SCREENING OF SELECTED POTATO VARIETIES FOR VIRUSES (PLRV AND PVY) THROUGH GROWTH CHARACTERISTICS AND ELISA TEST

BY

MST. ISRAT ZEREEN

REGISTRATION NO. 25137/00585

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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Approved by

Prof. Dr. M. Salahuddin M. Chowdhury
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Supervisor

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

Prof. Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
Sher-e-Bangla Agricultural University, Dhaka-1207

CERTIFICATE

This is to certify that thesis entitled, "**Screening of selected potato varieties for viruses (*PLRV* and *PVY*) through growth characteristics and ELISA test**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment 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 bearing, Registration No**. 25137/00585** 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: December, 2020

Dhaka, Bangladesh

Dr. M. Salahuddin M. Chowdhury
Professor
Department of Plant Pathology
Supervisor

Dedicated to my beloved Parents,

Husband and Children

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SAU, Dhaka ***The Author***

SCREENING OF SELECTED POTATO VARIETIES FOR VIRUSES (PLRV AND PVY) THROUGH GROWTH CHARACTERISTICS AND ELISA TEST

ABSTRACT

An experiment was conducted in glass house and laboratory of BADC to screen selected potato varieties viz. Asterix, Cardinal, Diamant, Lalpakri and Desiree against *PLRV* and *PVY* through growth characteristics and it was further confirmed through ELISA test. ELISA test followed by *in vitro* meristem culture performed in order to produce virus-free and sufficient amount of potato seed tubers. Among the selected potato varieties, the highest disease incidence of *PVY* and *PLRV* (1.22 and 1.89%) was recorded in Lalpakri and the lowest (0.33 and 0.44%) was in Asterix respectively. In Diamant, disease incidence of *PLRV* and *PVY* was 0.39% and 0.83% respectively. In Cardinal, disease incidence of *PLRV* and *PVY* was 1.56% and 1.11% respectively. In Desiree, disease incidence of *PLRV* and *PVY* was 0.44% and 1.22% respectively. Numbers of stems/hill of the virus free as well as infected cultivar were significantly different from each other. In both cases, Asterix showed the highest number of stems/hill followed by Cardinal, Desiree, Diament and Lalpakri. Numbers of nodes production of the virus free as well as infected cultivar were significantly different from each other. In both cases, Asterix showed the highest number of nodes production followed by Desiree, Cardinal, Diament and Lalpakri. The cultivars Asterix (12.12 tubers/plant) produced more tubers/plant followed by Desiree (10.43), Diamant (9.31), Cardinal (9.25) and Lalpakri (8.10). It was also observed that among the potato varieties tested against *PLRV* and *PVY*, Diamant produced 23.98 mm size tuber when infected with *PLRV* but *PVY* infection produced 21.43 mm size tuber. PVY infected Cardinal produced 22.88 mm size tuber but PLRV infection reduced size to 21.13 mm. Desiree produced 24.32 mm and 23.56 mm size tuber when infected with *PVY* and *PLRV*, respectively. Highest dry weight of whole plant was recorded in varieties Asterix (1.81 g) followed by Desiree (1.68 g), Diamant (1.66 g), Cardinal (1.63 g) and Lalpakri (1.55 g), respectively. Diamant produced 1.66 g dry weight/plant in *PLRV* and 1.52 g dry weight/plant in *PVY* infection. In *PVY* and *PLRV* infection Cardinal produced 1.60 g and 1.52 g dry weight/plant, respectively whereas Desiree produced 1.63 g dry weight/plant in *PVY* and 1.60 g dry weight/plant in *PLRV* infection. Asterix gave the highest percentage (92.7%) of survivability of plantlets followed by Lalpakri (90%), Desiree (87.5%), Cardinal (86.7%) and Diamant (71.7%). Asterix gave the highest percentage (99.70%) of virus free plantlets followed by Desiree (99.48%), Cardinal (99.47%), Diamant (99.10%) and Lalpakri (91.44%). From the results of this study it may be concluded that presence of viruses in potato plants hamper photosynthetic production and assimilation consequently all the growth characteristics which also depends on nature of viruses and potato varieties and Asterix are the most resistant to the both *PVY* and *PLRV*.

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LIST OF ABBREVIATIONS

FULL NAME	ABBREVIATIONS
Agro-Ecological Zone	AEZ
Archives	*Arch.*
Biological science	*Biosci.*
Biology	***Bio.***
Bangladesh Bureau of Statistics	BBS
Bangladesh Agricultural Development Corporation	BADC
International center for potato	ICIP
Centimeter	cm
Days after transplanting	DAT
The double antibody sandwish	DAS
Enzyme-linked immunosorbent assays	ELISA
Phosphate buffered saline+Tween	PBST
Phosphate buffered saline	PBS
Polyvinylpyrrolidone	PVP
Gram	g
Hectare	h
International	*Intl.*
Journal	*J.*
Kilogram	kg
Least significant differences	LSD
Meter	m
Murate of Potash	MP
Number	no.
Plant height	PH
Percent	%
Randomized Complete Block Design	RCBD
Relative Humidity	RH
Research	Res.
Square meter	m^2
Sher-e-Bangla Agricultural University	SAU
And Others	*et al.*
Potato virus X	*PVX*
Potato leaf roll virus	*PLRV*
Potato virus Y	*PVY*
Potato aucuba mosaic virus	*PAMV*
Potato mop-top virus	*PMTV*
Potato virus A	*PVA*
Potato virus M	*PVM*
Potato virus S	*PVS*
Tobacco streak viruses	*TSV*

Eggplant mottled dwarf virus	*EMDV*
Potato black rings pot virus	*PBRV*
Potato T virus	*PVT*
Potato virus U	*PVU*
Potato black rings pot virus	*PBRV*
Eggplant mottled dwarf virus	*EMDV*
Potato virus U	*PVU*
Potato T virus	*PVT*
Beet curly top virus	*BCTV*
Beet curly top virus	*BCTV*
Arracacha virus B	*AVB*
Tobacco streak viruses	*TSV*

CHAPTER I
INTRODUCTION

Potato (*Solanum tuberosum L.*) is grown under different agro-ecological zones. The tubers produced are used as cheap food, industrial raw material, animal feed and seed tubers. Potato plays an important role for sustainable world food security and it is among the four largest crops produced annually at high proportion of the global total area and yield after rice, wheat and maize (Wang *et al*., 2011). International center for potato (CIP) and its partners have shown potato as playing a dual role in food security, firstly as cash crop at the market and as food grown for consumption with great nutritive value (Devaux *et al*., 2014). As demographic growth continues to increase and causes not only steady hunger rates in developing countries, but also uncertainties in crop production yield. It has been highly recommended by FAO (2009), and was found to be one of the primary food sources for andean people (Lutaladio and Castaldi, 2009).

Potato is an herbaceous dicotyledonous plant and vegetative propagation using potato tuber seeds are the main used method by farmers (Otroshy, 2006). Among the main majors issue associated with vegetative clonal multiplication of potato-seed, susceptibility to viral, bacterial and fungal diseases are accounted (Loebenstein *et al.*, 2001). Poor seed quality results in poor yields and quality of progeny crops (Haverkort *et al*., 1991; Beukema & Van der Zaag, 1990). According to Bamberg *et al*., (2016), world potato-producing regions are fast infected by viruses and the use of infected plant seed tubers has been reported to be the main avenue of disease spread within pandemic regions (Legg *et al*., 2001; Legg and Thresh, 2003). However, farmers are unable to detect visually viral diseases symptoms, due to their variability and poor expression on leaves. Previous research has reported that about 40 species of viruses are infectious to potato worldwide (Volkonen, 2007). The yield reduction due to viral diseases may go up to 75%, and only infection caused by *Potato virus X* (*PVX*) alone can reduce the yield up to 15–30%; and a high proportion of tuber yield reduction has been reported to be caused by *Potato leaf roll virus* (*PLRV*) and some strains of *Potato virus Y* (*PVY*) (Mellor and Smith 1987). According to Loebenstein, (2001), at least 37 viruses occur worldwide in potato, these are *PLRV*, *Potato aucuba mosaic virus* (*PAMV*), *Potato mop-top virus* (*PMTV*), *Potato virus A* (*PVA*), *Potato virus M* (*PVM*), *Potato virus S* (*PVS*), *PVX* and

PVY to cite a few. And others are found in limited geographical areas of the world; these include *Arracacha virus B* (*AVB*), *Beet curly top virus* (*BCTV*), *Eggplant mottled dwarf virus* (*EMDV*), *Potato black rings pot virus (PBRV), Potato virus U (PVU), Potato T virus (PVT)* and *Ttobacco streak viruses (TSV). Among virus species mentioned above, PLRV, PVA, PVM, PVS, PVX,* and *PVY* have been reported to significantly affect cultivated potato and reduce potato crop production in general (Wang *et al*., 2011). Very recently, molecular characterization of *potato spindle tuber viroid (PSTVd*) isolates from potato has been done to reveal the viroid variants (Qiu *et al*., 2016). *PVS* is prevalent over the world but it is less important given that it only causes little reduction in yield production (Loebenstein, 2001).

However, *PVY* ranks fifth among the top ten of the most economically damaging plant viruses worldwide. Natural infection by *PVY* could cause a yield reduction upto 80% depending on the virus strain and potato cultivar (Sharma *et al.,* 2013). Further Davie *et al*., (2017) reported that *PVY* is the most prevalent potato virus worldwide due to some genetic modification that occurred in this viral species. Example is given to Potato tuber necrotic ringspot disease *PTNRD* which is a most prevalent disease induced by *PVY*, probably caused by emerging of the biological and genetic diversity of *PVY* leading to increased viral incidence in seed production (Karasev *et al*., 2013). Epidemiologically, the main viruses that are causing mosaics in potato plants are PVY (Kostiw 2011). *PLRV* and some strains of *PVY* frequently reduce tuber yield by 50-80% (Mellor *et al.,* 1987). Yield losses occur in potato due to *PLRV* up to 70%, (Mughal *et al.*, 1988). *PLRV* only infects members of the *Solanaceae* family and is globally distributed in areas where potatoes are grown (Chiunga, 2013). *PLRV* distribution occurs mainly through infected potato seed tubers and other vegetative parts including seedlings and micro-propagated plants (CABI, 2007). According to Moriones and Navas, (2000) the *PLRV* is one of the most devastating viral disease of cultivated potato in tropical and 18 subtropical regions of worldwide causing the losses up to 100 percent. Ajlan *et al*., (2007) reported that *PLRV* causes 96.90 % yield loss of potato plant in autum season

Effort has been and are still being made to eradicate virus propagation for establishing a national standard and producing certified seed potatoes from *in vitro* virus-free stock plantlets to certified seed tubers (Grade II) that are delivered to potato growers for

commercial production (Danci *et al*., 2012). Through *in vitro* technique, different types of disease free materials can be obtained such as: 1. plantlets or transplants, 2. microtubers, and 3. minitubers in large quantities (Hussey & Stacey, 1984; Jones, 1988; Gopal *et al*., 1998; Struik and Wiersema, 1999). It is, however essential to ensure that the supplied *in vitro* regenerated plants or microtubers are virus free for commercial potato cultivation. Unfortunately, there are no chemical agents like bactericides and fungicides that can use against plant viruses (Fakhrabad *et al*., 2012). Viral diagnosis is considered as one of the most valuable strategies for virus disease management. There are a number of systems available to detect viruses (Singh, 1999). Many certification authorities prefer visual detection of pathogens on the potato plants or seed tubers. Relying on such visual detection of symptoms is, however not always conclusive. In many cases plants may carry a latent infection which does not produce detectable symptoms (Banttari *et al.*, 1993). Serological procedures form the most reliable method for detection and quantitative assay of viruses. In this context the direct double antibody sandwich enzyme-linked mmunosorbent assay (DAS-ELISA) is used far and wide for virus detection (Clark and Adams, 1977). In the present investigation we have studied the presence or absence of the virus particles in greenhouse as well as *in-vitro* generated plants of five varieties namely Asterix, Diamant, Cardinal, Lalpakri and Desiree with two *PVY* and *PLRV* through growth characteristics and DAS-ELISA test.

Based on above comprehensive and systematic overview of previous studies related to the developed in *in vitro* methods and modified complex methods for potato virus elimination and detection methods the following objectives has been set to search in this research experiment.

1. To evaluate the level of resistance of selected varieties against *PLRV* and *PVY* through growth characteristics
2. To determine the incidence of *PLRV* and *PVY* through ELISA test
3. To establish virus free potato plantlets *in vitro* from plants through meristem culture and further detection

CHAPTER II

REVIEW OF LITERATURE

Potato (*Solanum tuberosum*) is an important and most widely grown vegetable crop in Bangladesh. Potato production in Bangladesh is under constant threat of potato leaf roll disease caused by *potato Leaf roll virus (PLRV*) and leaves drop streak, yellowing leaves and mosaic caused by *potato virus Y (PVY*). A lot of work has been done on various aspects of *PLRV* and *PVY* in Bangladesh and abroad and is reviewed as under:

2.1 Potato

Potato (*Solanum tuberosum*) is an herbaceous annual plant which belongs to the Solanaceae or "nightshade" family of flowering plants. It was originated in the Andes Mountains of South America more than 8000 years ago. The glob-948095

Bv./al total crop production of potato exceeds 376 million metric tons (Beuch, *et al,.2013*). It currently ranks the world's fourth most important food crop after maize, wheat, rice, and the first among root and tuber crops (Salazar, *et al*., 2003), Chiunga, *et al*., 2013). More than 6 billion people worldwide eat potato, which is produced in over 130 countries worldwide (Salazar, *et al*., 2003, Beuch, *et al*., *2013)*. Potato virus are widely distributed virus in potato all over the world as ranks the fifth among the top ten of the most economically damaging plant viruses worldwide.

