STUDIES ON SOME VIRAL DISEASES OF ECONOMICALLY IMPORTANT CROPS FROM SUB-HIMALAYAN WEST BENGAL AND THEIR MANAGEMENT STRATEGIES

Thesis submitted to the University of North Bengal
for the Award of Doctor of Philosophy
in
Botany

By
Bikram Saha

Supervisor
Dr. Aniruddha Saha
Co-supervisor
Dr. Dipanwita Saha

Department of Botany
University of North Bengal

December, 2013

Declaration

I, Bikram Saha hereby declare that the work embodied in my thesis entitled "**STUDIES ON SOME VIRAL DISEASES OF ECONOMICALLY IMPORTANT CROPS FROM SUB-HIMALAYAN WEST BENGAL AND THEIR MANAGEMENT STRATEGIES**" *has been carried out by me under the supervision of* ***Dr. Aniruddha Saha****, Associate Professor, Department of Botany, University of North Bengal and* ***Dr. Dipanwita Saha****, Assistant Professor, Department of Biotechnology, University of North Bengal for the award of the Degree of Doctor of Philosophy in Botany. I also declare that, this thesis or any part thereof has not been submitted for any other degree/Diploma either to this or other university.*

Bikram Saha

(Bikram Saha)

Date: 26th Dec, 2013

Place: Department of Botany,
University of North Bengal
Siliguri- 734 013

Department of Botany
University of North Bengal

DR. ANIRUDDHA SAHA
M.Sc.(Gold Med.),Ph. D., FNRS
Associate Professor

SILIGURI- 734013, W.B., FAX: 0353-2699001,
phone: +919832372105,
Email: asahanbu@yahoo.co.in

TO WHOM IT MAY CONCERN

This is to certify that the thesis entitled, "Studies on some viral diseases of economically important crops from sub-Himalayan West Bengal and their management strategies" submitted by Mr. Bikram Saha for the award of the degree of Doctor of Philisophy in Botany is based on the results of experiments carried out by him. Bikram has worked under my supervision at Department of Botany, University of North Bengal and Co-supervision of Dr. Dipanwita Saha, Department of Biotechnology, University of North Bengal. I am forwarding his thesis for the Ph. D. degree (science) of the University of North Bengal. He has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in his thesis.

Aniruddha Saha

(Aniruddha Saha)

Supervisor

Department of Biotechnology
University of North Bengal

Dr. Dipanwita Saha
M.Sc., Ph.D.
Assistant Professor

SILIGURI-734 013, West Bengal , India
Phone: 0353-2776354; FAX: 0353-2699001
Email: dsahanbu@yahoo.com
Mobile phone: 9434429800

TO WHOM IT MAY CONCERN

This is to certify that Mr. Bikram Saha has worked under my co-supervision at the Department of Biotechnology, University of North Bengal and the supervision of Dr. Aniruddha Saha, Department of Botany, University of North Bengal. His thesis entitled "Studies on some viral diseases of economically important crops from sub-Himalayan West Bengal and their management strategies" is based on his original work and is being submitted for the award of Doctor of Philosophy (Science) degree in Botany in accordance with the rules and regulation of the University of North Bengal.

Dipanwita Saha

(Dipanwita Saha) 26/12/2013
Co-Supervisor

Acknowledgement

I have much pleasure in expressing my deepest sense of gratitude to my supervisor Dr. Aniruddha Saha, Associate Professor, Department of Botany, University of North Bengal for his untiring guidance, help and involvement throughout the course of my academic endeavors. His incredible availability and pertinent comments largely improved the quality of the work. I never forget the numerous and passionate hours of discussion on and beyond my study topics with him and his confidence in me. I sincerely cannot express in words, my gratefulness to his tremendous support and able guidance throughout the course of this research work.

I would like to thank my co-supervisor Dr. Dipanwita Saha, Assistant Professor, Department of Biotechnology, University of North Bengal, for her inspiration, encouragement and guidance. I am grateful for her constructive suggestions which immensely helped me to sail through my academic endeavour successfully.

I am also indebted to Dr. S. C. Roy (Head), Department of Botany, University of North Bengal, Prof. A.P. Das, Prof. B.N. Chakraboorty, Prof. P.K.Sarkar, Prof. U. Chakraborty, Dr. A. Sen, Mr. P. Mandal and Dr. M. Choudhury of the Department for their valuable advice and encouragement throughout the course of this work.

I sincerely express my special thanks and regard to Dr. Kajal Kumar Biswas, Principal Scientist, Plant Virology unit, Division of Plant Pathology, IARI, New Delhi for his valuable suggestions and encouragement during the course of this study. I also like to thank Prof. D. K. Jha, Head, Department of botany, Gauhati University for his generous help during field survey in Gwahati, Assam.

I would like to express my deep sense of gratitude to the staff of Electron microscopy Laboratory, Cultivation of Sciences, Kolkata for their help in ultrastructural studies.

I also like to express my deep sense of gratitude to District Executive Officer, Paschim Banga Go-Sampad Bikash Sanstha, Siliguri Sub-Division for providing Liquid Nitrogen.

I appreciate the help and support of my senior colleagues namely Dr. Sourish Dasgupta, Dr. Parimal Mandal, Dr. Mehbub Isha, Dr. Chandrani Choudhury, Dr. Sankar Ghosh and Dr. Hari Kamal Barman.

I gratefully acknowledge the unconditional help and support that I received from Sima Mandal, Gargee Dhar Purkayastha, Tarun Mishra, Lopamudra Das, Ramasis Kumar, Hrisikesh Mandal, Bilok Sharma, Shibu Das, Piyali Sarkar, Prosenjit Chakraborty, Arnab Saha, Rajib Biswas, Biswanath Gan and Anand Sharma.

I also greatfully acknowledge University Grants Commission, New Delhi for their financial support for pursuing this work which would not have been possible, otherwise.

It would not have been possible for me to accomplish my PhD work without the constant love, affection and encouragement of my beloved parents (Mr. Bipul Kumar Saha and Mrs. Radha Saha) and younger brother (Arindam Saha).

Bikram Saha

Dated: 26/12/2013 ***(Bikram Saha)***

Abstract

The present work entitled “Studies on some viral diseases of economically important crops from sub-Himalayan West Bengal and their management strategies” consists of objectives such as i) Screening of viral diseases of economically important crops; ii) Studies on transmission and host range of the selected viruses; iii) Electron microscopic studies of selected viruses; vi) Detection of viral pathogens by molecular techniques; v) Management of selected viral diseases. At the onset of the work, brief reviews of literature in the lines of present objectives have been presented. The review has been divided in to some heads and subheads. Different experimental procedures and techniques used in the present work have been described in details in the materials and methods section. The work has been carried out after thorough survey of different viral diseases present in and around the crop growing areas of sub-Himalayan West Bengal. During field survey Leaf curl disease of tomato, Leaf curl disease of potato, Mosaic diseases of uncultivated plants and weeds as well as Mosaic and other diseases of papaya, bean and potato were found in the present study area. The occurrence and distribution of begomoviruses in tomato plants have been studied in sub-Himalayan West Bengal. The nearby sub-Himalayan Brahmaputra valley of Assam was also included in the study for comparison of the viruses present in the two areas. Electron microscopic observation of crude preparation of infected tomato leaves revealed the presence of isometric, pentagonal and ‘paired Geminivirus’ structures. The virus associated with TLCD was detected by PCR using *Begomovirus* specific primers. The results of the study confirmed the presence of TLCD (caused by *Begomovirus*) in the area. Whitefly transmission of the *Begomovirus* associated with TLCD samples of five locations, three from West Bengal and two from Assam were carried out. Results indicated that the TLCD in tomato was caused by whiteflie-transmitted *Begomovirus* species. The causal viruses could be transmitted both by whitefly inoculation technique as well as by mechanical transmission technique in some cases. Ten different plants cultivated around the tomato growing areas of sub-Himalayan West Bengal and Brahmaputra valley of Assam

were selected and tested for their potentiality as alternative host by mechanical sap inoculation. Out of the 10 different plants tested two plants failed to show symptoms and contained no detectable DNA of ToLCV. Four pairs of DNA-A specific *Begomovirus* primers were used to amplify the DNA-A genome. Only one isolate GUW-2 was amplified using all the four primer pairs among the tested samples and a complete genome of 2763 nucleotide of DNA-A was found. The complete genome was submitted in the GenBank (Accession no. JN676053). DNA-B was successfully amplified (using BF518 / BR16141 primer pair) from isolates COB-2 and SILIGURI-2. But DNA-β could not be amplified from any of the nine virus isolates of the present study (using Beta 01/Beta 02 primer pair). Seven TLCD samples, designated as isolates GUW-1, GUW-2, COB-2, SILIGURI-2, COB-5, COB-6 and RAI-1 from seven locations were randomly taken for molecular characterization of the virus. The phylogenetic analysis showed overall two genogroups are present among the isolates including present isolates. The present isolates COB-2, RAI-1 and SILIGURI-2 fell into one group (GR-1) and COB-5 and GUW-1 into another group (GR-2). The TLCD in these regions could be caused by ToLCNDV, ToLCPkV, ToLCRaV, ToLCBnV and TbCSV or mixture of different begomoviruses. Rolling circle amplification (RCA) technique was adopted to amplify full length genomes of the isolates where DNA-A was present but DNA-B was absent. This was done to detect the possibility of presence of DNA-β along with DNA-A. Out of five enzymes tested BamH1 could digest the amplicon by a single nick and was able to separate DNA-A and DNA-β in agarose gel. The isolates COB-5 and GUW-2 showed positive response on RCA and RFLP as two distinct bands of ~2.7kb and ~1.5kb was appeared after restriction digestion. For management of ToLCV (especially whitefly) diseases Leaf extracts of ten different plants were used. The results of the experiment revealed that the *Azadirachta indica* and *Clerodendron viscosum* leaf extracts could reduce disease incidence in tomato plants significantly.

Apical leaf curl disease causing viruses of potato were isolated and characterized from the fields of sub-Himalayan West Bengal. The disease

was successfully transmitted to healthy potato plants using both whitefly vector *B. tabaci* and mechanical sap inoculation. Three different types of primers for DNA-A and one primer for DNA-B were used for the purpose of molecular characterization of the virus. In phylogenetic analysis the virus sequences clustered with the ToLCNDV isolates. Some uncultivated virus infected plants like *Datura stramonium*, *Jatropha curcas*, *Croton bonplandianus*, *Acalypha indica*, *Ageratum conyzoides* were selected for detection of viruses. Molecular characterizations of the viruses were also performed using universal begomovirus primers for partial DNA-A genomes. Three sets of universal degenerate primers were used for amplification and confirmation of the partial coat protein, coat protein and core coat protein genes of viruses from the infected *J. curcas, D. stramonium, C. bonplandianus*, *A. indica* and *A. conyzoides* plants. Expected amplicons were found in the gels and the presence of the begomoviruses was confirmed. In order to know the relationship of begomoviruses infecting the plants in sub-Himalayan West Bengal the amplified products were cloned and sequenced. The viral gene isolate from *J. curcas* contained a nucleotide sequence of 495bp [Accession no. HQ597029 containing two ORFs (AV2 and AV1 partial)]. It showed highest 95% nucleotide sequence identity with *Jatropha mosaic India virus*- [Lucknow, strain SK-2, segment DNA-A (Accession no. HM230683)]. Virus from *D. stramonium* showed highest 99% nucleotide sequence identity with *Tomato leaf curl New Delhi virus* DNA-A complete genome (Accession no. AM850115). Three infected plant samples (*C. bonplandianus*, *A. indica* and *A. conyzoides*) gave positive virus gene amplification results when primer pair 'AV494 and AC1048' was used. In phylogenetic analysis they clustered with begomoviruses infecting papaya, tobacco, mesta and kenaf. The core CP gene of the begomovirus associated with *Ageratum* yellow vein disease of the present study area (JQ843097) showed its close relationship (99% nsi) with both *Tobacco curly shoot virus* infecting *Ageratum* plants in China (AJ971266, FN401522) and with *Tobacco curly shoot virus* of infected pepper (GU001879) plants (Qing *et al.*, 2010). The same core CP gene showed 98% nsi with *Ageratum enation virus* (JF728866) infecting *Ageratum* plants in India and clustering with both the begomoviruses.

Potyvirus caused mosaic diseases of some cultivated crops of sub Himalayan West Bengal were studied. Samples which showed distinct visible symptoms were subjected to RT-PCR and a positive reaction in PCR amplification of the target sequence (~650nt long) specific to potyvirus confirmed the presence of potyviruses. Transmission electron microscopic examination of crude preparation of papaya, potato and bean samples revealed the presence of flexuous rods. *Papaya ring spot virus* disease was successfully transmitted from infected papaya plant to healthy papaya and pumpkin plants.

Amplified CP sequence of virus isolate of papaya (677bp, Accession no. JX567310) showed nearest identity with *Papaya ring spot virus*. Amplified CP sequence of virus isolate of potato (801bp, Accession no. JX945850) showed nearest identity with *Potato virus Y*. Amplified CP sequence of the virus isolate of bean (576bp, Accession no. KC871565) showed nearest identity with *Bean common mosaic virus*. The isolates were also clustered with the same viruses in the phylogenetic tree.

List of tables

Contd…

Contd…

List of figures

List of Appendix

Abbreviations

(DI)DNA	Defective interfering DNA
µg	Microgram
µl	Microlitre
µm	Micrometer
^{0}C	Degree Celcious
aa	Amino acid
BLAST	Basic Local Alignment Search Tool
bp	base pair
ca.	Circa or approximately
CI	Cytoplasmic Inclusion
CLCuD	Cotton leaf curl Disease
cm	Centimeter
CP	Capsid protein or coat protein
CR	Common region
c-sense	Complementary sense
CTAB	Cetyl trimethyl ammonium bromide
d	Day(s)
DI	Defective interfering
DNA	Deoxyribonucleic acid
dNTPs	deoxyribonucleotide tri phosphates
ds	Double-stranded
dsRF	Double stranded replication fork
dT	Deoxythymine
EDTA	Ethylenediamine tetra acetic acid
ER	Endoplasmic reticulum
g	gram
G phase	Gap phase
h	hour
HCl	Hydrochloric Acid
HC-Pro	Helper Component Protein
ICMV	Indian casava mosaic geminivirus
ICTV	International Committee on Taxonomy of Viruses
IPTG	Isopropyl-β-Dithiogalactopyranoside
IR	Intergenic region
IR	Intergeneric region
kb	kilo bases
kD	kiloDalton
LB	Luria Bertain
M	Mole
m	Meter
mAmp	Miliampere
MEGA	Molecular evolutionary genetics analysis
mg	Miligram
min	Minutes
ml	Milliliter
mm	Millimeter
mM	Milimole
M-MulV	Moloney Murine Leukemia Virus
MOPS	3-(N-Morpholio) propanesulphonic acid
MP	Movement protein
mRNA	messenger RNA
NBRI	National Botanical Research Institute
NCBI	National Centre for Biotechnology Information
ng	Nanogram

Ng	nanogram	RCA	Rolling circle amplification
NI-a	Nuclear inclusion protein A	RCR	Rolling circle replication
NIa-VPg	Nuclear inclusion A linked VPg protein	RDR	Recombination dependent replication
NI-b	Nuclear inclusion protein B	RdRP	RNA dependent RNA polymerase
nm	Nanometer		
nsi /NSI	Nucleotide sequence identity	RNA	Ribonucleic acid
NSP	Nuclear shuttle protein	rpm	Revolution per minute
nt	Nucleotide	RT-PCR	Reverse transcription PCR
ORF	Open reading frame	ss	Single-stranded
Ori	Origin	TAE	Tris Acetate EDTA
PC-PCR	Print capture PCR	TBE	Tris Borate EDTA
PCR	Polymerase chain reaction	UTR	Untranslated region
PD	Plasmodesmata	V	Volt
PIPO	Pretty Interesting Potyviridae ORF	v-sense	Virion sense
		WFT	Whitefly transmitted
psi	Pounds per square inch	X-Gal	5-Bromo-4-Chloro-3-indolyl-β-D-galactopyranoside
PTA	Phosphotungstic acid		
RC	Replication complex		

CONTENTS

1. *Introduction*

India has a long history of agriculture. In worldwide farm output India has a significant role. Agriculture sector of India has a major contribution in Indian economy. A large number of crop plants are cultivated in India and some of them are unique in their own way (Meetei *et al.*, 2012). Agricultural crops of India, like other countries, are also threatened by several biotic and abiotic factors (AVRDC, 2012). Number of pests attack the crops and hinders the production or yield (Kataria and Kumar, 2012). Apart from pests several microscopic organisms/elements also cause a great annual loss to the agriculture sector. Among the microscopic elements viruses contribute a lot for the loss of crop yield (CRSP, 2012). Viruses belonging to family *Geminiviridae* are important among the viruses because of their capability of infecting a large number of economically important crops. These viruses are transmitted from plant to plant by specific insect host(s). One of such insect is *Bemisia tabaci* popularly known as whitefly (Wang *et al.*, 2012). Other insects such as leafhoppers and treehoppers are also causal agents.

Virus affected crops produce several diseases showing a wide range of symptoms. Plant viral diseases can in extreme cases reduce yields to zero leading to catastrophic effects on people. The yield reduction depends on many factors like crop variety, viral diseases, crop system and vector efficiency (in the case of vector-transmitted viruses). Some viral diseases have caused catastrophic losses in agriculture, such as hoja blanca on rice, *Citrus tristeza virus* and geminiviruses in many crops (Agrios, 1997).

Eupatorium lindleyanum, a plant very susceptible to a virus disease showing yellowing symptoms, is the earliest known written record of a virus disease found from a Japanese poem (Hull, 2002). Those symptoms have been shown to be caused by a geminivirus-satellite complex: *Eupatorium yellow-vein virus* (EpYVV) and a DNA β-satellite component (Saunders *et al.*, 2003).

The study of plant diseases, caused by viruses are generally separated into three phases. A descriptive phase known as “Classical Discovery Period” ranged from year 1883 to 1951. The second phase

evidenced by development of new techniques and further descriptions of virus properties during 1952-1983 (Early Molecular Era). The third phase (Recent Period) more techniques are available for studies on virus genome, gene functions, and plant transformation for resistance to virus diseases (Zaitlin and Palukaitis, 2000).

Amongst the biotic factors, virus diseases constitute a bulk of the diseases observed in all plant types, with variable symptoms including leaf curling and distortion, green or yellow foliar mosaic, stunting of plants, and reduced yields. The diseases caused by plant viruses can be devastating on crop yield.

Viruses are obligate parasites and they hijack host cellular machinery to replicate. A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein that is able to organize its own replication only within suitable host cells (Matthews, 1991; Hull, 2002). Hull (2002) reported that virus can usually be transmitted horizontally between hosts. Within cells, virus replication is (1) dependent on the host's protein synthesizing machinery, (2) organized from pools of the required materials rather than binary fission, (3) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane, and (4) continually giving rise to variants through various kinds of change in the virus nucleic acid.

Viruses lack the genetic information encoding the machinery essential to generate metabolic energy or for protein synthesis. As such, in older definitions of life, viruses are not alive. However, if the definition of an organism is broadened to “the unit element of a continuous lineage with an individual evolutionary history” (Luria *et al.,* 1978) then viruses are alive. Viruses replicate and spread, often at considerable speed and their evolutionary history can be traced by comparing species. They can also be observed to change in response to environment, hosts etc.

Virus species typically contains many variant types such as strains or serotypes (Van-Regenmortel, 1990). Historically virus taxonomy has been based on properties such as host range, particle morphology, and serological reaction, but it is now largely based on the type and sequence of the nucleic acid (Fauquet *et al.*, 2005).

Generally, a virus is an infectious and potentially pathogenic parasite in living organisms containing an RNA or DNA genome surrounded by a protective protein coat (Roossinck, 2011). Plant virus particles move between cells via plasmodesmata, then longer distances via the vasculature to cause infection throughout the host. The exact mechanism of transmission of virus is complex and involves one or more virus proteins, receptors within the maxillae or gut of the vector and receptors within the host plant (Hull, 2002).

There are many examples of economically important plant virus diseases. *African cassava mosaic virus* caused 50% yield losses to cassava (*Manihot esculenta*), one of the largest source of carbohydrate for human consumption, and led to starvation in Uganda in the 1990s. Annual losses of 60 million US$ were estimated during that period (Strange, 1993; Thresh and Cooter, 2005). Serious economic losses resulting from outbreaks of *Citrus tristeza virus* have also been reported from America (Wutscher, 1977). *Swollen shoot virus disease* resulted in the death of millions of cocoa (*Theobroma cacao)* trees which caused incalculable damage to economies as well as to social structure (Thresh, 1980, 1986).

Control of plant virus diseases is somewhat difficult task because of large number of varieties, wide host range of the species or varieties, several vectors, complex transmission processes and more so due to the common phenomenon of mixed infections. The lack of the rapid and accurate diagnostic technology makes the control of the virus disease more difficult. Virus diseases are controlled mainly by three approaches such as i) Elimination of virus sources by seed treatment, soil fumigation, destruction of infected plants (Agrios, 1987; Chamberlain, 1954; Cockbain *et al.*, 1976; Maloy, 1993). ii) Prevention of plant to plant transmission by vectors (Oku, 1994; Raccah, 1986; Thomason and McKenry, 1975). And iii) By virus resistant cultivars through selection and breeding (Garcia-Neria and Rivera-Bustamante, 2011; Borah and Dasgupta, 2012).

The introduction of new genotypes, cropping patterns and crops has aggravated the problem of viral disease in several areas including sub-Himalayan West Bengal (present study area). Although the use of resistant varieties has been found to be the most economical and practical, for

effective management of viral diseases but integrated approach is essential in sustainable agriculture. Development of integrated management practices also requires correct identification of the causative viruses because symptoms sometimes become misleading (Verma, 1993). Therefore, adequate understanding of the viruses and their vectors are essential. Considering the importance of the viral diseases in sub-Himalayan West Bengal, a place important for growing several horticultural and agricultural crops, the present work was undertaken with the following objectives.

Objectives:

1) Screening of viral diseases of economically important crops.
2) Studies on transmission and host range of the selected viruses.
3) Electron microscopic studies of selected viruses.
4) Detection of viral pathogens by molecular techniques.
5) Management of selected viral diseases.

2. Literature Review

The first and foremost thing to achieve successful agricultural produce is to make the crop plants healthy. The horticultural plants are infected by several viral pathogens. Detection of the viral pathogens is very important for taking necessary control measures. Study of vectors and their control are also important for successful production of horticultural produce. Though several works have been done for detection of different viruses attacking the horticultural crops throughout world still there are some areas where studies on viruses have not yet been done extensively. Hence, the understanding of the viral diseases of those areas is still need to be studied before going to formulate a disease control strategies. Sub-Himalayan West Bengal (present study area) and north-east India are such areas where detailed studies on viral diseases need to be carried out.

Hence, at the onset of the present study, it was considered to review the works of important plant viruses done throughout world in a selective and comprehensive manner. For convenience, the review has been divided into several subheads which are as follows.

Begomovirus

- Begomovirus Genome Structure and Viral-Encoded Proteins
- Replication of Begomoviruses
- Virus movement
- Criteria for species demarcation
- Insect vector
- Begomovirus infection
- Worldwide epidemics associated with begomoviruses
- Leaf curl disease of tomato
- Mosaic diseases of plants and weeds
- Pest management using botanicals

Potyvirus

- Potyvirus genome structure and viral encoded protein
- Potyvirus replication
- Virus movement
- Mosaic disease of papaya, bean and potato

2.1 Begomovirus

Begomovirus, type species *Bean golden mosaic virus* (BGMV), is the largest genus in the family. The viruses in this genus are transmitted by the whitefly (*Bemisia tabici*) and they infect primarily dicot plants. Most begomovirus species consist of a bipartite genome and few are monopartite. DNA-A encodes five proteins which are the CP, on the v-sense strand, and the Rep, TrAP (a transcriptional activator), REn, and C4 on the c-sense strand. DNA-B encodes two proteins: the nuclear shuttle protein (NSP) and the MP on the c-sense and v-sense strands, respectively (Stanley *et al.*, 2001, Gutierrez, 1999; Fauquet *et al.*, 2000; Briddon *et al.*, 1996).

Begomoviruses are small (ca. 18-30 nm) plant viruses with single-stranded circular DNA genomes that are encapsidated in twinned quasi-icosahedral particles. They belong to the *Geminiviridae* family. Begomoviruses are transmitted by whiteflies, and infect dicotyledonous plants; their genomes can be mono- or bi- partite (Lazarowitz, 1992). They cause significant and often total yield losses of important food and industrial crops in tropical and subtropical regions of the western and eastern hemispheres (Morales and Anderson, 2001; Navas-Castillo *et al.,* 1998; Polston and Anderson, 1997). High incidences of begomoviruses are associated with high populations of whiteflies and serious losses in several crops in the Americas and the Caribbean Basin (Brown and Bird, 1992; Morales and Anderson, 2001; Polston and Anderson, 1997).

The whitefly *Bemisia tabaci* is the vector of begomoviruses. When adults feed on infected plants; virus is usually transferred with food material through the salivary canal to the mid-gut and from the mid-gut it passes into the hemolymph. The virus is then circulated with normal hemolymph. It then passes into the salivary glands. As the whitefly feeds in healthy plants, the virus is transmitted with the saliva to the plant via the salivary canal (Czosnek *et al.*, 2002; Hunter *et al.*, 1998). The coat protein of begomoviruses has been shown to play an important role in the circulation of the virus in the vector (Hofer *et al.*, 1997).

Diverse symptoms develop in plants infected with begomoviruses. These symptoms are broadly of three types: (a) vein yellowing, (b) yellow mosaic, and (c) leaf curl. Large number of begomoviruses has been isolated in the last two decades or so. In the early 1960s about 27 such viruses were known (Varma, 1963), but more than 100 begomoviruses were reported by Fauquet *et al.* (2003), although a large number still remained to be classified during that time (Fauquet and Stanley, 2003). During the last two decades a large number of new begomoviruses causing mosaic and leaf curl symptoms have emerged in various parts of the World (Varma, 1990; Polston and Anderson, 1997; Padidam *et al.,* 1999; Rybicki and Pietersen, 1999).

Begomovirus Genome Structure and Viral-Encoded Proteins: Begomoviruses are mostly bipartite, but some Old World begomoviruses are monopartite. Bipartite begomoviruses have two components, designated A and B. Each component has ~2,600 nt. The DNA A of bipartite begomoviruses and monopartite begomoviruses have a very similar genome organization and encode 5-6 overlapping open reading frames (ORFs). The virion-sense strand (V) of DNA A encodes the coat protein (CP, AV1/V1) that encapsidate the viral ssDNA. The DNA of Old World begomoviruses encodes an additional ORF AV2/V2 that has been implicated in virus movement (Padidam *et al.,* 1996, Rigden *et al.*, 1993). The DNA A complementary-sense (C) strand encodes the replication-associated protein (Rep, AC1/C1), a transcription activator protein (TrAP, AC2/C2) and a replication enhancer protein (REn, AC3/C3). TrAP is involved in the control of both viral and host gene expression. Some DNA A of bipartite viruses and all monopartite viruses encode AC4/C4 that participates in cell-cycle control (Briddon and Stanley, 2006). The DNA B encodes two ORFs a virion-sense nuclear shuttle protein (NSP, BV1) and a complementary-sense movement protein (MP, BC1). The genes on the A component are involved in encapsidation and replication, whereas the genes on the B component are involved in the movement of virus through

the plant, host range, and symptom expression (Gafni and Epel, 2002; Lazarowitz, 1992).

CP gene, one of the five genes of DNA A component is transcribed in the viral sense or clockwise direction. The other four genes — replication-associated protein (Rep), transcriptional activator protein (TrAP), replication enhancer (REn), and AC4 — are transcribed in complementary sense or counterclockwise direction (Lazarowitz, 1992).

The two sets of genes overlap and are separated by an intergenic region (IR), which begins with the start codon of the Rep and ends with the start codon of the CP. This region does not encode any protein and its sequence varies widely among begomoviruses, except that there is a conserved GC-rich inverted repeat sequence, which has the potential to form a stem-loop structure (~30 nt) with the invariant nanomeric TAATATT(↓)AC sequence or loop of the stem-loop structure. The nanomeric sequence contains the initiation site (↓) of rolling circle DNA replication (Gutierrez, 2000; Laufs *et al.,* 1995), the TATA box, and the forward and inverted repeats.

Bipartite begomoviruses often spontaneously produce approximately half-sized defective DNA B components that function as defective interfering (DI) DNA. The DI DNA may have a biological role during infection to reduce the severity of the disease by competing with the genomic components for cellular resources (Stanley *et al.,* 1990). Monopartite begomoviruses have small circular single-stranded DNA satellites, named DNA β. These depend on begomoviruses for their proliferation and, in turn, they affect the accumulation and symptom expression of begomovirus (Mansoor *et al.,* 2003). DNA β satellites are viruses or nucleic acids that depend on a helper virus for replication but lack nucleotide sequence homology to the helper virus. Satellite viruses code for their own coat protein (Mayo *et al.*, 2005).

Replication of begomoviruses: Geminiviruses do not encode DNA polymerase and thus depend upon many host gene functions. These viruses replicate in differentiated cells that are in G phase and shut down most of their DNA replication activities. Geminivirus infection reactivates

the replication and converts the cell back to S phase of cell-cycle (Hanley-Bowdin *et al.,* 1999). Geminiviruses use both rolling circle (RCR) and recombination dependent replication (RDR) mechanisms for the replication of their genome (Gutierrez, 1999; Hanley-Bowdoin 2000, 2004; Gutierrez *et al.,* 2004). Upon entry into the plant cell, the virus particles are targeted to the nucleus where replication occurs and probably involves CP (Hull, 2002). RCR is a two step process. The first step is the synthesis of complementary-sense synthesis using the virion-sense as a template, to produce a ds RF intermediate which is later converted to genome sized circular DNA fragment. This process is thought to be primed by a short RNA molecule (Hanley-Bowdoin *et al.,* 1999). The isolation of oligo-ribonucleotides complementary to the 3' intergenic region from several mastreviruses, support the hypothesis (Hayes *et al.,* 1988). The host DNA polymerase completes the synthesis of complementary-sense strand synthesis. The synthesis of virion-sense strand is mediated by Rep nicking within TAATATTAC. The *Geminivirus* Rep protein is a site specific endonuclease that nicks and ligates the viral strand at the same position *in vitro* (Laufs *et al.,* 1995). The nonanucleotide sequence together with small iterated flanking sequence (called iterons) and intervening sequences form the origin of virion-sense DNA replication. The Rep protein shows specificity for the replication of their cognate genomes. Trans-replication of the DNA B component by DNA A encoded Rep is activated by Rep-iteron specificity. Because of this Rep specificity, most DNA A components are unable to trans-replicate heterologous DNA B (Hanley-Bowdin *et al.,* 1999).

Virus movement: Plant virus movement is a two step process, cell-to-cell movement through plasmodesmata (Pd) and long distance transport through phloem. Plant viruses mainly use two principal strategies for cell-to-cell movement. One involves binding of MP or MP complex with the viral genome, which is either RNA or DNA, and increasing size exclusion limit of Pd. The other is dependent on tubule formation (Lucas, 2006).

Plant viruses are challenged by the presence of the “cell wall” and they need to traverse this barrier while moving from an infected cell to an

adjacent cell. Hence, they employ the resident communication system, plasmodesmata (PD) which permit direct intercellular exchange of macromolecules (Lucas and Lee, 2004; McLean *et al.*, 1995). However, the PD openings are too small to permit passage of viral genomes or the viruses. Thus, the plant viruses encode one or more proteins, called movement proteins (MPs) that are essential for viral movement. MPs increase size exclusion limit (Haywood *et al.*, 2002; Tzfira *et al.*, 2000), interact with the endoplasmic reticulum and the cytoskeleton (Heinlein *et al.*, 1995, 1998) and also interact or modify diverse host factors to ensure successful spread (Lucas, 2006; Scholthof, 2005). DNA viruses replicate in the nucleus and have to cross the nuclear envelope to reach PD and subsequently move to the neighboring cell. Two mechanisms have been proposed for the movement of bipartite geminiviruses. A "relay race model" was proposed (Rojas *et al.*, 1998) and a "couple-skating model" has been proposed (Hehnle *et al.*, 2004).

