ASSESSMENT OF VARIETAL PERFORMANCE OF SELECTED TOMATO VARIETIES AGAINST *Tomato Yellow Leaf Curl Virus (TYLCV)* AND ITS MOLECULAR DETECTION THROUGH PCR

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Dedicated to my beloved parents and respected teachers

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CERTIFICATE

*This is to certify that the thesis entitled "**ASSESSMENT OF VARIETAL PERFORMANCE OF SELECTED TOMATO VARIETIES AGAINST TOMATO YELLOW LEAF CURL VIRUS (TYLCV) AND ITS MOLECULAR DETECTION THROUGH PCR**" 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 **MD. IBNE-SIAM-JOY, REGISTRATION NO.13-05417** 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: 08.10.2020

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ASSESSMENT OF VARIETAL PERFORMANCE OF SELECTED TOMATO VARIETIES AGAINST *Tomato Yellow Leaf Curl Virus (TYLCV)* AND ITS MOLECULAR DETECTION THROUGH PCR

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 2018-2020.The field experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. Genomic DNA extraction and PCR test was done in Molecular Biology and Plant Virology Laboratory following the standard protocol. In this experiment ten selected variety was selected for conducting the experiment. Among the ten selected varieties three varieties; BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19 showed moderately resistant reaction and lower disease severity against the *TYLCV*. Remaining selected varieties; BARI Tomato-17, BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato- 14, BARI Tomato-15, BARI Tomato-16 were showed susceptible reactions to highly susceptible and higher disease severity against *TYLCV*. In case of BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19 whitefly association was not increased with the increase of plant age. But it was noticed that in case of the remaining varieties whitefly association was increasing with the increase of plant age. Different growth parameters yield and yield contributing characters were also studied. Among the selected varieties the maximum number of leaves per plant was obtained in the variety BARI Tomato-11 and minimum was obtained in the variety BARI Tomato-9. The highest number of branches per plant was recorded in the variety BARI Tomato-11 and the lowest was recorded in the variety BARI Tomato-14. The maximum number of flowers was also obtained from BARI Tomato-11 and the minimum was observed from BARI Tomato-17. The maximum number of fruits was obtained from BARI Tomato-11 and the minimum was obtained from BARI Tomato-18. The maximum individual fruit weight was obtained from BARI Tomato-17 and minimum fruit weight was obtained from BARI Tomato-11. The highest yield per plant was obtained from BARI Tomato-17 and the lowest yield per plant was obtained from BARI Tomato-5. From molecular study through PCR test, it was revealed that results obtained on the basis of biological properties that was almost similar to PCR analyses to detect the *TYLCV*. Among the selected varieties, seven varieties; BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16 & BARI Tomato-17 gave the positive results in PCR test and shown sharp band at 520 bp fragment. Other varieties; BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19 gave negative result in PCR test. For the molecular detection of *Tomato Yellow Leaf Curl (TYLCV)*, PCR is the most reliable modern technique because it is simple, very specific and highly robust.

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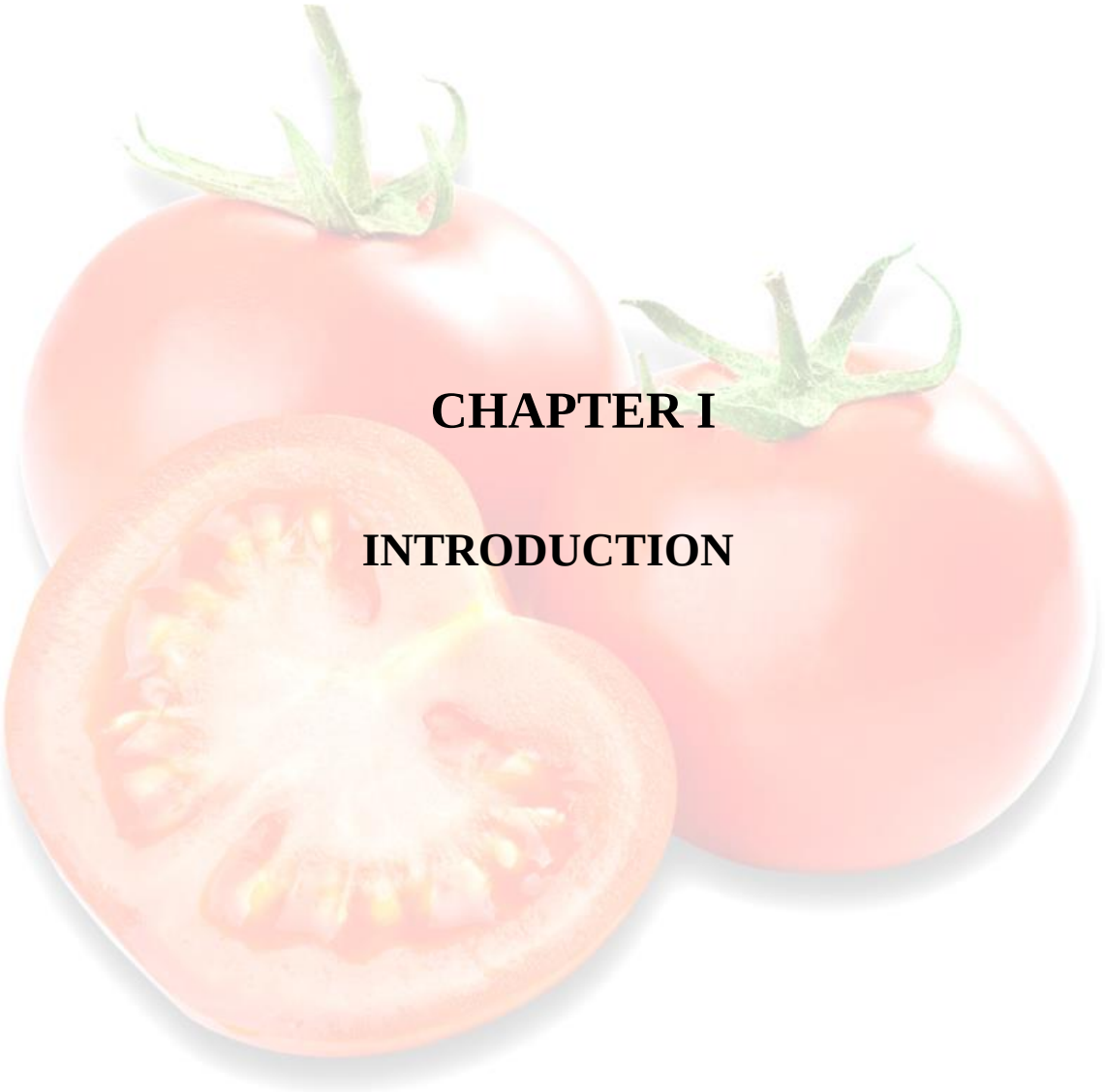
ABBREVIATIONS AND ACRONYMS

µg	Microgram
µl	Microliter
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
bp	Base pair
CV %	Percent Coefficient of Variation
DAT	Day after transplanting
et al.,	And others
FAO	Food and Agriculture Organization
FP	Forward primer
ICTV	International Committee on Taxonomy of Viruses
LSD	Least Significant Difference
mm	Millimeter
nm	Nanometer
PCR	Polymerase Chain Reaction
RCBD	Randomized Complete Block Design
RP	Reverse Primer
SRDI	Soil Resources Development Institute
ssDNA	Single-stranded DNA
TSP	Triple superphosphate
TYLCV	*Tomato Yellow Leaf Curl Virus*



CHAPTER I

INTRODUCTION

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Tomato (*Lycopersicon esculentum* Mill.) is a solanaceous self-pollinated vegetable crop. It is the second most important vegetable crop next to potato in the world (Choudhury, 1979). The cultivated types of tomato belong to *Lycopersicon esculentum* and are originated from South American Andes. Due to high adaptability of tomato plant to wide range of soil and climate, it is widely grown in our country (Ahamed, 1995). It is one of the most important, popular and nutritious vegetables that grown in Bangladesh and other countries. Present world production of tomato is about 170.8 million tons and total tomato growing area is 4.9 million hectares (FAOSTAT, 2016). As a cash crop, it has great demand in the International market (Solieman *et al.,* 2013). The best tomato growing areas in Bangladesh are Chittagong, Comilla and Rajshahi. In Bangladesh, the recent statistics shows that tomato was grown in 67535 acres of land and the total production was approximately 368121 metric tons during the year 2015-2016 and the average yield of tomato was 5451 kg/acre in winter season (BBS, 2016). It is used both as salad and to prepare curry. It is also used to make soups, pickles, conserves, ketchup's, juices, sauces etc. It is widely grown in both winter and summer season around all parts of the country (Haque *et al*., 1999). It also contains a large quantity of water, calcium and niacin all of which have great importance in the metabolic activities of human. It is also a good source of vitamin A, C, E and minerals (potassium, calcium, phosphorus, iron and zinc) that are very good for body and protect the body against diseases (Taylor, 1987). It is an excellent source of lycopene, carotenoids and polyphenolic compounds which are a powerful source of antioxidant and reduces the risk of prostate cancer (Hossain *et al.,* 2004). It is even present when tomatoes are cooked. Tomato also has medicinal value; the pulp and the juice of tomato is easily digestible and blood purifier. It is an important condiment in most diets

and a very cheap source of vitamins which referred as poor man's Orange (Frasher *et al.,* 1991).

In our country, the yield of tomato is not satisfactory in comparison with other tomato growing countries (Aditya *et al.,* 1999*).* Although the total cultivated area and production of tomato in our country have increased gradually over the last few years but the productivity is still very low (6.46 $tonha^{-1}$) compared to the average of the world yield (34.86 t ha^{-1}) as per (FAOSTAT,2016). Tomato production in our neighboring country India was 7873 kg/acre (Indian Horticulture Database, 2017) where as our production is 5451 kg/acre only.

The environment of India and Bangladesh almost same and thus the variation comes mainly due to pest and diseases infestation. There are many types of diseases occurs in tomato like viral, fungal, bacterial and nemic disease. Globally tomato is susceptible to more than 200 diseases, out of which 40 are caused by viruses (Martelli and Quacquarelli, 1982; Lukyanenko, 1991). However, the incidence and economic impact of virus infections in tomato varies greatly depending upon different factors like country, cropping method and the virus itself (Martelli and Quacquarelli, 1982).

In our country 16 different tomato viruses are identified. Among the viral diseases *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is the most devastating one and due to this viral disease yield loss may be raised upto 100% (Akanda *et al.,* 1991). *TYLCV* is also wide spread in many Mediterranean, Middle Eastern, American, African, and Asian countries. *TYLCV* is an ssDNA plant virus, which belongs to the family Geminiviridae of the genus Begomovirus (Czosnek and Laterrot, 1997). This viral disease is transmitted by whiteflies (*Bemisia tabaci*) and by grafting but not transmitted through mechanically. The disease was first reported in Israel and Jordan Valley in the early 1960s and is now economically significant in many countries (Jones *et al.,* 1993). The causal agent was described in 1964 and named as *Tomato yellow leaf curl virus TYLCV*) by Cohen and Harpaz in 1964. Since then, *TYLCV* has been reported from all over the

tropics, subtropics, the Mediterranean, the Caribbean's and the Americas (Czosnek and Laterrot, 1997, and Nakhla *et al.*, 1994). *TYLCV* threatens both commercial tomato productions in the fields and home garden which could be able to infect plants at any stage of plant growth (Gupta, 2000). In Bangladesh *TYLCV* incidence was first reported by (Akanda *et al*.,1991) based on symptomatology. Symptoms of *TYLCV* include stunted plant growth, chlorotic yellowing of leaves, and distortion of leaflets in a cupped down and inward shape or upward curling of the leaflet margins (Cohen and Lapidot, 2007). The impact of *TYLCV* on tomato production is very severe. If plants are infected at an early stage, they do not bear fruit and their growth becomes severely stunted and so 100% yield loss occurs.

Since the reports of *TYLCV*, many efforts have been made to characterize the virus systematically to manage the disease through manipulation of sowing dates, growing seedlings in net house, application of insecticides and so on (Paul, 2002; Rahman, 2003; Gupta, 2000; Azam, 2001; Akhter, 2003; and Sultana, 2001). Although the reports provide a number of information about *TYLCV* and its management in Bangladesh but none of the efforts could provide conclusive information about *TYLCV*. The frequent outbreak of disease epidemic and very high yield loss leading to a total crop failure have drawn the attention of the scientists to develop effective management program against *TYLCV* for profitable tomato production in many countries. Various strategies have been taken to manage the disease but all are not successful.

So far, there are many methods are reported for plant viruses' identification 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 to evaluate the varietal performance of tested tomato varieties against *Tomato Yellow Leaf Curl Virus* (*TYLCV*), and to detect *TYLCV* by modern molecular technique through PCR. In the current study aimed to identify, at first *TYLCV* through symptomological test and transmission method. Symptomatology based identification is possible but

it needs good skillness as well as plant pathological experience as because similar symptoms by other viruses may be intermingled that makes it difficult to differentiate. 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.