2.2 Potato Infecting Viruses

Potato is susceptible to a wide range of pathogens including bacteria, fungi, nematodes, viruses and viroid's (Mulder, *et al*., 2005). It is known to be infected by 175 diseases in addition to several physiological disorders (Islam, *et al*., 2013). Viruses beside *Phytophthora infestance* (the causative agent of late blight) are the most important ones among potato pathogens (Salazar, *et al*., 1996, Mulder, *et al*., 2005). Yield reduction of potato is attributed by many of these diseases, for example, the natural infection with *potato virus Y* could cause a yield reduction up to 80% depending on the virus strain and potato cultivars (Sharma, *et al*., 2013). Apart from late blight, viruses are the most demonstrable seed-borne agents that affect vigor, yield and tuber quality of potato (Salazar, *et al*., 1996, Wale, *et al*., 2008).

More than 40 viruses naturally infect cultivated potato (Table 1). Some of these viruses notably Potato leaf roll virus *(PLRV), Potato virus Y (PVY), Potato virus A (PVA), Potato virus X (PVX), Potato virus M (PVM), Potato virus S (PVS) and Potato aucubar*

mosaic virus (*PAMV*) occur worldwide in potato crops, while others are important only in some geographical areas (Altaleb, *et al*., 2011, Salazar, *et al*., 1996, Khan, *et al*., 2013. Abbas, *et al*., 2014, Naimer, *et al*., 1993).In order to spread successfully, Potato viruses need other agent to transfer from infected plants into other healthy ones, in a process called transmission. The transmission of any plant virus needs a cooperation of the virus, the transmitting agent, the plant and the environment under optimum conditions to achieve maximum efficiency (Wale, *et al*., 2008). Potato viruses are naturally transmitted via three ways. The first way is through the parts of plants that used for propagation. Vegetative propagation of infected tubers is considered as the main source of virus infection through which the viruses pass from one generation to the next.

Insects as vectors are the second way for virus transmission; these could be mites, aphids, nematodes or fungi. Among the insect vectors of potato viruses aphids are the most important ones, because the two most damaging viruses in the crop, *PVY* and *PLRV*, are transmitted via aphid species. Over 50 aphid species are known to transmit potato viruses; they transmit the largest number of viruses (Davie, *et al*., 2012). Another way for viruses transmission is mechanical transmission, by contact between infected and healthy plant parts, including contact with contaminated farm machinery, operator hands, or animals. The viruses which are most frequently encountered in potato fields such as *PVY, PVX, PVS, PVA, CMV, PVV* and *TMV*, are mechanically transmitted with the exception of *PLRV* which is transmitted only by insect vector (Naimer, *et al*., 1993, Altaleb, *et al*., 2011, Ahmad, *et al*., 2012) Virus infections cause plant disease by affecting their metabolism. In virology plant called primary diseased when they become infected during cultivation, while for plant that originated from infected tubers are secondary diseased plants. Plants with primary and secondary infection with the same virus usually show different symptoms (Salazar, *et al*., 1996). Viruses affect the potato plants in different ways; they cause rapid degeneration of potato tubers, foliar malformation, and mild or sever mosaic, stunted growth and wilting. The severity of viruses' symptoms on potato depends on many factors including, potato varieties, virus strain, time of infection, age of crop and environmental conditions (Wale, *et al*., 2008, Mulder, *et al*., 2005). Indeed most symptoms of potato viruses are masked at high temperatures, this makes indexing of virus by visual observation very difficult, and many plants will look healthy while they are actually infected. Thus, in order to manage viruses' diseases and control their spread, reliable virus detection methods are needed.

Table 1. Categorization of importance of potato viruses based in three characteristics (Modified from Salazar, *et al*., 1996)

Category	Viruses	Effect on yield	Risk analysis	Distribution
1	*PLRV, PVY, PVA*	Up to 90%	1	1
2	*APMoV, PVX, PAMV, PVV, PMTV, TRV, PYVV*	Up to 40%	1	2
3	*AMV, PYV, PBRV, TBRV, PVP, PVS, PRDV*	Up to 20%	1-2	2
4	*PVT, APLV, PVU, WPMV, EMDV, TNV, TRSV, AVB (O)*	Up to 10%	1-3	2
5	*SALCV, ToSWV, PYDV, TSV, BCTV, CMV, APLV, PYMV*	Up to 10%	2-3	2

According to the table Viruses in categories 1, 2 and some in category 3 can be considered the most economically important in potato production worldwide.

2.3 Potato Leaf Roll Viruses

Symptoms

Foliar symptoms of *PLRV* include leaf rolling, chlorosis (yellowing), reddening, 'leathering' of leaves, phloem necrosis, and stunting (Jutta, *et al*.2009; Alani, *et al*.2002) Plants infected early in the growing season may also be dwarfed, but if virus infection occurs late in the growing season foliar symptoms may not be exhibited. Potato plants develop resistance to foliar infection with plant age (Erik, *et al*., 1993*)* Many times, infection can be seen in a circular pattern in the field, and frequently surrounding what was most likely the original source of virus inoculums, an infected seed piece. Direct damage can also result from aphid feeding. Large numbers of aphids present can kill potato plants producing what are referred to as an 'aphid hole' in the field (Robert, *et al*., 2000*)*. *PLRV* travels through the phloem of the plant into tubers, reducing size and causing net necrosis. Net necrosis causes browning of the vascular system extending throughout the entire tuber. Tubers with net necrosis are unacceptable for processing into chips and fries, causing serious losses to growers and potato processors (Alphey, *et al*., 1986).

Figure 1. Foliar symptoms of *PLRV* include leaf rolling, chlorosis (Yellowing) and 'leathering' of leaves and stunting.

2. 4. Transmission of *PLRV*

Economically, *potato leaf roll virus (PLRV)* is one of the most important viruses affecting potato crops worldwide (Hunnius 1977; Peters and Jones 1981; Harrison 1984; Storch and Manzer 1985; van der Zaag 1987). The coat protein of luteoviruses is responsible for several viral characteristics including the serological properties (D'Arcy *et al*. 1989; van den Heuvel *et al*. 1990) and transmission specificity (Gildow and Rochow 1980b; Massalski and Harrison 1987). It is suspected that the read through protein joined to the coat protein at its C-terminal plays an important role in the virus transmission by vectors (Mayo *et al*., 1993). Woodford *et al*. (1994) indicated that the virus transmission by insects is a highly variable process because it involves interactions between the virus, vector and plant. In nature, *PLRV* is only transmitted by aphids. It can be transmitted by at least 11 aphid species (Kennedy *et al*., 1962; Kostiw 1980), of which the peach-potato aphid (*Myzus persicae* Sulz.) is the most efficient vector (Robert and Maury 1970; Robert 1971; Sylvester 1980).

PLRV can be artificially transmitted by stem grafting. *PLRV,* like other luteoviruses, is transmitted by aphids in a persistent manner, also referred to as a circulative one (Black 1959). The former term reflects the ability of the virus to remain in the infective form in the vector for a long time, usually to its death. This property is important from the point of view of virus epidemiology. The term 'circulative' refers to the circulation of the virus through Transmission and control of potato leaf roll virus 219 the aphid. The mechanism of the virus transmission by aphids has been intensively studied since the 1930s (Smith 1929; Elze 1931; Day 1955; Harrison 1958; Ponsen 1970, 1980). These studies were

instrumental in the understanding of the transport of luteoviruses through the vector's body. When ingested by the aphid, virus particles pass from the gut into the haemolymph where they can circulate through the aphid, enter the salivary glands and pass into the saliva, thereby entering a new plant when the insect feeds again. The luteovirus circulation in the vector has been studied mainly with regard to *PLRV* and *BYDV* (Gildow and Rochow 1980a; Gildow 1985, 1987).

There are differences of opinion between authors on the site of passage of the virus particles from the gut lumen into the haemocoel. Weidemann (1982) reported that the whole midgut, including the stomach, could be the site of the *PLRV* passage in *M. persicae*, while Garret *et al.,* (1993) found only the intestine involved in this process. Gildow (1985) demonstrated the hindgut as being the site for *BYDV* to pass into the haemocoel of *Rhopalosiphum padi* L. Garret *et al*. (1993) suggested that the differences between the results obtained in these three studies could be due to the different viruses, virus strains and aphid species or clones used.

2.5. *Potato virus Y (PVY)*

Symptoms

It is sometime referred to as latent potato mosaic virus. It show light yellow mottling with slightly crinkling on potato plants. It shows interveinal necrosis of top foliage. It shows stunting growth of diseases plants. Leaves may appear slightly twisting where strains of *PVY* combines.

Figure 2. *PVY* showing interveinal necrosis and light yellow mottling.

2.6. Transmission of *potato virus Y*

PVY may be transmitted to potato plants through grafting, plant sap inoculation and through aphid transmission. The most common manner of *PVY* infection of plant material in the field is through the aphid, and although aphids on their own can directly damage potato plants, it is their role as viral vectors which have the greatest economic impact. In cold climates aphids spend the winter either as wingless aphids giving birth to live young (*viviparae*) or as eggs. Hosts such as weeds and other crops serve as breeding grounds for these aphids and form a temporary area of colonization before the aphids migrate to the potato fields. In moderate climates, such as in South Africa, aphids are thought to reproduce asexually on weeds, other crops, indigenous plants and garden plants. This means that there are a number of aphids present year-round. The importance in effective and stringent monitoring of aphid populations is stressed in a review by Radcliffe and Ragsdale (2002) as *PVY* virions are introduced to potato fields almost solely by winged aphids from a virus source outside these fields. Wingless aphids have not yet been linked to the spread of *PVY* in potato fields. The green peach aphid (*Myzus persicae*) has been found to be most effective in its role as viral vector, butotherssuchas *Aphis fabae, Aphisgossypii, Aphisnasturtii, Macrosiphumeuphorbiae, Myzus(Nectarosiphon)certus, Myzus(Phorodon)humuli and Rhopalosiphum insertum* are also strongly associated with viral transmission

2.7. Detection methods of plant viruses

Viruses infect a wide range of plant species leading to cause many serious plant diseases in the world. The effect of viral diseases on plants quality and quantity leads to the loss of billions of dollars per year (Thresh, 2006; van der Vlugt, 2006). Unfortunately, there are no chemical agents like bactericides and fungicides that can use against plant viruses (Fakhrabad *et al*., 2012). Viral diagnosis is considered as one of the most valuable strategies for virus disease management. Thus to achieve an effective control, viruses must be accurately identified as an initial step of management. Diagnostic and detection techniques for plant viruses categorize based on: biological properties of the virus interaction with its host or vector, such as symptomatology and transmission tests and intrinsic properties of the virus itself, like serological and nucleic acid-based techniques (Fakhrabad *et al*. 2012; van der Vlugt, 2006).

2.8. Diagnostic methods based on biological properties

2.8.1 Symptomatology

Symptoms on plant usually used if they are characteristic of a certain disease (Hull. 2009). Symptomatology relies on visual inspection of the symptoms expressed by viruses in plant. Plant viruses cause many symptoms on plant like mosaic, mottling, stunting, leaves malformation, necrosis, chlorosis, yield reduction or a combination of these symptoms. However, many biotic and abiotic factors could affect the appearance of these symptoms. Moreover, some plants could exhibit virus-like symptoms as a result of unfavourable conditions. Although symptoms provide essential information about virus infection, we can't make a decision on symptomatology alone, as some viruses cause symptomless infection in plant. This method is usually done in conjunction with other confirmatory tests to achieve the maximum accuracy of viral diagnosis (Naidu & Hughes, 2003).

2.8.2 Biological assay (Transmission tests)

It is the one of the oldest methods for plant viruses' diagnosis. It is a traditional method that still uses in many laboratories as an important assay for virus detection and identification (Hull. 2009). It includes mechanical, graft, and vector transmission of the virus to susceptible herbaceous indicator plants. Mechanical transmission using sap inoculation to indicator plants can be done easily with minimum facilities. The characterizations of symptoms produced on these plants allow detection and identification of many viruses. Viruses that are not able to be transmitted via mechanical transmission as well as viruses of tree fruit can be diagnosed via vectors or grafting using suitable indicator plants. Although these assays are usually used for routine diagnosis of viruses, they may provide imprecise virus identification in addition they consume time and resources (Naidu & Hughes, 2003).

2.8.3 Microscopy

Electron Microscopy (EM) provides useful information about virus morphology. For stable viruses as well as viruses with filamentous and rod- shaped particles, rapid results can be observed using negative staining technique. Even though, it is not easy to detect viruses with isometric shape. Moreover virus particles that occur in low concentration, need to be concentrated in plant sap or captured using antibody-coated grids (Immunosorbent Electron Microscopy) to achieve high efficiency of virus visualization. EM is commonly used for virus detection when the needed facilities are readily

available, thus it cannot be used for rapid detection of multiple samples as it is very expensive and labor intensive method. Many plant viruses such as potyviruses form cylindrical inclusion bodies in infected cells. They develop large crystalline accumulations of virus particles, thus make their detection by EM or light microscopy a simple, rapid, and relatively inexpensive method to confirm viral infection. This cylindrical inclusions (CI) bodies are formed by a virus-encoded protein and can be considered as the most important phenotypic criterion for assigning viruses to certain genus level using selective stains (Danci *et al.*, 2009; Naidu & Hughes, 2003).