Criteria for Species Demarcation: Several taxonomic criteria for demarcating species of begomoviruses have been proposed by the International Committee on Taxonomy of Viruses (ICTV) based on the reliability and applicability of these criteria to the large number of characterized begomoviruses (Fauquet *et al.,* 2003). Nucleotide sequence comparison plays a much greater role in determining taxonomic status. Thus, for comparative analyses, only full-length DNA A sequences were considered, based on recombination events that readily occur among begomoviruses (Fauquet *et al.,* 2003; Pita *et al.,* 2001). A cut-off value of 89% of nucleotide sequence identity (NSI) of the A component was established to distinguish different species from strains (Fauquet *et al.,* 2003).

Insect vector: One reason for the appearance of epidemics is the spread of the vectors of geminiviruses (Rybicki and Pietersen, 1999). Geminiviruses have evolved a highly dependent relationship between their host plant and the whitefly vector. Whitefly has the potential to colonize a wide range of dicotyledonous species. There are numerous populations of *B. tabaci* that vary somewhat in their capacity to develop high population densities and

cause direct feeding damage, in the extent of their host ranges, and in the efficacy with which they can transmit geminiviruses (Bedford *et al.*, 1994; Brown *et al.*, 1995). The establishment of the B biotype in cotton-vegetable agro-ecosystems is the driving force behind the emergence of geminiviruses in cotton-vegetable agro ecosystems (Brown *et al.*, 1995). The increasingly wide distribution of higher population levels of whitefly vectors in agro-ecosystems were directly implicated with epidemics. An important consequence of all such epidemics has been co-related with incidence of multiple infections of viruses which in turn increased the chances of the emergence of new recombinant viruses (Harrison and Robinson, 1999).

Czosnek *et al.* (2002) reported that when an insect vector feeds on a virus infected plant a small subset of the virus population is acquired. Therefore, feeding of a single whitefly on multiple host plants adds different types of viruses to the pool, which was then transmitted to other host plants. A single insect could harbor ~600 million virions (approximately 1 ng viral DNA). The insect vector could retain begomoviruses (such as TYLCV) during its complete life time, but the transmission efficiency declined with time.

TYLCSV has been reported to be transmitted to the progeny of the vector through eggs and nymphs, though viral DNA lost its infection ability (Bosco *et al.*, 2004). There exists a direct relationship between the spread of begomoviruses and the increased population densities and high fecundity of the B biotype of *B. tabaci*, not only the virus spread within crops but also from wild plants and weeds. Distinct *B. tabaci* biotypes and genotypes are linked with begomovirus epidemics such as those related to the introduction of the B-biotype into the Western Hemisphere (Morales and Anderson, 2001). Other examples are the spread of CLCuD in the Indo-Pak sub-continent, influenced by the Indian genotype of *B. tabaci* (Ahmed *et al.*, 2010), cassava mosaic disease epidemic in Africa (Legg and Fauquet, 2004; Maruthi *et al.*, 2002) and spread of begomovirus infection with the silverleaf whitefly or sweet potato whitefly *B. tabaci* (B biotype) in the United States (Perring *et al.*, 1993). The comparison of an invasive B-

biotype *B. tabaci* in China with the native non-invasive ZHJ1 whitefly revealed that the B-biotype whitefly population displaced the native whitefly population when fed on plants infected with *Tomato yellow leaf curl China virus* (TYLCCNV) or *Tobacco curly shoot virus* (TbCSV) (Jiu *et al.*, 2006, 2007).

Whitefly species *Bemisia tabaci* (Biotype B) and *B. argentifolii* (Biotype Q) (Cohen *et al.*, 1992; Bellows *et al.*, 1994; Riley *et al.*, 1995; Legg, 1996), as well as *Trialeurodes vaporariorum* and *T. abutilonea* (Larsen and Kim, 1985) transmit geminiviruses. There is variation between whitefly biotypes in the ability to colonize plants and transmit geminiviruses (Rataul and Brar, 1989; Legg, 1996). *Bemisia tabaci* and *B. argentifolii* , transmit *African cassava mosaic*, *Bean golden mosaic*, *Bean dwarf mosaic*, *Bean calico mosaic*, *Tomato yellow leaf-curl, Tomato mottle*, and other begomoviruses in the Family: *Geminiviridae*. *B. tabaci* also infests other hosts such as *Commelina benghalensis* L. and *Euphorbia heterophylla* L. (Legg, 1996).

Since the first report (over 100 years ago) of *B. tabaci*, it became one of the most important pests worldwide in subtropical and tropical agriculture as well as in greenhouse production systems (Oliveira *et al.*, 2001). *B. tabaci* has gradually emerged as one of the major component in the potato seed production complex in India (Chandel *et al.*, 2010)

Begomovirus infection: During phloem feeding, viral particles enter in the cells, they become uncoated and viral DNA enters into the nucleus where viral DNA replication and transcription occur (Gafni and Epel, 2002). For monopartite begomoviruses CP is responsible for the transfer of viral DNA into the host cell nucleus and later into the cytoplasm. Bipartite begomoviruses do not need CP for movement and they use NSP to act as a shuttle for virus movement from the nucleus into the cytoplasm (Malik *et al.*, 2005). In the nucleus the complementary strand is synthesized following primer synthesis to produce a dsDNA intermediate, which serves as a template for transcription of viral proteins (Settlage *et al.*, 2005). Once the dsDNA is formed, bi-directional transcription starts with the help of

promoter sequences located in the IR. The viral transcripts are transported into the cytoplasm for translation (Hanley-Bowdoin *et al.*, 1999). The translated proteins enter into the nucleus to carry out replication, packaging and movement of viral DNA. The Rep protein of the *Begomovirus* binds to the *ori* and starts RCR mode of replication. After accumulation of ssDNA CP switch RCR and shuttles ssDNA into the cytoplasm. The CP starts packaging of the viral DNA to produce virions and the virus is either transported to the next cell through plasmodesmata or taken up by the whitefly to be transmitted to the next plant.

Worldwide epidemics associated with begomoviruses: Epidemics of begomoviruses have increased both in number and distribution (Brown and Bird, 1992; Harrison, 1985; Otim-Nape *et al.* 1997). They usually have a devastating impact on agricultural and horticultural crops. Several factors are responsible for outbreaks of begomovirus diseases, including the emergence of new viruses and whitefly biotypes, increased use of pesticides to control vector pests, monoculture, and the use of cultivars that are not tolerant or resistant to geminiviruses. Before the 1980s, begomoviruses infestation in the New World was a problem for legume production (Polston *et al.*, 1997).

Begomovirus have affected the crops like cucurbits, tomato, and cotton in the USA, Caribbean, Mexico, Central America, Brazil, and Venezuela. The above mentioned places have suffered from high incidences of begomoviruses with devastating economic consequences. Crops such as potato, melons, and beans are also affected. In 1990s geminiviruses destroyed up to 95% of the tomato harvest in the Dominican Republic. In 1991-92 they damaged tomato crop of $140 million in Florida (Fauquet and Stanley, 2003). In Sudan, epidemics of cotton leaf curl disease (CLCuD) threatened the cultivation of cotton during 1940s and 1950s (Idris and Brown, 2000). In Pakistan, CLCuD has acquired epidemic proportions (Mansoor *et al.*, 1999). A new ToLCD epidemic was also reported from India (Polston *et al.*, 1997).

Significant economic losses due to geminivirus were reported from Africa (in cassava) (Thresh *et al.,* 1998), from Pakistan (in cotton) (Briddon and Markham, 2000), and from Florida (in tomato) (Moffat, 1999).

In India, the emerging threat of the viruses belonging to the family *Geminiviridae* has been extensively addressed by Varma *et al.* (1992), Varma and Malathi (2003). The extent of yield loss caused by some geminiviruses has been estimated by Dasgupta *et al.* (2003) to be as high as 100%. Up to 96% loss in yield has been reported by *Bhendi yellow vein mosaic virus* (Pun and Doraiswamy 1999). In legumes (blackgram, mungbean and soybean together) the estimated yield losses was approximately $300 million per year (Varma and Malathi 2003).

Faria *et al.* (1997) reported that in São Paulo State, a tentative new begomovirus species (Tomato yellow vein streak virus) was responsible for 19 to 70% tomato crop loss. In the same region, severe ToYVSV epidemics were also observed in tomato (Colariccio *et al.*, 2001, Eiras *et al.*, 2002). About seven new species of begomovirus were identified on tomatoes in Brazil (Ribeiro *et al.*, 2003; Fernandes *et al.*, 2006). Rocha *et al.*, (2012) reported that in Brazil, a viral complex comprised of at least eight species is responsible for severe losses in tomato crops.

The bipartite begomoviruses *Mungbean yellow mosaic India virus* (MYMIV; Mandal *et al.*, 1997), *Mungbean yellow mosaic virus* (MYMV; Morinaga *et al.*, 1993), *Dolichos yellow mosaic virus* (DoYMV; Maruthi *et al.*, 2006) and *Horsegram yellow mosaic virus* (HgYMV) occur throughout southern Asia. They cause distinctive yellow mosaic symptoms in, as well as extensive losses to, grain legume production and have been collectively referred to as legume yellow mosaic viruses (LYMVs; Qazi *et al.*, 2007).

Chakraborty *et al.* (2003) reported that in Indian subcontinent, ToLCV is a major problem for tomato-growing regions as several reports on new strains have been documented including New Delhi, Lucknow, Bangalore, Varanasi, Mirzapur and so forth and posed a threat to crop productivity. Chandel *et al.* (2010) reported 40-70% infection of potato in Indo-Gangetic Plains of India by whitefly transmitted *Potato leaf curl virus* (PALCV).

Tomato and potato are two important crops and used as food throughout world. On the other hand *Jatropha* is also important for obtaining biodiesel. In this review the begomoviruses of these three plants have been given special emphasis. Hence, in the following paragraphs the important works of the previous scientists related to the three plants have been presented in a selective manner.

Leaf curl disease of Tomato: The genus *Lycopersicon* consists of nine closely related species. Of all the diseases reported on tomato, *Tomato leaf curl virus* (ToLCV), a geminivirus (Geminiviridae: subgroup-III) is the most important and destructive viral pathogen in many parts of India (Vasudeva and Samraj, 1948; Sastry and Singh, 1973; Saikia and Muniyappa, 1989; Harrison *et al.*, 1991). The incidence of ToLCV in tomato growing areas of Karnataka ranged from 17-100 per cent in different seasons and 50 to 70 per cent yield loss was in tomato Cv. Pusa Ruby grown in February- May (Saikia and Muniyappa, 1989). Yield loss exceeds 90 per cent, when infection occurred within four weeks after transplanting in the field (Sastry and Singh, 1973; Saikia and Muniyappa, 1989).

Tomato leaf curl diseases (TLCDs) occur in many tomato producing regions of the world. The disease is characterized by severe leaf curling, shrinking of tomato leaves and stunted plant growth. TLCD is caused by geminiviruses (genus *Begomovirus*, family *Geminiviridae*) and is transmitted by whitefly. Geminiviruses are a major constraint for the successful cultivation of tomato in tropic and subtropics (Czosnek *et al.*, 1988; Hong and Harrison, 1995). Several geminivirus species infecting tomatoes from old world have been characterized. Monopartite tomato infecting whitefly-transmitted Geminiviruses (WTGs), such as *Tomato yellow leaf curl virus* (TYLCV) from Israel (Navot *et at.*, 1991), *Tomato yellow leaf curl Sardinia virus* (TYLCSV-[Sic]) from Italy (Kheyr-Pour *et al.*, 1991) and *Tomato leaf curl virus* (ToLCV) from Australia (Dry *et al.*, 1993) are monopartite and therefore do not have a B component. On the contrary, *Tomato yellow leaf curl Thailand virus* (TYLCTHV) is a monopartite/bipartite virus that possesses a B component (Rochester *et*

al., 1990) which enhances symptom severity than the virus with A component alone.

A number of species or strains of tomato leaf curl geminiviruses have been reported to cause TLCD in India (Vasudeva and Samraj, 1948; Sastry and Singh, 1973; Muniyappa and Saikia, 1983; Saikia and Muniyappa, 1989; Harrison *et al.*, 1991; Kirthi *et al.*, 2002; Paximadis *et al.*, 2001; Ramappa *et al.*, 1998).

Reddy *et al.* (2005) reported *Tomato leaf curl New Delhi virus* (ToLCNDV) from Assam and West Bengal (Kolkata). Beside *Tomato leaf curl virus,* TLCD of tomato are also caused by *Tobacco curly shoot virus* (TbCSV) and *Tobacco leaf curl Yunnan virus* (TbLCYNV) in China (Li *et al.*, 2004).

The *Geminiviridae* are a family of plant viruses with circular single-stranded DNA (ssDNA) genomes encapsidated in twinned particles. Based on their genome arrangement and biological properties, geminiviruses are classified into four genera, *Mastrevirus, Curtovirus, Topocuvirus* and *Begomovirus* (Faquet *et al.*, 2003).

The largest genus of Geminivirus family is *Begomovirus*. Members of the genus *Begomovirus* are transmitted by whiteflies to a wide range of dicotyledonous plants and many have bipartite genomes, known as DNA-A and DNA-B. DNA-A has either one or two open reading frames (ORFs) in the virion sense (AV1, AV2) and up to four major ORFs in the complementary sense (AC1, AC2, AC3, AC4). The DNA-B component has one open reading frame in each of the virion (BV1) and complementary (BC1) orientation. The DNA-A and DNA-B components share little sequence similarity, except for ~170 nt of sequence in the intergenic region (IR), termed the common region (CR) (Hanley-Bowdoin *et al.*, 1999).

Some monopartite begomoviruses, such as *Ageratum yellow vein virus* (AYVV) and *Cotton leaf curl Multan virus* (CLCMV), have been proved to be associated with a satellite-like molecule, referred to as DNA-β (Briddon *et al.*, 2003; Cui *et al.*, 2004; Saunders *et al.*, 2004). DNA-β is a circular, single stranded molecule with approximately half the size of *Begomovirus* component and apart from the conserved sequence (TAATATTAC) common to all geminiviruses, shares negligible sequence

similarity to either DNA-A or DNA-B of bipartite begomoviruses (Saunders *et al.*, 2000).

Host range of whitefly transmitted geminiviruses is vast which include Kenaf (Paul *et al.*, 2006), *Dimorphotheca sinuta* (Raj *et al.*, 2007), *Urena lobata* (Chatterjee *et al.*, 2007), *Mimosa invasa* (Koraveih *et al.*, 2008), Bitter gourd (Rajinimala and Rabindran, 2007), *Datura stramonium* (Ding *et al.*, 2007), Papaya (Wu and Zhou, 2006), *Duranta repens* (Tahir *et al.*, 2006), Chilli (Shih *et al*, 2006), Methi (Raj *et al.*, 2001), common bean (Papayiannis *et al.*, 2007), *Ageratum conyzoides* (Wong *et al.*, 1993), potato (Garg *et al.*, 2001), *Capsicum annum* (Strenger *et al.*, 1990) of India and other parts of the world.

Muniyappa *et al.*, 2000 isolated ToLCV-Ban4 from infected tomato plants and the isolate was similar to the other three isolates from Bangalore previously sequenced and was closely related to ToLCV-Ban2. Phylogenetic analysis showed that the ToLCV isolates from Bangalore constitute a group of viruses separated from those of Northern India. They reported that single whitefly was able to acquire ToLCV-Ban 4 from infected tomato and to transmit the virus to tomato test plants, but five insects were necessary to achieve 100% transmission. Minimum acquisition access and inoculation access periods were 10 min and 20 min, respectively. A latent period of 6h was required for *B. tabaci* to efficiently infect tomato test plants.

According to Chatchawankanphanich and Maxwell (2002) the *Tomato leaf curl virus* (ToLCV) isolated from infected tomato plants with leaf curling and yellowing symptoms of Karnataka State was 2759 nucleotides (U28239) long and was organized similarly to that of other begomoviruses with monopartite genomes.

Li *et al.* (2004) reported that several begomoviruses were associated with tomato leaf curl disease in Yunnan provinces in China. 14 tomato samples showing leaf curl symptoms were grouped into four groups on the basis of their reaction with a panel of 16 monoclonal antibodies. Complete nucleotide sequence of 4 isolates corresponding to 4 group were done by them and they found that *Tomato yellow leaf curl China virus* (TYLCCV),

Tobacco curly shoot virus (TbCSV) and *Tomato yellow leaf curl Thailand virus* (TYLCTHV) were associated with the disease. They also reported the presence and absence of DNA-β in the four groups.

Maruthi *et al.* (2005) reported the diversity of Tomato leaf curl viruses (ToLCVs) from the two main tomato growing areas of Jessore and Joydebpur, Bangaladesh. *Tomato leaf curl New Delhi virus-Severe* (ToLCNDV-svr), *Tomato leaf curl Joydebpur virus-Mild* (ToLCJV-Mld) and *Tomato leaf curl Bangladesh virus* infecting tomato in Bangladesh was reported by them.

In the year 2005, Reddy *et al.* extracted total DNAs from 69 infected tomato plants and amplified coat protein gene (AV1) from 29 infected tomato plants using coat protein (CP) gene specific primers (CRv301 and CRc1152). Phylogenetic analysis of the CP sequences revealed five different tomato leaf curl begomoviruses (TLCBs) clusters each <88% identity to the others. Four clusters represented known Indian TLCBs whereas, one cluster contained sequence originating from Haryana State with most identity (89%) to the provisional *Begomovirus* species *Croton yellow vein mosaic virus*.

They further characterized 65 begomovirus positive samples by PCR with DNA β, DNA B, four Indian TLCB species specific primers, PALIc 1960/PARIv 722 (universal begomovirus primers) and by sequencing. The majority of samples represented monopartite TLCBs associated with DNA-β components. All four known TLCBs (*Tomato leaf curl Bangalore virus*, *Tomato leaf curl New Delhi virus*, *Tomato leaf curl Gujarat virus* and *Tomato leaf curl Karnataka virus*) appeared to be present throughout India. They found 13 samples with mixed begomovirus infection, a prerequisite for recombination.

Lefeuvre *et al.* (2006) reported a new tomato leaf curl virus from Seychelles archipelago. They used two primer set, (AV494 and AC1048) amplified the core region of the CP gene (approximately 550bp) and (VD360 and CP1266) amplified another fragment representing more than 90% of

the CP gene (approximately 900bp). According to them DNA-B and DNA-β were not associated with the isolate. Analysis of 522 core CP sequences showed between 88% and 89% nucleotide sequence identity with *Tomato leaf curl Mayotte virus* (ToLCMYTV) isolates (AJ865339 and AJ865340).

Maruthi *et al.* (2007) reported that at least five distinct species of tomato leaf curl viruses infected tomato and other host plants in Bangladesh. Phylogenetic analysis of their nucleotide sequences (~530 bases) from the intergenic region and capsid protein of DNA-A indicated the existence of five distinct begomoviruses.

Kumar *et al.* (2008) reported a distinct bipartite begomoviruses associated with tomato plants showing yellowing, curling and crumpling of the leaves from sub-temperate region in India. They used rolling circle amplification (RCA) using φ-29 DNA polymerase to amplify the DNA-A and DNA-B genome. The genome organization of their isolate was typical of an old world bipartite begomovirus and DNA-A showed <89% sequence identity with known begomoviruses. They named it *Tomato leaf curl Palampur virus* (ToLCPV).

Fazeli *et al.* (2009) classified eighteen Iranian begomovirus isolates (from tomato and weeds of Iran) into two major groups and two or three subgroup according to the 5'- proximal 200 nucleotides of the coat protein (CP) gene or the N-terminal 600 nucleotides of the Rep gene. They also indicated that the tomato producing areas in central, southern and southeastern Iran were threatened by begomoviruses originating from both the Mediterranean and the Indian subcontinent.

Pandey *et al.* (2010) described the genetic diversity of two isolates of monopartite Tomato leaf curl virus infecting tomato in two extreme regions (North and South) of Indian subcontinent. According to them the ToLCV-CTM and ToLCV-K3/K5 viruses were found to be monopartite, as neither DNA-B component nor betasatellite associated with begomovirus species. The complete nucleotide sequence of DNA-A genome of CTM exhibited highest sequence homology (88%) to Croton yellow vein mosaic virus

(AJ507777) and isolate K3/K5 (88.5%) to Tomato leaf curl Pakistan virus. This was less than the threshold value for demarcation of species in the genus *Begomovirus*. K3/K5 and CTM are considered to be novel isolates of *Tomato leaf curl virus*.

Andou *et al.* (2010) reported a severe leaf curling and yellowing symptoms of tomato plants on Ishigaki Island using PCR. They detected the presence of DNA-A and DNA satellite component. DNA sequencing analysis of the PCR products revealed that the symptomatic tomato plants were associated with *Ageratum yellow vein virus* (AYVV) infection. They successfully transmitted AYVV from the naturally infected weed host, *Ageratum conyzoides*, to healthy tomato plants by the insect vector *Bemisia tabaci* B biotype. This was the first report of AYVV occurrence in Japan.

Kumari *et al.* (2009) isolated a distinct begomoviruses from tomato plant showing severe leaf curling symptoms from Patna. Full-length genome (GenBank Accession No. EU862323) consisted of 2,752 nt and showed the highest identity (85.5%) with Tomato leaf curl Laos virus-[Laos] (GenBank Accession No. AF195782). The satellite DNA-β component (GenBank Accession No. EU862324) consists of 1349 nt and showed the highest identity (75.8%) with Tomato leaf curl Joydebpur betasatellite (GenBank Accession No. AJ96624). They named the isolate *Tomato leaf curl Patna virus* (ToLCPV) and β satellite as Tomato leaf curl Patna betasatellite (ToLCPaβ).

Anandhan *et al.* (2011) reported leaf curl disease of tomato from Uttarakhand, India and found 90% similarity with other isolates/strains of Tomato leaf curl New Delhi virus on the basis of CP gene sequence (EU847240). A satellite molecule, DNA β of ~1.4kb was also amplified using universal DNA β-specific primers and sequenced (EU847239).

Leaf curl disease of potato: The cultivation of potato is often affected due to attack of various diseases caused by fungi, bacteria, viruses and nematodes (Khurana *et al.*, 1998). The loss in potato yield due to one or more virus(s) infecting potatoes vary from low to very high. Generally severe

mosaic caused by *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV) alone can reduce the yields upto 70-80% while mild viruses, like PVX, PVS, PVM also depress the yields by 10-30% (Bhat *et al.*, 2010).

Interestingly, leaf curl disease of potato has been reported from India and Pakistan (Usharani *et al.,* 2003; Mubin *et al.,* 2009). Leaf curl disease of potato showed typical symptoms of begomoviruses and was reported to be transmitted by whitefly.

Potato plants grown from true seeds were naturally infected with the Solanum apical leaf curling virus (SALCV) at the high jungle site near San Ramon, Peru. Symptoms of SALCV infected potato plants include apical rolling, stunting, delayed emergence, weak plants, hair sprout and tuber dormancy. These symptoms superficially resembled certain aspects of unusually severe potato leaf roll virus infection or those of the aster yellows and stolbur mycoplasma disease. Up to 12 percent of the indexed plants were naturally infected with SALCV and SALCV was demonstrated in approximately half of the tuber that produced hair sprouts (Hooker *et al.*, 1985).

Garg *et al.* (2001) first reported the natural occurrence of geminivirus infecting potatoes in India and the virus is tentatively named as potato apical leaf curl begomovirus. Potato plants showing apical leaf curl and pinkish pigmentation were examined by them for the presence of geminivirus with electron microscope. The Clarified Virus Concentration (CVC) showed presence of a high concentration of geminate virus particles ca. 28X17nm. Though virion did not show trapping with Indian cassava mosaic geminivirus (ICMV) antisera, they exhibited excellent clamping with the antisera in liquid phase immune electron microscopy.

Usharani *et al.* (2003) reported that leaf curl disease in potato in northern India was caused by a strain of *Tomato leaf curl New Delhi virus* (ToLCNDV). Comparison of the complete nucleotide sequence of DNA-A revealed that it had 93-95% identity with that of ToLCNDV isolates and <75% identity with other Tomato leaf curl virus isolates and *Potato yellow*

mosaic virus. It was the first observation of a begomovirus causing a severe disease of potato in India.

Gawande *et al.* (2007) detected *Tomato leaf curl New Delhi virus* (ToLCNDV) causing potato apical leaf curl disease in potato using a simple, quick, economical, sensitive and reliable polymerase chain reaction called print-capture PCR (PC-PCR).

Mubin *et al.* (2009) reported a severe leaf curl disease of potato from Pakisthan and they found full-length begomovirus, betasatellite and alphasatellite molecules. The complete sequence of the begomovirus was 2,754bp (FM179613). Their strain showed 99% nucleotide sequence identity to an isolate of the Pakistan strain of Chili leaf curl virus (ChiLCV-PK[PK:Mul:98];AF336806). The betastellite amplified from potato (FM179615) was 1399bp in length and had the highest nucleotide sequence identity (91.1%) to chili leaf curl disease betasatellite (ChiLCB-[PK:MC:97]; AJ316032). The complete nucleotide sequence of the alphasetellite was determined to be 1369bp (FM179614) and was most closely related to tomato yellow leaf curl china alpha-satellite (TYLCCNA)-[CN:Yn89:02] (AJ579358), with 82% nucleotide sequence identity.

Venkatasalam *et al.* (2011) showed the utility the PCR technique in large scale screening for production of disease-free potato seed tuber. They could detect Tomato leaf curl New Delhi virus-[Potato] (ToLCNDV-[Pot] in most of the samples by PCR.

Mosaic disease of plants and weeds: Sharma *et al.* (2003) reported that yellow vein mosaic diseases (YVMD) became very destructive to the crops in India. In case of early infection the plants become stunted developing very few leaves and fruits. Bansal *et al.* (1984) reported the occurrence of yellow mosaic in different districts of Punjab and its incidence ranged between 1.25% and 20%.

It has also been reported that a number of common weeds serve as alternate hosts as well as reservoirs for many crop-infecting begomoviruses (Roye *et al.*, 1997; Sanz *et al.*, 2000). Several weeds frequently harbor

multiple viruses, resulting to the possibility of emergence of new recombinant strains (Mubin *et al.*, 2010; Umaharan *et al.*, 1998).

Rangaswamy *et al.* (2005) reported the presence of begomovirus infection in *Jatropha curcas* from northern India. The characteristic disease symptoms were chlorotic specks on leaves, curling and malformation of the leaves, severe reduction in leaf size and sterility of the plant.

Roye *et al.* (2005) reported tentative *Jatropha mosaic virus* (JMV) from common weed *Jatropha gossypifolia* with yellow mosaic symptoms from Jamaica.

Narayana *et al.* (2006) reported presence of naturally infected Jatropha plants with symptoms like mosaic, reduced leaf size, leaf distortion, blistering and stunting of diseased plants from Karnataka. The disease was successfully transmitted by graft-inoculation and whitefly transmission. They also reported the association of begomovirus in the diseased plants.

Tewari *et al.* (2007) observed severe mosaic symptoms on a large number of *J. curcas* plants in Uttar Pradesh. They successfully transmitted the disease by sap from the infected plants to healthy one using carborundum powder as an abrasive and also by cleft grafting but not by dodder.

Raj *et al.* (2008) reported the natural occurrence of a mosaic disease on *Jatropha curcas* growing in experimental plots of the National Botanical Research Institute (NBRI), Lucknow, India. They found their isolate was more close to *Indian* and *Sri lankan cassava mosaic virus* rather than *Jatropha mosaic virus.* Snehi *et al.* (2011) reported a severe yellow vein mosaic disease of *Jatropha gossypifolia* from agriculture fields of Lucknow, India. They found DNA A and DNA β satellite from the virus.

Gao *et al.* (2010) reported the complete nucleotide sequence of a Jatropha virus isolated from Dharwad, Southern India. Phylogenetic analysis of the virus suggested that it was a new strain of *Indian cassava mosaic virus*.

Ramkat *et al.* (2011) tested a total of 127 *Jatropha* samples from Ethiopia and Kenya by ELISA for RNA viruses and polymerase chain reaction for geminiviruses. Jatropha samples from 4 different districts in Kenya and Ethiopia (analyzed by ELISA) were negative for all three RNA viruses tested: *Cassava brown streak virus* (CBSV), Cassava common mosaic virus, Cucumber mosaic virus. Their efforts involved the amplification and sequencing of the entire DNA A molecules of 40 Kenyan isolates belonging to *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus*-Uganda.

Saunders *et al.* (2000) demonstrated that yellow vein disease of *A. conyzoides* was resulted from co-infection by DNA A (2,741 nt) of AYVV and a circular DNA β which was approximately half the size (1,347 nt) of DNA A.

Reddy *et al.* (2005) and Amin *et al.* (2002) reported that, the weed *Croton bonplandianum* (family Euphorbiaceae) across southern Asia frequently showed an intense yellow vein mosaic associated with begomoviruses. Briddon and Stanley (2006) reported a croton plant showing vein yellowing symptoms in a cotton field in Faisalabad (Punjab Province, Pakistan). Total nucleic acids were extracted and initial analysis by PCR with universal primers and limited sequencing suggested that the plant was infected with a virus related to *Croton yellow vein mosaic virus* (CYVMV).

Sivalingam *et al.* (2007) collected thirteen weeds and five cultivated crop plants were from Haryana, Punjab and Rajasthan and PCR amplified the viruses present in the plants, using coat protein gene of begomoviruses infecting cotton (CLCBV-CP). Seven weed species (*Croton bonplandianum, Tribulus terrestris, Cucumis* sp., *Solanum* sp., *Jatropha curcas*, wild sunflower and *Xanthium strumarium*) and three cultivated crop plants (chilli, mothbean and cluster bean) were positive for DNA amplification which indicated the presence of begomoviruses.

Xiong *et al.* (2007) reported that, *Ageratum conyzoides* plants exhibiting yellow vein symptoms collected near Haikou, Hainan Province, China, and contained begomoviral DNA-A-like molecules. Sequence alignment showed that AYVCNV has arisen by recombination among viruses related to *Ageratum yellow vein virus, Papaya leaf curl China virus*, and an unidentified *Begomovirus*. They also stated that all plants sampled contained molecules resembling DNA β.

Castillo-Urquiza *et al.* (2008) reported six novel viral species, of which three infecting tomato and three infecting weeds (*Blainvillea rhomboidea*, *Sida rhombifolia* and *Sida micrantha*) that are commonly associated with tomato fields. Six tentative species have also been described based on partial genome sequences.

Hussain *et al.* (2011) reported the complete sequences of a *Begomovirus* and an associated betasatellite, isolated from *Croton bonplandianum* of Pakisthan. The sequence of the begomovirus showed the highest level of nucleotide sequence identity (88.9%) to an isolate of *Papaya leaf curl virus* and thus, represented as a new species, for which they proposed the name *Croton yellow vein virus* (CYVV). The sequence of the betasatellite showed the highest levels of sequence identity (82 to 98.4%) to six sequences in the databases that have yet to be reported, followed by isolates of *Tomato leaf curl Joydebpur betasatellite* (48.7 to 52.5%). This indicated that the betasatellite identified by them (and the six sequences in the databases) was isolates of a newly identified species for which the name *Croton yellow vein mosaic betasatellite* (CroYVMB) was assigned.

Integrated pest management using botanicals: Use of insecticides has resulted in insecticide resistance or reduced susceptibility of the vectors in many cases. Frequent use of insecticides disrupts the ecology as well. Hence, monitoring of chemical use is necessary in agriculture. Studies indicate that aqueous extracts of *Melia azedarach,* botanicals from *Azadiracta indica*, *Trichilia pallida* show absolute control of vector (Rampersad 2003, Widham *et al.,* 1998).

Verma and verma, 1993 reported two fold increase in nodulation and grain yield with 50% per cent reduction in mungbean yellow mosaic incidence when dry leaf powder of *Clerodendron aculatum* was applied as soil drench in addition to six times foliar spray at weekly interval.

