than endemic because it has often struck suddenly and destroyed large areas of rice fields (Ling, 1977).

Tungro disease was observed in 1963 on many rice varieties at IRRI experimental farms and characterized and identified as virus disease (IRRI, 1967). Ling and Tiongco (1979) mentioned that rice tungro disease (RTD) was first studied in Japan and the Philippines before 1950. It is prevalent in Southeast Asian countries under different names. However

based on symptomatology, vector species, virus vector interaction, particle morphology of the virus and serological aspects the different names represent the same disease tungro (Ang *et al.*, 1983; Hibino *et al.*, 1978; Ling 1972 and Saito, 1977).

RT V can attack rice plant at all growth stages but most critical period of infection is the seedling stage of the plant and can cause 100% yield loss in case of susceptible variety (BRRI, 1983).

2.2.2. Symptoms

Different rice varieties express symptoms differently. In some moderately resistant varieties, the symptoms on the infected plants are conspicuous at one stage and may gradually disappear at later stages of growth. This is often termed as recovery from the disease (Ling, 1979).

Ou and Ling (1966) mentioned that the degree of stunting and discoloration varies with rice cultivar, environmental condition, age of the plant and strain of the virus.

Tungro infected plants express various symptom which is dependent on the susceptibility of the rice variety, age of the plant, the time of infection (Palomar and Ling, 1966), virus strain (Rivera and Ou 1965; Rivera and Ling 1971; Anjaneyulu and Vaktavatasalam, 1986) and the type of virus particle (Hibino *et al.*, 1978). The general symptoms are yellowing of leaves, stunting of plants, twisting of young leaves, reduction of tiller number, incomplete and delayed panicle emergence and delayed flowering (Anjaneyulu and John 1972; Ling and Tiongco, 1981). The symptom may vary from slight to severe stunting and yellow-orange to orange-red discoloration of leaves usually starting from the tips and gradually moving downwards (Saito *et al.*, 1986; Rao and Anjaneyulu, 1976). When rice plants are infected by tungro then leaves are slightly rolled outward and somewhat spirally twisted. Stunting is due both to shortening of the leaf sheath and the leaf blade.

Tillering is significantly reduced if the plants are affected at the early growth stage. Root development is poor, panicles are small and spikelets are sterile (Siwi *et al.*, 1987).

2.2.3. Diagnosis of tungro disease

Ling (1969) and Omura (1986) reported that for management of tungro. Disease prerequisite is a proper diagnosis of the disease. The first hand Identification is generally done by visual observation of the external symptom. Visual symptom may not always be reliable because viruses other than RTV may cause tungro like symptoms. Moreover, many other factors like soil nutrition, drought, weeds, and insect injury may cause tungro like symptoms.

2.2.4. The causal agent

Hibino *et al*, (1978) mentioned that tungro caused by two kinds of morphologically different virus particles have been identified from tungro infected plants: the rice tungro spherical virus (RTSV) and the rice tungro bacilliform virus (RTBV). Rice tungro bacilliform virus (RTBV) alone causes only mild symptom and rice tungro spherical virus (RTSV) causes only mild stunting of the plants. The symptoms are severe when RTBV and RTSV are present in the same infected plant (Hibino and Mariappan, 1983). Both the particles are restricted in phloem cells (Favali *et al.*, 1975; Saito, 1977).

2.2.5. The vector

The principal vector of the tungro virus is *Nephotettix virescens*. *Nephotettix nigropictus* and *Recilia dorsalis* have also been reported as vector of RTV (IRRI, 1969). Hsieh (1972) explained that the vector causes damage to rice plants directly by feeding on the growing plants thus greatly reducing its vigor, tiller number and ultimately its yield potential.

According to Miah (1976) there are two groups of the vector *Nephotettix virescens*, the transmitter group and non transmitter group. The transmitter group can transmit the virus in to the host plant. Whereas non transmitter group can not transmit.

Ling (1975) mentioned that the adult insect vector *Nephotettix virescens* appears to be efficient than the nymph in transmitting RTV in rice plants. The vector *Nephotettix virescens* carries more viruses from the susceptible cultivar than from the resistant cultivar during acquisition feeding (Mohana and Anjaneyulu 1984).

2.2.6. Varietal resistance

Karim (1978) reported that the resistance of a rice variety to tungro may be due to its resistance to the insect vector or to the virus or to both. The mechanism of resistance of a rice variety may either be due to the inactivation of the virus or the inhibition of the virus multiplication by substance or substances present in the rice plant. The resistance of the rice plant to rice tungro infection increase with age of the plant (Palomar and Ling 1966).

Mohana and Anjaneyulu (1984) mentioned that the resistance of a rice variety to tungro virus is not a permanent character of the plant but it changes with the certain factor. At low temperature (15° to 28°c), IR 20 loses its resistance against rice tungro virus in winter season but it maintains resistance during the summer season.

Flores *et al.* (1987) mentioned that at IRRI with the same GLH resistance gene (GLH 1) Pankhari 203 showed high resistance to tungro, while Jhingasail showed intermediate reaction. Similarly, T APL 796 (GLH 6) showed tungro resistance, while IR 36 (GIH 6) became susceptible.

Vidhyasekaran *et al.* (1987) reported that by rearing the vector for several generations on resistant variety, GLH resistant ADT 16 and IR 43 became susceptible to tungro.

Varietal resistance to tungro may not be stable. Observation in the Philippines revealed that IR50, IR54 and IR64 which had been highly resistant to GLH and also to tungro infection in the field succumbed to tungro infection in south Cotabato. The same trend was reported from Indonesia where rice varieties were resistant to tungro during release. But susceptible after 2- 3 of years intensive cultivation (Cabunagan *et al.*, 1987).

Miah (1984) mentioned that Ptb18 showed resistant reaction to rice tungro virus under the environmental condition of Bangladesh and India while it showed intermediate to resistant reaction at I RRI under the environmental condition of the Philippines. Rahman (1983) found that Variety Ptb18 was resistant to the vector but susceptible to the virus.

Variety IR 56 was found to be moderately reaction to RTV in the Philippines but resistant in Malaysia (IRRI, 1985). Varieties Kachamota and ARC 11554 were found resistant to

RTV in Malaysia and Indonesia. ARC 11554 was showed resistant to RTV in many countries (Miah, 1984).

2.3. Yield loss due to Tungro disease

Dai and Beachy (2009) reported that Rice tungro disease (RTD); caused by the confection 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 III

MATERIALS AND METHODS

The present study regarding “Assessment of the Virus –Vector Relationship of the Rice-Tungro Virus in the Selected Rice varieties” has been conducted during June to November 2020 at the central farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. Required materials used and methodology followed are described below under the following headings and subheadings.

3.1. Experimental site

Experimental site is situated at 23°77' North Latitude and 90°30' East latitude and at a height of 8.0 meters above sea level, which is AEZ-28 applies on this location (Madhupur Tract). The geological location of the site is presented on the map. (Appendix I).

3.2. Characteristic of Soil

The experiment was carried out on a red brown terrace soil that was shallow. The chosen area was a medium high land tract with the Tejgaon soil series. The experimental area is located above flood level and has an irrigation and drainage system. The soil has a silty clay texture and is made up of 26 percent sand, 43 percent silt, and 31 percent clay. The details of the soil characteristics that were recorded were reported in (Appendix II).

3.3. Climate of the Experimental site

The experimental site has a subtropical, damp, and humid environment. The climate is divided into three separate seasons: the winter season, which runs from November to February, the pre-monsoon or hot season, which runs from March to April, and the monsoon season, which runs from May to October. The research period's meteorological data, including air temperature, relative humidity, rainfall, and sunlight hour, has been reported in detail (Appendix III).