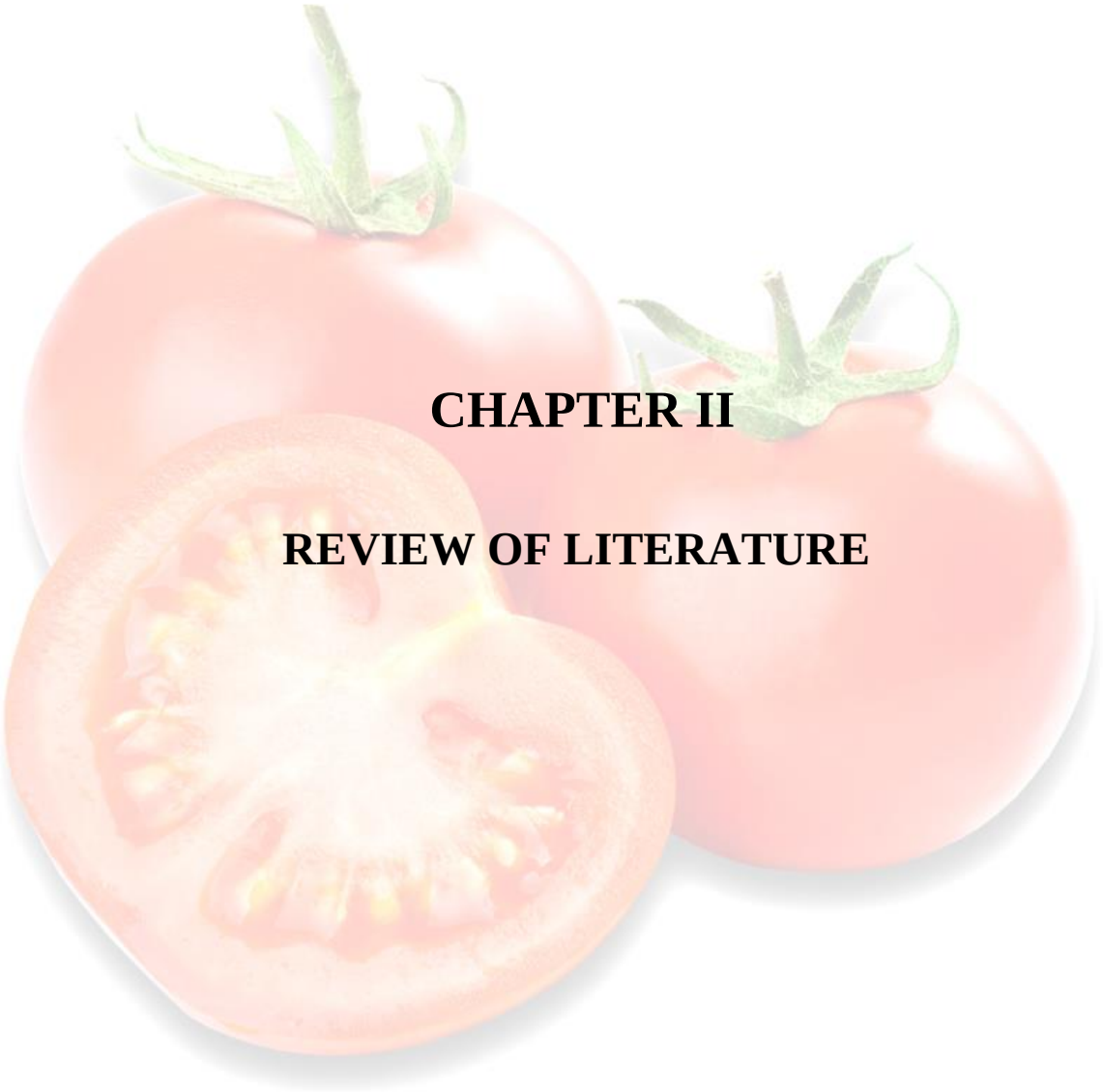
OBJECTIVES

The specific objectives of this study are given below:

i) To assess the varietal performance of selected tomato varieties against Tomato *Yellow Leaf Curl Virus (TYLCV)*

ii) To identify the TYLCV on the basis of biological properties

iii) To detect the TYLCV through modern molecular technique PCR.



CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop in Bangladesh. Tomato production in Bangladesh is under constant threat of Tomato yellow leaf curl disease caused by *Tomato Yellow Leaf Curl Virus* (*TYLCV*). A lot of work has been done on various aspects of *TYLCV* in Bangladesh and abroad and is reviewed as under-

2.1. About Tomato

Tomato originated from the Andean region, an area now located in parts of Chile, Colombia, Bolivia, Ecuador and Peru (Bai & Lindhout, 2007). Because tomato was first domesticated by the Mayas and the Aztecs (Barndt, 2008), Mesoamerica is considered as the birthplace for cultivated tomato. The word *tomato* existed in the native Mexican language nahuatl to describe plants bearing globose and juicy fruit (Blanca *et al*., 2012).

Tomato was introduced to Europe most probably from Mexico (Blanca *et al*., 2012) in the 16th century by Spanish conquistadors. Due to its resemblance with toxic *Solanum* species like belladonna and mandrake, the tomato was long used for ornamental purposes only appearing in cookbooks by the beginning of the 17th century. From Spain, the tomato reached Italy and England, whence British subsequently "exported" tomato to Asia, Middle East and North America (McCue, 1952; Bergougnoux, 2014).

Tomatoes are adapted to a wide range of environmental conditions, but in temperate areas low temperatures and short growing seasons can limit growth. Tomatoes prefer slightly acidic soils with a pH of 6.0 to 6.8. The tomato plant requires significant quantities of water, but not in excess, since tomato roots will not function under water-logged (anaerobic) conditions. (Cox and Tilth, 2009).

Sufficient moisture must be maintained to establish the plant and carry it through to fruit production. When the moisture level surrounding the roots is too high, epinasty, poor growth, late flowering, fewer flowers and lower fruit set occurs. Fruit disorders such as cracking and blossom-end-rot are common when water availability is inconsistent. Even under moderate water stress, photosynthesis is slowed because the movement of gases through the stomata is restricted and the movement of water up the xylem is slowed (Benton, 2008).

2.2 Morphology of Tomato

Tomato was classified by the Swedish botanist Carl Linnaeus in 1753 in the genus *Solanum* with the species ephitet *lycopersicum*. It belongs to the family Solanaceae, which contains over 3000 plant species, including many economically important plants such as potato, eggplant, peppers, petunia and tobacco. With 1250–1700 species, *Solanum* is the largest genus in the Solanaceae family. Tomato is botanically classified as the cultivated tomato *S. lycopersicum* and its twelve wild species. Wild tomato species have very small fruit while the modern cultivated tomatoes have a large variation in fruit size, ranging from less than 20 g for cherry tomato up to 500 g for the beef tomato (Bergougnoux, 2014).

Although usually cultivated as an annual crop, tomato is a perennial plant. It has bipinnate leaves, hairy stems and flowers with usually 5–7 petals (Blanca *et al*., 2012). Tomato is diploid (Nesbitt & Tanksley, 2002) and its genome size is approximately 900 Mb, comprising 12 chromosomes and 34,727 protein-coding genes (The Tomato Genome Consortium, 2012).

Tomato is cultivated for its fleshy fruit (Blanca *et al*., 2012). Botanically, tomato is a fruit berry, and not a vegetable (Bergougnoux, 2014). The fruit is a specialized organ that results from the development of the ovary after successful flower pollination and fertilization. It provides a suitable environment for seed maturation and dispersal (Chevalier *et al*., 2011). The fleshy fruit corresponds to the ovary and is composed of an epidermis, a thick pericarp (composed of exocarp, mesocarp and endocarp) and the placental tissues, which surround the

seeds. The pericarp is the outer wall of the gynoecium, and is composed of atleast two carpels, which determine the number of fruits locules (Bergougnoux, 2014).

2.3 About *TYLCV*

The virus belongs to genus Begomovirus and has a single-stranded DNA (ssDNA). The genomes are encapsidated in about 20X30 nm geminate particles (Goodman, 1977).

Among the viruses infecting tomato, *TYLCV* has the highest economical impact (Czosnek, 2007) and it is considered as one of the most devastating plant viruses worldwide (Hanssen *et al*., 2010; Péréfarres *et al*., 2012). Currently, 10 different Begomovirus species and their strains are associated with tomato yellow leaf curl disease (*TYLCD*) (Brown *et al*., 2015). Among them, *TYLCV* is the most dominant species and it is divided into different strains, among which the Israel (*TYLCV*) and mild (*TYLCV-Mld)* strains are most prevalent (Hanssen *et al*., 2010; Lefeuvre *et al*., 2010; Navas-Castillo *et al*., 2011).

Symptoms of *TYLCD* were first observed in the Jordan Valley in 1929 (Cohen & Lapidot, 2007). It took about 30 years before the virus was first described and found to be circulative and persistent in the insect vector (Cohen & Harpaz, 1964). During the 1970´s, the first electron micrographs (EM) were produced showing the novel geminate particle morphology of geminiviruses (Goodman, 1981) and it was discovered that the virions of Begomoviruses contain a genome of ssDNA (Goodman, 1977). EM observations of thin sections of *TYLCV*-infected tomato leaves also indicated that geminate particles are located in the nuclei of phloem parenchyma cells (Russo *et al*., 1980; Cherif & Russo, 1983). In the following decade, *TYLCV* virions were isolated and purified (Czosnek *et al*., 1988) and in 1991, the genome sequence of *TYLCV* was published (Navot *et al*., 1991).

TYLCV has a wide host range with more than 30 plant species in over 12 families, including vegetables and ornamentals as well as wild plants and weeds. The reservoirs for *TYLCV* vary among regions and because infection of other hosts

than tomato can be symptomless, reservoirs may not be obvious (Polston & Lapidot, 2007). In tomato, *TYLCV* can cause yield losses of up to 100% and induce symptoms such as upward curling, reduction and yellowing of leaves as well as flower abortion and overall reduction in growth (Díaz-Pendón *et al*., 2010; Navas-Castillo *et al*., 2011). *Tomato leaf curl virus* (*TYLCV*) is a group of whitefly-transmitted geminiviruses (Cohen and Harpaz, 1964; Czosnek *et al*., 1988), causing an extensive yield loss to tomato crops in many tropical and subtropical regions worldwide (Czosnek and Laterrot, 1997).

2.4 Disease symptoms

Tomato leaf symptoms include chlorotic margins, small leaves that are cupped, thick rubbery. The majority (90%) of flowers abscises after infection and therefore few fruits are formed. *TYLCV* is considered as a phloem limited virus (Ganif, 2003).

The various prominent symptoms of tomato leaf curl virus such as upward curling of leaf margins, stunting, reduction of leaf size, corrugated leaf, shortening of internodes and severe reduction in fruit yield, had been observed from Middle East (Makkouk and Laterrot, 1983). The upward leaf curling and interveinal and marginal chlorosis in tomato plants due to tomato leaf curl virus is reported by (Zhang *et al*., 2008).

(Avgelis *et al*., 2001) first reported that *TYLCV* in Greece. They described the disease symptom as leaf curling, reduced leaf size, yellowing, shortened internodes and a bushy appearance. Mechanical inoculation was unproductive while transmission was obtained by grafting on to healthy tomato plant.

It was reported that symptoms of stunting, curling and yellowing of leaf margins, and marked reductions in the number of fruits were observed in some greenhouse-grown tomato cv. Naxos plants in the province of Bari in Apulia, Italy, were observed in the being an isolate of *TYLCV-Sar*. The nucleotide sequence of the 580 bp amplicon shared 99.5% homology with a clone from a

Sicilian isolate and 97.5% with a clone from a Sardinian isolate of *TYLCV-Sar*. This is the first report of *TYLCV* in Apulia, Italy (Sialer *et al.*, 2001).

2.5. Virus Identification

A survey was conducted to determine the incidence of *Cucumber mosaic virus* (*CMV*), *Beet curly top virus* (*BCTV*), *Tomato yellow leaf curl virus* (*TYLCV*), *Tomato chlorotic spot virus* (*TcSV*), *Potato virus Y* (*PVY*), *Potato virus S* (*PVS*), *Tomato spotted wilt virus* (*TSWV*), *Tomato ringspot virus* (*TRSV*), *Tomato aspermy virus* (*TAV*), *Arabis mosaic virus* (*ArMV*), *Tobacco streak virus* (*TSV*), *Tomato bushy stunt virus* (*TBSV*), *Tobacco mosaic virus* (*TMV*), and *Tomato mosaic virus* (*ToMV*) on tomato (*Solanum lycopersicum*) in the major horticultural crop growing areas in the southeast and central regions of Iran. Samples of symptomatic plants were analyzed for virus infection by PCR (Polymerase Chain Reaction) using specific primer against the virus. ArMV and CMV were the most frequently found viruses, accounting for 25.6 and 23.4%, respectively, of the collected samples. *BCTV*, *TSWV*, *TMV*, *PVY*, *ToMV*, and *TYLCV* were detected in 6.1, 5.8, 5.6, 5, 4.8, and 1.6% of the samples, respectively. *TBSV*, *TAV*, *TSV*, *PVS*, and *TRSV* were not detected in any of the samples tested. Double and triple infections involving different combination of viruses were found in 13.9 and 1.7% of samples, respectively. This is the first report of *PVY* and *ArMV* as viruses naturally infecting tomato in Iran (Michael,2009).

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

Since *Tobacco mosaic virus (TMV)* was first recognized over a century ago, more than 1000 of plant viruses have been found (King *et al.*, 2011; Scholthof, 2000). It has been known that like other plant pathogens including bacteria, fungi, and

phytoplasma, plant viruses spread and cause major economic losses to many crops such as barley, Tomato, potato, rice, and wheat (Agrios, 2005; Ellis *et al.*, 2008; Strange, 2005).

Virus is ranked as the second most important plant pathogens following fungi (Vidaver and Lambrecht, 2004). Economic loss has been estimated more than several billion dollars per year worldwide because of plant viruses (Hull, 2002; Plant Viruses, 2003). The crop damages owing to viral diseases are difficult to predict, because it depends on region, virus strain, host plant cultivar/variety, and time of infection (Strange, 2005).

Symptoms of viral diseases include crinkling, browning of leaf tissues, mosaic, and necrosis. Sometimes, however, symptoms may not be visually detected because infection of plant viruses causes no symptoms (Bove *et al*., 1988; Vander Want and Dijkstra, 2006). In addition, plants can also display virus like symptoms when plants respond to unfavorable weather, nutritional imbalances, infection by other types of pathogens, damage caused by pests or abiotic agents and others (Vander Want and Dijkstra, 2006). Thus, viral disease diagnosis by symptoms is more difficult than other pathogens (Livenes *et al*., 2005). The diagnosis is the basis to manage plant diseases and to predict the crop loss by infection of plant pathogens (Vander Want and Dijkstra, 2006). Accurate diagnosis of virus diseases, is the first important step for crop management system (Aboul-Ata *et al.,* 2011).

As the internationalization of the domestic agricultural market, virus diagnostics is very essential to use high quality seed as well as virus free seeds (Lievens *et al.,* 2005; Wang *et al.,* 2011). The methods for detection and identification of viruses are critical in virus disease management (Aboul-Ata *et al*., 2011). Therefore, detection methods should be more convenient, effective, specific and permitted the use for detecting plant pathogens (McCartney *et al*.,2003).