Baranwala *et al.* (1997) reported that plant height and tomato yield was higher when *Clerodendron* leaf extract was applied to soil and or sprayed. The symptoms in *Clerodendron acculatum* treated plants were less than untreated plants. The foliar spray combined with soil application was most effective.

Nagaraju *et al.* (1997) reported that among Neemark (5.0%), NSKE (4.0%) leaf extract of *Azadirachta indica*, *Pongamia glabra*, *Ocimum sanctum*, *Vinca rosea*, *Phyllanthus niruri*, *Tagetes errecta* and *Spirocea oleracea* tested for their efficacy in checking transmission of *Pepper vein banding virus*. *S. oleracea* was found to be inhibitory, recording only 25 per cent transmission as against 86.60 per cent on untreated check. The virus concentration was reduced to great extent with less symptom production in *S. oleracea* treatment.

Somashekhara *et al.* (1997) reported that among neem products NSKE (4.0%) induced high whitefly mortality, while neem leaf extract and neem cake powder were ineffective against whiteflies. Further they observed 100 per cent mortality of *B. tabaci* up to eight days in Triazophos (0.15%) mixed with neemark/R.D.9 Replin/ or Aphhidin.

Singh and Awasthi, (2009) designed an experiment to test the efficacy of few botanical extracts on the yellow mosaic of mungbean. Maximum reduction in disease incidence recorded was 66.70 and 63.65 percent in mungbean and Urdbean, respectively by eight sprays of *Clerodendrum aculeatum*. Whereas, eight sprays of *Boerhaavia diffusa* root extract could reduce the disease incidence by 60.27 and 58.20 per cent followed by *Azadirachta indica* leaf extract by 42.43 and 42.92 percent in mungbean and urdbean, respectively.

The results of the experiment of Reddy *et al.* (2010) revealed that the botanicals like *Glyricidia* leaf extract (14.5%), *Clerodendron* leaf extract (13.37%) and also sorghum leaf extract (19.44%) were found effective in reducing the disease incidence and whitefly population activity at early stage of the crop up to 45 days after transplanting.

Singh *et al.* (2011) reported that the mungbean and urdbean crops were suffering from severe yellowing of the leaves due to vector borne virus. They also reported that the crops could be protected by the regular spraying with the aqueous leaf extract of *Azadirachta indica* or *Clerodendrum aculeatum* or the root extracts of the *Boerhaavia diffusa* separately.

From the literature it is evident that the study of begomoviruses needs special attention to control our crops effectively for sustainable production. The in depth knowledge of viruses will definitely help to formulate appropriate control measure to check the viral infestation as well as to check devastation in the fields. The molecular aspect of viruses will help us to differentiate closely related begomoviruses and will also help us to differentiate the virulent and avirulent isolates of the viruses.

2.2 Potyvirus

Potyviruses, a group established in 1959 (Brandes and Wetter, 1959), is the largest of the six genera in the *Potyviridae* family, and the type species is *Potato virus Y* (PVY). It is the largest and one of the most rapid growing of about 78 plant virus genera. Viruses belonging to this genus are responsible for severe disruption to many economically important crops. They are transmitted mechanically and by aphids in a non-persistent manner (Fauquet *et al.*, 2005; Gibbs *et al.*, 2003; Poutaraud *et al.*, 2004; Shukla *et al.*, 1994).

Potyviruses can induce a wide range of different symptoms in infected host plants. A combination of several symptoms is usually found in both natural and experimental hosts (Shukla *et al.,* 1994). In monocotyledonous species, longitudinal chlorotic or necrotic leaf streaks

are common while in dicotyledonous species common symptoms include chlorotic vein banding, mosaic, mottling, necrosis, and/or distortion of leaves are found. The severity of the symptoms depends on particular virus strain, the specific host genotype, vectors and environmental conditions (Matthews, 1991; Shukla *et al.*, 1994; Trigiano *et al.*, 2003).

Potyvirus genome structure and viral encoded protein: *Potyvius* particles are flexuous, filamentous, non-enveloped, rod-shaped, 680-900nm long and 11-13 nm wide. Understanding of the properties of viral genomes and the gene functions is essential for the understanding of the genetic basis of pathogenicity, and for generating effective detection and control strategies.

The *Potyvirus* genome consists of a single plus-stranded, positive sense RNA molecule of ~9.7kb, surrounded by about 2000 units of CP. The 5′ end of the genome carries a viral protein genome linked protein VPg and a poly adenine (poly-A) tail covalently bound to the 3′ end. The genome contains a single long ORF which is translated into a large 340-370 kiloDalton polyprotein and then cleaved by virus-encoded proteinases into ten functional proteins: N-terminal protein (P1 protein), helper component protein (HC-Pro), P3 protein, 6kDa protein 1 (6K1), cytoplasmic inclusion protein (CI), 6kDa protein2 (6K2), nuclear inclusion A linked VPg protein (NIa-VPg), nuclear inclusion a protein A (NI-a), nuclear inclusion protein B(NI-b) and capsid protein (CP) (Riechmann *et al., 1992*; Shukla *et al.*, 1994; Trigiano *et al.*, 2003). Tatineni *et al.*, (2009) reported a second ORF within the P3 coding region. That ORF encode another protein called P3N-Pretty Interesting Potyviridae ORF (PIPO) from a translation frameshift. This second ORF was first identified in TuMV and was then identified by sequence alignment in 48 viruses that represented all genera of *Potyviridae* family.

The study of *Potyvirus* genomes can also provide useful information on conserved and variable regions within genomes which could be used to design efficient oligonucleotides for detecting and distinguishing different potyviruses or strains for RT-PCR and microarray techniques.

Domier *et al.* (1987) predicted the functions of all the proteins contained within the potyvirus polyprotein. But experimental confirmation of the precise role of most proteins was still lacking (Revers *et al.*, 1999). Many potyvirus proteins have multiple functions (Atreya *et al.*, 1992; Berger and Pirone, 1986; Carrington and Freed, 1990; Dombrovsky *et al.*, 2005; Domier *et al.*, 1987; Edwardson *et al.*, 1993; Merits *et al.*, 1998; Riechmann *et al.*, 1992; Rodriguez-Cerezo and Shaw, 1991; Shahabuddin *et al.*, 1988).

The CP gene is best characterized gene in potyviruses. It is divided into three domains: the amino terminus, the core region, and the carboxy terminus. Both the amino and carboxy termini are variable, exposed on the surface of the protein in virus particles, are cleaved by trypsin, and contain the major virus specific epitopes. The CP functions in virus encapsidation and amplification, virus movement both cell-to-cell and long distance, and aphid transmission (Urcuqui-Inchima *et al.*, 2001).

Potyvirus replication

Like all other positive sense single stranded RNA viruses, the replication of potyviruses involves synthesis of negative-sense RNA complementary to the genomic RNA, which is used as template for synthesizing positive-sense progeny MA. Two potyviral proteins that play crucial role in the replication of the viruses are VPg and the NIb. Potyvirus NIb function as the RdRp and first catalyzes uridylylation of the VPg and covalently couples uridine nucleotide to the active site tyrosine residue of the VPg. Such a VPg act as the protein-primer of negative and positive strand RNA synthesis. The helicase activity of the CI might be involved in the unwinding of the double stranded replication intermediates.

The replication of positive-sense RNA viruses such as Potyviruses occurs in two steps both of which are catalysed by the viral RdRp NIb. In the first step, minus strand is synthesized which acts as a template to direct synthesis of genomic RNAs and in second step, positive sense genomic RNA molecule are synthesized (Whitham and Wang, 2004).

The VPg has been shown to interact with the viral 3'-end sequences to prime the negative strand RNA synthesis suggesting that VPg could be a putative primer for Potyvirus replication (Miyoshi *et al.*, 2006).

Replication occurs in replication complexes (RC) and requires a membranous environment. The source organelle for membrane can be the endoplasmic reticulum (ER), mitochondria or lysosome (Schaad *et al.*, 1997).

Since, the potyviral genome is positive sense; it must be replicated into a negative sense orientation which then acts as the template for positive sense genome synthesis. The RdRP cannot make nucleic acid *de novo*, it requires a primer-type structure to initiate synthesis (Teycheney *et al.*, 2000). It has been observed that the urydylylated VPg may act as the primer for the viral RNA synthesis.

Mathioudakis *et al.* (2012) reported that CP showed interaction of PepMV CP with HSP70 protein in tomato infected with *Pepino mosaic virus* (PepMV). CP has been found to be inhibitory to potyvirus replication at the early stage of infection. Therefore a J-domain protein called CPIP and HSP70 together have been shown to facilitate a degradation of CP to prevent early cessation of replication (Nagy *et al.*, 2011).

Virus movement

Proteins implicated in potyviral cell-to-cell movement include helper component-protease (HC-Pro) [Shen *et al.*, 2010], coat protein (CP) (Dolja *et al.*, 1994, 1995; Rojas *et al.*, 1997, Hofius *et al.*, 2007; Rodriguez-Cerezo *et al.*, 1997)], genome-linked protein (VPg) (Dunoyer *et al.*, 2004), and cylindrical inclusion protein (CI) (Rodriguez-Cerezo *et al.*, 1997; Carrington *et al.*, 1998; Roberts *et al.*, 1998). Indirect evidence suggests P3N-PIPO is also required for efficient cell-to-cell movement of the virus but not for RNA replication.

Criteria for species demarcation

Virus taxonomy is a complex task and the ICTV is the organization responsible to “categorize the multitude of known viruses into a single classification scheme that reflects their evolutionary relationships” (Khan and Dijkstra, 2002). In the 6th ICTV report (Murphy *et al.*, 1995),

previously established “plant virus groups” were given “genera” status and individual viruses listed as species within the genera.

Previous classification schemes were based almost entirely on phenotypic properties such as host range, symptomatology and serology often making it difficult to distinguish species and strains (Francki *et al.*, 1996; Noordam, 1973; Padidam *et al.*, 1995; Torrance and Mayo, 1997) and consequently sequence data has become increasingly important for virus classification (Adams *et al.*, 2005). Two key criteria for *Potyvirus* species demarcation in the 8th ICTV report (Fauquet *et al.*, 2005) are described as: (1) CP aa sequence similarity less than ca. 80%; (2) nt sequence identity of less than 85% over the whole genome. The report listed 111 definite *Potyvirus* species and 86 tentative species.

“Molecular criteria for genus and species discrimination within the family *Potyviridae*” was investigated by Adams *et al.* (2005). Complete sequences (187 numbers) belonging to the family *Potyviridae* and different individual genes including P1, CI and CP were used for the study. The author concluded that species demarcation criteria should be less than 76% nt identity and less than 82% aa similarity.

Mosaic disease of papaya, bean and potato:

Papaya ring spot virus (PRSV) infects all the stages of papaya plants. Leaves exhibit variety of peculiar symptoms depending upon the age of the plant and environmental conditions. The initial symptoms begin with light discolouration of leaves turning towards pale yellow. Mild mosaic, mosaic, mottling, chlorotic spots, chlorotic rings, vein clearing, leaf curling, blisters, leaf distortion, shoe string formation in leaves, pale oily greasy streaks on stem and ringspots are prominently seen on leaves, fruits, stem and hence the virus named as ringspot virus.

Papaya ring spot virus infects papaya in all the stages of its growth exhibiting a variety of symptoms based on age of the plant and environmental conditions. Cucurbits are also vulnerable to the infection of PRSV exhibiting a versatile nature of symptoms.

In field-collected samples of *Cucurbita maxima* a mild mosaic symptom was recorded which was confirmed as PRSV using DIBA

(Somowiyarjo, 1993). In contrast to this a strain of PRSV isolated from Taiwan was found to cause more severe symptoms than usual in cucurbits (Prowidentii, 1996). Dahal *et al.* (1997) also observed severe mosaic leaf distortions, blisters and shoestring on squash, while mosaic or yellow mosaic, leaf distortion and blisters were recorded on other cucurbits infected by PRSV. They also reported the 75 to 100 incidence of PRSV on papaya from Terai and inner Terai districts of Nepal.

Papaya Ring Spot Virus (PRSV) has been reported from every continent, wherever papaya is grown. Of the total 12 viruses reported on papaya, PRSV is the most destructive and has been the major constraint and limiting factor for its cultivation in all tropical and subtropical regions. In India the first report of PRSV was made by Capoor and Verma (1948) as papaya mosaic, subsequently it was reported in Bihar by Mishra and Jha (1955) as mosaic, from Madhya Pradesh by Garga (1963), Later Surekha *et al.* (1977) recorded incidence of PRSV in Udaipur of Rajasthan. From Marathwada region of Maharastra, Yemewar and Mali (1980) reported severe incidence of PRSV, from Punjab by Cheema and Reddy (1985) as papaya mosaic virus, from Andhra Pradesh by Susan John (1985) as papaya mosaic. Later Ramakrishna (1988) from Maharashtra stated that the virus causing mosaic, leaf distortion, shoe stringing and rings on fruits of papaya was PRSV – P, a member of potyvirus group. In Karnataka Byadgi *et al.* (1995) recorded a severe incidence of PRSV from northern parts of the state, later Shaikh (1996) and Hegde (1998) recorded the incidence of PRSV from Dharwad and Belgum districts of Karnataka, respectively. Jain *et al.* (2004) reported the presence of PRSV-P from the infected papaya plants cultivated in Bardhman district of West Bengal.

Khurana and Bhargava (1970) observed 75 – 100 percent incidence of PRSV in and around Ghorakhpur district of Uttar Pradesh. Singh *et al.* (2003) recorded the incidence of PRSV in every district of eastern Uttar Pradesh which ranged between 48 to 100 per cent.

Bean common mosaic virus (BCMV) (genus: *Potyviru*s, family: Potyviridae) is economically the most important virus infecting bean crops worldwide. It is aphid transmitted and seed-borne virus and represents a

complex of virus strains (Khan *et al.*, 1993, Mink *et al.*, 1994). BCMV is a monopartite flexuous rod shaped with positive sense ssRNA genome of about 10kb size. Earlier, identification and strain differentiation of BCMV were done based on their response on a series of *Phaseolus vulgaris* host differential cultivars (Drifhout, 1978). Later, on the basis of serological reactivity, isolates/strains of BCMV were divided into serogroups A and B (Vetten *et al.*, 1992). Phylogenetic analysis has established that viruses from the two different serotypes were actually belonged to two different species. Members of serotype A were placed in the species, Bean common mosaic necrosis virus (BCMNV) and members of serotype B were placed in the species, BCMV (Berger *et al.*, 1997).

The occurrence of BCMV on common bean has been reported from India by several authors (Muniyapa, 1976; Gupta and Chowla, 1990; Kapil *et al.*, 2011). Saqib *et al.*, (2005) reported the presence of BCMV in Western Australia by using universal potyvirus specific primers.

Potato virus Y (PVY) is a type member of the Potyvirus group, the largest group of plant viruses. It is a flexuous, helical, rod-shaped virus of approximately 700nm length, 12nm diameter and 3.4nm helical pitch. It has a single-stranded positive sense RNA genome of approximately 10kb length, encapsidated by about 2000 copies of a single coat protein of approximately 30kD size (Shukla *et al.*, 1994; Maki-Valkama, 2000; Syller, 2006; Urcaqui-Inchima *et al.*, 2001; Scholthof *et al.*, 2011). PVY is reported to be the most important potato virus and responsible for the major yield losses in India amongst the other potato viruses (Khurana *et al.*, 1987). Classically, PVY has been grouped into three main clusters of parental strains, PVY^{O}, PVY^{N} and PVY^{C} based on the host hypersensitive response, genome sequence and/or serological properties (Singh *et al.*, 2008).

The detection of PVY by using polymerase chain reaction has been reported by Singh (1998), Singh (1999) and Fakhrabad *et al.* (2012). Ghosh and Bapat (2006) used RT-PCR method to detect PVY in tobacco and potato using CP gene specific primers and reported that RT-PCR is more efficient

method of virus detection. Volkov *et al.* (2009) detected PVY in potato and wild plants of far-east Russia by RT-PCR technique.

Sharma *et al.* (2011) reported that coat protein region is unable to delineate the strain of the same viruses but can clearly distinguish different viruses of the family *Potyviridae*.

3. Materials and Methods

3.1 Plant Materials

3.1.1 Tomato plants

Collection of infected plants: Tomato plants showing chlorosis, leaf curling, distortion, yellowing and/or stunted growth were collected from the tomato growing fields across sub-Himalayan plains of northeast-India. More than 50 collection spots were demarcated for collection of samples.

Maintenance of infected plants: Some infected plants were selected on the basis of severity of the symptoms and were uprooted from the fields with some soil attached to their roots. The uprooted plants were immediately placed in plastic pots containing prepared soil. All such pots were covered with mosquito net specially prepared to cover each pot. All the pots thus procured were labeled and were maintained in a net house (specially built for keeping the infected plants) in the experimental garden of the Department of Botany, University of North Bengal.

Collection of healthy plants and seeds: Seeds of Pusa Ruby variety (Susceptible to ToLCD) of tomato were procured from seed sellers of Bagdogra, Siliguri.

Maintenance of the plants: Collected tomato seeds were sown about 1.25 cm deep in seed-bed (either on earthen tray or directly on soil). Seedlings (approximately 10 cm long) of tomato plants from the earthen tray were finally transferred to earthen pots (16 cms in diameter and 12 cms in height) filled with soil-farm yard manure mixture 9:1 (100g of farm yard manure were mixed with 900 g of fine soil). Plants grown directly on soil were also transferred to experimental plots. The plants were maintained in the garden at natutal temperature (25^0C-30^0C) and light. pH of soil was about 5.6.

Optimum moist condition required for tomato seed germination was maintained to avoid damping off and other diseases. All the plants were watered as and when required to keep optimum moist condition.

3.1.2 Potato plants

Collection of infected plants: Severe leaf curl disease of potato was found in the cultivated potato fields of sub-Himalayan West Bengal. The affected plants were severely stunted with apical leaf curl and crinkled leaves and also showed typical conspicuous mosaic typical of *Begomovirus* infection. Samples from different regions of sub-Himalayan West Bengal were collected and immediately brought to the laboratory.

Maintenance of infected plants: Some infected plants were transferred to the experimental garden following the procedure as stated in case of tomato plants in section (3.1.1). Pots with infected plants were covered with mosquito net specially prepared to cover the pot. All the pots thus procured were labeled and were maintained in a separate net house (specially built for keeping the infected plants) in the experimental garden of Department of Botany of University of North Bengal.

Collection of healthy plant propagules: Potato tubers were collected from from Leuchipukri Krishi Bhander, Siliguri. Kufri Jyoti variety was selected on the basis of its year round production and susceptibility to viral diseases.

Maintenance of plants: Potato tubers of the Kufri Jyoti variety were sown in experimental plots (size: 1 m long, 1m wide and 10 cm high) as well as in pots (16 cms in diameter and 12 cms in height). For potato plants experimental plots and pots were filled with soil-farm yard manure mixture 9:1 (100g of farm yard manure were mixed with 900 g of fine soil). Pots were of same size

The plants in pots were grown under natural conditions of daylight and temperature (15-25^0 C) during winter and maintained throughout the growing season. The plants were watered regularly with ordinary tap water to avoid drying of soil. For maintenance of plants (both infected and healthy) in the experimental garden each pot was supplemented with 120-150 gm N, 45 gm P_20_5 and 100 gm K_2O.

3.1.3 Papaya plants

Collection of infected plants: Small infected plants showing symptoms like severe mosaic, ring spot symptom on the fruit, severe leaf deformation and/or stunted plant growth, were collected from the papaya growing fields of sub-Himalayan West Bengal.

Some infected plants were collected and were sown in the pots (16 cms in diameter and 12 cm in height). All the plants were covered with mosquito net. All the pots thus procured were labeled and were maintained in a net house (specially built for keeping the infected plants) in the experimental garden of Department of Botany, University of North Bengal.

Collection of healthy plants: Seeds of papaya plants of Honeydew variety were collected from Matigara, Siliguri.

Maintenance of papaya plants: The seedlings were raised in nursery beds 3m long, 1m wide and 10 cm high as well as in pots (16 cm in diameter and 12 cm in height). The seeds were treated with 0.1% Monosan (phenyl mercuric acetate) and were sown at 1 cm depth and in rows (10 cm apart). The nursery beds were covered with polythene sheets to protect the seedlings. About 15cm tall seedlings were transferred to pots containing sand, soil and compost in 2:2:1 ratio (w/w). All the pots were of same size.

3.1.4 Collection of other infected plants and weeds

Bean and potato plants showing severe mosaic symptoms were collected from the different cultivated areas of sub-Himalayan West Bengal. *Datura stramonium* and *Jatropha curcas* plants showing chlorosis, leaf curling, distortion, yellowing and stunted growth and *Croton bonplandianum*, *Acalypha indica* and *Ageratum conyzoides* showing typical begomovirus infecting symptoms of yellowing of the vain and mosaic were collected from the sub-Himalayan plains of East-India. Some infected plants were collected and maintained in the experimental garden.

3.1.5 Collection and maintenance of plants used in host range study

Plants like *Cucumis sativus*, *Lycopersicon esculentum* (cv. Pusa Ruby), *Capsicum annum* (cv. Akasmoni) and *Phaseolus vulgaris* (cv. Improved Tender Green), *Nicotiana rustica* (cv. Hookah), *Pisum sativum* and *Solanum tuberosum*, *Cucurbita pepo, Solanum melongena* and *Brassica oleracea* var. *botrytis* were collected from different nurseries of Siliguri and were used for virus transmission and host range studies.

3.2 Preservation of samples (leaves) for experiments

Leaves of the infected plants were cut with sterile blade to avoid contamination. Cut leaves were poured in zip packs and marked according to the area and date. Leaves were then washed with running distilled water to avoid any trace of dust. Similarly, healthy leaves were also collected. Washed leaves were dried with blotting paper and weighed. Dried leaves were packed in a fresh air tight plastic pack and stored at -20˚C for future experiments.

3.3 Insect transmission of virus

Collection and maintenance of whiteflies: White flies are abundant in the infected fields of tomato and potato. Whenever any disturbance is faced by the whiteflies, they come out of bush and try to fly in the upward direction. Hence, a trap was prepared to collect the white flies suggested by Ssekyewa (2006) and Reddy (2006). The trap was simply a plastic jar whose mouth is tied with a piece of mosquito net and the base of the jar is open. The trap was placed on the infected site and as soon as white flies starts moving inside the trap, another piece of mosquito net is placed at the base of the trap-jar and tied with thread. Thus collected whiteflies were carried to the experimental garden and released in some tomato plants dedicated to maintain the whiteflies. These white flies were used for experimental transmission of tomato leaf curl virus as and when required.

Rearing cage for whitefly: A wooden cage (45 x 45 x 30 cm) was constructed and muslin cloth was fixed on three sides with adhesive (fevicol) and the top and the front was covered with glass. The front glass

can be easily moved on the grooves made in the wooden framework. This frame was kept on the wooden rectangular base. In each cage, healthy pumpkin plants were grown in polyethylene bags and were used for maintenance of whiteflies.

Cages used for acquisition of virus by the insects: The 20 cm long plastic bottle with 7.5 cm diameter at one end and tapering towards the narrow mouth was used to prepare cages to ToLCV acquisitions. The bottom portion of such bottles was removed with the help of a sharp knife and was covered with muslin cloth. The whiteflies were collected into the bottle and the virus infected tomato branch was inserted into the bottle through the narrow mouth and then closed with cotton plug. In all the experiments 24 hours acquisition access feeding period was given. After the acquisition access period, the viruliferous whiteflies were taken and were used for inoculation.

Cages used for inoculation of seedlings: Plastic bottles of 15.0 cm long with 5.0 cm diameter and also plastic vials of 7 cm long with 3 cm diameter were taken. With the help of sharp knife black muslin cloth was fixed to the top portion with the help of adhesive (fevicol), which helped to avoid accumulation of excess moisture inside the cage and also prevents escape of whiteflies. A small grove of about 0.6 cm was made on the cages, which helped to release the viruliferous whiteflies into the cages placed over the young seedling. For grown up seedlings / bigger sized cages were used or cages were plugged with cotton after inserting the young leaflets into the tube and the cages were tied to bamboo sticks with a rubber band.

Transmission by whitefly and symptom development: Whiteflies were collected by a modified trap as suggested by Ssekyewa (2006) from infected fields of tomato. Collected whiteflies were released in some healthy tomato plants and were checked for virus infectivity. If the tomato plants were found without symptoms, even after 20 days, then the flies were considered virus free (Essam El-Din *et al.,* 2004).Then the flies were maintained in pumpkin plants in a separate net house. These white flies were used for experimental transmission of tomato leaf curl virus as and when required.

Virus-free white flies were used as vectors in transmission experiment and insect transmission was done following the techniques as described by Ghanem *et al.* (2001). About twenty insects were allowed to feed on infected tomato plants in an insect proof cage for 24 hours (acquisition access period). After 24 hours all the 20 insects were transferred to a healthy plant, duly covered by a mosquito net. The insects were allowed to feed for 72 hours on healthy tomato plants. After 72 hours all the insects were removed carefully from the plant by shaking the plants and the insect-free plants were left for symptom development up to sixty days. Generally, symptoms start appearing after 20 days but severe symptom development was found after 30 days.

3.4 Mechanical transmission

Mechanical transmission of *Tomato leaf curl virus*: Leaves of the infected plants (tomato) were crushed in 0.1 M potassium phosphate buffer, pH 7.0 (1:2, w/v) containing 0.15% sodium sulphite following the method of Chakraborty *et al.* (2003). The resultant sap was used for inoculation of the healthy plants in the following way. Ten different plants were selected for transmission test. Initially, the young leaves of test plants were dusted with carborundum powder (400mesh). A cotton-wrapped stick was soaked in the sap-inoculums and then rubbed on the leaves where carborundum was previously applied. Inoculated plants were kept in net house up to 60 days with proper care for symptom development. The visible symptoms appeared following artificial transmission experiment confirmed the success of mechanical transmission of the virus. However, the presence of virus was further confirmed by PCR amplification of the viral coat protein from the infected leaves of the test plants.

Limited host range study of *Tomato leaf curl virus*: Generally viruses survive in a wide host range and cause disease in one or two most preferable host known as target host. The host range test of tomato leaf curl virus was performed in ten different cultivated plants growing in and around tomato growing fields of north Bengal and Brahmaputra valley of Assam. The plants were of four families such as Solanaceae, Fabaceae, Cucurbitaceae and Brassicaceae). The test was performed by two different

ways (Insect transmission and mechanical transmission). External symptoms were observed for a long period of time (up to 60 days). The hosts which showed symptoms were subjected to PCR amplifications for confirmation of the presence of virus.

Mechanical sap inoculation of *Papaya ringspot virus*: Mechanical sap inoculation of papaya ring spot virus to healthy papaya and pumpkin plants was done following the method of Reddy *et al.* (2007). Potassium phosphate buffer (0.1M, pH 7.0) was used for the mechanical inoculation of papaya ring spot virus to the test plants. Young tender leaves from the infected papaya plants showing good symptoms were collected, washed thoroughly under tap water to remove dirt and blotted dry. The leaf samples were pulverized in a clean sterilized mortar and pestle by adding chilled buffer at 1 ml per gram of leaf tissue. After through maceration, the pulp was squeezed through sterile absorbent cotton or muslin cloth. The resultant extract was used as "standard inoculums" for mechanical sap inoculation.

Young healthy, vigorously growing test plant seedlings (papaya and pumpkin) grown from healthy seeds and maintained in insect proof glass house were selected. A pinch of celite (600 mesh) was added to the standard inoculum before inoculation as an abrasive. A small piece of sterilized absorbent cotton wool soaked in the standard inoculum was gently rubbed over the upper surface of the leaves, gently and unidirectionally. The plants were labeled and kept under observation for symptom expression up to 60 days.

3.5 Electron microscopic study

Preparation of sample: Crude tissue extract of the samples (papaya and bean) were treated following the Brandes 'Dip method' with certain modifications. Details of the procedure are as follows. Infected leaves from papaya and bean plants were grind with 0.1M phosphate buffer pH 7.2 in a mortar & pestle. Initially a small amount of buffer was poured in the mortar and pestle and the leaves were crushed to paste. Finally buffer (1ml/g of fresh tissue) was added to the tissue homogenate and mixed thoroughly. One drop of the resultant homogenate was mixed with a drop

of 2% PTA (pH 7.0). Twenty microlitre drop of the mixture was placed on a copper coated carbon grid for 30 sec. The drop was socked with a filter paper strip and the grid was allowed to air dry.

Electronmicroscopic photography: The prepared grid was examined under JEOL JEM-2010 electron microscope Indian Association for Cultivation of Science, Kolkata and photographs were taken at suitable magnification.

3.6 Molecular detection

Extraction of total DNA of virus infected plants: Total DNA was extracted from the Infected and healthy plants. Cetyl trimethyl ammonium bromide (CTAB) method (Dellaporta *et al.*, 1983) modified by Sharma *et al.*, (2003) was followed for extraction of DNA. One gram of plant tissue was dipped in 5 ml of alcohol and after 30 minute alcohol was evaporated. CTAB-DNA extraction buffer was prepared by mixing Tris-100mM [pH8], 20mM EDTA [pH8], 1.4M NaCl, 2% CTAB and 2µl/ml β-mercaptoethanol. The tissue was then homogenized with pre-warmed (at 60˚C) 2% CTAB-DNA extraction buffer. The resultant homogenate was incubated at 60˚C for 1h, in a water bath. During incubation, occasionally the homogenate was mixed by gentle swirling. After incubation, homogenate was removed from the water bath and 0.6 volume of chloroform-isoamylalcohol (24:1) was added. The homogenate was mixed thoroughly by inversion of the up side down and again down side up. After 15 minutes the mixture was centrifuged at 15,000 rpm for 10 min. Aqueous phase was transferred to another tube and DNA was precipitated by using 0.6 volume of isopropanol. DNA was subjected to formation of pellet at 12,000 rpm in 1.5 ml centrifuge tubes. DNA was washed with 70% ethyl alcohol and dried overnight. Dried DNA was dissolved in $T_{10}E_1$ buffer [pH 8]. RNAse (2.5 µl) was added to 0.5 µl of crude DNA and mixed gently and incubated at 37˚C for 1h. After this 0.3 ml of chloroform-isoamylalcohol (24:1) was added and was mixed thoroughly. Finally the resultant mixture was centrifuged for 15 min at 15,000 rpm. Supernatant was removed except the white interface layer. DNA was reprecipitated from the supernatant by mixing absolute alcohol (supernatant: Alcohol:: 1:2). DNA was pelleted by centrifugation

and then washed with 70% alcohol. Dried DNA was redissolved in $T_{10}E_1$ buffer [pH 8] and stored at -20˚C for further use. All extracted DNAs were diluted 10-fold in sterile distilled deionised water before amplification through PCR as recommended by Reddy *et al.* (2005).

Agarose gel electrophoresis of extracted DNA: The frame of the gel-casting unit was cleaned and sealed with a tape to form a mould. The frame was placed on a flat platform to ensure a flat and level base; the comb was then positioned parallel to the open edge of the frame about 2 mm above the surface. Agarose powder was added to TAE buffer (1X) and was dissolved by melting at 100ºC, the solution was cooled to 50ºC; about 25 µl of the ethidium bromide (0.5 µg/ml final concentration) was added to the gel and then poured into the gel frame and allowed to set. After setting the gel, it was transferred to the gel tank such that the wells were towards the negative pole. The gel tank was filled with TAE buffer just enough to cover the surface of the gel. 10 µl of the PCR product was mixed with 5 µl of (6X) loading dye and loaded to the wells of submerged gel along with marker DNA (Lambda DNA, *EcoR I* and *Hind III* double digest, MBI, Fermentas) using a micropipette. The electrophoresis apparatus connected to the power supply and electrophoresis was carried out at 50 V for 45 minutes or up to deep blue dye migrated to the reaches three-fourth of the gel. It was then visualized on UV transilluminator and photographs were taken by Nikon D3000 camera.