3.4. Variety Selection

In total 12 mostly cultivated Aman rice varieties were selected which are as follows-

| SI No | Variety |
|-------|-------------|
| 1. | BRR1 Dhan34 |
| 2. | BRR1 Dhan72 |
| 3. | BRR1 Dhan80 |
| 4. | BRR1 Dhan71 |
| 5. | BRR1 Dhan87 |
| 6. | BRR1 Dhan75 |
| 7. | BRR1 Dhan70 |
| 8. | BR11 |
| 9. | BRR1 Dhan51 |
| 10. | BRR1 Dhan49 |
| 11. | BRR1 Dhan30 |
| 12. | Binadhan-7 |

3.5. Seed collection

Seed were collected from Bangladesh Agricultural Development Corporation, (BADC) Gabtoli Dhaka.

3.6. Seedling Preparation

3.6.1. Seed Soaking

Seed were soaked in 12 plastic pots separately with tap water for overnight and maintained carefully.



Fig. 1: Soaking of selected rice varieties seed

3.6.2. Sprouting of seeds

Seeds were removed from the water before being placed in 12 different gunny bags and maintained at room temperature for 48 hours to sprout before being planted in a seed bed.



Fig. 2: Sprouting of selected rice varieties seed

3.6.3. Seed bed preparation and seed sowing

In the Agronomy farm of Sher-e-Bangla Agricultural University, Dhaka, the seed bed was prepared by puddling the soil using a power tiller and harrow. Manuring was not necessary because the earth was rich in organic matter. Sprouted seeds were planted in a damp seedbed. The seedlings were cared properly for adequately. Weeds were pulled out of the seed bed and irrigation was applied as needed.

3.7. Field experiment

3.7.1. Land preparation and application of cow dung

With the help of a power tiller and a harrow, the soil was prepared. The land was initially ploughed and opened on July 19, 2020. The final ploughing was done using a power tiller then the soil surface was laddered to make it level. Finally weeds and stubbles were cleared from the land.

3.7.2. Fertilizer application

Fertilizers were applied according to fertilizer recommendation guide- 2012 (BARC). the plots were applied with 25 kg urea, 6 kg mop, 6 kg tsp, 6 kg gypsum, 5 kg zinc oxide, 2 kg boric acid. During final site preparation, all fertilizers were integrated with the soil except 2/3 urea. The remaining urea was applied in two equal installments at 30 and 45 days following transplantation.

3.7.3. Design and layout

The experiment was carried out in randomized complete block design (RCBD) with 3 replication. The replication was represented by blocks. Each block contained 12 unit plots, for a total of 36 plots (12 X 3=36). Each unit plot measured $3 \times 2.0 = 6.0 \text{ m}^2$. The distance between plot to plot and block to block was 1 m.

3.7.4. Seedling transplantation

Seedlings were carefully pulled from the seed bed and put into the main field. The distance between rows to row was kept at 25 cm, while the distance between hill to hill was kept at 20 cm. In each individual hill, four seedlings were replanted.

3.7.5. Intercultural operation

Intercultural operation was done when necessary. Weeding was done properly in first block and in 2nd block partially. In 3rd block no weeding was done.

3.7.6. Parameter assessed

The following parameter were assessed-

1. Disease incidence(%)
2. Tiller number
3. Plant height
4. Panicle length
5. Grain per Panicle
6. Fresh seed weight
7. Dry seed weight

3.7.7. Assessment of the disease incidence in the field

Each of the plots was investigated for recording of disease incidence of tungro virus. Data was recorded visually by observing typical symptom. The data on the following parameters were recorded; symptom on leaf blade or leaf sheath,% disease incidence , number of tiller/hill, high of tiller, number of panicle, length of panicle, grain weight before drying, grain weight after drying. Incidence: % disease incidence was estimated by using the following formula (Rajput and Bartaria, 1995)



Fig. 3: Collection of data of disease incidence from field

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

3.7.8 Tiller number

For counting the tiller number, in total 5 plants were selected from each of the plot and number of tiller was counted.

3.7.9. Plant height

For measurement the plant height in total five hill were selected from each of the plot and plant height was measured by using the measurement scale.

3.7.11. Panicle length

For measurement of the panicle length five plants were selected from individual tiller and panicle length was measured by measuring scale.

3.7.12. Grain per Panicle

For counting grain per panicle five panicles were selected from each of the tiller of individual plot and counted grain.

3.7.13. Fresh weight

For fresh weight measurement, Grain was weighted of every plot separately by digital weight machine just after harvesting.

3.7.13. Dry weight

For dry weight measurement, grain was dried under sunlight for two days after that dry weight was kept by digital machine.



Fig. 4: Data of dry weight from individual plot

3.8. Pot experiment

3.8.1. Pot preparation

Earthen pot was prepared for pot experiment. Soil was collected from the same field where the field experiment was conducted.

3.8.2. Seedling transplantation

Seedlings were carefully pulled up from the seedbed and placed into the pot. Three seedlings were put into each of the pots.



Fig. 5: Seedling transplantation in pot

3.7.5. Intercultural operation

Intercultural operation such as gap filling, weeding, watering, fertilization etc. were done when and where necessary.

3.8.3. Collection of tungro virus infected rice plants

Tungro infected rice plants were collected from Chindina Upazilla, Cumilla district on 24/09/2020. The infected plants were uprooted carefully and put in the synthetic net bag.



Fig. 6: collection of tungro infected rice plant

Rearing procedure of GLH

GLH is frequently collected from the field to begin the rearing. The main items needed for GLH rearing are a greenhouse and rearing cages. The green house should be well-lit and provide a suitable atmosphere for growing plants around the year. Pan trays, rearing cages, and earthen pots should be available in the greenhouse. The size of the trays is determined by the greenhouse's dimensions as well as the volume of the materials to be employed. The trays must be deep enough to cover the potted plants' roots. The size of the trays was determined by the greenhouse's dimensions as well as the volume of the materials to be employed. The trays should be deep enough to cover the potted plants' roots. Use mesh in your raising cages. The plants (food plants of 10 days) with earthen pot sit in water in a bottomless cage put in a water pan tray. To offer the best humidity, potted plants were placed in around 8 cm of water inside the cage. Then, within the cage, inoculate the gathered GLH in a feeding plant. Hoppers are often collected with an aspirator, with Mylar also being used to move hoppers from one plant to another.

3.8. 4.Collection of GLH and inoculation with collected tungro +ve rice plants

Green leaf hopper (GLH) were collected from rearing house and inoculated with collected tungro infected rice plants immediately and covered with synthetic net carefully.



Fig. 7: Collection of GLH and inoculated with tungro infected rice plant

3.8.5. Inoculation with variety

GLH was kept with tungro affected rice plant for 24 hours for artificial infection with virus then GLH with tungro affected rice is was released in shed for inoculation with virus.



Fig. 8: Release of GLH in net house

3.8.6. Opening and collection of data from shed

The first shed was opened after 10 days of inoculation with GLH and data was collected.



The 2nd shed was opened after 20 days of inoculation and data was collected.



3rd shed was opened after 25 days of inoculation and data was collected.



Fig. 9: Collection of data from net house

3.8.8. Data analysis

Data from field experiments were analyzed using computer based software STATISTIX-10. Treatment means was compared through LSD range test at 5% level of significance. In control condition variety was categories according to disease rating scale of RTVD. Disease was identified by visual basis, observing the typical symptoms of RTVD. The disease incidence reaction was assessed by using the following disease rating scale described by). Ali *et al.*, (2005). Disease rating scale of RTVD has been reported in (Appendix IV).

