TRANSMISSION OF VIRUSES CAUSING MAIZE LETHAL NECROSIS DISEASE (MLND) THROUGH SEED IN COMMERCIAL HYBRIDS AND THEIR MOLECULAR DETECTION THROUGH RT- PCR

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CERTIFICATE

*This is to certify that thesis entitled, "**TRANSMISSION OF VIRUSES CAUSING MAIZE LETHAL NECROSIS DISEASE (MLND) THROUGH SEED IN COMMERCIAL HYBRIDS AND THEIR MOLECULAR DETECTION THROUGH RT-PCR**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in PLANT PATHOLOGY** embodies the result of a piece of bona-fide research work carried out by **RUBAIYA ISLAM**, Registration no. **13-05443** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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The Author

Transmission of Viruses Causing Maize Lethal Necrosis Disease (MLND) through Seed in Commercial Hybrids and Their Molecular Detection through RT-PCR

ABSTRACT

An experiment was conducted in pot and laboratory condition under the Department of Plant Pathology of Sher-e-Bangla Agricultural University, Dhaka-1207 during 2020-2021.The pot experiment was carried out in a Completely Randomized Design (CRD) with ten replications. RT-PCR test was done in Molecular Biology and Plant Virology Laboratory. In this experiment ten varieties/lines *viz*., Tain 927, Jonaki 707, Rocket 55, Konok 51, Kohinoor, Line A.S, Line A, Line 984, Khoi Bhutta and Sweet corn were selected for conducting the experiment. Among the ten tested varieties/lines, Tain-927, Rocket-55 and Line A were showed tolerant. Variety Khoi bhutta was showed highly susceptible against Maize Lethal Necrosis Disease (MLND). Remaining varieties were showed susceptible to moderately susceptible. Molecular study was also performed for the detection of targeted maize viruses that cause MLN disease. From molecular study through RT-PCR test, it was revealed that results obtained on the basis of biological properties. All the selected varieties/lines gave the positive results in RT-PCR test against *Maize Chlorotic Mottle Virus* but showed negative result against *Sugarcane Mosaic Virus*. Different morpho-physiological, yield and yield contributing characters were also studied. Among the selected varieties, the maximum height of plant was obtained in the variety Tain-927 and minimum was obtained in the variety Khoi bhutta. The highest chlorophyll content was recorded in the variety Konok-51 and the lowest was recorded in the variety Kohinoor. The maximum number of fruits fruit weight and length was obtained from variety Tain-927 and the minimum was obtained from variety Khoi bhutta. Both disease incidence and severity show negative relation with plant height and chlorophyll content. From the study, it may be concluded that maize viruses were transmitted through hybrid seeds.

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LIST OF ABBREVIATIONS

AEZ	Agro-Ecological Zone
BBS	Bangladesh Bureau of Statistics
CV %	Percent Coefficient of Variance
CRD	Completely Randomized Design
DAS	Days After Sowing
eds.	editors
et al.	et alia (and others)
etc.	et cetera (and other similar things)
FAO	Food and Agricultural Organization
FP	Forward Primer
L.	Linnaeus
LSD	Least Significant Difference
i.e.	id est (that is)
MLND	Maize Lethal Necrosis Disease
MCMV	*Maize Chlorotic Mottle Virus*
MoP	Muriate of Potash
mm	Millimeter
nm	Nanometer
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
RP	Reverse Primer
SAU	Sher-e-Bangla Agricultural University
SRDI	Soil Resources and Development Institute
SCMV	*Sugarcane Mosaic Virus*

CHAPTER I

INTRODUCTION

INTRODUCTION

The maize (*Zea mays* L) is the staple food and critical for food security in many countries of the world. It belongs to the family Poaceae. It also known as corn, one of the most important and widely grown cereal crops in the world after wheat and rice (FAOSTAT, 2017). This cereal crop was first domesticated by indigenous peoples in southern Mexico about 10,000 years ago. (Benz, 2001).

Maize not only being consumed directly by humans but also used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup (Foley,2019). Maize has six major types such as- dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn (Franklin, 2013). Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels, while field corn varieties are used for animal feed, various corn-based human food uses (including grinding into cornmeal or masa, pressing into corn oil, and fermentation and distillation into alcoholic beverages like bourbon whiskey), and as chemical feedstocks. Maize is also used in making ethanol and other biofuels.

Maize is very popular due to its high nutritional significance enriched with abundant amount of macronutrients like starch, fiber, protein and fat along with micronutrients like vitamin B complex, ß-carotene and essential minerals, i.e. magnesium, zinc, phosphorus, copper, etc. In 100 g maize, there are calories 96%, water 73%, protein 3.4 g, carbs 21 g and sugar 4.5 g (USDA, 2019).

Maize has a great impact on worldwide cereal consumption. It accounts for 40% of the overall cereal production and 30% of total food caloric intake

(Ekpa *et al*., 2019; Santpoort, 2020). In Bangladesh, maize is grown all over the country. But Bandarban, Rangamati, Dinajpur, and Rajshahi districts are the major maize producing areas. The production of maize in Bangladesh increased from 1,552 thousand tones in 2010 to 4,100 thousand tones in 2019 growing at an average annual rate of 11.72% (BBS 2020).

The low yield of maize is attributed to a combination of constraints among which diseases play a major role. Worldwide, more than 50 viruses have been reported in maize causing an array of symptoms in single or mixed infections (Redinbaugh and Zambrano 2014). Of these, at least a dozen viruses have been considered important in terms of prevalence and economic losses in several maize producing areas (Redinbaugh and Pratt 2008). In Bangladesh, two major viral diseases caused by *Maize Dwarf Mosaic Virus (MDMV)* and *Maize Chlorotic Mottle Virus (MCMV)* are causing yield reduction at a great extent.

Maize Chlorotic Mottle Virus (MCMV) is comparatively more severe when it synergistically combined with certain numbers of *potyviridae* viruses to ultimately causing deadly disease called Maize Lethal Necrosis Disease (MLND).(Awata *et al*., 2019; Kiambi *et al*., 2019).

Virus transmission through seed is common in many annual crops and the viruses are also vectored by insects in a non-persistent or persistent manner (Mengesha, 2019). This mode of transmission is epidemiologically critical since the virus is associated with the planted seed resulting in infected plants randomly dispersed in the whole field (Nordenstedt *et al*., 2017; Cobos *et al*., 2019). Transmission of viruses causing MLN disease through seed has been reported (Mahuku *et al*., 2015; Mekureyaw, 2017; Shango *et al*., 2019).

Invasion of seeds by viruses interferes with plant normal physiological activities, morphology, reduction of plant vigour and negatively affect overall crop productivity (Sevik and Balkaya, 2015; Mengesha, 2019).

OBJECTIVES

The present study was undertaken to achieve the following specific objectives:

-To observe the transmission of viruses through seeds of Maize Lethal Necrosis Disease (MLND) based on symptoms and morphological appearances,

-To study the status of the intensity level caused by MLND in terms of disease incidence and disease severity and

-To detect viruses causing MLND through molecular detection through RT-PCR

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Maize is one of the oldest and most important crop in the world. It is the highest yielding grain crop having multiple uses. Because of more nutritious status, it could be good source of nutrients for under nourished and mal-nourished population in Bangladesh.

The production of maize in terms of quantity and quality is becoming a threat in Bangladesh due to pathogen attack like fungi, bacteria, viruses etc. Viral disease is one of the major constraints among which Maize Lethal Necrosis Disease is a new one and threatening the yield performance.

Several researches have been done on various aspects of MLND which is reviewed as under-

2.1. About maize

The word maize is derived from the Arawak Indian word ma-hiz. The Spanish. Equivalent, maize, is still in use in the Spanish-speaking countries of America. In the United States and Canada maize is known as corn and is considered as an important cereal food crop.

Maize is a cultigen; human intervention is required for it to propagate. Whether or not the kernels fall off the cob on their own is a key piece of evidence used in archaeology to distinguish domesticated maize from its naturally-propagating teosinte ancestor (Benz, (2001).

The plant was domesticated and cultivated in the Americas long before the Europeans reached the New World. Little is known about the origin and development of the cultivable variety of maize. It is said that maize was

developed through favorable mutation of the wild species *Zea mexicana*. Most historians believe maize was domesticated in the Tehuacán Valley of Mexico.

Maize is a facultative short day plant (Karl, J.R. (January 2002) and flowers in a certain number of growing degree days > 10 °C (50 °F) in the environment to which it is adapted.(Paliwal,(2000). Maize grows well in sandy loam and heavy clay loam type of soils having pH in between 5.5 and 8.5. A temperature in between 12'C and 29'C seems to be favorable for its growth.

2.2. Morphology of maize

Maize (bhutta) a cereal crop, *Zea mays* of the family Graminae, order Cyperales. It belongs to the family Poaceae. The maize plant is often 3 m (10 ft) in height *(*Wellhausen, Edwin John (1952) .The stem is commonly composed of 20 internodes (Stevenson, J. C.; Goodman, M. M. (1972) of 18 cm (7 in) length *(*Wellhausen, Edwin John (1952).

The leaves arise from the nodes, alternately on opposite sides on the stalk *(*Willy H. Verheye, ed. (2010) and have entire margins. Typical corn plants develop 18 to 22 total leaves, silk appears about 55 days after emergence, and mature in around 125 days after emergence (Ritchie *et al*., 1993).

The apex of the stem ends in the tassel, an inflorescence of male flowers. When the tassel is mature and conditions are suitably warm and dry, anthers on the tassel dehisce and release pollen. Maize pollen is anemophilous (dispersed by wind), and because of its large settling velocity, most pollen falls within a few meters of the tassel.

Ears develop above a few of the leaves in the midsection of the plant between the stem and leaf sheath where female inflorescences are developed and tightly enveloped by several layers of ear leaves commonly called husks.

Elongated stigmas, called silks, emerge from the whorl of husk leaves at the end of the ear. They are often pale yellow and 18 cm (7 in) in length, like tufts of hair in appearance. At the end of each is a carpel, which may develop into a "kernel" if fertilized by a pollen grain.

The pericarp of the fruit is fused with the seed coat referred to as "caryopsis", typical of the grasses, and the entire kernel is often referred to as the "seed". The cob is close to a multiple fruit in structure, except that the individual fruits (the kernels) never fuse into a single mass. The grains are about the size of peas, and adhere in regular rows around a white, pithy substance, which forms the cob. The maximum size of kernels is reputedly 2.5 cm (1 in) (Alexander (1961).

An ear commonly holds 600 kernels. They are of various colors: blackish, bluish-gray, purple, green, red, white and yellow. When ground into flour, maize yields more flour with much less bran than wheat does. It lacks the protein gluten of wheat and, therefore, makes baked goods with poor rising capability. A genetic variant that accumulates more sugar and less starch in the ear is consumed as a vegetable and is called sweet corn. Young ears can be consumed raw, with the cob and silk, but as the plant matures (usually during the summer months), the cob becomes tougher and the silk dries to inedibility. By the end of the growing season, the kernels dry out and become difficult to chew without cooking.

2.3. About MLND

Maize lethal necrosis disease (MLN disease, MLND, corn lethal necrosis) is a viral disease affecting maize (corn) predominantly in East Africa, Southeast Asia and South America, which was recognised in 2010.

Maize Lethal Necrosis Disease (MLND) is a disease of maize caused by coinfection of maize with *Maize Chlorotic Mottle Virus (MCMV*) and one of several viruses from the Potyviridae, such as *Sugarcane Mosaic Virus, Maize Dwarf Mosaic Virus, Johnsongrass Mosaic Virus* or *Wheat Streak Mosaic Virus.* (FAO. June 2013).

MCMV was first reported in Peru in 1974. MLN was subsequently found in the USA .The disease was later reported from several countries across the Americas, Asia, and Africa. After the first detection of MLN in Kenya in September 2011, followed by confirmation of the pathogens involved in the disease in Africa (Wangai *et al*., 2012), *MCMV* was recorded across the region over the next 3–4 years.

MLN had a devastating effect not only on the maize crop and the livelihoods of the resource-poor farmers in the affected countries, but also on other key factors in the maize seed/grain value chain, especially small- and medium-enterprise (SME) seed companies and processors.