Extraction of RNA: Total RNA from the healthy and infected papaya, bean and potato plants was extracted using GeneiPure Total RNA extraction Kit. Plant sample (100mg) stored at -20ºC was used for this purpose. The leaf samples were placed in pre-chilled autoclaved mortar and pestle and small volume of liquid nitrogen was added and the samples were grind to a fine powder. 600 µl lysis buffer containing 6 µl of β-mercaptoethanol were added to the powder and mixed thoroughly to form homogenous lysate and transferred to fresh 1.5 ml microcentrifuge tubes. The lysates were cleared by centrifugation at 10,000 rpm at room temperature for 3 minutes and the pellets were discarded. The cell lysates were then filtrated through filtration column by centrifugation for 1 minute

at 10,000 rpm at room temperature and flow through were collected to a new 1.5 ml microcentrifuge tubes. 300 µl of absolute ethanol was added to the flow through (lysate) and mixed immediately by repeated pipetting. The lysate was transferred to the GeneiPure™ RNA column placed in a 2 ml collection tube and centrifuged for 1 minute at 10,000 rpm at room temperature. Then the column was washed with Wash buffer I followed by centrifugation at 10,000rpm for 15 seconds and Wash buffer II followed by centrifugation at 10,000rpm for 15 seconds). Extracted RNA was eluted by using 50µl elution buffer and eluted RNA was stored at -20°C for further use.

Agarose gel electrophoresis of extracted RNA: Agarose gel electrophoresis was done using the Genei RNA gel electrophoresis kit (Genei, Bangalore) for visualization of extracted RNA. 4-5 µl extracted RNA was run on agarose gel in 1X MOPS buffer. 2 µl of 0.5mg/ml ethidium bromide was added to the RNA along with 14 µl RNA sample buffer. The gel was run at 50V till the dye front bromophenol blue reaches three-fourth of the gel.

cDNA synthasis: cDNA was synthesized from 9µg RNA with M-MuLV RT-PCR kit (Genei, Bangalore). Oligo (dT) $_{18}$ primer and M-MuLV reverse transcriptase were used in this purpose. The product was directly used for PCR amplification within 2 hours.

Primers used: The diseased samples, which showed leaf curl symptoms, were used for DNA extraction. From the extracted DNA, amplification of begomovirus specific DNA sequence was done using PCR amplification technique. The primers as proposed by Reddy *et al.* (2005) were used in the present study. Four, different primer pairs were used in the study. DengA/DengB primer pair which targets a ~530bp region that start at the DNA-A origin of replication and continues to the 5' end of the AV1 (coat protein) gene was used. CRV301 and CRC1152 which amplify the coat protein gene of all mono- and bipartite Indian TLCB DNA-A sequences (available in GenBank) were used as second primer pair. To amplify from position '722' within CP gene through the AC2 and AC3 gene to position ~1960, the third primer pair PALIc1960 and PARIv722 was

used. Fourth primer pair BF518 and BR16141were used to amplify the partial DNA-B sequence (BV1 and BC1 gene) as reported by Reddy *et al.* (2005). Primers PALIv1978 and PARIc496 were used to amplify AC1 ORF and AV1 ORF (Rojas *et al.*, 1993). AV494 and AC1048 were also used in the present study which amplify core coat protein gene of all mono- and bipartite Begomovirus DNA-A sequences available in GenBank (Wyatt and Brown, 1996). 'CP9502' specific for 3' end of *Potyvirus* genomes and 'CPuP' which pairs with the coat protein region of *Potyvirus* genomes primer pair as described by Singh *et al.* (2007) was used for *Potyvirus* amplification. All the primer pairs with their sequence data and product size have been shown in Table 1.

Table1: Primers used for DNA amplification and sequencing

Primer name	Sequence data[a]	Product size (bp)
Deng universal primers Deng A Deng B	5'TAATATTACCKGWKGVCCSC3' 5'TGGACYTTRCAWGGBCCTTCACA3'	~530
CP primers CRv301 Crc1152	5'ATGKCSAAGCGWCCRGCAGA3' 5'TTWARAATGTAAWWKGAGCAG3'	~870
Rojas universal primers PALIc1960 PARIv722	5'ACNGGNAARACNATGTGGGC3' 5'GGNAARATHTGGATGGA3'	~1280
Rojas universal primers PALIv1978 PARIc496	5'GCATCTGCAGGCCCACATYGTCTTYCCNGT3' 5'AATACTGCAGGGCTTYCTRTACATRGG3'	~1300
Core coat protein primers AV494 AC1048	5'GCYATRAGRAAGCCMAG3' 5'GGRTTDGARGCATGHGTACATG3'	~570
DNA-B primers BF518 BR16141	5'GAGTTTCCGYTTGTGGAAGWCCCGGAAGT3' 5'YTKGAAYTTYGGTCTGTKG3'	~1120
DNA-β primers Beta01 Beta02	5'GGTACCACTACGCTACGCAGCAGCC3' 5'GGTACCTACCCTCCCAGGGGTACAC3'	~1350
Potyvirus primers CPuP CP9502	5'TGAGGATCCTGGTGYATHGARAAYGG3' 5'GCGGATCCTTTTTTTTTTTTTTTT3'	~650

[a]Degeneracy N=A+C+G+T, R=A+G, H=A+T+C, K=G+T, W=A+T, V=A+C+G, S=C+G, B=G+T+C, Y=C+T, M=A+C, D=A+T+G

PCR amplification using *Begomovirus* specific primers: Polymerase chain reactions (PCRs) were performed in 25 µl reaction mixtures containing 2 µl of template DNA, 0.5 units of *Taq* polymerase (Genie, Bangalore), 1.5mM $MgCl_2$, 0.2mM dNTPs, 1 pmol of each forward and reverse primers in 1X reaction buffer (*Taq* buffer B). The amplification was carried out using a Gene Amp 2400 thermal cycler PCR system (Perkin Elmer).

Amplification programme consisted of one initial cycle of denaturation at 94˚C for 1 min, annealing at 52˚C for 1min 30sec and extension at 72˚C for 2min. After the initial cycle, 40 cycles of PCR were performed of denaturation (94˚C for 50sec), annealing (at 52˚C for 45sec) and extension (at 72˚C for 1min 30sec). After that amplification programme was continued for 10 minutes at 72˚C.

Agarose gel electrophoresis: Amplified products were electrophoresed at 5V/cm through 1.5% (w/v) agarose gels in 1X Tris Acetic acid EDTA (TAE) electrophoresis buffer and was visualized under UV transilluminator following ethidium bromide staining of the gel (30min in 0.5mg/ml ethidium bromide). The molecular weight of the amplified products was calculated using standard molecular weight DNA markers.

PCR amplification using *Potyvirus* specific primers and Agarose gel electrophoresis: PCR amplification was done following the method of Singh *et al.* (2007) using 'CP9502' specific for 3' end of *Potyvirus* genomes and 'CPuP' which pair with the coat protein region of *Potyvirus* genomes primer pair. The PCR mixture (25 µl) contained 2 µl cDNA, 0.2 µg of each primer, 10X PCR buffer, 10mM dNTP mix and 1.5U *Taq* polymerase (Genei, Bangalore). The mixture was heated at 94°C for 2 min, followed by 35 cycle of 94°C for 1 min, 54°C for 2 min and 72°C for 1 min and finally, 1 cycle of 72°C for 10 min.

Amplification products were electrophoresed at 5V/cm through 1.5% (w/v) agarose gels in 1X Tris Acetic acid EDTA (TAE) electrophoresis buffer, and visualized under UV transilluminator following ethidium bromide staining of the gel (30min in 1 µg/ml ethidium bromide).

Cloning of PCR products: The PCR amplified DNA fragment was cloned into the pGEM-T vector using pGEM-T easy cloning kit (Promega, USA) following the manufacturer's protocol. The T/A cloning method was suitable for cloning of PCR fragments amplified with primers that carry dG or dC at their 5' ends. The pGEM-T vector was prepared by cutting the pGEM-T easy Vector with EcoRV and adding a 3' terminal thymidine to both ends. These single 3'-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases. The high-copy number pGEM-T contains T7 and SP6 RNA polymerase promoters flanking, a multiple cloning region within the α-peptide coading region of enzyme β-galactosidase. Insertation activation of the α-peptide allows recombinant clones to be directly identified by colour screening on indicator plates.

Preparation of Competent Cells for Transformation: The component cells of *Escherichia coli* strain JM109 were prepared by calcium chloride method as described by Sambrook and Russel, (2001). JM109 cells were grown overnight on the LB media and single colony of this culture was inoculated into 30 ml of LB broth. The cells were grown at 37°C on a rotary shaker at 100 rpm till Optical Density reached 0.3 to 0.4 at 600nm. They were then aseptically transferred to a sterile chilled centrifuge tube and were immersed in ice for 15 min. Then the cells were pelleted by centrifugation at 5000 rpm for 5 min. at 4°C. The pelleted cells were resuspended in 15 ml ice cold 0.1 M CaCl2 and incubated on ice for 30 min. The cells were again recovered by centrifugation at 5000 rpm for five minutes at 4°C. The pellet obtained was resuspended in 6 ml of 0.1M CaCl2 and one ml of 100 per cent glycerol. The cells were then stored in chilled 1.5 ml microfuge tubes in aliquots of 200 µl and quick frozen by keeping it in 0°C.

Preperation of ligation mixture: Ligation reaction was set up using pGEM-T Easy vector and 2X rapid ligation buffer. In 0.5 ml PCR tubes 5 µl 2X rapid ligation buffer, 1 µl pGEM-T Easy vector, 2 µl purified PCR product and 1 µl T4 ligase were added. The final volume was adjusted to 10

µl by adding deionized water. For control set, 2 µl control insert DNA was used instead of purified PCR product. All the tubes were incubated overnight at 4°C.

Transformation: Then 2 µl of ligation mixture was added to each of the sterile 1.5 ml microcentrifuge tube on ice and 50 µl competent cells were transferred carefully in to each tube. The tubes were flicked gently to mix and were placed on ice for 20 minutes. The cells were subjected to heat-shock for 45-50 seconds in a water bath at exactly 42°C. After that they were immediately returned to ice for 2 minutes. 950 µl LB broth was added to the tubes containing cells transformed with ligation reactions and 900 µl to the tubes containing cells transformed with control insert DNA at room temperature and incubated for 1.5 hours at 37°C with shaking (~150rpm). After that 100 µl of each transformation culture were plated onto duplicate LB/amplicillin/IPTG/X-Gal plates. The plates were incubated overnight (16-24 hour) at 37 °C followed by overnight incubation at 4 °C to facilitate blue colour development. The vector contains the ampicillin resistant gene for antibiotic selection and *lac*Z gene that allowed blue/white selection of recombinant colonies by a α-complementation.

Screening of clones: White colonies were screened for positive insert and were grown in LB plates containing ampicillin, IPTG and X-Gal. PCR was carried out from the grown colony followed by boiling lysis using specific primers to confirm the colony carrying the insert.

3.7 Sequencing of the cloned insert

After successful confirmation of the presence of expected insert in the clone, the plates were directly sent for sequencing. The sequencing was done from automated DNA sequencing service from Genei, Bangalore and from Eurofins Genomics India Pvt Ltd. Sequencing was done in both directions using SP6 forward and T7 reverse primers.

3.8 Submission of sequence and Phylogenetic analysis

The nucleotide sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) to obtain the correct sequence. The nucleotide and deduced amino acid sequences were compared with the corresponding

sequences of other isolates in the GenBank database (http://www.ncbi.nlm.nih.gov) using the BLAST program (Altschul *et al.*, 1997). The sequence data from the three amplified PCR products were assembled and analyzed using CLUSTAL W from MEGA 4.0 version software. The sequence was submitted to GenBank with proper annotations. The accessions of the GenBank have also been received.

Preparation of phylogenetic tree: The sequences were compared with the equivalent sequences from a range of other geminiviruses present in GenBank. Multiple sequence alignment was carried out using the software ClustalW in MEGA *version* 4.0 (Tamura *et al.*, 2007). The evolutionary tree was constructed using the Neighbor-Joining method (Saitou and Nei, 1997). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004).

3.9 Rolling-circle amplification (RCA)

Techniques for the single-step amplification of whole genomes have been developed into powerful tools for phylogenetic analysis, epidemiological studies and studies on genome organization. Recently, the bacteriophage phi29 DNA polymerase has been used for the efficient amplification of circular DNA viral genomes without the need of specific primers by rolling-circle amplification (RCA) mechanism (Johne *et al.*, 2009)

Detection and full length genome amplification: For initial detection, polymerase chain reaction (PCR) was performed from the total DNA using begomovirus group specific primer pair DengA and DengB (Reddy *et al.*, 2005). Full length genome of tomato leaf curl virus was amplified using TempliPhi™ DNA Amplification kit (GE Healthcre, USA). In the reaction mixture 0.5µl (10-20ng) of DNA was mixed with 5 µl of sample buffer, denatured for 5 min at 95°C and cooled down to room temperature. To this mixture 5 µl reaction buffer and 0.2 µl of enzyme mix were added and the reaction was run for 18h at 30°C as proposed by Packialakshmi *et al.* (2010).

Restriction digestion and agarose gel electrophoresis: 1.5µl of RCA product was digested with five types of restriction enzyme such as BamH1, PstI, HinDIII, EcoR1 and HpaII for 2 hour (Haible *et al.*, 2006; Kumar *et al.*, 2008). Restriction products were run on 2% agarose gel and were observed in UV transilluminator. Photographs of the bands in the agarose gels were taken and were compared with standard DNA ladders.

3.10 Management of vectors of some viral diseases by plant extract

Collection of plants for extraction of botanicals: Several plant materials (leaf) were collected from foothills of north eastern Himalayas (sub-Himalayan West Bengal). The plants were selected on the basis of easy availability in the growing areas of sub Himalayan West Bengal. Plants were collected, identified and voucher specimens have been deposited in the herbarium, Department of Botany, University of North Bengal.

Preparation of leaf extract: The leaves of *Eucalyptus* sp., *Clerodendron* sp., tea (*Camellia sinensis*), neem (*Azadirachta indica*), *Datura* sp., *Xanthium* sp., *Polyalthia* sp., *Crotalaria* sp., *Mitracarpus* sp. and *Piper* sp. were used for extraction. The leaf extracts were prepared following the method of Reddy *et al.* (2010). Leaf materials (500 gm of each) were washed thoroughly in running tap water to remove any trace of dust particles. Then the leaf materials were pulverized in a mortar and pestle and mixed with water in a 1:1 ration (W/V). The mixture was squeezed by using double layered muslin cloth and filtrate was diluted at 1:4(20%) ratio and the diluted extracts were used for field spay following the method of Reddy *et al.* (2010).

Field application: For spraying the tomato field was divided into 11 plots small plots and each plot contained 20 plants. In one plot Sterile distilled water was sprayed and it was treated as control plot. The other ten plots were sprayed with ten different leaf extracts of the ten plants used for the purpose. All the sprayings were done at 10 days intervals after sowing and up to 60 days. The concentration of the spray solute was 20%. The

percent disease incidence of ToLCV {(No. plants infected/total No. of plants in plot) X 100} were recorded.

3.11: List of major chemicals used

In addition to the common laboratory reagents, following chemicals were used during the work:

Chemicals	**Company**
10x Taq DNA buffer B	Genie, Bangalore
2% PTA (Phosphotungstic acid)	CDH Pvt. Ltd., New Delhi, India
6x loading dye	Genie, Bangalore
Acetic acid	SRL Pvt. Ltd., Mumbai, India
Agarose	SRL Pvt. Ltd., Mumbai, India
Ampicilin	HiMedia, Mumbai, India
Bam H1	Promega Corporation, USA
Calcium chloride	HiMedia, Mumbai, India
Carborundum powder (400 mesh)	CDH Pvt. Ltd., New Delhi, India
Celite (600 mesh)	CDH Pvt. Ltd., New Delhi, India
Chloroform	E. Merck, Mumbai, India
CTAB	CDH Pvt. Ltd., New Delhi, India
DEPC-treated water	Chromus Biotech, Bangalore, India
dNTP mix	Genie, Bangalore
Eco RI	Promega Corporation, USA
Eco RV	Promega Corporation, USA
EDTA	SRL Pvt. Ltd., Mumbai, India
Ethydium bromide	SRL Pvt. Ltd., Mumbai, India
Ethyl alcohol	JHI Co. Ltd., China
Forward and Reverse Primer	Sigma, USA
Glycerol	SRL Pvt. Ltd., Mumbai, India
HinDIII	Promega Corporation, USA
HPa II	Promega Corporation, USA

Contd...

Contd...

Chemicals	**Company**
IPTG	Promega Corporation, USA
Isoamyl alcohol	E. Merck, Mumbai, India
Isopropanol	SRL Pvt. Ltd., Mumbai, India
JM 109	Promega Corporation, USA
$MgCl_2$	Genie, Bangalore
M-MuLV reverse transcriptase	Genie, Bangalore
M-MuLV RT-PCR kit	Genie, Bangalore
NaCl	E. Merck, Mumbai, India
Oligo $(dT)_{18}$ primer	Promega Corporation, USA
pGEM- T easy cloning kit	Promega Corporation, USA
pGEM-T vector	Promega Corporation, USA
Phi 29 DNA polymerase	Promega Corporation, USA
PstI	Promega Corporation, USA
RNA gel electrophoresis kit	Genie, Bangalore
RNase	Promega Corporation, USA
Sodium Sulphite	E. Merck, Mumbai, India
T_4 ligase	Promega Corporation, USA
Taq DNA polymerase	Genie, Bangalore
TempliPhi™ DNA amplification kit	GE Healthcare, USA
Total RNA extraction kit	Genie, Bangalore
Tris	SRL Pvt. Ltd., Mumbai, India
X- Gal	Promega Corporation, USA
β-marcaptoethanol	SRL Pvt. Ltd., Mumbai, India

3.12: Composition of Buffers, solutions and media used

Potassium phosphate buffer [0.1M; pH- 7.0]

Stock solution A:

KH_2PO_4	136.09 g
Distilled water	1000 ml

Stock solution B:

K_2HPO_4	174.18 g
Distilled water	1000 ml

38.5 ml of stock solution A was added to 61.5 ml of stock solution B and distilled water was added to obtain a final volume of 1000 ml (pH 7.0).

2X CTAB DNA extraction buffer (for per gram of leaf tissue):

Tris (1M):	500µl (pH 8.0)
NaCl (5M):	1.4ml
EDTA (0.5M):	200µl (pH 8.0)
β-marcaptoethanol:	10µl
Sterile water:	2.89ml
CTAB:	100mg

TE buffer:

Tris-HCl	10 mM
EDTA	1 mM
Final pH	8.0

TAE buffer (**composition of 50X TAE)**:

Tris base	242 g
Glacial acetic acid	57.1 ml
EDTA (0.5 M)	100 ml
Distilled water (final volume made up to)	1000 ml
Final pH	8.0

To make 1X TAE buffer, 1 ml 50X stock buffer was diluted in 49 ml distilled water to make final volume 50 ml.

Ethidium bromide stock solution (1 ml):

0.5mg ethidium bromide was dissolved in 1 ml distilled water; Stored in dark bottle at 4˚C.

1x MOPS buffer

10x MOPS Buffer Composition:

200 mM MOPS, pH 7.0	41.9 g
80 mM Sodium Acetate	4.1 g
10 mM EDTA, pH 8.0	3.7 g

Then the final volume was made up to 1.0 liter with distilled water. Then 100 ml of 10X MOPS buffer and 20 ml 37% Formaldehyde was mixed with 880 ml distilled water to make 1X MOPS buffer.

Luria-Bertani (LB) Agar

To make Luria-Bertani (LB) Agar 25.0 g of powder was mixed with distilled water. Then agar powder was added at the rate of 1 % i.e., 10 g and the final volume was made up to 1.0 liter with distilled water. Then it was autoclaved at 15 psi pressure at 121º C for 15 minutes.

Luria-Bertani (LB) Broth, Miller

To make Luria-Bertani (LB) Broth 25.0 g of powder was mixed with distilled water to make the final volume of 1.0 liter. Then it was autoclaved at 15 psi pressure at 121º C for 15 minutes.

Ampicillin stock solution (100 mg/ml)

One gram of ampicillin was dissolved in 10 ml of sterile distilled water. Solution was filter sterilized using Whatman poly ethersulfone membrane (0.2 µm pore size), stored in aliquots at -20°C.

X-Gal stock solution (20 mg/ml)

200 mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside was dissolved in 10 ml of N, N-dimethylformamide. The solution was stored at -200C in a dark bottle.

IPTG stock solution (100 mM)

240 mg of IPTG (Isopropyl-β-Dthiogalactopyranoside) was dissolved in 10 ml of sterile distilled water. The solution was filter sterilized and stored in aliquots at 4˚C.

LB +Ampicillin+X-gal+IPTG plates

Autoclaved LB agar medium was allowed to cool to 50-55ºC. Required volume of ampicillin stock solution was added to the medium to reach the final concentration of 100 µg/ml. The medium was gently mixed and poured on 90-mm size petri plates. The plates were allowed to solidify and dried open under laminar for 30 min. 40 µl of X-Gal stock solution (20mg/ml) and 40 µl of 100 mM IPTG stock solution were spread evenly over each plate with sterile glass spreaders.

4. Results and Discussions

4.1 PART I: Tomato leaf curl disease (TLCD)

In tropical and subtropical climate *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) transmit numerous geminiviruses (Chandel *et al.,* 2010). Begomoviruses are a major constraint for the successful cultivation of tomato in tropic and subtropics (Kumar *et al.,* 2012). Several begomovirus species infecting tomatoes in India have been characterized (Kumari *et al.,* 2011; Chakraborty *et al.,* 2003; Tiwari *et al.,* 2010,13). Chakraborty *et al.* (2003) described Tomato leaf curl virus (ToLCV) as a threatening virus of tomato. Tomato infecting begomoviruses of north India have bipartite genome while that of south India have monopartite genome (Borah and Dasgupta, 2012). Since, the first report of occurrence TLCD from India (Vasudeva and Samraj, 1948) several reports of Indian occurrence of the disease have been published. *Tomato leaf curl New Delhi virus* (ToLCNDV) has been reported from Assam and Kolkata (Reddy *et al.,* 2005). TLCD of tomato are also caused by *Tobacco curly shoot virus* (TbCSV) and *Tobacco leaf curl Yunnan virus* (TbLCYNV) in China (Li *et al.,* 2004). Association of DNA-β with the Tomato leaf curl diseases was reported by Sivalingam *et al.* (2010) from several places of India.

Cultivation of tomato plants almost round the year in sub-Himalayan West Bengal and Brahmaputra valley of Assam is well known due to its climate. As a result chances of infection of the plants by begomoviruses are also more in the area. Hence, the occurrence and distribution of begomoviruses in tomato plants was thought to be studied primarily in sub-Himalayan West Bengal. As similar agro climatic zone is also prevail in the Brahmaputra Valley of Assam, it was also thought to study the area for comparison and analysis of the viruses with that of sub Himalayan West Bengal. A map of the area of study has been presented in Fig. 1

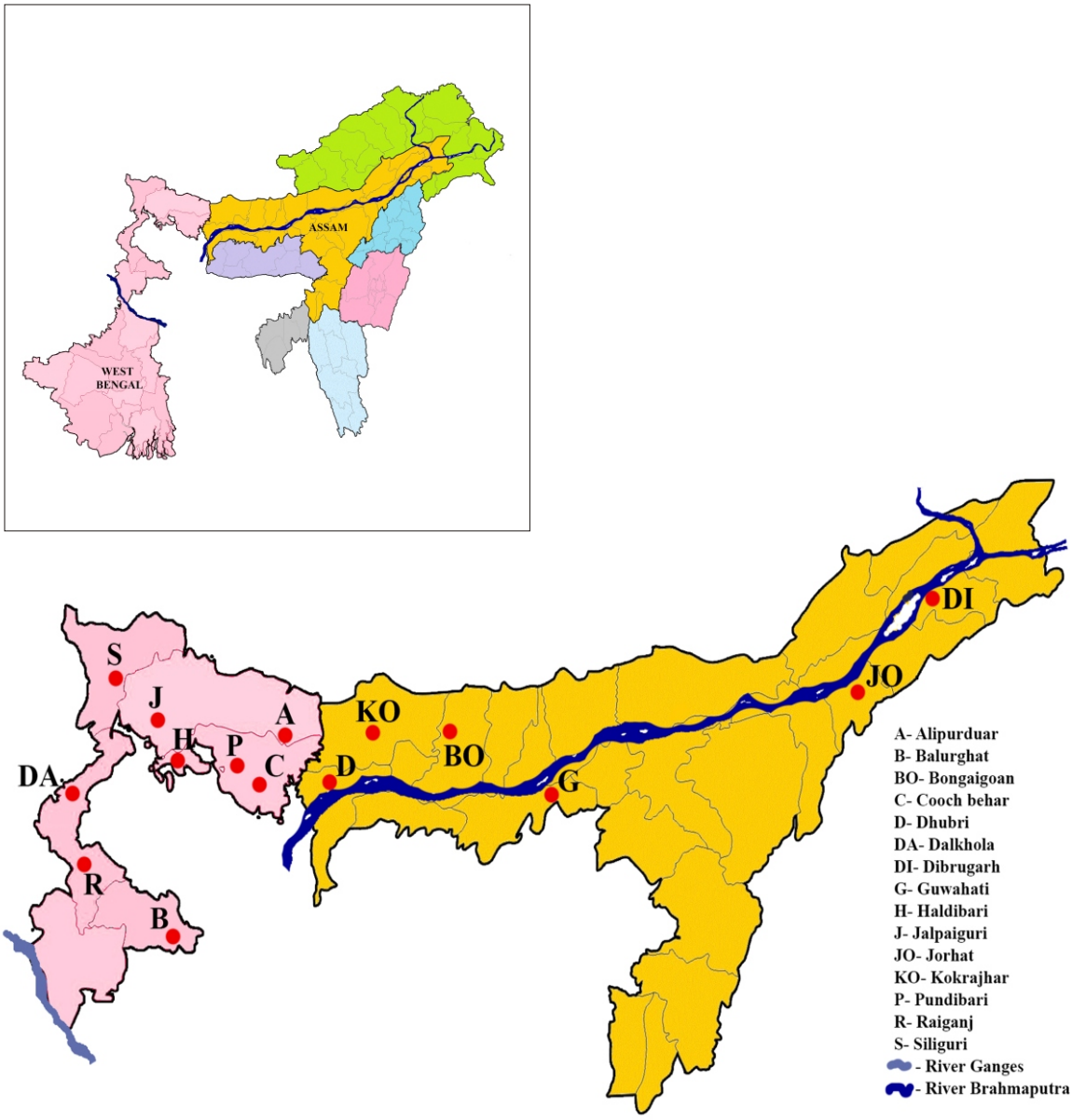

Fig. 1: The map of sub-Himalayan West Bengal and Assam. Collection spots of virus affected plant samples shown by red points.

Survey, disease incidence and symptomatology: Different kinds of disease symptoms; mild to severe leaf curling (downward and upward), leaf blistering, leaf crinkling and leaf narrowing of leaves with stunted growth were observed in the field conditions (Fig. 2a, 2b, 2c, 2d, 2e & 2f). Samples which showed distinct visible symptoms were subjected to polymerase chain reaction (PCR). A positive reaction in PCR amplification of a target sequence (530nt long) specific to begomovirus confirmed the presence of begomovirus in the samples tested (Table 2 and 3). The severely infected plants produced no fruit; however, less infected plants produced fruits of significantly reduced sizes. The disease incidence was found to be varied from location to location and region to region. Disease incidence of about 18-83% was found in sub Himalayan West Bengal and it was recorded as 14-71% in Assam (Table 2 and 3). Results as evidenced from Table-2 and 3, indicated high incidence of TLCD in sub-Himalayan West Bengal and Brahmaputra valley of Assam.

Morphology of virus particles: Electron microscopic examination (Fig. 3b) of crude preparation of infected tomato leaves revealed the presence of isometric, pentagonal and 'paired Gemini virus' structures. The dimension of the virus particles were 20 X 30 nm when negatively stained with 2% phosphotungstic acid (PTA) pH 7.0. The bipartite structures of our study are very much similar with that of characteristic geminate particles of begomovirus as suggested by Czosnek *et al.* (1988).

Detection of Begomovirus by whitefly transmission and polymerase chain reaction (PCR): The virus associated with TLCD was detected by PCR using Begomovirus specific primers, DengA/ DengB. Altogether 15 diseased samples (Table 2 & 3) were tested. All the samples showed positive PCR reaction amplifying desired size, 530 nt sequence fragment of CP gene of Begomovirus. The results confirmed that TLCD was caused by certain species of Begomovirus. Seven samples (5 from West Bengal and 2 from Assam) were selected for Molecular characterization. GenBank accessions were also done for all the seven samples. The accession numbers have been presented in the table 4.

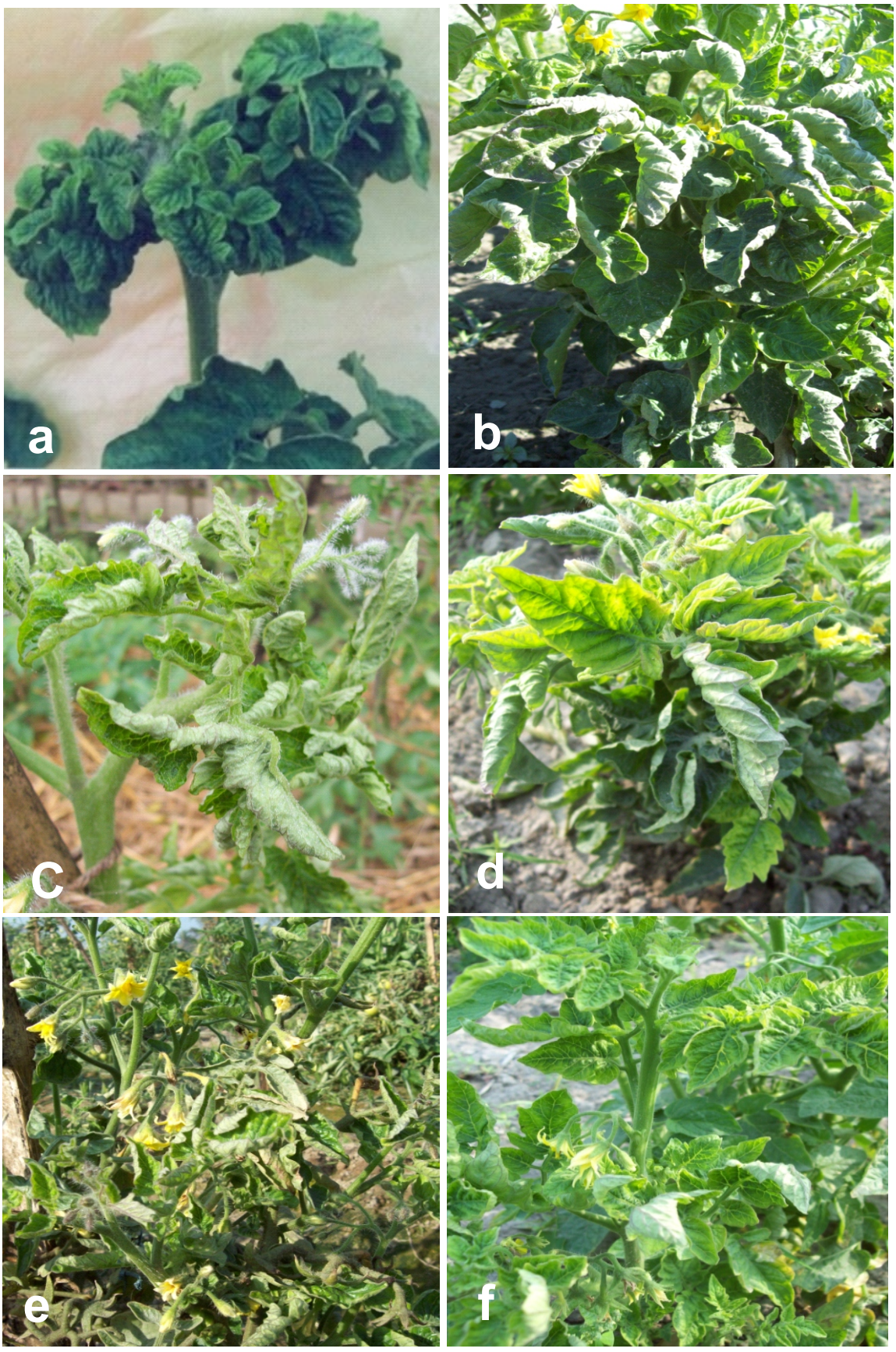

Fig. 2: Naturally infected tomato plants of sub-Himalayan West Bengal and Assam showing TLCD. (a) Severe leaf curl from Coochbehar, West Bengal, (b) Leaf curl from Guwahati, Assam, (c) Leaf curl with stunted growth from Siliguri, West Bengal, (d) Leaf curl from Haldibari, West Bengal, (e) Leaf curl from Kokrajhar, Assam, (f) Leaf curl from Dalkhola, West Bengal.

