FIELD OBSERVATION OF SELECTED MUNGBEAN CULTIVARS AGAINST *Mungbean Yellow Mosaic Virus (MYMV)* AND ITS MOLECULAR DETECTION

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This is to certify that the thesis entitled **"FIELD OBSERVATION OF SELECTED MUNGBEAN CULTIVARS AGAINST *MUNGBEAN YELLOW MOSAIC VIRUS (MYMV)* AND IT'S MOLECULAR DETECTION"** *submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in PLANT PATHOLOGY****, embodies the results of a piece of bona-fide research work carried out by* ***TANJILA HASAN, REGISTRATION NO.13-05457*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated:09.01.2022
Dhaka, Bangladesh

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DEDICATED
TO
MY BELOVED FAMILY
AND
RESPECTED TEACHERS

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The Author

Field Observation of Selected Mungbean Cultivars against *Mungbean Yellow Mosaic Virus (MYMV)* and It's Molecular Detection

ABSTRACT

An experiment was conducted in field and laboratory condition under the Department of Plant Pathology of Sher-e-Bangla Agricultural University, Dhaka-1207 during 2020-2021. The field experiment was carried out in Randomized Complete Block Design (RCBD) with three replications to observe the field performances of ten selected mungbean cultivars and molecular study was done in Molecular Biology and Plant Virology Laboratory. From the field experiment results, it was revealed that three varieties namely BARI mung-7, BARI mung-8 and BINAmoog-9 showed moderately resistance and lower disease severity against *Mungbean yellow mosaic virus (MYMV)*. Among the remaining selected varieties, four varieties namely BARI mung-5, BARI mung-6, BINAmoog-5 and BINAmoog-8 showed moderately susceptible whereas BU mug1, Sonamug and Chaitamug appeared as susceptible to highly susceptible and higher disease severity against *MYMV*. Field study results were approved by the molecular study through PCR test. All the tested varieties remained PCR positive which revealed that every single variety was *MYMV* infected. Different growth parameters and yield contributing characters were also studied. Among the tested varieties, the tallest plant found in BARI mung-7 and the shortest was in Chaitamug. Maximum number of branches per plant was obtained in the BARI mung-7 and minimum was obtained in BU mug1. The highest and the lowest total chlorophyll content were recorded in BARI mung-7 and Chaitamug respectively. The highest number of pods per plant was recorded in BARI mung-8 and the lowest was recorded in Sonamug. Maximum pod length was observed in BARI mung-7 and minimum length was observed in Chaitamug. In terms of number of seed per pod, the highest and lowest results were found in BARI mung-8 and Chaitamug respectively. Pod sterility percentage was maximum in Chaitamug and minimum in BARI mung-7. The highest weight of 1000-seed was recorded in BARI mung-6 and the lowest was recorded in BARI mung-8.The highest yield per plot was obtained from BARI mung-7 and the lowest yield per plot was obtained from Chaitamug. From the study, it was observed that minimum disease incidence and severity were appeared on BARI mung-7 in natural field condition and gave the best performances in respect of yield and yield contributing characters.

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ABBREVIATIONS AND ACRONYMS

µg	Microgram
µl	Micro Litre
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
BINA	Bangladesh Institute of Nuclear Agriculture
BBS	Bangladesh Bureau of Statistics
bp	Base-pair
BSMRAU	Bangladesh Sheikh Mujibur Rahman Agricultural University
CV%	Percent Coefficient of Variation
DAS	Day after sowing
DI	Disease incidence
et al.,	All others
etc.	Etcetera
FAO	Food and Agricultural Organization
g	Gram
hrs.	hours
FP	Forward primer
ICTV	International Committee on Taxonomy of Viruses
LSD	Least significant differences
mm	Millimeter
MoP	Muriate of potash
MYMV	Mungbean Yellow Mosaic Virus
ppm	Parts per million
PCR	Polymerase Chain Reaction
PDI	Percent disease index
SAU	Sher-e-Bangla Agricultural University
RCBD	Randomized complete block design
SRDI	Soil Research and Development Institute
TSP	Triple super phosphate

CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

Mungbean, commonly known as green gram or moog dal, an Asiatic species of the pan-tropical genus *Vigna* belongs to the family Leguminosae and sub-family Papilionaceae, and botanically recognized as *Vigna radiata* (L.) Wilczek. Mungbean plant is a deep rooted, erect or semi-erect, hairy annual herbaceous plant containing alternate, usually tri-foliate but occasionally quadra- or pentafoliate leaves. Inflorescence is an axillary or terminal raceme with a cluster of 10-20 flowers. Flowers are papilionaceaus. Pods are linear, cylindrical, slightly bulged over the seeds, 5.0-9.0 cm long, beaked slightly, dark brown or black in colour with bristles, containing 5-10 seeds. Seeds are small, globular with colors ranging from yellow, green or light brown (Banglapedia, 2021). Though mungbean is originated in India or the Indo-Burmese region, it is a vital crop grown throughout Asia, Australia, West Indies, South and North America, tropical and subtropical Africa; because of its adoptability to a large number of cropping systems (Karthikeyan *et al*, 2004). Being an important short-duration legume, mungbean is grown extensively in major tropical and subtropical countries of the world. The agro-ecological condition of Bangladesh is favorable for growing this crop. The optimum temperature ranges from 20°- 35°C depending upon season (BARC, 2013). Mungbean is also drought tolerant crop and can grow with a minimum supply of nutrients.

Mungbean is one of the most important pulse crops of Bangladesh. It is considered as the best of all pulses from the nutritional point of view. So mungbean is considered as poor man's protein (Mian, 1976). It is an excellent source of protein (24.5%) with high quality of lysine (460 mg g^{-1} N) and tryptophan (60 mg g^{-1} N). It also contains remarkable quantity of ascorbic acid and riboflavin (0.21 mg $100g^{-1}$) (Azadi *et al*., 2013). Mungbean also contain

vitamins and minerals. Mungbean contains carbohydrate (51%), protein (24-26%), minerals (4%) and vitamins (3%) (Khan, 1981). Mungbean seeds are used either whole or split into in preparation of 'dal', a soup porridge eaten with a cereal or other traditional cuisines, and is a main protein source for vegetarian diet in the country. It contains 24.5g protein and 59.9g carbohydrate, 75 mg calcium, 8.5 mg iron and 49 mg β-carotene per 100g of split dual (Bakr *et al*., 2004). Generally grain legumes are limited by the low sulfur containing amino acids like cysteine and methionine still both of these amino acids are comparatively more in mungbean (Engel, 1978). The seeds are used to prepare sweets, bean sprouts, starch noodles, mungbean soup and are also fried in oil to be eaten as snack. Young seedlings make nice vegetable. The green plant is used as fodder in many areas. It is also a good green manure crop. The dried stems and pod walls remaining after threshing are also used as cattle feed. Besides providing protein in the diet, mungbean has the remarkable quality of helping the symbiotic root rhizobia to fix atmospheric nitrogen and hence to enrich soil fertility (Anjum *et al*., 2006) which not only enables it to meet its own nitrogen requirement but also benefits the succeeding crops (Ali, 1992).

In Bangladesh, mungbean is traditionally cultivated twice in a year; it is grown annually on an area of about 2.308 lakh hectares of land and a about 1.28 lakh m tons of grains are produced in winter and an area of about 0.337 lakh hectares of land and a about 0.41 lakh m tons of grains are produced in summer (Krishi dairy, 2021). In Barisal and Patuakhali this crop is widely cultivated (Banglapedia, 2021). It is well suited to a large number of cropping systems and constitutes an important source of cereal based diets, worldwide, covering more than six million hectares per annum. However, Asia, alone, accounts for 90% of world's mungbean production. India is the world's largest mungbean producer accounting for about 65% of world's acreage and 54% of its global production (Singh, 2011).

There are numerous reasons for low yield but yield losses due to insect biotic stresses (insects, nematodes, fungi, virus etc.) are distinct one. Pathogenic

organism like fungus and virus are responsible for occurring severe yield loss of mungbean. So far a total of sixteen diseases of mungbean have been recorded in Bangladesh (Nene, 1973). Of which foot and root rot (*Fusarium oxysporum, Sclerotium rolfsii*), Cercospora leaf spot (*Cercospora crunta*) and *mungbean yellow mosaic virus (MYMV)* are most damaging to the crop (Faruq and Islam, 2010). Mungbean is also affected by a dozen of insect pests such as pod borer, leaf miner, jassids, foliage caterpillar, cut worm, aphids and white fly (Ayub, 1987). Different species of sucking insect pests like aphid, jassid, leaf hopper and whitefly are of the major importance (Islam *et al*, 2008). These insect pests not only reduce the vigor of the plant by sucking the sap but transmit diseases particularly viral disease and affect photosynthesis as well (Sachan *et al*., 1994).

Mungbean yellow mosaic virus is the most destructive disease of mungbean in the Indian subcontinent and adjacent areas of Southeast Asia (Jayasekera and Ariyarantoe, 1988; Bakar ,1991). It is the most damaging disease of mungbean in Bangladesh (Jalaluddin and Shaikh, 1981). It was first identified in India in 1955 and is naturally transmitted by whitefly (*Bemisia tabaci* Genn), but not by mechanical inoculation or by seed (Nariani, 1960). It is found to spread the begomoviruses, the major hazard to the flourishing production of mungbean in India, Sri Lanka, Pakistan, Bangladesh, Papu New Guinea, Philippines and Thailand (Honda *et al*. 1983).

The characteristic symptoms of the disease appear as yellow patches on mungbean leaves coalesced to form a larger patch that develops into a yellow mottle; eventually the entire leaf may turn yellow. The green areas appear as dark green islands interspersed in yellow chlorotic areas; the infected leaf blade appears wavy. Sometimes the leaves may become malformed and wrinkled. Maturity is delayed in the diseased plants and flowers and pods production are severely reduced. Seeds developed from severely infected plants are small and immature (Poehlman, 1991).

Yield loss due to *MYMV* was estimated as 63% (Anon. 1984). Estimation of actual losses due to *MYMV* in farmer's field is difficult as these losses vary from year to year and from variety to variety (Krisnareddy, 1989). The incidence and severity of yellow mosaic disease is considered to be directly related to the availability and abundance of insect vector and also depend upon the time of infection (Dhingra and Ghosh, 1993). Winter mungbean genotypes are highly susceptible to MYMV and caused 67-100% yield (Jalaluddin and Shaikh, 1981). According to Bakr *et. al.* (2004) *MYMV* causes up to 85% yield loss when infection starts from the 4th week of sowing.

For successful cultivation of mungbean, *MYMV* management must be prioritized while trying to develop and release improved high yielding mungbean cultivars. Till today high and fairly stable resistant varieties of mungbean against *MYMV* infections are not available in Bangladesh. Some resistant and tolerant cultivars have been released by Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) which depends on cultural and environmental factors to remain healthy. Reports on management of *MYMV* are scanty. The control of insect vector is an important tactic for managing yellow mosaic disease of mungbean. Some chemicals were found to be effective in reducing the incidence of yellow mosaic disease (Borah, 2012). Though injudicious application of these chemicals pollute the environment and cause health hazards but other alternate approaches like using plants extracts and cultural practices individually cannot do well against the vector. Integrated disease management system and development of *MVMY* resistant variety can pave the way to solve this major problem. For developing the management strategy and resistant variety, it is a pre-requisite to identify the virus appropriately.

There are many methods reported for plant virus detection viz. biological properties, physiological properties or in-vitro properties, intrinsic properties, Serological test and modern molecular techniques. The purpose of the present study was also to evaluate the varietal performance of tested mungbean

varieties against *Mungbean Yellow Mosaic Virus* (*MYMV*), and to detect *MYMV* by modern molecular technique through PCR. The current study is carried out to identify *MYMV* through symptom test at first. Symptom based identification is possible but it needs good skill as well as plant pathological experience as because similar symptoms may appear due to other stresses. PCR is the modern molecular technique for detection of most of the plant viruses because it is more reliable, simple, very specific and highly robust.

Keeping all these facts in mind the present study was undertaken to achieve the following specific objectives

- to assess the varietal performance of selected mungbean varieties against *Mungbean Yellow Mosaic Virus (MYMV)*,
- to identify *MYMV* on the basis of visual symptoms and
- to detect *MYMV* through Polymerase Chain Reaction (PCR).

CHAPTER II
REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mungbean is one of the most important pulse crops, which received much attention of the researchers throughout the world. A notable number of research works on different aspects of mungbean production have been performed extensively in South East Asian countries, especially in the Indian subcontinent for its yield and quality improvement. In Bangladesh, research workers started research on mungbean in the early eighty decade and focused on the improvement of this short-duration pulse crop to fulfill the protein demand. Bangladesh Agricultural Research Institute (BARI) has released its' first mungbean variety (BARI mung-1) in 1982 and Bangladesh Institute of Nuclear Agriculture (BINA) has released its' first mungbean variety (BINAmoog-1) in 1992. These two leading research institutes have already released 17 mungbean varieties. Recently Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) has started research work on varietal development and improvement of this crop and released 6 varieties. Mungbean (Vigna radiata L. Wilczek) suffers from many diseases of which yellow mosaic disease causes severe damage to the crop. Findings of various experiments related to the study of the performance of different varieties of mungbean against *MYMV* in home and abroad have been reviewed and discussed in this chapter.

2.1 Yellow Mosaic Disease on Mungbean and Its Symptoms

Mungbean is a vital, environment-friendly food grain crop with a rich source of proteins, minerals and vitamins besides carbohydrates. Considering nutritive and economic value, it ranks next to cereals. Being a member of Fabaceae family, mungbean plays a pivotal role in the restoration of soil fertility by

atmospheric nitrogen fixation through symbiosis with *Rhizobium* species (Anjum *et al*.,2006), and also play an important part in the sustainability of agricultural production system. In addition, characteristics like rapid growth, early maturity and easily digestibility without flatulence further add to its value in various cropping systems. Mungbean is cultivated in different regions of Bangladesh. Although large amounts of mungbean is extensively cultivated in Bangladesh but its production has not yet been increased substantially. The main reason for low yield is the susceptibility to various diseases of which yellow mosaic disease caused by *Mungbean Yellow Mosaic Virus (MYMV)* is the most damaging one (Nariani, 1960). *Mungbean Yellow Mosaic Virus* is transmitted by the vector, the whitefly (*Bemisia tabaci*).

In the end of 1940s, Yellow mosaic disease of lima bean (*Phaseolus lunatus*) and later in *Dolichos lablab* were reported in Pune, India (Capoor and Varma, 1948; Capoor and Varma, 1950). Then a notable number of researches were carried out to find out the host range of *Yellow Mosaic Virus*. Experiments brought to light a remarkable finding; host range of yellow mosaic disease included numerous cultivars of all groups of legumes. In mungbean, mosaic disease was first identified as a problem at the experimental farm of the Indian Agricultural Research Institute, New Delhi in India in 1955. The yellow mosaic on mungbean is a viral disease, was first described by Nariani (1960) and Nene (1968) named the virus as *Mungbean yellow Mosaic Virus (MYMV).* Based on the reports of discovery of *MYMV* disease in India, other neighboring countries also confirmed its presence in their own territories. It was confirmed in Pakistan (Ahmad,1975), Sri Lanka (Shivanathan,1977), Bangladesh (Jalaluddin and Shaikh 1981) and Thailand (Thongmeearkom *et al.* 1981). Between 1960 and 1980, most of the mungbean yellow mosaic disease research was involved on managing the disease by controlling vector population with insecticides, and resistance breeding.

MYMV was recorded in 1955 at India agricultural research institute, New Delhi (Nariani,1960). The earliest symptoms of *MYMV* infection in susceptible

cultivars as reported by Nariani, are the appearance of yellow specks on young leaves, with the next emerging trifoliate leaf exhibiting irregular yellow and green patches, and slight puckering and reduced leaf size.

Nene(1969) observed that in case of severe infection only very few number of pods were produced.