A lot of methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods and so on (Lopez *et al.*, 2008; Makkouk and Kumari, 2006; Webster *et al.*, 2004).

2.6. About PCR

The polymerase chain reaction (PCR) is widely used in plant pathology for the diagnosis of plant diseases, allowing the detection of very small amounts of the disease agent in the infected plant, and also the cloning of genomic fragments of the pathogen (Henson and French, 1993). The PCR usually requires the purification of the target DNA although it has been demonstrated that plant DNA can be amplified from crude extracts of leaves (Klimyuk *et al.*, 1993), from aqueous extracts of tissues squashed on a membrane (Langridge *et al.*, 1992) and even from leaf and root pieces (Berthomieu and Meyer, 1991). *TYLCV* DNA can be amplified from nucleic acids isolated from tomato plants and from individual whiteflies by PCR (Navot *et al.*, 1992).

The Polymerase Chain Reaction (PCR) methods are highly effective as a tool for rapid and large-scale diagnostics of *TYLCV*-infected samples (Briddon and Markham, 1995). Apart from being a tool useful for detection, the products of the diagnostic PCR reactions are suitable for further characterization of the viruses (Briddon and Markham, 1995).

2.7. *TYLCV* Identification

Tomato yellow leaf curl *(TYLCV)* is one of the most devastating viral diseases of cultivated tomato (*Lycopersicon esculentum*) in tropical and subtropical regions worldwide, and losses of up to 100% are frequent. In many regions, *TYLCV* is the main limiting factor in tomato production. The causal agents are a group of geminivirus species belonging to the genus Begomovirus of the family Geminiviridae, all of them named *Tomato yellow leaf curl virus (TYLCV)*. There has been almost 40 years of research on *TYLCV* epidemics and intensive research programmes have been conducted to find solutions to the severe problem caused by these viruses. (Moriones and Navas-Castillo, 2000).

The major tomato virus having monopartite single-stranded DNA is *Tomato Yellow Leaf Curl Virus (TYLCV)*. Symptoms caused by this virus are chlorotic and leathery leaves, leaf curling, blistering, reduced leaf size, shortened internodes, chlorosis of leaf margins, rounding of leaflets, flower abscission and poor bearing (Cohen and Nitzany, 1966: Yassin, 1982; Makkouk *et al.*, 1983; Thomas, 1984).

There are three distinct *TYLCV*s based on nucleotide sequence comparisons. It is also considered that viruses of the genus Begomovirus, which have nucleotide sequence similarity levels below 90 % are distinct from each other (Padidam *et al.,* 1995), although later on ICTV reported that this can only be concluded when complete genome sequences have been compared (Fauquet *et al.,* 2003), and not on the basis of the intergenic region (IR) or coat protein gene alone. Similarity comparisons have previously been done on the basis of the intergenic region and partial sequences for other *TYLCV*s including isolates from Egypt and Israel, which are similar but different from isolates from Spain (GenBank No. L 277081) and Sicily (GenBank No. Z28390) (Noris *et al.*, 1993).

2.8. Incidence and distribution of *TYLCV*

TYLCV was present in almost all fields of Belgaum, Dharward, Haveri districts of Karnataka with percent disease incidence of 4 to 100 % in rabi and 60 to 100 % during summer season. (Reddy *et al*., 2011).

TYLCV is quite general in the tropics. In Africa, it has been reported from South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et al*., 1982; AVRDC, 1987; Czosneck *et al.*1990; Nakhla *et al.*, 1993; Nono-Womdim *et al*, 1994; Chiang *et al*., 1996). It is also widespread in the rest of the Old World and in the New World, e.g., in South East Asia and East Asia, the Americas and the Mediterranean (Green and Kallo, 1994; Chiang *et al.*, 1996; Polston and Anderson, 1997; Czosnek and Laterrot, 1997).

A survey of tomato and pepper viruses was conducted in Sudan during the last ten years. It covered Central, Northern, Eastern, Southeastern and Western regions of Sudan. The results revealed the presence of many mosaic - inducing virus and virus like agents. *Cucumber mosaic virus (CMV), Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Tomato yellow leaf curl virus (TYLCV)* and *Potato virus Y (PVY)* were all found to infect both tomato and pepper (Elshafie *et al.,* 2005).

In the semi-tropical climatic zone of Egypt, indicated that at the beginning of Spring and early Summer (February - April), when tomato plants have just established, *TYLCV* incidence is very low (Moustafa, 1991). The latter becomes high towards the end of Summer (September – mid-October), and then coincides with peak whitefly population density (Riley *et al.,* 1995).

This is followed by high *TYLCV* incidence and severe damage in the fall (Autumn) when production losses rise to 80% and almost all plants are infected. Similarly, Cohen and Antignus (1994) observed that in the Jordan Valley, the spread of *TYLCV* was significantly correlated with *B. tabaci* population size. As in Egypt, peak whitefly population occurred between the first week of September and Mid-October. In Tanzania, *TYLCV* symptoms and whitefly vector presence are reported to be most common during November to February (Nono-Womdim *et al.,* 1996).

2.9. Transmission of *TYLCV*

Three-quarters of all known plant viruses are transmitted by insect vectors (Hogenhout *et al.,* 2008). Until recently, *TYLCV* was known to be transmitted only by *B. tabaci* or artificially via grafting, particle bombardment or agroinoculation using Agrobacterium tumefaciens (Stanley *et al.,* 2001; Scholthof *et al.,* 2011). Notably, *TYLCV* was recently reported to be also seed transmissable with floral infection and seed transmission rates of 20–100%. Importantly, virus was detected in the embryos of the seeds by PCR (Kil *et al.,* 2016).

More than 230 plant virus and viroid diseases are transmitted through seeds (Sastry, 2013) and infected seeds can be the initial source of inoculum for subsequent vector mediated transmission (Ali & Kobayashi, 2010). In addition, seed transmission enables survival of viral inoculum between growing seasons and virus diseases may be disseminated worldwide through exchange of seeds having undetected infections (Sastry, 2013).

TYLCV is transmitted by a whitefly (*Bemisia tabaci Gennadius*) of the Family Aleyrodidae (Gerling and Mayer, 1995). *Bemisia tabaci* occurs in biotypes A and B. Biotype B is more common than A and is regarded by some as a separate species designated *B. argentifolii* (Bellows *et al.,* 1994). Others continue to regard it as a biotype of *B. tabaci* even though there are many more biotypes, which include biotype Q (Demechelis *et al.*, 2000).

In some circumstances, the incidence and rate of spread of *TYLCV* are directly proportional to the whitefly population present in the environment (Mansour *et al.,* 1992; Mehta *et al.,* 1994). Both adults and larvae can acquire the virus by feeding on infected plants with a minimum access and acquisition period (AAP) of 15 minutes. The virus has a latent period of 21-24 hours, and persists for 10 to 20 days in viruliferous B. tabaci adults (Cohen *et al.*, 1966).

Whiteflies are vectors of viruses causing many diseases in the tropics and subtropics. The whiteflies are a snow-white insect measuring about 1mm in length (Bohmfalk *et al.,* 2006). The adult whitefly starts laying eggs immediately after emerging from the nymph. Eggs are laid underneath leaves to protect them from adverse weather conditions such as rainfall and direct solar radiation (Marks, 2006).

2.10. Screening

Twenty-three tomato accessions were screened for resistance to *Tomato yellow leaf curl virus* under field conditions and examined that accessions of the wild species *Lycopersicon pimpinellifolium, Lycopersicon hirsutum,* and *Lycopersicon peruvianum* showed variance in their response to infection,

however *Lycopersicon chilense* showed highest degree of resistance against the disease (Zakay *et al.,* 1991).

Twenty tomato genotypes were screened for resistance against *Tomato yellow leaf curl virus (TYLCV)* in Madhya Pradesh, India and reported that the cultivars Hisar Anmol and Hisar Gaurav were resistant to tomato leaf curl disease (Rai *et al.,* 2001).

Ten tomato cultivars were screened against *TYLCV* at 45 days after planting and observed that among all the cultivars Punjab Chhuhara showed higher degree of resistance against tomato leaf curl virus (Sajeed *et al*., 2002).

A total of 34 tomato genotypes were screened for resistance to *TYLCV* under glasshouse and field conditions and found that *Lycopersicon hirsutum* LA1777 and PI 390659 were best sources of resistance to the virus (Maruthi *et al*., 2003).

Total 22 cultivars of tomatos were screened against *TYLCV* in Faizabad and out of 22 cultivars screened, none of the cultivars was found resistant against the disease. However, Hisar Anmol was found moderately resistant to the virus, while three cultivars were categorized as moderately susceptible and 18 were found susceptible to tomato leaf curl virus (Yadav and Awasthi, 2009).

The screening of tomato germplasm against *TYLCV* was done in Ghana. The researcher evaluated 30 accessions against the disease under field conditions at 30, 45 and 60 days after transplanting and found that no accession provided complete resistance to tomato leaf curl virus (Osei *et al.,* 2012).

Thirty-two tomato genotypes were screened for resistance against *tomato leaf curl disease* during rabi season at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and Vegetable research farm, Varanasi, Uttar Pradesh. It was observed that one wild accession, H-88-78-1 showed immune reaction against *TYLCV*, three genotypes viz., Hissar Lalima, TLBRH-6 and NS-515 showed resistant reaction and eight genotypes viz., Hissar Anmol, Kishi Vishesh, Kashi Amrit, Kashi Sharad, KS-17, KS-118, Avinash-2 and US-1008 were found moderately resistant against tomato leaf curl virus (Singh, 2014).

Twenty-seven tomato varieties/lines were screened for the source of resistance against *tomato yellow leaf curl virus (TYLCV)* under field conditions and found that three varieties were highly susceptible, six were susceptible, four were moderately susceptible, six were moderately resistant and eight were resistant. No variety/line was highly resistant or immune against tomato leaf curl virus disease (Zeshan *et al.*, 2016).

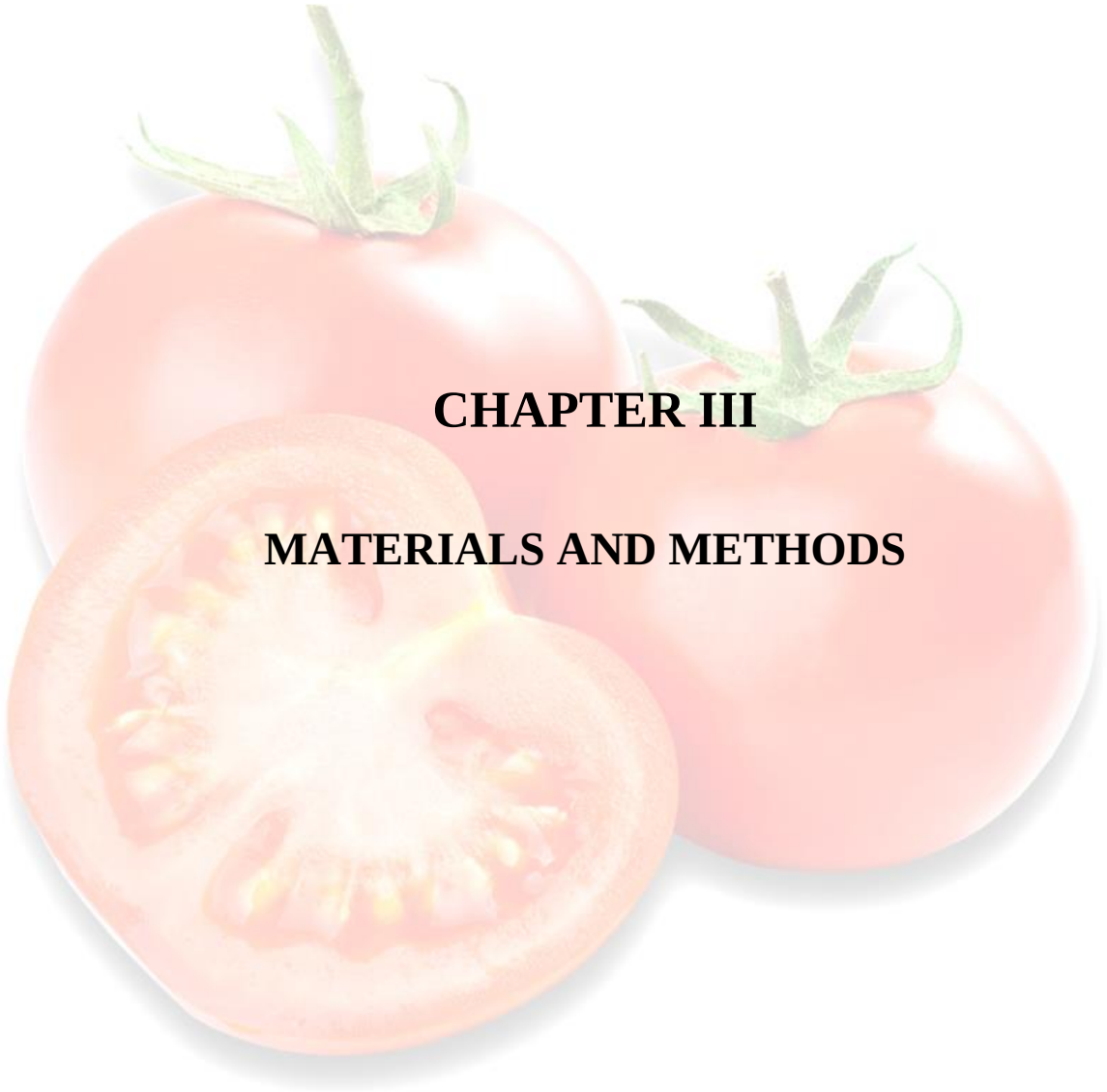
2.11. Yield Loss

Water deficits improved the quality of fruits, increased soluble solids and acidity and that water stress throughout the growing season significantly reduced yield and fruit size, but plants stressed only during flowering showed fewer but bigger fruits than completely non-stressed plants (Nuruddin *et al.*, 2003).

The *tomato yellow (TYLCV) leaf curl* virus (is one of the most devastating viral disease of cultivated tomato *(Lycopersicon esculentum)* in tropical and subtropical regions of worldwide causing the losses up to 100 per cent (Moriones and Navas, 2000).