2.8.4 Serological Methods

Serology is one of the most easiest and specific methods for virus diagnosis that gives a rapid and precise outputs. All of serological assays depend on the virus coat protein properties and fall into two types, solid phase assays (ELISA, western immuno-blotting) and liquid phase assays (precipitation and agglutination tests). Serological techniques which include enzyme- linked immunosorbent assay (ELISA) and tissue immuno-blotting assay considered as powerful tools for plant viruses detection. These techniques are based on an antigen-antibody reaction between virus specific coat proteins (epitopes) and anti-viral antibodies that raised in mammalian systems like rabbits and mice, therefore it can be visualized using several detection means such as enzyme-labelled antibodies (Hampton *et al*., 1990).

2.8.5. i Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay is the most significant and popular advance among serological tests, especially in virus detection (Lopez *et al*. 2003). In 1977 ELISA was introduced to plant virology by Clark and Adams. Since then it has been very common for plant virus detection in plant material, insect vectors, seeds and vegetative propagules (Clark & Adams 1977). ELISA is widely used for the detection of plant viruses due to its simplicity, adaptability, reliability, sensitivity in addition to its economy, thus it is used to test large number of samples in a relatively short period of time (Batool *et al*., 2011; Naidu & Hughes, 2003). The basic principle of the ELISA based on immobilizing the antigen onto a solid surface, or trapping antigen by specific antibodies, and probing with specific immunoglobulins attached to an enzyme label. The positive reaction is detected by adding the suitable substrate. The enzyme converts substrate to product, which can be easily visualized due to its color. Many variations of ELISA have been developed. These variations are categorized into, "direct" and "indirect" forms of ELISA. Both categories

have the same underlying theory and the same final results, meanwhile they differ in the way of detection for the antigen-antibody complex. The disadvantages of ELISA are the time of extraction for plant tissue which can take several hours as well as the incubation times required for samples and antibodies in order to be adhered into microtiter wells (Naidu & Hughes, 2003; Lima *et al*., 2012).

ii. Tissue blotting immunosorbent assay (TBIA)

TBIA is a variation of dot-blot immuno assay (DBIA) which is used for virus detection in both plants and vectors. It was first used in 1990 (Lin, *et al*., 1990). Since then it became widely used as simple and reliable method for virus detection (Chang, *et al*., 2009). TBIA has relatively the same technique as ELISA except that a freshly-cut edge of plant or vector is used and spotted onto a membrane instead of using a micro titer plate. Moreover, in ELISA a soluble substrate is used for color development, while a precipitating one is usually used for virus detection in TBIA (Hsu & Lawsow, *et al*., 1990).Although TBIA has some advantages over ELISA, as there is no need for tissue extraction and membranes can be blotted directly in the field and in addition to be processed at a later date, TBIA has several disadvantage is that the results are qualitative, rather than being quantitative as well as it is difficult to observe weak positive reactions due to the interference of sap components with the subsequent diagnostic reaction (Makkouk, *et al*.,1994, Naid, *et al*., 2003).

2.8.6 Nucleic Acid-based Methods

Nucleic acid-based techniques are used extensively for plant viruses' detection and identification, mainly after the advent of the polymerase chain reaction (PCR). Although serological techniques are widely used for virus detection, they have certain drawbacks if compared with nucleic acid-based techniques. They are based on the viral coat proteins and its antigenic properties, which represents only about 10% of the total virus genome (Gould, *et al*., 1983) thus they neglect the rest of the virus genome. While in nucleic acid-based detection methods any region in the virus genome can be targeted and diagnosed. Moreover, in some cases serological procedures cannot be used particularly for the detection of viroid's, satellite RNAs, viruses which lack particles (e.g., *Groundnut rosette virus (GRV),* viruses which occur as extremely diverse serotypes (e.g., Indian and African *Peanut clump virus and Tobacco rattle virus) and viruses that are difficult* to purify (Naidu, *et al*., 2003). Polymerase chain reaction (PCR) is an extremely

specific, sensitive and versatile in vitro method that have a great potential to amplify trace amounts of targeted nucleic acid using specific primers to the region of amplification, and thermostable DNA polymerase (Gould, *et al*., 1983.Lepez, *et al*., 2003). It consists of three step cycles: denaturation in which the two complementary strands of the double-stranded DNA are separated at high temperature (usually 94-95 °C), annealing of two oligonucleotide primers to their complementary sequences in the opposite strands of the target DNA (annealing temperature depends on the nucleotide composition and length of the primer, usually 35-65 °C), and elongation or extension of each primer through the target region (usually at 72 °C) using a thermo-stable DNA polymerase (e.g., Taq polymerase). Each DNA strand made in one cycle will use as a template for synthesis of a new DNA strand in the next cycle. The procedure is repeated many times (30-40 cycles) by an automated thermal cycler until sufficient product of amplicon is produced. This procedure is directly applied for amplification of plant viruses with DNA genomes (eg. Caulimo, Gemini and Badnaviruses) using gene-specific primers to the region of amplification. While in plant viruses with RNA genomes, the targeted RNA must be converted to a complimentary DNA (cDNA) using reverse transcription (RT) prior the beginning of PCR procedure. In addition to its usefulness as a diagnostic technique, PCR is used by many research laboratories in the world for many purposes including, molecular characterization (Alfaro-fermade, *et al*., 2008), DNA comparisons between related pathogen species as well as in evolutionary studies (Rossink, *et al*., 2001). Although PCR can achieve a very high sensitivity and specificity, different comparative assays have been reported a failure of PCR amplification to correctly diagnose infected and non-infected plant material. Although PCR can achieve a high sensitivity and specificity, different comparative assays a failure of PCR amplification to correctly diagnose infected and non- infected plant material has been reported. This failure could be a consequence of the "carry-over" contamination of amplicons that can be responsible for false-positive results and inhibitor components in sample extracts which is the main reason for false negatives (Naidu, *et al*., 2003).

2. 9 Yield losses

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). 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). The *PLRV* is one of the most devastating viral diseases of cultivated potato in tropical and 18 subtropical regions of worldwide causing the losses up to 100 per cent (Moriones and Navas, 2000). It was reported that 96.90 % yield loss of potato plant due to *PLRV* in autum season (Ajlan *et al*., 2007). *PVY* ranks fifth among the top ten of the most economically damaging plant viruses worldwide. Natural infection by *PVY* could cause a yield reduction upto 80% depending on the virus strain and potato cultivar (Sharma *et al*., 2013). Further Davie *et al*., (2017) reported that *PVY* is the most prevalent potato virus worldwide due to some genetic modification that occurred in this viral species. Example is given to potato tuber necrotic ringspot disease *PTNRD* which is a most prevalent disease induced by *PVY*, probably caused by emerging of the biological and genetic diversity of *PVY* leading to increased viral incidence in seed production (Karasev *et al*., 2013). Epidemiologically, the main viruses that are causing mosaics in potato plants are *PVY* (Kostiw 2011). *PLRV* and some strains of *PVY* frequently reduce tuber yield by 50-80% (Mellor et al., 1987).

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out in glass house and laboratory condition during 2019-2020 to screen out the selected potato varieties against major potato viruses through growth characteristics and ELISA test. The material used and methodology adopted during the study are being summarized under heading and sub-heading.

3.1. Experimental site

The experiment was conducted in glass house of Bangladesh Agriculture Development Corporation (BADC), Dhaka-1207, during the period of October 2019 to April 2020. The experimental area was situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon. 1988).

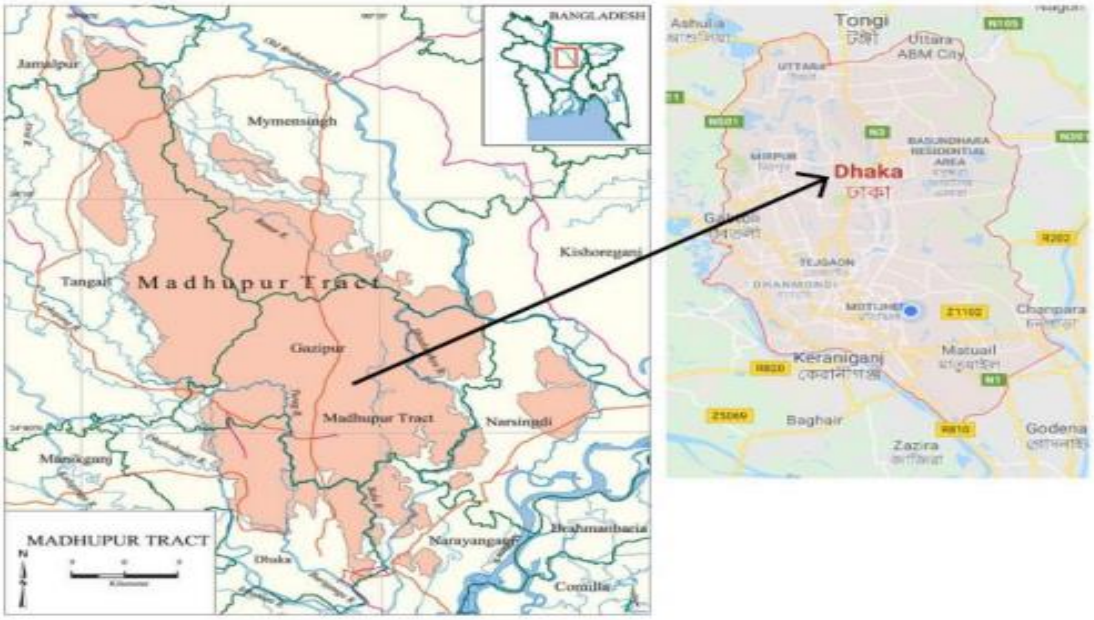


Figure 3. Madhupur tract, AEZ No-28.

3.2. 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 28^0 C to 32^0 C in summer, falling to 20^0 C in winter, with extreme lows of 10^0 C. Rainfall ranges between 1,000 mm and 1,500 mm annually, heavy rainfall in kharif season (May-September) and scanty in rabi season (October-March) . Severe storms are unusual but tornadoes have struck the southern areas. During the month of December, January and February there was no rainfall. During the period of investigation the average maximum temperature was 32^0 C and average minimum temperature was 20^0 C. 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.

3.3. Planting materials

Total five potato varieties viz. Asterix, Diamant, Cardinal, Lalpaki and Desiree were selected to conduct the research. Seed tubers were collected mainly from Bangladesh Agriculture Development Corporation (BADC), Dhaka. Tuber samples were collected from plants of all varieties showing virus-like symptoms as well as from symptomless ones which grown in the glass house whereas samples were also collected from *in-vitro* raised plantlets of all varieties from BADC, Dhaka;.

Table 2. Characteristics of the potato varieties used in the study

Variety/ Characteristics	**Astarix (BARI-25)**	**Diamant (BARI-7)**	**Cardinal (BARI-8)**	**Lalpakri (Local)**	**Desiree**
Days to maturity	90-95 days	90-95 days	90-95days	90-100 days	80-100 days
Dormancy	60-70 days	50-60 days	50-60 days	60-70 days	60-70 days
Skin colour	Red	Whitish brown	Light red	Red	Red
Flesh colour	Light yellow	Light yellow	Yellowish	Yellow	Creamy
Growth habit	Erect	Long-erect	Erect	Erect	Erect
Shape	Oval-elongated	Elongated	Oval	Round	Elongated
Yield (ton/h)	25-35	25-35	25-35	20-25	25-35

3.4. Experimental design, soil preparation and seed tuber planting

The experiment was carried out in a complete randomized design (CRD) with three replications and each variety contains 40 trays and each tray contains 12 plants. The soil was collected from BADC field, Dhaka. Soil was mixed with Furadan 5G and kept for one day by covering the whole soil with polythene sheet to sterilize the soil. Then it was mixed with desired amounts of fertilizers and cowdung. Finally, soil was poured in tray and the seed tubers were sown in individual row and proper care was taken for better germination and seedling development. The treatments were assigned randomly to unit trays of the glasshouse.

3.5. Intercultural operations

3.5.1. Gap filling

Gap filling was done after one week of transplanting. For gap filling seed tubers were taken from same source.

3.5.2. Weeding

Three hand weeding was done. First one was done at 20 DAT (Days after Transplantation) and second and third were done at 40 and 60 DAT, respectively.

3.5.3. Manure and Fertilizer application

During the final tray/pot preparation approx. 15 kg cowdung, 2 kg Urea, 1 kg TSP (Triple Super phosphate) and 0.80 kg MOP (Murate of Potash) were mixed with soil. All the fertilizers were applied in basal dose except urea. The rest half doses of Urea (175 kg ha^{-1}) were applied at top dressings as 30 DAP followed by earthing up and light irrigation.

3.5.4. Irrigation water and drainage

Irrigation was done when it was necessary. The trays were irrigated by a watering cane and excess water also drained out from pot after heavy rain.

3.6. Data recorded and assessed

All experimental plants were selected for data collection and data of the following parameters were recorded.

a. Viral incidences in potato varieties
b. Number of stems/hill of different potato varieties
c. Stem length (cm) of different potato varieties
d. Number of leaves of different potato varieties
e. Fresh and dry weight (g) of aerial part/plants
f. Height (cm) of plant of different potato varieties
g. Number of nodes production in different potato varieties
h. Number of tuber production in different potato varieties
i. Size of tuber in different potato varieties
j. Weight (g) of tuber in different potato varieties
k. Dry matter of whole plant in different potato varieties
l. Yield performance

3.6.1. Identification of *Potato leaf roll (PLRV)* and *Potato virus Y (PVY)* calculation of disease incidence

Disease was identified by visual basis, observing the typical symptoms of *PLRV* and *PVY*. Symptoms like leaf mottling, mosaic and necrosis were observed in plants for *PVY*. Symptoms like paleness and upward rolling of young leaves, especially at the base, stunted growth and yellowing of leaf margins were recorded for *PLRV*. Percent disease incidence was calculated by using the following formula and disease incidence reaction percentage was measured by using the rating scale presented in table 3.