Nair *et al.* (1974) observed that due to this disease a necrotic centre may develop in the yellow spots in some cultivars. In the infected cultivars, they observed on reduction in number and size of pods.

Singh and Singh (1979) reported that *MYMV* causes disease in a variety of leguminous crops, but the most seriously affected are blackgram, mungbean and soybean in the Indian subcontinent. During a survey in 1973 and 1974, which were favorable years for the spread of *MYMV*, the incidence of yellow mosaic in mungbean was recorded as more than 60% in six districts in Haryana state.

In mungbean, *MYMV* infection results considerable decrease in chlorophyll and DNA contents and increase in RNA, phenols, free amino acids, sugars and enzymes, was reported by Chhabra *et al.*, (1981).

Thongmeearkom *et al.* (1981) described the steps of disease development in *MYMV* infected plants. They stated that the whitefly delivers the virus through proboscis to the phloem cells of the host plant where it gets multiplied. In leaf cells, the virus particles often form loose aggregates that sometimes fill the nuclei of infected phloem cells. In mungbean, hypertrophied nucleoli, aggregates of virus particles and fibrillar bodies appear in the nuclei of phloem cells as early as two days before the appearance of the symptom. Virus particles are often scattered in distribution but occasionally form aggregates having a paracrystalline or double cylindrical arrangement in the vacuoles of infected sieve elements. It causes yellow-coloured spots scattered on young leaves followed by yellow mosaic pattern.

Singh *et al.* (1982) reported that chlorosis, stunting and fewer branches and premature shedding of leaves are the most common symptoms of mungbean yellow mosaic disease.

Fakir (1983) first reported yellow mosaic disease of mungbean in Bangladesh. He gave a detailed description and some recommendations for the management of the disease.

Ahmed (1985) described the chronological development of symptoms of the disease as appearance of scattered yellow spots on young leaves which eventually turn to large irregular green and yellow mosaic with light stunting of emerging trifoliate leaves associated with occasional puckering of green area along with increase in size of yellow area in subsequent emerging leaves which completely turned to yellow mosaic in some plants. Disease plants mature late. Pods were stunted, curled and frequently contained small, immature seeds.

Bakr (1991) reported that the symptoms in the disease appear on the leaves as minute yellow specks that may expand and cover the entire area. Mixture of irregular yellow green patches could be observed on the leaves. Pods are smaller in size and borne small shriveled seeds.

Poehlman (1991) observed the yellow patches on mungbean leaves, which coalesced to form larger patches that developed into a yellow mottle; eventually the entire leaf could turn yellow. Maturity was delayed in the disease plants and flower and pod production were severely reduced. In some cases pods become sterile. Seeds that developed on severely infected plants were small and immature.

Malathi and John (2008) reported that due to *MYMV* infection the yellow leaves slowly dry and wither. Infected plants bear few flowers and pods with some immature and deformed seeds, thus affecting the yield both qualitatively and quantitatively. Pods of the infected plants are reduced in size and turn yellow in color. In severe cases, other plant parts become completely yellow.

2.2 Causal Organism: *Mungbean Yellow Mosaic Virus (MYMV)*

MYMD is caused by begomoviruses, popularly recognized as geminiviruses which are the leading and the most significant genus within the family, Geminiviridae. Geminiviruses are plant viruses characterized by twin icosahedral particles (Thomas *et al*. 1986).

Based on sequence identity analyses, the bipartite begomovirus isolates, namely, *Mungbean Yellow Mosaic Virus (MYMV)*, *Mungbean Yellow Mosaic India Virus (MYMIV) And Horse Gram Yellow Mosaic Virus (HgYMV)* are recognized as the causal agents of MYMD in different regions of the world (Qazi *et al*. 2007; Malathi and John 2008; Ilyas *et al.* 2010). Among these, *HgYMV* is the least studied. Its complete sequence is available in the databases but no detailed studies are conducted on this virus. The other two pathogens namely, *MYMV* and *MYMIV*, occur across the Indian subcontinent. The northern, central and eastern regions of India are dominated by *MYMIV* infestations (Usharani *et al*. 2004) but *MYMV* is more ubiquitous in the southern (Karthikeyan *et al*. 2004; Girish and Usha 2005) and western regions of the country. In Bangladesh, *Mungbean Yellow Mosaic Virus* and *mungbean Yellow Mosaic India Virus*, these two strains causes yellow mosaic disease on mungbean.

Geminiviruses (subgroup I), the genome consist of a single circular ssDNA of about 2.6-2.8 kb, and these viruses are transmitted by leafhoppers. Subgroup II geminiviruses, with the exception of tobacco yellow dwarf virus, infect monocotyledonous plants and include, maize streak virus and wheat dwarf virus. In other geminiviruses (subgroup III), the genome consist of two circular ssDNAs off about equal size (2.4 -2.8kb each). Most of these viruses are transmitted by whiteflies of the genus *Bemisia*. Subgroup III geminiviruses infect only dicotyledonous and include many geminiviruses that causes devastating losses in many crops, particularly in the tropical and subtropical regions where high population of *Bemisia* (whiteflies) are common. (Brown and Bird, 1992).

MYMV possesses monopartite or bipartite circular; single- stranded DNA genomes (Ahmed *et al*. 1991, Navot *et al*. 1991). Bipartite begomoviruses contains ss-DNA genomes of approximately 2.7 kb. The majority of the begomoviruses are bipartite and the genomic components are referred to DNA-A and DNA-B (Stanley 1983) which are about 2800 nucleotides in length (Qazi *et al.* 2007). DNA-A is involved in various nuclear functions. It encodes for all the factors required for viral DNA replication and DNA helicase (Choudhury *et al.* 2006) and the replication enhancer protein, regulation of gene expression and encapsidation or insect transmission (Raghavan *et al.* 2004). There are numerous DNA-B components associated with yellow mosaic viruses (John *et al*. 2008). The DNA-B component encodes two genes. They are the nuclear shuttle protein (NSP) and the movement protein (MP) act together to move the virus from one cell to the other within the plant (Shivaprasad *et al.* 2005).

2.3. Transmission and Epidemiology of Disease

MYMV is transmitted principally by the polyphagous leaf sucking pest in a persistent (circulative) manner. Nariani (1960) reported that the virus causing yellow mosaic of mungbean is transmitted by the whitefly (*Bemisi tabaci*).

Nene (1971) observed that *MYMV* could be acquired from and transmitted to *Phaseolus mungo* by whitefly adults after 15 minutes feeding period. The *MYMV*-vector relationship was thoroughly studied by Nene (1973). He studied the life history of the vector, its maintenance, multiplication and dispersal on *Vigna radiata.* The minimum acquisition-feeding period for whitefly using susceptible mungbean variety was 15 minutes. The minimum inoculation period was 10 minutes. It took 4 hours for a whitefly after it was allowed the acquisition feeding to transmit the virus successfully. Starvation prior to acquisition feeding increased the efficiency of transmission. The whitefly co-transmits the virus in 100% of the plants inoculated.

Butter (1977) also studied the life history of the vector (*Bemisia tabaci*), its maintenance, multiplication and dispersal on cotton. He found that the females laid between 38 and 106 eggs in their total life span on the lower surface of leaves. The hatching period was between 24 and 48 hours. The total life cycle from egg to adult stage ranged from 13 to 72 days. Population builds up from April to October when the average maximum temperature ranged from 21 to 35℃.

Murugeson and Chelliah (1977) reported that *MYMV* could be transmitted successfully by a single infectious *Bemisia tabaci* but maximum infection was given by 10 flies per plant. Infection was ensured when vector had a pre-acquisition starvation period of 24 hours.

According to Chenulu *et al*. (1979) *MYMV* is transmitted by the whitefly in circulatory manner. Pre-acquisition and pre-inoculation starvations either increase the efficiency of transmission or have no effect. Isolates from Bangladesh, Pakistan and Sri Lanka have similar characteristics.

The only report on mechanical transmission by Honda *et al.* (1983) presented the mechanical transmission of the isolate of *MYMV* of Thailand and they obtained the highest transmission rates with 0.1 M potassium or sodium phosphate buffer of pH 7.8. Thereafter no one reported any infestation caused by mechanical transmission.

Basu (1986) also confirmed that whitefly is an effective vector of *MYMV*. So far, no intraspecific diversity has been identified mainly due to host and season correlated variation in pupal morphology.

Grewal (1988) took attempts to transmit the disease by sap inoculation by rubbing freshly extracted sap of mosaic affected leaves on the healthy young seedlings of mungbean. However, the disease could not be transmitted in this manner.

Brunt *et al.* (1990) reported that the virus was transmitted in nature by an insect vector belonging to the Aleyrodidae: *Bemisia tabaci* in a non-persistent

manner. Helper virus was not apparently required for transmission. Non-vector transmission was apparently not by mechanical inoculation; not by seed; not by pollen.

Patel and Srivastava (1990) observed that the number of *Bemisia tabaci* infestation was 1.67-2.67 per 10 leaves with corresponding amount of yellow mosaic virus infected plants ranged between 0.90- 2.47%.

Aftab *et al.* (1992) reported that there is a positive correlation between the increasing rate of mungbean yellow mosaic virus disease and increase in the whitefly population.

Dhingra and Ghosh (1993) also studied on the efficiency of *B. tabaci* in transmission of mungbean yellow mosaic geminirvirus in reciprocal inoculation test of five different hosts. They reported that the maximum percentages of virus transmission occurred when the test and source plants were of the same species. Mungbean and urdbean were better tested as source plants than French bean (*P. vulgaris*) and pigeon pea for the test and/or the vector. They also described that the virus transmission percentage increased with the increase in the number of adult whitefly or test plant and that the nymphs were less efficient vectors than the adults.

Nath (1994) studied the relationship between disease incidence and population size of B. tabaci in the crop sown. He observed a positive correlation between incidence and population size of *B. tabaci*. Nath (1994) also studied the effect of the weather parameters on the population of whitefly and the incidence of yellow mosaic virus on green gram. He reported a simple positive significant correlation between the disease incidence and the population of the fly, temperature, the relative humidity, rainfall and the number of rainy days necessary for the infection

Dantre *et al.,* (1996) studied on a yellow mosaic virus disease of soybean and mungbean and reported that the mungbean yellow mosaic Gemini virus was transmitted by whitefly (*Bemisia tabaci*) but not through sap or seed.

According to Malathi and John (2008), a single viruliferous adult is capable of transmitting the dreadful virus and it can transmit the virus within an acquisition and inoculation access period of 24 hours. Acquisition and inoculation by whitefly adults can be affected in a minimum of 15 minutes. The insect may attain the virus after a single bite and its transmission efficiency increases with time on the source plant of virus as well as on the healthy mungbean plant.

The most efficient female and male adults in a population can retain infectivity for 10 and 3 days, respectively. Thus, the female adults are three times more efficient as vectors than males. Neither female nor male adults can retain infectivity throughout their life span. Nymphs of *B. tabaci* can acquire the virus from diseased leaves and the virus does not pass through eggs of *B. tabaci*. Nevertheless, the cropping seasons highly influence the vector population. Whitefly is found to be high during summer season compared to spring and rainy seasons. They thrive best under hot and humid conditions and the population also towers with higher temperatures. Moreover, the spread of *MYMV* on a local (within and between fields) and a regional basis reflects its dispersal. Its population is correlated with disease incidence. High populations appear on the crop that are 20-30 days old leading to higher disease incidence (on the 45th day). Spring and rainy seasons attribute to unfavorable conditions for the multiplication of the whitefly. Therefore, the disease incidence is not high during those seasons.

Islam *et al*. (2008) conducted an experiment on seven recommend varieties of mungbean to know the population dynamics of whitefly under existing environmental conditions and its impact on incidence of *Mungbean yellow mosaic virus* (*MYMV*) and yield. The peak population was found at 32°C and 80% relative humidity. The lowest percent of *MYMV* infected plant was found in BARI mungbean-6 and a positive relationship was found between whitefly population and incidence of *MYMV* disease. The highest yield of mungbean

was obtained from BARI mung-6 and there was a strong negative relationship between the *MYMV* infection and yield of mungbean.

2.4. Host and Source plant

The host range of most of the isolates of *MYMV* has been restricted to leguminous species, except for an isolate studied by Nene (1973) which could also infect four nonleguminous common weeds, viz. *Brachiariaramosa, Cosmosbipinnatus, Ecllpta alba and Xanthium strumarium.*

2.5. Response of Mungbean Plant to *Mungbean Yellow mosaic virus*

Khatri *et al.* (2003) was conducted survey and determined the spread of yellow mosaic virus (YMV) disease and extensive damage was caused by the disease on mothbean (*Vigna aconitifolia*). They further observed that *MYMV* was the most important disease of mothbean in the region during both years.

Bashir (2005) screened 276 lines of mungbean and out of which 10 showed resistance lines against *MYMV*.

Shad *et al.* (2006) found that there was no resistant line against *MYMV* and identified of seven susceptible and 247 as highly susceptible lines.

Yellow mosaic is reported to be the most destructive viral disease not only in Pakistan, but also in India, Bangladesh, Srilanka and contiguous areas of South East Asia (Biswass *et. al*., 2008. John *et. al.*, 2008).

Awasthi and Shyam,(2008) reported that there were 30 susceptible and 43 highly susceptible genotypes of mungbean in there study. Great variation in genotype response to *MYMV* represents variability in their genetic makeup.

Iqbal *et al*. (2011) observed one hundred genotypes/lines of mungbean germplasm against *MYMV* during summer season under field conditions at NARC, Islamabad. The germplasm were categorized in to resistant and

susceptible depending upon severity of disease. Response of mungbean accessions to *MYMV* was determined and none of the genotype/line was found to be highly resistant to disease. Four genotypes/lines i.e. 014043, 014133, 014249, 014250 were found as resistant. Eight were moderately resistant and 30 were moderately susceptible. Remaining 30 accessions were classified as susceptible and 43 as highly susceptible accessions.

2.6. Effect of *Mungbean Yellow mosaic virus* on yield and yield components

Reduction in yield in legumes due to *MYMV* depends on the time of infection and severity of the disease. If highly susceptible varieties of black gram or soybean are infected within three weeks of sowing, no yield is obtained. Infection of these species during the fourth, fifth, sixth, seventh and eighth week results in yield reductions up to 85, 60, 44, 28 and less than 10%, respectively. Yield is significant by decreased when infection occurs up to 50 days after sowing. Reduction in the number of pods plant, seeds/ pod and seed weight is the main contributing factors for the decrease in yield (Nene 1973).

Marimuthu *et al*., (1981) found that healthy mungbean cultivars Mung and B-105 gave yield 6.5 and 5.14 g seed/plant, respectively, while yields were decreased to 4.4 and 2.03 g in severely infected plants due to *MYMV*.

Singh *et al.* (1982) observed that early infected plants had more severe symptoms than the late infected ones. They also established that chlorosis, stunting and reduced branching contributed to yield loss. Ahmed (1985) observed 85% *MYMV* incidence both in summer and winter pulse verities.

Ahmad (1991) observed that *mungbean yellow mosaic virus* infection causes maximum growth reduction (62.94%) and yield loss (83.9%) for (*vigna radiata*) on which symptoms appeared 20 days after 11 sowing (DAS). For plants on which symptoms appeared 30 or 40 DAS growth parameters and yield were less affected. It is concluded that early crop infection reduced yield more than late infection.

Chamderand Singh (1991) observed that plant height and fresh weight reduced upto 38.2% and 28.5% respectively for *MYMV* infection on mungbean and Urbean. The shape, size and appearance of pods and seeds of plants were considerably distorted although seed germ inability was found to be unaffected.

MYMV infection affects grain yield when the plant having infection up to 50 days after planting. The color, texture, size and germination of the seeds were found to be affected. Yellow mosaic caused 16% yield loss in mungbean and 10% yield loss (Fakir, 1983). Reduced plant height and fresh shoot weight were reported along with yield loss up to 66%.