Tomato yellow leaf curl virus is a geminivirus transmitted by whitefly (*Bemisia tabaci*). It causes most destructive disease of tomato throughout the Mediterranean region, the Middle East and the tropical regions of Africa and Central America. It is also reported from Japan, Australia and the USA. In many cases yield loss can be up to 90% reported by (Ganif, 2003).

It has been reported that water deficit stress increases the flower abortion, thus affects the fruits settings. The low marketable fruit yield obtained for some tomato varieties might be due to non-development of flowers. It was observed that only 50% of the flowers produced developed into fruits, thus sink size was a limiting factor to fruit production in tomato (Olaniyi *et al.*, 2010).



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 2018-2020, to ascertain the incidence, severity of *Tomato Yellow Leaf Curl Virus* (TYLCV) and its detection through PCR. The material used and techniques adopted during the study are being summarized hereby with some headings and sub-headings.

3.1. Experimental Site

The experiment was conducted at central research field, Plot No. 01, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, during the period of October 2018 to April 2019. The experimental area situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon, 1988). (Appendix- I).

3.2. Soil Characteristics

The soil characteristics of the experiment field was 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. (Appendices- II).

3.3. Climate

The climate of the Modhupur Tract varies slightly from north to south, the northern reaches being much cooler in winter. Average temperatures vary from

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 32^0C and average minimum temperature was 20^0C. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment was collected from Bangladesh Meteorological Department, Agargaon, Dhaka. (Appendices-III).

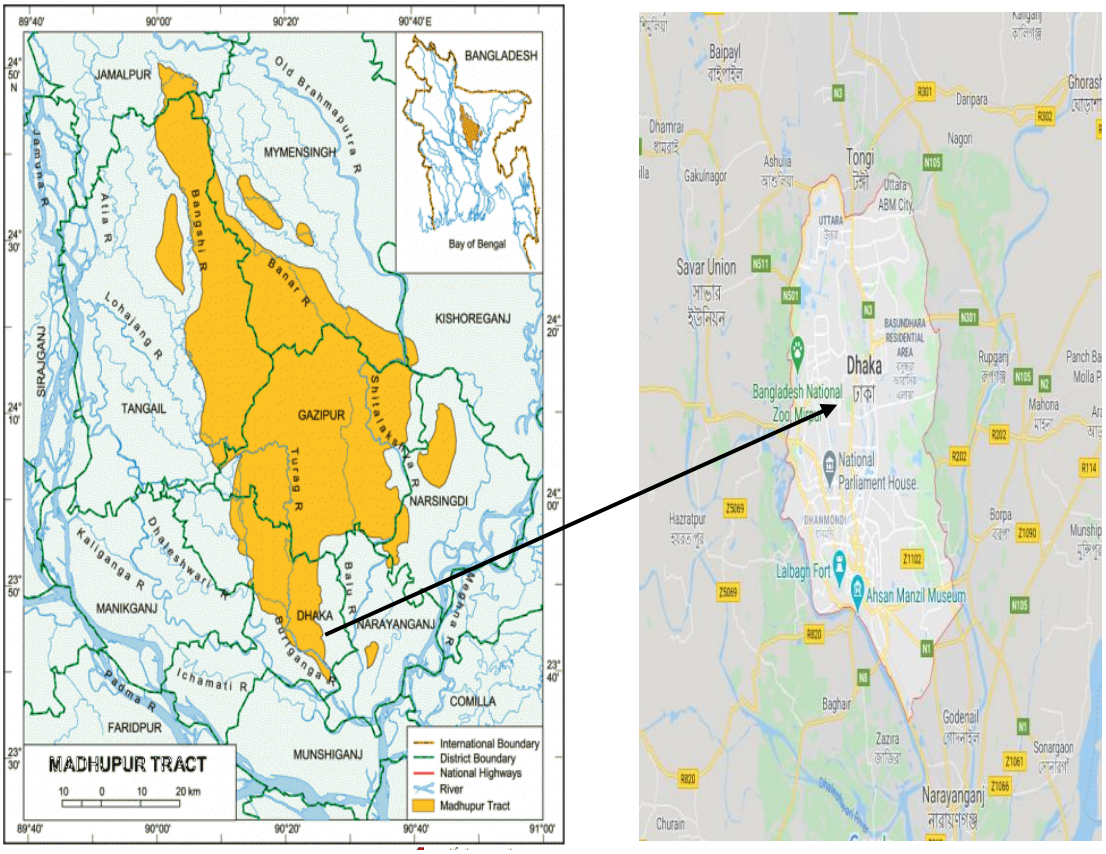


Figure 1. Madhupur Tract, AEZ No. 28

3.4. Planting Material

In total ten tomato assortments were chosen to conduct the examination. Seeds were gathered predominantly from Bangladesh Agricultural Research Institute (BARI), Gazipur. Selected varieties of tomato are popular varieties of BARI and most cultivated all over the country. Name of selected tomato varieties used in the present study are mentioned in table 1.

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

SL No.	Name of variety	Origin
01	BARI Tomato-5	BARI
02	BARI Tomato-8	BARI
03	BARI Tomato-9	BARI
04	BARI Tomato-11	BARI
05	BARI Tomato-14	BARI
06	BARI Tomato-16	BARI
07	BARI Tomato-17	BARI
08	BARI Tomato-18	BARI
09	BARI Tomato-19	BARI
10	BARI Tomato-15	BARI

3.5. Experimental Design

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.

3.6. Seedling Preparation

For the seedlings preparation, seeds were soaked overnight in distil water. Seedlings were grown in a seed bed of the experimental field of SAU. The soil of seed bed was mixed with Furadan 5G and covered the whole soil with polythene sheet to sterilize the soil. Then it was mixed with desired amount of fertilizers and cowdung. Finally, the seeds were sown in individual row and proper care was taken for better germination and seedling development. Some seedlings were found damping off diseased then Cupper oxychloride (Semco) was treated in the tray @ 1g/L water.

Figure 2. Raising of seedling in seed bed

3.7. Land Preparation and Transplanting of Seedling

The selected land for the experiment was first opened on 10 October, 2018 by power tiller and expose to the sun for a week. After one week the land was ploughed and cross-ploughed several times with a power tiller and laddering was done to obtain good tilt. Weeds and stubbles were removed and the large clods were broken into smaller pieces to obtain a desirable tilth of soil for sowing of

seeds. After removal of the weeds, stubbles and dead roots, the land was leveled and the experimental plot was separated to make the unit plots and were prepared as 10 cm raised beds. Solid and uniform measured 30 days old seedlings were removed independently from the seed beds. The seed beds were watered before evacuating the seedlings to limit the root injury. The seedlings were relocated in the pits of the exploratory plots toward the evening of 11 November, 2018 keeping up a dividing of 40 cm and 60 cm between the lines and plants, individually.

Figure 3. Seedling transplanting to the main field

Light water system was given following relocating by utilizing a watering stick. So as to hole filling and to check the outskirt impact, some additional seedlings were likewise relocated around the fringe zone of the test field.

Figure 4. Watering of transplanted seedlings into the experimental field

3.8 Intercultural Operations

3.8.1. Gap filling

Gap filling was done after one week of transplantation. The seedlings were taken from the same source and a minor gap filling was done where it was necessary.

3.8.2. Weeding

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

3.8.3. Manure and Fertilizer management

The following doses of manure and fertilizers were used

Manure/fertilizer	Doses/ha
Cow-dung	10 ton
Urea	400 kg
TSP	250 kg
MP	200 kg

3.8.4. Irrigation and drainage

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

3.8.5. Staking

When the plants were well established, staking was given to each plant by bamboo sticks to keep plants erect.

3.9. Identification of *Tomato Yellow Leaf Curl Virus* (*TYLCV*)

Identification of the virus was done on the basis of the biological properties such as symptomology and host-transmission. Visual observation was done by observing the typical symptoms of *TYLCV* infection like cupping, downward and upward curling, marginal chlorosis and mottling of the infected leaf, remarkable reduction of leaf area, smaller sized leaflets and severe stunting of plant (Sinistera *et al.,* 2000). The disease incidence of *TYLCV* was calculated on the basis of the appearance of typical symptoms of the virus. This was done by counting the plants observed everyday starting from the transplanting to harvesting date. The plants were inspected every alternate day morning to note the visual appearance and also to count the insect vectors.

3.10. Inspection of Insect Vectors (whitefly) Association

TYLCV is vectored by insect vectors whitefly. In this study, the inspection of whitefly association was done by using yellow trap method. 6×6 inch yellow color board was used that was polished with sticky oil. The whitefly was counted and number was recorded as per plot so that whitefly association with each variety could be measured. Whitefly association data was taken at an interval 15 days in each marked plot that was started from 30 DAT.

Figure 5. Yellow Trap used for inspection of insect vectors whitefly association in each plot.

3.11. Estimation of Disease Incidence

Disease incidence, which measures the extent of proportion of a disease within a given field (Agrios 2005), was estimated by using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of disease plant}}{\text{Number of total plants observed}} \times 100$$

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

Table 2. Disease Rating Scale of *TYLCV*

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

3.12. Calculation of Disease Severity

Symptom development was evaluated according to the symptom severity scale described by Lapidot and Friedmann, 2002. Disease severity was calculated by the following formula (calculation given in appendix-IV) and following disease rating scale (Table 3):

$$\text{Disease Severity} = \frac{\textbf{Sum total of disease rating}}{\textbf{Total number of observation} \times \textbf{Maximum grade in the scale}} \times 100$$

Table 3. Disease severity rating scale of *TYLCV* to determine disease severity

Grading Scale	Symptoms
0	No visible symptoms, healthy plant.
1	Very slight yellowing of leaflet margins on apical leaf.
2	Some yellowing and minor curling of leaflet ends
3	A wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop
4	very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops

3.13. Molecular Detection of *TYLCV* through PCR

The molecular detection was done at Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology. For molecular detection of *TYLCV* through PCR, the diseased and healthy leaves samples were collected from the experimental field. Molecular detection was done via two molecular protocols. Firstly, total genomic DNA was extraction from infected leaves as well as healthy leaves samples then performed the PCR test by using total DNA as template to detect the *TYLCV*.

3.13.1 Primer Designing

For detection of *TYLCV, Coat-protein (CP)* gene specific primers were designed using primer-3 version 0.4.0 software. (Deng *et.al* 1994). Primers were made to amplify the conserved/less mutating genomic segment and tested for primer specificity in-silico by applying BLAST, provided by, to reduce the chance of non-specificity. The 3′sequence primers with no similarity to viral sequences or other origin sequences were marked as selected. Primers were synthesized commercially and primer pair used in the study is presented in table 4.

Table.4 Primer pair used in the present study to amplify *TYLCV* at 520 bp fragment

Primers	**Primer Sequences 5'-3'**	**Tm of Primers (^{0} C)**	**Amplicon size (bp)**
TYLCV 520-FP	TAATATTACCGGACCGC	55	520
TYLCV 520- RP	TGGAGCTTGCAAGGCCCTTCACA	55	

3.13.2. DNA extraction kit Collection

DNA extraction Kit was collected from molecular biology reagents merchants (Advanced Bioscience, Bangladesh). The name of the DNA extraction kit was GF-1 plant DNA extraction kit.

Figure 6: GF-1 DNA extraction kit

3.13.3. Kit Components

Table.5 GF-1 DNA extraction kit component

Components	Amount
GF-1 columns	50
Collection Tubes	50
Plant Tissue Lysis Buffer (Buffer PL)	18ml
Plant Genomic Binding Buffer (Buffer PB)	35ml
Wash Buffer	24ml
Elution Buffer	10ml
Proteinase	1.05ml

3.13.4 DNA Extraction Protocol

Total DNA was extracted from tomato leaf samples (given in appendix-V) DNA was extracted using Nucleic acid extraction kit (GF-1 kit). Kit protocol was as follows-

Homogenization

Grind leaf tissue sample in liquid nitrogen into fine powder.

Tissue Lysis

Add 280 µl Buffer PL. Vortex sample 30 second. Add 20 µl Proteinase K. Incubate 65℃,1-2hr.

Centrifugation

Centrifuge at 14,000 rpm for 5 min. Transfer supernatant into new tube.

Removal of RNA

Add 20 µl RNase A and Incubate at 37℃ for 5 minute.

Homogenization

Add 2 volumes Buffer PB and mix thoroughly. Incubate at 65℃ for 10 minutes.

Addition of Ethanol

Add 200 µl absolute ethanol and mix immediately.

Loading to column

Transfer sample to column.

Centrifuge

Centrifuge At 10000 rpm for 1 minute. Discard Flow Through.

Column Washing

Add 650 µl Wash Buffer.

Centrifuge

At 10000 rpm for 1 minute. Discard flow through.

Column Drying

Elution

Transfer column to a new micro centrifuge tube. Add 50-100 µl Elution Buffer or water. Stand for 2 minutes.

Centrifuge

Store DNA at -20°C

3.13.5. Genomic DNA analysis with Agarose gel (1%)

Genomic DNA was extracted from leaf sample. For conformation and quantification of genomic DNA, extracted DNA was analyze in 1% agarose gel.

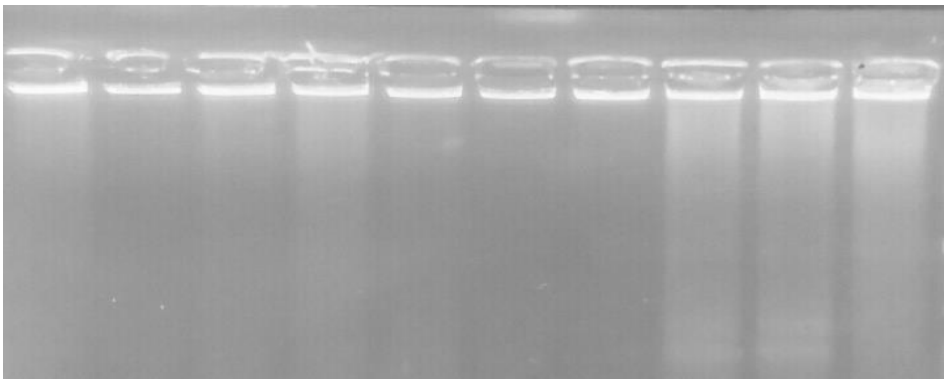


Figure 7: Genomic DNA was analyzed in 1% agarose gel.