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plant}}{\text{Number of total plants observed}} \times 100. \text{ (Agrios 2005)}$$

Table 3. Disease incidence rating scale of *PLRV* and *PVY* (Ali *et al*. 2005)

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

3.6.2. Number of stems/hill

Stems were counted of the plant from the virus free as well as infected cultivar at an age of 60 DAT.

3.6.3. Stem length

Stem length of the cultivars were measured by a meter scale from the ground to longest tip of the plant in centimeter from the virus free as well as infected cultivar at an age of 60 DAT.

3.6.4. Number of leaves/plant

The leaves of each plant were counted from the virus free as well as infected cultivar at an age of 60 DAT.

3.6.5. Fresh weight and dry weight (g)

Fresh weight of aerial part/plants were taken immediately in destructive method and dry weight of aerial part/plants were taken of the same areal part of the plant after drying at 70°C for 7 days of different potato varieties from the virus free as well as infected cultivar at an age of 60 DAT.

3.6.6. Number of nodes/hill

The number of nodes production of different potato varieties from the virus free as well as infected cultivar at an age of 60 DAT.

3.6.7. Height of plant (cm)

The height of the plant of different potato varieties were taken after harvesting.

3.6.8. Number of tubers/plant

The number of tuber production in different potato varieties were counted after harvesting.

3.6.9. Size of tuber/plant (cm)

The Size of tuber in different potato varieties was measured by a centimetre scale after harvesting.

3.6.10. Weight of tuber/plant (gm)

The weight of tuber in different potato varieties individually was measured by a digital balance meter in gram (g). A mean weight was taken of collected tuber from each tuber as per variety

3.6.11. Weight of dry matter/plant (gm)

The weight of different potato varieties were measured by a digital balance meter in gram (g) after harvesting.

3.6.12. Yield/plant(kg)

Number of tubers/plant and weight of tubers/plant of different potato varieties were recorded after harvesting. Total yield per plant was measured in kg.

3.7. ELISA kit collection

ELISA kits were collected from the local supplier. The brand name of the ELISA chemicals was “Life Science” and “Agdia”

Figure 4. ELISA kit.

3. 8. Identification of *Potato leaf roll virus (PLRV)* and *Potato virus Y (PVY)*

The relative amount of viral titters in leaves was determined through DAS-ELISA assay as described by Clarks and Adams (1977b). Leaves sample was collected from each plant at 60 days after sowing and tested by DAS-ELISA. Samples were considered positive for the presence of *PVY* or *PLRV* when antiserum reacted produce yellowish color with the infected plant samples. The absorbance value at 405 nm was taken and were greater than twice the mean of comparable healthy control plants recorded 2 h after adding the substrate and was measured using a lab detect microplate reader running Capture 96, Inc software (Kohl and Ascoli 2017).

3.9. Meristem culture for virus elimination and multiplication

Meristems cultures were performed according to Waswa *et al*. (2017) with some modifications. Apical meristem tips (domes with 1-2 leaf primordia) were excised in sterile conditions and transferred to Petri plates on 10 ml of solid MS medium. The excised explants were surface-sterilized by soaking in water with liquid detergent for 30 min and then immersed in 70% ethanol for 5 s and later in a solution of 14% sodium hypochlorite and 2 drops of tween-20 for 3 min (Lizaragga *et al*., 1989). The disinfected explants were rinsed three times in sterile distilled water and introduced *in vitro* for plantlet regeneration. From sterilized shoot tips, immature leaves and leaf primordial were snapped off. Then the isolated meristems (0.3 mm) were quickly transferred in the media. In this level data were recorded on quick responses on the establishment of

primary meristem of the studied genotypes. After 3-4 week of inoculation of meristem, the developed meristems were sub cultured on semi-solid medium for 3-5 weeks for shoot development and root formation. Data were recorded again on different parameter. Before massive shoot multiplication, DAS-ELISA was done of developed plantlets for checking virus freeness. The plantlets, which were free from viruses showed no color development. The single node with axillary bud of virus free plantlets were used for massive propagation in MS semi-solid medium for shoot and root formation. The standard tissue culture media containing 6 g agar, 100 mg ascorbic acid, 100 ml macro nutrient stock solution containing 12 ml/l $MgS0_4$, 50 ml/l calcium chloride, NH_4NO_3 (35 g/200 ml), KNO_3 (40 g/200 ml) and KH_2PO_4 (3.5 g/200 ml), 10 ml micro nutrient stock solution containing KI (0.02 g/200 ml), H_3BO_3 (0.1 g/200 ml), $MnSO_4.H_20$ (0.5 g/200 ml), $ZnS0_4.7H_20$ (0.2 g/200 ml), $Na_2MoO_4.2H_2O$ (0.005 g/200 ml), $CuSO_4.5H_2O$ (0.005 g/10 ml), $CoCl_2.6H_2O$ (0.005 g/10 ml), 5 ml $FeSO_4.7H_2O$, 100 mg myoinosotol, 10 ml vitamins, 1 ml folic acid, 4 ml L-Arginine and 30 g sucrose in 1000 ml of distilled water; pH 5.8. The regenerated plantlets were sub-cultured 3 times every 4 weeks to obtain adequate numbers of plantlets per variety.

3.10. Statistical analysis of data

The data was analysed by using the statistical analysis software "Statistix-10". The mean value was compared according to LSD range test at 5% level of significance. Tables, bar diagram, and photographs were used to present the data as and when necessary for comparing different parameters.

CHAPTER IV

RESULTS AND DISCUSSION

The main target of the study was to identify the viruses infecting potato in order to produce healthy potato plantlets for further multiplication of virus free seed of five varieties. Maximum incidence of both the tested viruses was recorded in different potato varieties grown in glass house at BADC station. All these infected plants were roughed out to limit virus spreading and produce virus free potato seed. Data were recorded on percent of disease incidence, stem length, number of stems per hill, number of leaves, number of nodes, fresh and dry weight of aerial part, whole plant height, number of tubers/plant, size and weight of tuber/plant, dry weight/plant, survivability and virus freeness based on ELISA test.

4.1. Disease incidence (%) of PLRV and *PVY* in selected potato varieties

The highest disease incidence (1.22 and 1.89%) was recorded in Lalpakri and the lowest incidence (0.33 and 0.44%) in Asterix for *PVY* and *PLRV*, respectively. *PLRV* Incidence in Diamant was 0.39% but *PVY* was 0.83%. Asterix showed the most resistant to the both *PVY* and *PLRV*. Results are presented in figure 5.

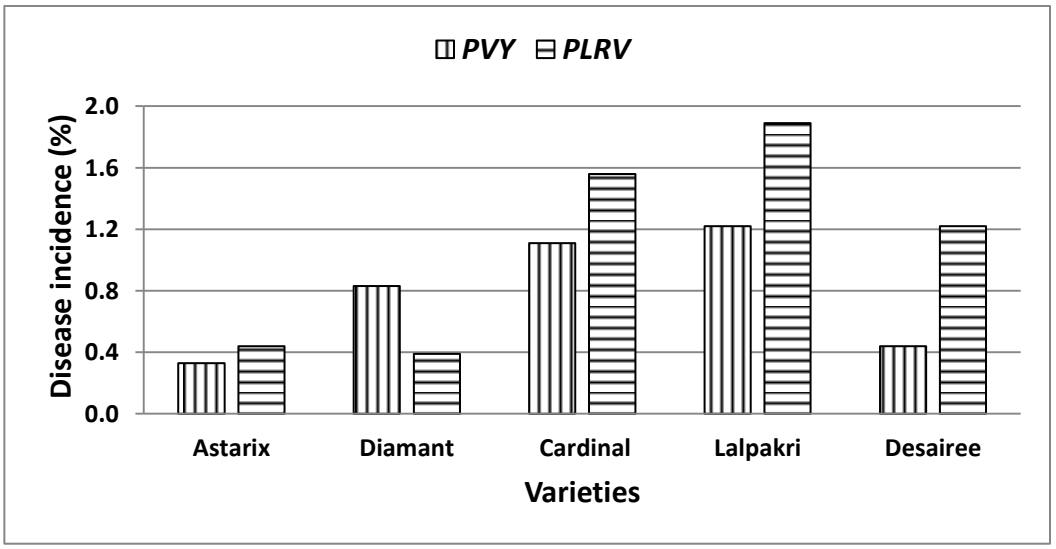
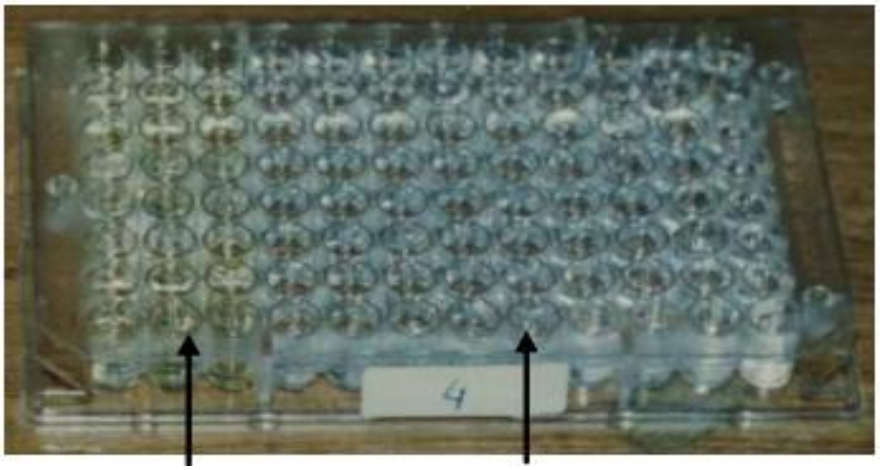


Figure 5. Viral incidences in tested potato varieties grown in glass house condition

4.2. DAS-ELISA test for disease (%) incidence of *PVY* in green house growing plants

Symptoms like leaf mottling, mosaic and necrosis were observed in infected plants. It was tested through DAS-ELISA test that antiserum reacted with the samples taken from all five varieties grown in glass house (Figure 6).



***PVY*(+ve)** ***PVY*(-ve)**

Figure 6. Viral disease incidences of *PVY* in different potato varieties grown in glass house conditions. Positive results of DAS-ELISA were judged based on the development of yellow colour in the wells of ELISA plates.

4.3. DAS-ELISA test for disease incidence of *PLRV* in green house growing plants

Viral symptoms like paleness and upward rolling of young leaves, especially at the base, stunted growth and yellowing of leaf margins were recorded from the samples taken from glass house of Asterix, Cardinal, Desiree, Diamant and Lalpakri (Figure 7). It was tested through DAS-ELISA test that antiserum reacted with the samples taken from all five varieties grown in glass house

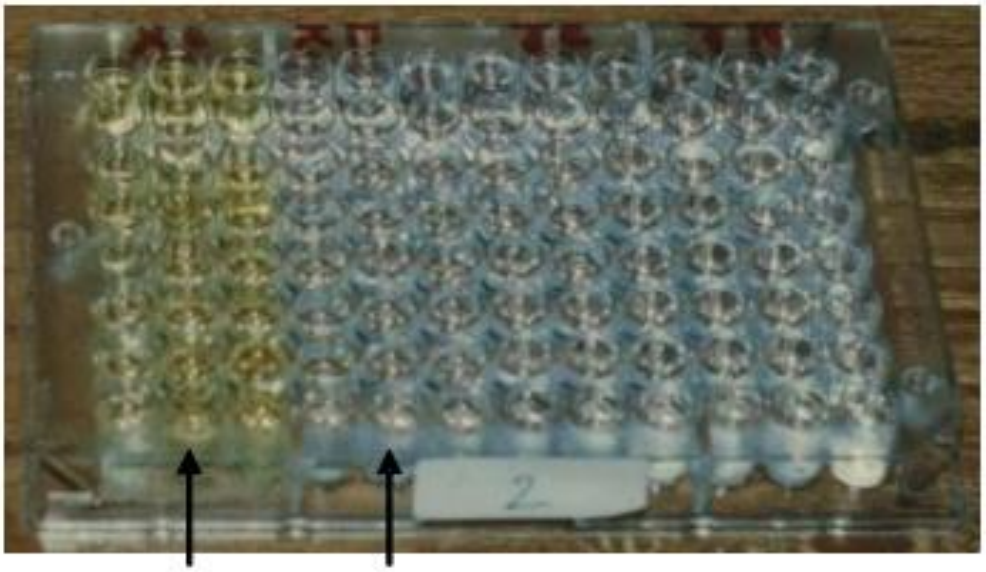


Figure 7. Disease incidence of *PLRV* in different potato varieties grown in glass house. Positive results of DAS-ELISA were judged based on the development of yellow colour in the wells of ELISA plates.

4.4. Numbers of stems/hill in selected potato varieties against *PLRV* and *PVY*

Numbers of stems/hill of the virus free as well as infected hills were significantly different from each other except Cardinal and Desiree. In both cases, Asterix (5.8) showed the highest number of stems/hill than Cardinal (5.3), Desiree (5.2), Diamant (4.5) and Lalpakri (3.3) (Table 4).