Whitefly transmission of the Begomoviruses associated with TLCD samples of five different locations (3 from West Bengal and 2 from Assam) were also carried out (Table 5). TLCD of all the samples were transmitted to healthy tomato (var. Pusa Ruby) seedlings with transmission efficiency of 50-80% depending on the samples. Typical TLCD symptoms were produced in the inoculated plants within 18-30 days after whitefly inoculation. The present data indicated that the TLCD in tomato was caused by whitefly-transmitted Begomovirus species. Some white flies used in the present study have been shown in Fig. 3a

Table 2: Occurrence tomato leaf curl disease (TLCD) complex in different regions of sub-Himalayan West Bengal

State	**Area/location**	**Field symptoms**	**PCR**	**Percent disease incidence**
West Bengal	Coochbehar	Downward LC, Chl, no fruit	+	28-83
	Siliguri	Upward LC, Chl, no fruit	+	24-61
	Haldibari	Severe LC	+	20-56
	Jalpaiguri	Mild LC	-	20-33
	Pundibari	LC, St	+	28-43
	Raiganj	Upward LC	+	30-56
	Balurghat	LC	+	43-63
	Dalkhola	Leaf rolling, LC	+	20-54
	Alipurduar	Mild LC	+	18-33

+ = presence of the virus; - = absence of the virus.

*Randomly one isolate from each location were taken for molecular based study. Symptom codes: LC: Leaf curl; ST: Stunting; Chl: Chlorosis; LC: Leaf crinkle; LR: Leaf rolling

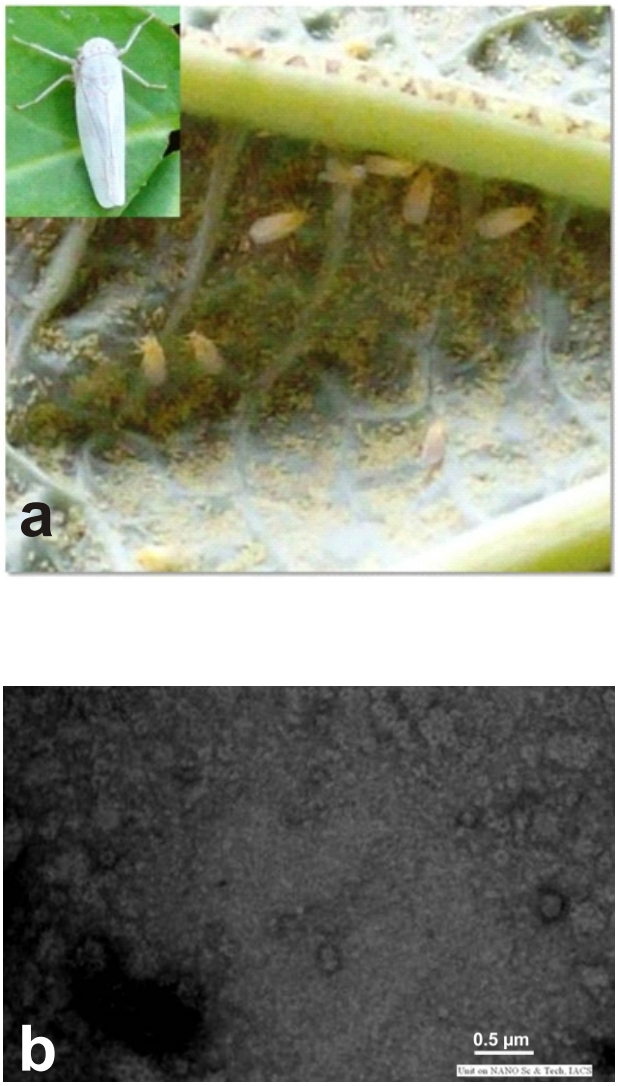

Fig. 3: (a) Whiteflies associated with the *Begomovirus* infected tomato leaves of Coochbehar, (b) Transmission Electron Microscopic photograph of *Tomato leaf curl virus.*

Table 3: Occurrence of tomato leaf curl disease (TLCD) complex in different areas of Brahmaputra valley of Assam

State	Area/ location	Field symptoms	PCR	Percent disease incidence
Assam	Dibrugarh	LC, St, small fruit	+	20-31
	Bongaigaon	Mild LC, normal fruit	+	35-44
	Jorhat	LC, small fruit	+	14-20
	Guwahati	Mild LC, Leaf crinkle	+	45-71
	Dhubri	Mild LC, St	+	30-50
	Kokrajhar	LC	+	29-43

+ = presence of the virus; - = absence of the virus.

*Randomly one isolate from each location were taken for molecular based study. Symptom codes: LC: Leaf curl; ST: Stunting; Chl: Chlorosis; LC: Leaf crinkle; LR: Leaf rolling.

Table 4: Isolates selected for Molecular characterization with their accession numbers

State	Location	Isolate code	Accession Number of GenBank
West Bengal	Coochbehar	COB-2	HM856625
	Siliguri	SILIGURI-2	HQ557032
	Haldibari	COB5	JF509102
	Pundibari	COB-6	JF825867
	Raiganj	RAI-1	JF825865
Assam	Guwahati	GUW-1	JF825866
	Dhubri	GUW-2	JN676053

Table 5: Transmission of TLCD complex in tomato plants through whitefly inoculation in greenhouse condition

Source of virus	No. of plants inoculated	Symtoms appeared	Days taken to appear symptoms	No. of pl infected/ no. of pl inoculated (% trasmission)
Guwahati	Tomato cv. Pusa Ruby	Severe LC, St, Chl	20-29	8/10 (80)
Kokrajhar	Do	LC, Chl	20-28	3/6 (50)
Coochbehar	Do	LC, St, Chl	21-31	6/9 (67)
Siliguri	Do	Mild LC, St	18-28	6/12 (50)
Raiganj	Do	LC, poor growth, Chl	20-30	5/8 (62)

LC: Leaf curl, Chl: Chlorosis, St: Stunting

Comparison of insect transmission and mechanical transmission

Two different types of transmission experiments were performed for transmission of begomovirus complexes in tomato. In one set of experiment white fly was used for transmission of the virus. In another set of experiment carborundum powder along with sap (leaf extract) of infected plant was used. Details of the experimental setup have been described in materials and methods (sections-3.1.8 & 3.1.9). Results presented in Table 6 distinctly showed that viruses could be transmitted both by the mechanical transmission technique and by white fly inoculation technique. In 'white fly transmission technique' all the 20 plants tested showed characteristic visible leaf curl symptom similar to that of tomato 'infecting begomovirus complex', after 20 days of inoculation. Eight out of 20 plants tested for mechanical transmission, showed similar visible symptoms as found in case of white fly transmitted plants after 20 days of incubation. Transmission efficiency of the two techniques was compared by percent

transmission as suggested by Ghanem *et al.* (2001). Percent transmission efficiency was calculated by the following formula:

$$\text{Transmission efficiency} = \frac{\text{No. of plants showed visual symptoms}}{\text{No. of plants inoculated by the technique}} \times 100$$

Table 6: Transmission of TLCD complex and percent symptom development by the virus following two different techniques (mechanical inoculation and white fly inoculation)

Mode of transmission	No. of plants inoculated	No. of plants showed visible Symptoms (after 60 days)	% transmission efficiency of the experiment.
Mechanical inoculation	20	8	40
Control	20	0	0
Inoculation by white fly	20	20	100
Control	20	0	0

Determination of limited host range

Ten different plants cultivated around the tomato growing areas of north Bengal and Brahmaputra valley of Assam were selected and tested for their potentiality, if any, as alternative host by mechanical sap inoculation. Out of the 10 different plants tested two plants failed to show symptoms and contained no detectable DNA of ToLCV when amplified by using DengA/DengB primers. For limited host range test, the plants were collected from the nearby cultivated plots. All the plants were observed carefully and only the healthy plants were used for the experiment. Five plants of each species and/or variety were mechanically inoculated with the sap of infected tomato plants using carborundum powder. Under identical conditions, in a separate net house, sterile distilled water and carborundum powder was applied in control plants. Details of the

inoculation technique have been described under materials and methods (Section-3.1.9). Seedlings with 2-3 leaves were selected for inoculation. Successful transmission was assessed by visual observations after 20 days. Plants mechanically inoculated with sap of infected plants produced leaf curl symptoms on young leaves. In *Cucumis sativus*, *Lycopersicon esculentum* (cv. Pusa Ruby), *Capsicum annum* (cv. Akasmoni) and *Phaseolus vulgaris* (cv. Improved Tender Green) disease symptoms appeared as leaf curl (Fig. 4a, 4b, 4c & 4d). In *Nicotiana rustica* (cv. Hookah) local lesion was developed after inoculation (Fig.4e). *Pisum sativum* and *Solanum tuberosum* (Fig.4f) could not tolerate the mechanical inoculation and the plants died due to distortion of the leaves. No symptoms were developed in case of *Cucurbita pepo, Solanum melongena* and *Brassica oleracea* var. *botrytis.*

DNA sequencing

In the present study four pair of DNA-A specific begomovirus primers were used to amplify the DNA-A genome. Expected amplicon was obtained from all the isolates using DengA/DengB and PALIc1960/PARIv722 primers (Fig.5). Primer pair CRV301/CRC1152 was not able to amplify the isolates HALDI-1 and SILGURI-1. Only the isolate GUW-2 was amplified using all the four primer and a complete genome of 2763 nucleotide of DNA-A was submitted in the GenBank (Accession no. JN676053). DNA-B was successfully amplified (using BF518 / BR16141 primer pair) from isolates COB-2 and SILIGURI-2. But DNA-β could not be amplified from any of the nine virus isolates of the present study (using Beta 01/Beta 02 primer pair). Nucleotide sequence identity (nsi) of partial DNA-A, coat protein (CP) genes and partial DNA-B of present begomovirus complexes with other begomoviruses have been presented in Tables 7, 8, 9 and 10.

The partial DNA-A [Pre-coat protein (AV2), coat protein (AV1), AC5 protein (AC5), replication enhancer protein (AC3) and transcription activator protein (AC2) gene] (HM856625) and DNA-B (nuclear shuttle protein (BV1) and movement protein (BC1) genes) (JN390433) of the Tomato leaf curl disease (TLCD)- associated begomovirus complex, isolate

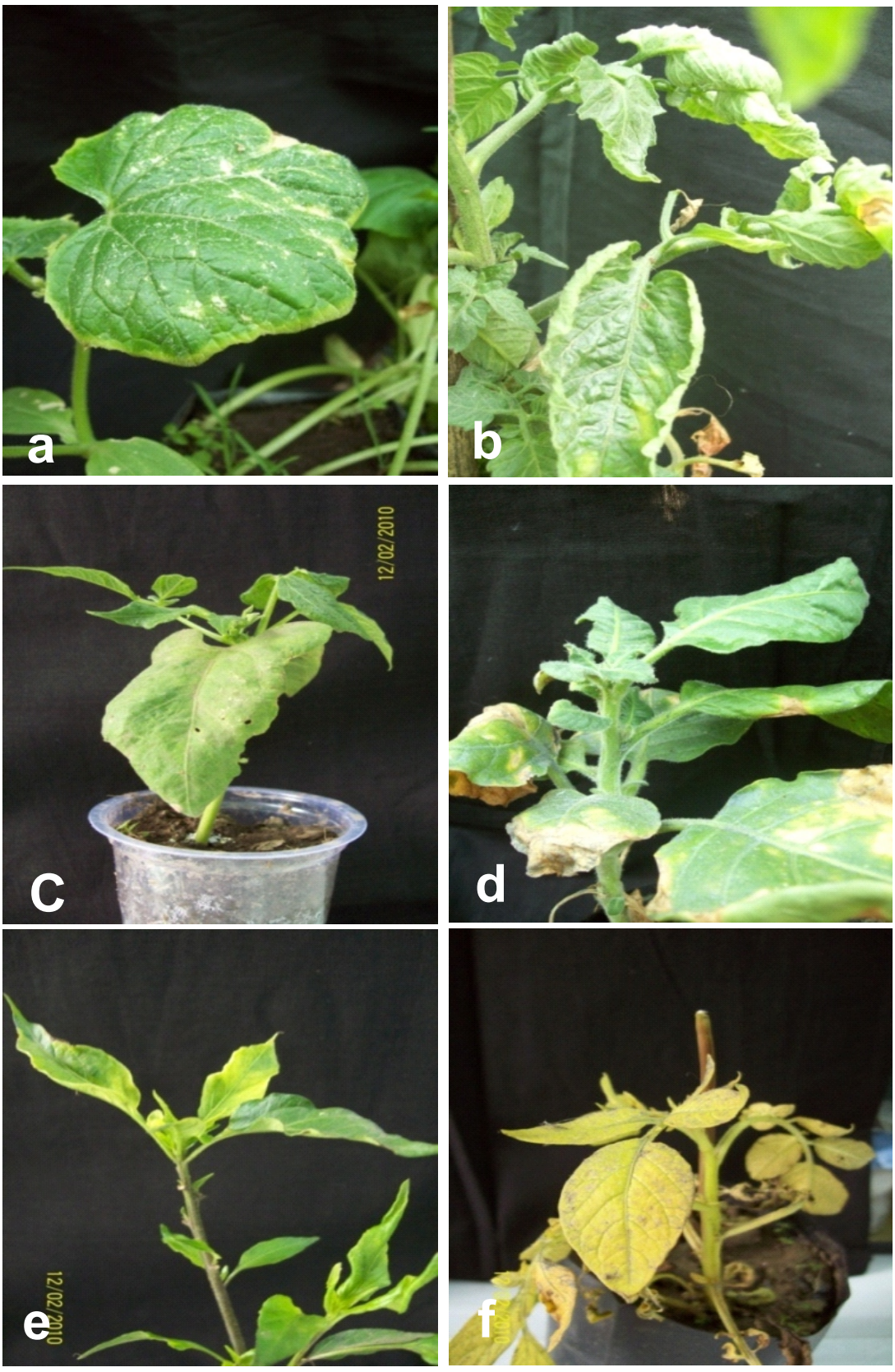

Fig. 4: Symptoms of virus infections in different host species mechanically transmitted through sap inoculation technique. (a) Infected *Cucumis sativus*, (b) Infected *Lycopersicon esculentum*, (c) Infected *Capsicum annum*, (d) Infected *Phaseolus vulgaris*, (e) Infected *Nicotiana rustica*, (f) Infected *Solanum tuberosum.*

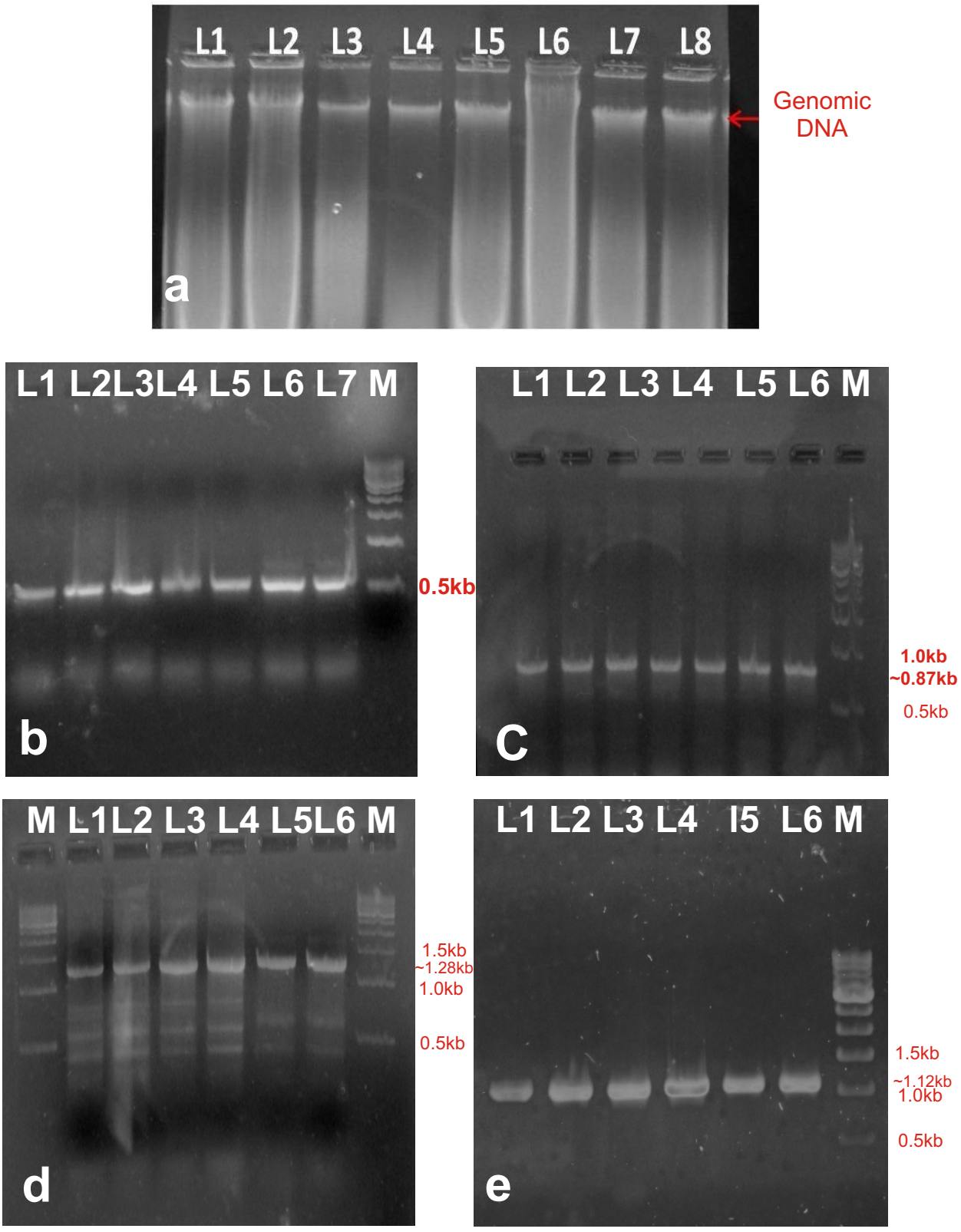

Fig. 5: Agarose gel electrophoresis (1.5%) of (a) extracted DNA from infected tomato plants, (b) PCR amplicons using primer pair Deng A/Deng B, (c) PCR amplicons using primer pair CRv301/Crc1152, (d) PCR amplicons using primer pair PALIc1960/PARIv722, (e) PCR amplicons using primer pair BF518/BR16141 from infected tomato plants, M: DNA Ladder 500bp.

COB-2 showed highest nucleotide sequence identity (nsi) with *Tomato leaf curl New Delhi virus* (AY286316, 99%) (unpublished; sequence deposited in the GenBank from India in 2006) and *Tomato leaf curl New Delhi virus* DNA-B (FN356024, 99%) (unpublished; sequence deposited in the GenBank from Himachal, India in 2009) respectively.

The *Begomovirus* associated with isolate COB-6 (Accession no. JF825867) containing pre-coat protein (AV2), coat protein (AV1), replication enhancer protein (AC3), and transcription activator protein (AC2) genes, complete; and replication associated protein (AC1) gene, partial and isolate COB-5 (Accession no. JF509102) for coat protein gene showed its maximum identity with *Tobacco curly shoot virus* (GU001879, 96%) (unpublished; sequence deposited in the GenBank in 2010) which infect pepper in China.

The *Begomovirus* associated with the isolates SILIGURI-1 and HALDI-1 both containing partial coat protein gene showed 98% nucleotide sequence identity with *Tobacco curly shoot virus* (Accession no. AJ420318 and AF240675) isolated from China.

The coat protein genes of begomovirus disease complex associated with the isolates RAI-1, GUW-1 and COB-5 showed maximum nucleotide sequence identity with tomato leaf curl New Delhi virus (EU375489, 99%), Tomato leaf curl Bangladesh virus (AF188481, 98%) and Tobacco curly shoot virus (GU001879, 98%) respectively.

GUW-2 isolate was found to be monopartite and only DNA-A was found to occur. The complete nucleotide sequence analysis of DNA-A of GUW-2 (JN676053) showed maximum nucleotide sequence identity with *Tomato leaf curl Ranchi virus* (GQ994095, 97%) (sequence deposited in the GenBank from Ranchi, India in 2010) which infected tomato in Ranchi, India.

Table 7: Nucleotide sequence identity (nsi) of partial DNA-A, coat protein (CP) genes and partial DNA-B of the begomoviruses of Coochbehar district of West Bengal with that of some reported sequences of Genbank

Begomovirus complexes under study	Characterized sequence	Accession no.	Highest identity with	% identity
isolate COB-2	AV2, AV1, AC5, AC3 and AC2 gene	HM856625	*Tomato leaf curl New Delhi virus* - [India: 2006] (AY286316)	99%
isolate COB-2	BV1 and BC1 genes	JN390433	*Tomato leaf curl New Delhi virus* DNA-B [India:Himachal:2009] (FN356024.1)	99%
isolate COB-6	AV2, AV1, AC3 and AC2 genes, complete and AC1 gene, partial	JF825867	*Tobacco curly shoot virus* [China: 2010] (GU001879)	96%
isolate COB-5	AV1 gene	JF509102	*Tobacco curly shoot virus* [China: 2010] (GU001879)	98%
isolate HALDI-1	AV1, AC3, and AC2 genes, partial	HM856627	*Tobacco curly shoot virus* [China:2003] (AF240675)	96%

Table 8: Nucleotide sequence identity (nsi) of partial DNA-A and coat protein (CP) genes of the begomoviruses of the sub-Himalayan plains of Darjeeling district of West Bengal with that of some reported sequences of Genbank

Begomovirus complexes under study	Characterized sequence	Accession no.	Highest identity with	% identity
isolate SILIGURI-1	pre-coat protein (AV2) gene, complete and coat protein (AV1) gene, partial	HQ597031	*Tobacco curly shoot virus* [China: 2013] (HF569281)	98%
isolate SILIGURI-1	(AV1) gene, partial; (AC3) and (AC2) genes, complete; and (AC1) gene, partial	JN676055	*Tobacco curly shoot virus* [China:2005] (AJ420318)	98%
isolate SILIGURI-2	AV3, AV2, AV1, AC5, AC3, and AC2 genes, complete and AC1 gene, partial	HQ597032	*Tomato leaf curl New Delhi virus*-India [India/New Delhi/Chilli/2009] (HM007113)	96%

Table 9: Nucleotide sequence identity (nsi) of coat protein (CP) genes of the begomoviruses of Uttar Dinajpur district of West Bengal with that of a reported sequence of Genbank

Begomovirus complexes under study	Characterized sequence	Accession no.	Highest identity with	% identity
RAI-1	Coat protein gene	JF825865	*Tomato leaf curl New Delhi virus* [India: Ganzaria, Lucknow: 2008] (EU375489)	99%

Table 10: Nucleotide sequence identity (nsi) of DNA-A and coat protein (CP) genes of the begomoviruses of the Brahmaputra valley of Assam with that of some reported sequences of Genbank

Begomovirus complexes under study	Characterized sequence	Accession no.	Highest identity with	% identity
isolate GUW-1	Coat protein gene	JF825866	*Tomato leaf curl Bangladesh virus* [Bangladesh: 2003] (AF188481)	98%
isolate GUW-2	Complete genome	JN676053	*Tomato leaf curl Ranchi virus* [Ranchi:2010] (GQ994095)	97%

Analysis of virus nucleotide sequence and phylogeny

Seven TLCD samples, designated as isolates GUW-1, GUW-2, COB-2, SILIGURI-2, COB-5, COB-6 and RAI-1 from seven locations were randomly taken for molecular characterization of the virus. Nucleotide sequence identity matrix of tomato leaf curl disease causing begomoviruses with other begomoviruses (isolates/species) based on CP gene have been presented in table 11. Sequence alignment showed that CP gene sequence of isolate GUW-1 was identical with GUW-2 and COB-5 with COB-6 (100% nt identity). Therefore, five present isolates COB-2, COB-5, GUW-1, RAI-1 and SILIGURI-2 were analyzed further for sequence comparison with other 16 species/isolates begomoviruses (Table 12). The sequence analysis showed that the present isolates shared 79-98% nt identity among them and 79-99% nt identities with other isolates. The present isolates COB-2, RAI-1 and SILIGURI-2 were related to each other sharing 94-98% nt identity and they were distant from isolates COB-6 and GUW-1 by sharing 79-81%. The isolate COB-6 and GUW-1 shared 94% nt identity between them.

The phylogenetic analysis showed overall two genogroups among all the isolates including present isolates (Fig. 6 & 7). The present isolates COB-2, RAI-1 and SILIGURI-2 fell into one group (GR-1) and COB-5 and GUW-1 into another group (GR-2).

The sequence analysis determined that COB2, RAI-1 and SILIGURI-2 are similar with begomoviruses like *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Tomato leaf curl Pakisthan virus* (ToLCPkV) showing 96-99% nt identity. The isolate COB-5 is related to *Tobacco curly shoot virus* (TbCSV) of China and Uttakhand sharing 98% nt identity and isolate GUW-1 to *Tomato leaf curl Ranchi virus* (ToLCRaV) and *Tomato leaf curl Bangladesh virus (*ToLCBnV) showing 98% identity. Thus, it could be concluded that the TLCD in these regions could be caused by ToLCNDV, ToLCPkV, ToLCPkV, ToLCBnV and TbCSV or mixture of different begomoviruses.

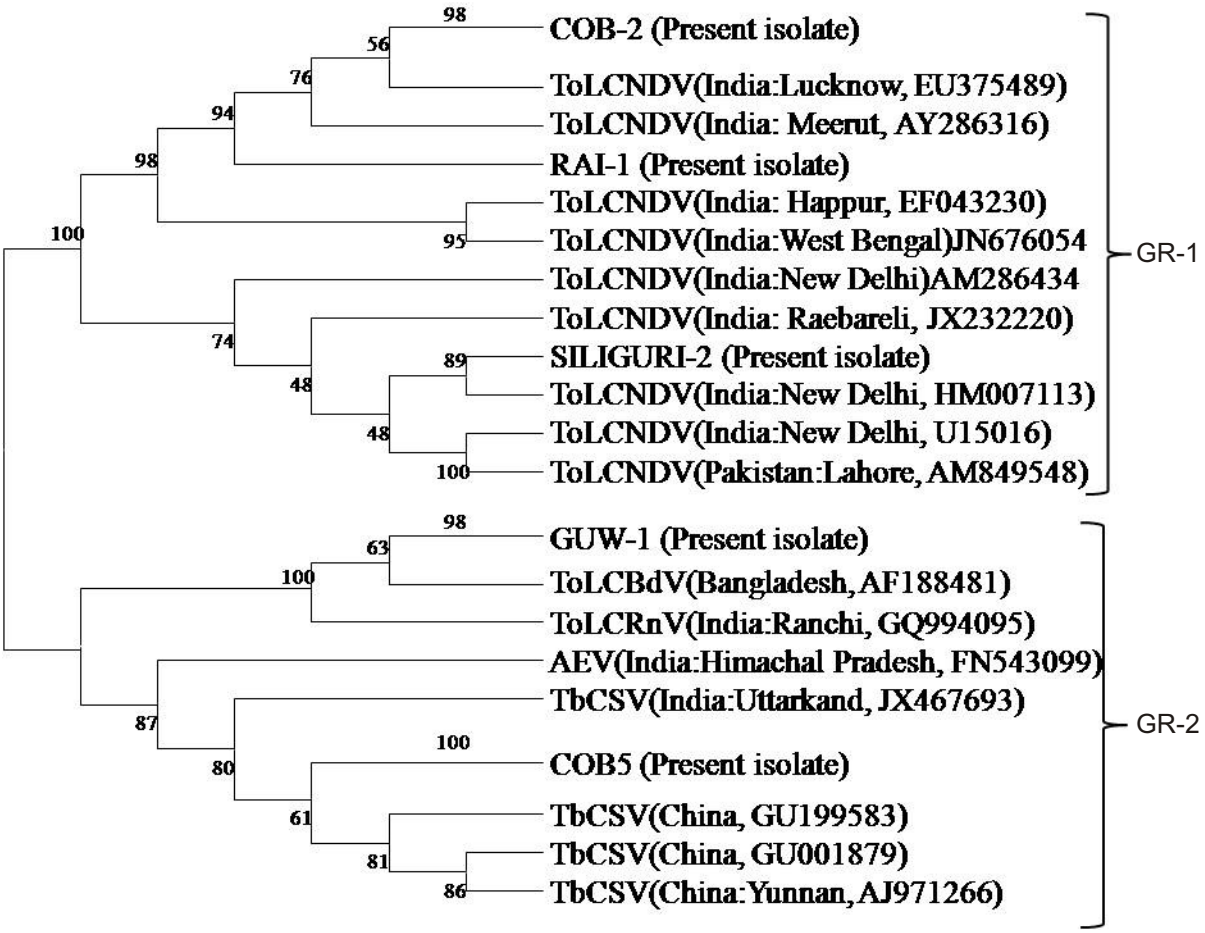

Fig. 6: Phylogenetic relationship of tomato leaf curl disease causing begomoviruses with other *Begomovirus* isolates/species based on CP gene using maximum parsimony in MEGA version 4.0 program. The significance of the nodes was estimated with 100 bootstrap repetitions.

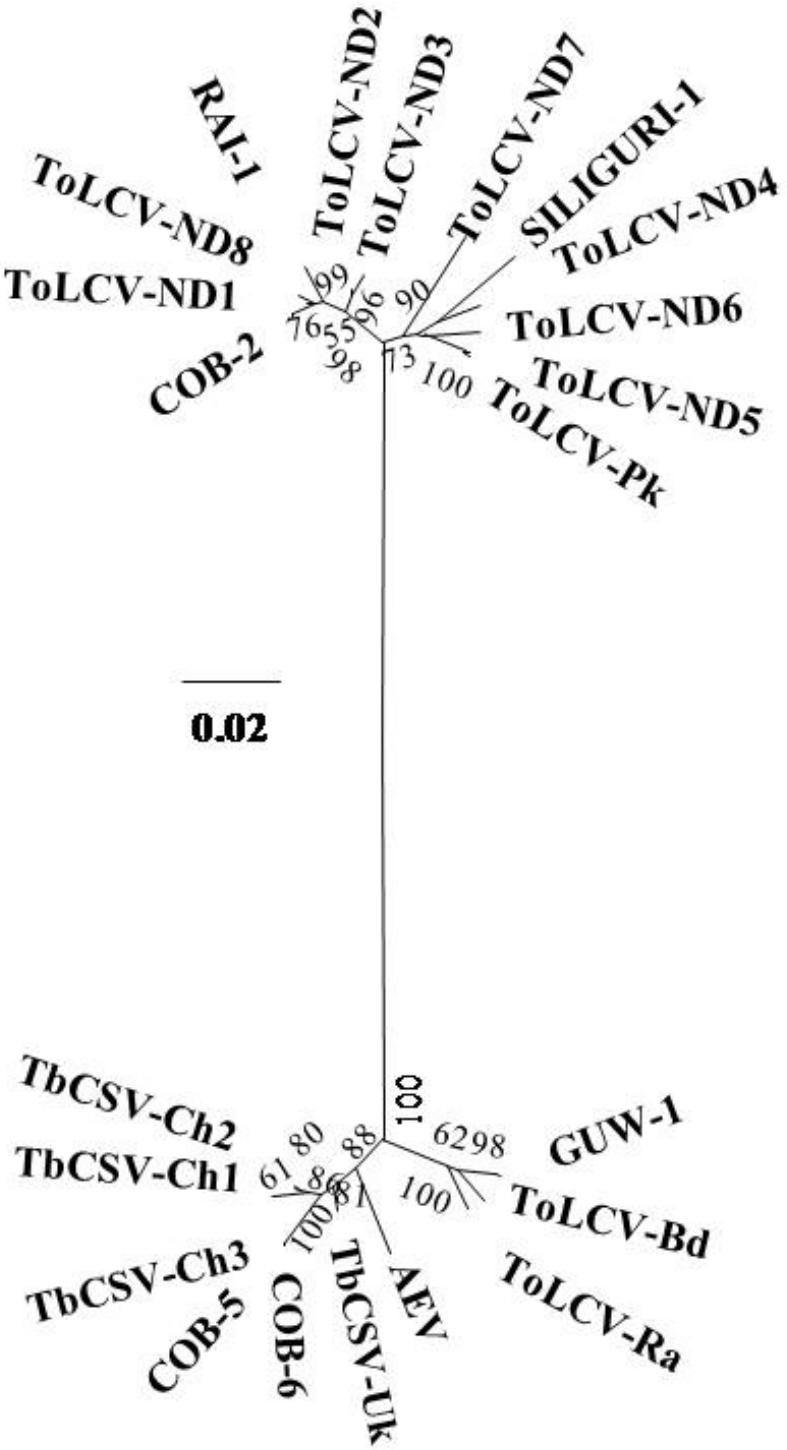

Fig. 7: The Coochbehar isolates (COB-5 & COB-6) and COB-2 distinctly differs in two separate groups as evident from the phylogenetic analysis of the present ToLCV isolates.