2.4. DISEASE SYMPTOMS

Maize leaves show symptoms include mosaic, chlorosis and eventually necrosis, resulting in either plant stunting or death (Niblett and Claflin, 1978; Uyemoto *et al*., 1980, 1981). Leaves of infected plants become yellow from

the tip and margins to the centre. Older leaves (bottom of plant) remain green. Ears and leaves dry up and sometimes look like a mature plant. The whole plant dies and maize cobs remain without kernels.

MLND symptoms can be confused with symptoms of nutrient deficiency but plants affected by MLND appear only in some areas and are scattered or clumped in a field while nutrient deficiency appears on many plants over large areas of a field (www.plantwise.org. *Retrieved 2020-06-22*.).

In the early stages, MLND causes long yellow stripes on leaves. Unlike maize streak virus disease though, the streaks of MLND are wider. As the disease advances, the maize leaves become yellow and dry out from the outside edges towards the midrib.

MLND can also cause dwarfing and premature aging of the plants. Finally, the entire plant dries out and dies. Dead plants can then be seen scattered across the field among healthy looking plants. Late infected plants don't tassel and tend to produce poor grain filled cobs (www.plantwise.org. *Retrieved 2020-06-22*).

2.5. VIRUS IDENTIFICATION

Viruses are very tiny compared to other groups of plant pathogens like fungi and bacteria which can be visualized through microscopes but plant viruses are too small to observe using light microscopes and they can be seen only using a transmission electron microscope and are made of a coat protein and a type of nucleic acid, DNA or RNA based on the nucleic acid core carrying genetic information (Ellis *et al*., 2008).

There are a total of more than 40 reported maize viral diseases worldwide. Five of them have reportedly occurred in China. They are maize rough dwarf disease, maize dwarf mosaic disease, maize streak dwarf disease, maize crimson leaf disease, maize wallaby ear disease and corn lethal necrosis disease.

In Ethiopia, four types of viruses were reported to infect maize. These are: *Maize Streak Virus (MSV), Sugarcane Mosaic Virus (SCMV), Maize Dwarf Mosaic potyvirus (MDMV),* and *Maize Mottle Chlorotic Stunt Virus (MMCSV)* (Tewabech *et al*., 2002).

Virus is ranked as the second most important plant pathogens following fungi (Vidaver and Lambrecht, 2004).The crop damages owing to viral diseases are difficult to predict, because it depends on region, virus strain, host plant cultivar/variety, and time of infection (Strange, 2005).

Symptoms of viral diseases include crinkling, browning of leaf tissues, mosaic, and necrosis. In addition, plants can also display virus like symptoms when plants respond to unfavorable weather, nutritional imbalances, infection by other types of pathogens, damage caused by pests or abiotic agents and others (Vander Want and Dijkstra, 2006).

In July 2014, cases of maize infection by Maize Lethal Necrotic Disease was reported from eastern rift valley regions of Ethiopia and confirmed by ELISA test. Currently, MLND infestation has spread to enormous areas of the country. Both *MCMV* and *SCMV* are transmitted through mechanical means and are known to be seed transmitted. In addition to this, *MCMV* can be

experimentally transmitted by thrips and beetles while *SCMV* is vectored by aphids (Cabanas *et al*., 2013).

Accurate diagnosis of virus diseases, is the first important step for crop management system (Aboul-Ata *et al*., 2011). Therefore, detection methods should be more convenient, effective, specific and permitted the use for detecting plant pathogens (McCartney *et al*.,2003).

2.6. INCIDENCE AND DISTRIBUTION OF MLND

MCMV was first reported in Peru in 1974 (Castillo and Hebertt, 1974). MLN was subsequently found in the USA (Niblett and Claflin, 1978). The disease was later reported from several countries across the Americas, Asia, and Africa, including Argentina (Teyssandier *et al*., 1982), Thailand (Klinkong and Sutabutra, 1982; Uyemoto, 1983), Mexico (Delgadillo and Gaytán, 1987), China (Xie *et al*., 2011), Kenya (Wangai *et al*., 2012), Uganda (Mahuku et al., 2015a,b), Rwanda (Adams *et al*., 2014), D.R. Congo (Lukanda *et al*., 2014), Ethiopia (Mahuku *et al*., 2015b), Taiwan (Deng *et al*., 2014), Ecuador (Quito-Avila *et al*., 2016), and Spain (Achon *et al*., 2017). After the first detection of MLN in Kenya in September 2011, followed by confirmation of the pathogens involved in the disease in Africa (Wangai *et al*., 2012), MCMV was recorded across the region over the next 3–4 years.

In Rwanda, it was first reported in 2013 and was found endemic in all maize-growing districts (Adams *et al*., 2014). The disease was officially reported in

the Democratic Republic of Congo (DRC) predominantly in the western provinces of the north and south Kivu in 2014 (Lukanda *et al*., 2014).

In Ethiopia, maize plants with MLN symptoms were first observed in 2014 prompting surveillance efforts which led to the first report (Mahuku *et al*., 2015b). There are reports of MLN in Southern Sudan (Mahuku *et al*., 2015a,b, unpublished results) and Burundi (Ministry of Agriculture surveillance reports, 2017).

A high incidence (50-80%) and severity (1 - 4 using 1-5 scale) of MLND were found in symptomatic randomly selected plants at South Tigray, Raya Azabo district. Among six surveyed zones in Oromia region East Shoa and Jimma were highly affected zones with highest incidence (20-100 %) and severity rate (2.5), while the remaining zones were moderately affected by the disease. (Girma Demissie *et. al*., 2018).

2.7. Transmission

Virus transmission through seed is common in many annual crops and the viruses are also vectored by insects in a non-persistent or persistent manner (Mengesha, 2019). This mode of transmission is epidemiologically critical since the virus is associated with the planted seed resulting in infected plants randomly dispersed in the whole field (Nordenstedt *et al*., 2017; Cobos *et al*., 2019). Seed infection ensures that the virus survives between the cropping growing seasons and therefore, a source of primary inoculum for initiation of secondary spread in epidemics especially presence of large number of insect vectors (Redinbaugh and Stewart, 2018).

Transmission of viruses causing MLN disease through seed has been reported (Mahuku *et al*., 2015; Mekureyaw, 2017; Shango *et al*., 2019). Passage of the virus through seed is determined by the ability of the virus to move from plant entry point to the reproductive organs, replicate and produce infected

progenies (Cobos *et al*., 2019). The virus infects the host either through seed coat or embryo while a few contaminate the seed surface (Kil *et al*., 2016; Montes *et al*., 2020).

Under field conditions, *MCMV* and *SCMV* are known to be transmitted from plant to plant by several insect vectors (Jiang et al., 1992; Cabana et al., 2013; Ford et al., 2004) with alternative host plants in maize fields acting as inoculum sources of the MLN-causing viruses (Nelson et al., 2011). *MCMV* is transmitted by thrip and chrysomelid beetles (Zhao et al., 2014) and while *SCMV* is transmitted by aphids mainly *Myzus perscae* and *Aphis gossypii* (Cabana et al., 2013). Research also indicates that contaminated seed and soil transmit *MCMV* and *SCMV* though at very low rate (Jensen et al., 1991a; Zhang et al. 2011b; Mahuku et al., 2015b). Transmission of *MCMV* through seed was first reported in America by Jensen et al. (1991b) and recently in Africa by Mahuku et al. (2015a). Transmission of *SCMV* has been reported (Li et al., 2011) but it is not being considered as a major concern (Redinbaugh & Stewart, 2018). *MCMV* and *SCMV* either appear singly or in mixed infections (Guadie et al., 2018b).

Invasion of seeds by viruses interferes with plant normal physiological activities, morphology, reduction of plant vigour and negatively affect overall crop productivity (Sevik and Balkaya, 2015; Mengesha, 2019). Passing of a virus in seed through crop generations can lead to increased virus seed transmission resulting in virus accumulation and virulence (Pagan *et* al. 2014; Kiruwa *et al*., 2020).

2.8. Yield Loss

Maize is a strategic cereal crop in sub-Saharan Africa and a staple crop for more than 70 million people, with its cultivation covering more than 25 million ha in sub-Saharan Africa (Mudde et al., 2018). Among the diseases, maize lethal necrosis (MLN) disease has emerged as the single most important production constraint in maize (Demissie et al., 2016; X. Xie et al., 2016; Qin Yang et al., 2017).

MLN is expected to threaten maize production especially in developing countries. It has been estimated that highly MLN-affected areas can experience a massive yield loss (Wangai et al., 2012). Due to dependence of farmers to maize as their main food crop, shortage in its supply can be synonymous with food insecurity.Considering each individual MLN-causing viruses, Mahuku et al., (2015b) indicated that MCMV is considered a primary disease-causing virus behind almost all MLN cases. The MCMV alone has a big potential to establish in warm arid, semi arid and sub-humid tropics (Isabirye and Rwomushana, 2016). Since MCMV is new to the crop system, plants seems not to be prepared for attack i.e. plants have little or lacks resistance to the pathogen, and thus the additional weakened effect by the potyviruses or other viruses such as Maize mosaic virus and Maize rayado fino virus and/or abiotic stress favor their full colonization to maize host (Nelson et al., 2011).

Swaziland, Burundi, and Rwanda will lose 100% each and Uganda 88.1% in terms of national maize production area. In a synergistic interaction of *MCMV* with a potyvirus, higher damage to maize crop are expected as it is clear that effects are higher when in combination compared to when *MCMV* or a potyvirus infects the host individually (Xia et al., 2016).

CHAPTER III
MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was carried out under the field condition at central farm of Sher-e-Bangla Agricultural University, Dhaka during December, 2020 to April, 2021 to ascertain the incidence, severity of Maize Lethal Necrosis Disease (MLND) and the subsequent varietal performance affected by the disease. The material used and techniques adopted during the study are being summarized hereby with some headings and sub-headings.

3.1. Experimental Site and Duration

The experiment was conducted at central research farm (beside weather station) of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, during the period of November 2020 to April 2021 in pots. The experimental area situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon, 1988) (Appendix IA).

3.2. Soil Characters

The soil used in the experimental pots was silt loam. Soil was contained low level of nutrients and non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment, soil samples were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU) at a depth of a 0 to 30 cm and analyzed in the Soil Resources Development Institute (SRDI), Farmgate, Dhaka (Appendix IB & II).

3.3. Climate

The climate of the Modhupur Tract varies slightly from north to south, the northern reaches being much cooler in winter. Average temperatures vary from 19 28℃ to 32℃ in summer, falling to 20℃ in winter, with extreme lows

of 10°C. Rainfall ranges between 1,000 mm and 1,500 mm annually, heavy rainfall in Kharif season (May-September) and scanty in Rabi season (October-March). Severe storms are unusual but tornadoes have struck the southern areas. During the month of December, January and February there was no rainfall. During the period of investigation, the average maximum temperature was 32°C and average minimum temperature was 20°C. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment was collected from Bangladesh Meteorological Department, Agargaon, Dhaka (Appendices-III).

3.4. Collection of Commercial Hybrids Seeds and Selection

Commercial hybrids maize seeds was collected from authorized seed traders and approved seed stockist. Controls consisted of certified seeds was also collected. The varieties (Tain-927, Jonaki-707, Rocket-55, Konok-51, Kohinoor) were collected from Siddik bazar, Dhaka. Line A.S., Line A and Line 984 were collected from Department of Agronomy, Sher-e- Bangla Agricultural University. Khoi Bhutta and Sweet corn variety were collected from Department of Plant Pathology, Sher-e- Bangla Agricultural University.

Seeds were selected from the collected seeds on the basis of dry inspection for further experimental studies. The selected seeds were sorted to obtain lots of relatively uniform seed size and dried to a moisture content of 15%.Name of selected maize varieties used in the present study are mentioned in table 1.

Table 1: Name and origin of maize varieties/lines used in the present Study

Sl. No.	Variety/Line	Origin
01	Tain 927	Siddik Bazar, Dhaka
02	Jonaki 707	Siddik Bazar, Dhaka
03	Rocket 55	Siddik Bazar, Dhaka
04	Konok 51	Siddik Bazar, Dhaka
05	Kohinoor	Siddik Bazar, Dhaka
06	Line A.S	Dept. of Agronomy, SAU
07	Line A	Dept. of Agronomy, SAU
08	Line 984	Dept. of Agronomy, SAU
09	Khoi Bhutta	Dept. of Plant Pathology, SAU
10	Sweet corn	Dept. of Plant Pathology, SAU

3.5. Lab Experiment

3.5.1. Experimental Design

The experiment was carried out in a Completely Randomized Design (CRD) with three (03) replications for each variety. Each replication contained three (03) petridishes and each petridish contained four (04) seeds. So, total number of seeds for each replication in each variety was twelve (12).