CHAPTER IV

RESULTS

The main objective of the research was to screening of resistant variety against tungro disease of rice and assessment virus-vector Relationship of the selected varieties .To obtain the goal, the disease incidence was estimated at the field condition naturally. Besides the selected Rice varieties were artificially inoculated with virus affected Green Leaf Hopper in control condition at different day after inoculation (DAI). The incidence of tungro disease at field was estimated by visual observation and in control condition Virus-Vector Relationship 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. Disease Incidence features of selected rice varieties against tungro disease in field

From the study, it was found that the tested varieties showed significant variation in features of percent disease incidence against rice tungro disease (Figure-10). Among the tested varieties there was no disease incidence found in BRRi dhan80, BRRi dhan87, Binadhan-7, BRRi dhan30 and BRRi dhan49. Among the tested varieties, the highest disease incidence was recorded in BRRi dhan34 and BR11 which was 53.34% followed by BRRi dhan70 which was 46.67%. Among the tested varieties, the lowest disease incidence was found in BRRi dhan51 which was (13.35%). Remaining BRRi dhan71, BRRIdhan75 and BRRi dhan72 were statistically similar. These results are presented in figure-10

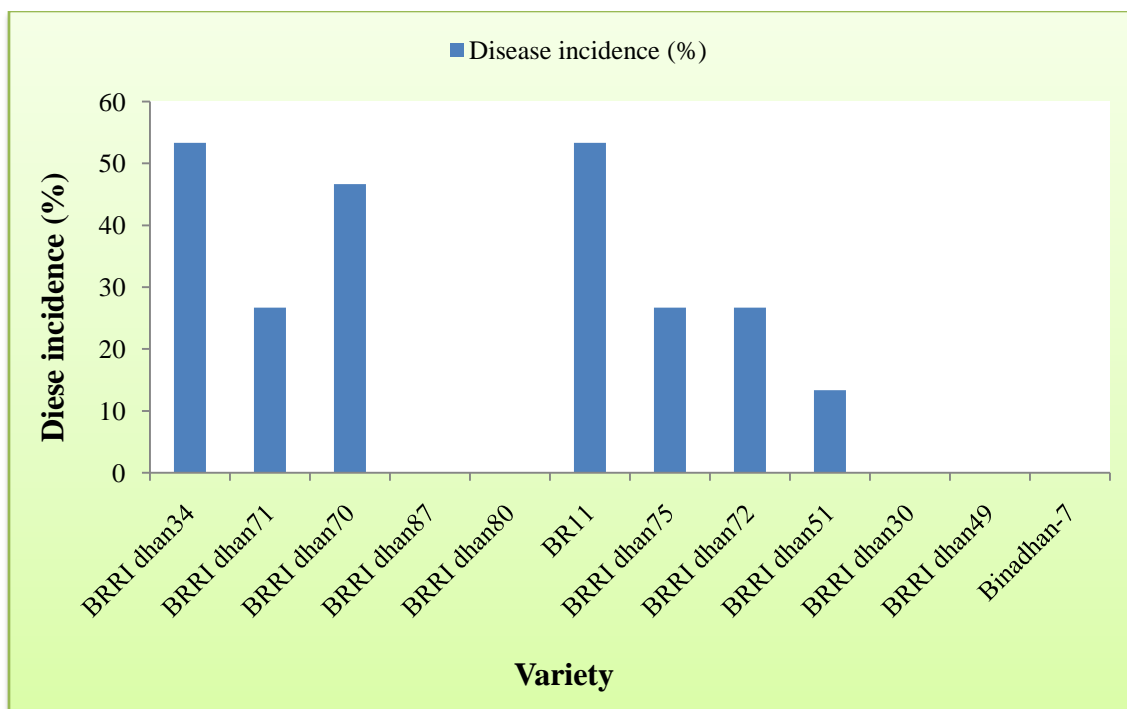


Fig. 10: Performance of disease incidence in different rice varieties against tungro disease in field

4.2. Disease Incidence of tungro in control condition (net house)

After inoculation with viruliferous insect, Green leaf Hopper (GLH), disease incidence was recorded at 10, 20 and 25 Day after Inoculation (DAI) (acquisition period). At 10 days of inoculation (acquisition period) with GLH, the highest disease incidence (38.47%) was recorded in BRR1 dhan34 followed by BRR1 dhan71, BRR1 dhan70 and BRR1 dhan72 which were 31.25%, 30.77% and 29.42%, respectively (Fig.11). There was no disease incidence found in BRR1 dhan87, BRR1 dhan49 and Binadhan-7. Almost similar disease incidence was recorded in BRR1 dhan11, BRR1 dhan75 and in BRR1 dhan72 which were 25%, 25% and 29.42% ,respectively .In BRR1 dhan80 and BRR1 dhan51 incidence were 13.345 and 11.12% which was close to each other. At 10 DAI, the lowest incidence (9.09%) was found in BRR1 dhan30.

At 20 DAI (acquisition period) with GLH the highest disease incidence (55.56%) was found in BRR1 dhan71 followed by BRR1 dhan34 which was (53.85%) and BRR1 dhan11 (50%). There were no incidence in BRR1 dhan49 and Binadhan-7. Almost similar disease incidence was found in BRR1 dhan70, BRR1 dhan75 and BRR1 dhan72 which were

37.5%, 31.58% and 38.47%, respectively. BRRi dhan80, BRRi dhan51 and BRRi dhan30 disease incidence was found 20%, 14.28% and 25%, respectively.

At 25 DAI (acquisition period) with GLH the highest disease incidence (66.67%) was found in BRRi dhan71 followed by BRRi dhan34, BRRi dhan11 and BRRi dhan75 which were 58.46%, 53.85% and 57.15%, respectively. Almost similar disease incidence was found in BRRi dhan70 and BRRi dhan72 incidence which were 43.75% and 46.67%, respectively. No disease was observed in BRRi dhan49 and Binadhan-7. Remaining BRRi dhan87, BRRi dhan80, BRRi dhan51 and BRRi dhan30 disease incidence were found 18.18%, 27.78%, 17.69% and 30.29%, respectively.