Table 4. Number of stems/hill of different potato varieties grown in glass house conditions

Cultivar	Number of stems/hill		
	Virus free plant	*PVY*	*PLRV*
Asterix	5.8±0.10 a	5.8±0.10 a	5.8±0.10 a
Diamant	4.5±0.08 c	4.5±0.08 c	4.5±0.08 c
Cardinal	5.3±0.11 b	5.3±0.11 b	5.3±0.11 b
Lalpakri	3.3±0.06 d	3.3±0.06 d	3.3±0.06 d
Desiree	5.2±0.09 b	5.2±0.09 b	5.2±0.09 b
$LSD_{0.05}$	0.4	0.4	0.4

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.5. Stem length in selected potato varieties against *PLRV* and *PVY*

Stem length (cm) of the virus free as well as infected plants were significantly different from each other except Diamant and Lalpakri (Table 5). In virus free cases, Asterix (23.45 cm) showed the highest stem length than Cardinal (22.37 cm), Desiree (22.12 cm), Diamant (20.30) and Lalpakri (20.22) (Table 5). *PLRV* infected Diamant produced 18.22 cm long stem but *PVY* infection produced 17.22 cm long stem (Table 5). *PLRV* infected Cardinal produced 18.17 cm long stem but *PVY* infection produced 19.37 cm long stem (Table 5). Stem length of Desiree was 18.82 cm in *PLRV* and 19.62 cm in *PVY* infection (Table 5).

Table 5. Stem length (cm) of different potato varieties grown in glass house conditions

Cultivar	Stem length (cm)		
	Virus free plant	*PVY*	*PLRV*
Asterix	23.45±0.31 a	21.45±0.31 a	20.45±0.31 a
Diamant	20.30±0.36 d	17.22±0.36 d	18.22±0.36 d
Cardinal	22.37±0.43 b	19.37±0.43 b	18.17±0.43 b
Lalpakri	20.22±0.52 d	17.30±0.52 d	16.30±0.52 d
Desiree	22.12±0.29 bc	19.62±0.29 bc	18.82±0.29 bc
$LSD_{0.05}$	1.08	1.80	1.62

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.6. Number of leaves of different potato varieties grown against *PLRV* and *PVY*

Numbers of leaves of the virus free as well as infected cultivar were significantly different from each other except Asterix and Cardinal when infected with *PVY* (Table 6). In both *PVY* and *PLRV* cases, Asterix (53.6 and 51.83) showed the highest numbers of leaves than Cardinal (51.3 and 47.75), Desiree (48.1 and 45.70), Lalpakri (41.1 and 38.57) and Diamant (22.8 and 24.63) (Table 6).

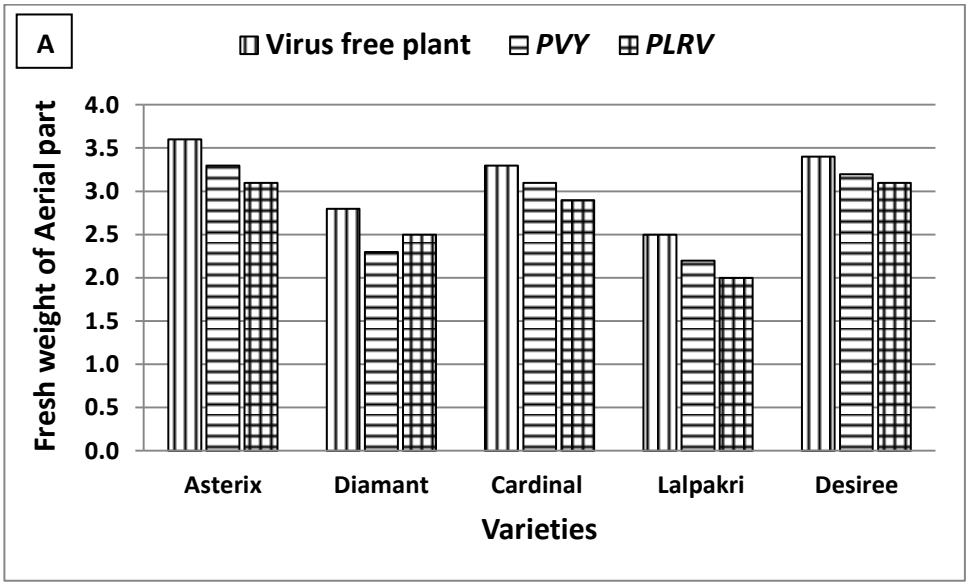
Table 6. Number of leaves of different potato varieties grown in glass house conditions

Cultivar	Number of leaves/hill		
	Virus free plant	*PVY*	*PLRV*
Asterix	65.87±0.75 a	53.6±0.31 a	51.83±0.03 a
Diamant	32.54±0.81 e	22.8±0.14 d	24.63±0.02 e
Cardinal	59.76±0.65 b	51.3±0.21 a	47.75±0.04 b
Lalpakri	47.86±0.68 d	41.5±0.12 c	38.57±0.01 d
Desiree	53.77±0.71 c	48.1±0.22 b	45.70±0.03 c
$LSD_{0.05}$	6.10	2.2	4.32

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.7. Fresh and dry weight (kg) /gm of different potato varieties against *PLRV* and *PVY*

Dry weights of aerial part/plants of the virus free as well as infected cultivars were significantly different from each other except Cardinal and Desiree (Figure 8). In all cases, Asterix showed highest dry weight of aerial part/plants than Desiree, Cardinal, Diament and Lalpakri (Figure 8). Diamant and Lalpakri and Asterix, Cardinal and Desiree were statistically similar for fresh weight of aerial part/plants under the virus free as well as infected condition (Figure 8). Diamant produced 2.61 kg and 0.61 g fresh and dry weight of aerial part/plant when infected with *PLRV* but 2.3 kg fresh and 0.59 g dry weight per plant when infected with *PVY* (Figure 8). In *PVY* infection Cardinal produced 3.1 kg fresh and 0.71 g dry weight but in *PLRV* produced 2.9 kg fresh and 0.68 g dry weight per plant whereas Desiree produced 3.2 kg fresh and 0.71g dry weight in *PVY* and 2.9 kg fresh and 0.68 g dry weight per plant in *PLRV* infection (Figure 8).



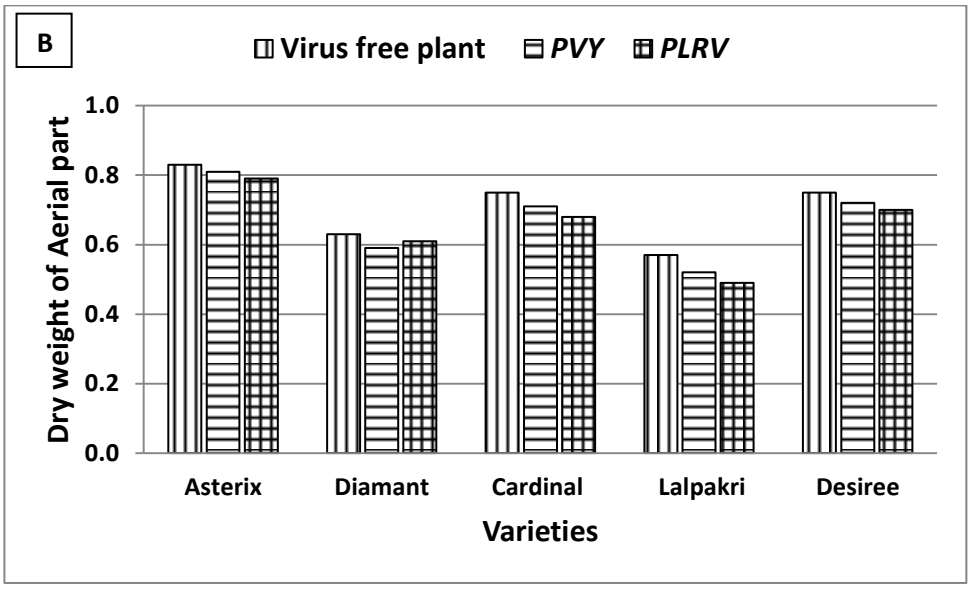


Figure 8. Fresh weight of aerial parts/plant (A) and dry weight of aerial parts/plant (B) of different potato varieties grown in glass house conditions.

4.8. Plant height of different potato varieties against *PLRV* and *PVY*

Plant height was also affected by both cultivar and virus infection: the shortest plants were found for Lalpakri whereas plant length was highest for Asterix (Table 7). Plant heights of the virus free as well as infected cultivar were significantly different from each other except Desiree, Cardinal and Diamant when infected with *PVY* whereas cardinal and Desiree were same for *PLRV* (Table 7). In both cases, Asterix showed highest plant

height than Desiree, Cardinal, Diament and Lalpakri (Table 7). Plant height of Diamant was 45.54 and 44.09 cm when infected with *PLRV* and *PVY*, respectively whereas Cardinal was 40.53 and 44.90 cm respectively. Desiree produced 45.21 cm tall plant when infected by *PVY* but 39.09 cm by *PLRV* infection (Table 7).

Table 7. Height of plant of different potato varieties grown in glass house conditions

Cultivar	Plant height (cm)		
	Virus free plant	*PVY*	*PLRV*
Astarix	59.33±0.65 a	49.43±0.91 a	47.47±0.98 a
Diamant	52.00±0.12 d	44.09±0.68 bc	45.54±0.93 b
Cardinal	55.33±0.74 c	44.90±0.72 bc	40.53±0.76 c
Lalpakri	46.24±0.58 e	40.43±0.62 d	38.35±0.86 cd
Desiree	56.00±0.49 b	45.21±0.46 b	39.02±0.65 c
$LSD_{0.05}$	3.32	4.20	1.92

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.9. Numbers of nodes production in different potato varieties against *PLRV* and *PVY*

Numbers of nodes production of the virus free as well as infected cultivar were significantly different from each other (Table 8). In both virus free as well as infected cases, Asterix showed highest number of nodes production than Desiree, Cardinal, Diament and Lalpakri (Table 8). There was no significant difference between Cardinal and Diamant in virus freeness; Asterix and Desiree, Cardinal and Diamant when infected with *PVY* whereas Diamant and Desiree were same when infected with *PLRV* (Table 8).

Table 8. Number of nodes production in different potato varieties grown in glass house conditions

Cultivar	Number of nodes/stem		
	Virus free plant	*PVY*	*PLRV*
Astarix	11.00±0.24 a	7.78±0.36 a	7.12±0.41 a
Diamant	8.00±0.16 c	6.65±0.31 b	6.13±0.37 b
Cardinal	8.41±0.32 c	6.58±0.41 b	5.09±0.34 c
Lalpakri	7.45±0.41 d	6.16±0.36 c	4.63±0.28 d
Desiree	9.34±0.35 b	7.53±0.28 a	6.10±0.18 b
$LSD_{0.05}$	1.65	0.24	0.98

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.10. Tuber/plant in selected potato varieties

The cultivars Asterix (12.12 tubers/plant) produced more tubers/plant than the Desiree (10.43), Diamant (9.31), Cardinal (9.25) and Lalpakri (8.10) (Table 9). There was no significant difference between Cardinal, Diamant and Lalpakri in virus freeness as well as when infected with *PVY*; whereas Cardinal and Desiree were same when infected with *PLRV*. The ranking order for the number of tubers produced from varieties was Asterix>Desiree>Diament>Cardinal>Lalpakri (Table 9).

Table 9. Number of tuber production in different potato varieties grown in glass house conditions after eight weeks of planting

Cultivar	Number of tubers/plant		
	Virus free plant	*PVY*	*PLRV*
Astarix	12.12±0.36 a	9.38±0.64 a	8.54±0.81 a
Diamant	9.31±0.41 c	7.25±0.73 c	7.91±0.93 b
Cardinal	9.25±0.53 c	7.25±0.65 c	6.15±0.75 c
Lalpakri	8.10±0.46 c	7.17±0.75 c	5.12±0.64 d
Desiree	10.43±0.36 b	8.63±0.64 b	6.33±0.46 c
$LSD_{0.05}$	1.68	0.74	0.62

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.11. Tuber size in selected potato varieties

Largest tubers were recorded for the cultivar Asterix (28.76 mm) while Lalpakri (26.42 mm) produced smallest tubers (Table 10). There was significant effect of cultivar as well as virus infection on the individual size of tuber. There was statistically no significant difference between Cardinal and Diamant; Lalpakri and Desiree in virus freeness; Cardinal, Diamant and Lalpakri when infected with *PVY*; Cardinal and Lalpakri; Diamant and Desiree when infected with *PLRV* (Table 10). The ranking order for the size of tubers produced from varieties was Asterix>Diamant>Cardinal>Desiree>Lalpakri (Table 10).

Table 10. Size of tuber in different potato varieties grown in glass house conditions after eight weeks of planting

Cultivar	Size of tuber (mm)		
	Virus free plant	*PVY*	*PLRV*
Astarix	28.76±0.21 a	25.90±0.32 a	24.79±0.18 a
Diamant	27. 65±0.32 b	21.43±0.36 c	23.98±0.16 b
Cardinal	27.43±0.24 b	22.88±0.26 c	21.13±0.21 c
Lalpakri	26.42±0.26 c	22.67±0.41 c	21.73±0.25 c
Desiree	26.65±0.25 c	24.32±0.28 b	23.56±0.12 b
$LSD_{0.05}$	1.10	1.56	0.80

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.12. Tuber weight in different potato varieties

Heaviest (8.12 g) tubers were recorded for the cultivar asterix while Lalpakri produced lightest (7.40 g) tubers (Table 11). There also was significant effect of cultivar as well as virus infection on the individual weight of tubers as well as yield of tubers. The ranking order for the weight of tubers produced from varieties was Asterix>Diamant>Cardinal>Desiree>Lalpakri (Table 11). There was statistically no significant difference between Diamant, Cardinal, Lalpakri and Desiree in virus freeness; Asterix, Desiree and Cardinal, Diamant and Lalpakri when infected with *PVY;* Cardinal, Lalpakri and Asterix, Diamant and Desiree when infected with *PLRV* (Table 11). In *PLRV* and *PVY* infection, Diamant produced 6.85 g and 6.58 g/tuber, respectively.