3.5.2. Evaluation of Percentage Germination

Germination test was carried out using a sample of 12 seeds for each variety. The seeds were surface sterilized in 2% sodium hypochlorite solution, rinsed thrice in distilled sterile water and sown on moist paper towels in petridishes. The seeds were incubated at 25℃ and the seeds which had germinated was

counted after every alternative day from the 5th days after sowing until germination ceased. Seeds germination was determined when the radicle was about 2 mm in length as described by Sivritepe *et al*. (2016).

3.5.3. Evaluation of Seedling Emergence

Seedling emergence was determined by counting the number of seedlings emerged in each treatment replicate every three consecutive days commencing the 7th day after placing until no additional seedlings emerged (Aliloo *et al.,* 2011).

3.6. Net house Experiment

3.6.1. Pot Collection and Preparation

Earthen pots were collected from Rayer Bazar, Dhaka. Then pots were filled with soil containing loam soil-sand-manure (2:2:1 v/v) potting medium.

3.6.2. Seed sowing

Seeds were sown on December 04, 2020. Five (5) seeds were sown on each pot.

3.6.3. Intercultural Operations

3.6.3.1. Thinning and Gap filling

Thinning was done after emergence of seedling and three plants were kept in each pot .Gap filling was done in pots where required. The seedlings were taken from the same source and a minor gap filling was done where it was necessary.

3.6.3.2. Weeding

Weeding were accomplished as and whenever necessary to keep the crop free from weeds, for better soil aeration and to break the soil crust. It also helps in conservation of soil moisture. Four weeding were done manually at 15, 30, 45 and 55 DAS to keep the plots free from weeds.

3.6.3.3. Manure and Fertilizer management

Dried cowdung mixed loamy soil was used in pots for this study. The following doses of fertilizers were used –

Fertilizers	Doses/ Pot
Nitrogen	25 g
Phosphorus	25 g
MoP	15 g
Sulphur	2 g
Zinc	1 g

3.6.3.4. Irrigation and drainage

Irrigations were given throughout the growing season as and when necessary. Stagnant water effectively drained out at the time of excess water.

3.6.3.5. Insecticide application

Sevin WP 85 was applied in pots for preventing ants from destroying seeds for germination.

3.6.4. Parameters Assessed

All experimental plants were selected and mean data of the following parameters were recorded. The following parameters were assessed:

Morphological parameters

- Percentage of germination
- Seedling emergence
- Plant height (cm)
- Seedling weight (g)

Disease related parameters

- Number of leaves as per growth stages
- Estimation of disease incidence (%)
- Estimation of disease severity (%)

Physiological parameter

- Chlorophyll content (µg/g)

Molecular study

- Primer Designing
- Sample collection and RNA extraction
- cDNA synthesis
- RT-PCR amplification
- Gel electrophoresis and documentation

Yield and yield contributing characters

- Cob length (cm)
- Individual fruit weight (g)

Morphological parameters

3.6.4.1. Percentage of germination

Percent seed germination was determined using the last count of seeds which germinated as proportion of the total number of seed sown. Germination test was carried out using twelve (12) seeds for each replication in each variety. The seeds were surface sterilized in 2% sodium hypochlorite solution, rinsed thrice in distilled water and sown on moist blotting paper in petridishes. The seeds which had germinated was counted after every alternative day from the 5^{th} days after sowing until germination ceased. Seed germination was determined when the radicle was 2 mm in length as described by Sivritepe *et.al*. (2016).

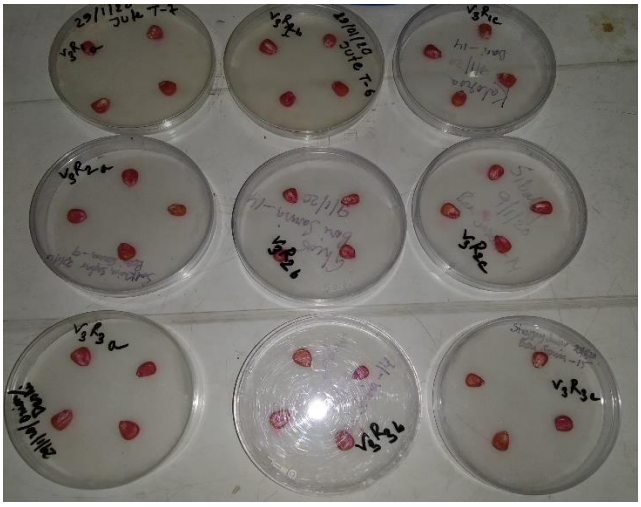


Figure 1. Seeds on moist petridishes

3.6.4.2. Seedling emergence

Percentage seedling emergence was computed with the values obtained from counting divided by the total number of seeds drilled.

A **B**

Figure 2. Seed sowing (A) and seedling emergence (B) in pot

3.6.4.3. Plant height (cm)

Plant height was recorded weekly on 10 randomly selected and tagged plants per variety at 24 DAS, 32 DAS, 37 DAS, 44 DAS, 51 DAS.

3.6.4.4. Seedling weight (g)

Individual seedling weight was measured by a digital balance meter in gram (g). The weight of fresh and dry seedlings both are taken. A mean weight was taken of collected seedlings from each plant as per variety and replication.

Disease related parameters

3.6.4.5. No. of leaves as Per Growth Stage

Different growth stages are numbered 0 to 10. Growth stage 0 lasts from planting of the seed up to when the seedling is just visible above the soil surface. Growth stage is reached when the plant is biologically mature. (Jéan du Plessis *et. al.*, 2003).

Stage 0 : from planting to seed emergence

Stage 1 : four leaves completely unfolded

Stage 2 : eight leaves completely unfolded

Stage 3 : twelve leaves completely unfolded

Stage 4 : sixteen leaves completely unfolded

Stage 5 : silk appearance and pollen shedding

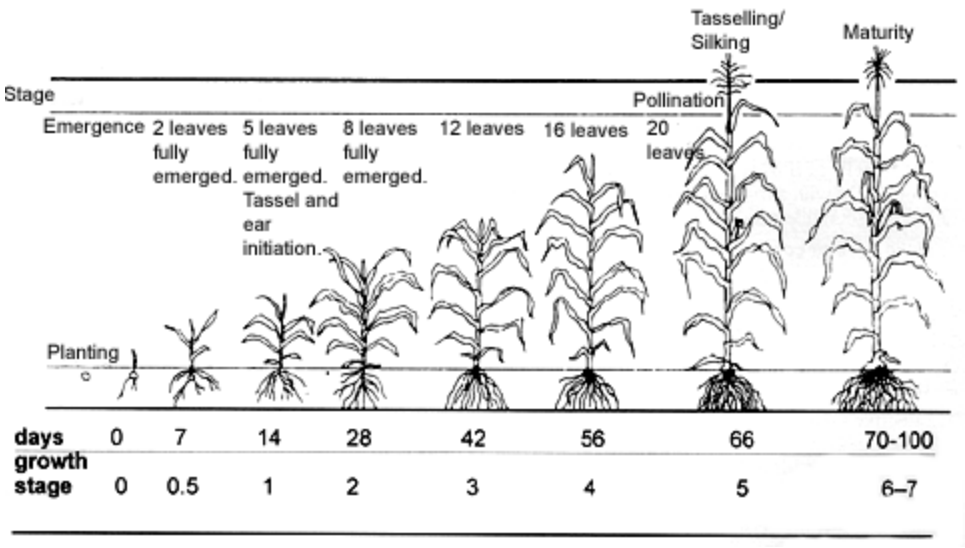
Stage 6 : green mealie stage

Stage 7 : soft dough stage

Stage 8 : hard dough stage

Stage 9 : physiological maturity

Stage 10 : biological maturity



Source: Claxton, R. A. (1976)

Figure 3. Different growth stages of maize plant

3.6.4.6. Number of infected leaves per plant

The infected leaves of each plant were counted from 30 DAS and continued up to 70DAS.

3.6.4.7. Estimation of Disease Incidence

Visual assessment of MLN disease incidence and severity was done weekly commencing on the 42nd post-emergence for five weeks on 10 plants per variety.

Percentage disease incidence was calculated by counting the number of symptomatic plants and expressed as a percentage of the total number of plants in each treatment.

Disease was identified by visual basis, observing the typical symptoms of MLND. The disease incidence reaction was assessed by using the following disease rating scale described by). Ali *et al*., (2005).

Table 2. Disease Rating Scale of MLND

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%

3.6.4.8. Estimation of Disease Severity

Disease severity was calculated using the formula according by Bock *et al*. (2020):

$$\text{Percent severity} = \frac{\Sigma(n \times v) \times 100}{V \times N}$$

Where ,

n= Number of plants in each category

v= Numerical value of symptoms category/code

N= Total number of plants

V= Maximum numerical value of symptoms category

Physiological parameter

3.6.4.9. Chlorophyll content

i) The selected leaf samples were collected and kept in separate polythene bag.

ii) After collection, the leaf samples were immediately taken to the crop physiological laboratory for subsequent analysis.

iii) Around 20 mg leaf sample was weighed and poured into glass vial containing 20 ml 80% acetone solution.

iv) The glass vials are kept into dark condition for 48 hours.

v)After 48 hours chlorophyll was determined by using double beam spectrophotometer at 663 and 645 nm wave length and chlorophyll was determined by using the following formula (according to Witham *et. al.*, 1996).

$$\text{Chlorophyll (a+b)g}^{-1}\text{ leaf tissue} = \frac{[20.2\ (D645)+8.02\ (D663)]\times v}{1000\times w}$$

Where,

D = optical density regarding of the chlorophyll extract at wave length of 663 and 645 nm

v = Final volume (ml) of the 80% acetone with chlorophyll extract

w = Weight of fresh sample in g

Weighing of leaves

Addition of Acetone

After keeping under dark condition for 48 hr

Placement of glasstubes into spectrophotometer

Figure 4. Different steps of chlorophyll determination by using acetone

Molecular study

The molecular detection was done at Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology. Molecular detection was done by following three molecular steps. At first, RNA was extracted from infected leaves as well as healthy leaves samples, then cDNA synthesis was dine and finally performed the PCR test by using cDNA as template to detect the MLND.

3.6.4.10. Primer Designing

For detection of MLND, Coat-protein (CP) gene specific primers were designed using primer-3 version 0.4.0 software. (Deng *et.al* 1994). Primers were made to amplify the conserved/less mutating genomic segment and tested for primer specificity in-silico by applying BLAST, provided by, to reduce the chance of non-specificity. The 3′sequence primers with no similarity to viral sequences or other origin sequences were marked as selected. As MLND is caused by combination of Maize Chlorotic Mottle Virus (MLND) and a potyvirus, Sugarcane Mosaic Virus (SCMV), two primers were synthesized commercially and primer pair used in the study is presented in table 2.

Table 3. Primer pair used in the present study to amplify MLND at 1066 bp fragment for MCMV and 1192 bp fragment for SCMV

Virus	Sequence	Bp	Tm	Amplicon (bp)
MCMV	Forward- TGGAAAACATTGCTGTTGGA Reverse - CAGGACTCTGCCAGAAGGAC	20bp 20bp	58°C	1066
SCMV	Forward- GCACAGGGATCAAGGAAGAA Reverse- TGTCCTGCAGACTGGTTCAC	20bp 20bp	58°C	1192

Preparation of stock solution of primers

To prepare stock solution of forward primer and reverse primer, 250 µl DEPC treated water was added in each primer.

Preparation of working solution of primers

To prepare working solution of forward primer and reverse primer, 10 µl of forward primer was added with 90 µl DEPC treated water and 10 µl reverse primer was added with 90 µl DEPC treated water to make 100 µl volume.

3.6.4.11. Sample collection and RNA extraction

For molecular detection of MLND through PCR, the diseased and healthy leaves samples were collected from the experimental site.