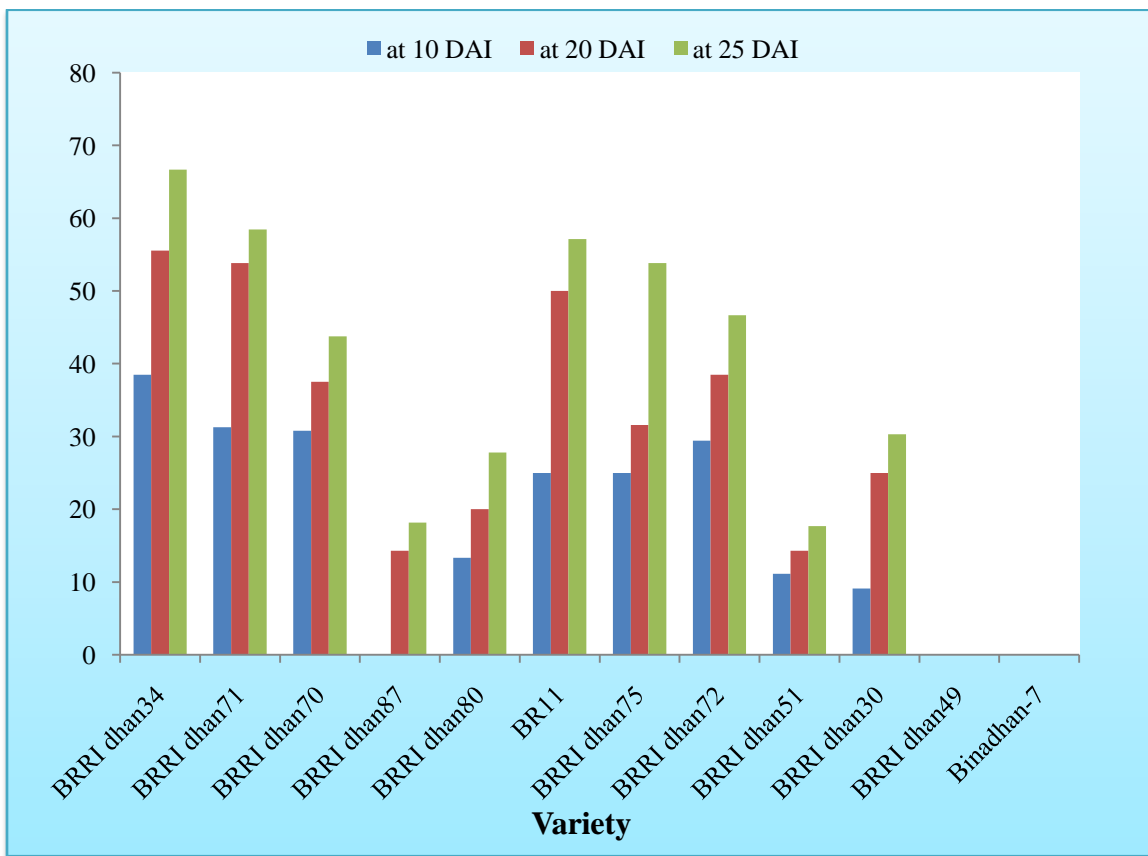


Fig. 11: Performance of tested rice varieties on disease incidence against Tungro

Disease in control condition at DAI

4.2.1 Disease reactions of selected rice varieties against tungro disease in control condition(net house) at DAI (different day after inoculation)

In different day after inoculation the varieties showed significant variant in terms of disease reaction features. At 10 DAI (acquisition period) most of the varieties showed moderately resistant to tolerant. BRRRI dhan87, BRRRI dhan49 and Binadhan-7 showed immune to tungro virus at 10 day after inoculation with viruliferous insect Green leaf Hopper. BRRRI dhan30 showed highly resistant at 10 DAI. At 20 day after inoculation (acquisition period) most of the varieties showed tolerant to moderately susceptible. . BRRRI dhan49 and Binadhan-7 remain immune at 20 day after inoculation with GLH. Reaming BRRRI dhan87, BRRRI dhan80, BRRRI dhan51 and BRRRI dhan30 showed moderately resistant at 20 day after inoculation. At 25 day after inoculation (acquisition period) BRRRI dhan51 showed moderately susceptible to susceptible. BRRRI dhan11 and BRRRI dhan75 become tolerant to moderately susceptible. BRRRI dhan43, BRRRI dhan87, BRRRI dhan72 and BRRRI dhan51 remain same as 20 DAI. BRRRI dhan49 and Binadhan-7 showed immune at 25 DAI. BRRRI dhan30 become moderately resistant to tolerant.

Table 1: Disease reaction features of tested rice varieties against tungro disease in control condition at DAI (different day after inoculation)

| Variety | At 10 DAI | Disease reaction | At 20 DAI | Disease reaction | At 25 DAI | Disease reaction |
|--------------|--------------|---------------------|--------------|---------------------|--------------|---------------------|
| BRRRI dhan34 | 38.47 | T | 55.56 | MS | 66.67 | S |
| BRRRI dhan71 | 31.25 | T | 53.85 | MS | 58.46 | MS |
| BRRRI dhan70 | 30.77 | T | 37.5 | T | 43.75 | T |
| BRRRI dhan87 | 0 | I | 14.28 | MR | 18.18 | MR |
| BRRRI dhan80 | 13.34 | MR | 20 | MR | 27.78 | T |
| BR11 | 25 | MR | 50 | T | 57.15 | MS |
| BRRRI dhan75 | 25 | MR | 31.58 | T | 53.85 | MS |
| BRRRI dhan72 | 29.42 | T | 38.47 | T | 46.67 | T |
| BRRRI dhan51 | 11.12 | MR | 14.28 | MR | 17.69 | MR |
| BRRRI dhan30 | 9.09 | HR | 25 | MR | 30.29 | T |
| BRRRI dhan49 | 0 | I | 0 | I | 0 | I |
| Binadhan-7 | 0 | I | 0 | I | 0 | I |

4.3. Effect of tungro disease on growth, yield and yield contributing character of tested varieties

Yield contributing character such as plant height, tiller number, panicle length, grain per panicle, fresh and dry weight have been affected due to tungro disease of rice.

4.3.1. Plant height of tested varieties due to tungro disease of rice

The plant height in different tested varieties was measured before panicle initiation stage. Among the tested varieties, the tiller number was ranged from 84.27 to 102.27 (Table 2). The highest plant height was found in BRRIdhan72 (102.27) which was statistically identical with BRRIdhan30 (100.47) and BRRIdhan34 (100.07). The lowest plant height was found in BRRIdhan51 (84.27) which were statistically different from all other tested varieties. In remaining varieties; BRRIdhan70 (98.93), BRRIdhan75 (98.13), Binadhan-7 (98.13), BRRIdhan80 (96.67), BR11 (95.80), BRRIdhan49 (95.60), BRRIdhan71 (93.07) and BRRIdhan87 (90.33) have no significant different with each other.

4.3.2. Tiller number of tested varieties due to tungro disease of rice

The tiller number in different tested varieties was counted before panicle initiation stage. Among the tested varieties, the tiller number was ranged from 12 to 17 (Table 2). The highest tiller number was found in BRRIdhan34 (17) and BRRIdhan87 (17) both was statistically similar with BRRIdhan51 (16) and Binadhan-7 (16). The lowest tiller number was found in BRRIdhan72 (12) which was statistically different from all others tested varieties. In remaining varieties; BRRIdhan71(14),BRRIdhan70(14),BRRIdhan80(15), BR11(15), BRRIdhan75(15), BRRIdhan30(15) and BRRIdhan49(15) have no significant difference with each other.

4.3.3. Panicle length of tested varieties due to tungro disease of rice

The panicle length in different tested varieties was measured and significant variant was found. The panicle length was ranged from 20.36 cm to 27.05 cm (Table 2). The highest panicle length was measured in BRRIdhan75 (27.05cm) which was statistically identical with BR11 (26.59cm), BRRIdhan87 (26.63cm), BRRIdhan71 (26.52cm), BRRIdhan34 (26.37cm), BRRIdhan80 (25.33cm), BRRIdhan72 (24.95cm) and BRRIdhan70

(24.09cm). The lowest panicle length was found in BRRIdhan51 (21.28cm), Binadhan-7 (22.32cm) and BRRIdhan30 (22.47cm).

Table 2. Effect of tungro disease on plant height, tiller number and panicle length of tested varieties

| Variety | Plant height (cm) | Tiller number | Panicle length (cm) |
|------------|-------------------|---------------|---------------------|
| BRRIdhan34 | 100.07 ab | 17.00 a | 26.37ab |
| BRRIdhan71 | 93.07 ef | 13.67 ab | 26.52 a |
| BRRIdhan70 | 98.93 bc | 14.00 ab | 24.09 bc |
| BRRIdhan87 | 90.33 f | 17.00 a | 26.63 a |
| BRRIdhan80 | 96.67 cd | 15.00 ab | 25.33 ab |
| BR11 | 95.80 de | 15.00 ab | 26.59 a |
| BRRIdhan75 | 98.13 bcd | 15.00 ab | 27.05 a |
| BRRIdhan72 | 102.27 a | 12.00 b | 24.95 ab |
| BRRIdhan51 | 84.27 g | 16.00 a | 21.28 d |
| BRRIdhan30 | 100.47 ab | 15.00 ab | 22.47 cd |
| BRRIdhan49 | 95.60 de | 15.34 ab | 20.36 d |
| Binadhan-7 | 98.13 bcd | 16.34 a | 22.32 |
| CV (%) | 7.16 | 13.28 | 5.80 |

4.3.4. Grains per panicle of tested varieties due to tungro disease of rice

The grains per panicle in different tested varieties were counted. Among the tested varieties, the grain per panicle was ranged from 98 to 186 (Table.3). The highest grains per panicle were found in BRRIdhan34 (186) which were statistically identical with BRRIdhan75 (152). The lowest grain per panicle was found in BRRIdhan49 (98) which were statistically different from all other tested varieties. In the others varieties, the grain per panicle was found statistically identical with each other.