3.13.6 PCR amplification

PCR was conducted in a reaction volume (25 µl) containing 14.3 µl of sterile water, 2.5 µL of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.1) and 0.1% Triton™ X 100), 2 µl of dNTP (2.5 mM), 2 µL of FP/ RP (10 µM), 1.0 µL of MgCl (25 mM), 0.2 µl of Taq polymerase (5 IU µL) and 1 µL of template DNA (diluted 1:25 in water). Following thermal cycle programme was performed, 1 cycle (4 min at 94°C), 30 cycles (1 min at 94°C, 1 min at 55°C and 2 min at72°C) and 1 cycle (10 min at 72°C). PCR products were stored at -20°C.

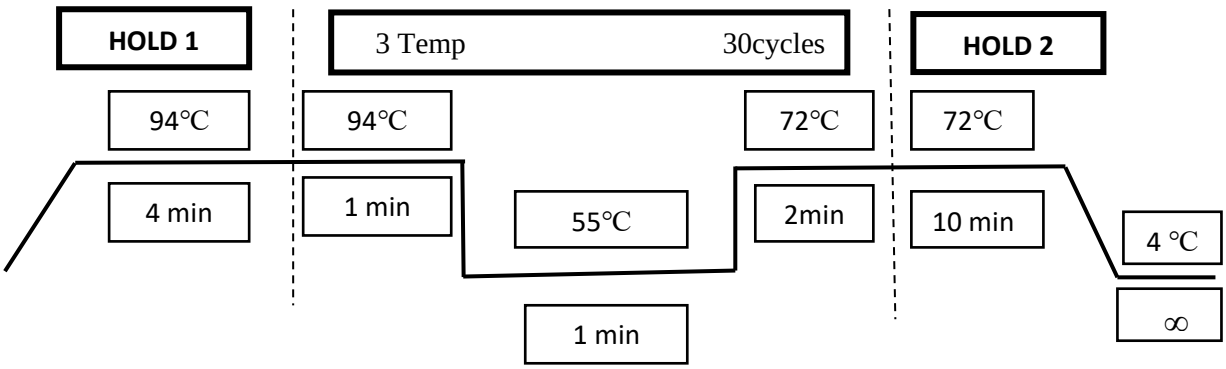


Figure 8. PCR cycling conditions to amplify *TYLCV-CP* gene fragment

3.13.7 Agarose gel electrophoresis and gel documentation

PCR products (25 µl of each) were subjected to 1% (w/v) agarose gel electrophoresis with 2 µl of loading dye at 80 volts for 1 hour in TBE buffer and stained with ethidium bromide (0.5 mL) and visualized under UV trans-illuminator and gel documentation system. The results were verified against DNA marker (Vivantis).

3.14. Parameters Assessed

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

a. No of leaves/plant
b. No of infected leaves/plant
c. No of branches/plant
d. No of flowers/plant
e. No of fruits/plant
f. Fruits diameter
g. Individual fruit weight
h. Shoot length
i. Root length
j. Yield/plant

3.14.1 No of leaves/plant:

The leaves of each plant were counted from 30 DAT and continued up to 60DAT. Only adult leaves are counted excluding the very young leaves and buds.

3.14.2 No of infected leaves/plant

The infected leaves of each plant were counted from 30 DAT and continued up to 60DAT.

3.14.3. No of infected branches/plant

The number of branches of each plant was counted from 30 DAT and continued up to 60DAT. As the branch was counted at adult age so there was no young branch considered in the counting.

3.14.4 No of flowers/plant:

The number of flowers of each plant was counted from 30 DAT and continued up to 60DAT. Only the healthy flowers were considered and the data was recorded.

3.14.5 No of fruits/plant

The number of fruits of each plant was counted and mean number of tomato fruits of each variety were recorded.

3.14.6 Fruits diameter

Mean diameter of collected tomatoes from each plant as per variety were measured by a slide calipers in centimeter (cm).

3.14.7 Individual fruit weight

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

3.14.8 Shoot length

Shoot length of the plant of was measured by a meter scale from the ground to longest tip of the plant in centimeter (cm) at last harvesting time stage.

3.14.9 Root length

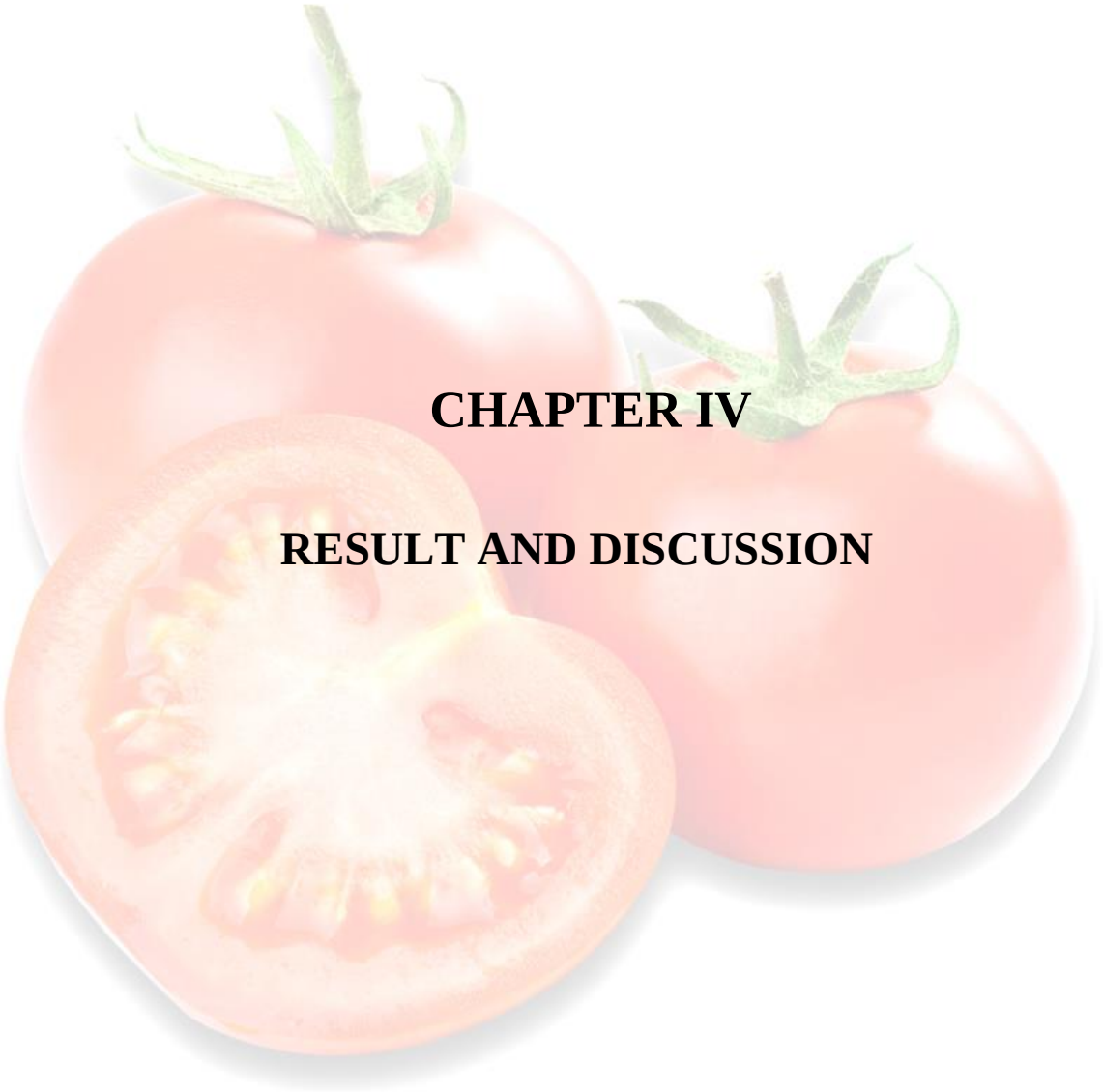
Root length of the plant of was measured by a meter scale in centimeter (cm) while the plant was uprooted.

3.14.10 Yield/plant

Every time tomato was harvested followed by measuring the weight and diameter and data was recorded. Total yield per plant was measured in kg and the diameter was measured in cm.

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

RESULT AND DISCUSSION

CHAPTER IV
RESULTS AND DISCUSSION

This chapter represents the experimental results. The evaluation of tomato varieties against *Tomato yellow leaf curl virus* (*TYLCV*) viz. BARI Tomato-5, BARI Tomato-8, BARI Tomato-09, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16, BARI Tomato-17, BARI Tomato-18, BARI Tomato-19 under field condition was done. Results were compiled based on disease incidence (%), disease severity (%) and morphological parameters. *TYLCV* identification was confirmed by molecular detection through PCR test.

4.1. Disease incidence (%) of *TYLCV* in selected tomato varieties

The effect of different varieties on disease incidence (%) of *Tomato yellow leaf curl virus* (*TYLCV*) was observed based on disease rating scale of *TYLCV* as present in Table 2 in methodology section. According to disease incidence rating scale followed in this study, BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16 were showed highly susceptible and BARI Tomato-17 was showed susceptible against the *TYLCV* in filed condition. And among the selected varieties, only three varieties; BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19 were showed moderately resistant against *TYLCV*. (Table 6.)

Table 6: Disease incidence (%) of selected tomato varieties against *Tomato Yellow Leaf Curl Virus (TYLCV)*

Variety	Disease Incidence (%)	Level of Resistance/Susceptibility
BARI Tomato-5	16.67	Moderately Resistant
BARI Tomato-8	100	Highly Susceptible
BARI Tomato-9	83.33	Highly Susceptible
BARI Tomato-11	83.33	Highly Susceptible
BARI Tomato-14	100	Highly Susceptible
BARI Tomato-16	83.33	Highly Susceptible
BARI Tomato-17	66.67	Susceptible
BARI Tomato-18	16.67	Moderately Resistant
BARI Tomato-19	16.67	Moderately Resistant
BARI Tomato-15	83.33	Highly Susceptible

4.2. Disease severity (%) of *TYLCV* in selected tomato varieties

The effect of different varieties on Disease severity (%) of *Tomato yellow leaf curl virus* (*TYLCV*) was observed based on disease severity rating scale of *TYLCV* as presented in Table 3 in methodology section. According to disease severity rating scale that followed in this study, the highest disease severity (67.46% and 76.39%) was found in BARI Tomato-14 and BARI Tomato-16 respectively, on the basis of grading scale it was showed the very severe plant

stunting and yellowing with severe leaf curling, cupping and margin chlorosis. The other varieties, BARI Tomato-8, BARI Tomato-11, BARI Tomato-17 and BARI Tomato-15 were showed the moderate disease severity (32.3%,32.1%, 42.5% and 39.4% respectively), while BARI Tomato-5, BARI Tomato-9, BARI Tomato-18 and BARI Tomato-19 were showed lower disease severity (9.1%, 28.3%, 10.6% and 9.4% respectively). According to the disease severity grading scale these varieties are showed very slightly yellowing, some yellowing with minor curling and a wide range of leaf yellowing, curling, cupping and not shown chlorosis symptom. Disease severity is shown graphically in figure 5.

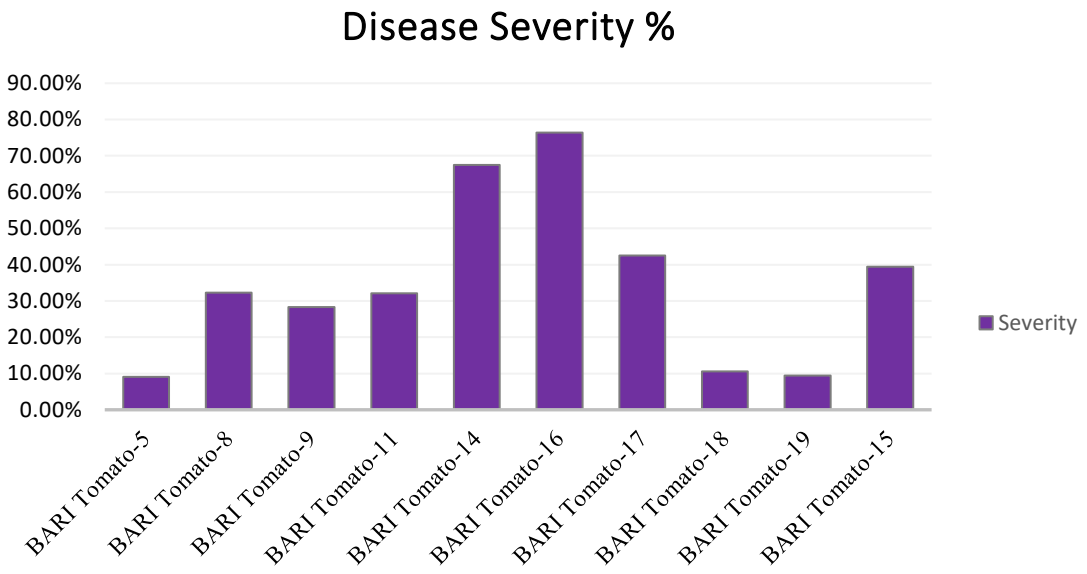


Figure 9: Graphical representation of disease severity (%) of different tomato varieties against *Tomato Yellow Leaf Curl Virus (TYLCV)*

4.3. Incidence of whitefly association per plot

Whitefly plays an important role for transformation *TYLCV*. For counting of whitefly per plot, yellow trap was used. It was observed that in the varieties of BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-16. BARI Tomato-17, and BARI Tomato-15, the whitefly association was increased with the increase of plant age. In the varieties of BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19 BARI, the whitefly association was not increase with the increased of plant age. As clearly shown in figure 10.