Table 4. Chemicals used for RNA extractions

Components	Amount
Trizol	1 ml
Chloroform	0.2 ml
Isopropanol	0.5 ml
Ethanol	1 ml
DEPC treated water	20 µl
Agarose gel	1 %

RNA extraction protocol

RNA was extracted from maize leaf samples and the protocol was as follows:

Homogenization

Grind leaf tissue sample in liquid nitrogen into fine powder

↓

Tissue Lysis

Shift ground samples to 1.5 ml tube and add 1 ml Trizol

↓

Keep it for 5 minutes at room temperature for dissociation of nucleoprotein complex

↓

Add of 0.2 ml chloroform and shake for 30-45 seconds before pouring

↓

Put it in room temperature for 5-10 minutes

↓

Centrifugation

Centrifuge at 13,000 rpm at 4℃ for 15 minutes

↓

Upper portion of this centrifuged solution (RNA rich) poured into another eppendorf

↓

Precipitation achieved by pouring 0.5 ml isopropanol

↓

Incubation

Incubate it for 10 minutes

↓

Centrifugation

Centrifuge at 13,000 rpm at 4℃ for 10-12 minutes

↓

Remove supernatant and collect pellet

↓

Addition of Ethanol

Washing of pellet by adding 1 ml ethanol

↓

Re-suspend sample through pipetting

↓

Centrifugation

Centrifuge at 10,000 rpm at 4℃ for 5-6 minutes

↓

Remove supernatant and collect pellet

↓

Drying of pellet for 10 minutes (RNA containing)

↓

Resuspension

Resuspend RNA by 20 µl DEPC treated water

↓

Put it for -70℃

Quantification of RNA by spectrophotometer

↓

Quality confirmed in 1% Agarose gel

Selection of leaf sample

Collection of sample

Addition of liquid nitrogen

Grinding of leaf sample

Figure 5. Selection and grinding of leaf sample

3.5.4.12. Agarose gel preparation

RNA was extracted from leaf sample and for confirmation of RNA, extracted RNA was analyze in 1% agarose gel.

Table 5. Preparation of 500 ml 5X TBE Buffer

Components	Amount
Tris-Cl	27g
Boric Acid	13.75 g
EDTA	2.32g
Distilled water	Upto 500ml

Preparation of 500 ml 1X TBE Buffer

400 ml distilled water was added into 100 ml 5X TBE Buffer to make 1X TBE Buffer.

100 ml gel preparation (1% gel)

60 ml TBE buffer was taken from prepared 1X TBE buffer in a conical flask and 0.6g agarose gel was added into it. Then buffer and gel containing flask was heated in oven for 1.5 minutes and then kept for cooling. After cooling, 2 μl Ethidium Bromide was added in it.

Gel electrophoresis

Loading dye	20 μl
DEPC treated water	60 μl
Sample	4 μl
Dye	2 μl

Figure 6. Placement of loading dye with sample into agarose gel

3.6.4.13. cDNA synthesis

The following protocol was used for cDNA synthesis-

1. RNA sample and primer were mixed in sterile RNase-free microfuge tube.

Components	Volume
Total RNA	Upto 4 μg
Reverse Primer	1 μl
Nuclease-free water	To a volume of 6 μl

2. Sample RNA was denatured for 5 minutes at 65℃, spun briefly and put promptly on ice.

3. The following components were added-

Components	Volume
ProtoScripts II Reaction Mix (2X)	10 μl
ProtoScript II Enzyme Mix (10 X)	2 μl

4. The 20 μl cDNA synthesis reaction was incubated at 42℃ for one hour.
5. The cDNA product was then stored at -20℃ .

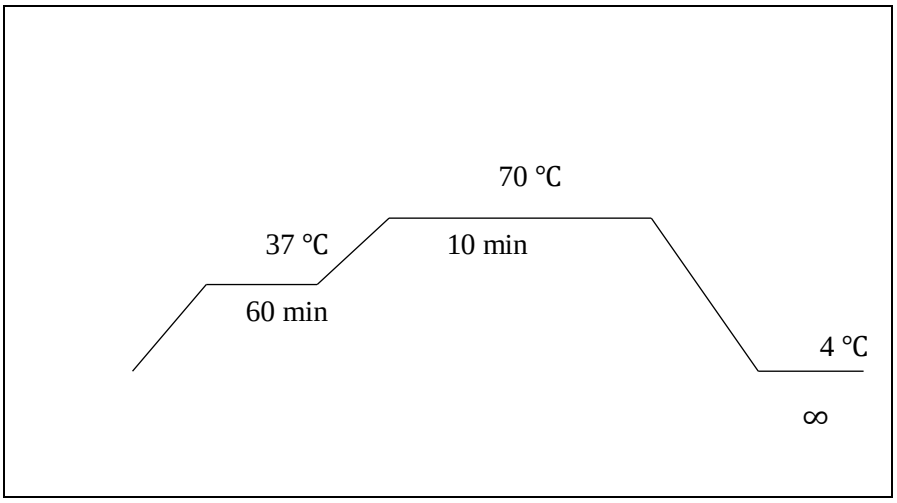


Figure 7. Cycling profile for cDNA synthesis

3.6.4.14. RT-PCR

RT-PCR was done for detection of viruses. Following thermal cycle programme was performed, 1 cycle (4 min at 94°C), 30 cycles (1 min at 94°C, 1 min at 55°C and 2 min at72°C) and 1 cycle (10 min at 72°C). PCR products were stored at -20°C.

Table 6 . PCR composition

Components	Amount
PCR master mix	4 µl
cDNA	3 µl
Forward primer	2 µl
Reverse primer	2 µl
PCR water	9 µl

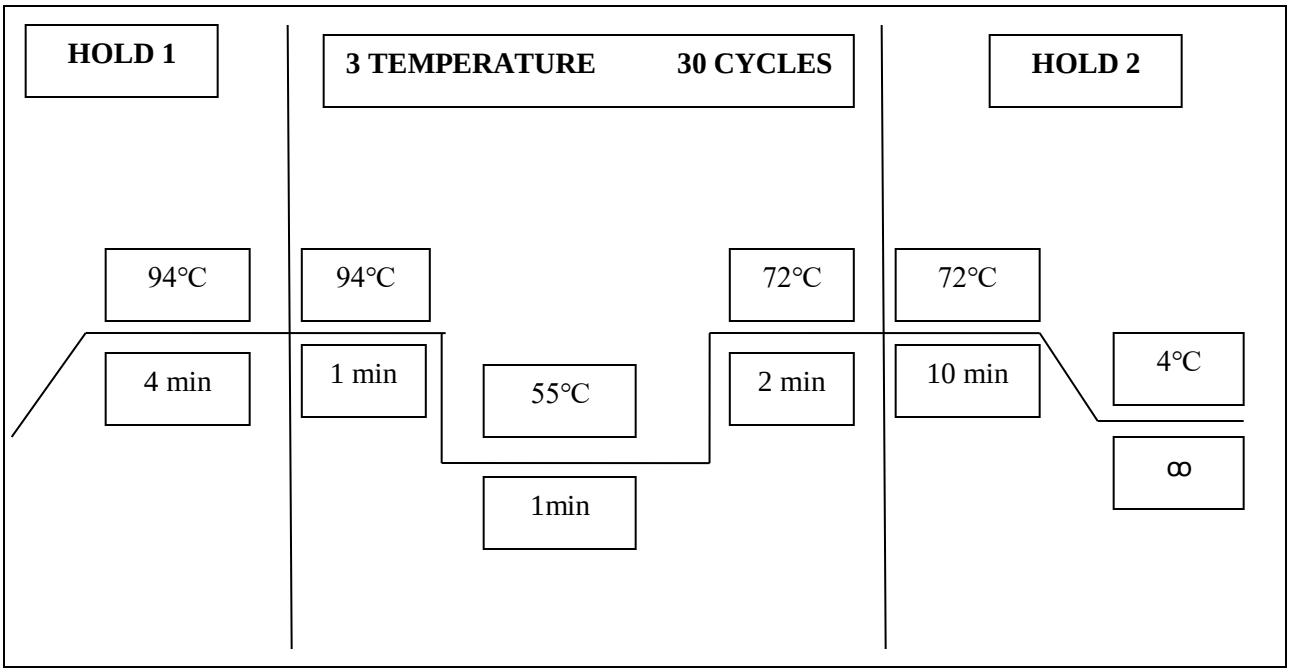


Figure 8. RT-PCR cycling for detection of viruses

Yield and yield contributing characters

3.6.4.15. Cob length (cm)

Cob length was measured by a meter scale in centimeter (cm) at last harvesting time stage.

3.6.4.16. Individual fruit weight (g)

Individual fruit weight was measured by a digital balance meter in gram (g). A mean weight was taken of collected fruits from each plant as per variety and replication.

Figure 9. Fruit (cob) of maize

3.7. Evaluation of Plant Growth and Yield

Plant height was recorded weekly on 10 randomly selected and tagged plants per pot commencing on the 21st day post-emergence until 50% tasseling. .At physiological maturity, cobs from the ten tagged plants per plot in the field and three plants in the polythene bag were harvested separately for measurement of cob length. The cobs were shelled and dried to 15 % moisture content for determination of grain weight.

3.8. Evaluation of Seedling, Cob Length and Grain Weight

Seedling and grain both were weighed in fresh and dry condition. Weight of fresh seedlings were recorded according to each variety and replication. Then they were kept in paper bags separately according to variety and replication. Drying of seedlings were done at the laboratory of Department of Soil Science, Sher-e-Bangla Agricultural University. The seedlings were dried in air oven at 105°C for 48 hours. Right after 48 hours, seedlings were taken for weighing again to record dry weight. After harvesting, cob length were recorded against each variety and replication by using centimeter scale. The weight of fresh grains were recorded and kept for sun drying for two days.

Then the weight of dry grains were recorded against each variety and replication.

3.9. Statistical analysis of data

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

CHAPTER IV

RESULTS AND DISCUSSION

Results

Results were compiled based on morphological parameters, disease related parameters, physiological parameters and yield contributing parameters. Molecular detection was also done for MCMV and SCMV. The analyses of variance (ANOVA) of the data on different parameters were done (Appendix VI). The results have been presented and discussed in this chapter under the following headings:

4.1. Percentage of seed germination

Percent seed germination of selected maize varieties and lines were found to be differed in the table no 7 and figure 10. The highest germination percentage was observed in variety Tain-927 (91.63%) followed by Konok-51 (91%), Rocket-55 (83.3%), Jonaki-707 (74.97%) and Khoi bhutta (74.96%). The lowest germination was recorded in Line A.S. (41.65%) preceded by Line A (49.98%), Line 984 (58.31%), variety Kohinoor (66.64%) and Sweet corn (66.64%).

A **B**

Figure 10. Seed germination with 2 mm radicle at 4 DAS (A) and at 7 DAS (B)

Table 7. Percentage of germination of selected maize varieties and lines against Maize Lethal Necrosis Disease (MLND)

Variety/Line	Percentage of germination
V_1 (Tain 927)	91.63
V_2(Jonaki 707)	74.97
V_3(Rocket 55)	83.33
V_4 (Konok 51)	91
V_5 (Kohinoor)	66.64
V_6 (Line A.S)	41.65
V_7 (Line A)	49.98
V_8 (Line 984)	58.31
V_9(Khoi Bhutta)	74.97
V_{10} (Sweet Corn)	66.64

4.2. Percentage of seedling emergence

Data on seedling emergence was recorded on counting the number of seedlings emerged in each treatment (variety/lines) every three consecutive days commencing 7th day after sowing (DAS), until no additional seedlings emerged. From the results, it was observed that seedling emergence significantly varied at 7DAS in varieties and lines. At 7 DAS, the highest seedling emergence was recorded in variety, Kohinoor (70.60%) followed by in variety Tain-927 (59.10%),Rocket-55(57%), Line A (47.40%), Line 984 (47.50%) and variety Sweet corn (48.40%).The lowest seedling emergence was recorded in variety Jonaki-707 (29.70%) preceded by Konok-51 (37.80%), Khoi Bhutta (39.60%) and Line A.S (40.80%).