Table 3: Effect of tungro disease on grains per panicle of tested varieties

| Variety | Grains per Panicle (no) |
|-------------|-------------------------|
| BRRi dhan34 | 186a |
| BRRi dhan71 | 142 bc |
| BRRi dhan70 | 117cd |
| BRRi dhan87 | 126 bcd |
| BRRi dhan80 | 125 bcd |
| BR11 | 127 bcd |
| BRRi dhan75 | 152 ab |
| BRRi dhan72 | 138 bc |
| BRRi dhan51 | 113 cd |
| BRRi dhan30 | 118 bcd |
| BRRi dhan49 | 98 d |
| Binadhan-7 | 116 cd |
| CV (%) | 15.90 |

4.3.5. Fresh weight of tested varieties due to tungro disease of rice

After harvesting, the fresh weight in different tested varieties was measured. The highest fresh weight was found in Binadhan-7 (2.66 kg) which was statistically identical with BRRi dhan49 (2.66 kg), BRRi dhan30 (2.61 kg), BRRi dhan51 (2.56 kg) and BRRi dhan71 (2.05 kg). The lowest fresh weight was found in BRRi dhan75 (1.27 kg) which was statistically identical with BRRi dhan70 (1.36 kg), BRRi dhan72 (1.41 kg), BRRi dhan34 (1.64 kg), BRRi dhan87 (1.87 kg), BRRi dhan80 (1.82 kg) and BR11 (1.98 kg)

Table 4: Effect of tungro disease on fresh and dry weight of tested varieties

| Variety | Fresh weight per plot(kg) | Dry weight per plot(kg) |
|-------------|---------------------------|-------------------------|
| BRRi dhan34 | 1.6317 cde | 1.1670 bc |
| BRRi dhan71 | 2.0453 abc | 1.3977 b |
| BRRi dhan70 | 1.3597 de | 0.8093 c |
| BRRi dhan87 | 1.8613 cde | 1.2550 bc |
| BRRi dhan80 | 1.8123 cde | 1.1670 bc |
| BR11 | 1.9733 bcd | 1.3690 b |
| BRRi dhan75 | 1.2693 e | 0.7820 c |
| BRRi dhan72 | 1.4087 cde | 0.8460 c |
| BRRi dhan51 | 2.5550 ab | 2.2330 a |
| BRRi dhan30 | 2.6050 ab | 2.1593 a |
| BRRi dhan49 | 2.6523 a | 2.4030 a |
| Binadhan-7 | 2.6597 a | 2.4737 a |
| CV | 19.51 | 20.32 |

4.3.6. Dry weight of tested varieties due to tungro disease of rice

After sun drying, the dry weight in different tested varieties was measured. The highest dry weight was found in Binadhan-7 (2.48 kg) which was statistically identical with BRRi dhan49 (2.41 kg), BRRi dhan51 (2.24 kg) and BRRi dhan30 (2.16 kg) (Table.4). The lowest dry weight was found in BRRi dhan75 (0.79 kg) which was statistically identical with BRRi dhan70 (0.81 kg) and BRRi dhan72 (0.85 kg). Remaining varieties, BRRi dhan71 (1.4 kg), BR11 (1.4 kg), BRRi dhan34 (1.17 kg), BRRi dhan87 (1.26 kg) and BRRi dhan80 (1.17 kg) have no significant difference with each other.

4.4 Relationship study

4.4.1. Relationship between disease incidences in field condition with yield (dry weight)

From the result it revealed that relationship between disease incidence and yield of the tested varieties were negatively co-related. When the disease incidence is high the yield of the varieties becomes low.

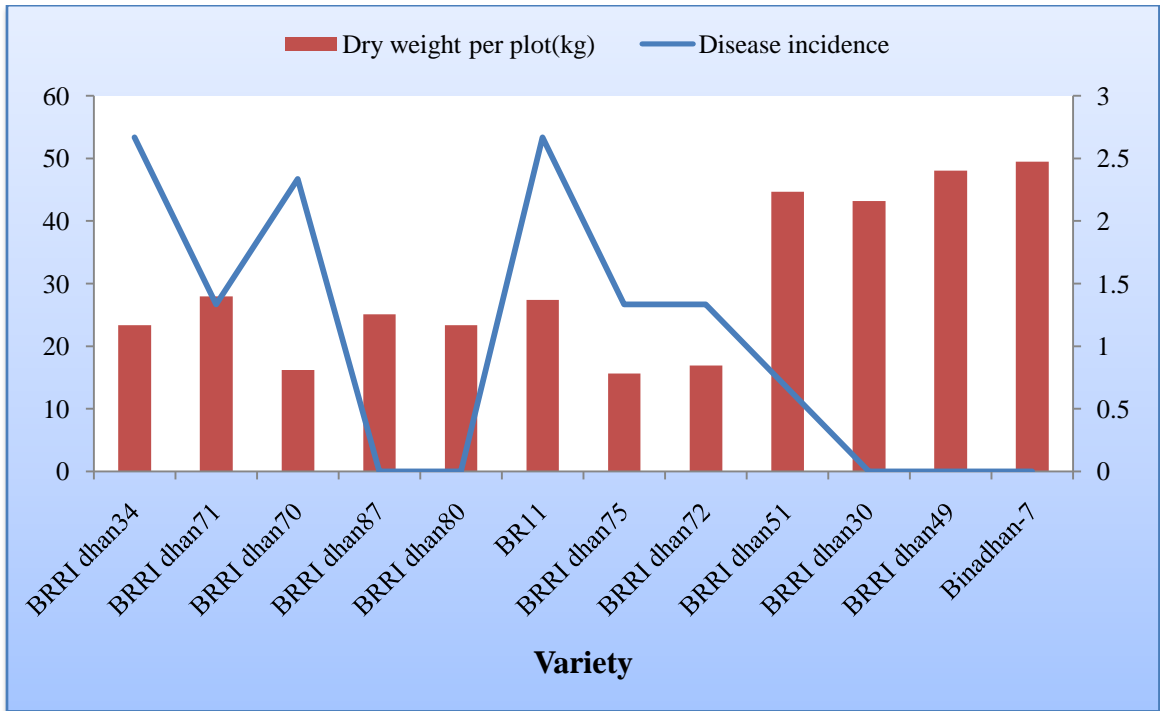


Figure 12: Relationship between Tungro disease incidence and dry weight in selected rice varieties in field