Bakar (1991) described yellow mosaic virus as the most serious limiting factor in mungbean and black gram cultivation and can attack the crop at any stage of growth, however, losses are severe when it attacks at an early stage.Total loss had been reported when the crop was infected by *MYMV* within 1-2 weeks after germination. 63% and 20-30% losses were recorded 3 and 4-7 weeks of age.

Varma and Sandhu (1992) studied that yield loss models based on components like number of pods per plant, severity of disease and stage of infection by *MYMV* could predict yield loss very close to the actual loss in black gram. These of such a model would provide better estimates of losses due to the virus in different crops.

Sachan *et al.* (1994) found a drastic reduction in the infection of *YMV* when whitefly attack was reasonably controlled. The yellow mosaic virus caused 30-70% yield loss.

Gill (1999) reported that *MYMV* infection in the early growth stages of mungbean reduced yields significantly more than that of infection at the flowering stage.

Babu *et al.* (2004) reported that infection of (*Vigna radiata*) plants by *MYMV* caused significant reduction in number of pods/plant, seed yield and 100-seed wt. When healthy and infected leaves were compared a reduction in the contents of chlorophyll and functional chloroplast cells was evident in the

latter. Soluble N and reducing sugars accumulated to a greater extent in infected leaves and the rate of photosynthesis was reduced.

Ganapathy and Karuppiah (2004) reported that the reduction in grain yield by *MYMV* ranged from 39.9 to 51.5% in black gram varieties. They also observed that reduction in plant height, pods/plant, 100-seed weight and crop growth rate contributed to decreased grain yield.

Usharani *et al.,* 2004 stated that mungbean yellow mosaic virus disease (*MYMV*) is a major problem in production of mungbean leading to high grain yield losses from 10-100 percent.

Yaqoob *et. al*. (2005) observed variations in reduction of growth components and subsequent yield loss by *MYMV* among the cultivars under natural condition.

The economic impact of *MYMV* on yield depends upon the time of virus infection and is related to the plant development. Early infection by the virus gives the highest reduction in yield. If the infection occurs after three weeks from planting, then the yield loss surmounts to 100%. However, the losses will be meagre if infestation occurs after eight weeks from planting. Over a broad geographic range, the yield reductions between 10% and 85% have been reported (Khattak *et al.* 2000; Varma and Malathi 2003; Kang *et al.* 2005)

Gupta and Pathak (2009) reported that the yellow mosaic virus disease of black gram [*Vigna mungo* (Linn.) Hepper] caused by *Mungbean yellow mosaic Gemini virus* and transmitted by whitefly (*Bemisia tabaci* Genn.) is most serious in northern states of India, particularly, Bundelkhand Zone of Madhya Pradesh. *Mungbean yellow mosaic virus (MYMV)* causes yield loss up to 80 % and is becoming problematic in mungbean growing areas.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

The present study was carried out under the field condition at central 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, severity of *Mungbean Yellow Mosaic Virus* (*MYMV*) and its detection was done through PCR. This chapter includes a brief description of the experimental site, experimental period, climatic condition, crop or planting materials, land preparation, experimental design and layout, crop growing procedure, treatments, intercultural operations, data collection and virus detection along with statistical analysis with some headings and sub-headings.

3.1. Experimental Site

The experiment was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka. The experimental field is located at the 23º74′ N latitude and 90º35′ E longitude with an elevation of 8.2 meter from sea level. (Appendix- I).

3.2 Experimental period

The experiments were carried out during the kharif- I season from March, 2020 and to June, 2021. Seeds were sown on 19th March, 2020 and were harvested on 7th -21st June 2020. Lab work was done May-June, 2021.

3.3 Soil Characteristics

The soil characteristics of the experiment field were a medium high land which belongs to the Modhupur tract, Agro Ecological Zone no 28. The soil texture was silt loam, low level of nutrients, non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment, soil samples 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-II).

3.4 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 28℃ to 32℃ in summer, falling to 20℃ in winter, with extreme lows of 10℃. 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℃ and average minimum temperature was 20℃. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka. (Appendix-III).

3.5. Planting Material

In total ten mungbean cultivars were chosen to conduct the examination. Seeds were gathered predominantly from Bangladesh Agricultural Research Institute (BARI), Gazipur; Bangladesh Institute Nuclear Agriculture (BINA), Mymensingh; Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur; Lalmonirhat and Patuakhali districts. Name of selected mungbean varieties used in the present study are mentioned in table 1.

Table 1. Name and origin of tomato varieties used in the present study

Treat. No.	Name of the Varieties	Origin	Year of release
1	BARI mung-5	BARI	1997
2	BARI mung-6	BARI	2003
3	BARI mung-7	BARI	2010
4	BARI mung-8	BARI	2015
5	BINAmoog-5	BINA	1998
6	BINAmoog-8	BINA	2010
7	BINAmoog-9	BINA	2012
8	BU mug1	Dept. of Agronomy, BSMRAU.	2000
9	Sonamug	Patuakhali district	-------
10	Chaitamug	Lalmonirhat district	-------

3.6. Experimental Design and Layout

The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications and each variety contains 3 plots. The total number of unit plots was 30. Total experimental area was 200 m^2, allocated by the authority. Approximately 0.25 m space was left as border in each side. Plots were 3×1.5 m^2 along with 0.5m spacing in each side. (Appendices- IV).

• Total plot area: 200m^2

• Number of plot: 30

• Plot size: 4.5 m^2

• Block to block distance: 0.50m

• Plot to boundary distance: 0.25 m

• Plot to plot distance (Lengthwise): 0.5 m

• Plot to plot distance (breath wise): 0.5 m

• Plant to plant spacing: 10 cm

• Row to row spacing: 30 cm

3.7. Cultivars used as treatments:

i. V_1 = BARI Mungbean-5

ii. V_2 = BARI Mungbean-6

iii. V_3 = BARI Mungbean-7

iv. V_4 = BARI Mungbean-8

v. V_5 = BINAmoog-5

vi. V_6 = BINAmoog-8

vii. V_7 = BINAmoog-9

viii. V_8= BU mug1

ix. V_9= Sonamug

x. V_{10}= Chaitamug

3.8. Land Preparation

The allocated plot for the experiment was ploughed and cross-ploughed several times by power tiller followed by laddering to obtain a good tilth. Weeds and stubbles were removed and the large clods were broken into smaller pieces to obtain a desirable condition of soil for sowing of seeds. Finally, the land was leveled and the total area was divided into the unit plots in accordance with the experimental design mentioned in the following section.

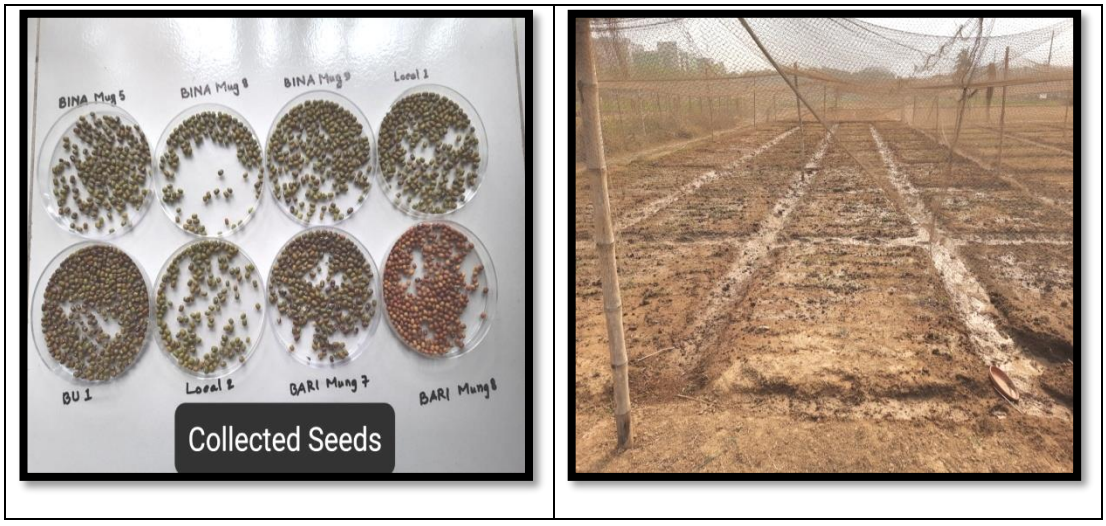

Plate 1. Collected seeds of different varieties and Prepared land for seed sowing.

3.9. Fertilizers application

As mungbean is a leguminous crops, so additional Nitrogenous fertilizer was not used during the experimental period. The sources of P_2O_5, K_2O were triple superphosphate (TSP), muriate of potash (MOP), were applied, respectively for maintaining soil health. The entire amounts of TSP, MOP were applied during the final land preparation. Well decomposed cow dung (10 t ha^{-1}) was also applied during final land preparation. The fertilizers were mixed well with the soil by spading and individual unit plots were leveled.

3.10. Sowing of seeds

Seeds were sown in the main field on the 19th March, 2020 having line to line distance of 30 cm and plant to plant distance of 10 cm. Seed sowing was done at a depth of 5-6 cm and the seeds were covered by loose soil with the help of hand.

3.11. Intercultural practices

Various intercultural operations such as thinning of plants, weeding and irrigation were accomplished whenever required to keep the plants healthy and the field weed free. Special care was taken to protect the crop from birds especially after sowing and germination stages. The field was irrigated with 2days interval due to scorching heat of sun.

3.12. Harvesting

The crop was harvested three times according to maturity on 7th -21st June 2020.The harvested crop of each plot was bundled separately. Harvested pods were threshed and grains were dried to protect from pathogenic attack. Grains were recorded plot wise and the yields were expressed in gram (g) as per plot and per ha.

3.13. Collection of Field experimental data

The following parameters were considered for data collection.

Disease incidence and severity related Parameter

a. Percent disease incidence/ plot
b. Percent disease incidence/Plant
c. Percent Disease index

Morphophysiological Parameter

a. Plant height (cm)
b. No. of primary branch/ plant
c. Chlorophyll content

Yield and yield contributing Parameter

a. No. of pod /plant
b. Pod length (cm)
c. No. of seed/pod
d. Pod sterility
e. 1000-seed weight
f. Yield (g/plot)
g . Yield (kg/ha)

3.13.1. Percent disease incidence/ plot

At first typical symptoms of *MYMV* were studied. The Mungbean plants were inspected every day until harvest and the symptoms appeared in the mungbean plants were noted. Total number of plants was counted from each plot and the infected plant having mosaic symptoms were observed carefully during the data collection. Data on mosaic disease incidence were recorded at an interval of 15 days commencing from first incidence and continued up to 3 times.

The growth stage of the plants were categorized as follows

1) Early stage- 3weeks after seed sowing

2) Mid stage- 2 weeks after early stage, and

3) Late stage- after mid stage up to harvest.

The disease incidence was expressed in percentage on the basis of stage as well as total i.e., average of three stages. The percent disease incidence per plot was calculated using the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected plant}}{\text{Total number of plants checked}} \text{ X } 100$$

3.13.2. Percent disease incidence/Plant

Incidence of mosaic diseases were recorded at before and after flowering. Ten plants were randomly selected from each plot and the mosaic symptoms on leaves were observed carefully for the collection of data. Data on mosaic disease incidence were recorded at an interval of 15 days commencing from first incidence and continued up to 3 times.

The percent disease incidence per plant was calculated using the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \text{ X } 100$$

3.13.3. Percent Disease index

Severity of mosaic diseases were recorded from ten plants which were randomly selected in each plot and observed carefully for the collection of data. Data on mosaic disease severity were recorded at an interval of 15 days commencing from first severity and continued up to 3 times.

The percent disease index was calculated using the following formula:

$$\text{PDI} = \frac{\text{Sum of all disease rating}}{\text{Total number of leaves observed X Highest grade in scale}} \text{ X100}$$

Table 2. Description of symptoms, disease score and PDI and criteria of Classification of genotypes into different responses groups (AVRDC Scale)

Score	Symptom description	PDI (%)	Response
1	No visible symptoms on leaves	1.01–10.00	Highly resistant (HR)
2	Small yellow specks with restricted spread covering up to 5% leaf area	10.01–25.00	Resistant (R)
3	Yellow mottling covering 5.1–15% leaf area	25.01–40.00	Moderately resistant (MR)
4	Yellow mottling and discoloration of 15.1–30% leaf area	40.01–60.00	Moderately susceptible (MS)
5	Pronounced yellow mottling and discoloration of leaves (covering 30.1–75% of area) and pods, reduction in leaf size and stunting of plants	60.01–80.00	Susceptible (S)
6	Severe yellow discoloration covering >75% of foliage, stunting of plants and reduction in pod size	>80.00	Highly susceptible (HS)

3.13.4. Plant height (cm)

The plant height was measured from the ground level to the top of the plant. Heights of 5 plants randomly selected plants from each plot were measured. It was done at the flowering stage of the crop (at 45 DAS).

3.13.5. Number of primary branch/ plant

Primary branches were counted at the flowering stage. Branches of 5 randomly selected plants from each plot were counted and averaged.

3.13.6. Chlorophyll content

Following protocol [Witham's Acetone Method, 1996] was run to determine chlorophyll content.

- ✓ The randomly selected leaf samples were collected and kept in separate polythene bag.

- ✓ After collection, the leaf samples were immediately taken to the laboratory for subsequent analysis.
- ✓ Around 20 mg leaf samples was weighed and entered into glass vial containing 20 ml 80% acetone solution.
- ✓ The glass vials were kept into dark condition for 48 hours.
- ✓ 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.

Chlorophyll (a+b) mg g^{-1} leaf tissue = $\frac{[20.2\ (D645) + 8.02\ (D663)]\ X\ V}{1000\ X\ W}$

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

Data were presented in tabular and graphically.

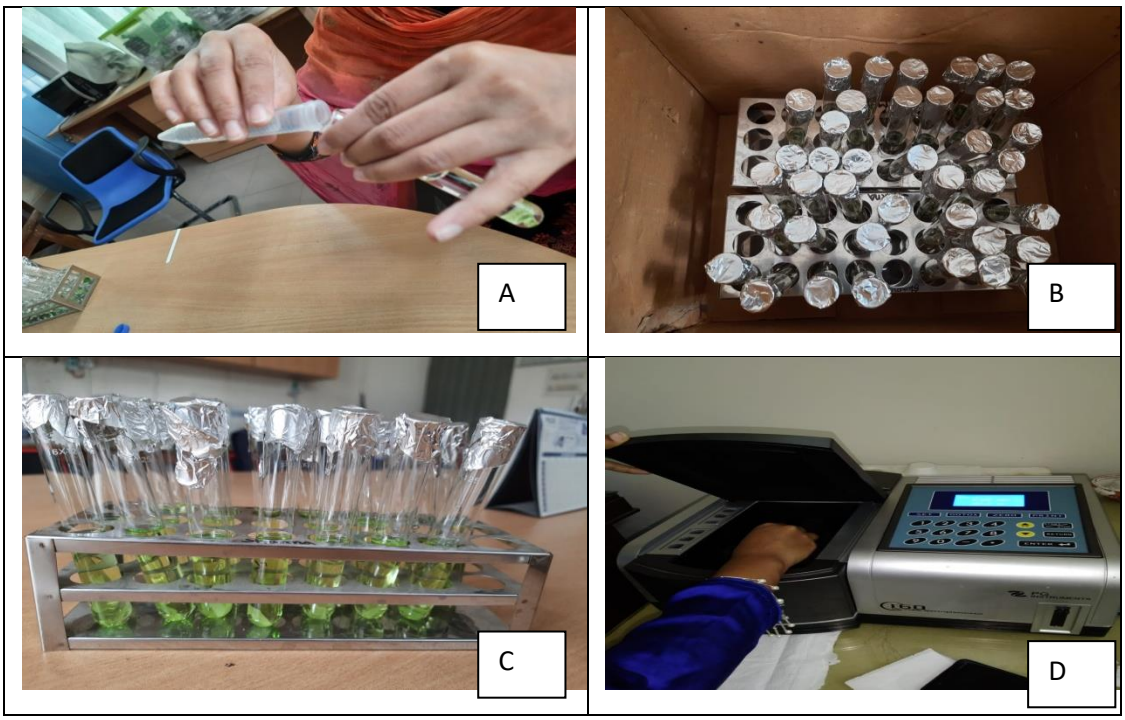

Plate 2. Different steps [A-D] of determination of chlorophyll content by acetone method.