Cardinal produced 6.89 g/tuber when infected with *PVY* but 5.70 g/tuber in *PLRV* infection whereas Desiree was 7.01 g/tuber in *PVY* and 6.66 g/tuber in *PLRV* infection (Table 11).

Table 11. Weight of tuber in different potato varieties grown in glass house conditions after eight weeks of planting

Cultivar	Weight of tuber (g)		
	Virus free plant	*PVY*	*PLRV*
Astarix	8.12±0.23 a	7.45±0.35 a	7.22±0.42 a
Diamant	7.70±0.16 b	6.58±0.31 b	6.85±0.35 a
Cardinal	7.65±0.21 b	6.89±0.29 b	5.70±0.31 b
Lalpakri	7.40±0.26 b	6.71±0.22 b	5.65±0.29 b
Desiree	7.60±0.25 b	7.01±0.18 a	6.66±0.24 a
LSD	0.41	0.43	0.36

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.13. Yield performance (kg) i.e; number of tubers/plant and weight of tubers/plant of different potato varieties grown in glass house after harvesting

In virus free condition, Asterix (22.87 tubers/plant and 1.84 kg tubers/plant) showed highest yields per plant than desiree (21.21 tubers/plant and 1.76 kg tubers/plant), cardinal (19.33 tubers/plant and 1.58 kg tubers/plant), diamant (20.43 tubers/plant and 1.71 kg tubers/plant) and lalpakri (19.17 tubers/plant and 1.49 kg tubers/plant kg tubers/plant) (Figure 9 A&B). Diamant produced 19.21 tubers/plant and 1.62 kg tuber/plant in *PVY* infection but 18.17 tubers/plant and 1.57 kg tuber/plant in *PLRV*. Cardinal when infected with *PVY* produced 17.31 tubers/plant and 1.51 kg tuber/plant but when *PLRV* it produced 16.16 tubers/plant and 1.43 kg tuber/plant whereas Desiree produced 20.23 tubers/plant and 1.71 kg tuber/plant and 19.75 tubers/plant and 1.67 kg tuber/plant in *PVY* and *PLRV* infection, respectively (Figure 9 A&B).

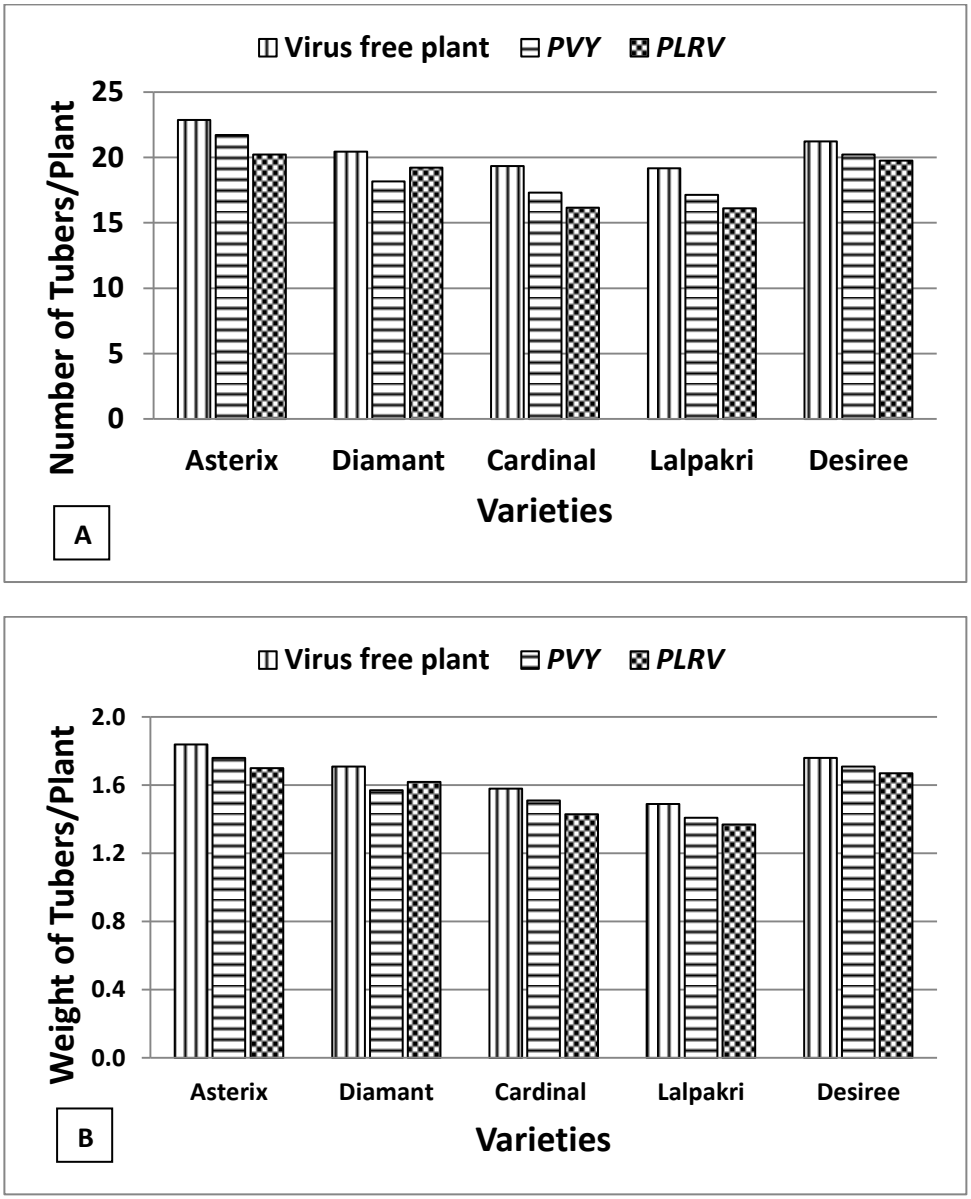


Figure 9. Yield performance (kg) i.e.; number of tubers/plant (A) and weight of tubers/plant (B) of different potato varieties grown in glass house conditions after harvesting

4.14. Dry matter of whole plant of selected potato varieties:

Highest dry weight of whole plant was recorded in varieties Asterix (1.81 g) followed by Desiree (1.68 g), Diamant (1.66 g), Cardinal (1.63 g) and Lalpakri (1.55 g), respectively. There also was significant effect of cultivar as well as virus infection on the dry weight of whole plant. The ranking order for the dry weight of whole plant different varieties was Asterix>Desiree>Diament> Cardinal>Lalpakri. There was statistically significant differences present between Asterix, Desiree and Diamant in virus freeness and when infected with *PVY*; Asterix, Desiree and Diamant, Cardinal when infected with *PLRV*. Diamant produced 1.66 g dry weight/plant in *PLRV* and 1.52 g dry weight/plant in *PVY* infection. In *PVY* and *PLRV* infection Cardinal produced 1.60 g and 1.52 g dry weight/plant, respectively whereas Desiree produced 1.63 g dry weight/plant in *PVY* and 1.60 g dry weight/plant in *PLRV* infection.

Table 12. Dry matter of whole plant in different potato varieties grown in glass house conditions after harvest

Cultivar	Dry matter (g)/plant		
	Virus free plant	*PVY*	*PLRV*
Asterix	1.81±0.11 a	1.76±0.06 a	1.68±0.13 a
Diamant	1.66±0.09 b	1.52±0.07 c	1.66±0.11 a
Cardinal	1.63±0.08 c	1.60±0.08 b	1.52±0.07 c
Lalpakri	1.55±0.12 d	1.51±0.09 c	1.48±0.08 d
Desiree	1.68±0.07 b	1.63±0.11 b	1.60±0.09 b
LSD	0.12	0.12	0.02

The data were presented as means ±SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

4.15. Shoot regeneration and virus elimination in potato by meristem culture of potato

Meristem culture is a procedure in which apical or axillary growing tips (0.1-0.3 mm) are dissected and allowed to grow into plantlets on artificial nutrient media under controlled conditions. The meristem culture technique for virus elimination is essentially based on the principle that many viruses are unable to infect the apical/axillary meristems of a

growing plant and that a virus free if a small (0.1-0.3 mm) piece of meristematic tissue is propagated. Asterix gave the highest percentage (92.7%) of survivability of plantlets followed by Lalpakri (90%), Desiree (87.5%), Cardinal (86.7%) and Diamant (71.7%). Diamant showed the lowest percentage (71.7%) of survivability of plantlets (Table 13). In this study, Asterix was found better than other varieties (Table 13). Asterix gave the highest percentage (99.70%) of virus free plantlets followed by Desiree (99.48%), Cardinal (99.47%), Diamant (99.10%) and Lalpakri (91.44%) (Table 13). Lalpakri showed the lowest percentage (91.44%) of virus free plantlets.

Table 13. Shoot regeneration and virus elimination by meristem culture of potato

Cultivar	Shoot regeneration (%)	Frequency of virus free plants (%)		Mean
		PVY	*PLRV*	
Astarix	92.7	99.60	99.80	99.70
Diamant	71.2	99.12	99.09	99.10
Cardinal	86.7	99.50	99.44	99.47
Lalpakri	90.0	89.96	92.93	91.44
Desiree	87.5	99.50	99.46	99.48

The data were presented as means SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

Virus freeness was further confirmed by DAS-ELISA (Figure 10) showed the microtiter wells where single yellow colour was developed which was for Lalpakri that indicated the absence of *PVY* and *PLRV* viruses in all other varieties tested from regenerated plantlet.

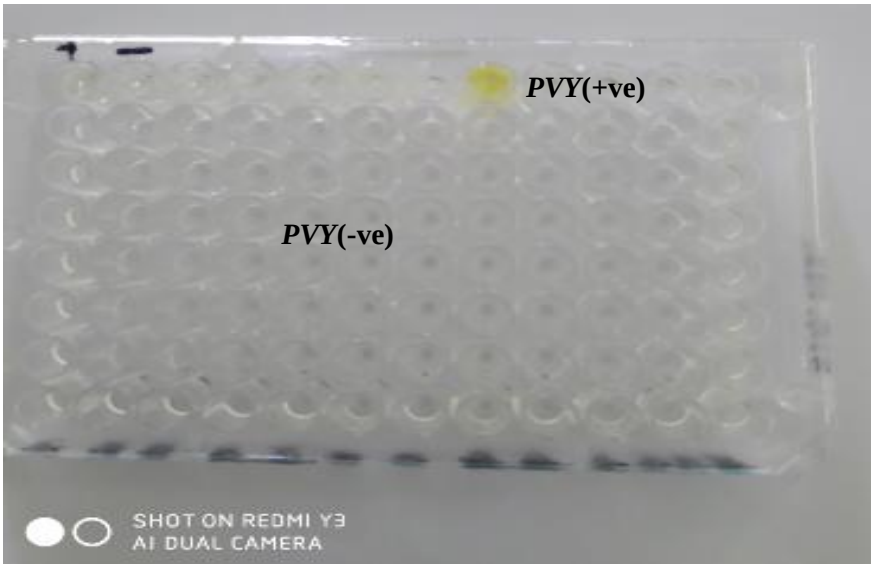


Figure 10. Viral incidences of different potato varieties grown in *in vitro*. Positive results of DAS-ELISA were judged based on the development of yellow colour in the wells of ELISA plates.

4.16. Shoot length, number of leaves and microtubers/plantlets of different potato varieties grown in *in vitro*

Plantlets of Asterix were relatively taller (16.14 cm) than other varieties while Desiree registered the shortest (14.98 cm) (Table 14). Insignificant differences were observed for shoot length among the varieties. Conversely, taller plantlets have been produced in other studies where plant hormones were used. Asterix produced more leaves (13.74) compared to other varieties followed by Diamant (13.43) and Lalpakri (12.2) the least mean number of leaves. None of the varieties was significantly different from others for producing number of leaves/plantlet (Table 14). These results indicate that all test varieties were able to produce sufficient number of leaves to support the photosynthetic process and eventual establishment in the *in vitro* as well as soil media. The number of microtubers strongly differed among the five various cultivars. Lalpakri the local variety produced the lowest (16.68) number of microtubers. The highest numbers of microtubers were found in Asterix (21.40). The ranking order for the number of microtubers/plantlet of different varieties was Asterix>Desiree>Cardinal> Diament>Lalpakri (Table 14).

Table 14. Shoot length, number of leaves and Microtubers/plantlets of different potato varieties grown in *in vitro*

Cultivar	Shoot length (cm)	Number of leaves/plantlets	Number of microtubers/plantlets
Asterix	16.14±0.51 a	13.74±0.53 a	21.40±0.41 a
Diamant	15.89±0.57 a	13.43±0.45 a	18.80±0.35 b
Cardinal	16.65±0.49 a	12.96±0.37 b	19.64±0.28 b
Lalpakri	15.43±0.46 a	12.22±0.42 c	16.68±0.31 c
Desiree	14.98±0.36 a	12.88±0.29 b	20.87±0.42 a
$LSD_{0.05}$	0.50	0.30	0.52

The data were presented as means SE and means sharing dissimilar letters in a column are statistically significant and similar letters are non-significant (P>0.05).