CHAPTER V

DISCUSSION

From the result it was observed that all the tested varieties were infected with tungro disease except BRR1 dhan30, BRR1 dhan80, BRR1 dhan87, Binadhan-7 and BRR1 dhan49 in field condition. Among the varieties the highest incidence was found in BRR1 dhan34 and BR11 and the lowest disease incidence was recorded in BRR1 dhan51. Disease Incidence features was varied significantly in tested rice varieties against tungro disease in control condition, at 10 days after inoculation the highest disease incidence was found in BRR1 dhan34 and lowest was found in BRR1 dhan30. No tungro disease symptom was appeared in BRR1 dhan87, BRR1 dhan49 and Binadhan-7. At 20 days after inoculation the highest disease incidence was found in BRR1 dhan34 followed by BRR1 dhan71 and BR11. Latif *et al.*, (2011) reported that among the inbreeds (35), BR10 and BR11 were susceptible to tungro. In contrast, BR5, BR22, BR23, BRR1 dhan27 and BRR1 dhan31, BRR1 dhan32, BRR1 dhan37 and BRR1 dhan38 were moderately resistant to tungro. Lowest disease incidence was found in BRR1 dhan51 and BRR1 dhan87, no tungro disease symptom was appeared in BRR1 dhan49 and Binadhan-7. At 25 days after inoculation the highest disease incidence was found in BRR1 dhan34 followed by BRR1 dhan71 and BR11 and lowest disease incidence was found in BRR1 dhan51. There was no tungro disease symptom appears in BRR1 dhan49 and Binadhan-7. Islam *et al.*, (2001) reported that inbreed varieties- BR 25, BRR1 Dhan 28, BRR1 Dhan 31 and BRR1 Dhan 32 are moderately resistance to tungro while BR 11, BR 22, BRR1 Dhan 34, BRR1 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 study of disease reaction feature, it was revealed that disease incidence was increased with the increase of day after inoculation in the tested varieties under controlled condition, it was noted that most of the tested varieties was appeared the tungro disease symptom at 20 day after inoculation except BRR1 dhan49 and Binadhan-7. It means 20 days is the optimum acquisition period for transmission the tungro virus through insect vectors, GLH. These results are agreed with previous report Twenty-one days old

seedlings were inoculated through mylar cage methods. In each pot, 10 seedlings were grown and inoculated with the green leafhopper (@ 3insect/seedling) which was previously feed tungro infected plants for 24 hours. After 24 hours of inoculation, seedlings were transplanted in the net house for disease scoring. Disease incidence and disease severity were scored by following standard protocols at 21 days after inoculation (latif *et al.*, 2021)

In case of tiller number of tested varieties, the highest tiller number was found in BRRi dhan34 and in BRRi dhan87. The lowest tiller number was found in BRRi dhan72. In case of plant height of tested varieties, highest plant height was found in BRRi dhan72 and lowest height was found in BRRi dhan51. From the result it was observed that, the highest grain per panicle was found in BRRi dhan34 and lowest grain per panicle was found in BRRi dhan49. In case of panicle length of tested varieties, the highest panicle length was found in BRRi dhan75, BRRi dhan87, BR11, BRRi dhan34 and BRRi dhan71 and lowest panicle length was found in BRRi dhan49. From the result it was observed that, the highest fresh and dry weight was found in Binadhan-7 and BRRi dhan49 and lowest weight was found in BRRi dhan75.

CHAPTER VI

SUMMARY AND CONCLUSION

Rice is the most important food crop of the world including Bangladesh. Almost half of the world population lives on rice and over 90% of the world rice are consume in Asia. Different diseases and pests hamper rice production. Among the major diseases, rice tungro disease is one of the most serious diseases. It is one of the most damaging, destructive and complex viral diseases of rice which is transmitted by the insect vector Green Leaf Hopper (GLH). In severe cases, Tungro susceptible varieties infected at an early growth stage yield loss may be up to 100%. The present study was conducted to assess the selected rice varieties against tungro disease through the study of virus-vector relationship.

From the present study, it was observed that among the tested varieties, the highest disease incidence was found in BRRRI dhan34 and BR11 and the lowest disease incidence in BRRRI dhan51 in the field condition. No tungro disease symptoms was appeared in the varieties; BRRRI dhan80, BRRRI dhan87, BRRRI dhan30, BRRRI dhan49 and Binadhan-7. In virus-vector relationship study for optimization the acquisition period, most of the varieties were infected with tungro disease except BRRRI dhan49 and Binadhan-7. In inoculation study with different days after interval, it was noted that most of the tested varieties except BRRRI dhan49 and Binadhan-7 were showed tolerant to susceptible when inoculated with viruliferous insect vector GLH. From the study it was also found that 20 days is the optimum acquisition period for transmission of tungro virus through insect vector. From the relationship study between disease incidence and yield, the result was negatively co-related. Severely infected with tungro disease BRRRI dhan34 and BR11 gave lower yield whereas Binadhan-7 and BRRRI dhan49 gave satisfactory yield performance in field condition, From the study it may be concluded that, among the tested rice varieties BRRRI dhan49 and Binadhan-7 was showed resistance to tungro viruses. From the virus-vector relationship study it may be concluded that 20 days is the optimum acquisition period for transmission of tungro viruses to through insect vector GLH. Moreover, further studies need to be carried out on other varieties and in different agro-ecological zone of our country.

CHAPTER VII

REFERENCES

- Ali, S., Khan, M.A., Habib, A., Rashed, S. and Iftikhar, Y. (2005). Correlation of environmental conditions with Okra yellow vein mosaic virus and Bemisiatabaci population density. *International Journal of Agriculture and Biology* **7**: 142-144.
- Ang, O.C., Min, C. P., Kim H. N., Omura, T., Usogi, T. and Saito, Y. (1983). Morphology and serological relationship of Penyakit merah virus in Malaysia and rice tungro virus in the Philippines. *Intern. Rice Res. Newslet.* **8**(6): 10.
- Anjaneyulu, A. and John, V. T. (1972). Strains of tungro virus. *Phytopathol.* **62**(10): 1116-1119.
- 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.
- BER (Bangladesh Economic Review), 2010. Department of Finance, Ministry of Finance, Government of the People's Republic of Bangladesh, Dhaka
- BBS (Bangladesh Bureau of Statistics). (2019). *Yearbook of Agriculture Statistics*. Bangladesh Bureau of Statistics, Ministry of planning Govt. people's Repub. of Bangladesh. Dhaka. pp. 123-127.
- Bergonia, H. T. (1978). Control measure to prevent tungro virus outbreak. *Plant Prat. Newslet.* **7** (2): P-4.
- Bethell, D., & Huang, J. (2004). Recombinant human lactoferrin treatment for global health issues: Iron deficiency and acute diarrhea. *BioMetals*, **17**, 337–342.
- BRRI. (Bangladesh Rice Research Institute). *Ann. Rep. for 1983*
- Burlando, B., & Cornara, L. (2014). Therapeutic properties of rice constituents and derivatives (*Oryza sativa L.*): A review update. *Trends in Food Science & Technology*, **40**, 82–98.

- 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., Hibino, H., Sama, S and Rizvi, S. A. (1987). Resistance of rice plants to *Nephotettix virescens* in relation to rice tungro- associated viruses. In Proc. workshop on rice tungro virus. AARD- Marous Res. Inst. for food crops.
- 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.
- Crawford, G. W., & Shen, C. (1998). The origins of rice agriculture: Recent progress in East Asia. *Antiquity*, 72, 858–866.
- 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.
- 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.
- DAE (Department of Agricultural Extension), (2010). Government of the People's Republic of Bangladesh, Dhaka
- Fairhurst, T. and Dobermann, A., 2002. Rice in the global food supply. *World*, 5(7,502), pp.454-34.
- Fakir, G. A. (1982). An annotated list of seed borne diseases in Bangladesh Agricultural Information Service. Dhaka, Bangladesh. pp. 15-22.
- FAOSTAT. (2003). Worldwide rice area harvest and production. FAO Statistical Yearbook. Finance, Government of the People's Republic of Bangladesh, Dhaka
- Favali, M. A ., Pellegrine, S and Bassi, M. (1975). Ultra-structural alterations induced by rice tungro virus in rice leaves. *Virology* 66: 502-507.