Table 8. Seedling emergence in selected Maize varieties at 7 DAS against Maize Lethal Necrosis Disease (MLND)

Variety/Line	Seedling Emergence (%) at 7DAS
V_1 (Tain 927)	59.10 b
V_2(Jonaki 707)	29.70 e
V_3(Rocket 55)	57.00 b
V_4 (Konok 51)	37.80 d
V_5 (Kohinoor)	70.60 a
V_6 (Line A.S)	40.80 d
V_7 (Line A)	47.40 c
V_8 (Line 984)	47.50 c
V_9(Khoi Bhutta)	39.60 d
V_{10} (Sweet Corn)	48.40 c
CV (%)	9.63

4.3. Seedling weight (g)

Seedlings were weighed at 15 DAS in fresh condition. Then the seedlings were dried for two days as described in methodology section.

At 15 DAS, in fresh condition, the highest seedling emergence was recorded in Line A (4.82 g) and variety Sweet corn (4.61 g) followed by in Line A.S (4.36 g).The lowest seedling weight in fresh condition was recorded in variety Tain-927 (1.97 g) preceded by Jonaki-707 (3.02 g).Seedling emergence recorded in Line 984 (3.75 g), variety Konok-51 (3.80 g) and Khoi bhutta (3.93 g) were statistically identical. Similarly, seedling emergence recorded

in variety Rocket-55 (3.47 g) was statistically identical with Kohinoor (3.32 g).

Data recorded on dry seedlings showed the highest seedling emergence in variety Khoi bhutta (0.91 g) and Rocket-55 (0.90 g). The lowest data was recorded in variety Tain-927 (0.30 g) preceded by statistically similar data of variety Kohinoor (0.66 g) and Line 984 (0.61 g). The seedling emergence of variety Jonaki-707 (0.85 g) and Line A.S (0.82 g) were statistically identical with variety Khoi bhutta (0.91 g) and Rocket-55 (0.90 g). Variety Konok-51 (0.73 g) and Sweet corn (0.73 g) were statistically identical in terms of seedling emergence.

Table 9. Seedling weight of selected Maize varieties at 15 DAS against Maize Lethal Necrosis Disease (MLND)

Variety/Line	**Seedling fresh weight (g)**	**Seedling dry weight (g)**
V_1 (Tain 927)	1.97 e	0.30 e
V_2(Jonaki 707)	3.02 d	0.85 ab
V_3(Rocket 55)	3.47 cd	0.90 a
V_4 (Konok 51)	3.80 bc	0.73 bc
V_5 (Kohinoor)	3.32 cd	0.66 cd
V_6 (A.S)	4.36 b	0.82 ab
V_7 (A)	4.82 a	0.56 d
V_8 (984)	3.75 bc	0.61 cd
V_9(Khoi Bhutta)	3.93 bc	0.91 a
V_{10} (Sweet Corn)	4.61 a	0.73 bc
CV (%)	19.11	21.31

4.4. Symptomology

- In the early stage, long yellow stripes on leaves were observed.
- Leaves of infected plants became yellow from the tip and margins to the center.
- Ears and leaves dried up.
- MLND also caused dwarfing and premature aging of the plants.
- The whole plant died eventually and maximum maize cobs remained without kernels.

Figure 11. Typical Symptoms of MLND

4.5. Disease incidence (%) of MLND in selected maize varieties

Data on disease incidence ws recorded at 30, 45 and 70 days after sowing (DAS). From the results, it was observed that disease incidence significantly varied at different days after sowing (table no 10).

At 30 DAS, the highest disease incidence was recorded in variety, Khoi Bhutta (65.00%) and Line 984 (57.00%) followed by variety Jonaki-707 (47.67%), Kohinoor (48.00%) and Line A.S (47.67%).The lowest disease incidence was recorded in variety Tain-927 (32.33%) and Rocket-55 (29.33%).Disease incidence (45.00%) recorded in the variety Sweet corn was statistically identical with Jonaki-707, Kohinoor and Line A.S. In Line A, disease incidence (37.66%) was statistically identical with variety Rocket-55 and Tain-927.

At 45 DAS, the highest disease incidence was recorded in variety, Khoi Bhutta (70.00%) followed by in variety Jonaki-707(46.33 %) and Kohinoor (52.67%).The lowest disease incidence was recorded in Tain-927 (37.00%) and Rocket-55 (35.00%).Disease incidence recorded in Line 984 (62.00%) was statistically identical with variety Khoi bhutta, in Line A (47.00%) and Line A (47.00%) were statistically identical with variety Tain-927 and Rocket-55. Disease incidence recorded in variety Konok-51 (61.00%) and Sweet corn (53.33%) were statistically similar with Khoi Bhutta, Jonaki-707, Kohinoor, Line 984 and Line A.S.

At 70 DAS, the highest disease incidence was recorded in variety, Khoi Bhutta (75.00%), Konok-51(67.66%) and Line 984 (67.67%) followed by in variety Jonaki-707 (57.33%), Kohinoor (58.67%) and Sweet corn (58.33%).The lowest disease incidence was recorded in Tain-927 (38.00%) and Rocket-55

(38.00%) preceded by Line A (47.67%).Disease incidence recorded in the Line A.S. (51.00%) was statistically identical with Line A, variety Jonaki-707, Kohinoor and Sweet corn.

Table 10. Disease incidence of different Maize varieties at 30 DAS, 45 DAS and 70 DAS against Maize Lethal Necrosis Disease

Variety/Line	Disease Incidence (%)		
	30 DAS	**45 DAS**	**70 DAS**
V_1 (Tain 927)	32.33 d	37.00 f	38.00 d
V_2(Jonaki 707)	47.67 b	46.33 d	57.33 b
V_3(Rocket 55)	29.33 d	35.00 f	38.00 d
V_4 (Konok 51)	59.66 a	61.00 abc	67.66 a
V_5 (Kohinoor)	48.00 b	52.67 cd	58.67 b
V_6 (Line A.S)	47.67 b	50.00 de	51.00 bc
V_7 (Line A)	37.66 cd	41.00 ef	47.67 c
V_8 (Line 984)	57.00 a	62.00 ab	67.67 a
V_9(Khoi Bhutta)	65.00 a	70.00 a	75.00 a
V_{10} (Sweet Corn)	45.00 bc	53.33 bcd	58.33 b
CV (%)	6.39	6.34	5.09

The effect of different varieties on disease incidence (%) of Maize Lethal Necrosis Disease (MLND) was observed based on disease rating scale of MLND as present in methodology section. Among the varieties/lines, variety Tain-92, Rocket-55 and Line A were tolerant, Khoi bhutta was highly susceptible, variety Jonaki-707, Kohinoor, Sweet corn and Line A.S. were moderately susceptible, variety Konok-51 and Line 984 were susceptible. According to disease incidence rating scale followed in this study (table-11).

Table 11. Disease rating scale to determine disease incidence of MLND

Variety/Line	Disease Incidence (%) at 70 DAS	Level of Resistance/Susceptibility
V_1 (Tain 927)	38.00	Tolerant
V_2(Jonaki 707)	57.33	Moderately Susceptible
V_3(Rocket 55)	38.00	Tolerant
V_4 (Konok 51)	67.66	Susceptible
V_5 (Kohinoor)	58.67	Moderately Susceptible
V_6 (Line A.S)	51.00	Moderately susceptible
V_7 (Line A)	47.67	Tolerant
V_8 (Line 984)	67.67	Susceptible
V_9(Khoi Bhutta)	75.00	Highly Susceptible
V_{10} (Sweet Corn)	58.33	Moderately Susceptible

4.6. Percent Disease Index (PDI)

Data on percent disease index (PDI) was recorded at 30, 45 and 70 days after sowing (DAS). From the results, it was observed that PDI significantly varied at different days after sowing (table-12).

At 30 DAS, the highest PDI was recorded in variety Sweet corn (67.67%) and the lowest PDI was recorded in variety Tain-927 (37.00%). Percent Disease Index (PDI) of variety Khoi bhutta (60.00%), Rocket-55(47.67%) and Line 984 (53.00%) were statistically identical with variety Sweet corn. Variety Konok-51 (46.00%), Kohinoor (39.33%), Jonaki-707 (39.67%), Line A(37.67%) and Line A.S (43.00%) showed statistically identical of Percent Disease Index (PDI).

At 45 DAS, the highest PDI was recorded in variety Sweet corn (71.00%) followed by Khoi Bhutta (65.00%) which was statistically identical with Sweet corn. The lowest PDI was recorded in variety Tain-927 (40.0%), Jonaki-707(42.33%), Kohinoor (41.00%) and Line A (43.00%). PDI of Line 984 (57.67%), variety Konok-51 (54.33%), Rocket-55 (50.00%) were statistically identical with variety Tain-927, Jonaki-707, Kohinoor, and Line A.

At 70 DAS, the highest PDI was recorded in variety Khoi bhutta (77.66%) and Sweet corn (70.00%) followed by Line 984 (61.00%).The lowest PDI was recorded in variety Jonaki-707 (46.33%). Percent Disease Index (PDI) of variety Konok-51 (63.00%) and Line A.S(56.33%) were statistically identical with Line 984. Moreover, PDI of Line A (47.67%), variety Tain-927 (48.67%), Kohinoor (47.67%) and Rocket-51 (54.67%) were statistically identical with variety Jonaki-707.

Table 12. Percent Disease Index (PDI) of different Maize varieties at 30 DAS, 45 DAS and 70 DAS against Maize Lethal Necrosis Disease

Variety/ Line	Percent Disease Index (%)		
	30 DAS	**45 DAS**	**70 DAS**
V_1 (Tain 927)	37.00 f	40.00 e	48.67 de
V_2(Jonaki 707)	39.67 def	42.33 e	46.33 e
V_3(Rocket 55)	47.67 cd	50.00 cde	54.67 cde
V_4 (Konok 51)	46.00 cde	54.33 cd	63.00 bc
V_5 (Kohinoor)	39.33 def	41.00 e	47.67 de
V_6 (Line A.S)	43.00 def	46.33 de	56.33 cd
V_7 (Line A)	37.67 ef	43.00 e	47.67 de
V_8 (Line 984)	53.00 bc	57.67 bc	61.00 c
V_9(Khoi Bhutta)	60.00 ab	65.00 ab	77.66 a
V_{10} (Sweet Corn)	67.67 a	71.00 a	70.00 a
CV (%)	6.46	6.90	5.39

4.7. Number of leaves at different growth stages

Due to Maize Lethal Nacrosis Disease (MLND), number of leaves were reduced at different growth stages of maize plants. At 56 days, the growth of leaves almost stopped and showed different result at selected maize varieties/lines. At 56 days, the highest number of leaves was observed in

variety Tain-927 (16 leaves) and lowest was in variety Khoi Bhutta (8 leaves) (table-13).

Table 13. Number of leaves of selected maize varieties/lines at different growth stages

Varieties/ Lines	**Days and number of leaves**					
V_1 (Tain 927)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	8	12	16
V_2(Jonaki 707)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	7	10	12
V_3(Rocket 55)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	7	11	12
V_4 (Konok 51)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	3	6	10	12
V_5 (Kohinoor)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	6	8	10
V_6 (Line A.S)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	7	12	14
V_7 (Line A)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	8	12	14
V_8 (Line 984)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	8	10	12
V_9(Khoi Bhutta)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	6	8	8
V_{10} (Sweet Corn)	**Days**	**7**	**14**	**28**	**42**	**56**
	Number of leaves	2	4	8	10	12

4.8. Plant Height (cm)

Plant height was recorded weekly commencing 21st day post-emergence until 50% tasseling. Data was recorded at 24 DAS, 31 DAS, 38 DAS, 45 DAS and 52 DAS (table-14).

At 24 DAS, the highest plant height recorded in variety Tain-927 (13.00 cm), Rocket-55 (12.22 cm), Kohinoor (11.70 cm) and Line A (13.11) followed by variety Jonaki-707 (9.26 cm), Line A (9.39cm) and Line 984 (8.81cm). The lowest plant height was recorded in variety Konok-51 (6.73cm).The plant height recorded in variety Khoi bhutta (6.73cm) and Sweet corn (7.93cm) were statistically identical with variety Jonaki-707 (9.26 cm), Line A (9.39cm), Line 984 (8.81cm) and Konok-51 (6.73cm).