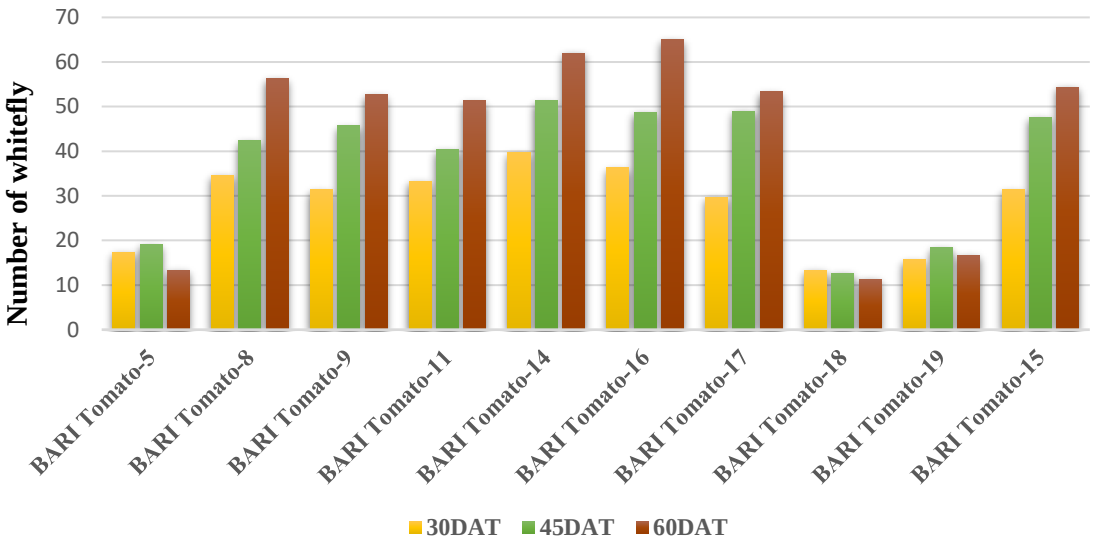
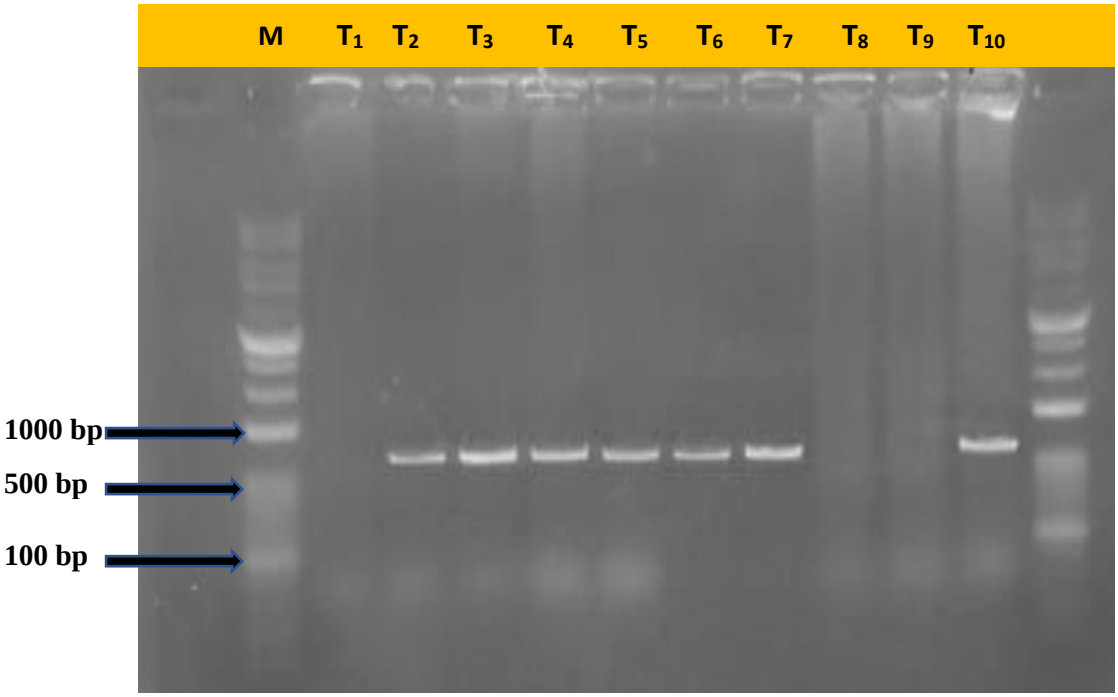


Figure 10: Graphical representation of whitefly association per plot in selected tomato varieties.

4.4. *TYLCV* Detection through PCR

Now a day molecular detection through PCR is reliable technology. After PCR amplification through optimized protocol as describe in methodology section, the samples of PCR product were loaded in agarose gel (1.5%). DNA ladder (100 bp) were used in between two side of the samples loaded from ten tomato varieties. From the gel documentation (figure 10), it was depicted that sample from seven varieties; BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16 and BARI Tomato-17 showed PCR positive result and gave sharp band at 520 bp that indicates virus present in the seven varieties. On the other hand, samples from the three varieties; BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19, don't gave any band in gel documentation, its means samples were not amplified that indicates virus negative result.



T1: BARI Tomato-5, T2: BARI Tomato-8, T3: BARI Tomato-9, T4: BARI Tomato-11, T5: BARI Tomato-14, T6: BARI Tomato-16, T7: BARI Tomato-17, T8: BARI Tomato-18, T1: BARI Tomato-19, T1: BARI Tomato-15, M-DNA marker

Figure 11: PCR amplification to detect *TYLCV*, samples from ten tomato varieties

Table 7. PCR test for *TYLCV* detection

SL. No.	Variety	Result
1.	BARI Tomato-5	Not detected
2.	BARI Tomato-8	Detected
3.	BARI Tomato-9	Detected
4.	BARI Tomato-11	Detected
5.	BARI Tomato-14	Detected
6.	BARI Tomato-16	Detected
7.	BARI Tomato-17	Detected
8.	BARI Tomato-18	Not detected
9.	BARI Tomato-19	Not detected
10.	BARI Tomato-15	Detected

4.5. The morphological features which are identical, in-relation to yield and yield contributing character in tomato against *Tomato yellow leaf curl virus (TYLCV)*

4.5.1. Number of leaves and branches per plant in selected tomato cultivars

In terms of number of leaves per plant was showed significant variance among the tested tomato varieties. The maximum number of leaves per plant was obtained in the variety BARI Tomato-11 (70.00) followed by variety BARI Tomato-14(63.00), BARI Tomato-8 (58.33), BARI Tomato-5 (55.33), BARI Tomato-16 (55.00), BARI Tomato-15(52.00), BARI Tomato-18 (51.33), BARI Tomato-17 (49.67), BARI Tomato-19 (48.33) and BARI Tomato-9 (46.67). Among the varieties; BARI Tomato-11, BARI Tomato-14, BARI Tomato-8, BARI Tomato-5, BARI Tomato-18 & BARI Tomato-9, showed statistically significant difference, while there was no statistically significant difference among the varieties; BARI Tomato-5 & BARI Tomato-16, BARI Tomato-18 & BARI Tomato-15, BARI Tomato-17 & BARI Tomato-19.

In terms of number of branches per plant was showed significant variance among the selected tomato varieties. The maximum number of branches per plant was recorded in the variety BARI Tomato-11 (11.667), followed by BARI Tomato-19(9.000), BARI Tomato-5 (7.667), BARI Tomato-18(7.667), BARI Tomato-8(7.000), BARI Tomato-16(6.667), BARI Tomato-17(5.667), BARI Tomato-15 (5.667), BARI Tomato-9 (5.000) &BARI Tomato-14 (4.667). There was significant difference among the varieties; BARI Tomato-11, BARI Tomato-19 and BARI Tomato-9. But there was no significant difference among the varieties; BARI Tomato-5, BARI Tomato-18, BARI Tomato-8, BARI Tomato-16, BARI Tomato-17 and BARI Tomato-15 each and other. Results are presented in Table 8.

Table 8: Number of leaves and branches per plant in selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV)*

Variety	Number of leaves/Plant	Number of branches /Plant
BARI Tomato-5	55.33 d	7.67 bc
BARI Tomato-8	58.33 c	7.00 cd
BARI Tomato-9	46.67 g	5.00 e
BARI Tomato-11	70.00 a	11.67 a
BARI Tomato-14	63.00 b	4.67 e
BARI Tomato-16	55.00 d	6.67 cd
BARI Tomato-17	49.67 ef	5.67 de
BARI Tomato-18	51.33 e	7.67 bc
BARI Tomato-19	48.33 fg	9.00 b
BARI Tomato-15	52.00 e	5.67 de
CV (%)	2.83	13.26
LSD (0.05)	2.67	1.61

4.5.2. Number of flowers and fruits per plant in selected tomato cultivars

In terms of number of flowers per plant was showed significant variance among the tested tomato varieties. The maximum number of flowers was obtained from BARI Tomato-11 (185.33) and the minimum number of flowers was observed from BARI Tomato-17 (47.67). Among the varieties there were significant difference between BARI Tomato-11, BARI Tomato-16 & BARI Tomato-15 than other varieties. There was no significant difference among BARI Tomato-5 & BARI Tomato-9, BARI Tomato-8 & BARI Tomato-19, BARI Tomato-14, BARI Tomato-17 & BARI Tomato-18.

In terms of number of fruits per plant was showed significant variance among the tested tomato varieties. The maximum number of fruits was obtained from BARI Tomato-11 (151.67) and the minimum number of fruits obtained from

BARI Tomato-18 (23.00). Among the varieties there were significant difference between BARI Tomato-11 & BARI Tomato-16 than the other varieties. There were no significant difference BARI Tomato- BARI Tomato-15 BARI Tomato-19, BARI Tomato-9, BARI Tomato-5, BARI Tomato-8, BARI Tomato-14, BARI Tomato-17, BARI Tomato-18 were statistically similar. Results are presented in Table no 9.

Table 9: Number of flowers and fruits per plant in selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV).*

Variety	No of flowers/Plant	No of fruits/Plant
BARI Tomato-5	53.00 ef	28.67 e
BARI Tomato-8	63.00 d	25.33 ef
BARI Tomato-9	54.67 e	30.00 de
BARI Tomato-11	185.33 a	151.67 a
BARI Tomato-14	48.00 f	26.33 ef
BARI Tomato-16	101.33 b	48.67 b
BARI Tomato-17	47.67 f	23.33 f
BARI Tomato-18	48.33 f	23.00 f
BARI Tomato-19	62.00 d	35.00 cd
BARI Tomato-15	74.33 c	40.00 c
CV (%)	4.83	6.80
LSD Value(0.05)	6.1096	5.0370

4.5.3. Fruits diameter (cm), Individual fruit weight (g) and yield per plant (kg) in tested tomato varieties

In terms of fruit diameter (cm) per plant was showed significant variance among the selected tomato varieties. The maximum fruit diameter was obtained from BARI Tomato-17 (7.9333) & the minimum fruit diameter was obtained from BARI Tomato-11 (2.1667). Among the varieties there were significant

difference between BARI Tomato-17, BARI Tomato-8, BARI Tomato-5 & BARI Tomato-11. There was no significant difference between BARI Tomato-9, BARI Tomato-14, BARI Tomato-16, BARI Tomato-18, BARI Tomato-15& BARI Tomato-19.

In terms of individual fruit weight per plant was showed significant variance among the selected tomato varieties. The maximum individual fruit was weight obtained from BARI Tomato-17 (184.40) and minimum fruit weight was obtained from BARI Tomato-11 (8.13). Among all the varieties there were significant difference because all the varieties fruits weight was different from each other.

In terms of yield per plant was showed significant variance among the selected tomato varieties. The highest yield per plant was obtained from BARI Tomato-17 (4.3000) and the lowest yield per plant was obtained from BARI Tomato-5 (1.1167). Among the varieties BARI Tomato-17, BARI Tomato-9, BARI Tomato-16 were statistically different from others. There was no significant difference between BARI Tomato-5, BARI Tomato-11, BARI Tomato-8, BARI Tomato-15, BARI Tomato-14, BARI Tomato-19 & BARI Tomato-18. Results are presented in table no10.

Table 10: Fruits diameter (cm), Individual fruits weight (g) and yield (kg) per plant in selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV)*

Variety	Fruits diameter	Individual fruit weight	Yield (kg)
BARI Tomato-5	4.3667 f	38.97 i	1.1167 g
BARI Tomato-8	6.9333 b	106.57 c	2.7000 d
BARI Tomato-9	6.3667 c	130.00 b	3.8867 b
BARI Tomato-11	2.1667 g	8.13 j	1.2167 g
BARI Tomato-14	6.4000 c	88.40 d	2.3200 e
BARI Tomato-16	5.1667 de	70.00 f	3.4033 c
BARI Tomato-17	7.9333 a	184.40 a	4.3000 a
BARI Tomato-18	5.6000 d	77.73 e	1.7867 f
BARI Tomato-19	4.9333 e	58.87 h	2.0600 ef
BARI Tomato-15	5.5000 d	67.43 g	2.6933 d
CV (%)	5.49	1.45	7.36
LSD Value (0.05)	0.5212	2.0645	0.3216

4.5.4. Shoot length (cm) and root length (cm) in selected tomato varieties

In this study shoot length showed significant variance among the tested tomato varieties. The highest shoot length was obtained from BARI Tomato -9 (104.27) and the lowest shoot length was obtained from BARI Tomato-16(81.07). Among the varieties there were no significant difference between all the varieties.

In this study root length showed significant variance among the tested tomato varieties. The highest root length was obtained from BARI Tomato -9 (22.800) and the lowest root length was obtained from BARI Tomato-17 (16.533). Among

the varieties there were no significant difference between all the varieties and statistically identical. Results are presented in table no 11.