CP genes in the genus begomoviruses are the most conserved (Brown *et al.,* 2001). Therefore, analysis of CP gene sequence, could only detect Begomovirus genus level. However, for provisional demarcation of some species of Begomovirus, CP gene sequences have also been used earlier when complete sequence of DNA molecule of the Begomoviruses were not known (Brown *et al.*, 2001; John *et al.,* 2006; Santoso *et al.*, 2008; Samad *et al.,* 2009; Haider *et al.,* 2007). Because of the growing number of recognized species under this genus, derivation of the complete nucleotide sequence of the virus is necessary to distinguish species under begomovirus (ICTV, 2012). Although, analysis of CP gene could not be accurate for identification of virus species under genus Begomovirus, but in the present study, CP gene based method provided valuable information about the causes of TLCD in tomato crops of Sub-Himalayan Tarai regions of West Bengal and Brahmaputra valley of Assam. This is for the first time molecular characterization of Begomoviruses causing TLCD of this region has been reported.

The present study showed that TLCD in tomato crop caused by Begomovirus complex in Sub-Himalayan Tarai regions of West Bengal and Brahmaputra valley of Assam of North east of India are very common and the incidence of this disease is quite high. The high disease incidence may be attributed to prevalence of whitefly vector, particularly whitefly biotype B, warm tropical climatic conditions supporting year round survival of the whitefly, intensive cultivation of crops and polyphagous nature of the whitefly serving path of sustenance of begomovirus in alternative hosts.

The taxonomy of the Begomovirus causing TLCD in tomato in these regions is still not clear, until the complete genome of the viruses will be sequenced and analyzed. However, the present study would definitely help to detect the Begomoviruses infecting tomato crops, understand the epidemiology of this disease and design management strategy of the disease and its efficient insect vector for economic production of tomato crop in these regions.

Table 11: Nucleotide sequence identity matrix of tomato leaf curl disease causing begomoviruses with other begomovirus isolates/species based on CP gene with selected begomoviruses

	COB-2	COB-6	COB-5	SIL-2	RAI-1	GUW-1	GUW-2	To-R	Tb-C	To-B	Ae-I	Tb-I	To-I1	To-I2	To-I3	To-I4	To-I5	To-I6	To-I7	To-I8	Tb-C1	Tb-C2	To-I9
COB-2	0	81	81	94	98	81	81	81	81	81	81	81	99	98	98	96	96	96	96	95	81	81	99
COB-6		0	100	79	81	94	95	95	98	95	97	98	81	81	81	81	81	81	81	79	98	98	80
COB5			0	79	81	94	95	95	98	95	97	98	81	81	81	81	81	81	81	79	98	98	80
SIL-2				0	94	79	80	80	80	80	79	80	94	95	94	96	96	96	95	94	80	79	94
RAI-1					0	81	81	81	81	81	81	81	99	98	98	96	96	96	96	95	81	81	99
GUW-1						0	100	98	94	98	95	95	81	81	81	81	81	81	81	80	94	94	81
GUW-2							0	98	95	98	95	95	81	81	81	81	81	81	81	80	95	94	81
To-R								0	95	98	95	95	81	82	81	81	81	81	81	80	95	94	81
Tb-C									0	95	96	98	81	82	81	81	81	81	81	80	100	99	81
To-B										0	95	95	81	81	81	81	81	81	81	80	95	94	81
Ae-I											0	96	81	81	81	81	81	81	81	80	96	96	81
Tb-I												0	81	82	81	82	82	82	82	80	98	98	82
To-I1													0	99	98	96	96	96	96	95	81	81	99
To-I2														0	99	96	97	97	96	96	82	81	99
To-I3															0	96	96	96	96	95	81	81	98
To-I4																0	98	98	97	96	81	81	96
To-I5																	0	100	98	96	81	81	96
To-I6																		0	98	96	81	81	96
To-I7																			0	96	81	81	96
To-I8																				0	80	80	95
Tb-C1																					0	99	81
Tb-C2																						0	81
To-I9																							0

*To-R=ToLCRnV-CP (India :Ranchi) GQ994095; Tb-C=TbCSV-CP (China) GU001879; To-B=ToLCBdV-CP (Bangladesh)AF188481; Ae-I=AEV-CP (India:Himachal Pradesh)FN543099; Tb-I=TbCSV-CP(India:Uttarkand) JX467693; To-I1=ToLCNDV-CP (India:Lucknow)EU375489; To-I2=ToLCNDV-CP (India)EF043230; To-I3=ToLCNDV-CP (India:West Bengal)JN676054; To-I4=ToLCNDV-CP(India:New Delhi)HM007113; To-I5=ToLCNDV-CP(India:New Delhi)U15016; To-I6=ToLCNDV-CP(Pakistan:Lahore)AM849548; To-I7=ToLCNDV-CP(India)JX232220; To-I8=ToLCNDV-CP(India:New Delhi)AM286434; Tb-C1=TbCSV-CP(China:Yunnan)AJ971266; Tb-C2=TbCSV-CP(China)GU199583; To-I9=ToLCNDV-CP(India)AY286316

Table 12: The sequence of CP genes of other begomoviruses used in the present study for sequence analysis of the present virus isolates

Sr. No.	Name of the virus	Source country	Source crop	Described as in the present study	Acc. No.
1	*Tomato leaf curl New Delhi virus*	India (Lucknow)	Potato	ToLCV-NDI	EU375489
2	*Tomato leaf curl New Delhi virus*	India (Happur)	Potato	ToLCV-ND2	EF043230
3	*Tomato leaf curl New Delhi virus*	India (West Bengal)	*Datura stramonium*	ToLCV-ND3	JN676054
4	*Tomato leaf curl New Delhi virus*	India (New Delhi)	chilli	ToLCV-ND4	HM007113
5	*Tomato leaf curl New Delhi virus*	India (New Delhi)	Tomato	ToLCV-ND5	U15016
6	*Tomato leaf curl New Delhi virus*	India (Raebareli)	Tomato	ToLCV-ND6	JX232220
7	*Tomato leaf curl New Delhi virus*	India (New Delhi)	pumpkin	ToLCV-ND7	AM286434
8	*Tomato leaf curl New Delhi virus*	Pakistan (Lahore)	*Solanum nigrum*	ToLCV-ND-9	AM849548
9	*Tomato leaf curl New Delhi virus*	India (Meerut)	Potato	ToLCV-ND-8	AY286316
10	*Tobacco curly shoot virus*	China (Panzhihua)	Pepper	TbCSV-Ch1	GU001879
11	*Tobacco curly shoot virus*	Cjina (Yunan)	*Ageratum conyzoides*	TbCSV -Ch2	AJ971266
12	*Tobacco curly shoot virus*	China (Chongqing)	*Alternanthera philoxeroides*	TbCSV -Ch3	GU199583
13	*Tobacco curly shoot virus*	India (Uttara-khand)	Tomato	TbCSV -UK	JX467693
14	*Tomato leaf curl virus*	Bangladesh	Tomato	ToLCV-Bd	AF188481
15	*Tomato leaf curl virus*	India (Ranchi)	Tomato	ToLCV-Ra	GQ994095
16	*Ageratum enation virus*	India (H.P)	*Zinnia sp*	AEV-Hp	FN543099

RCA analysis

Universal DNA-A specific primers and universal DNA-B specific primers successfully amplified the partial DNA-A and DNA-B genomes from the isolates under study. But DNA-β specific primer pair could not be able to amplify the DNA-β genome from the present isolates. Under the circumstances, Rolling circle amplification (RCA) technique (Kumar *et al.*, 2008) was adopted to amplify full length genomes of the isolates where DNA-A were present but DNA-B were absent. This was done to detect the possibility of presence of DNA-β along with DNA-A. Following amplification of the full length genome (amplicon of high molecular weight) by RCA was also subjected to digestion by restriction enzymes (*Bam*H1, *Pst*I, *Hin*DIII, *Eco*R1 and *Hpa*II). One of enzyme BamH1 could digest the amplicon by a single nick and was able to separate DNA-A and DNA-β in agarose gel. The isolates COB-5 and GUW-2 showed positive response on RCA and RFLP as two distinct bands of ~2.7kb and ~1.5kb were appeared after restriction digestion (Fig. 8).

Control of disease

Leaf extracts of ten different plants were prepared and it was sprayed on the plants in 10 different plots. In one plot sterile distilled water was sprayed. Details of the spray procedure have been described in the Materials and methods (section 3.1.11). All the plants were subjected to white fly inoculation. Disease appeared in most of the plants but degrees of incidences were significantly low in cases of Neem leaf extract and *C. viscosum* leaf extract-sprayed plants. The results of the experiment revealed that the Neem leaf extract and *C. viscosum* leaf extract could reduce disease incidence in tomato plants. Neem extract could reduce disease incidence up to 90% where as *C. viscosum* leaf extract could reduce disease incidence up to 80% (Table 13). The reduction of the disease incidence was due to reduced population of insects as observed after application of the plant extracts. Successful inhibition of infection of some viruses on some crops has been reported by several workers (Verma *et al.,*

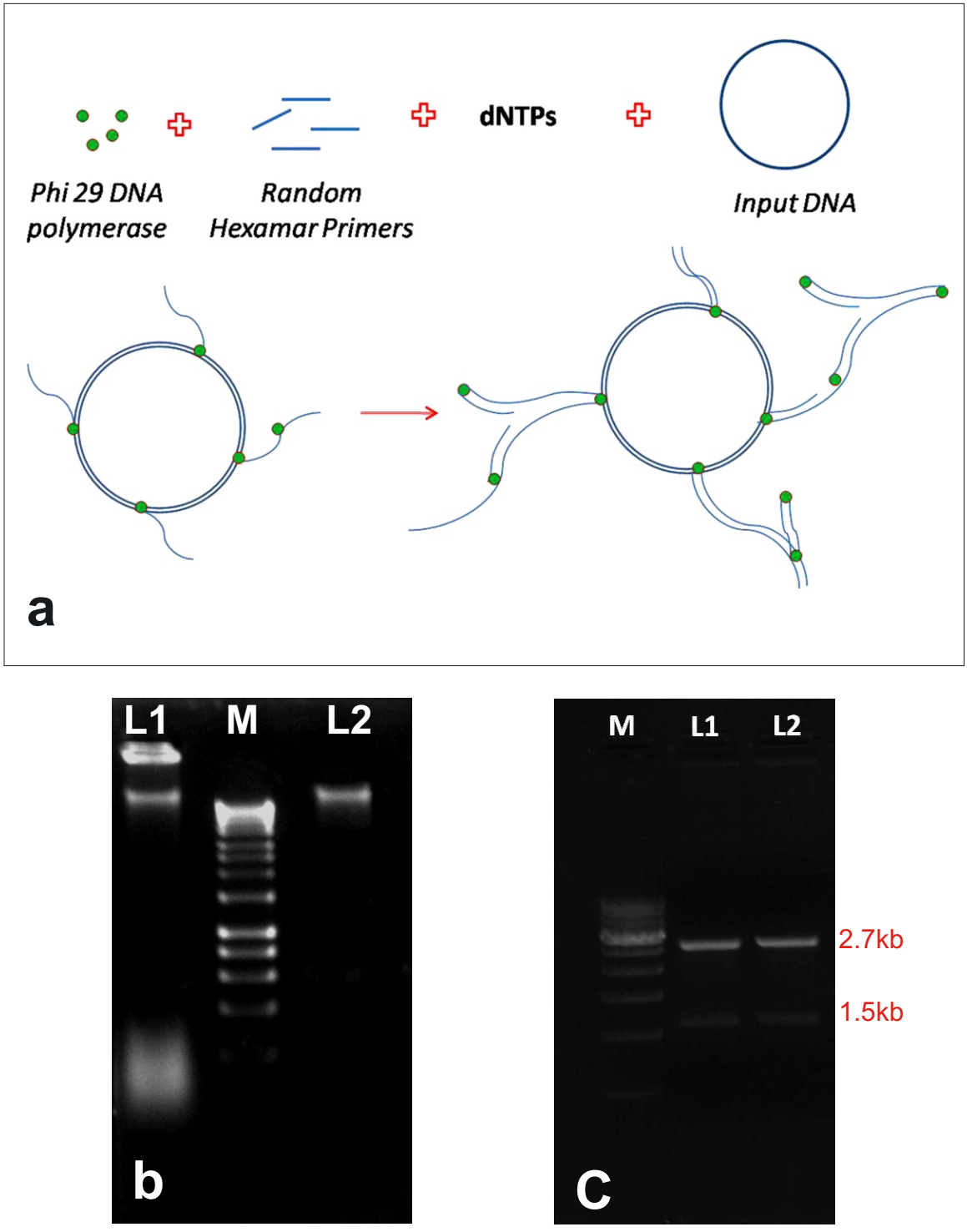

Fig. 8: (a) Schematic representation of the TempliPhi amplification process where random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers. When the DNA polymerase reaches a downstream extended primer, strand displacement synthesis occurs. The displaced strand is rendered single-stranded and available to be primed by more hexamer primer. The process continues, resulting in exponential, isothermal amplification. (b) agarose gel electrophoresis of RCA (rolling circle amplification) amplified products (L1=COB-5 sample, L2= GUW-2 sample). (c) RCA products (L1= COB-5, L2=GUW-2 and M= 500bp marker) after restriction digestion with BamH1.

1985; Barakat, 1988; Singh *et al.,* 1988a; Alexandre *et al.,* 1989 and Shaikh, 1996).

Table13: Effect of botanicals on ToLCV disease incidence

Serial numbers of Treatments	Leaf extracts of	% disease incidence	% disease reduction
1	*Eucalyptus tereticornis*	95	5
2	*Clerodendron viscosum*	20	80
3	*Camellia sinensis*	100	0
4	*Azadirachta indica*	10	90
5	*Datura stramonium*	100	0
6	*Xanthium strumarium*	100	0
7	*Polyalthia longifolia*	100	0
8	*Crotalaria pallida*	95	5
9	*Mitracarpus hirtus*	100	0
10	*Piper betle*	95	5
Control	Water	100	0

Leaves were extracted in sterile distilled water; Spraying were done at 10 days intervals.

Sub-Himalayan plains of east India is a geographically isolated area. In West Bengal it is surrounded by Himalayas in one side and the river Ganges in other side. In Assam it is the Brahmaputra valley which is limited by Himalayas in the north and by hills of Meghalaya in the south. This area is suitable for production of tomato throughout the year. Whiteflies are one of the most important pests worldwide in subtropical and tropical agriculture as well as in green house production systems. It adapts easily to new host plants as well as in new geographical regions and has now been reported from all over the world (Chandel *et al.,* 2010).

The occurrence and genomic pattern of tomato infecting begomovirus complexes from several places of sub-Himalayan plains of east India have been shown in the present study. CP genes are most conserved gene in *Geminiviridae* (Wyatt and Brown, 1996). For preliminary virus identification CP gene sequences can be used (Brown *et al.,* 2001). For establishing new begomovirus species and for definite classification complete sequence of DNA-A is necessary (Fauquet *et al.*, 2008). As the CP genes are highly conserved among begomoviruses, the identity of the begomoviruses can be predicted through CP gene sequence analysis when complete DNA A sequences is unavailable (Santoso *et al.,* 2008; Samad *et al.,* 2009; Haider *et al.,* 2007; John *et al.,* 2006).

In phylogenetic tree the begomovirus complexes isolated from seven different places were clustered in a single group. Isolate COB-2 and isolate COB-6 clustered with each other in phylogenetic tree when coat protein gene analysis was done. But COB-2 showed highest nucleotide sequence identity with ToLCNDV and COB-6 showed highest nucleotide sequence identity with TbCSV. The above observation led us to conclude that COB-2 and COB-6 although close to each other in phylogenetic tree analysis but they differed in nucleotide sequence alignment. Hence, recombination could have been take place in begomovirus complexes. Similarly recombination may be present in GUW-1 and GUW-2 isolates. Interestingly, begomovirus complexes associated with isolate COB-5 is

clustered with TbCSV which infect pepper in China. This indicates that the present isolate may be a strain of TbCSV. These results support the fact that the begomovirus complexes present with the isolates in Sub-Himalayan plains of east India may be an isolate of tomato infecting begomoviruses present in nearby states or may be a recombinant new strain. Survey of sub-Himalayan East Indian plains demonstrated that the distribution of TLCD and whitefly *B. tabaci* biotype B were evident in the present study area. Stonor *et al.* (2003) while studying occurrence of TLCV in Australia showed that spread of the disease was by *B. tabaci* biotype B in some area while another area was infested by another biotype which was not so severe in transmitting diseases.

Whitefly-transmitted (WFT) geminiviruses, or subgroup II of *Geminiviridae*, cause serious diseases in vegetable and fibre crops and are emerging viral pathogens in tropical and sub-tropical regions (Brown, 1994; Brown and Bird, 1992). Identification of WFT geminiviruses by traditional virological methods has proven difficult. Many of the most economically important WFT geminiviruses are not readily mechanically transmitted. Therefore, use of definitive bioassay hosts for virus identification and evaluation of host range and other biological properties has been very difficult in many cases.

In the present study, screening of ten plant species by mechanical sap inoculation has proved that tomato infecting begomovirus complexes may transmit by mechanical way. Eight out of ten different plants were infected by the virus, indicated the wide presence of the virus. Among the ten potential crops, tomato was found to be the major host. Further, analysis of begomovirus complexes needs whole genome sequence analysis.

Chakraborty *et al.* (2003) were unable to transmit *Tomato leaf curl Gujarat virus* (ToLCGV)-[Var] from tomato to okra, cotton, *Nicotiana glutinosa* by mechanical transmission but El-Dougdoug *et al.* (2009) successfully transmitted *Squash leaf curl virus* (SqLCV) in members of the family Cucubitaceae, Fabaceae, and Chenopodiaceae by syringe injection and also by whitefly transmission. El-Dougdoug *et al.* (2006) was also

successful in transmitting *Tomato yellow leaf curl virus* (TYLCV) from infected tomato plants to healthy tomato and *Datura stramonium* by syringe injection using a buffer (0.1 M phosphate buffer pH 7.2 and infected sap, 1:1::W/V) as reported by Allam *et al.* (1994). Essam El-Din *et al.* (2004) transmitted Tomato mechanically by using Piven's buffer (Piven *et al.*, 1995) but they could not transmit the virus mechanically by using Abdel-Salam's buffer (Abdel-Salam, 1990).

Presently, recombination between *Begomovirus* DNA-A molecules has been experienced (Fondong *et al.,* 2000; Kirthi *et al.,* 2004; Maruthi *et al.,* 2004; Padidam *et al.,* 1999). Although, CP sequence is not only sole representative of the full DNA-A genome and could be inaccurate for species demarcation. Still, it has been adopted by several scientists to enable wider scale studies (Reddy *et al.,* 2005; Paul *et al.,* 2012). Warm tropical climate of India supports year round survival of the whitefly vector and intensive cultivation of crops. This has led to severe attack of several crops by begomoviruses and more so due to several recombinants of the virus present in a geographic region. The north Bengal plains and Brahmaputra valley of Assam is unique in geography and in climate and that led us to study the occurrence and distribution of tomato infecting begomoviruses. It has also been shown that the polyphagous nature of the vector Bemisia tabaci helped in sustenance of begomovirus causing TLCD in alternative hosts growing in and around tomato fields (Borah and Dasgupta, 2012).

The results of the present study revealed that tomato infecting begomovirus complexes are geographically highly diverse among themselves throughout north-east Indian plains. On the basis of CP genes we have got tomato leaf curl disease causing begomoviruses throughout north-east Indian plains but we have also experienced that recombination in the nucleotide sequences happened in the past naturally.

Several begomovirus infecting tomato plants causing tomato leaf curl diseases have been characterized from northern and southern India and several reports on new strain have been documented. Kumari *et al.* (2010)

reported a new begomovirus species causing tomato leaf curl disease in Ranchi, India and Reddy *et al.* (2005) reported only one begomovirus infecting tomato in Assam and WB states of north-east India. From the above studies it was evident that works on begomovirus infecting tomato was not done extensively and that has been done in the present. Thus present work significantly adds informations from sub-Himalayan east-Indian plains to the existing knowledge of occurrence and distribution of the virus.

4.2 PART II: Potato leaf curl disease

Potato (*Solanum tuberosum* L.) is one of the important and widely cultivated vegetable crops of India. India's total production of potato ranks fourth in the world although in cultivated-area wise it ranks third (Bansal and Trehan, 2011). In India, West Bengal is the 2nd largest producer of potato. The cultivation of potato is often affected due to attack of various diseases caused by fungi, bacteria, viruses and nematodes (Khurana *et al.*, 1998). Severe mosaic caused by *potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV) alone can reduce the yields up to 70-80% while mild viruses, like PVX, PVS and PVM can also depress yields by 10-30% (Bhat *et al.*, 2010). Leaf curl disease of potato showed typical symptoms of begomoviruses. Leaf curl disease of potato has been reported from India and Pakistan (Usharani *et al.*, 2004; Mubin *et al.*, 2009). *Bemisia tabaci* Biotype B was found to be a major vector for transmission of the begomoviruses in India (Banks *et al.*, 2001; Narayana *et al.*, 2006, 2007; Maruthi *et al.*, 2007).

In the present study, potato apical leaf curl disease (PALCD) was found to be one of the major diseases of potato observed during the survey of sub-Himalayan West Bengal. On the basis of suspected symptoms (apical leaf curl) of begomovirus infection the study was undertaken to confirm the presence of Begomovirus. The study includes collection of the infected samples of the sub Himalayan West Bengal, detection of the virus in the infected samples and their molecular characterization.

Symptoms and disease incidence

An apical leaf curl disease was observed during field survey of sub Himalayan West Bengal. In potato fields of Coochbehar (West Bengal, India) the incidence of the disease was about 40 to 45 % (Table-14). The percent disease incidence was calculated by total number of plants shown symptoms divided by total number of plants (in a field of 100 square meters) x 100.

Table 14: Occurrence of potato apical leaf curl disease (PALCD) in different regions of sub-Himalayan West Bengal

State	Area/location	Field symptoms	PCR	Percent disease incidence
West Bengal	Jalpaiguri	Mild ALC	-	20-33
	Siliguri	ALC, Chl, no tuber	-	24-30
	Haldibari	Mild ALC, no tuber	-	10-15
	Coochbehar	ALC, Lc, Chl	+	40-45
	Pundibari	Lc	-	28-32
	Raiganj	Upward Lc	-	30-46
	Balurghat	Mild ALC, Chl	-	43-63
	Dalkhola	Leaf rolling, Lc	-	20-54
	Alipurduar	Mild ALC	-	18-33

+ = presence of the virus; - = absence of the virus. Symptom codes: LC: Leaf curl; ST: Stunting; Chl: Chlorosis; Lc: Leaf crinkle; LR: Leaf rolling; ALC: Apical leaf curl

*Randomly one isolate from each location were taken for molecular based study

The characteristic symptoms observed on naturally infected potato plants were apical leaf curl associated with crinkled leaves and mosaic (Fig. 9b). It was also observed that the size of the potato tubers of the infected plants were significantly smaller than that of healthy plants of the same fields.

Transmission efficiency

The disease was successfully transmitted to healthy potato plants using both whitefly vector *B. tabaci* and mechanical sap inoculation using sap of infected potato leaves. In case of white fly transmission technique all the 20 plants tested showed characteristic visible symptom of upward curling of margins of potato leaves. Upward curling was visible after about 10 days of inoculation. Severe symptoms of leaf curling were observed after about 30 days of inoculation. In case of mechanical transmission, the inoculated plants showed distinct visible symptoms of leaf curl after 20 days of incubation. The symptoms resulted both from artificial insect

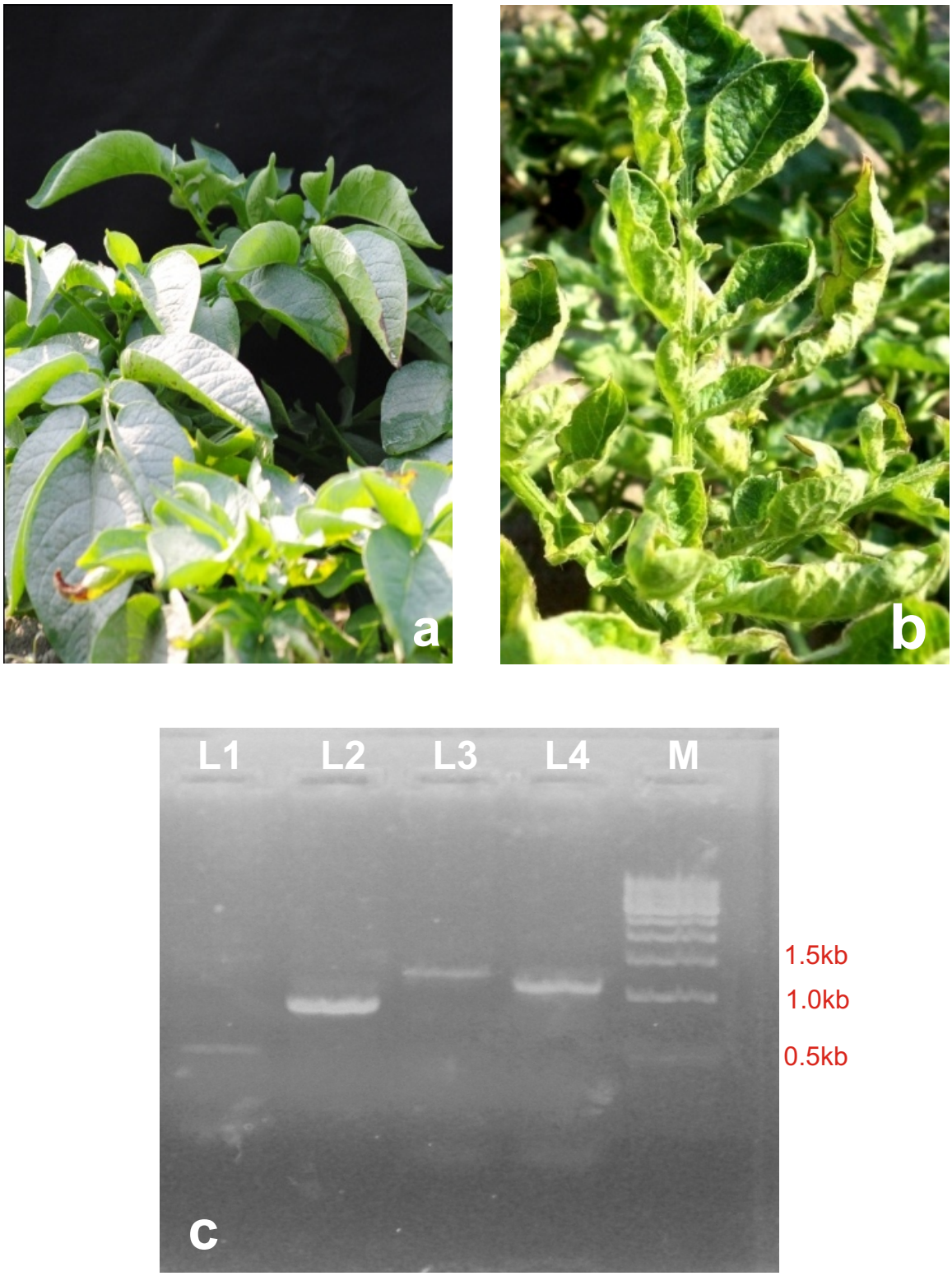

Fig. 9: (a) Healthy potato plants in field,(b) Mosaic and curling of potato leaves and (c) agarose gel electrophoresis of PCR amplified products using four set of primers (L1=Deng A/Deng B, L2= CRv301/Crc1152, L3=PALIc1960/PARIv722, L4= BF518/BR16141). M= DNA Ladder 500bp.

inoculations and mechanical sap inoculations were of similar nature (Table-15).

Table 15: Transmission of PALCD and percent symptom development by the virus in two different techniques (mechanical inoculation and whitefly inoculation)

Mode of transmission	No. of plants inoculated	No. of plants showed visible Symptoms (after 60 days)	Percent transmission efficiency of the experiment.
Mechanical inoculation	10	4	40
Control	20	0	0
Inoculation by white fly	10	10	100
Control	20	0	0

PCR amplification

DNA were extracted from the infected samples collected from different places of the present study area. Specific genes of the suspected virus were amplified by polymerase chain reaction (PCR). The amplified products were allowed to run on agarose gel. Molecular weights were determined by using standard DNA-molecular weight markers (500bp ladder). Three different types of primers for DNA-A and one primer for DNA-B were used for the purpose of amplification as suggested by Reddy *et al.* (2005). The expected sizes of the amplified DNA-A by the three primers were ~530bp, ~870bp and ~1280bp. Similarly, amplified DNA-B was ~1120bp long. The expected sizes of the amplicons were in conformity with that of Reddy *et al.* (2005). Hence, the samples were considered to contain virus with the expected DNA genomes. The samples containing viral DNA were cloned and then sequenced.

Sequencing and phylogenetic analysis

Following cloning and sequencing, the sequences were analyzed using BLAST at the NCBI website (http://www.ncbi.nlm.nih.gov/). All the sequences thus obtained were again subjected to multiple sequence alignment using CLUSTAL W in MEGA *version* 4.0 software. The common regions from all the sequences were omitted and a sequence of 1539 nucleotide long containing pre-coat protein, coat protein, AC5, AC3, AC2 and partial AC1 gene of DNA-A segment and 1001 nucleotide long containing BV1 and BC1 genes (partial) were submitted to the GenBank after proper annotations. The accession numbers for these submissions are HQ597033 for DNA-A and JN390432 for DNA-B (Table-16). Basic local alignment search tool (BLAST) analysis of the virus showed highest sequence identity with *Tomato leaf curl New Delhi virus* (ToLCNDV) isolates. In phylogenetic analysis also the virus sequences clustered with the ToLCNDV isolates (Fig. 10 & 11). Twenty five different virus sequences from GenBank were used for the study (Table 17).

Table 16: Sequences of *Begomovirus* isolated from potato of the present study.