At 31 DAS, the highest plant height was found in variety Tain-927 (20.22cm) and Rocket-55 (18.96cm). The lowest height was recorded in variety Konok-51 (9.66cm). Data collected on height of variety Kohinoor (7.25cm), Jonaki-707 (15.11cm) and Line A.S (15.30cm) were statistically identical with Tain-927 (20.22cm) and Rocket-55 (18.96cm). The plant height recorded in variety Sweet corn (12.89 cm), Khoi bhutta (11.18cm), Line A (13.40cm) and Line 984 (14.14cm) were statistically identical with variety Konok-51 (9.66cm).

At 38 DAS, the highest plant height was measured in variety Tain-927 (35.00cm), Rocket-55 (30.03cm) and Kohinoor (29.42cm). The lowest height was found in variety Jonaki-707 (22.08cm), Konok-51 (20.32cm), Khoi bhutta (22.11cm), Sweet corn (23.00cm), Lina A.S (17.55cm), Line A (20.11cm) and Line 984 (21.55cm).

At 45 DAS, the highest plant height was recorded in variety Tain-927 (54.00cm) followed by variety Rocket-55 (43.48cm), Kohinoor (42.81cm) and Line 984 (41.55cm). The lowest height was found in Line A.S (28.15cm) preceded by variety Konok-51 (33.44cm). The data recorded in variety Khoi bhutta (36.63cm) and Sweet corn (36.81cm) were statistically identical with Rocket-55 (43.48cm), Kohinoor (42.81cm), Line 984 (41.55cm) and Konok-51 (33.44cm).The plant height of variety Jonaki-707 (32.51cm) and Line A (31.92cm) were statistically identical with Line A.S (28.15cm) and variety Konok-51 (33.44cm).

At 52 DAS, the highest plant height was recorded in variety Tain-927 (87.29cm) followed by variety Jonaki-707 (65.66cm), Rocket-55(66.15cm), Kohinoor (65.81cm) and Line 984 (65.41cm). The lowest data of plant height was recorded in Line A.S (44.00cm) preceded by variety Konok-51 (52.44cm), Khoi bhutta (55.19cm), Sweet corn (52.40cm) and Line A (52.22cm).

Table 14. Plant height (cm) of different Maize varieties at 24 DAS, 31 DAS, 38 DAS, 45 DAS and 52 DAS against Maize Lethal Necrosis Disease (MLND)

Variety/ Line	Plant height (cm)				
	24 DAS	**31 DAS**	**38 DAS**	**45 DAS**	**52 DAS**
V_1 (Tain 927)	13.00 a	20.22 a	35.00 a	54.00 a	87.29 a
V_2(Jonaki 707)	9.26 b	15.11 bc	22.08 b	32.51 cd	65.66 b
V_3(Rocket 55)	12.22 a	18.96 a	30.03 a	43.48 b	66.15 b
V_4 (Konok 51)	6.73 c	9.66 e	20.32 b	33.44 c	52.44 c
V_5 (Kohinoor)	11.70 a	17.25 ab	29.42 a	42.81 b	65.81 b
V_6 (Line A.S)	13.11 a	15.30 bc	17.55 b	28.15 d	44.00 d
V_7 (Line A)	9.39 b	13.40 cd	20.11 b	31.92 cd	52.22 c
V_8(Line 984)	8.81 b	14.14 cd	21.55 b	41.55 b	65.41 b
V_9(Khoi Bhutta)	8.18 bc	11.18 de	22.11 b	36.63 bc	55.19 c
V_{10} (Sweet Corn)	7.93 bc	12.89 cd	23.00 b	36.81 bc	52.40 c
CV (%)	7.15	7.17	8.43	7.05	2.66

4.9. Chlorophyll content (µg/g)

From table-15, it was revealed that at 52 DAS, chlorophyll content of the plant was showed significant variance among the tested maize varieties. In healthy leaves, the maximum chlorophyll content was obtained in the variety Tain-927 (5.47 µg/g) followed by Konok-51 (4.7 µg/g), Sweet corn (4.51 µg/g) and Line A (4.10 µg/g). The lowest chlorophyll content obtained in variety Rocket-55 (2.45 µg/g) preceded by Jonaki-707 (2.93 µg/g), Khoi bhutta (2.81 µg/g), Line A.S (2.81 µg/g), Kohinoor (3.51 µg/g) and Line 984 (3.76 µg/g).

In diseased leaves, the maximum chlorophyll content was obtained in the variety Konok-51 (3.54 µg/g) followed by variety Tain-927 (3.51 µg/g) and Line A (2.64 µg/g). The minimum chlorophyll content was obtained in variety Jonaki-707 (1.54 µg/g) and Kohinoor (1.55 µg/g) preceded by variety Rocket-55 (2.16 µg/g), Line A.S (2.21 µg/g), Line 984 (2.30 µg/g),variety Sweet corn (2.35 µg/g) and Khoi bhutta (1.85 µg/g).

Table 15. Leaf chlorophyll content in selected maize varieties against Maize Lethal Necrosis Disease (MLND)

Variety/ Line	Chlorophyll content (µg/g)	
	Chlorophyll content in healthy leaf (µg/g)	Chlorophyll content in diseased leaf (µg/g)
V_1 (Tain 927)	5.47 a	3.51 b
V_2(Jonaki 707)	2.93 e	1.54 f
V_3(Rocket 55)	2.45 f	2.16 d
V_4 (Konok 51)	4.7 b	3.84 a
V_5 (Kohinoor)	3.51 d	1.55 f
V_6 (Line A.S)	2.81 e	2.21 d
V_7 (Line A)	4.10 c	2.64 c
V_8(Line 984)	3.76 d	2.30 d
V_9(Khoi Bhutta)	2.81 e	1.85 e
V_{10} (Sweet Corn)	4.51 b	2.35 d
CV (%)	2.46	4.12

4.10. RT-PCR test for *MCMV* and *SCMV* detection

RT-PCR is more reliable and robust technique for the detection of plant viruses. In this study, *MCMV* and *SCMV* gene specific primers were used to detect the viruses of Maize Lethal Necrosis Disease. From the RT-PCR result, *MCMV* was found to be positive whereas *SCMV* was not detected through the RT-PCR. From the RT-PCR result of *MCMV*, it was proved that only one type of virus was dominant to cause the MLND here in Bangladesh. RT-PCR result is presented in table 16.

Table 16. RT-PCR test result for virus detection

Variety/line	RT-PCR Result	
	MCMV	SCMV
V_1 (Tain 927)	Detected	Not Detected
V_2(Jonaki 707)	Detected	Not Detected
V_3(Rocket 55)	Detected	Not Detected
V_4 (Konok 51)	Detected	Not Detected
V_5 (Kohinoor)	Detected	Not Detected
V_6 (Line A.S)	Detected	Not Detected
V_7 (Line A)	Detected	Not Detected
V_8(Line 984)	Detected	Not Detected
V_9(Khoi Bhutta)	Detected	Not Detected
V_{10} (Sweet Corn)	Detected	Not Detected

4.11. Individual fruit weight (g)

Individual fruit (cob) was weighed in fresh and dry condition. From table-17, it was observed that data recorded in fresh condition was recorded highest in variety Tain-927 (143.10 mg) followed by) Line A.S (98.80 mg), Sweet corn (96.00 mg) and Rocket-55 (95.80 mg). The lowest weight was recorded in variety Khoi Bhutta (46.30 mg) and Jonaki-707 (50.10%) preceded by Konok-51 (73.50 mg). Weight of individual cob in fresh condition of Line A (78.20 mg) and Line 984 (77.30 mg) were statistically identical with variety Konok-51, Sweet corn, Rocket-55 and Line A.S. Similarly, weight of individual fruit of variety Kohinoor (66.10 mg) was statistically identical with Konok-51, Khoi bhutta and Jonaki-707.

After sun drying if two consecutive days, data recorded of individual fruit weight was highest in variety Tain-927 (132.30 mg) followed by Line A.S (90.20 mg). The lowest fruit weight was recorded in variety Khoi bhutta (39.30 mg) and Jonaki-707 (41.60) preceded by Konok-51 (64.70 mg). Individual fruit weight of variety Rocket-55 (87.00 mg) and Sweet corn (86.00 mg) were statistically identical with Line A.S. Again, dry weight of fruit recorded in variety Kohinoor (56.40 mg), Line A (68.00 mg) and Line 984 (70.50 mg) were statistically identical with Konok-51, Jonaki-707 and Khoi Bhutta.

Table 17. Individual fruit weight of selected Maize varieties against Maize Lethal Necrosis Disease (MLND)

Variety/Line	Grain fresh weight (g)	Grain dry weight (g)
V_1 (Tain 927)	143.10 a	132.30 a
V_2(Jonaki 707)	50.10 d	41.60 e
V_3(Rocket 55)	95.80 b	87.00 bc
V_4 (Konok 51)	73.50 c	64.70 d
V_5 (Kohinoor)	66.10 cd	56.40 de
V_6 (Line A.S)	98.80 b	90.20 b
V_7 (Line A)	78.20 bc	68.00 cd
V_8 (Line 984)	77.30 bc	70.50 bcd
V_9(Khoi Bhutta)	46.30 d	39.30 e
V_{10} (Sweet Corn)	96.00 b	86.00 bc
CV (%)	31.54	33.91

4.12. Cob Length (cm)

The length of cob of selected maize varieties and lines was recorded highest in variety Tain-927 (16.70 cm) followed by Line A.S (13.10 cm). The lowest length of cob was recorded in variety Khoi bhutta (9.20 cm), Jonaki-707 (7.83 cm) and Konok-51 (7.50 cm). Con length recorded in variety Rocket-55 (15.90 cm), Kohinoor (15.90 cm), Line A (15.80 cm), Line 984 (15.00 cm)

and variety Sweet corn (13.80 cm) were statistically identical with variety Tain-927 and Line A.S (table-18).

Table 18. Cob Length (cm) of selected Maize varieties against Maize Lethal Necrosis Disease (MLND)

Variety/Line	Cob Length (cm)
V_1 (Tain 927)	16.70 a
V_2(Jonaki 707)	7.83 d
V_3(Rocket 55)	15.90 ab
V_4 (Konok 51)	7.50 d
V_5 (Kohinoor)	15.90 ab
V_6 (Line A.S)	13.10 c
V_7 (Line A)	15.80 ab
V_8 (Line 984)	15.00 abc
V_9(Khoi Bhutta)	9.20 d
V_{10} (Sweet Corn)	13.80 bc
CV (%)	22.10

4.13. Relation between Disease incidence (%) and Percent Disease Index (PDI) with plant height (cm)

From the relationship study, it was revealed that plant height became reduced with the increase of disease incidence (at 70 DAS). It means that viral disease effect on plant height directly. From the figure 13, it was depicted that disease incidence and plant height were negatively related. In figure 14, it was observed that disease severity was also negatively related with plant height.