Table 11: Shoot length (cm) and Root length (cm) in tested tomato varieties against *Tomato yellow leaf curl virus (TYLCV)*

Variety	Shoot length	Root length
BARI Tomato-5	92.43 abcd	19.733 abc
BARI Tomato-8	83.67 cd	16.700 cd
BARI Tomato-9	104.27 a	22.800 a
BARI Tomato-11	89.33 bcd	17.467 bcd
BARI Tomato-14	97.50 ab	19.800 ab
BARI Tomato-16	81.07 d	18.400 bcd
BARI Tomato-17	81.30 d	16.533 d
BARI Tomato-18	94.97 abc	19.100 bcd
BARI Tomato-19	84.87 bcd	17.233 bcd
BARI Tomato-15	92.03 abcd	18.967 bcd
CV (%)	8.82	9.60
LSD Value(0.05)	13.643	3.0739

Discussion

Tomato (*Lycopersicon esculentum* Mill.) is an important and widely grown vegetable crop. It is a good source of antioxidant, vitamin A, C, E and minerals. It also reduces the risk of cancer. Its juice is a good blood purifier. The crop suffers from many fungal, viral, bacterial and nematode diseases which causes reduction in the yield and quality of tomato fruit. Among the viral diseases, *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is the most important one which limits the tomato production to a great extent. The disease caused a serious loss in tomato production. Therefore, the present experiment was carried out to evaluate the incidence and severity level of *Tomato Yellow Leaf Curl Virus* (*TYLCV*) against the tested tomato germplasms, and to screen the resistance/tolerance of selected tomato germplasms against *TYLCV* by molecular detection through PCR. The result generated during the course of investigation is discussed here.

Among the selected tomato varieties, most of the selected tomato varieties were showed highly susceptible (83.33-100%) to *TYLCV* except BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19. Among the tested varieties, the highest disease incidence (100%) was found in two varieties; BARI Tomato-8 and BARI Tomato-14. All selected tomato plants were infected and appear the remarkable symptoms of *TYLCV*. So according to disease incidence rating scale used in this study both varieties are highly susceptible to *Tomato yellow leaf curl virus (TYLCV)* in natural field condition. Moderate to higher disease incidence (66.67%-83.33%) was recorded in BARI Tomato-17, BARI Tomato-9, BARI Tomato-11, BARI Tomato-16 and BARI Tomato-15. According to disease incidence rating scale these varieties are susceptible to highly susceptible against *TYLCV*. The lowest disease incidence (16.67%) was found in BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19. According to disease incidence rating scale these varieties are moderately resistant against *TYLCV*. The findings estimated in the current study that agreement with two recent published reports on the disease incidence in cultivated tomato varieties (Reddy *et al.,* 2011; Zeshan *et al.,* 2016).

Among the selected tomato varieties, the highest disease severity was recorded in BARI Tomato-16 (76.39%) followed by BARI Tomato-14(67.50%), BARI Tomato-17 (52.50%), BARI Tomato-15 (39.40%), BARI Tomato-8 (32.30%), BARI Tomato-11(32.10%), BARI Tomato-9(28.30%). The lowest disease severity was found in BARI Tomato-5(9.10%) preceded by BARI Tomato-19(9.40%) and BARI Tomato-18(10.16%). From the disease incidence and severity analysis, it was revealed that BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19 showed moderately resistance and lower disease severity against *Tomato yellow leaf curl virus (TYLCV)* at certain level of plant growth. The results of disease incidence and severity of the present study match with the previous study that was conducted by Yadav and Awasthi, (2009).

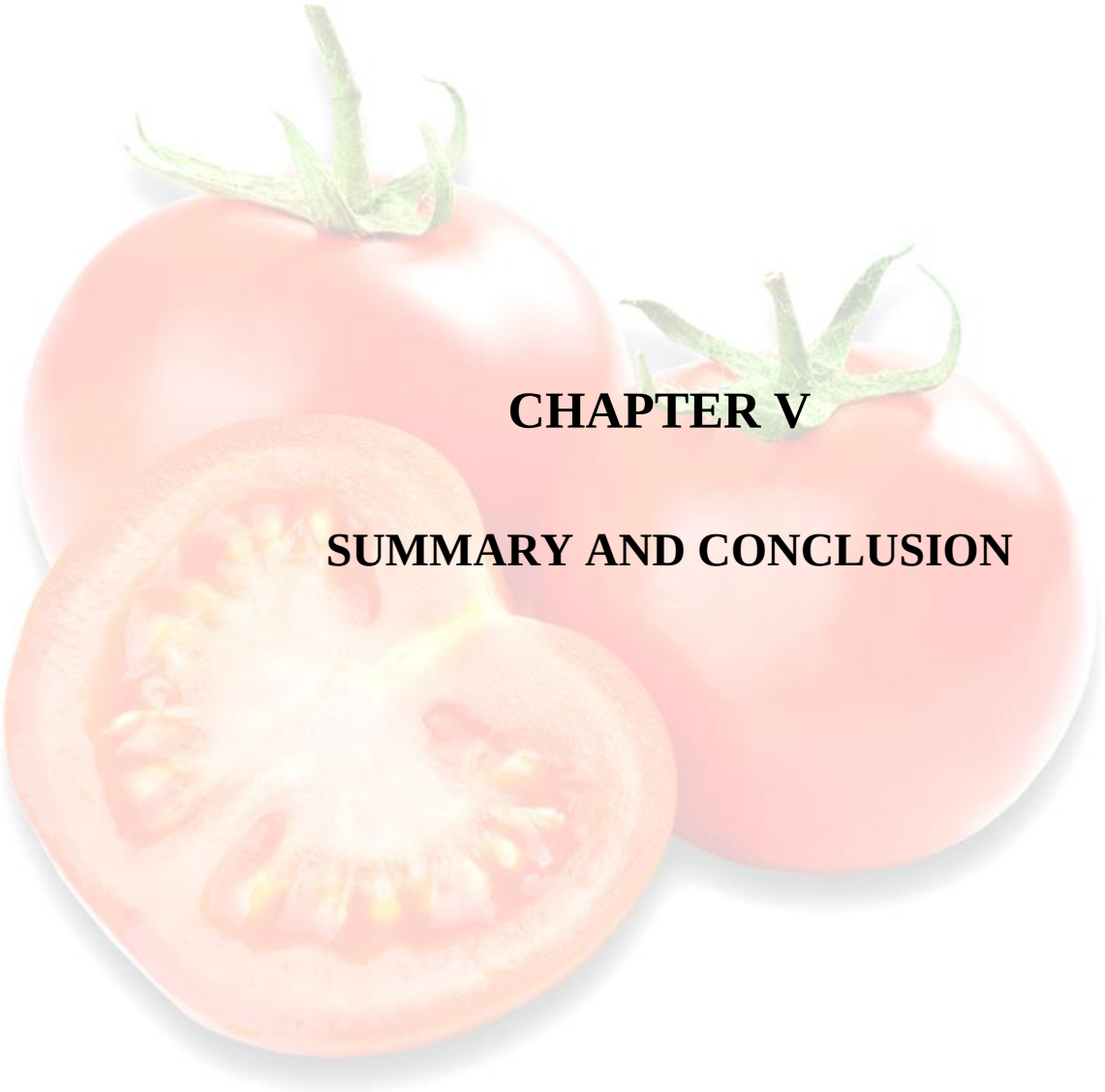
For management of specific virus, transmission of the virus is major concern. Among the tomato viruses *TYLCV* is most common and major threat of tomato cultivation. According to publish reports regarding plant virus transmission, this plant virus is mainly transmitted by insect vectors whitefly. Early infection of whitefly and appear the *TYLCV* typical symptoms that causes drastic reduction of all the growth contributing character of all the tomato varieties. The extent of damage in different growth contributing characters was largely dependent upon the stage of infection of *TYLCV*, condition of growing seedlings and tomato varieties. In the present study, it was noticed that the insect vectors whitefly association was increasing day by day with increases of plant age. This trend was observed in all selected tomato varieties except BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19, where whitefly association was almost similar throughout the growing season. Almost same phenomenon of whitefly association with the *TYLCV* infection was noted by Gupta, (2000).

From the PCR test to detect that *TYLCV* through molecular detection, it was revealed that among selected tomato varieties viz. BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16, BARI Tomato-17 were infected by *Tomato yellow leaf curl virus (TYLCV*) and gave positive result in PCR test. Remaining three varieties; BARI

Tomato-5, BARI Tomato-18 and BARI Tomato-19 gave negative result in PCR test. So, infection was there but expression of viral genome not in these three tomato varieties. From both lab and field findings, it may be concluded that although the varieties were infected but showed moderately resistance against *TYLCV*. Results from the present study are agreement with the study of (Samarakoon *et al*., 2012).

The infected tomato plant shows different morphological responses against different morphological features. The yield of individual variety depends on the number of leaves, branch, flowers and fruits per plant. The maximum number of leaves per plant was obtained in the variety BARI Tomato-11 and minimum number of leaves per plant was obtained in the variety BARI Tomato-9. The highest number of branches per plant was recorded in the variety BARI Tomato-11 and the lowest number of branches per plant was recorded in the variety BARI Tomato-14. The maximum number of flowers was obtained from BARI Tomato-11 and the minimum number of flowers was observed from BARI Tomato-17. The maximum number of fruits was obtained from BARI Tomato-11 and the minimum number of fruits obtained from BARI Tomato-18. In the present study the yield contributing parameters seemed to be affected to varying extent depending on the viral infection, Number of whiteflies per leaf, growing condition and tomato variety. Similar observations were recorded by Ajlan *et al.,* (2007); Olaniyi *et al.,* (2010) and Ganif, (2003).

From the findings of this study, it is revealed that out of ten varieties BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19 showed better performance compared to other varieties against *Tomato yellow leaf curl virus (TYLCV)*. Both disease incidence and severity were lower in these varieties and virus was not detected.



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 2018-2020, to ascertain the incidence and severity of *Tomato Yellow Leaf Curl Virus (TYLCV)* and its molecular detection through PCR**.** In total ten Tomato varieties were tested in this study against *TYLCV*. 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 to prevent the infestation of insect vector whitefly.

The experiment was aimed to assess the varietal performance of tested tomato varieties against *Tomato Yellow Leaf Curl Virus (TYLCV)* and identify the *TYLCV* on the basis of biological properties. Genomic DNA was extracted from leaf samples of the tested varieties to detect the *TYLCV* through modern molecular technique PCR.

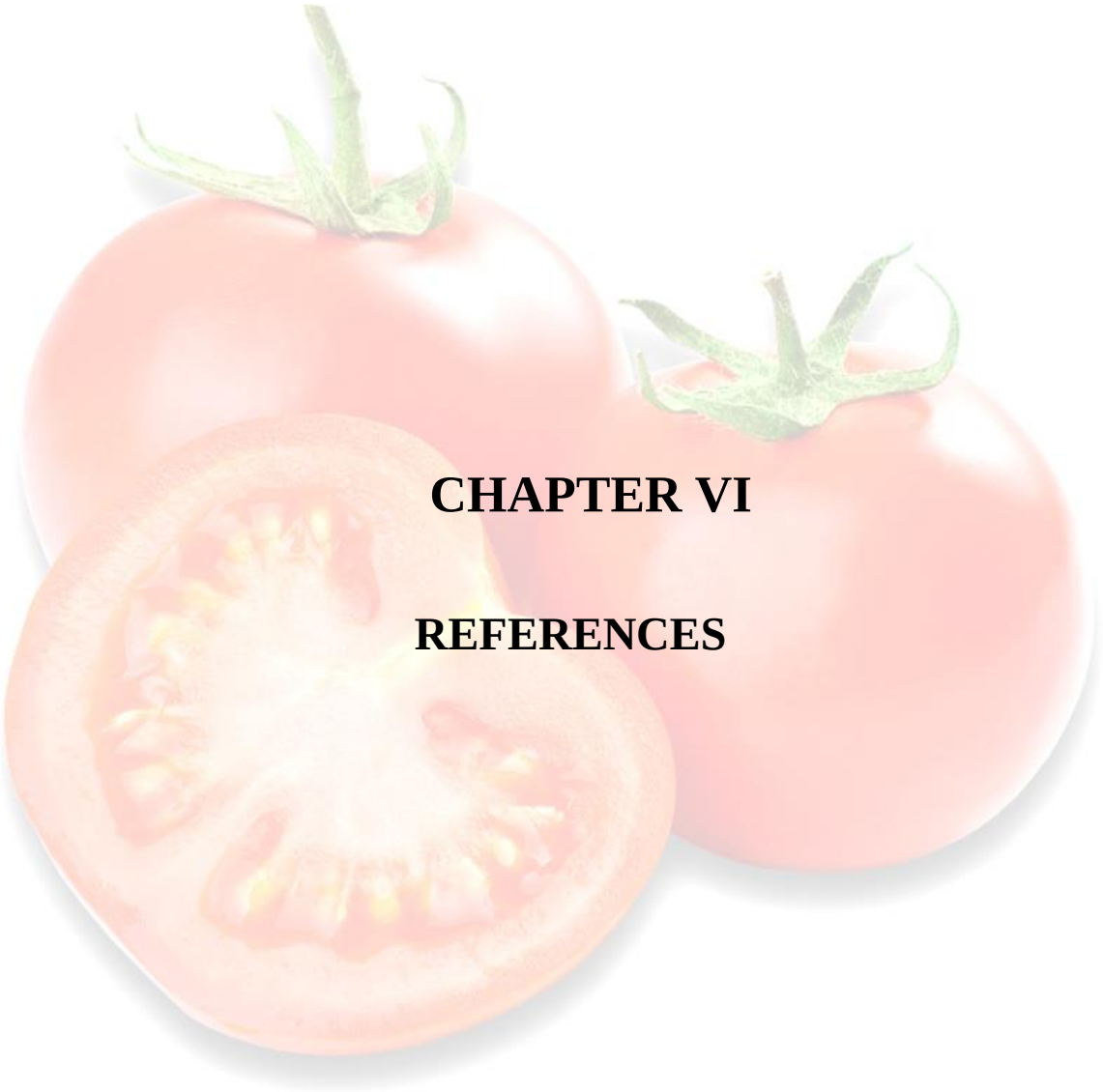
From the study it was observed that among the ten selected varieties, BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19 showed lower disease incidence and severity up to 60 DAT. Remaining varieties; BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato- 14, BARI Tomato-15, BARI Tomato-16 & BARI Tomato-17 were showed high disease incidence and severity at 30DAT as well as up to 60 DAT.

Among the ten selected varieties, whitefly association was varied significantly. In case of BARI Tomato-5, BARI Tomato-18 & BARI Tomato-19 whitefly association was not increased with the increase of plant age. But it was noticed that in BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato- 14, BARI Tomato-15, BARI Tomato-16 & BARI Tomato-17 whitefly association was increasing with the increase of plant age.