- Flores, Z. M., Cabunagan, R. C., Jonson G. E. and Hibino, H. (1987). Resistance to rice tungro-associated viruses of rice varieties with deherent genes for green leafhopper resistance. *Int. Rice Res. Newslet* **12** (5): 11.
- Goufo, P., & Trindade, H. (2014). Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol, and phytic acid. *Food Sciences and Nutrition*, *2*, 75–104.
- Herd, 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.
- Hibino, H. and Mariappan, V. (1983). Rice tungro virus complex: Current Research and Future Plans. Paper presented at the Int. Rice Conf. 18-22 Apr.1983
- Hibino, H., Roechan, M. and Sudarisman, S. (1978). Association of two types of virus particles with Penyakit habang (tungro disease) of rice in Indonesia. *Phytopath.* *68*: 1412-1416
- Hsieh, C. Y (1972). Population ecology of the rice green leafhopper, *N. vtrescen* (Dist). MS thesis (unpublished), UPLB at Los Banos, Philippines. p87.
- IRRI (International Rice Research Institute) (1967). *Ann. Rep. for 1966*. Los Banos, Leguna, Philippines.
- Islam, M. J., Hassan, M. S., Islam M. R. and Badshah M. A. (2001). Post Flood Rehabilitation and Adaptive Research Support Project. BRRI, Comilla. P p.30-31
- John, V.T. (1968). Identification and characterization of tungro, a virus disease of rice in India. *Plant Dis. Rept.* *52*: 871-875.

- 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.
- Juliano, B. O. (1993). Rice in human nutrition (FAO food and nutrition series. No. 26). Rome: Food and Agriculture Organization of the United Nations (Chapter 4)
- Karim, A. N. M. (1978). Varietal resistance of rice to green leafhopper *N. virescens* (Dist): Sources, mechanism and genetics of resistance. Ph.D thesis (unpublished). UPLB, Los Banos, Philippines.
- 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.
- Ling, K C. (1972). Rice virus diseases. The Intern. Rice Res. Inst. Los Banos Laguna. Philippines
- Ling, K. C. (1967). Testing rice varieties for resistance to tungro disease. *Prot. Symp.* April, 1967. IRRI. pp. 277-291.
- Ling, K. C. (1969). Testing rice varieties for resistance to tungro disease. *Proc. Symp. On virus diseases of the rice plants.* John Hopkins Press Baltimore. P. 255-277.
- Ling, K. C. (1975). Environmental epidemiology of rice tungro disease. Effect of Virus source on disease incidence. *Phil. Phvtopath.* 11: 46-57.
- Ling, K. C. and Tiongco, E. R. (1981). Rice virus disease in the Philippines. *Integrated Pest management.* Phil. Phytopathol. Soc. inc. Los Banos. Philippines.
- Ling, K. C. and Tiongco. (1979). Rice virus diseases in the Philippines. *International Rice Res. Inst.* Philippines
- Ling, K.C. (1977). Recent studies on rice tungro disease at IRRI. *Trop. Agric. Res Ser.* No. 10.

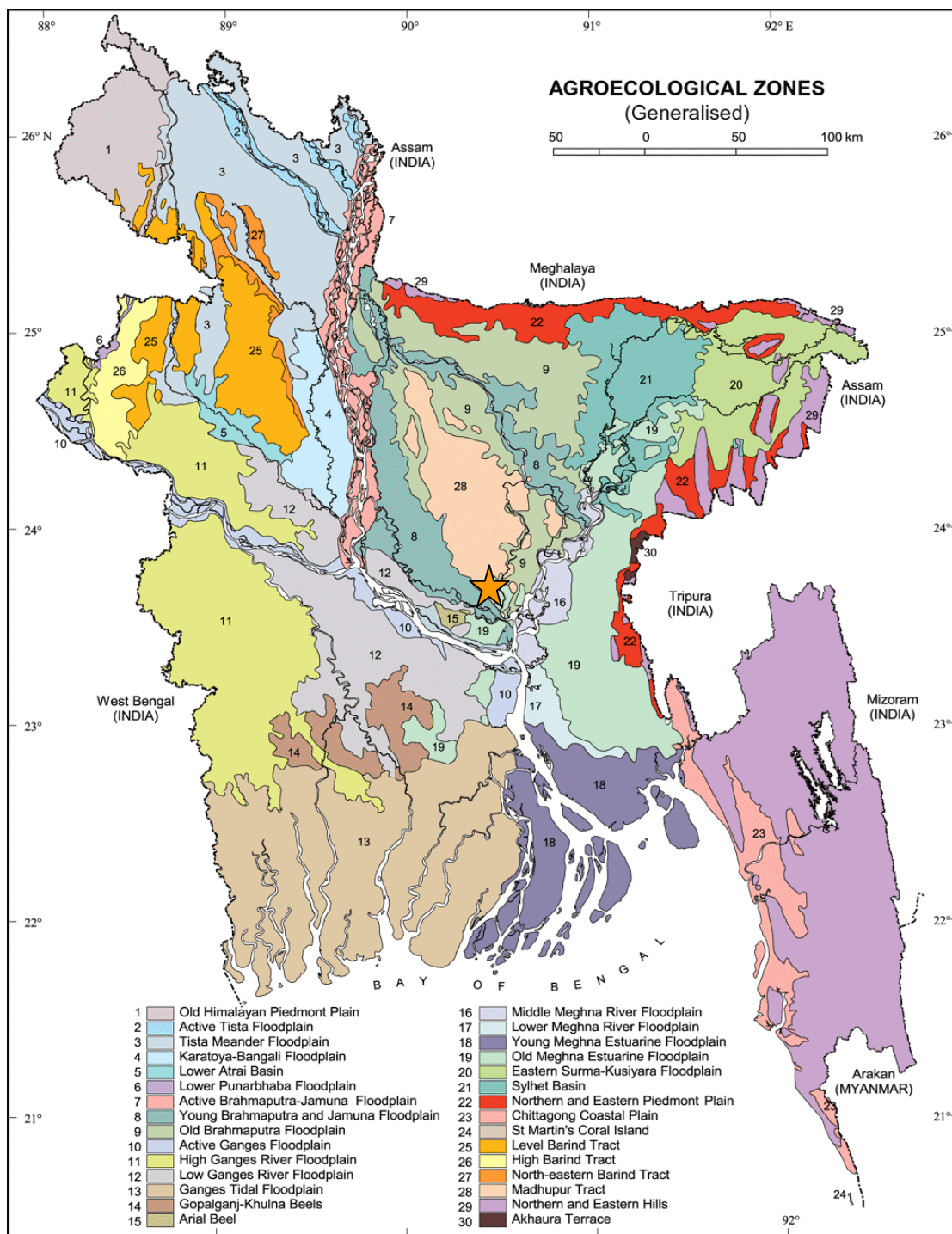
- Ling, K. C. (1979). Rice virus diseases The Intern Rice Res. Inst. Fourth Printing. Ios Banos Laguna. Philippines. p.10.
- Marmey, P., Brian, B., Emmanuel, J., De Kochko, A., Ching, A.O., Piere, Y., Gary, S., Roger, N. B. and Claude, M.F. (1999). RTBV open reading frame 3 encodes a single 37-Kda coat 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.
- Miah, S A. (1976). Possible forecasting of outbreak of tungro virus disease of rice. A paper presented in the 3rd Bangladesh Annu Conf. held in BAU. Myrnensingh, Jan. 23-26. 1976.
- Miah, S. A. (1984). Comments on RTV Collaborative Project A Paper presented in IRRC- 1984 held at CRRT Bhubaneswar, India. Oct. 1984.
- Miah, S.A (1973). Present status of rice diseases. Country report and research notes on virus diseases. *The Rice Path Newslet.* 2/73.
- 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.
- Mohana, G. and Anjaneyulu, A. (1984). Effect of meteorological factors on symptomatology and aquisition of rice tungro virus by *N virescens*. *IRRN* (6): 9.
- Muralidharan, K., Krishnaveni, D., Rajarajeswari, N.V.L. and Prasad, A.S.R., 2003. Tungro epidemics and yield losses in paddy fields in India. *Current science*, 85(8), pp.1143-1147.
- Nahar, M. A., Rahman, M. M., Akanda, S. I. and Miah, S. A. (1985). Epidemiology of tungro in relation to vector population and varietal resistance. Abstr. 10th Ann. Bangladesh, Sci. Conf. Mar. 1985.
- Nuque, F. L. and Miah, S. A. (1969). A rice virus disease resembling tungro in East Pakistan. *Plant Dis. Rept.* 53 (11): 880-890.