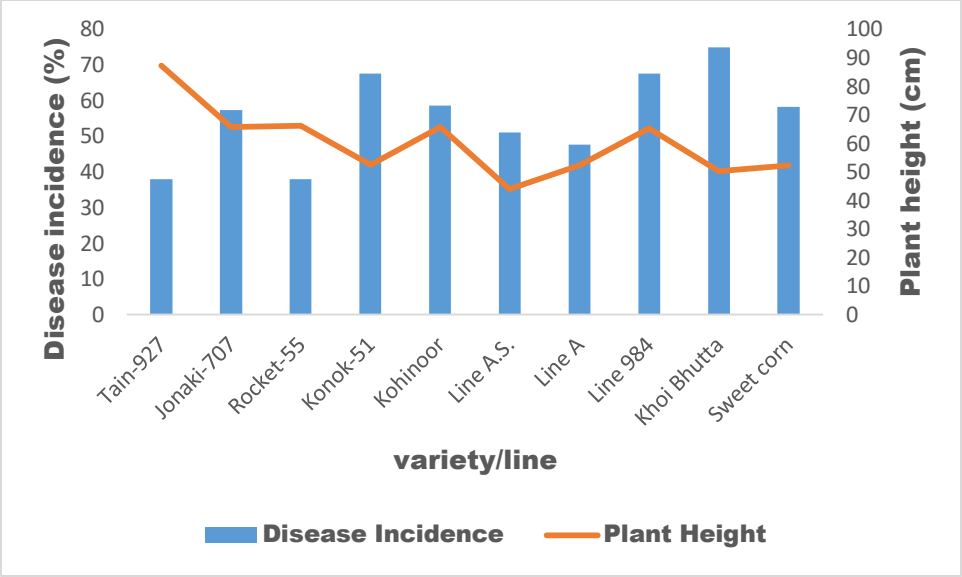


Figure 12. Relation between disease incidence at 70 DAS and plant height (cm) at 52 DAS

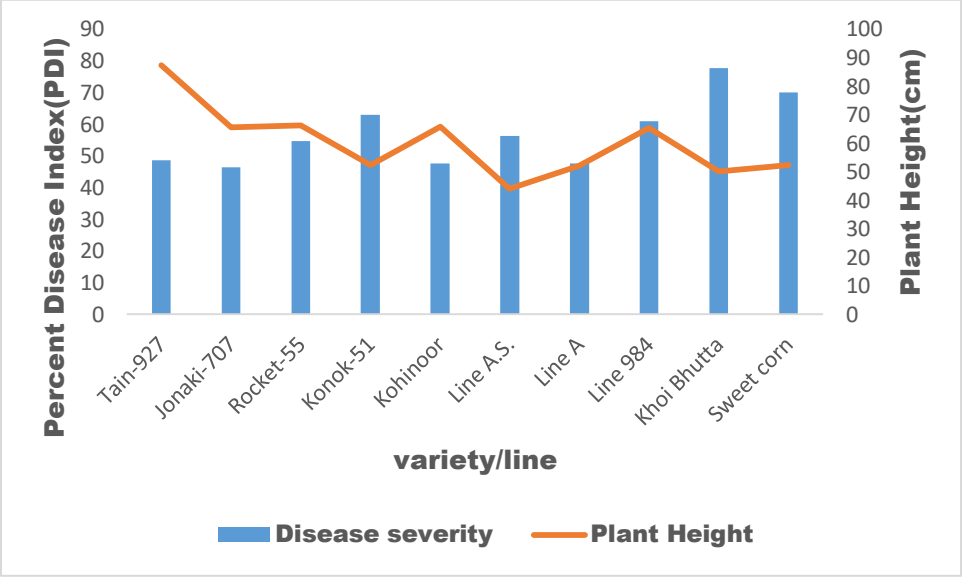


Figure 13. Relation between percent disease index (PDI) at 70 DAS and plant height(cm) at 52 DAS

4.14. Relation between Disease incidence (%)and Percent Disease Index (PDI) with chlorophyll content against varieties/lines

From the relationship study, it was revealed that chlorophyll content (µg/g) was reduced with the increase of disease incidence as well as percent disease index both. In figure 14, it was depicted that disease incidence and chlorophyll content were negatively related. In figure 15, it was showed that percent disease index was negatively related with chlorophyll content.

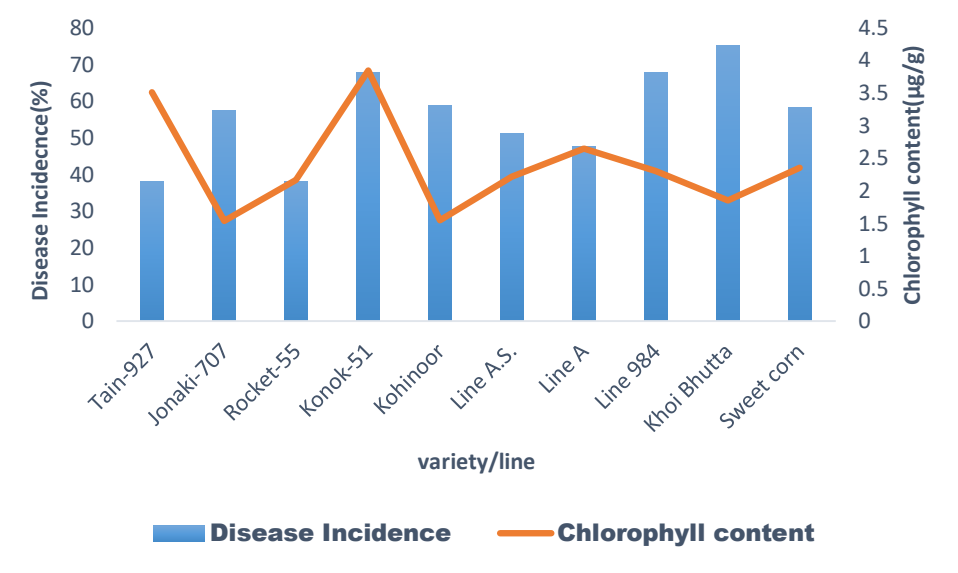


Figure 14. Relation between disease incidence (%) at 70 DAS and chlorophyll content at diseased leaf

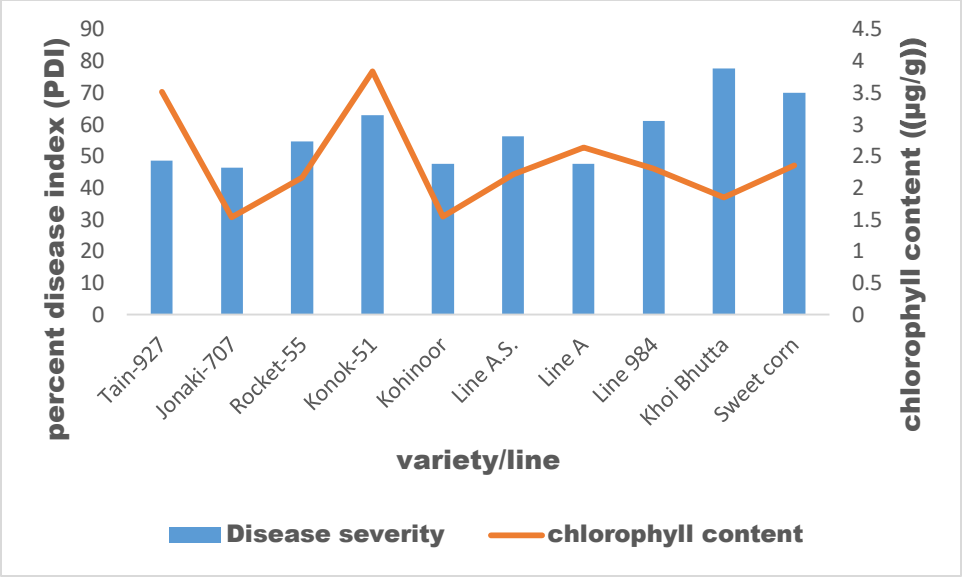


Figure 15. Relation between percent disease index (PDI) at 70 DAS and chlorophyll content at diseased leaf

DISCUSSION

Cereal grains provide humankind with more nourishment than any of the food class in the whole world and meet nearly half of the total caloric requirement. Only wheat, rice and maize are providing as major cereal food sources accounting for 94% of all cereal consumption (FAO, 2017). Maize is introduced in the cropping pattern of Bangladesh. More than 90% of maize is used as poultry feed and the remaining in fish sector and as human food products. The country has a great potentiality to improve and expand the maize production. But there lies some constraints which hinders the production at a great extent. Farmers cultivating maize are not completely aware of the benefits of maize cultivation. They are not interested to invest for maize cultivation as they do not have proper information on maize farming and marketing techniques (Faruk, 2008).

There are no sufficient literatures available regarding the study on seed quality in Bangladesh. A study in Nepal, it was observed that due to lack of quality seeds maize production was hampered 54% (Sanjiv *et.al*. 2018). Moreover, lack of proper interactions between farmers and extension service officers, lack of advanced technologies and trainings are also problems in maize production. Due to incidence of diseases and infestation of pests cause a great damage to maize production. There are many biotic and abiotic factors that hinders production of maize throughout the world include Bangladesh. Among the abiotic stresses salinity, water logging, metal toxicity, nutrient deficiency are major and among the biotic stresses, diseases like ear rot, northern leaf blight, stem rot, mosaic disease, rough dwarf disease, maize lethal necrosis disease are the causal agents (Fangpin, 2014). In Bangladesh, every year a large amount of hybrid maize seeds are imported from other

country to meet the national demand (Bangladesh Seed Market - Growth, Trends, COVID-19 Impact, and Forecasts, 2021 - 2026). Due to lack of proper inspection, new virus like *Maize Chlorotic Mottle Virus* causing Maize Lethal Necrosis Disease (MLND) are introduced in Bangladesh.

In the present study, maize viruses that can be transmitted through the seed were studied. For conducting the study, in total seven hybrid varieties and three lines were tested in pot experiment. Among the selected maize varieties/lines, the highest disease incidence (75% at 70 DAS) of MLND was found in variety Khoi Bhutta and according to disease rating scale used in this study, it showed highly susceptible. Among the tested varieties, the lowest disease incidence (38%) was recorded in Tain-927 and Rocket-55 and according to disease rating scale used in this study, it showed tolerant to MLND at 70 DAS. All selected maize varieties and lines were infected and appear the remarkable symptoms of MLND. According to disease incidence rating scale used in this study, moderate to higher disease incidence (51.00%-58.67%) was recorded in variety Jonaki-707, Kohinoor, Sweet corn and Line A.S. According to disease incidence rating scale these varieties are moderately susceptible against MLND. Variety Konok-51 and Line 984 was showed disease incidence (67.66%) and susceptible against MLND. The results found in the current study agreement with recent published study on the disease incidence in maize varieties (Girma *et.al*., 2018).

Among the tested maize varieties/lines, at 70 DAS, the highest PDI was recorded in variety Khoi bhutta (77.66%) lowest PDI was recorded in variety Jonaki-707 (46.33%). The results of disease incidence and severity of the present study match with the previous study that was conducted by (Girma *et.al*., 2018).High severity of MLN disease infection reduced pollen

production and the few produced pollen was of low viability. Such result was commenced with published paper of Mekureyaw, 2017.

Transmission of virus through seed is a major concern. In this study, the seeds collected from local market showed Maize Lethal Necrosis Disease (MLND) which is caused due to combined infection of *Maize Chlorotic Mottle Virus* and *Sugarcane Mosaic Virus.* But only Maize chlorotic mottle virus (MCMV) was detected in seedlings. Infection of seed with seed borne pathogens, including viruses contributes to low seed quality with an overall effect on yield. Our findings are supported with the two recent published research articles (Redinbaugh and Stewart 2018; Asiimwe *et al*., 2019). Besides, according to Mahuku *et al.* (2015), *MCMV* was detected in seeds harvested from *MCMV*-infected maize plants and purchased from the market at 46% and 72%, respectively.

From the RT-PCR results to detect the viruses of MLND, it was found that all selected hybrid maize varieties/lines were infected by *Maize Chlorotic Mottle Virus (MCMV)* and gave positive result in RT-PCR test whereas no amplification was found in case of Sugarcane *Mosaic Virus (SCMV)* via RT-PCR test. Results from the present study are supported with the previous study, where it was reported that seed harvested from MLN-infected maize inbred lines were infected with *MCMV*, although *Sugarcane Mosaic Virus (SCMV*) was not detected (Gatunzi, 2018).

The infected maize plant shows different morphological responses against different morphological features. Due to viral infection, the chlorophyll content was reduced. From our study, it was observed that in diseased leaves, the maximum chlorophyll content was obtained in the variety Konok-51 (3.54 µg/g) and the minimum chlorophyll content was obtained in variety Kohinoor (1.55 µg/g) Presence of viruses in the host cell results in reduction of

ribosomal proteins, proteins related to stress responses and alteration of redox homeostasis (Xia et al., 2018; Choudhury et al., 2019; Dang et al., 2020). Existence of the virus genome in the cell is also responsible for the reduction, clustering and change in structure of the chloroplast (Xhao et al., 2016; Bhattacharyya and Chakraborty 2018), accompanied with changes in the content and ratio of chlorophyll A and B. Disruption and inhibition of chloroplast development and function has been suggested to cause typical photosynthesis-related symptoms, such as chlorosis and mosaic (Sholeh et al., 2019; Terefe and Gudero 2019).

Maize plants infected at early stages develop yellow stripe from the tip to the margin. Such symptoms were found in recent published papers Teresa *et.al*., (2020); Awata *et. al* (2019) .