3.13.7. Number of pod /plant

Pods were counted at the ripening stage. Pods of 5 randomly selected plants from each plot were harvested separately in three times. Total number of harvested pods in each time from each of the plant were counted and averaged.

3.13.8. Pod length (cm)

It was done after harvest. Firstly 5 plants per plot were selected randomly. Then length of 5 pods from each plant were measured and averaged.

3.13.9. Number of seed/pod

It was done after harvest. Firstly 5 pods per plot were selected randomly. Number of seeds pod^{-1} was counted. Sum of the seeds of the selected pods from each plot were counted and averaged.

3.13.10. Pod sterility

Pods were plucked from ten plants that selected randomly from each replicate plot. More than 50% unfilled pods were considered as sterile pod. Pod sterility was determined by calculating following formula:

$$\% \text{ Pod sterility} = \frac{Number\ of\ sterile\ pods/plant}{Total\ number\ of\ pods/\ plant} \text{ X } 100$$

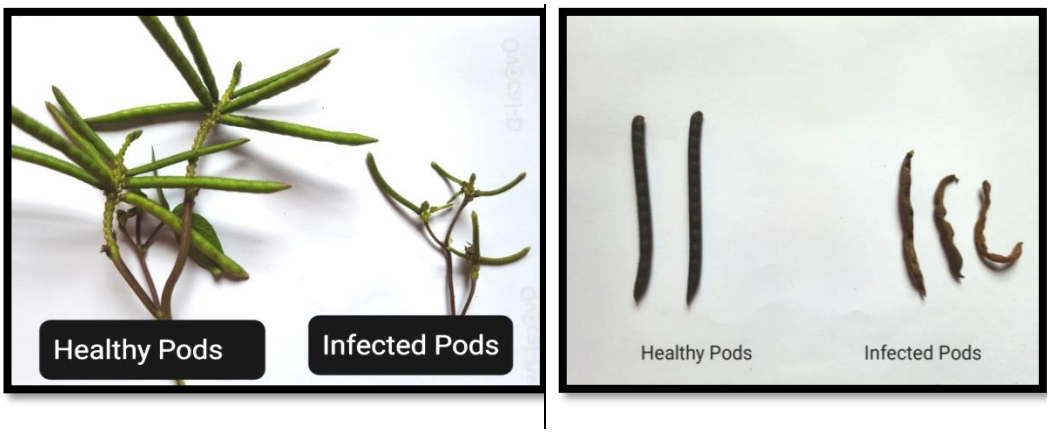

Plate 3: Healthy and Infected Pods

3.13.11. 1000-seed weight

Firstly harvested pods were dried and threshed. Then 1000 seeds from each plot were counted and weighed.

3.13.12. Yield (g/plot)

Harvested pods were dried and threshed firstly. Grains obtained from each plot (4.50 m^2) were dried, weighed carefully.

3.13.13. Yield (Kg/ha)

Grain weight (g) obtained from each plot (4.50 m^2) was converted to kg ha^{-1}.

3.14. Molecular Detection of *MYMV*

3.14.1.Primer Designing

Commercially synthesized primer pair used in the study is presented in table 3.

Table 3. Primer pair used in the present study to amplify *MYMV* at 900 bp fragment

Primers	Primer Sequences 5'-3'	T_m of Primers (°C)	Amplicon size (bp)
MYMV-CP-F	ATGGGKTCCGTT GTATGCTTG	59.4	900
MYMV-CP- R	GGCGTCATTAGC ATAGGCAAT	59.4	

3.14.2.List of Chemicals:

• CTAB extraction buffer
• 2% β-mercaptoethanol.
• Phenol:chloroform:isoamyl alcohol (P:C:I) (25:24:1).
• Chloroform:isoamyl alcohol (C: I) (24:1).
• 70% ethanol.
• 100% isopropanol (chilled).
• RNase
•3 M sodium acetate and
• TE buffer

3.14.3.Preparation of DNA Extraction Chemicals

- **2.5%CTAB extraction buffer**

  CTAB (also called hexadecyltrimethylammonium bromide) is a cationic detergent that facilitates the separation of polysaccharides during purification while additives, such as polyvinylpyrrolidone, aid in inactivating polyphenols. CTAB based extraction buffers are widely used when purifying DNA from plant tissues. The EDTA in CTAB works as a chelating agent in DNA extraction. It chelates the metal ions present in the enzymes, metal ions work as a cofactor to increase the catalytic activities of an enzyme. To prepare 2.5% CTAB extraction buffer, the following chemicals were dissolved in distilled water and mixing the chemicals thoroughly with the help of heating at 65℃ temperature in hot water bath.

  - 2.5% CTAB buffer
  - 20 mM Ethylenediaminetetraacetic Acid (EDTA),
  - 100 mM Tris-HCl (pH 8),
  - 2% w/v polyvinylpyrolidine (PVP), and
  - 1.4 M NaCl

- **2% β-mercaptoethanol**

  1ml of β-mercaptoethanol was dissolved into 50 ml distilled water to prepare 2% β-mercaptoethanol solution which was added to the pre-heat CTAB extraction buffer just before using. β-mercaptoethanol is widely used for retarding oxidation of biological compounds in solution and to reduce these disulfide bonds and irreversibly denature the proteins.

- **Phenol : chloroform : isoamyl alcohol (25:24:1)**

  Phenol remains in semi-solid condition in normal room temperature. At first phenol was heated to convert it into liquid form and then the amount of 25ml was measured by measuring cylinder. After that 24 ml of chloroform and 1 ml of isoamyl alcohol were measured and thoroughly mixed into previously measured phenol to prepare P:C:I solution. Finally the solution was preserved in glass jar for using it when

necessary. During DNA extraction process, a mixture of phenol: chloroform: isoamyl alcohol (25:24:1) is added to promote the partitioning of lipids and cellular debris into the organic phase, leaving isolated DNA in the aqueous phase.

- **Chloroform : isoamyl alcohol (C: I) (24:1)**

  24 ml of chloroform and 1 ml of isoamylalcohol were measured, mixed and preserved to make it ready for future using purpose. In DNA extraction method, Isoamyl alcohol helps in reducing foaming between interphase. It prevents the emulsification of a solution. The liquid phase contains DNA and the organic phase contains lipid, proteins and other impurities.

- **70% ethanol**

  70 ml of ethanol was diluted into distilled water and made the volume of 100ml. DNA is washed with 70% ethanol to remove some (or ideally all) of the salt from the pellet. DNA is a polar molecule due to its highly charged phosphate backbone. If enough ethanol is added, the electrical attraction between phosphate groups and any positive ions present in solution becomes strong enough to form stable ionic bonds and DNA precipitation.

- **100% isopropanol (chilled)**

  100% pure isopropanol was kept in refrigerator and made it chilled for using it in DNA extraction protocol. The use of chilled isopropanol increases the rate of precipitation of DNA and allows it to flocculate and settle very easily and quickly.

- **RNase**

  RNase (30 mg RNase dissolved in 1 ml of buffer containing 100 µl of 1 M Tris, pH 8.0, 30 µl of 5 M NaCl and remaining sterile millipore

water, kept for 15 min in hot water bath). Ready to use RNase was collected commercial chemical company and preserved it in -20℃ refrigerator**.** RNase is used to remove RNA during procedures for the isolation of plasmid and genomic DNA.

- **3 M sodium acetate**

  Sodium Acetate (3 M) is commonly used during DNA and RNA extraction. Sodium acetate, which is a salt, helps precipitate nucleic acids. 24.61 g of sodium acetate was added to 100ml distilled water to prepare 3M sodium acetate. Adjust the pH to 5.2 with glacial acetic acid. In DNA precipitation, a salt (sodium acetate) reacts with DNA. It breaks up into Na^+ and $(CH_3COO)^-$. The positively charged sodium ion to neutralize negatively charged PO_3^- of the DNA. Hydrophilic nature of DNA helps it to dissolve it in water but by reacting with sodium acetate, DNA becomes less hydrophilic.

- **TE buffer [10 mM Tris and 1mM EDTA, pH 8.0]**

  To prepare 10X TE buffer, 15.759 g of Tris-Cl and 2.92 g of EDTA were dissolved into 800ml of distilled water then adjusted the pH at 8.00. Finally distilled water was added up to volume 1L. After that the solution was converted in 1X. The pH of TE buffer is slightly basic however water have slightly acidic pH. This basic pH of TE buffer makes DNA more soluble and EDTA helps to protect from the DNase. TE buffer is also preferable over H_2O to store the DNA samples.

3.14.4. Sample Collection

The molecular detection was done at Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. For molecular detection of *MYMV* through PCR, the diseased and healthy leaves samples were collected from the experimental field.

3.14.5.DNA Extraction Protocol

Total genomic DNA was extracted following modified CTAB method according to Darshan *et. al.* (2017):

- 100 mg of fresh leaves were collected and rinsed with distilled water twice and dried.
- 1.0 ml of 2.5% CTAB buffer (preheated at 65℃ for at last 10 min) was added and ground quickly leaves without liquid nitrogen, using pre-chilled mortar-pestle.
- The homogenate were transferred into 2.0 ml microcentrifuge tubes followed by addition of 500µl of CTAB buffer and 30µl 2% β-merceptoethanol and mixed well by inversion of tubes.
- The tubes were incubated at 65℃ in water bath for 45-60 min along with continuous mixing after every 15 min and subsequently cool down by keeping them at room temperature (RT) for 10-15 min.
- The tubes were centrifuged at 11000 rpm for 10 min at 27-30℃.
- The supernatant was transferred into new fresh tubes and addition of equal volume of P:C:I(25:24:1) to each tube and mixed by inverting followed by centrifuge at 11000 rpm for 10 min at 27-30℃.
- 5µl DNase free RNase was added in supernatant of each tube and incubate at 37℃ for 20min
- Equal volume of C:I(24:1) was added to the tubes and centrifuged of tubes at 11000 rpm for 10 min at 27-30℃.
- The upper aqueous phase was transferred to a fresh tube and repeat C:I treatment
- The upper aqueous phase were transferred to new fresh tubes containing 150µl of 3M sodium acetate and add equal amount of 100% chilled isopropanol to the tube followed by gentle mixing by inverting. Thick and bright white pellets of DNA were observed. The tubes were kept at -20℃ for 2 hours or precipitation of DNA.

- The tubes were centrifuged at 10000 rpm for 15 min at 4℃
- The supernatant were discarded after centrifugation and wash pellets with 200μl of 70% ethanol by centrifuging tubes at 7000 rpm for 5 min at 27-30℃ . The ethanol wash was repeated and the supernatant was discarded.
- The pellets were air-dried at room temperature or 45min. Finally the pellets were dissolved in 200µl of 1X Tris-EDTA (1X TE) buffer at room temperature and store at -20℃ for further use.

3.14.6.Genomic DNA analysis with Agarose Gel

Genomic DNA was extracted from leaf sample. Extracted total genomic products (5µl of each) were subjected to 0.8% (w/v) agarose gel electrophoresis with 2 µl of loading dye at 100 volts for 45min in TBE buffer and stained with ethidium bromide (1µl) and visualized under UV trans-illuminator and gel documentation system for confirmation (Figure 1).

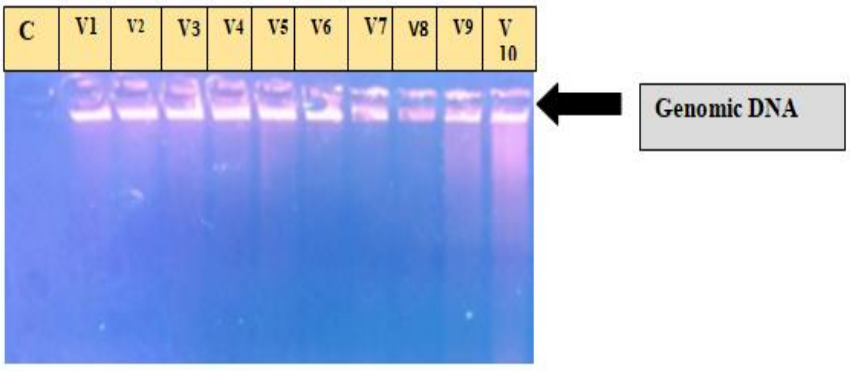

Figure 1. Genomic DNA was analyzed in 0.8% agarose gel.

3.14.7.PCR amplification

PCR was conducted in a reaction volume (25 µl) containing 15.3 µl of sterile water, 2.5 µl of 10X PCR buffer, 2 µl of dNTP (2.5 mM), 2 µl of forward primer, 2µl of reverse primer, 0.2 µl of Taq polymerase and 1 µL of template DNA (diluted 1:25 in water). Following thermal program was performed. Then the PCR products were stored at -20°C.(figure-2)

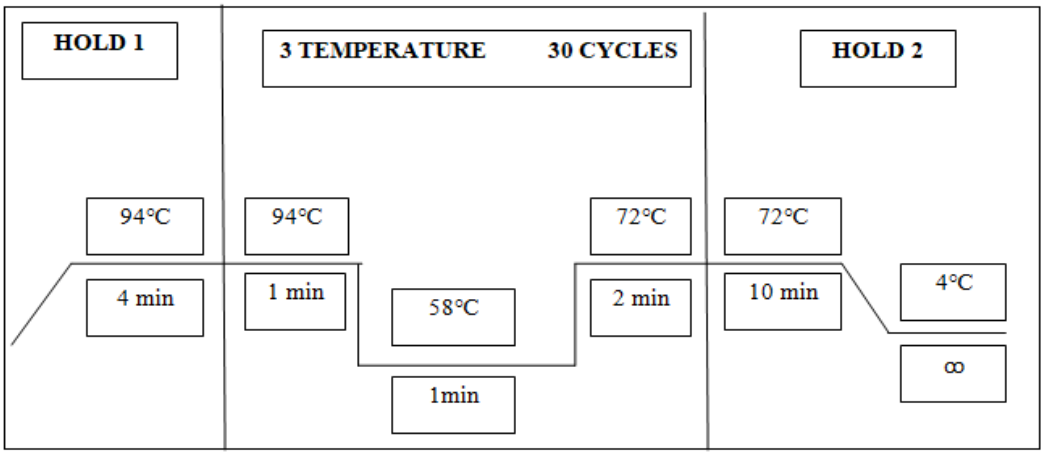

Figure 2. PCR cycling conditions to amplify *MYMV-CP* gene fragment

3.14.8.Agarose Gel Electrophoresis

PCR products (25 µl of each) were subjected to 0.8% (w/v) agarose gel electrophoresis with 2 µl of loading dye at 100 volts for 45min in TBE buffer and stained with ethidium bromide (1µl) and visualized under UV trans-illuminator and gel documentation system. The results were verified against DNA marker.

3.15. 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

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted to observe the varietal performance of mungbean cultivars against *Mungbean yellow mosaic virus (MYMV)* and to detect the *MYMV* through PCR. In total ten mungbean cultivars viz., BARI mung-5, BARI mung-6, BARI mung-7, BARI mung-8, BINAmoog-5, BINAmoog-8, BINAmoog-9, BU mug1, Sonamug, and Chaitamug were selected and observed their morphological and yield performance against *MYMV*. Molecular detection was also performed in this study. The results of the present study have been presented, discussed and compared as far as possible with the results of other research.

4.1. Response of Tested Mungbean Cultivars against *Mungbean Yellow Mosaic Virus*

4.1.1. Disease incidence (%) per plot

Percent disease incidence per plot was recorded at 30, 45 and 60 days after sowing (DAS). From the result, it was revealed that disease incidence (%) per plot was varied significantly. Results are presented in Table 4.