DISCUSSION

From the disease incidence study Diamant was found comparatively less sensitive to *PLRV* (0.39%) but moderate sensitive to *PVY* (0.83%). Cardinal was moderate sensitive to *PVY* (1.11%) but relatively more sensitive to *PLRV* (1.56%) whereas Desiree was less sensitive to *PVY* (0.44%) and highly sensitive to *PLRV* (1.22) (Figure 5). Asterix showed the most resistant to the both *PVY* and *PLRV* (Figure 5). Sample of symptomed plant was tested through DAS-ELISA test and antiserum reaction observed differently from all five varieties grown in glass house (Figure 6 & 7). It may be concluded from the study that presence of viruses in potato plants depends on nature of viruses as well as potato varieties. Ahmad *et al.* (2003) also detected *PVY* at different percentage on different varieties. Mughal and Khalid (1985), who detected eight potato viruses including *PVY* (Fig 6). Rodriguez and Jones (1978) also observed similar responses from field grown potato plants.

The ranking order for the number of stems/hill produced from varieties was Asterix>Cardinal> Desiree>Diament> Lalpakri (Table 4). Therefore, it is clear that the effects of cultivar were highly significant for the numbers of stems/hill (Table 4).

Diamant was comparatively less sensitive to *PLRV* representing by producing 18.22 cm long stem than moderate sensitive to *PVY* producing 17.22 cm long stem (Table 5). Cardinal was moderate sensitive to *PVY* when it produced 19.37 cm long stem but highly sensitive to *PLRV* produced 18.17 cm long stem whereas Desiree was less sensitive to *PVY* produced 19.62 cm long stem but highly sensitive to *PLRV* when it produced 18.82 cm long stem (Table 5). Asterix was the most resistant to the both *PVY* and *PLRV* (Table 5). From the investigation it may be concluded that presence of viruses in potato plants hamper stem length which also depends on nature of viruses and potato varieties.

Diamant was comparatively less sensitive to *PLRV* produced 24.63 leaves/hill than moderate sensitive to *PVY* produced 22.8 leaves/hill (Table 6). Cardinal was moderate sensitive to *PVY* produced 51.3 leaves/hill but highly sensitive to *PLRV* produced 47.75 leaves/hill whereas Desiree was less sensitive to *PVY* produced 48.1 leaves/hill than highly sensitive to *PLRV* produced 45.70 leaves/hill (Table 6). Increase numbers of leaves increases the total leaf area which in turn increased the amount of solar radiation

intercepted and more photoassimilate might have been produced and assimilated to tubers. In this connection, Moorby and Morris (1967) reported that nitrogen fertilizer plays a significant role in the production of stem and axillary branches, which resulted in greater numbers of leaves in field.

Diamant was comparatively less sensitive to *PLRV* representing by 2.61 kg and 0.61 g fresh and dry weight of aerial part/plant, respectively but moderate sensitive to *PVY* when it produced 2.3 kg fresh weight and 0.59 g dry weight per plant (Figure 8). Cardinal was moderate sensitive to *PVY* when it produced 3.1 kg fresh weight and 0.71 g dry weight but infection by *PLRV* produced 2.9 kg fresh weight and 0.68 g dry weight per plant which representing high sensitivity whereas Desiree was less sensitive to *PVY* when it produced 3.2 kg fresh weight and 0.71g dry weight but highly sensitive to *PLRV* when it produced 2.9 kg fresh weight and 0.68 g dry weight per plant (Figure 8).

Diamant was comparatively less sensitive to *PLRV* representing by plant height 45.54 cm whereas moderate sensitive to *PVY* because it produced 44.09 cm plant height (Table 7). Cardinal was moderate sensitive to *PVY* when it produced 44.90 cm height but infection by the *PLRV* it height reduced to 40.53 cm representing high sensitivity whereas Desiree was less sensitive to *PVY* when it produced 45.21 cm tall plant but *PLRV* infection produced 39.09 cm tall plant indicating high sensitivity (Table 7).

The ranking order for the number of nodes produced from varieties was Asterix>Desiree>Diament>Cardinal>Lalpakri (Table 8). Numbers of nodes production of the virus free as well as infected cultivar were significantly different from each other (Table 8). Therefore, this study proved that the effects of cultivar were highly significant for the numbers of nodes production (Table 8).

Diamant was comparatively less sensitive to *PLRV* representing by producing 7.91 tubers/plant but moderately sensitive to PVY when it produced 7.25 tubers/plant (Table 9). Cardinal was moderate sensitive to *PVY* representing by producing 7.25 tubers/plant but infection by *PLRV* produced 6.15 tubers/plant indicating high sensitivity whereas Desiree was less sensitive to *PVY* when it produced 8.63 tubers/plant but *PLRV* infection produced 6.33 tubers/plant which representing high sensitivity (Table 9). The ranking

order for the number of tubers produced from varieties was Asterix>Desiree>Diament>Cardinal>Lalpakri (Table 9).

There was significant effect of cultivar as well as virus infection on the individual size of tuber (Table 10). Diamant produced 23.98 mm size tuber when infected with *PLRV* but *PVY* infection produced 21.43 mm size tuber (Table 10). *PVY* infected Cardinal produced 22.88 mm size tuber but *PLRV* infection reduced size to 21.13 mm (Table 10). Desiree produced 24.32 mm and 23.56 mm size tuber when infected with *PVY* and *PLRV*, respectively (Table 10). It could say that Diamant was comparatively less sensitive to *PLRV* representing by producing 23.98 mm size tuber but *PVY* infection produced 21.43 mm size tuber indicating moderate sensitivity (Table 10). Cardinal was moderate sensitive to *PVY* representing by producing 22.88 mm size tuber but highly sensitive to *PLRV* representing by producing 21.13 mm size tuber whereas Desiree was less sensitive to *PVY* because it produced 24.32 mm size tuber but highly sensitive to *PLRV* representing by producing 23.56 mm size tuber (Table 10).

There also was significant effect of cultivar as well as virus infection on the individual weight of tubers as well as yield of tubers (Table 11). Diamant was comparatively less sensitive to *PLRV* representing by 6.85 g/tuber weight but moderate sensitive to *PVY* representing by producing 6.58 g/tuber weight (Table 11). Cardinal was moderate sensitive to *PVY* because produced 6.89 g/tuber weight but highly sensitive *PLRV* infection because produced 5.70 g/tuber weight whereas Desiree was less sensitive to *PVY* when it produced 7.01 g/tuber weight but highly sensitive to *PLRV* when it produced 6.66 g/tuber weight (Table 11).

Diamant, Cardinal, Desiree and Lalpakri were statistically similar for number of tubers but Diamant and Desiree for weight of tubers under the virus free condition (Figure 9 A&B). Cardinal and Lalpakri were statistically similar for number of tubers but Asterix and Desiree similar for weight of tubers under *PVY* infected condition (Figure 9 A&B). On the other hand, *PLRV* infection showed Asterix, Diamant and Desiree and Cardinal and Lalpakri were similar for number of tubers production but significantly different for weight of tubers except Asterix and Desiree (Figure 9 A&B). Diamant was comparatively less sensitive to *PLRV* representing by producing 19.21 tubers/plant and

1.62 kg tuber/plant than moderate sensitive to *PVY* when it produced 18.17 tubers/plant and 1.57 kg tuber/plant (Figure 9 A&B). Cardinal was moderate sensitive to *PVY* when it produced 17.31 tubers/plant and 1.51 kg tuber/plant but infection by the highly sensitive *PLRV* it produced 16.16 tubers/plant and 1.43 kg tuber/plant whereas Desiree was less sensitive to *PVY* when it produced 20.23 tubers/plant and 1.71 kg tuber/plant than highly sensitive to *PLRV* when it produced 19.75 tubers/plant and 1.67 kg tuber/plant (Figure 9 A&B).

There also was significant effect of cultivar as well as virus infection on the dry weight of whole plant. Diamant was comparatively less sensitive to *PLRV* representing by 1.66 g dry weight/plant but moderate sensitive to *PVY* representing by producing 1.52 g dry weight/plant (Table 12). Cardinal was moderate sensitive to *PVY* when it produced 1.60 g dry weight/plant but infection by the highly sensitive *PLRV* produced 1.52 g dry weight/plant whereas Desiree was less sensitive to *PVY* when it produced 1.63 g dry weight/plant than highly sensitive to *PLRV* when it produced 1.60 g dry weight/plant (Table 12).

The meristem explants from greenhouse grown *PVY* and *PLRV* affected potato plant were subjected to culture media. Different varieties showed significant difference for survival of meristem derived plantlets and production of virus free plantlets derived from meristem of potato (Table 13). Asterix was found better than other varieties in case of survivability of plantlets (Table 13). However, as it appears, the higher survival percentage of meristem derived plantlets may not be a varietal character rather it depends on many other factors such as size of meristems, method of excision of meristem, degree of temperature and precautions followed during inoculation. The most delicate part of plant is the meristematic zone, naturally which is very liable to injury or death. That may be the reason of low survival rate of plantlets derived from meristems in tissue culture. Khanom (1984) investigated that among the local varieties Lalpakri gave the highest (96.00) survival percentage followed by Lalsheel.

In general, larger the size of the meristem, better the chances of its survival *in vitro*, whereas, smaller the size of the meristem, better the chances of its being virus free.

Asterix gave the highest percentage (99.70%) of virus free plantlets and Lalpakri the lowest (91.44%) (Table 13).

A very narrow variation was found in freeing *PVY* and *PLRV* between varieties which ranged from 99.70 to 91.44% indicating negligible role of varieties in obtaining plantlets free from viruses (Table 13). In the present investigation 0.2 to 0.5 mm size of meristem was used. So, care should be taken during excision of meristem including size need to be considered for successfully eliminate more *PVY* and *PLRV*. From present study it may be concluded that presence of viruses in potato plants depends on nature of viruses, potato varieties and size of meristem. Irrespective of varieties, size of the meristem was a factor for obtaining virus free plantlets. Size of meristem (Smith and Mellor, 1968) and size of explant (Zhuk, 1978) have critical role in virus elimination, so 0.3 to 0.7 mm long meristem with one or two leaf primordia produced high proportion of virus free plantlets (Table 13). Irrespective of varieties, size of the meristem was a factor for obtaining virus free plantlets. Bokx (1972) attached similar importance to the size of the meristem for virus elimination in potato. Virus freeness was further confirmed by DAS-ELISA (Figure 10) showed the microtiter wells where single yellow colour was developed which was for Lalpakri that indicated the absence of *PVY* and *PLRV* viruses in all other varieties tested from regenerated plantlet.

Plantlets of Asterix were relatively taller (16.14 cm) than other varieties while Desiree registered the shortest (14.98 cm) (Table 14). Insignificant differences were observed for shoot length among the varieties. Asterix produced more leaves (13.74) compared to other varieties followed by Diamant (13.43); and Lalpakri (12.2) the least mean number of leaves (Table 14). None of the varieties was significantly different from others for producing number of leaves/plantlet (Table 14). These results indicate that all test varieties were able to produce sufficient number of leaves to support the photosynthetic process and eventual establishment in the *in vitro* as well as soil media. More leaves per plantlet can be obtained with the use of growth regulators (Zaman *et al*. 2001 and Nuwagira 2013) however; this may not be necessary depending on the size of the culture vessel and the number of plantlets per vessel. The number of microtubers strongly differed among the five various cultivars. Lalpakri the local variety produced the lowest (16.68) number of microtubers. The highest numbers of microtubers were found in

Asterix (21.40). The ranking order for the number of microtubers/plantlet of different varieties was Asterix>Desiree>Cardinal> Diament>Lalpakri (Table 14). Generally each plantlet or explant can produce one microtuber with a weight of 0.2–0.7 g and 3–10 mm diameter (Struik & Lommen, 1990). Recently some larger microtubers with higher weight are produced in some laboratories (10 g or more).

CHAPTER V
SUMMARY AND CONCLUSIONS

Bangladesh is belonging to developing countries, where virus diseases are of major concern which lowers the yields of potato and requires the development of suitable, sensitive and reliable detection method and supply disease free seed potato to the grower for sustainable potato production. Control of potato viruses is not easy & their spread is thus best be minimized by deterrent measures. As potato is a vegetatively propagated crop, infectivity increases through subsequent generations. The most effective approach to control viruses in potato includes the development of sensitive, simple and economical detection method in any particular region, multiplication and supply sufficient amount of virus free seed potato. Use of disease free seed is one of the durable and economical methods for increasing sustainable potato production in Bangladesh.

The present study was conducted to screen selected potato varieties viz. Asterix, Cardinal, Diamant, Lalpakri and Desiree, against *PLRV* and *PVY* on the basis of growth characteristics and through ELISA test followed by *in vitro* meristem culture performed in order to produce virus-free mini-tuber and sufficient amount of potato seed tubers. For *PVY*, symptoms like leaf mottling, mosaic and necrosis were observed in infected plants whereas for *PLRV* upward rolling of young leaves, especially at the base, stunted growth and yellowing of leaf margins were recorded. It was further confirmed through ELISA test where antiserum reacted with the infected samples taken from all five varieties. The highest disease incidence of *PVY* and *PLRV* (1.22 and 1.89%) was recorded in Lalpakri and the lowest (0.33 and 0.44%) in Asterix respectively. In Diamant, disease incidence of *PLRV* and *PVY* was 0.39% and 0.83% respectively. In Cardinal, disease incidence of *PLRV* and *PVY* was 1.56% and 1.11% respectively. In Desiree, disease incidence of *PLRV* and *PVY* was 0.44% and 1.22% respectively.