The yield of individual variety depends on the number of leaves, plant growth and fruits per plant. From our study, it was observed that plant height was maximum reduced in variety Khoi bhutta and the lowest reduction was observed in variety Tain-927. Because infection of plants with viruses reduces the stomata density in the leaf area and in turn minimizes the uptake of carbon dioxide for photosynthesis (Choudhury et al., 2019). At 56 days, before tasseling, the maximum number of leaves in diseased plants was observed in variety Tain-927 and the minimum number of leaves was observed in Khoi bhutta. The highest yield was found in variety Tain-927 and lowest yield was observed in variety Khoi bhutta. Our study agreed with the findings of published paper of Mengesha et al., (2019).

CHAPTER V

SUMMARY AND CONCLUSION

Summary and Conclusion

The present study was carried out at central research farm of Sher-e-Bangla Agricultural University, Dhaka as well as in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology during 2020-2021, to ascertain the incidence and severity of Maize Lethal Necrosis Disease (MLND) and its molecular detection through RT-PCR. In total ten maize varieties/lines were tested in this study against MLND. The pot experiment was carried out in Completely Randomized Design with ten replications. All the tested varieties/lines were remaining in natural conditions and transmission of MLND through seed was observed in terms of disease incidence, severity and other yield contributing parameters.

The experiment was aimed to assess the varietal performance of tested maize varieties/lines against Maize Lethal Necrosis Disease (MLND) and identify the causal virus on the basis of biological properties. Genomic RNA was extracted from leaf samples of the tested varieties to detect the MLND through modern molecular technique RT-PCR.

From the study it was observed that among the ten selected varieties/lines, variety Tain-927, Rocket-55 and Line A showed lower disease incidence and severity up to 70 DAS. Variety Khoi bhutta showed higher disease incidence and severity among other varieties/lines. Among remaining varieties; variety Jonaki-707, Kohinoor, Sweet corn, Konok-51, Line 984 and Line A.S showed moderately susceptible to susceptible disease incidence and severity at 70 DAS.

Among the selected ten maize varieties/lines, chlorophyll content was also observed where the highest chlorophyll content was found in variety Tain-927 and the lowest one was found in variety Khoi bhutta.

The morphological features which are identical to yield and yield contributing characters was also studied. The number of leaves per plant was counted at 56 DAS before tasseling and the highest number was observed in the variety Tain-927.The highest plant height at 52 DAS was found in variety Tain-927 and the lowest plant height was in Line A.S. The maximum length of cob was obtained from variety TAin-927 and the minimum number in Khoi bhutta. The highest yield per plant was obtained from Tain-927 and the lowest yield in Khoi bhutta.

From molecular study through RT-PCR test, it was revealed that results obtained on the basis of symptomology found almost similar to RT-PCR analyses where *MCMV* was detected. Among the tested varieties, all varieties/Lines gave the positive results in PCR test against *MCMV* but show negative result against *SCMV*.

From the above findings on different parameters studied, it can be concluded that Maize Lethal Necrosis Disease (MLND) transmission occurred through seeds. Among the selected varieties/lines, variety Tain-927 showed lower disease incidence and severity whereas Khoi bhutta showed lower results. *Maize Chlorotic Mottle Virus (MCMV)* and a potyvirus, *Sugarcane Mosaic Virus* are responsible for Maize Lethal Necrosis Disease (MLND). From the symptoms and RT-PCR test, we can conclude that *MCMV* was detected.

From the findings of this study, it was revealed that out of ten tested varieties/lines, varieties namely Tain-927, Rocket-55 and Line- A showed better performance in terms of morpho-physiological features as well as yield and yield contributing characters as compared to other tested varieties/lines against Maize Lethal Necrosis Disease (MLND).

CHAPTER VI
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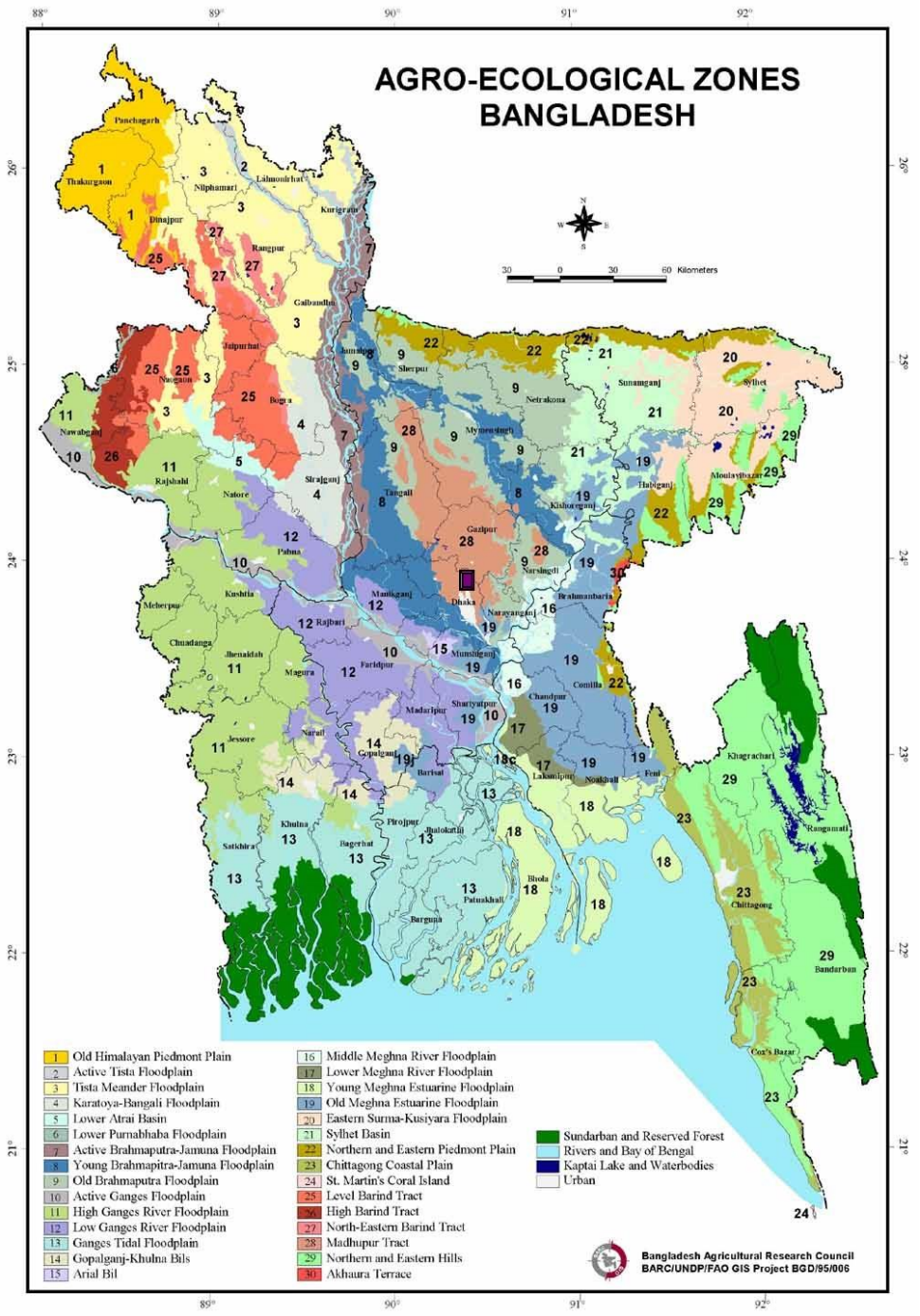
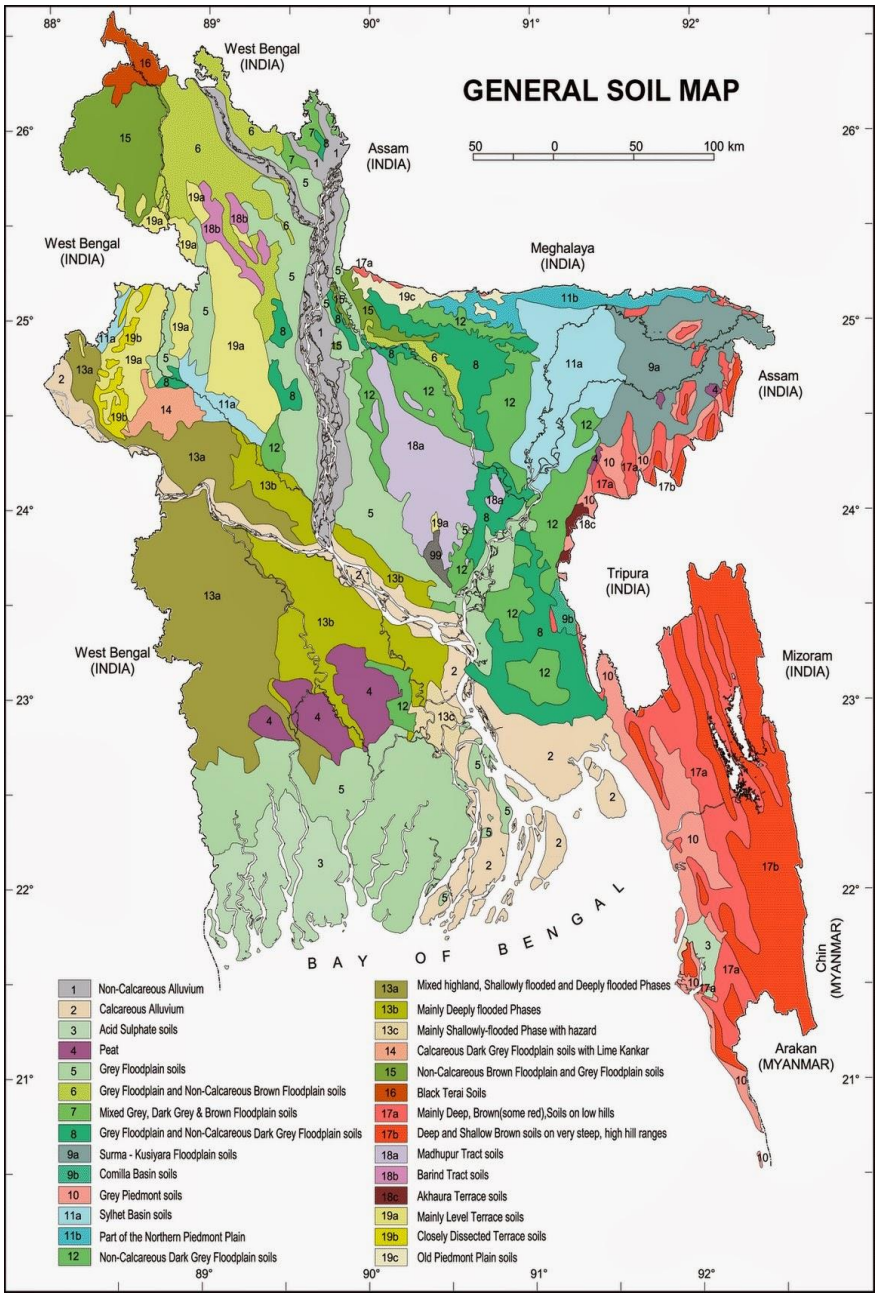
APPENDICES

APPENDIX I (A): Map showing the experimental sites under study



The experimental site under study

Appendix I(B): Map showing the general soil sites under study



Appendix II: Characteristics of soil of experimental site is analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

Morphological characteristics of the experimental soil collected from SAU Research field

Morphological features	**Characteristics**
Location	Experimental field, SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained

Physical and chemical properties of the initial soil

Characteristics	**Value**
% Sand	27
% Silt	43
% clay	30
Textural class	Silty-clay
pH	5.5
Organic carbon (%)	0.43
Organic matter (%)	0.75

Total N (%)	0.075
Available P (ppm)	21.00
Exchangeable K (meq/ 100 g soil)	0.11
Available S (ppm)	43

Source: SRDI, 2018

Appendix III: Monthly average of Temperature, Relative humidity, total Rainfall and sunshine hour of the experiment site during the period from November 2020 to April 2021

Year	Month	Temperature			Relative Humidity (%)	Rainfall (mm)	Sunshine (Hour)
		Max (°C)	Min (°C)	Mean (°C)			
2020	November	32	24	29	65	42.8	349
	December	27	19	24	53	1.4	372
2021	January	27	18	23	50	3.9	364
	February	30	19	26	38	3.1	340
	March	35	24	31	38	19.6	353
	April	38	25	33	54	292.4	315