Sl No.	Accession Number	Title	Host	Status
1	HQ597033	Tomato leaf curl New Delhi virus isolate COB-3 pre-coat protein (AV2), coat protein (AV1), AC5 (AC5), AC3 (AC3), and AC2 (AC2) genes, complete cds; and AC1 (AC1) gene, partial cds	*Solanum tuberosum*	Published on 25-May-2011
2	JN390432	Tomato leaf curl New Delhi virus isolate COB-3 nuclear shuttle protein (BV1), partial cds and movement protein (BC1), partial cds	*Solanum tuberosum*	Published on 13-Dec-2011

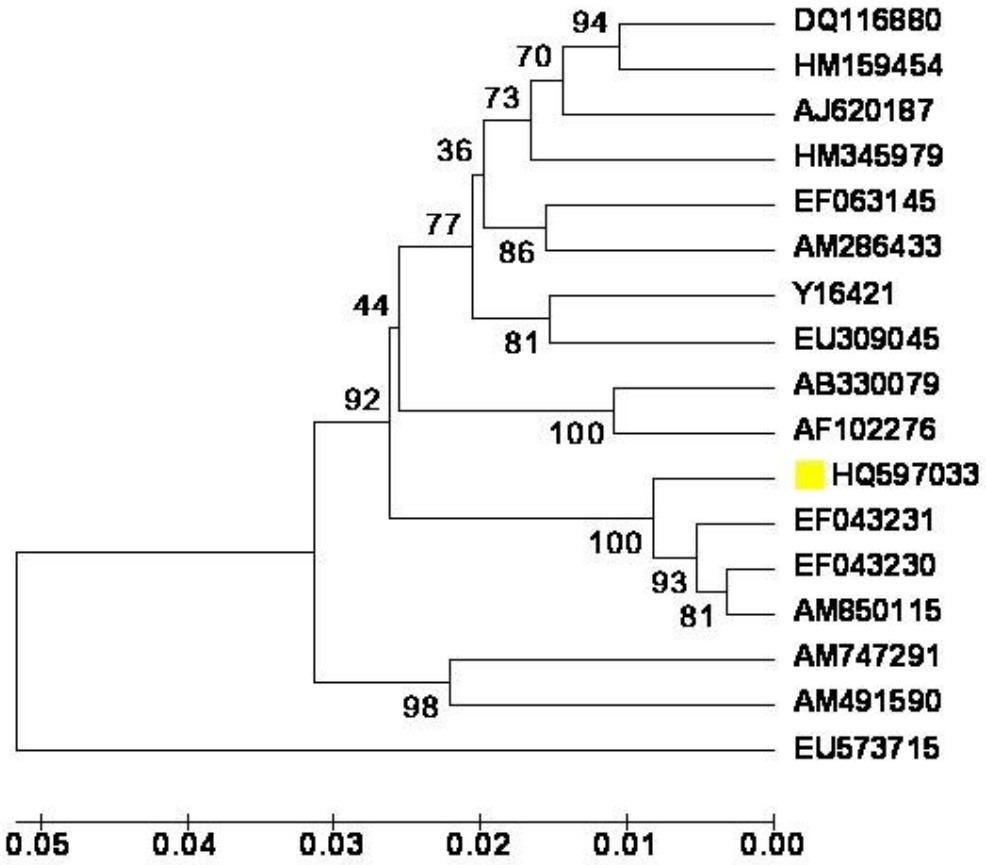

Fig. 10: Phylogenetic relationship of *Tomato leaf curl New Delhi virus* recorded from cultivated potato in sub-Himalayan West Bengal, India with other *Begomovirus* isolates/species based on DNA-A containing pre-coat protein (AV2), coat protein (AV1, AC5, AC3,AC2 and partial AC1) gene using maximum parsimony in MEGA version 4.0 program. The significance of the nodes was estimated with 100 bootstrap repetitions.

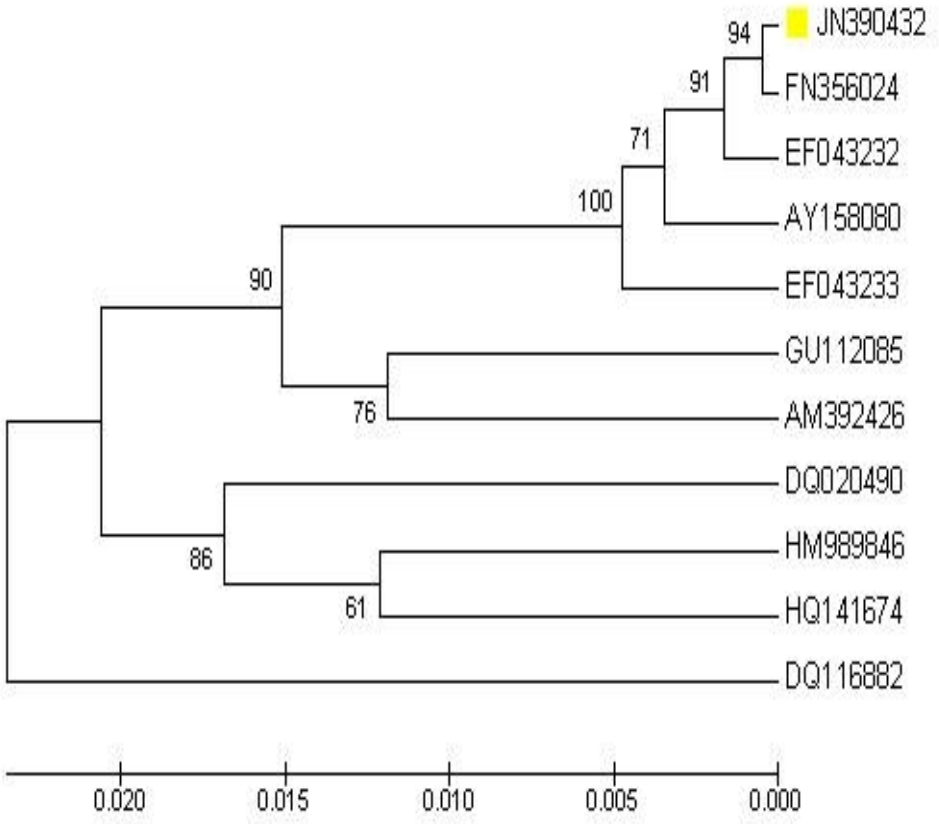

Fig. 11: Phylogenetic relationship of *Tomato leaf curl New Delhi virus* recorded from cultivated potato in sub-Himalayan West Bengal, India with other *Begomovirus* isolates/species based on DNA-B containing BV1 and BC1 gene using maximum parsimony in MEGA version 4.0 program. The significance of the nodes was estimated with 100 bootstrap repetitions.

Table 17: Different virus sequences from GenBank used for the study

Accession No.	Virus Name	Host	Area
DQ116880	*Tomato leaf curl New Delhi virus* – chili pepper	*Capsicum annuum*	Pakistan: Khanewal
HM159454	*Tomato leaf curl New Delhi virus*- Severe	*Lycopersicon esculentum*	India
AJ620187	*Tomato leaf curl New Delhi virus*	*Solanum nigrum*	Pakistan
HM345979	*Tomato leaf curl New Delhi virus*	*Lycopersicon esculentum*	India: Maharashtra, Pune
EF063145	Tomato leaf curl New Delhi virus - India	*Gossypium hirsutum*	India
AM286433	*Tomato leaf curl New Delhi virus* [Pumpkin: New Delhi]	*Cucurbita maxima*	India:New Delhi
Y16421	*Tomato leaf curl New Delhi virus*- [Lucknow]	Not mentioned	India: Lucknow
EU309045	*Tomato leaf curl New Delhi virus*- Chilli pepper	*Capsicum annuum*	India
AB330079	*Tomato leaf curl New Delhi virus*- [cucumber: Thailand]	*Cucurbita maxima*	Thailand
AF102276	*Tomato leaf curl New Delhi virus* - [Luffa]	*Luffa acutangula*	Thailand
EF043231	*Tomato leaf curl New Delhi virus* - [Potato]	*Solanum tuberosum*	India
EF043230	*Tomato leaf curl New Delhi virus* - [Potato]	*Solanum tuberosum*	India
AM850115	*Tomato leaf curl New Delhi virus*	*Solanum tuberosum*	India:Himachal
AM491590	*Bitter gourd yellow vein Virus*- [Pakistan: Lahore: 2004]	*Momordica charantia*	Pakistan:Lahore
EU573715	*Squash leaf curl China virus* - [Pumpkin: Varanasi]	*Cucurbita maxima*	India:Varanasi
FN356024	*Tomato leaf curl New Delhi virus*	*Solanum tuberosum*	India:Himachal
EF043232	*Tomato leaf curl New Delhi virus* - [Potato]	*Solanum tuberosum*	India
AY158080	*Tomato leaf curl New Delhi virus* - [Potato]	*Solanum tuberosum*	India
EF043233	*Tomato leaf curl New Delhi virus* - [Potato]	*Solanum tuberosum*	India
GU112085	*Tomato leaf curl New Delhi virus* [India: Bangalore: OY135: 2005]	*Abelmoschus esculentus* cultivar Arka Anamika	India: Bangalore, Karnataka
AM392426	Tomato leaf curl New Delhi virus [Multan: Duranta repens]	*Duranta repens*	Pakistan:Punjab, Multan

Contd...

Table 17(contd...): Different virus sequences from GenBank used for the study

DQ020490	*Tomato leaf curl New Delhi virus* - Bitter Gourd	*Momordica charantia*	India
HM989846	*Tomato leaf curl New Delhi virus*-JLH13	*Luffa acutangula*	India: New Delhi
HQ141674	*Tomato leaf curl New Delhi virus*-JID17	*Lycopersicon esculentum*	India: Pune
DQ116882	*Tomato leaf curl New Delhi virus* - chili pepper	*Capsicum annuum*	Pakistan: Khanewal

The most conserved gene of Geminiviridae is CP gene. For provisional identification of virus and to infer geographic and vector relationship CP gene sequences can be used (Brown *et al.*, 2001). But for virus identification complete sequence of at least DNA-A is necessary (Fauquet *et al.*, 2008). In the present study the phylogenetic analysis of CP sequences along with pre-coat protein (AV2), AC5, AC3, AC2 and partial AC1 were sequenced to identify the virus and to compare with different sequences of begomoviruses of GenBank. Additionally, DNA-B of the virus containing partial BV1 and BC1 were also sequenced. All the above sequences were compared with 16 closely related sequences of begomovirus present in the GenBank. Amplified DNA-A showed highest 98% nucleotide sequences identity with that of *Tomato leaf curl New Delhi virus* (ToLCNDV). Amplified DNA-B showed highest 99% nucleotide sequence identity with the ToLCNDV. In phylogenetic analysis also the virus sequence is clustering with the ToLCNDV isolates. It is a first record of begomovirus infection of cultivated potato in sub-Himalayan West Bengal of Eastern India.

Apical leaf curl of potato has been reported by some authors. From, Hissar, Lakra (2002) reported heavy yield losses in susceptible potato varieties due to a virus, which they reported as apical leaf curl virus. Similar observation was recorded by Garg *et al.* (2001) also. Presence of 40-75% disease incidence of PALCV was reported by Venkatasalam *et al.* (2005) from some cultivars of Indo-Gangetic plains. *Tomato leaf curl new Delhi virus* from potato apical leaf curl diseased samples was detected by Gawande *et al.* (2007) following print capture PCR. The association of begomovirus with apical leaf curl disease of potato was reported by

Venkatasalam *et al.* (2011). They used ToLCNDV specific primer pair and ToLCNDV specific probe. The association of geminivirus (white fly transmitted begmovirus) with leaf curl disease of potato by using polyclonal antibody of *Indian cassava mosaic virus* in immunoelectron microscopy test has also shown by Garg *et al.* (2001). They named the disease as potato apical leaf curl virus based on symptomology of the virus. Using DAS ELISA test Singh and Venkatasalam *et al.* (2005) reported the affinity of leaf curl disease of potato towards tomato yellow leaf curl virus. The affinity also led them to assume that the virus was a *Geminivirus*.

Usharani *et al.* (2004) reported a leaf curl disease in potato caused by a strain of *Tomato leaf curl New Delhi virus* having accession numbers AY286316 [DNA-A (partial)] and AY158080 [DNA-B (partial)]. Our virus isolate having accession number JN390432 (DNA-B) showed homogeneity with DNA-B sequence under same cluster. DNA-A (accession number HQ597033) of the virus showed unrelatedness while clustering. This is a first observation of begomovirus causing a severe disease of potato in north-Eastern India. The nucleotide sequence data indicated that the causal organism is a virus closely related to ToLCNDV. The virus isolate showed sap transmissibility and also could infect potato plants when artificial insect transmission test was conducted. Chandel *et al.* (2010) also showed that polyphagous pest *B. tabaci* could infect numerous fiber, food, vegetable and ornamental plants in north western plains of India. Kumar *et al.* (2012) also reported that whiteflies could infect a wide spectrum of plants including potato, by ToLCV. With the above findings, it is likely that our strain is, probably, a new strain of ToLCNDV which infects cultivated potato in sub-Himalayan Eastern India through whitefly vectors. While studying diversity of a bipartite begomoviruses, Jyothsna *et al.* (2013) showed that ToLCNDV could infect potato and cucurbits showing almost similar symptoms as found in tomato, growing in nine different places of northern India. It was also noticed that infection occurred by the present strain in a geographic region of Coochbehar which is infested with white fly vector. Among the ten geographic region of sub-Himalayan West Bengal, studied, Coochbehar being the farthest from the Himalayas and having slightly different climatic conditions than the other nine regions indicated

that geographic condition is also a factor for virus infection and disease development in potato. In Coochbehar, tomato and potato are grown in nearby fields and the place is infested with several strains of ToLCNDV (isolated from tomato leaves). Most likely the viruses from the tomato plants serve as reservoirs for infection of the potato plants in the area. Similar observation was found by Sohrab *et al.* (2013). They reported that ToLCNDV and Potato apical leaf curl disease (PALCD) in the northern India could easily be transmitted by whitefly to potato showing PALCD. The overlapping planting and harvesting of the crops was possibly the reason of transmission (Sohrab *et al.*, 2013). Thus our results of the present study significantly similar with that of Sohrab *et al.*, (2013).

4.3 PART III: Begomoviruses of some noncultivated plants and weeds

High incidence of begomoviruses transmitted by white flies (*Bemisia tabaci*) is one of the formidable biotic constraints of crop production in the sub-Himalayan West Bengal and Brahmaputra valley of Assam. Most of the present study areas have a climate that is conducive to year–round vegetable production. Substantial losses due to viral diseases have also been experienced in the area. Salati *et al.* (2002) also reported the probable contribution of begomoviruses to the epidemic disease status of cultivated and non-cultivated plants. Other than epidemiological importance, weeds that harbor dual or multiple begomovirus infections may facilitate recombination between the constituent begomoviruses, resulting in the emergence of recombinant viruses (Mendez-Lozano *et al.,* 2002). It is believed that the emergence of tomato-infecting begomoviruses was the result of horizontal transfer of indigenous viruses that infect wild or weed hosts by the new biotype of the whitefly vector. Following entry into new host the indigenous viruses would have rapidly evolved via recombination and pseudorecombination, giving rise to the species currently detected in the field (Castillo-Urquiza *et al.,* 2008). It has also been reported that a number of common weeds serve as alternate hosts as well as reservoirs for many crop-infecting begomoviruses (Roye *et al.*, 1997; Sanz *et al.*, 2000). Several weeds frequently harbor multiple viruses, resulting in the possibility of emergence of new recombinant strains (Mubin *et al.*, 2010; Umaharan *et al.*, 1998).

The agro-climatic conditions of north-east India has ideal conditions for plant viruses to attack. Although it has been established that weeds can play an important role in the emergence of plant viral epidemics affecting crops in different parts of the world (McLaughlin *et al.*, 2008; Rojas *et al.*, 2000) but they are still largely neglected. Only limited work has been carried out to characterize the begomovirus complexes associated with different weed species in India (Paul *et al.,* 2012).

Considering the above informations, the present study was under taken. Uncultivated virus infected plants like *Datura stramonium*, *Jatropha curcas*, *Croton bonplandianus*, *Acalypha indica*, *Ageratum conyzoides* were

selected for detection of viruses (Fig. 12 & 13). Molecular characterizations of the viruses were also thought to be performed using universal begomovirus primers for partial DNA-A genomes. Before going into details of the molecular studies, occurrence of the diseases caused by begomoviruses were studied in different locations. The study was made on the basis of observations of typical symptoms of begomoviruses. Representative samples of each location were subjected to PCR amplifications for expected size of amplicons. The samples which showed band of expected size in agarose gel were considered PCR positive [*i.e.* presence of begomovirus(s)]. All together, eight different locations were selected on the basis of severity of viral diseases. In the entire places virus affected plants were found but representative samples of four different places could amplify the virus genes by the primers used in the study (Table-18). Further, experiments were conducted by those PCR positive samples only.

PCR detection of the virus

Three sets of universal degenerate primers were used for amplification and confirmation of the partial coat protein, coat protein and core coat protein genes of viruses from the infected *J. curcas, D. stramonium, C. bonplandianus*, *A. indica* and *A. conyzoides* plants. Expected amplicon of ~530 bp was successfully amplified using DengA and DengB primers from the samples to confirm the presence of begomovirus. For provisional identification of *Begomovirus* from the infected samples, CRV301 and CRC1152 primer pair was used for amplification of coat protein and core coat protein genes. An expected viral amplicon of ~870bp was successfully amplified only from infected plant species *D. stramonium*. On the other hand, an expected viral amplicon of ~575 bp was successfully amplified from infected *C. bonplandianus*, *A. indica* and *A. conyzoides* with symptoms of 'yellowing of the vain' and 'mosaic'.

Fig. 12: *Croton bonplandianus,* (a) healthy plant; (b) naturally virus infected plant. (c) *Ageratum conyzoides* (healthy plant), (d) Naturally virus infected *A. conyzoides* plant.

Fig. 13: (a) *Acalypha indica* plant (healthy), (b) *A. indica* plant (infected), (c) *Datura stramonium* twigs (healthy), (d) *D. stramonium* twigs (infected), (e) *Jatropha curcas* leaf (healthy), (f) *J. curcas* leaf (infected).

Table 18: Occurrence of begomoviruses of some noncultivated plants and weeds of different regions of sub-Himalayan West Bengal.

State	Area/location	Host	Field symptoms	PCR*	Disease incidence
West Bengal	Coochbehar	*Datura stramonium*	Upward LC	+	38-89
	Siliguri	*Jatropha curcas*	Upward LC, Chl, no fruit	+	24-61
	Haldibari	*Croton bonplandianus*	Severe mosaic	+	30-86
	Jalpaiguri	*Acalypha indica*	Mild LC	-	20-33
	Pundibari	*Ageratum conyzoides*	LC, ST	-	28-43
	Raiganj	*Ageratum conyzoides*	Upward LC	-	30
	Balurghat	*Ageratum conyzoides*	Severe mosaic	+	50-63
	Dalkhola	*Croton bonplandianus*	Leaf rolling, LC	-	20-54
	Alipurduar	*Datura stramonium*	Mild LC	+	18-33

LC: Leaf curl, ST: Stunting, Chl: Chlorosis, LR: Leaf rolling
* One isolate from each location were taken randomly for molecular based study

Analysis of partial coat protein, coat protein and core coat protein gene sequences:

In order to know the relationship of begomoviruses infecting the plants in sub Himalayan West Bengal the amplified products were cloned following blue white screening (Fig.14c) and then sent for sequencing. Sequenced genes were subjected to annotations. After proper annotations and multiple sequence alignment, the sequences were submitted in the GenBank and subsequently accession numbers were assigned by the GenBank to each valid samples. The viral gene isolate from *J. curcas* contained a nucleotide sequence of 495bp [Accession no. HQ597029 containing two ORFs (AV2 and AV1 partial)]. It showed highest 95% nucleotide sequence identity with *Jatropha mosaic India virus*-[Lucknow, strain SK-2, segment DNA-A (Accession no. HM230683)]. Bird (1957) and Roye *et al.* (2006) also reported the occurrence of *Jatropha mosaic virus* from a weed plant (*Jatropha gossypifolia*) from Puerto Rico and Jamaica respectively. The occurrence of begomovirus infection in *Jatropha curcas*

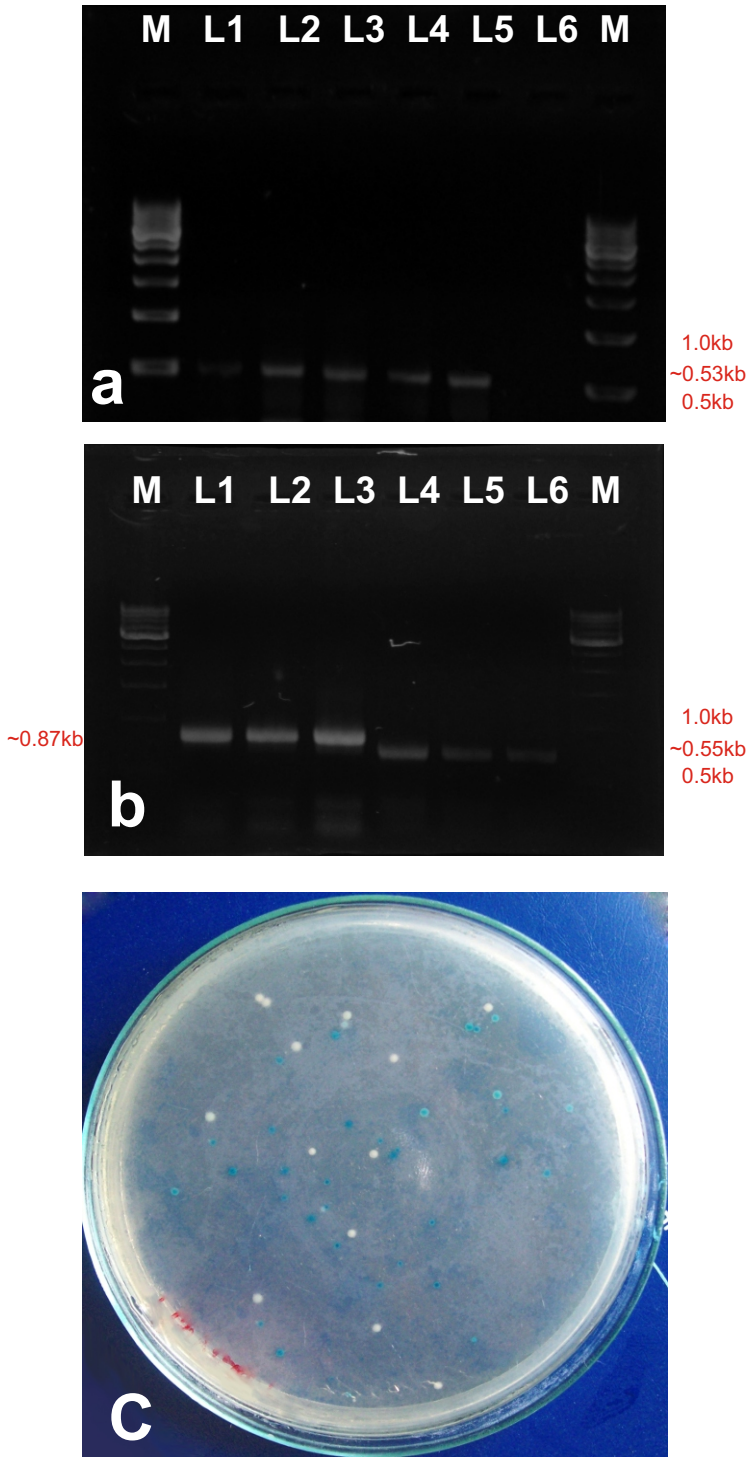

Fig.14 : (a) Agarose gel electrophoresis of PCR amplified products of different plant samples using Deng A/Deng B universal primers. [L1=*Croton bonplandianum*, L2=*Datura stramonium*, L3=*Jatropha curcas*, L4=*Acalypha indica*, L5=*Ageratum conyzoides*] (b) Agarose gel electrophoresis of PCR amplified products of *Datura stramonium* plant sample [(L1-L3; using primer pair CRv30/Crc1152) and agarose gel electrophoresis of PCR amplified products of *Acalypha indica* (L4), *Croton bonplandianum* (L5) and *Ageratum conyzoides* (L6) using primer pair AV494/AC1048, M= DNA Ladder 500bp. (C) Blue-white screening of clones in LB plates containing ampicillin, IPTG and X-Gal.

causing mosaic and stunting of the plant has been reported by several workers (Raj *et al.,* 2008; Tewari *et al.,* 2007; Gao, 2010). From northern India, *Jatropha mosaic India virus* (JMIV) has been reported by Narayana *et al.* (2006). They reported mosaic, leaf distortions, curling and blistering of *J. curcas* plants infected by JMIV.

In the present study, similar symptoms have also been observed in *J. curcas* growing in sub-Himalayan West Bengal. *Jatropha* is a perennial weed plant genus and serve as continuous source of viral inoculums.

Virus from *D. stramonium* having a nucleotide sequence of 771bp (Accession no. JN676054) contained a single ORF of full coat protein (AV1) gene. It showed highest 99% nucleotide sequence identity with *Tomato leaf curl New Delhi virus* DNA-A complete genome (Accession no. AM850115). Presence of begomovirus infection in *Datura* plant species was reported from India and other parts of the world (Costa, 1955; Ding *et al.,* 2007; Marwal *et al.,* 2012; Fiallo-Olive *et al.,* 2013).

Three infected plant samples (*C. bonplandianus*, *A. indica* and *A. conyzoides*) gave positive virus gene amplification results when primer pair 'AV494 and AC1048' was used. The GenBank accession numbers of the amplified products were JQ811770 (for 516 nucleotide amplicon from *Acalypha indica*), JQ796374 (for 555 nucleotide amplicon from *Croton bonplandianus*) and JQ843097 (for 522 nucleotide amplicon from *Ageratum conyzoides*). The core cp gene of *Acalypha* yellow vein disease-associated complex showed highest (98%) nucleotide sequence identity (nsi) with *Jatropha mosaic India virus* [isolate Katarniaghat segment DNA-A (accession no. JN135236)] and clustered with the same in the phylogenetic tree.

The core CP gene of the begomovirus associated with *Croton* yellow vein disease in the present study area (JQ796374) showed its close relationship (97% nsi) with *Croton yellow vein mosaic virus* (FN645898) which infects *Acalypha* plants in India (Zaffalon *et al.,* 2012) and *Croton* plants in India (accession no. JN817516). But in phylogenetic analysis it clustered with begomoviruses infecting papaya, tobacco, mesta and kenaf. The core CP gene of the begomovirus associated with *Ageratum* yellow vein disease of the present study area (JQ843097) showed its close relationship

(99% nsi) with both *Tobacco curly shoot virus* infecting *Ageratum* plants in China (AJ971266, FN401522) and with *Tobacco curly shoot virus* of infected pepper (GU001879) plants (Qing *et al.,* 2010). The same core CP gene showed 98% nsi with *Ageratum enation virus* (JF728866) infecting *Ageratum* plants in India and clustering with both the begomoviruses (Fig. 15). Begomovirusvirus-sequences of some noncultivated plants and weeds of the present study have been presented in Table 19. A list of virus-sequences along with their hosts and accession numbers used in phylogenetic analysis has been presented in Table 20.

Table 19: A list of *Begomovirus* sequences of some noncultivated plants and weeds of the present study

Accession No.	Virus Name	Host	Area of collection
JN676054	*Tomato leaf curl New Delhi virus*	*Datura stramonium*	Coochbehar
HQ597029	*Jatropha mosaic India virus*	*Jatropha curcas*	Siliguri
JQ796374	*Croton yellow vein mosaic virus*	*Croton bonplandianus*	Haldibari
JQ811770	*Jatropha mosaic India virus*	*Acalypha indica*	Jalpaiguri
JQ843097	*Tobacco curly shoot virus*	*Ageratum conyzoides*	Balurghat

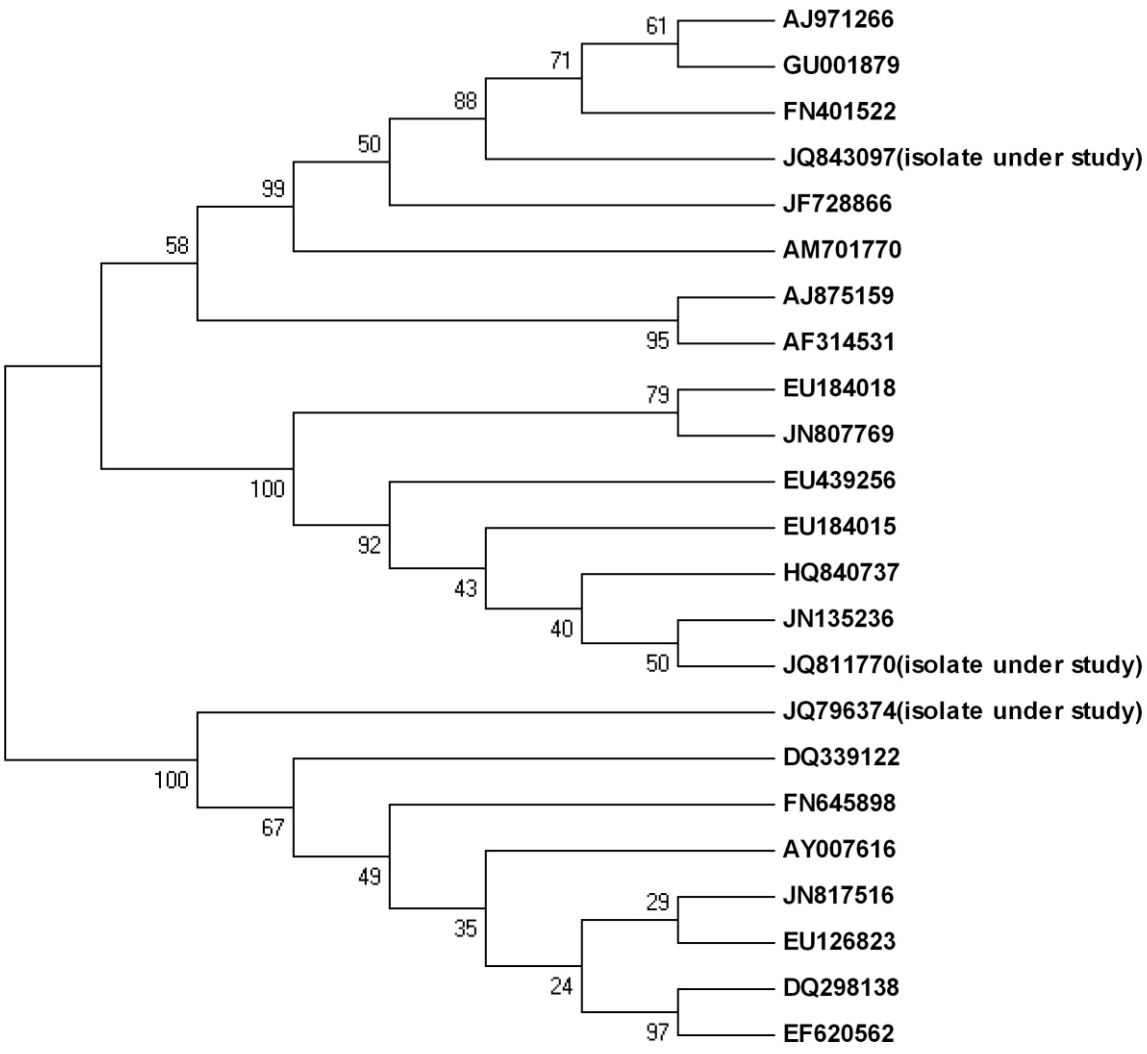

Fig. 15: Most parsimonious tree showing the relationship of core coat protein gene (JQ843097, JQ811770 and JQ796374) of isolate with other GenBank-published *Begomovirus* sequences using MEGA version 4.0 program. Numbers at nodes indicate the bootstrap percentage scores out of 1000 replicates.