At 30 DAS, the plot wise disease incidence was ranged from 2.22% to 7.56%. Minimum disease incidence (2.22%) was found in V_3 (BARI mung-7) and V_7 (BINAmoog-9), which was statically similar each and other. Maximum disease incidence was found in V_9 (Sonamug; 7.56%) followed by V_{10} (Chaitamug; 6.00%) which was statistically different each and other. The results obtained from the rest of the varieties, provided intermediate results compared to the highest and the lowest value and did not show statistical variance.

At 45 DAS, maximum disease incidence (26.00%) was found in V_{10} (Chaitamug) and minimum disease incidence (10.66%) was found in V_3 (BARI Mung-7). Among the other cultivars, the second highest disease incidence (19.11%) was found in V_9 (Sonamug) which was identical with V_1 (BARI mung-5, 18.00%). The results obtained from the rest of the varieties, viz; BARI

mung-6 (11.55%), BINAmoog-9 (12.22%), BU mug-1 (12.66%), BINAmoog-5(12.89%), and BINAmoog-8 (13.33%) were statistically identical to V_{10} (Chaitamug), which showed the minimum disease incidence.

At 60 DAS, maximum disease incidence (42.77%) was found in V_{10} (Chaitamug) followed by Sonamug (35.78%), BARI mung-5 (30.89%); whereas Chaitamug, Sonamug and BARI mung-5 were statistically different. Minimum disease incidence (16.44%) was found in V_3 (BARI Mung-7). The remaining tested varieties were showed intermediate disease incidence (%) which was statistically identical to each and other.

Table 4. Disease incidence per plot of different Mungbean cultivars at 30, 45, and 60 DAS against *Mungbean yellow mosaic virus*

Cultivar	%Disease incidence / plot		
	30 DAS (before flowering)	45 DAS (at flowering)	60 DAS (at Ripening)
V_1 (BARI mung-5)	4.22 cd	18.00 b	30.89 c
V_2 (BARI mung-6)	3.55 de	11.55 cd	23.55 ef
V_3 (BARI mung-7)	2.222 f	10.66 d	16.44 g
V_4 (BARI mung-8)	2.89 ef	11.55 cd	26.22 de
V_5 (BINAmoog-5)	4.00 cde	12.89 cd	27.11 cde
V_6 (BINAmoog-8)	3.77 cde	13.33 c	26.66 de
V_7 (BINAmoog-9)	2.222 f	12.22 cd	21.55 f
V_8 (BU mug1)	4.89 bc	12.66 cd	28.66 cd
V_9 (Local -1)	7.56 a	19.11 b	35.78 b
V_{10} (Chaitamug)	6.00 b	26.00 a	42.77 a
CV (%)	16.54	9.20	8.35

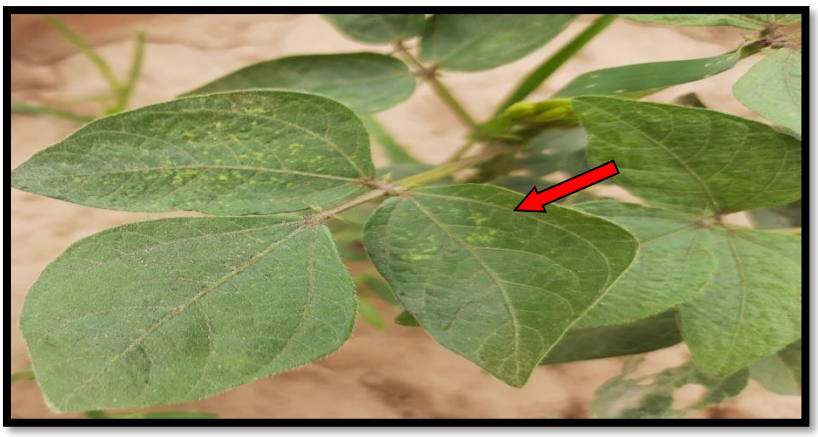

Figure 3. Disease Incidence at 30 DAS

Figure 4. Disease Incidence at 45 DAS

Figure 5. Disease Incidence at 60 DAS

4.1.2. Disease incidence (%) per plant

Disease incidence of tested Mungbean cultivars against *Mungbean yellow mosaic virus* were also measured at 30 DAS, 45 DAS and 60 DAS differed significantly (Table 5).

At 30 DAS, the lowest disease incidence was found in V_7 (BINAmoog-9; 3.49%) followed by V_3 (BARI Mung-7; 3.55%), and V_4 (BARI Mung-8; 3.56%); which was statistically identical to each and other. The highest disease incidence was found in V_{10} (Chaitamug; 7.06%); the second highest disease incidence was found in V_9 (Chaitamug; 6.05%) which was statistically identical to V_2 (BARI mung-6; 6.15%).

At 45 DAS, the highest disease incidence (30.47%) was found in V_{10} (Chaitamug) followed by V_9 (Local -1; 25.32%) and the lowest disease incidence (10.14%) was found in V_4 (BARI Mung-8) followed by V_6 (BINAmoog-8; 12.24%), V_3 (BARI mung-7; 12.78%); where Sonamug and BINAmoog-8 was statistically different to each other, BINAmoog-8 and BARI mung-7 was statistically identical. The results obtained from the remaining varieties *viz;* BARI mung-5, BARI mung-6, BINAmoog-5 and BINAmoog-9 showed intermediate results which was statistically identical to each and other.

It was observed that disease incidence among the tested varieties was increased with the increase of plant age. During final data collection at 60 DAS, the highest disease incidence (50.99%) was found in V10 (Chaitamug) followed by Sonamug (40.81%), BARI mung-5 (38.82%); which showed statistically significant difference among their values. The lowest disease incidence (20.69%) was found in V_3 (BARI Mung-7) followed by V_7 (BINAmoog-9; 26.28%), V_6 (BINAmoog-8; 29.35%); showing statistically significant differences to each and other.

Table 5. Disease incidence per plant of different Mungbean cultivars at 30,45 and 60 DAS against *Mungbean yellow mosaic virus*

Cultivar	%Disease incidence/ plant		
	30 DAS (before flowering)	45 DAS (at flowering)	60 DAS (at ripening)
V_1 (BARI mung-5)	4.28 d	18.22 c	38.82 c
V_2 (BARI mung-6)	6.15 b	15.53 de	31.92 f
V_3 (BARI mung-7)	3.55 e	12.78 f	20.69 i
V_4 (BARI mung-8)	3.56 e	10.14 g	31.91 f
V_5 (BINAmoog-5)	4.50 d	15.98 d	34.89 e
V_6 (BINAmoog-8)	5.48 c	12.24 f	29.35 g
V_7 (BINAmoog-9)	3.49 e	14.78 e	26.28 h
V_8 (BU mug1)	5.71 bc	17.79 c	37.16 d
V_9 (Local variety -1)	6.05 b	25.32 b	40.81 b
V_{10} (Chaitamug)	7.06 a	30.47 a	50.99 a
CV (%)	6.23	2.91	2.72

4.1.3. Percent Disease Index (PDI)

Under the present study, percent disease index of different Mungbean cultivars viz., BARI mung-5, BARI mung-6, BARI mung-7, BARI mung-8, BINAmoog-5, BINAmoog-8, BINAmoog-9, BU mug1, Sonamug, Chaitamug were measured against *Mungbean yellow mosaic virus* at 30, 45 and 60 DAS. Results are presented in Table 6.

Initially, at 30 DAS, there was no significant difference among the tested varieties. Maximum disease severity (11.33%) was found in V_{10} (Chaitamug). Minimum disease severity (4.67%) was found in V_3 (BARI Mung-7).

At 45 DAS, 15 days interval from first day of data collection, PDI in tested varieties was varied significantly. V_9 (Sonamug) showed the highest disease

severity (33.33%) and V_{10} (Chaitamug) showed the second highest disease severity (32.67%) followed by BARI mung-5 (29.33%), BU mug-1 (26.67%), BINAmoog-5 (24.67%), BARI mung-6 (24.00%), andBINAmoog-8 (23.33%); which were statistically identical with each and other. Minimum disease severity was found in V3 (BARI mung-7; 18.67%) which was statistically identical with BINAmoog-9 (20.00%) and BARI mung-8 (20.67%).It was observed that disease severity among the tested varieties was increased with the increase of plant age. During final data collection at 60 DAS, the highest disease incidence (82.00%) was found in V_{10} (Chaitamug) followed by Sonamug (77.33%), BU mug-1 (62.67%) which showed statistically significant different result. Minimum disease incidence (32.67%) was found in V_3 (BARI Mung-7) which was statistically identical to BINAmoog-9 (33.33%) and BARI mung-8 (34.67%). The remaining tested varieties showed intermediate result which was identical to each and other.

Table 6. Disease severity of different mungbean cultivars at 30, 45, and 60 DAS against *Mungbean yellow mosaic virus*

Cultivar	PDI (disease severity) with 15 days interval		
	30DAS (before flowering)	45DAS (at flowering)	60DAS (at ripening)
V_1(BARI mung-5)	6.00 cd	29.33 b	57.33 d
V_2(BARI mung-6)	6.00 cd	24.00 d	44.00 d
V_3(BARI mung-7)	4.67 d	18.67 e	32.67 e
V_4(BARI mung-8)	4.67 d	20.67 e	34.67 e
V_5(BINAmoog-5)	8.00 bc	24.67 cd	47.33 d
V_6(BINAmoog-8)	5.33 d	23.33 d	45.33 d
V_7(BINAmoog-9)	5.33 d	20.00 e	33.33 e
V_8(BU mug1)	8.66 b	26.67 c	62.67 c
V_9(Sonamug)	10.00 ab	33.33 a	77.33 b
V_{10}(Chaitamug)	11.33 a	32.67 a	82.00 a
CV (%)	18.32	5.41	7.84

Figure 6. Disease severity at 30DAS

Figure 7. Disease severity at 45DAS

Figure 8. Disease severity at 60DAS

4.1.4 Reaction of mungbean cultivars against *MYMV*

According to the AVRDC PDI Scale, it was revealed that Percent Disease Index of the tested varieties ranges from highly susceptible to moderately resistance (Table 7). Among the tested varieties, Chaitamug collected from Lalmonirhat showed highly susceptibility (82.00%) and Sonamug (77.33%), BU mug-1 (62.67%), BARI mung-5 (57.33%) and BARI mung-6 (44.00%) showed susceptibility to *MYMV*. The remaining tested varieties, BARI mung-7 (32.67%), BINAmoog-9 (33.33%) and BARI mung-8 (34.67%) were moderately resistant. All tested mungbean varieties were infected and appear the remarkable symptoms of *MYMV* (Table 7).

Table 7. Response of selected mungbean cultivars against *Mungbean yellow mosaic virus (MYMV)*

Tested varieties	PDI at 60DAS	Plant response to *MYMV*
V_1(BARI mung-5)	57.33	Moderately Susceptible
V_2(BARI mung-6)	44.00	Moderately Susceptible
V_3(BARI mung-7)	**32.67**	**Moderately resistant**
V_4(BARI mung-8)	**34.67**	**Moderately resistant**
V_5(BINAmoog-5)	47.33	Moderately Susceptible
V_6(BINAmoog-8)	45.33	Moderately Susceptible
V_7(BINAmoog-9)	**33.33**	**Moderately resistant**
V_8(BU mug1)	62.67	Susceptible
V_9(Sonamug)	77.33	Susceptible
V_{10}(Chaitamug)	**82.00**	**Highly Susceptible**

4.2. *MYMV* Detection through PCR

Now a day molecular detection through PCR is reliable technology. After PCR amplification through standard protocol as describe in methodology section, the samples of PCR product were loaded in agarose gel (0.8%). DNA ladder (100bp) was used in between two sides of the samples loaded representing ten tested mungbean varieties. From the gel documentation (Figure 9), it was confirmed that all the tested varieties showed PCR positive result (Table-8) and gave sharp band at 900 bp that indicates virus present in the ten tested varieties.

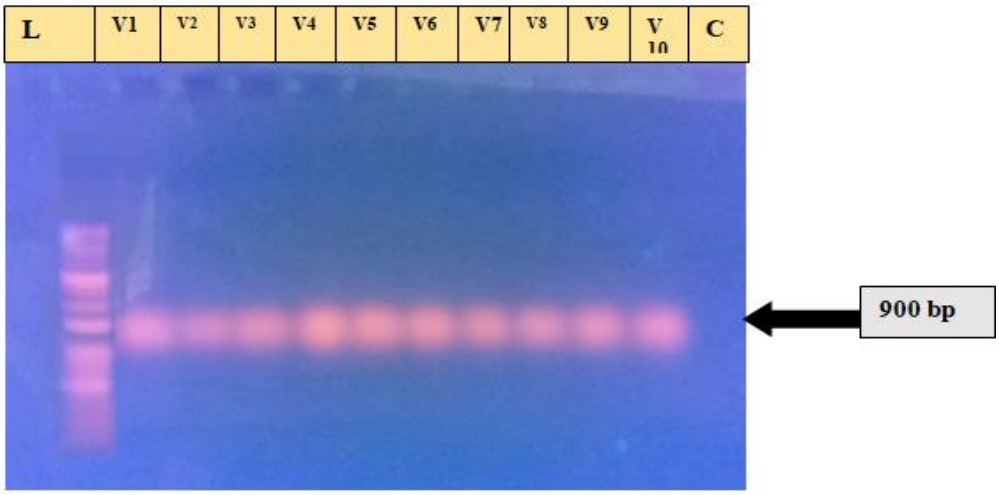

Figure 9. PCR amplification to detect *MYMV* representing tested Mungbean varieties where L denotes 2kbp DNA ladder; V1 to V10 denote gel images of of PCR products obtained from different mungbean varieties and C denotes control.

Table 8. PCR test for *MYMV* detection

Sl. No.	Tested varieties	Result
1	BARI mung-5	Detected
2	BARI mung-6	Detected
3	BARI mung-7	Detected
4	BARI mung-8	Detected
5	BINAmoog-5	Detected
6	BINAmoog-8	Detected
7	BINAmoog-9	Detected
8	BU mug1	Detected
9	Sonamug	Detected
10	Chaitamug	Detected

4.3. Morphological features related to yield contributing character

4.3.1. Plant height (cm)

Plant height was recorded once in time at flowering stage (45 DAS).From the study it was observed that significant variation of the plant height was found among the tested varieties (Table-9). The shortest plant was recorded in Chaitamug (49.43cm). On the other hand, the tallest plant was obtained in the variety BARI mung-7 (59.20cm) followed by variety BARI mung-6(57.80cm), BARI mung-8 (56.30cm), BINAmoog-8 (54.40cm), Sonamug (53.06cm), BINAmoog-9 (43.33cm), BINAmoog-5 (42.87cm), BU mug-1 (42.23cm), BARI mung-5 (42.00cm). Among the tested varieties, BARI mung-7, BARI mung-6, BARI mung-8, BINAmoog-8, Sonamug and Chaitamug showed statistically significant difference; while there was no significant difference was found in the varieties; BINAmoog-9, BINAmoog-5, BU mug-1, and BARI mung-5.

4.3.2. Number of branches per plant

Number of branches per plant was significantly influenced by the different tested varieties. The data were recorded at flowering stage (45 DAS). Maximum number of branches per plant was recorded in the variety BARI Mung-7 (4.27), followed by BARI mung-8 (4.07), BARI mung-6 (3.47), Chaitamug(3.07), and BINAmoog-8(2.80); whereas statistically identical result was shown by BARI mung-7 and BARI mung-8. Minimum number of branches per plant was recorded in the variety BU mug-1(2.00) followed by BINAmoog-9(2.47), Sonamug (2.53), BINAmoog-5 (2.53) and BARI mung-5 (2.67). There was significant difference between the varieties BU mug-1 and BINAmoog-9; but there was no significant difference among the varieties BARI mung-5, BINAmoog-5, Sonamug and BINAmoog-9. Results are presented in Table 9.