- Omura, T. (1986). Detection of rice viruses in individual vectors by serological methods. In Trop. Agris. Res. Series No. 19 TARC. Tsukuba, Ibraki, Japan. pp. 183 -186.
- Ou, s fl, Rivera. C. T., Navarathan. S. J. and Goh. K. G (1965) Virus nature of Penyakit merah disease of rice in Malaysia. Plant Vis. Rept. 49:778-782.
- Ou, S. H. and Ling, K. C. (1966). Virus disease of rice in the South Pacific. FAO Plant Protec. Bull. 14. 113.
- Ou, S.H. (1985). Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 61-96
- Palomar, M. K. and Ling, K. C. (1966). Growth and yield of rice plants inoculated with tungro virus. Phil. phytopath. 2: 17.
- Peng, S.B. and Yang, J.C. (2003). Current status of the research on high yielding and high efficiency in resource use and improving grain quality in rice. China J. Rice Sci. 17: 275–280.
- Rahman, M. M. (1983). Resistance mechanism of eight rice varieties to tungro virus complex and its vector *Nephotettix virescens*. M. S. thesis. Univ. of the Philippines at Los Banos. Laguna, Philippines
- Rajput, R.L. and Bartaria, A.M. (1995). Reaction of rice cultivars to brown spot. Agricultural Science Digest Journal.15 (4): 205-206.
- Rao, G. M. and Anjaneyulu, A. (1976). Influence of nitrogen nutrition on tungro diseased plants of different rice cultivars. *Oryza* 13 (2): 73- 79.
- Rivera, C. T. and Ling, K. C. (1971). Transmission studies of a new strain of rice tungro virus. *phil. Phytopath.* 7: 10-17.
- Rivera, C. T. and OU, S. H. (1965). Leafhopper transmission of tungro a disease of rice plant. Plant dis. Repr. 49: 127-131.

- Saito, Y ., Hibino, H., Omura, T. and Inoue, H. (1986). Rice tungro virus. In virus diseases of rice and legumes in the tropics (eds. T. Kaiwara and S. Kanno). Tropical Agri. Res. Centre, Tsukuba, Ibarakin 305, Japan. pp. 3-13.
- Saito, Y. (1977). Interrelationships among waika disease, tungro and other similar diseases of rice on Asia. In Tropical Agri. Res. Series No. 10. TARC Tsukuba Ibaraki, Japan. pp 129- 135.
- 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.
- Sen, S., & Chakraborty, R. (2017). Food in health preservation and promotion: A special focus on the interplay between oxidative stress and pro-oxidant/antioxidant. In H. U. Shekhar, Z. H. Howlader, & Y. Kabir (Eds.). Exploring the nutrition and health benefits of functional foods (pp. 265–300). USA: IGI Global.
- Shahjahan, A. K. M., Rahman. M. M. and Miah. S. A. (1992). Status and future research need for tungro in Bangladesh Paper presented at the Int. Rice Res Conf., I RRI. Los Banos. Philippines.
- Siwi. S. S., Kortohadjono, A., Hamoto, S. and Diratmaja, A. (1987). The green leafhopper genus *Nephotettix matsumara*. In Proc. Workshop on rice tungro virus. Ministry of Agric. Maros Res. Inst. for food Crops. Indonesia.
- Tantera, D.M. (1986). Present status of rice and legume disease in Indonesia. *Trop. Agris. Ser.No. 19. TARC, Tsukuba, Japan.*
- Vidhyasekaran, P ., Saivaraz, K., Lewin, H. D. and Chelliah, S. (1987) Reaction of IR and ADT varieties to green leafhopper (GLH) and tungro (RTV). *Int. Rice Res. Newslet.* **12**(5): 12.
- 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

Appendix I: Map showing the experimental sites under study



A. Morphological characteristics of the experimental field

| Morphological features | Characteristics |
|-------------------------------|---------------------------------------------------------------|
| Location | Sher-e-Bangla Agricultural University Research Farm, Dhaka |
| AEZ | AEZ-28, Madhupur Tract |
| General Soil Type | Shallow Red Brown Terrace Soil |
| Land type | Medium high land |
| Soil series | Tejgaon |
| Topography | Fairly leveled |

B. The initial physical and chemical characteristics of soil of the experimental site (0 - 15 cm depth)

| Physical characteristics | |
|---------------------------------|----------------|
| Constituents | Percent |
| Sand | 26 |
| Silt | 43 |
| Clay | 31 |
| Textural class | Silty clay |
| Chemical characteristics | |
| Soil characters | Value |
| pH | 6.1 |
| Organic carbon (%) | 0.48 |
| Organic matter (%) | 0.86 |
| Total N (%) | 0.09 |
| Available P (ppm) | 21.56 |
| Exchangeable K (me/100 g soil) | 0.14 |

Source: Soil Resource and Development Institute (SRDI), Farmgate, Dhaka

Appendix III: Monthly record of air temperature, relative humidity and total rainfall of the experimental site during the period from June- November, 2019

| Month (2019) | Air temperature (degrees Celsius) | | Relative humidity (%) | Total rainfall (mm) |
|-----------------|-----------------------------------|---------|--------------------------|------------------------|
| | Maximum | Minimum | | |
| June | 36.2 | 25.5 | 82 | 380 |
| July | 36.4 | 23.8 | 82 | 573 |
| August | 35.9 | 23.2 | 80 | 475 |
| September | 34.0 | 22.7 | 78 | 460 |
| October | 31.8 | 21.3 | 70 | 250 |
| November | 29.20 | 18.75 | 50 | 38 |

Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka- 1212

Appendix IV: Disease Rating Scale of RTVD

| Scale | Rating | Incidence Range (%) |
|-------|------------------------|---------------------|
| 0 | Immune | 0% |
| 1 | Highly Resistant | 1-10% |
| 2 | Moderately Resistant | 11-25% |
| 3 | Tolerant | 26-50% |
| 4 | Moderately Susceptible | 51-60% |
| 5 | Susceptible | 61-70% |
| 6 | Highly Susceptible | 71-100% |

**Appendix V: Analysis of variance of the data on disease incidence at field condition
(%) of tested rice varieties**

| Source of variation | Degrees of freedom | Mean square of disease incidence at field condition (%) |
|---------------------|--------------------|---------------------------------------------------------|
| Replication | 2 | 3811.11 |
| Treatment | 11 | 1174.75* |
| Error | 22 | 368.69 |

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VI: Analysis of variance of the data on Growth parameters of tested rice varieties

| Source of variation | Degrees of freedom | Mean square of growth parameters | | |
|---------------------|--------------------|----------------------------------|---------------|----------------|
| | | Plant height | Tiller Number | Panicle length |
| Replication | 2 | 2.51 | 11.69 | 28.96 |
| Treatment | 11 | 74.24** | 6.14* | 16.49** |
| Error | 22 | 2.71 | 4.03 | 2.01 |

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VII: Analysis of variance of the data on yield contributing and yield parameters of tested rice varieties

| Source of variation | Degrees of freedom | Mean square of yield contributing and yield parameters | | |
|---------------------|--------------------|--------------------------------------------------------|--------------|------------|
| | | Grains/panicle | Fresh weight | Dry weight |
| Replication | 2 | 7413.19 | 1.24 | 1.04 |
| Treatment | 11 | 1550.03** | 0.82** | 1.21** |
| Error | 22 | 426.29 | 0.15 | 0.09 |

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level