Numbers of stems/hill of the virus free as well as infected cultivar were significantly different from each other except Cardinal and Desiree. In both cases, Asterix showed the highest number of stems/hill followed by Cardinal, Desiree, Diament and Lalpakri. Stem length (cm) of the virus free as well as infected cultivar were significantly different from each other. In virus free cases, Asterix (23.45 cm) showed the highest stem length followed by Cardinal (22.37 cm), Desiree (22.12 cm), Diamant (20.30) and Lalpakri

(20.22). *PLRV* infected Diamant produced 18.22 cm long stem but *PVY* infection produced 17.22 cm long stem. *PLRV* infected Cardinal produced 18.17 cm long stem but *PVY* infection produced 19.37 cm long stem. Stem length of Desiree was 18.82 cm in *PLRV* and 19.62 cm in *PVY* infection. Numbers of leaves of the virus free as well as infected cultivar were significantly different from each other. In both *PVY* and *PLRV* cases, Asterix (53.6 and 51.83) showed the highest numbers of leaves followed by Cardinal (51.3 and 47.75), Desiree (48.1 and 45.70), Lalpakri (41.1 and 38.57) and Diamant (22.8 and 24.63). Dry weights of aerial part/plants of the virus free as well as infected cultivars were significantly different from each other. In all cases, Asterix showed the highest dry weight of aerial part/plants followed by Desiree, Cardinal, Diament and Lalpakri. Diamant produced 2.61 kg and 0.61 g fresh and dry weight of aerial part/plant when infected with *PLRV* but 2.3 kg fresh and 0.59 g dry weight per plant when infected with *PVY*. In *PVY* infection Cardinal produced 3.1 kg fresh and 0.71 g dry weight but in *PLRV* produced 2.9 kg fresh and 0.68 g dry weight per plant whereas Desiree produced 3.2 kg fresh and 0.71g dry weight in *PVY* and 2.9 kg fresh and 0.68 g dry weight per plant in *PLRV* infection.

Numbers of nodes production of the virus free as well as infected cultivar were significantly different from each other. In both cases, Asterix showed the highest number of nodes production followed by Desiree, Cardinal, Diament and Lalpakri. There was no significant difference between Cardinal and Diamant in virus freeness; Asterix and Desiree, Cardinal and Diamant when infected with *PVY* whereas Diamant and Desiree were same when infected with *PLRV*. The cultivars Asterix (12.12 tubers/plant) produced more tubers/plant followed by Desiree (10.43), Diamant (9.31), Cardinal (9.25) and Lalpakri (8.10). There was no significant difference between Cardinal, Diamant and Lalpakri in virus freeness as well as when infected with *PVY*; whereas Cardinal and Desiree were same when infected with *PLRV*. The largest tubers were recorded for the cultivar Asterix (28.76 mm) while Lalpakri (26.42 mm) produced the smallest tubers. There was significant effect of cultivar as well as virus infection on the individual size of tuber. There was statistically no significant difference between Cardinal and Diamant; Lalpakri and Desiree in virus freeness; Cardinal, Diamant and Lalpakri when infected with *PVY;* Cardinal and Lalpakri; Diamant and Desiree when infected with *PLRV*. Diamant produced 23.98 mm size tuber when infected with *PLRV* but *PVY* infection

produced 21.43 mm size tuber. PVY infected Cardinal produced 22.88 mm size tuber but PLRV infection reduced size to 21.13 mm. Desiree produced 24.32 mm and 23.56 mm size tuber when infected with *PVY* and *PLRV*, respectively. Highest dry weight of whole plant was recorded in varieties Asterix (1.81 g) followed by Desiree (1.68 g), Diamant (1.66 g), Cardinal (1.63 g) and Lalpakri (1.55 g), respectively. Diamant produced 1.66 g dry weight/plant in *PLRV* and 1.52 g dry weight/plant in *PVY* infection. In *PVY* and *PLRV* infection Cardinal produced 1.60 g and 1.52 g dry weight/plant, respectively whereas Desiree produced 1.63 g dry weight/plant in *PVY* and 1.60 g dry weight/plant in *PLRV* infection.

In virus free condition, Asterix (22.87 tubers/plant and 1.84 kg tubers/plant) showed the highest yields per plant than desiree (21.21 tubers/plant and 1.76 kg tubers/plant), cardinal (19.33 tubers/plant and 1.58 kg tubers/plant), diamant (20.43 tubers/plant and 1.71 kg tubers/plant) and lalpakri (19.17 tubers/plant and 1.49 kg tubers/plant kg tubers/plant). Diamant produced 19.21 tubers/plant and 1.62 kg tuber/plant in *PVY* infection but 18.17 tubers/plant and 1.57 kg tuber/plant in *PLRV*. Cardinal when infected with *PVY* produced 17.31 tubers/plant and 1.51 kg tuber/plant but when *PLRV* it produced 16.16 tubers/plant and 1.43 kg tuber/plant whereas Desiree produced 20.23 tubers/plant and 1.71 kg tuber/plant and 19.75 tubers/plant and 1.67 kg tuber/plant in *PVY* and *PLRV* infection, respectively. Asterix gave the highest percentage (92.7%) of survivability of plantlets followed by Lalpakri (90%), Desiree (87.5%), Cardinal (86.7%) and Diamant (71.7%). Diamant showed the lowest percentage (71.7%) of survivability of plantlets. Asterix gave the highest percentage (99.70%) of virus free plantlets followed by Desiree (99.48%), Cardinal (99.47%), Diamant (99.10%) and Lalpakri (91.44%). Lalpakri showed the lowest percentage (91.44%) of virus free plantlets. A very narrow variation was found in freeing *PVY* and *PLRV* between varieties which ranged from 99.70 to 91.44% indicating negligible role of varieties in obtaining plantlets free from viruses. The different varieties were cleaned and multiplied through meristem culture. Plantlets of Asterix were relatively taller (16.14 cm) than other varieties while Desiree registered the shortest (14.98 cm). Asterix produced more leaves (13.74) compared to other varieties followed by Diamant (13.43) and Lalpakri (12.2) the least mean number of leaves. These results indicate that all test varieties were able to produce sufficient number of leaves to support the photosynthetic process and eventual establishment in the

in vitro as well as soil media. Lalpakri the local variety produced the lowest (16.68) number of microtubers. The highest numbers of microtubers were found in Asterix (21.40). From the results of this study and overall performance of different varieties; varieties could be ranked as follows Asterix>Desiree>Cardinal> Diament>Lalpakri and Asterix was found better than other varieties. However, further study needed for recording and documenting the responses of the different varieties to different viruses through symptomology, growth characteristics, ELISA and molecular technique.

CHAPTER VI

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CHAPTER VII

APPENDICES

APENDIX 1.

Protocol of ELISA test

1. Dilute coating antibody in coating buffer as recommended on the bottle label and add 100µl to the required number of wells for the test.
2. Wrap the plate tightly in cling film or place in a plastic box with some damp paper towels and close the box. Incubate the plate at 37^0C for 4 hours.
3. Wash the plate three times with phosphate buffered saline + Tween 20 (0.05%) – PBST. To do this fills the wells of the plate with PBST and invert to remove the buffer. Repeat twice; pat the plate dry on paper towels.
4. Extract the samples by grinding 1g of tissue with10ml of general extraction buffer in a mortar and pestle for an alternative method of grinding. Then filter the sample through a layer of muslin (or similar fine cotton gauze). If this is not available then allow the plant material settle and use the supernatant in the test. In some cases the recommended ratio of sample to buffer may have to be reduced to allow a clear signal to be obtained if the plant material is not highly infected.
5. Add 100µl of each sample, positive and negative control to the coated wells. ADGEN recommended that all samples and controls are tested in duplicate. Remember, 1 ADGEN UNIT= 2 TEST WELLS. ADGEN positive and negative controls are reconstituted by adding 2ml of distilled/deionised water and gently shaking. Any unused reconstituted control may be stored at -20^0C. However, the performance of the positive controls may decrease when stored in this manner.
6. Wrap the plate as described in (2) above and incubate at 4^0C overnight (at least 16 hours).
7. Wash the plate as described in (3) above.
8. Dilute the antibody-enzyme conjugate as recommended on the bottle label in conjugate buffer and add 100µl to each test well.
9. Wrap as in (2) above and incubate at 37^0C for 1hour.
10. Wash four times as described in (3) above. An extra wash is included at this stage to ensure that all unbound antibody-enzyme conjugate is removed from the wells.

11. Prepare the substrate just before use – add pNPP at 1mg/ml to substrate buffer (one 5g tablet in 5ml of buffer). Alternatively use one of the ADGEN liquid substrates. All of these substrates may change color when exposed to light and should be protected from light to prevent this occurring.

12. Add 100µl of prepared substrate to each test well.

13. Wrap the plate as in (2) above and incubate in the dark ar room temperature for 1 hour.

14. Read the absorbance using a spectrophotometer at 405nm (for pNPP and ADGEN Yellow) or 595-650nm (for ADGEN Blue). Alternatively positive and negative samples may be scored visually although this may not be as accurate as using a spectrophotometer. A positive sample may be determined as one which gives an absorbance value which is greater than the absorbance values of the negative control. A negative sample is one which gives an absorbance value which is the same as, or less than the negative control. Visually a positive sample will give a darker color than the negative control and a negative sample will give a similar or lighter color to the negative control

APENDIX 2.

Reagents required for DAS-ELISA test:

1. Coating antibody (codes in the ADGEN catalogue ending in -01/-02
2. Conjugate (ADGEN codes ending in -03/-04)
3. Coating buffer (ADGEN codes 02-001/02-002)
4. Phosphate buffered saline + Tween 20 (ADGEN code 02-003)
5. Extraction Buffer (ADGEN codes 02-004 - 02-016 depending on antigen of interest)
6. Conjugate buffer (ADGEN codes 02-008/02-009)
7. pNNP tablets (ADGEN code 0-001/0-002)
8. Substrate buffer (ADGEN code 02-010/02-011)
9. Alternatively for your convenience ADGEN supply a DAS ELISA buffer pack (02-017/02-018) and prepared liquid substrates which are stable, convenient and easy to use (03-003/03-004) and for enhanced detection in your assay choose ADGEN blue liquid substrate system (03-005/03-006)

APENDIX 3.

Recommended buffer for DAS-ELISA

3.1. Coating buffer (Carbonate buffer)

Name of Chemical	Amount
Sodium carbonate	1.59g
Sodium hydrogen carbonate	2.93g
Up to 1 litre with dH_2O was made. The pH of this buffer is 9.6 and does not require to be adjusted.	

3.2. Phosphate buffer saline (PBS) ×10

Name of Chemical	Amount
Sodium chloride	80g
Potassium dihydrogen orthophosphate	2g
Disodium Hydrogen orthophosphate	11.5g
Potassium chloride	2g
Made up to 1litre with dH_2O.The pH of this solution when diluted to 1×s is 7.2	

3.3. Wash buffer (PBS+Tween 20)

Name of Chemical	Amount
Phosphate Buffer Saline	1 litre
Tween 20	0.5m

3.4. General extraction buffer

Name of Chemical	Amount
Polyvinylpyrrolidone (PVP)	20g
Ovalbumin	2g
Sodium sulphite (anhydrous)	1.3g
Sodium azide	0.2g
Tween 20	0.5mL
Sodium Chloride	8g

Potassium diHydrogen orthophosphate	0.2g
Disodium Hydrogen orthophosphate	1.15g
Potassium chloride	0.2g

Made up to 1 litre with distilled/deionised water. This buffer can be difficult to get into solution and it is easier if the PVP is mixed into a "paste" with a small volume of water before adding the other components and the remainder of water.

3.5. Conjugate buffer

Name of Chemical	**Amount**
Bovine serum albumin	0.2g
PBST	100 mL

3.6. Substrate buffer (Diethanolamine buffer 1M)

Name of Chemical	**Amount**
Dietanolamine	90.39g
Dietanolamine-HCl	19.82g
Magnesium chloride	0.1g

Made up to 1litre with dH2O. The pH of the buffer is 9.8 and it does not require to be adjusted. (The diethanolamine and dietanolamine-HCl are liquids 32 however; it is easier to weight them out than to measure their volumes as they are extremely viscous.)

pNPP was added to the above buffer at 1mg/ml to make up the substrate for alkaline phosphatase.

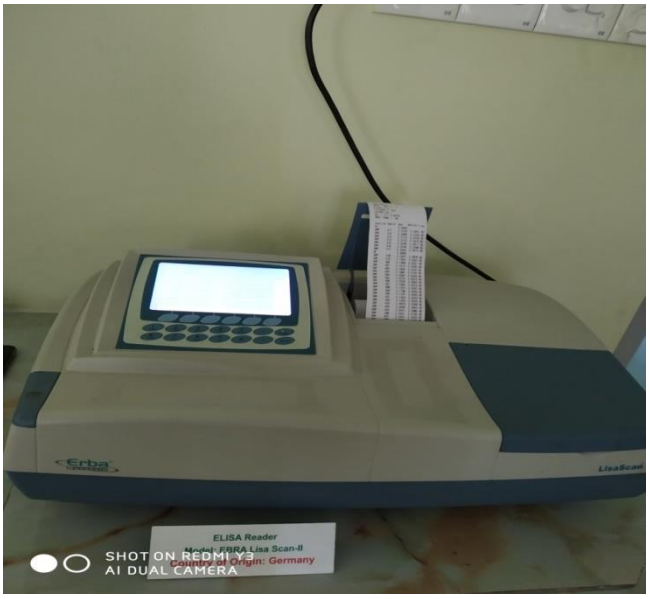
APENDIX 4. Some photographs of the research work

A. Potato meristem cutting

B. Wash microtitre plate by microtitre plate washer

C. Wash microtitre plate with pbst

D. Digital stir is used for buffer solution stirring



E. Take reading by ELISA reader

F. Result of ELISA test