Table 9. Plant height and Nnmber of branches per plant in tested mungbean varieties against *Mungbean yellow mosaic virus (MYMV)*

Cultivar	Plant Height(cm)	No of Branch/ Plant
V_1 (BARI mung-5)	42.00 h	2.67 de
V_2 (BARI mung-6)	57.80 b	3.47 b
V_3 (BARI mung-7)	59.20 a	4.27 a
V_4 (BARI mung-8)	56.30 c	4.07 a
V_5 (BINAmoog-5)	42.87 fg	2.53 e
V_6 (BINAmoog-8)	54.40 d	2.80 d
V_7 (BINAmoog-9)	43.33 f	2.47 e
V_8 (BU mug1)	42.23 gh	2.00 f
V_9 (Sonamug)	53.06 e	2.53 e
V_{10} (Chaitamug)	39.43 i	3.07 c
CV (%)	0.89	4.80

4.3.3. Number of pods per plant

The result (Table-10) showed that the mungbean varieties differed significantly from each other in number of pods per plant. Maximum number of pods was obtained from BARI mung-8 (29.53) followed by BINAmoog-9 (28.00), BINAmoog-8 (26.40), and BARI mung-7 (25.67); the previously mentioned varieties were statistically different to each and another. Minimum number of pods was observed from BINAmoog-5 (15.47) followed by Sonamug (16.33), Chaitamug (18.33), BARI mung-5 (17.33), BARI mung-6 (18.73), and BU mug-1 (19.20). Among the other varieties, there was statistically significant difference between BINAmoog-5 and Sonamug; whereas Chaitamug, BARI mung-5, BARI mung-6, and BU mug-1 showed no statistically significant difference.

4.3.4. Pod length (cm)

Significant influence was found in case of pod length among the different varieties under the present study (Table-10). Results indicated that the highest pod length (9.20cm) was observed in V_3 (BARI Mungbean-7) which was statistically identical with V_4 (BARI mung-8; 9.00cm) and V_7 (BINAmoog-9; 8.50cm) and V_6 (BINAmoog-8; 8.23cm). The lowest pod length (5.23 cm) was obtained in V_{10} (Chaitamug) followed by V_5 (BINAmoog-5; 5.33cm) and V_1 (BARI mung-5; 5.46cm) which were identical to each and other. The remaining tested varieties, V_8 (BU mug-1; 6.50cm) and V_9 (Sonamug; 6.40cm) showed intermediate result which were identical to each and other.

4.3.5. Number of seeds per pod

Significant variation was observed for number of pods plant-1 among the different varieties (Table-10). The highest number of seeds per pods (13.10) was observed in V_4 (BARI Mung-8) which was closely followed by V_3 (BARI mung-7). The lowest number of seeds per pod (6.91) was obtained from V_{10} (Chaitamug) which was identical with BARI mung-5 (8.20), BINAmoog-5 (7.89), Sonamug (7.36), and BU mug-1 (7.19) .The results obtained from the rest of the varieties, were intermediate type compared to highest and lowest value. Among them, BARI mung-6 (10.68) and BINAmoog-9 (10.50) were shown no significant differences.

4.3.6. Percent pod sterility

Significant effect in percent pod sterility was observed in tested mungbean varieties (Table-10). The pod sterility under different varieties ranged from 6.74% to 24.49% while the highest sterile pod was recorded in Chaitamug mungbean variety which was collected from Lalmonirhat. The second highest pod sterility was found in V_5 (BINAmoog-5; 20.24%) followed by V_1 (BARI mung-5; 19.61%) and V_9 (Sonamug; 18.93%), which were identical to each other. In contrast, the lowest sterile pod was found in BARI Mung-7 (6.74%) followed by BARI mung-8 (8.12%), BINAmoog-9 (8.46%); there was no

statistically significant difference among the varieties. The results obtained from the rest of the varieties, were intermediate type compared to highest and lowest value.

Table 10. Number of Pod/Plant, Pod Length, Number of Seed/ Pod, Percent pod sterility in tested mungbean cultivars against *Mungbean yellow mosaic virus (MYMV)*

Cultivar	No. Pod/ plant	Pod length (cm)	No. Seed / pod	% pod sterility
V_1(BARI mung-5)	17.33 f	5.46 e	8.20 e	19.61 b
V_2(BARI mung-6	18.73 ef	8.46 bc	10.68 c	12.09 d
V_3(BARI mung-7)	25.67 d	9.20 a	12.24 b	6.74 f
V_4(BARI mung-8)	29.53 a	9.00 ab	13.10 a	8.12 ef
V_5(BINAmoog-5)	15.47 h	5.33 e	7.89 ef	20.24 b
V_6(BINAmoog-8)	26.40 c	8.23 c	9.53 d	9.57 de
V_7(BINAmoog-9)	28.00 b	8.50 bc	10.5. c	8.46 ef
V_8(BU mug1)	19.20 e	6.50 d	7.19 g	15.61 c
V_9(Sonamug)	16.33 g	6.40 d	7.36 fg	18.93 b
V_{10}(Chaitamug)	18.33 f	5.23 e	6.91 g	24.49 a
CV (%)	1.7	4.95	3.53	11.12

4.4. Physiological features related to yield contributing character:

Chlorophyll content:

Chlorophyll content was measured through following Witham -formula, also known as 'Acetone method' for chlorophyll content determination. At 45DAS, chlorophyll content of the plant was showed significant variance among the tested mungbean varieties. The maximum chlorophyll content was obtained in the variety BARI mung-7 (2.7040 µg/g) followed by variety BARI mung-6 (2.2881 µg/g), BINAmoog-9(2.2813 µg/g), BARI mung-8 (2.1517 µg/g), BINAmoog-8 (1.9163 µg/g) and the lowest chlorophyll content was obtained in Chaitamug (1.0833 µg/g), all of the varieties showed statistically significant difference. Results are presented in Table 11.

Table 11. Leaf chlorophyll content in selected mungbean varieties against *Mungbean yellow mosaic virus (MYMV)*

Varieties	Chlorophyll Content (µg/g)
V_1 (BARI mung-5)	1.2860 i
V_2 (BARI mung-6)	2.2881 b
V_3 (BARI mung-7)	2.7040 a
V_4 (BARI mung-8)	2.1517 d
V_5 (BINAmoog-5)	1.8093 f
V_6 (BINAmoog-8)	1.9163 e
V_7 (BINAmoog-9)	2.2813 c
V_8 (BU mug-1)	1.5130 g
V_9 (Sonamug)	1.3210 h
V_{10} (Chaitamug)	1.0833 j
CV (%)	0.82

4.5. Yield Data Obtained from Tested Mungbean Varieties

4.5.1. 1000 seed weight

Variety had a significant effect in 1000 seed weight and it was also observed in studied mungbean (Results are presented in Table 12). The 1000 seed weight under different varieties ranged from 33.64g to 49.22 g while highest weight was recorded in BARI Mung-6 (49.22 g) followed by BARI Mung-7 (45.83 g) and BINAmoog-9 (41.82 g). In contrast, the lowest weight of 1000 seed (g) was found in BARI Mung-8 (33.64 g) which followed by BINAmoog-8 (37.97 g) and Chaitamug (38.06 g). The results obtained from the rest of the varieties, were intermediate type compared to highest and lowest value.

4.5.2. Yield (g plot^{-1})

Significant variation was found in case of yield per plot, among the different varieties under the present study (Table 12). The highest yield (1000.7 g plot^{-1}) was observed in V_3 (BARI Mung-7) followed by BINAmoog-9 (883.70 g plot^{-1}), BARI mung-8 (766.40 g plot^{-1}), BARI mung-6 (756.90 g plot^{-1}). The lowest yield (330.60 g plot^{-1}) was obtained from V_{10} (Chaitamug) from Lalmonirhat. The results obtained from the rest of the varieties, BINAmoog-8 (686.60 g polt^{-1}), BINAmoog-5 (569.00 g polt^{-1}), BU mug1 (502.90 g polt^{-1}), BARI mung-5 (451.5 g plot^{-1}), and Sonamug (427.50 g plot^{-1}) provided intermediate results compared to the highest and the lowest value. Results are presented in Table 12.

4.5.3. Yield (kg ha^{-1})

Significant variation was found in case of yield (Kg ha^{-1}), among the different varieties under the present study (Table-12). The highest yield (2254.5 Kg ha^{-1}) was observed in V_3 (BARI Mung-7) followed by BINAmoog-9 (1963.90 Kg ha^{-1}), BARI mung-8 (1703.10 Kg ha^{-1}), BARI mung-6 (1682.0 Kg ha^{-1}). The lowest yield (734.70 Kg ha^{-1}) was obtained from V_{10} (Chaitamug) from Lalmonirhat. The results obtained from the rest of the varieties, BINAmoog-8 (1525.8 Kg ha^{-1}), BINAmoog-5 (1264.5 Kg ha^{-1}), BU mug1 (1117.50 Kg ha^{-1}), BARI mung-5 (1003.3 Kg ha^{-1}), and Sonamug (734.70 Kg ha^{-1}) provided intermediate results compared to the highest and the lowest value. Results are presented in Table 12.

Table 12. Thousand seed weight, Yield per plot and Yield per Hectare (estimated) in selected mungbean varieties against *Mungbean yellow mosaic virus (MYMV)*

Cultivar	1000-seed wt.	Yield (g/plot)	Yield (Kg/ ha)
V_1 (BARI mung-5)	39.57 d	451.5 g	1003.3 g
V_2 (BARI mung-6)	49.22 a	756.9 c	1682.0 c
V_3 (BARI mung-7)	45.83 b	1007.8 a	2254.5 a
V_4 (BARI mung-8)	33.64 f	766.4 c	1703.1 c
V_5 (BINAmoog-5)	39.37 d	569.0 e	1264.5 e
V_6 (BINAmoog-8)	37.97 e	686.6 d	1525.8 d
V_7 (BINAmoog-9)	41.82 c	883.7 b	1963.9 b
V_8 (BU mug1)	41.70 c	502.9 f	1117.5 f
V_9 (Sonamug)	46.45 b	427.5 g	950.1 g
V_{10} (Chaitamug)	38.06 e	330.6 h	734.7 h
CV (%)	1.34	2.26	2.30

4.6. Relationship between DI and PDI with morphophysiological features and Yield Contributing Characters

Relationship between morphological features of different mungbean varieties with disease incidence and severity at 60 DAS

The data revealed that there was significant negative correlation between plant height and number of branches with disease development. The tallest plant (59.20cm) and the highest number of branch (4.27) were observed in V_3 (BARI mung-7) which showed minimum disease incidence (plot wise, 16.44% and plant wise, 20.69%) and severity (PDI, 32.67%). On the other hand, the shortest plant (39.43 cm) was found in V_{10} (Chaitamug) which showed maximum disease incidences (plot wise, 42.77% and plant wise, 50.99%) and disease severity (PDI, 82.00%). (The result is illustrated in figure 10 and figure 11)

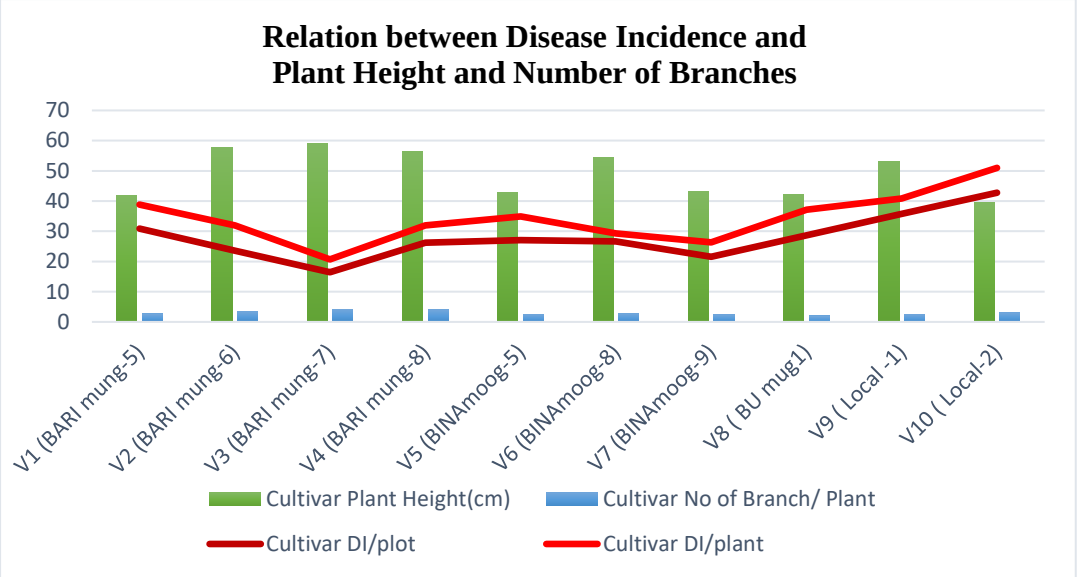

Figure 10. Plant height and Number of branches of different mungbean varieties in relation to disease incidence

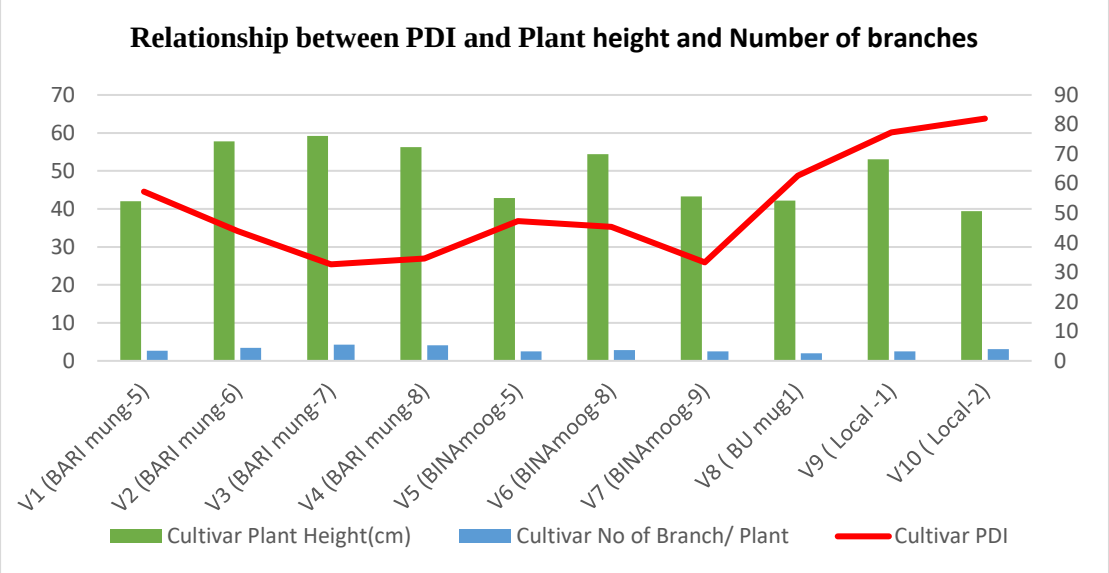

Figure 11. Plant height and Number of branches of different mungbean varieties in relation to disease severity

Relationship between Chlorophyll Content of different mungbean varieties with disease incidence and severity at 60 DAS

In tested varieties, chlorophyll content at 45DAS was varied from 2.7040 (BARI mung-7) to 1.0833 (Chaitamug) µg/g, which was closely related to disease incidence and disease severity. Maximum disease incidences (plot wise, 42.77% and plant wise, 50.99%) and disease severity (PDI, 82.00%) was

found in V_{10} (Chaitamug) which variety have minimum chlorophyll content. On the other hand, minimum disease incidences (plot wise, 16.44% and plant wise, 20.69%) and disease severity (PDI, 32.67%) was observed in V_3 (BARI mung-7) which contained maximum chlorophyll content.(the result is illustrated in figure 12 and figure 13)