The morphological features which are identical to yield and yield contributing characters was also studied. The highest number of leaves per plant was counted in the variety BARI Tomato-11 and the lowest number of leaves in BARI Tomato-9. The highest number of branches per plant was recorded in the variety BARI Tomato-11and the lowest number of branches in BARI Tomato-14. The maximum number of flowers was counted from BARI Tomato-11 and the minimum number in BARI Tomato-17. The maximum number of fruits was obtained from BARI Tomato-11 and the minimum number in BARI Tomato-18. The maximum fruit diameter was measured from BARI Tomato-17 and the minimum in BARI Tomato-11. The maximum individual fruit weight was measured from BARI Tomato-17 and minimum in BARI Tomato-11. The highest yield per plant was obtained from BARI Tomato-17 and the lowest yield in BARI Tomato-5. The highest shoot length was measured from BARI Tomato -9 and the lowest in BARI Tomato-16. The highest root length was measured from BARI Tomato -9 and the lowest in BARI Tomato-17.

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 *TYLCV*. Among the tested varieties, seven varieties; BARI Tomato-8, BARI Tomato-9, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Tomato-16 & BARI Tomato-17 gave the positive results in PCR test and shown sharp band at 520 bp fragment. Other varieties; BARI Tomato-5, BARI Tomato-18 and BARI Tomato-19 gave negative result in PCR test.

From the above findings on different parameters studied, it can be concluded that BARI Tomato-5, BARI Tomato-18 and BARI Tomato- 19 showed lower disease incidence and severity up to certain growth stage of the tomato plants. For the molecular detection of *Tomato Yellow Leaf Curl (TYLCV)*, PCR found to be the most reliable modern technique because it is simple, very specific and highly robust.



CHAPTER VI

REFERENCES

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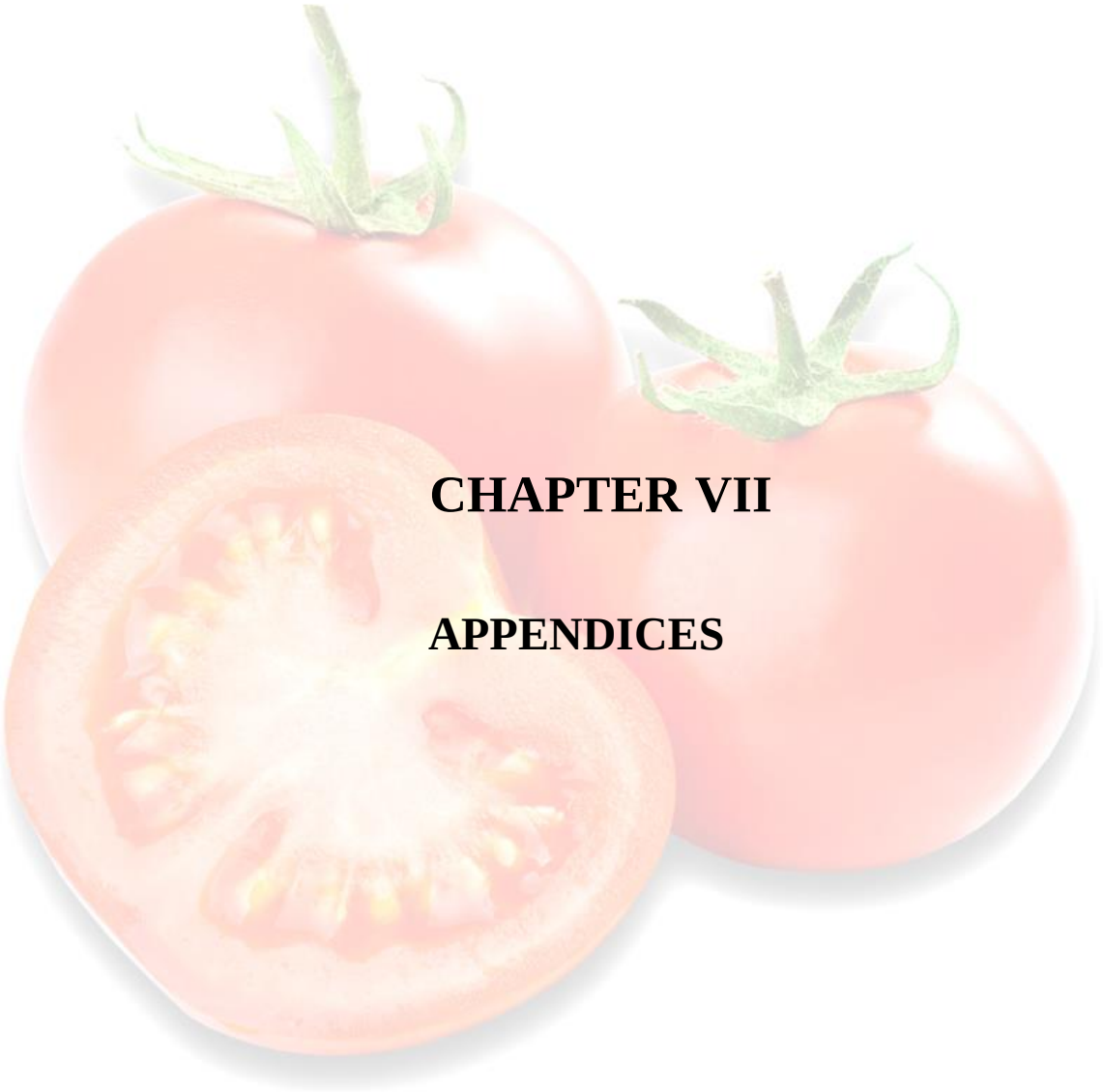


CHAPTER VII

APPENDICES

CHAPTER VII

APPENDICES

Appendix-I. Map showing the experimental site under study

Appendix-II. Physiochemical properties of soil of the experimental field

Characteristics	Value
Sand (%)	25.67
Silt (%)	53.86
Clay (%)	20.48
Texture	Silty loam
pH	5.7-7.1
Organic carbon (%)	0.30
Organic matter (%)	0.55
Total N (%)	0.028
Phosphorus(µg/g soil)	23.59
Exchangeable K Milliequivalents/100 g soil	0.61
Sulphur (µg/g soil)	28.45
Boron (µg/g soil)	0.06
Zinc (µg/g soil)	2.32

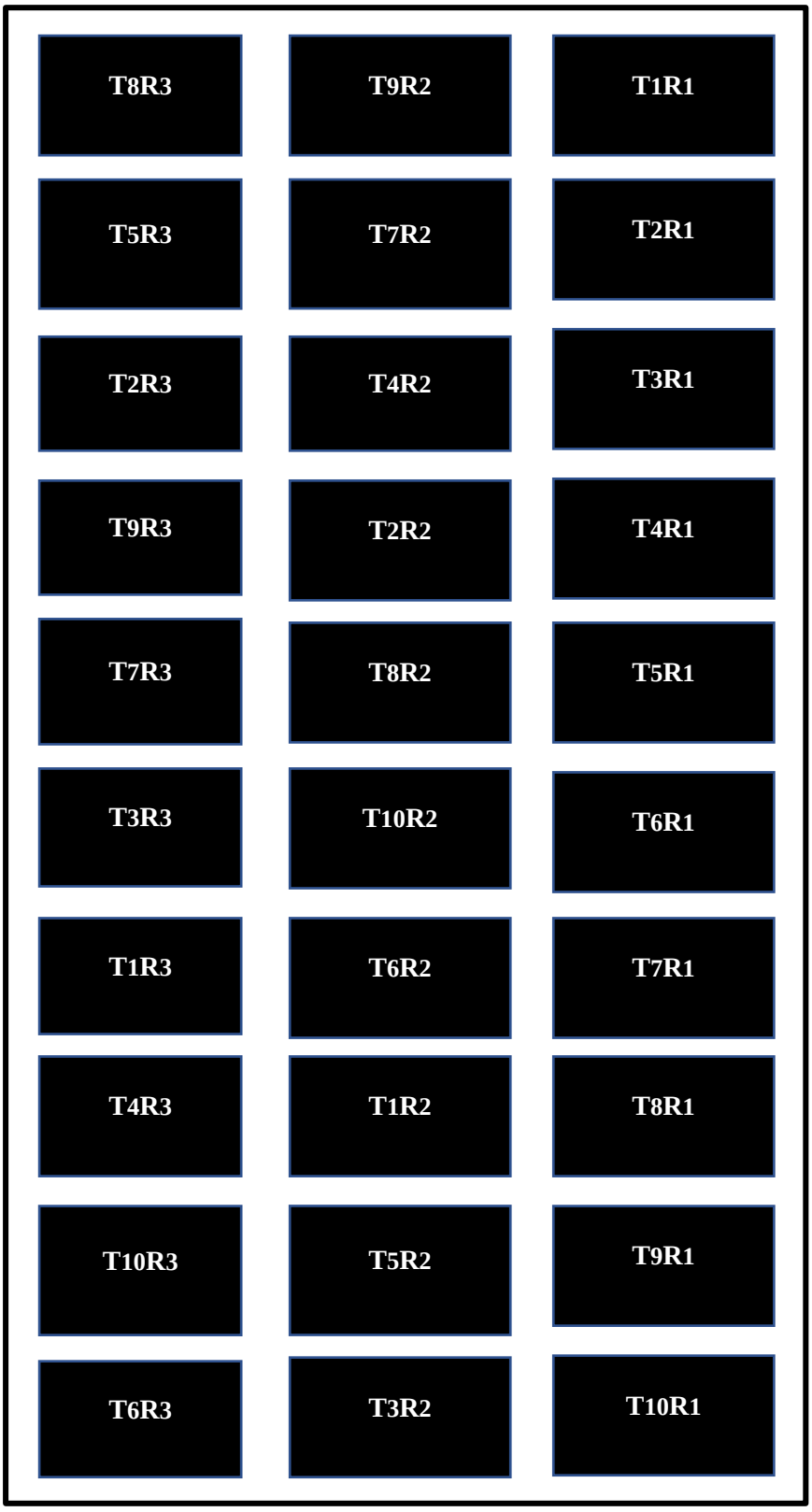
Source: Soil Resources Development Institute (SRDI), Dhaka-1207.

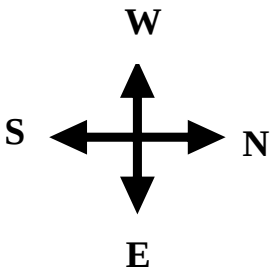
Appendix-III. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour of the experimental period (October 2018- March 2019)

Month	Average RH (%)	Average Temperature (ºC)		Total Rainfall (mm)	Average Sunshine hours
		Min.	Max.		
October	79	26	31	175	6
November	68	22	30	35	8
December	72	16	28	15	9
January	68	15	25	7	9
February	56	17	30	25	8
March	55	20	33	65	7

Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka-1207.

Appendix IV. Layout of the experiment field





Appendix-IV. Disease severity calculation (BARI Tomato-16)

$$\% \text{ Disease Severity} = \frac{\textbf{Sum total of disease rating} \times \textbf{100}}{Total\ number\ of\ observations \times Maximum\ grade\ in\ scale}$$

Sum of total disease rating:

Disease grade	Frequency (diseased leaf)	Disease rating
0	0	0
1	4	4
2	12	24
3	12	36
4	26	104
		Total=168

Sum of total disease rating= 168

Total no of observation= 55

Maximum grade in the scale= 4

$$\%\text{Disease Severity} = \frac{168}{55 \times 4} \times 100$$

$$= 76.4$$

Disease Severity of BARI Tomato-16

Appendix-V: DNA was extracted from tomato leaf

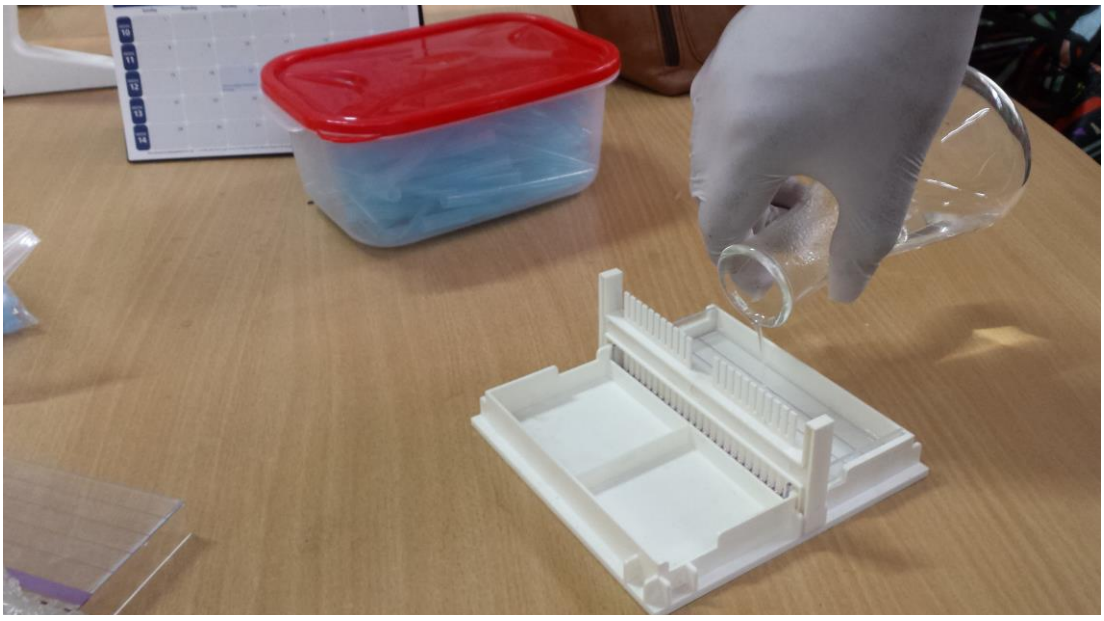
Eppendorf Tube for DNA extraction

Application of kit Chemical to the leaf sample

Centrifuge the leaf sample

Genomic DNA collection by micropipette

PCR the extracted sample



Agarose gel preparation

PCR product loaded in gel documentation system