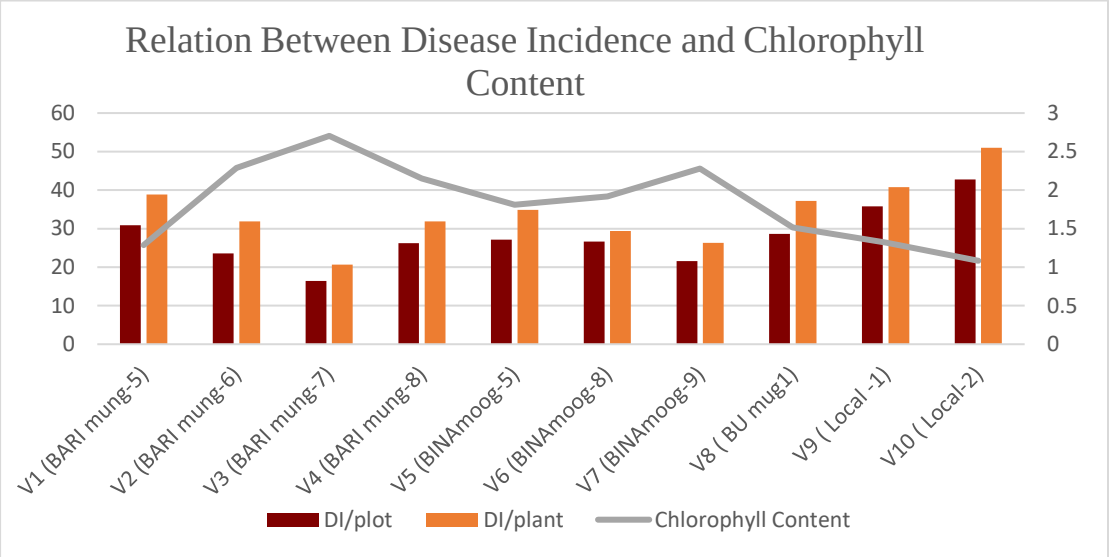

Figure 12. Chlorophyll content of different mungbean varieties in relation to disease incidence

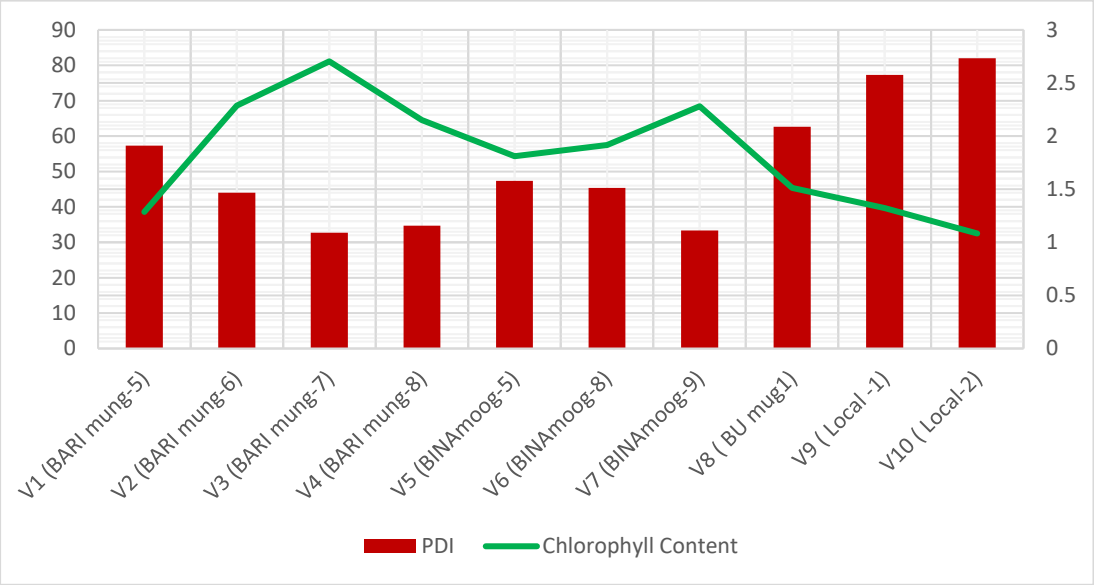

Figure 13. Chlorophyll content of different mungbean varieties in relation to disease severity

Relationship between disease incidence and severity at 60 DAS with yield contributing characteristics of different mungbean varieties

Pod sterility which is one of the most important yield contributing factors of mungbean differed significantly among the different varieties in relation to disease development. The highest number of sterile pod (24.49%) was found in Chaitamug which was the most infected (PDI, 82.00%) variety. On the contrary, the lowest number of sterile pod (6.74%) was found in BARI mung-7 which was the least infected (PDI, 32.67%) among the tested varieties. (the result is illustrated in figure 14 and figure 15)

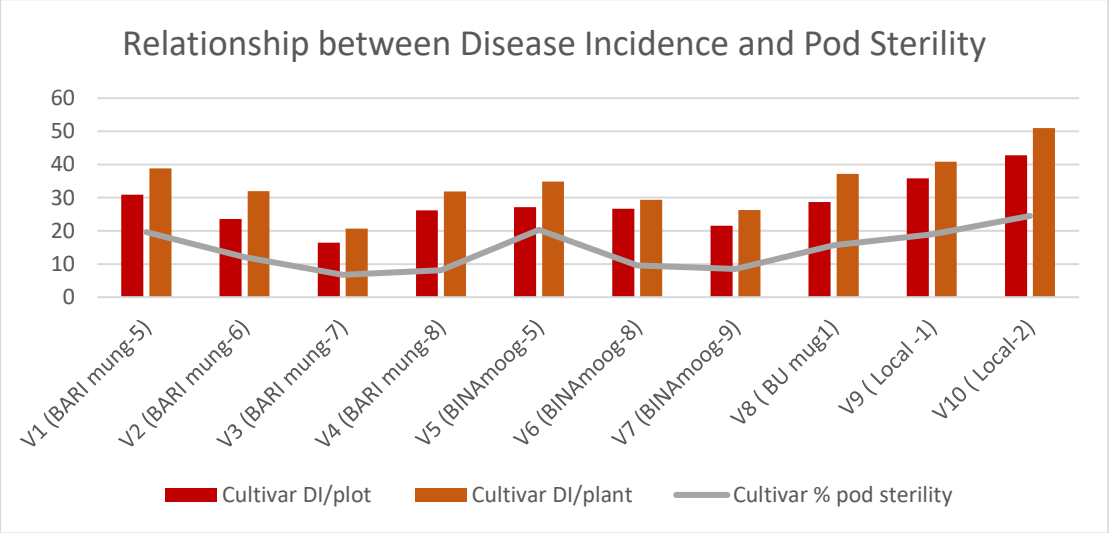

Figure 14. Pod sterility percentage of different mungbean varieties in relation to disease incidence

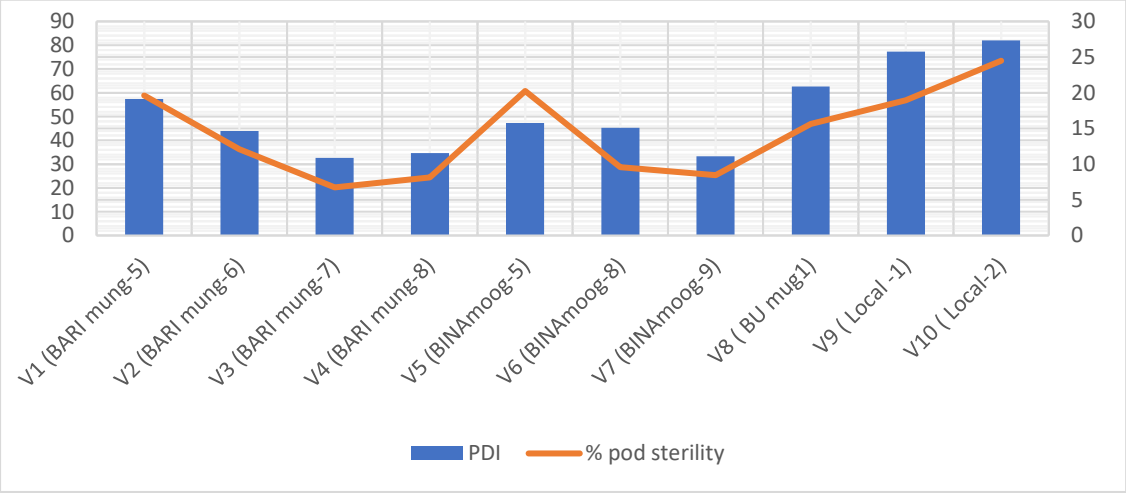

Figure 15. Pod sterility percentage of different mungbean varieties in relation to disease severity

The present experimental results revealed that Variety had a significant effect in 1000-seed weight and it was also observed in tested mungbean varieties. The 1000 seed weight under different varieties ranged from 33.64 g to 49.22g while highest weight was recorded in BARI mung-6 (49.22 g) and the lowest weight of 1000 seed (g) was found in BARI mung-8 (33.64 g). The variation in 1000-seed weight might be due to variation in the genetic make-up of the varieties.

The present experimental results revealed that Yield ($kgha^{-1}$) of tested mungbean varieties was related to disease incidence and severity. Lower quantity of yield was observed with increased disease incidence and severity. Significant variation was also observed for number of seed yield Kg ha^{-1} among the different varieties. Results revealed that the highest seed yield (2254.50 kg ha^{-1}) was observed in V_7 (BARI mung-7) and the lowest seed yield (734.7 kg ha^{-1}) was obtained from V_{10} (Chaitamug). Plot wise minimum disease incidence was found in V_3 (BARI mung-7) at all growth stages (2.22, 10.66 and 16.44 % at 30, 45 and 60 DAS respectively) where the highest disease incidence found in V_{10} (Chaitamug; 42.77 %) at 60 DAS. Considering disease incidence per plant, minimum disease incidence (31.91%) was observed in minimum disease incidence was V_3 (BARI mung-7) and V_{10} (Chaitamug) showed maximum disease incidence. Likewise Minimum disease severity was found in V_3 (BARImung-7) (4.67, 18.67 and 32.67 % at 30, 45 and 60 DAS respectively) whereas the maximum disease severity (82.00%) was found in V_{10} (Chaitamug) at 60 DAS. (the result is illustrated in figure 16 and figure 17)

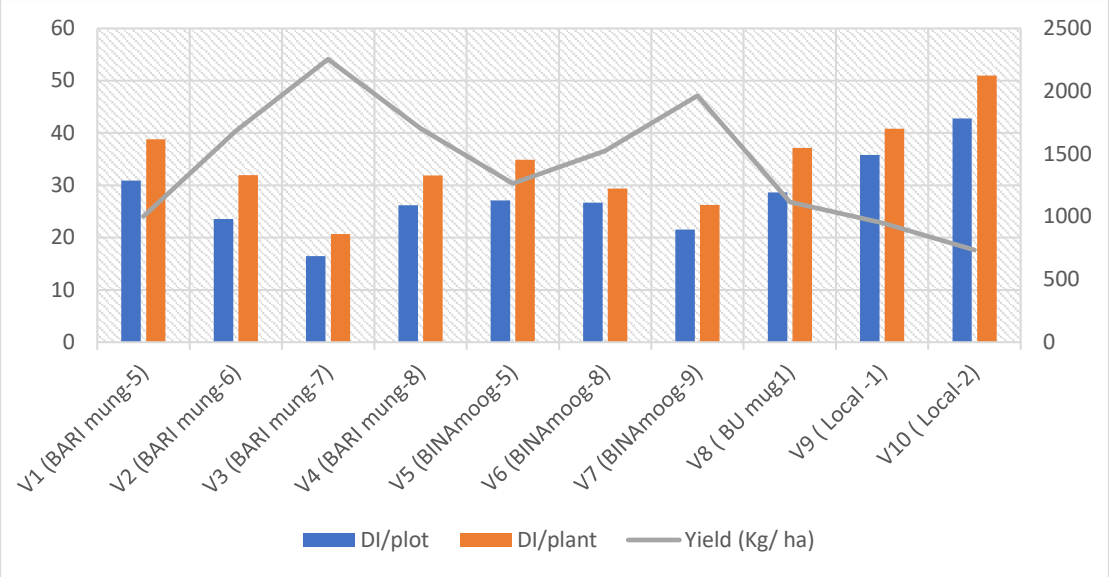

Figure 16. Yield of different mungbean varieties in relation to disease incidence

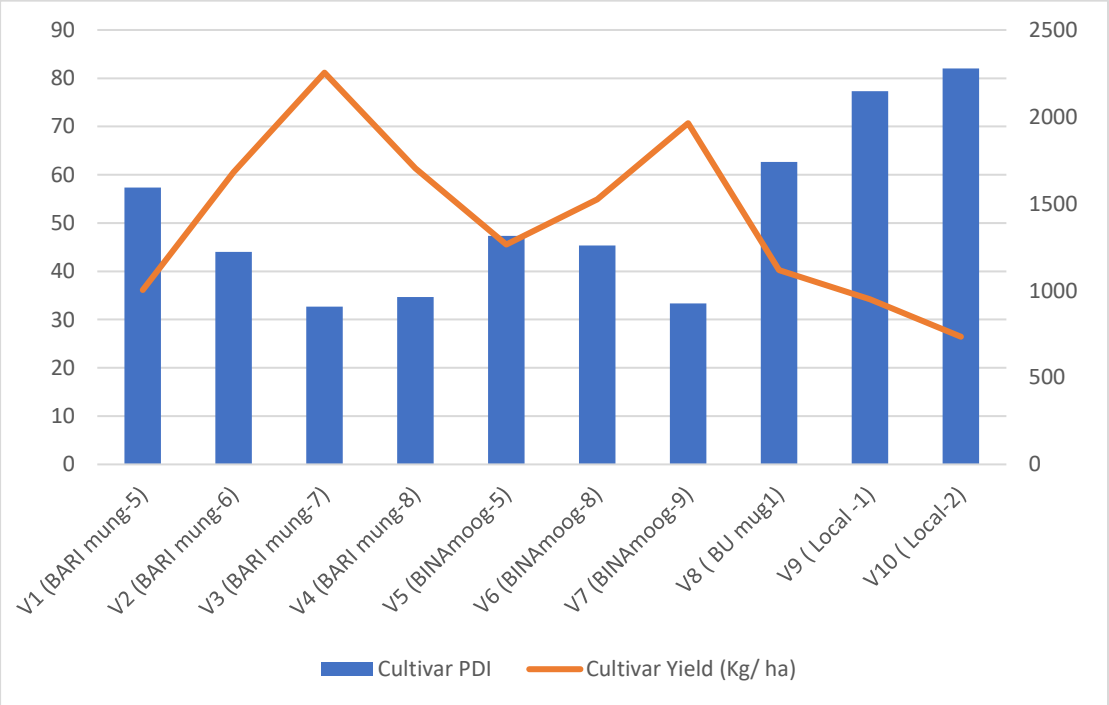

Figure 17. Yield of different mungbean varieties in relation to disease severity

4.7. DISCUSSION

In Bangladesh various types of pulse crops are grown, among which grass pea, lentil, mungbean, chickpea, field pea and cowpea are important. Mungbean is grown extensively in our country and other major tropical and subtropical countries of the world, due to its low input requirement and short growing period. Yellow mosaic is reported to be the most destructive viral disease in mungbean not only in Bangladesh, but also in India, Pakistan, Srilanka and adjacent areas of South East Asia (Bakar, 1981; Malik, 1991). *MYMV* causes irregular yellow and green patches on mature leaves and complete yellowing of leaves. Affected plants produce less number of pods and flowers and few seeds. This disease is destructive, wide-spread and inflicts heavy loss annually. Therefore, the present experiment was carried out to study the level the resistance of selected mungbean cultivars against *MYMV* through observation of disease incidence and disease severity. Molecular detection of *MYMV* through PCR was also a part of the study.

The differences among the varieties for disease incidence (%) and disease severity (%) were significantly different at all growth stages. Plot wise minimum disease incidence was found in BARI mung-7 at all growth stages (2.22, 10.66 and 16.44 % at 30, 45 and 60 DAS respectively) where finally the highest disease incidence found in Chaitamug; 42.77 %. Disease incidence per plant also showed accordant findings. Minimum disease incidence (31.91%) was observed in minimum disease incidence was BARI mung-7 and Chaitamug showed maximum disease incidence. Likewise Minimum disease severity was found in BARImung-7 (4.67, 18.67 and 32.67 % at 30, 45 and 60 DAS respectively) where finally the maximum disease severity (82.00%) was found in Chaitamug. Tested mungbean varieties in response to *MYMV* under field conditions may perhaps be associated to genetic makeup. Favorable environmental conditions for the disease development and the presence of enormous vector population in the field also have vital role on disease development. The results of disease incidence and severity of the present study

agree with the previous study that was conducted by Farghali and Hussein (1995) and Mishra (2002).

The tallest plant (59.20cm) and the highest number of branch (4.27) were observed in BARI mung-7 which showed minimum disease incidence (plot wise, 16.44% and plant wise, 20.69%) and severity (PDI, 32.67%). On the other hand, the shortest plant (39.43 cm) was found in Chaitamug which showed maximum disease incidences (plot wise, 42.77% and plant wise, 50.99%) and disease severity (PDI, 82.00%). This dissimilarity in plant tallness and branching might be attributed to the interaction with pathogen. The results of the present study agree with the previous study that was conducted by Farghali and Hussein (1995) and Mishra (2002).

In tested varieties, chlorophyll content at 45DAS was varied from 2.7040 (BARI mung-7) to 1.0833 (Chaitamug) µg/g, which was closely related to disease incidence and disease severity. Maximum disease incidences (plot wise, 42.77% and plant wise, 50.99%) and disease severity (PDI, 82.00%) was found in Chaitamug whereas minimum disease incidences (plot wise, 16.44% and plant wise, 20.69%) and disease severity (PDI, 32.67%) was observed in BARI mung-7. There was a significant reduction of in chlorophyll content in infected leaves. This reduction in the chlorophyll content may be due to virus infection inhibits the formation of plastids in young growing leaves in turn it reduced the plant photosynthetic ability so ultimately affects the chlorophyll content of infected leaf. Results were agreement with the reports of Singh and Mall (1973) Similar, results were also reported by Dhillon *et al.* (1976) who observed reduction in chlorophyll content of *Calendula officinalis* leaves infected with potato yellow dwarf disease.

Pod sterility which is one of the most important yield contributing factors of mungbean differed significantly among the different varieties in relation to disease development. The highest number of sterile pod (24.49%) was found in Chaitamug which was the most infected (PDI, 82.00%) variety. On the contrary, the lowest number of sterile pod (6.74%) was found in BARI mung-7

which was the least infected (PDI, 32.67%) among the tested varieties. The finding is in close conformity with the finding of Ahmad (1991).

The present experimental results revealed that Variety had a significant effect in 1000-seed weight and it was also observed in tested mungbean varieties. The 1000 seed weight under different varieties ranged from 33.64 g to 49.22g while highest weight was recorded in BARI mung-6 (49.22 g) and the lowest weight of 1000 seed (g) was found in BARI mung-8 (33.64 g). The variation in 1000-seed weight might be due to variation in the genetic make-up of the varieties. Aslam *et al.* (2004) and Hussain *et al.* (2008) also reported significant differences for 1000-seed weight.

The present experimental results revealed that Yield ($kgha^{-1}$) of tested mungbean varieties was related to disease incidence and severity. Decreased yield was observed with increased disease incidence and severity. The highest seed yield (2254.50 kg ha^{-1}) was observed in BARI mung-7 and the lowest seed yield (734.7 kg ha^{-1}) was obtained from Chaitamug. It was clearly revealed that a drastic reduction in yield was observed due to the infection of *MYMV* and yield loss rapidly increases in relation to increase of disease incidence and disease severity which is in close conformity with the previous findings of Marimuthu *et al.* (1981); Awasthi and Shyam, (2008) and Sachan *et al.* (1994).

CHAPTER V

SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

The present study was carried out under the field condition at central 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 *Mungbean Yellow Mosaic Virus (MYMV)* and its molecular detection through PCR**.** In total ten Mungbean varieties were tested in this study against *MYMV*. The field experiment was carried out in Randomized Complete Block Design with three replications. All the tested varieties were remaining in natural conditions without insecticide application.

The experiment was aimed to assess the varietal performance of tested mungbean varieties against *Mungbean Yellow Mosaic Virus (MYMV)* and identify the *MYMV* on the basis of biological properties. Genomic DNA was extracted from leaf samples of the tested varieties to detect the *MYMV* through modern molecular technique PCR. From molecular study through PCR test, it was revealed that results obtained on the basis of biological properties found almost similar to PCR analyses to detect the *MYMV*. Among the tested varieties, all of the tested varieties; V_1 (BARI mung-5), V_2 (BARI mung-6), V_3 (BARI mung-7), V_4 (BARI mung-8), V_5 (BINAmoog-5), V_6 (BINAmoog-8), V_7 (BINAmoog-9), V_8 (BU mug1), V_9 (Sonamug), V_{10} (Chaitamug) gave the positive results in PCR test and shown sharp band at 900bp fragment.

Among the all varieties significant influence was found in case of plant height, Number of branch plant $^{-1}$, pods plant^{-1}, pod length and seed yield ha^{-1} among the different varieties under the present study. Results indicated that the highest plant height (59.20 cm) was observed in V_3 (BARI mung-7) where the lowest plant height (39.43 cm) was obtained from V_{10} (Chaitamug). Results also showed that the highest number of branch plant $^{-1}$ (4.27), considerably higher number of pods plant^{-1} (25.67), the highest pod length (9.20 cm) and the highest seed yield (2254.50 kg ha^{-1}) was observed in V_3(BARI mung-7) where

the lowest pod length (5.23 cm) and seed yield (734.70 kg ha^{-1}) the second lowest number of pods $plant^{-1}$ (18.33) was obtained from V_{10} (Chaitamug) variety. Statistically significantly effect was also found in case of pod sterility. The highest number of sterile pod (24.49%) was found in Chaitamug and the lowest number of sterile pod (6.74%) was found in BARI mung-7 among the tested varieties. Again, practices with different cultivars, the lowest disease incidence per plot (2.22, 10.66 and 16.44 % at 30, 45 and 60 DAS respectively) was found in V_3 (BARImung-7) where V_{10} (Chaitamug) showed the second highest disease incidence per plot (6.00%) at 30 DAS but at 45 and 50 DAS V_{10} (Chaitamug) showed the highest disease incidence per plot (26.00% and 42.77% respectively). In case of disease incidence per plant, V_3 (BARI mung-7) showed minimum results (3.55, 12.78 and 20.69 % at 30, 45 and 60 DAS respectively). On the contrast, V_{10} (Chaitamug) showed maximum results (7.06, 30.47 and 50.99 % at 30, 45 and 60 DAS respectively) at all the growth stages. The minimum percent disease index (4.67, 18.67 and 32.67 % at 30, 45 and 60 DAS respectively) was originated in V_3 (BARI mung-7) where maximum percent disease index (11.33%, 32.67% and 82.00%) was found in V_{10} (Chaitamug) at 30, 45 and 60 DAS.

From the field study, it may be concluded that BARI mung-7 showed minimum disease incidence and severity and gave the best performances in respect of yield and yield contributing characters.

CHAPTER VI

REFERENCES

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CHAPTER VII
APPENDIX

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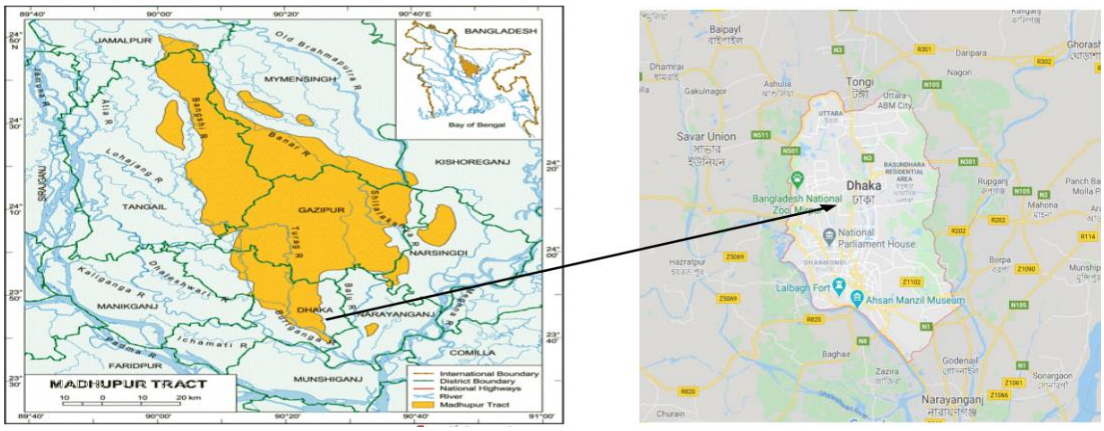
Appendix-I. Madhupur Tract, AEZ No. 28



Appendix-II: Particulars of the Agro-ecological Zone of the Experimental Site.

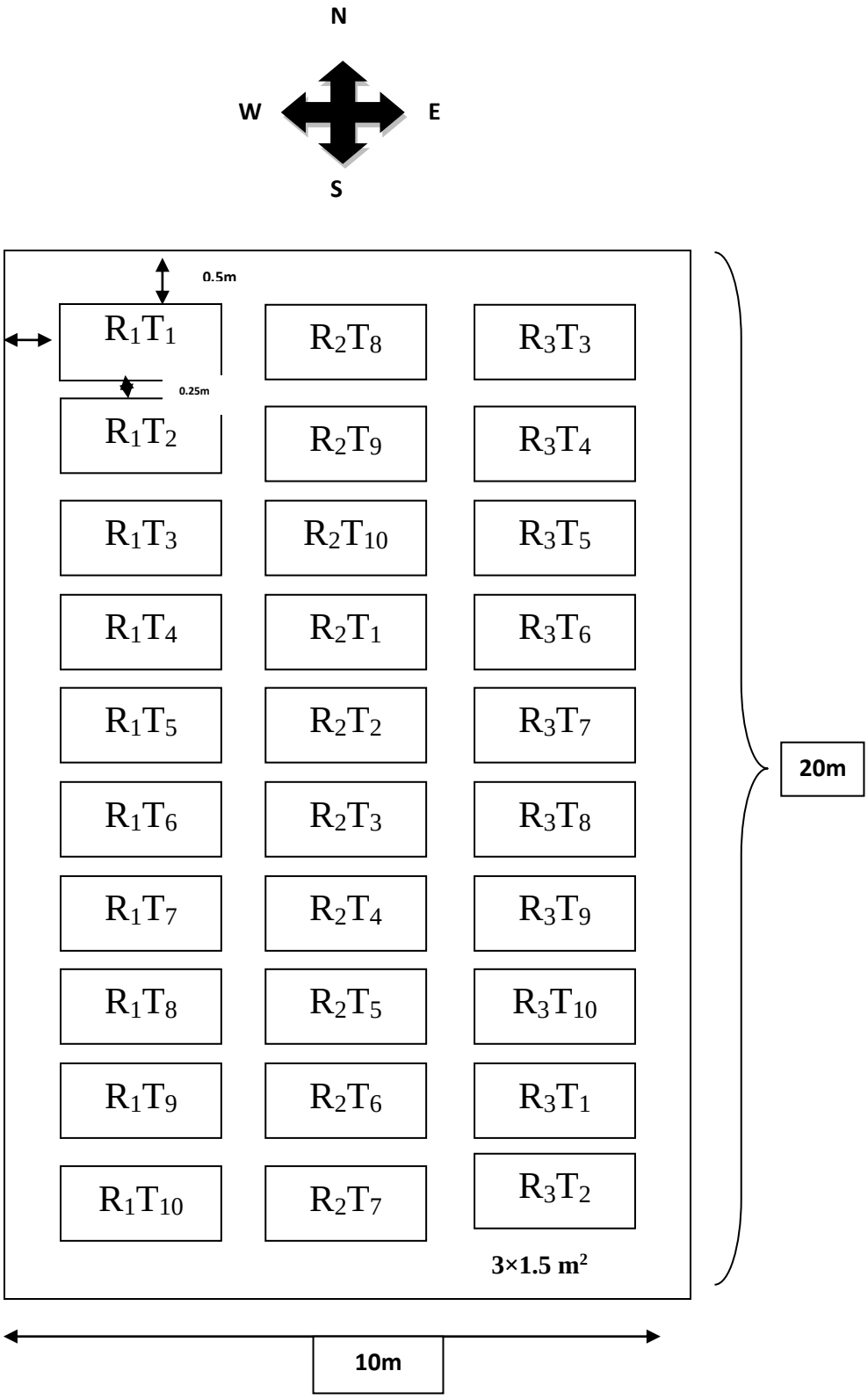
Agro-ecological region	: Madhupur Tract (AEZ-28)
Land Type	: Medium high land
General soil type	: Non- Calcareous Dark gray floodplain soil
Soil series	: Tejgaon
Topography	: Up land
Location	: SAU Farm, Dhaka
Field level	: Above flood level
Drainage	: Fairly good
Firmness (consistency)	: Compact to friable when dry.

Appendix III. Monthly average air temperature, relative humidity and total rainfall of the experimental site during the period from March to June, 2021

Month (2021)	* Air temperature°C		* Relative humidity (%)	* Rainfall (mm) (total)
	Maximum	Minimum		
March	31.7	20.5	65	25
April	33.4	23.2	67	78
May	34.7	25.9	70	185
June	35.4	24.9	80	277

* Monthly average
Source: Bangladesh Meteorological Department (Climate and weather division) Agargoan, Dhaka-1207.

Appendix IV: Layout of the field experiment: (RCBD)

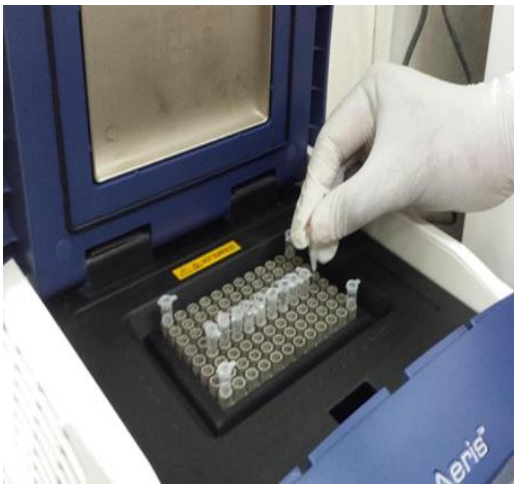


Appendix V: Photos which were captured during DNA Extraction

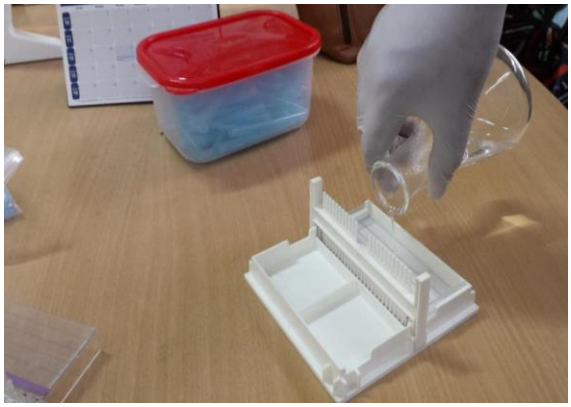
Leaf sample collection

Chemicals used for DNA extraction

Eppendorf tubes containing extracted gemonic products



PCR the extracted genomic products



Agerose gel preparation

PCR product loading on gel documentation system

Appendix VI: Field Data collection