Table 20: Other *Begomovirus* sequences used in phylogenetic analysis with the viruses of the present study

Accession No.	Virus Name	Host	Country
AJ971266	*Tobacco curly shoot virus*	*Ageratum conyjoides*	China
GU001879	*Tobacco curly shoot virus*	Pepper	China
FN401522	*Tobacco curly shoot virus*	*Ageratum conijoides*	China
JF728866	*Ageratum enation virus*	*Ageratum conyjoides*	India
AM701770	*Ageratum enation virus*	*Brassica rapa*	Pakistan
AJ875159	*Tomato leaf curl Joydebpur virus*	Tomato	Bangladesh
AF314531	*Pepper leaf curl Bangladesh virus*	Chilli	Bangladesh
EU184018	*Acalypha yellow mosaic virus*	*Acalypha Indica*	India
JN807769	*Acalypha yellow mosaic virus*	*Acalypha Indica*	India
EU439256	*Indian cassava mosaic virus* [Lucknow]	*Acalypha Indica*	India
EU184015	*Clerodendron yellow mosaic virus*	*Clerodendron* sp.	India
HQ840737	*Jatropha mosaic India virus* [Lucknow]	*Jatropha curcas*	India
JN135236	*Jatropha mosaic India virus*	*Jatropha curcas*	India
DQ339122	Whitefly transmitted Indian begomovirus	*Phyllanthus niruri*	India
FN645898	*Croton yellow vein mosaic virus*	*Acalypha* sp.	India
AY007616	*Tobacco leaf curl virus*	Tobacco	India
JN817516	*Croton yellow vein mosaic virus*	*Croton bonplandianus*	India
EU126823	*Papaya leaf curl virus*	*Carica papaya*	India
DQ298138	*Mesta yellow vein mosaic virus*	*Hibiscus subdariffa*	India
EF620562	*Kenaf leaf curl virus*	*Hibiscus cannabinus*	India

Although, weeds are reservoirs of begomoviruses that infect crop plants and act as “melting pots” that may have yield new viruses/virus strains by recombination and component exchange due to their frequently harboring multiple viruses, it has been largely neglected in the study of plant viruses (Mubin *et al.,* 2010). Besides cultivated plants, many weed species are also hosts for begomoviruses (Assuncao *et al.,* 2006) and characterization of those weed-infecting begomoviruses is, therefore important for elucidating their ecology and evolutionary behavior. In the present study, the genomic pattern of begomoviruses of different uncultivated crops has been done with the universal begomovirus specific primers. Several workers used DengA and DengB universal primers to prove the presence of whitefly transmitted begomoviruses (Narayana *et al.,* 2006; Raj *et al.,* 2008; Reddy *et al.,* 2005). As coat protein genes are highly conserved among begomoviruses, the identity of the begomoviruses can be predicted through CP gene sequence analysis when complete DNA-A sequence is unavailable. Reddy *et al.* (2005) used CRv301 and CRv1152 to detect the diversity and distribution of tomato infecting begomoviruses in India. The core CP primers have been illustrated to amplify a fragment for most, if not all, begomoviruses irrespective of Old or New World origin and that makes it possible to detect the viruses rapidly, which in turn helps (by prediction of provisional species) to identify the affiliation of the viruses by comparing with reference begomovirus core CP sequences (Wyatt and Brown, 1996; Brown *et al.,* 2001). All begomoviruses code for coat protein which act as protective coat of the virus particle and also determine vector transmissibility of the viruses by whitefly vector (*B. tabaci*). Several authors have reported that the CP gene is highly conserved among begomoviruses originating from the same geographical region and which helped selected local vector populations to adapt themselves for transmission of viruses while sucking of sap (McGrath and Harrison, 1995; Maruthi *et al.,* 2002). Smaller fragments comprising the core coat protein gene (core CP) sequences have also been used to establish provisional species identification due to their highly conserved nature of CP gene sequences (Govindappa *et al.,* 2011). Besides coat protein and core coat protein genes, the 200 nucleotides at the 5' region of the CP gene is highly variable and has been proposed as an informative region for prediction of taxonomic relationships of *Begomoviruses* (Padidam *et al.,* 1995; Brown *et al.,* 2001), although definite species assignment requires sequencing of complete

DNA-A, especially due to the high recombination rate of begomovirus genome (Preis and Jeske, 2003). The presence of recombinant event in the isolated viruses of the present study cannot be nullified. However, considering the high degree of identity among most isolates and previously characterized begomoviruses, tentative species assignments are possible quite reliably as suggested by Rodriguez-Pardina *et al.* (2006). In the present study area *Jatropha*, *Datura*, *Croton*, *Ageratum* and *Acalypha* plants are naturally grown as uncultivated crops and these plants have been taken into consideration for analysis of begomovirus complexes. From the present study, it is evident that *Jatropha* mosaic begomovirus complexes, Tomato leaf curl begomovirus complexes (specially Tomato leaf curl New Delhi virus), Croton yellow vein mosaic begomovirus complexes and Tobacco curly shoot begomovirus complexes (specially Tobacco curly shoot virus) are present in the study area. The associated begomovirus complexes not only infected uncultivated crops but there are a number of reports of association of these viruses with crop infection. *Tomato leaf curl New Delhi virus* is a major pathogen of tomato in India (Padidam *et al.*, 1995; Srivastava *et al.,* 1995). *Tomato leaf curl New Delhi virus* not only infects tomato but also other cultivated crops (Jyothsna *et al.,* 2013). Similarly, *Tobacco curly shoot virus* was also isolated from crops like tomato, pepper, common bean etc. (Li *et al.,* 2005; Qing *et al.,* 2010; Venkataravanappa *et al.,* 2012). Interestingly, Croton yellow vein mosaic begomovirus complexes, a major pathogen of *Croton* and of other uncultivated weeds (Snehi *et al.,* 2011; Zaffalon *et al.,* 2012; Paul *et al.,* 2012) and also of tomato (Pramesh *et al.,* 2013) was also present in the present study area. This study support the fact that uncultivated crops grown around the crop growing fields and associated weeds serve as a reservoir of multiple crop infecting begomoviruses. It was also evident from the study that similar symptoms were not produced by same viruses in different plants. Based on the above analysis, it may be suggested that the existence of genetic diversity of uncultivated crop infecting begomoviruses in sub-Himalayan north-east Indian plains need further investigation. Such knowledge will aid the development of control strategies for virus protection of cultivated crops.

4.4 PART IV: *Potyvirus* caused mosaic diseases of some cultivated crops of sub Himalayan West Bengal

Potyvirus is the largest among the six genera (*Potyvirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus*, *Tritimovirus* and *B*ymovirus) of the family Potyviridae. Some economically important species are included in this family.

Aphid-transmitted potyviruses and specifically the genus *Potyvirus* are one of the most successful groups of plant pathogens in the world. The genus has a worldwide distribution throughout higher plants. Several members are important pathogen on plants. *Papaya ring spot virus* (PRSV) has been considered the most damaging virus infecting papaya worldwide. *Turnip mosaic virus* (TuMV) is ranked second most important virus infecting field grown vegetables. *Bean common mosaic* virus infecting beans and potato infected by *Potato virus Y* are found in several places of India. PRSV is commonly grouped into two types: Type P (PRSV-p) infects cucurbits and papaya, whereas type W (PRSV-w) infects cucurbits but not papaya. The latter type was previously referred to as WMV-1.

Reports of potyvirus is available from southern part of West Bengal but no such report is available from sub Himalayan West Bengal, although, symptoms of potyvirus was experienced during survey of the fields of the present study area. The presence of the symptoms of potyviruses during survey, prompted us to detect and analyze the viruses present in the three plants (Papaya, Bean and Potato) of the present study area (Fig.16, 17a & 17c).

Survey, disease incidence and symptomatology:

Different kinds of disease symptoms like severe mosaic, ring spot symptom on the fruit, severe leaf deformation and stunted plant growth were observed in the field conditions. Samples which showed distinct visible symptoms were subjected to RT-PCR and a positive reaction in PCR amplification of the target sequence (~650nt long) specific to potyvirus confirmed the presence of potyviruses. About 41- 91% disease incidence in case of papaya, 28-83% in case of bean and 17% to 56% in case of potato have been recorded from Sub-Himalayan of West Bengal. On the basis of

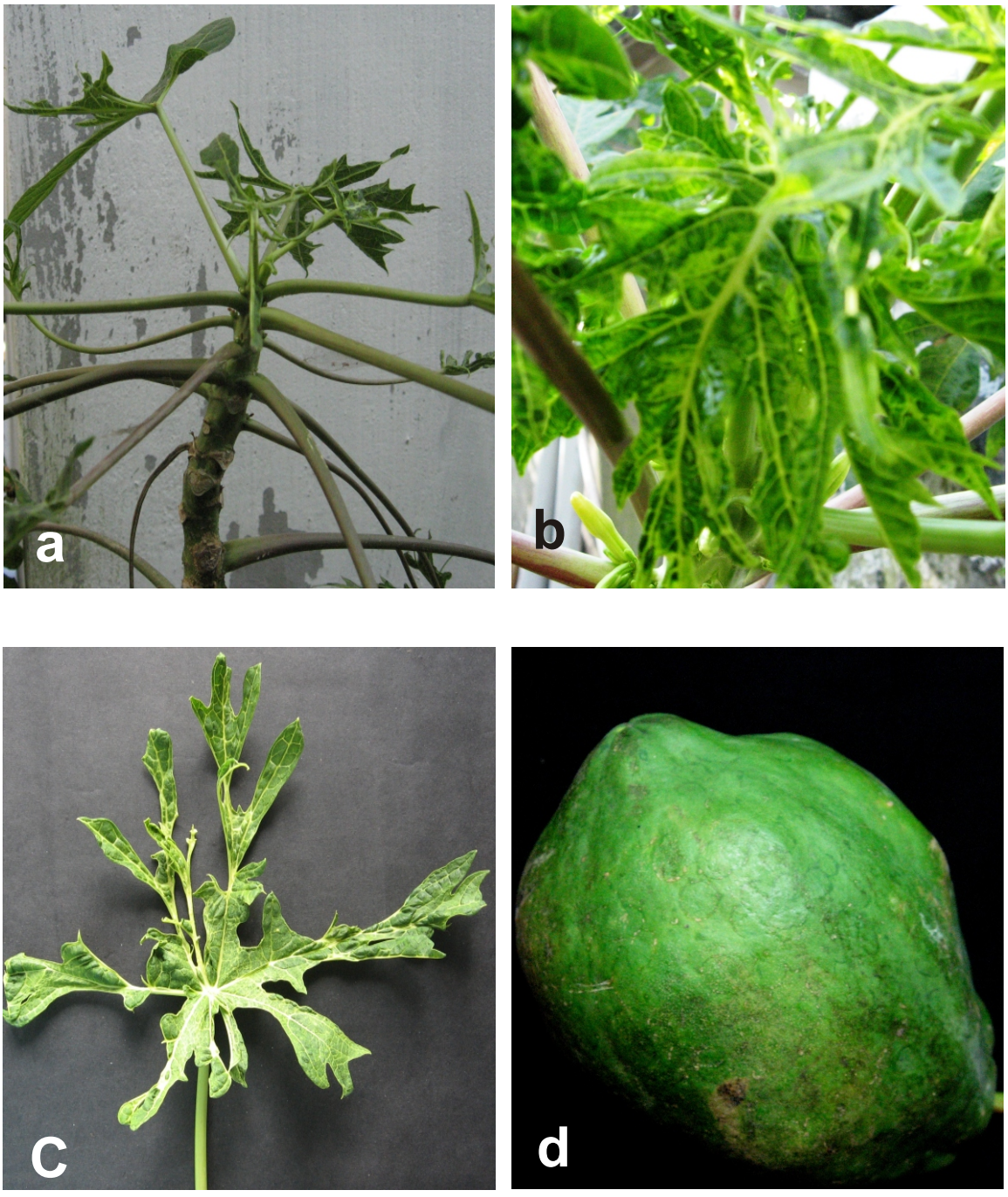

Fig. 16: Naturally infected papaya plant showing (a) severe mosaic, (b) stunted plant growth, (c) severe leaf deformation and (d) ring spot symptom on the fruit.

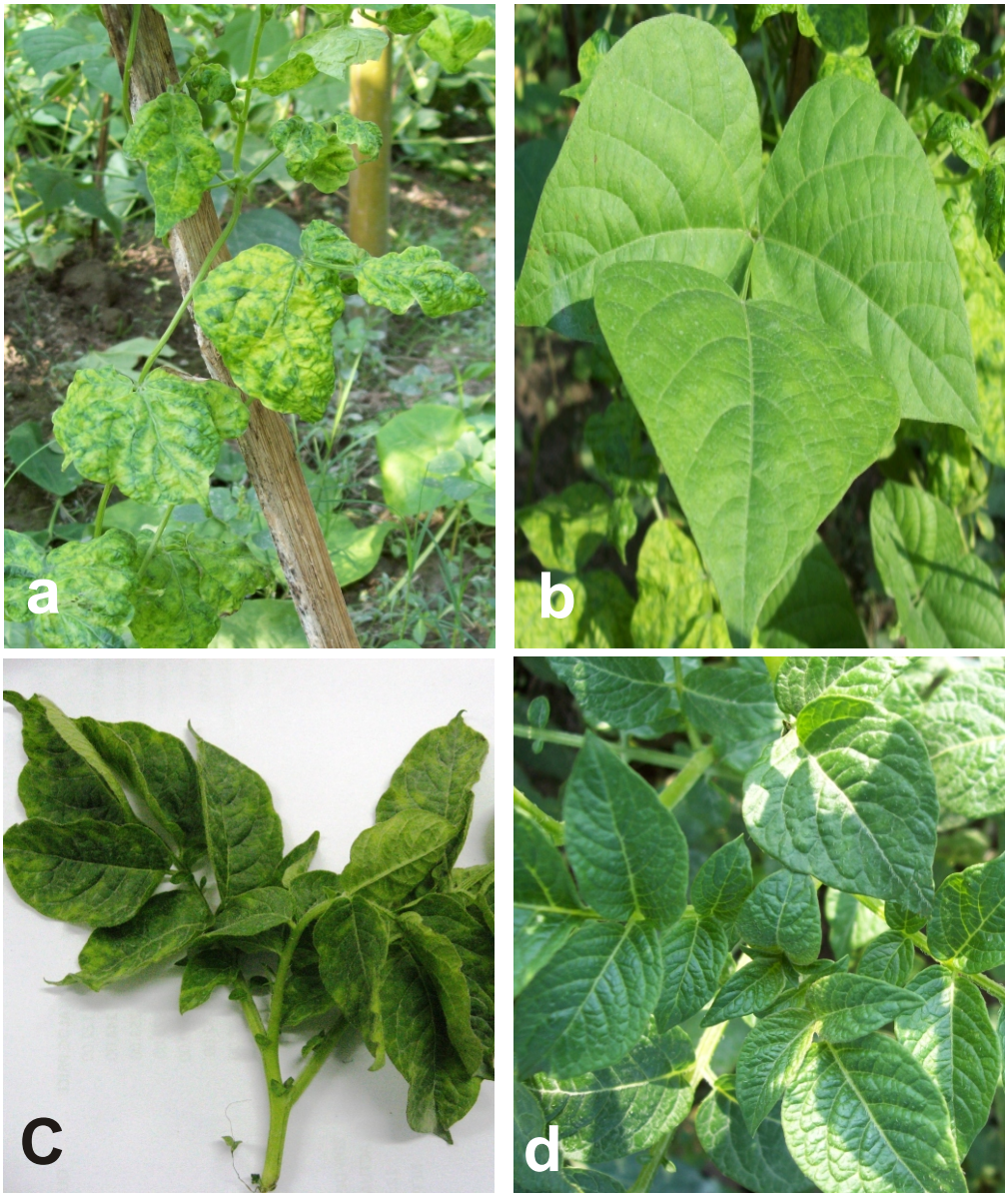

Fig. 17: Naturally infected (a) bean plant with blister and leaf mosaic, (b) healthy bean leaves, (c) potato plant with leaf mosaic and (d) healthy potato plant.

symptoms and PCR amplifications, occurrences of each of the three diseases were computed and have been presented in the tables 21, 22 and 23.

Table 21: Occurrence of *Papaya ring spot virus* (PRSV) in sub-Himalayan West Bengal

State	Area/location	Field symptoms	PCR	Disease incidence
West Bengal	Coochbehar	SM	+	41-56
	Siliguri	RS, SM, ST	+	41-91
	Haldibari	SLD	+	50-80
	Jalpaiguri	SLD	+	56-83
	Pundibari	Mosaic	-	28-43
	Raiganj	Mosaic	-	25-50
	Balurghat	RS (on the fruit)	+	43-63
	Dalkhola	SM	+	20-54

SM=severe mosaic, RS= ring spot, ST=stunted plant, SLD=Severe leaf distortion

* One isolate from each location were taken randomly for molecular based study

Table 22: Occurrence of *Bean common mosaic virus* (BCMV) in sub-Himalayan West Bengal

State	Area/location	Field symptoms	PCR	Disease incidence
West Bengal	Coochbehar	MS, ST	+	28-83
	Siliguri	MS, ST	+	34-61
	Haldibari	MS	-	30-56
	Jalpaiguri	MS	-	43-63
	Pundibari	SM	+	28-43
	Raiganj	SM	+	30-38
	Balurghat	SM	+	43-63
	Dalkhola	MS	+	40-54
	Alipurduar	MS, ST	+	28-33

SM=severe mosaic, MS= mosaic, ST=stunted plant

* One isolate from each location were taken randomly for molecular based study

Table 23: Occurrence of *Potato virus Y* (PVY) in sub-Himalayan West Bengal

State	Area/location	Field symptoms	PCR	Disease incidence
West Bengal	Coochbehar	Mosaic	+	17-43
	Siliguri	Mosaic	+	24-44
	Haldibari	Mosaic	-	20-46
	Jalpaiguri	Mosaic	-	20-33
	Pundibari	Mosaic	+	28-43
	Raiganj	Mosaic	+	32-50
	Balurghat	Mosaic	-	43-56
	Dalkhola	Mosaic	+	20-54
	Alipurduar	Mosaic	+	18-33

Morphology of virus particles

Transmission electron microscopic examination of crude preparation of papaya and bean samples revealed the presence of flexuous rods (Fig.18). The flexuous rods of our study were very much similar with that of characteristic flexuous rods of *Potyvirus* as suggested by Anjaiah *et al.* (1989).

Mechanical sap inoculation

Under artificial conditions 20 number of papaya plants of (one month old plants) were mechanically sap inoculated using 0.1M potassium phosphate buffer (pH 7.0). Symptoms were developed 15 days after inoculation. The symptoms were first developed on newly emerging leaves. The characteristic symptoms were vein clearing, chlorotic spots and chlorotic rings. Later these plants produced various types of symptoms including leaf reduction to shoe string, leaf distortion, puckering, mosaic patterns on leaves and stunted growth, 48-55 days after inoculation. Similar experiments were also performed in 20 number of young pumpkin plants (with 3-4 leaved stage).

Similar types of symptoms on leaves, stems and fruits of infected papaya plants have been reported by several workers (Linder *et al.*, 1945; Bhandari, 1952; Conover, 1962; Dahal *et al.*, 1997; Roy *et al.*, 1999) confirming to produce various types of symptoms, typically ring spot being prominent on leaves and fruits.

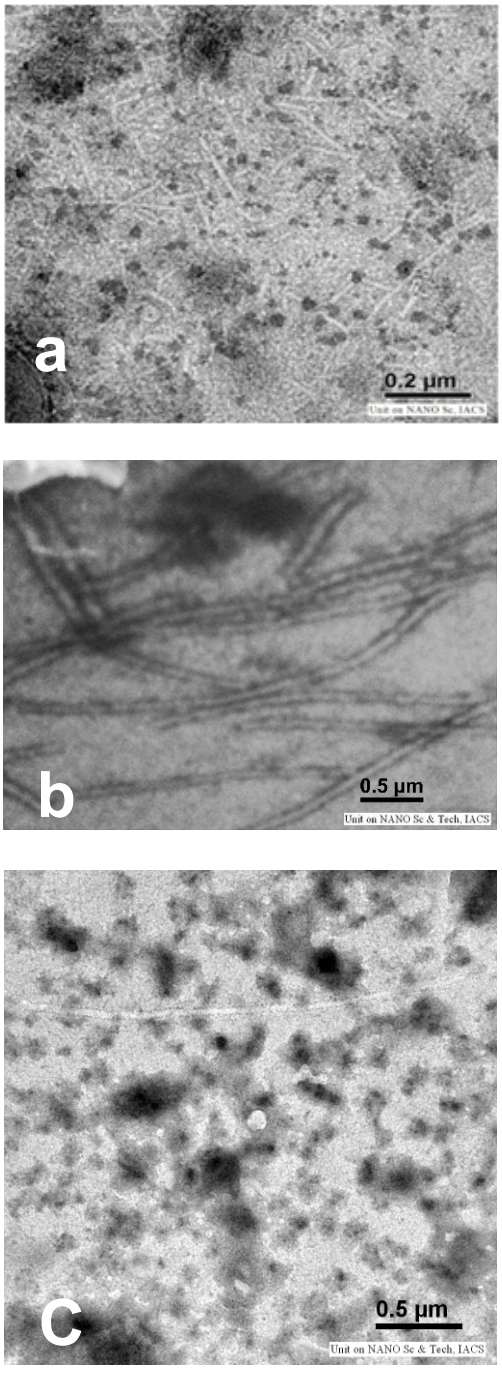

Fig. 18: Transmission Electron Microscopic photograph of (a) *Bean common mosaic virus,* (b) *Papaya ringspot virus* type P and (c) *Potato virus Y.*

The virus under study was readily sap transmissible from papaya to papaya and from papaya to pumpkin plant (Fig.19). Mosaic symptoms were produced in pumpkin plant when mechanical sap inoculation was done (Fig. 19d).

Similar type of result was reported by Lima and Gomes (1975); Yemewar and Mali (1980); Roy *et al.* (1999). Based on above mentioned results, it is evident that present isolate of PRSV can be successfully transmitted to papaya and member of Cucurbitaceae family.

PCR amplification

RNA were extracted and specific genes were amplified in PCR. The amplified products were allowed to run on agarose gel. The PCR products of papaya, bean and potato samples were amplified by specific primers and molecular weight were determined by using standard DNA-molecular weight markers. The primer was "CP9502' and 'CPuP'. The expected size of the amplified RNA by the primer as mentioned above was ~650bp. The results of our experiments were compared with the standard results as suggested by Singh *et al.* (2007). The samples were considered to contain virus when the molecular weight of the amplified products matched with standard reported molecular weights. The results of some amplified samples in presence of DNA-molecular weight markers have been presented in the Fig. 20.

Nucleotide sequencing and analysis

The 3'- terminal regions of papaya (677) (Accession no. JX567310), potato (801) (Accession no. JX945850) and bean (576) (Accession no. KC871565) isolates were cloned and sequenced. The sequenced regions each contained a single open reading frame (ORF) of 450 and 453 nucleotides that could potentially encode partial coat protein of 150 and 151 amino acids of PRSV and PVY isolates respectively. The ORF was followed by an UTR of 206 and 328 nucleotides excluding the poly (A) tail in PRSV and PVY isolates. On the other hand the bean isolate contained a single open reading frame of 449 nucleotides and encode a product similar to polyprotein and the isolate also contains an UTR of 126 nucleotides. When the nucleotide sequences of the isolates were compared with those of other potyviruses, there was 92% nucleotide identity with *Papaya ring spot*

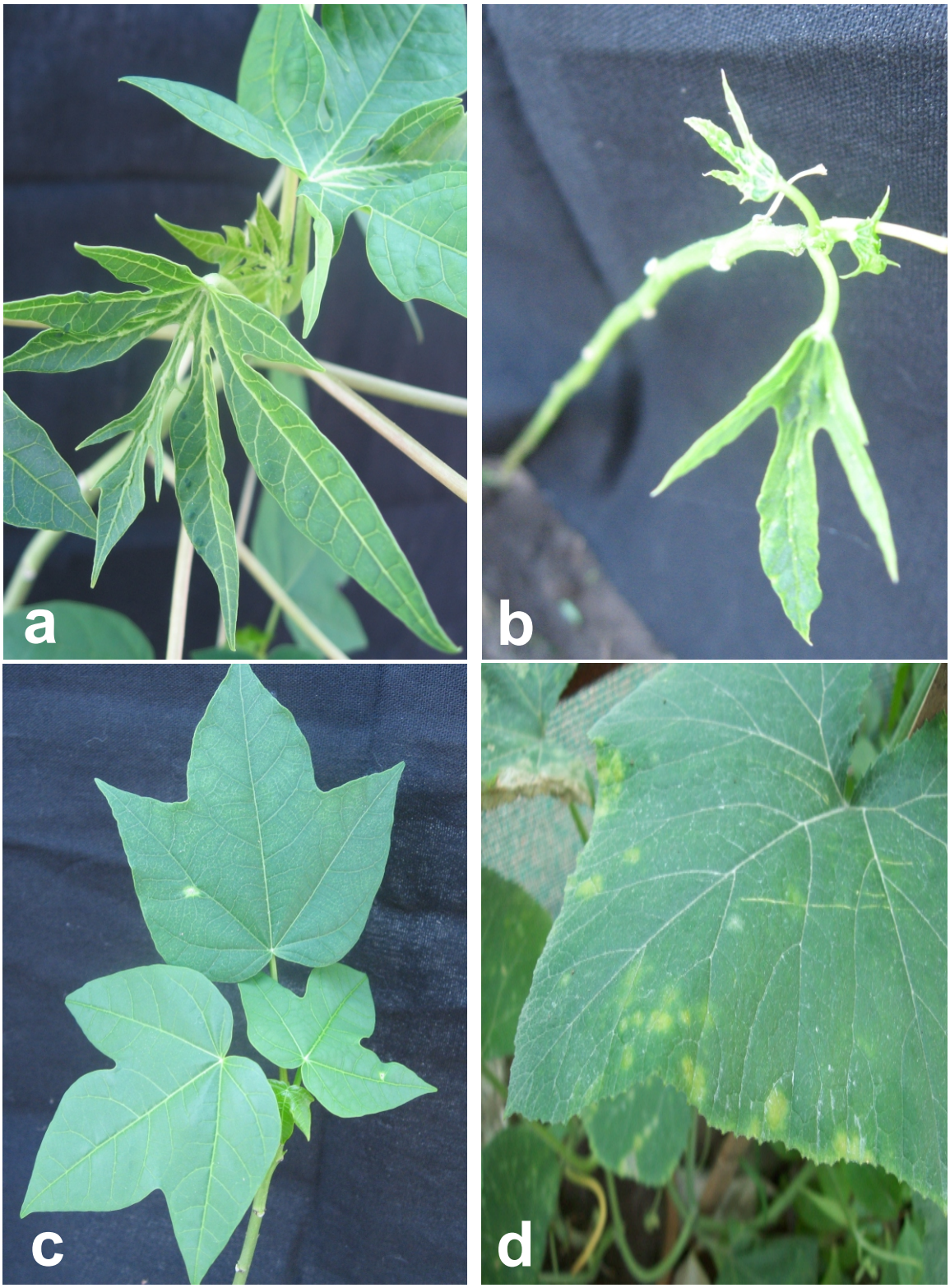

Fig. 19: (a) and (b) Mild mosaic and leaf deformation developed on papaya plant following mechanical sap transmission, (c) Healthy papaya plant, (d) Mosaic symptom developed on leaf of pumpkin plant following mechanical sap transmission.

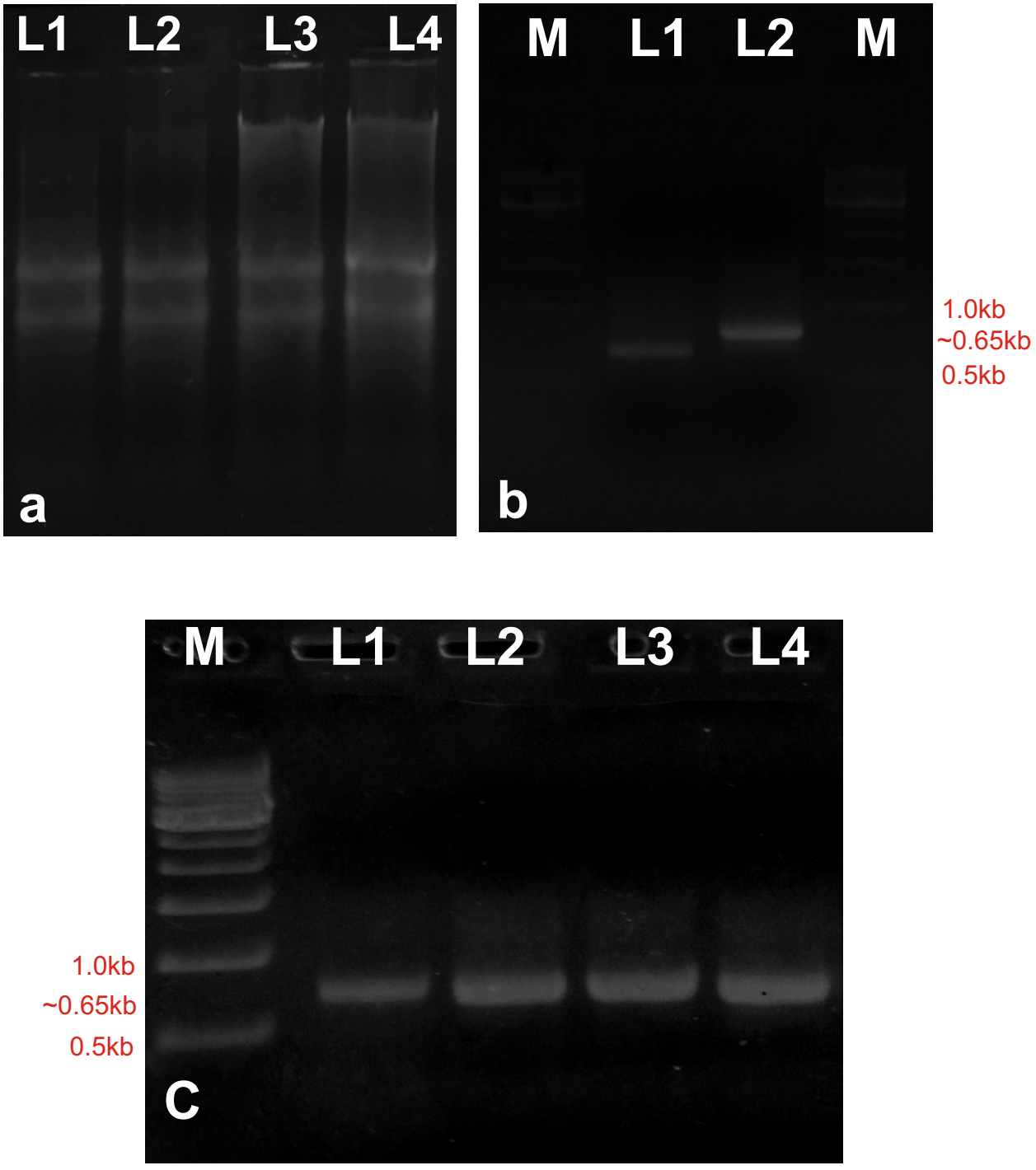

Fig. 20: (a) Agarose gel electrophoresis of total RNA extracted from infected plants using “total RNA extraction kit” (L1 & L2 =papaya, L3= common bean and L4= potato leaf samples). (b) Agarose gel electrophoresis of PCR products of the cDNA using “RT-PCR kit” from infected common bean (L1) and potato (L2). M=500bp DNA marker. (c) Agarose gel electrophoresis of PCR products of the cDNA using “RT-PCR kit” from papaya (L1-L4). M=500bp DNA marker.

virus when query coverage was 98% and 91% when query coverage was 100% in case of papaya isolate. Similarly potato isolate showed 99% nucleotide identity with *Potato virus* Y when query coverage was 98% and 100% when query coverage was 97%. On the other hand bean isolate showed 98% nucleotide sequence with Bean common mosaic virus. The isolates were also clustered with the same viruses in the phylogenetic tree (Fig. 21). The details of the sequences of the present study have been presented in the table 24. The other sequences of the GenBank that have been used for phylogenetic studies have also been presented in table 25.

Table 24: The sequences of the CP genes of the present study

Sl No.	Accession Number	Title	Host	Status
1	JX945850	*Potato virus Y* isolate SLGPVY1 coat protein gene, partial cds	*Solanum tuberosum*	Published on 25-Dec-2012
2	JX567310	*Papaya ringspot virus* P isolate SLGPRSV1 coat protein gene, partial cds.	*Carica papaya*	Published on 11-Nov-2012
3	KC871565	*Bean common mosaic virus* isolate Coochbehar polyprotein-like mRNA, partial sequence	*Phaseolus vulgaris*	Published on 30-JUL-2013

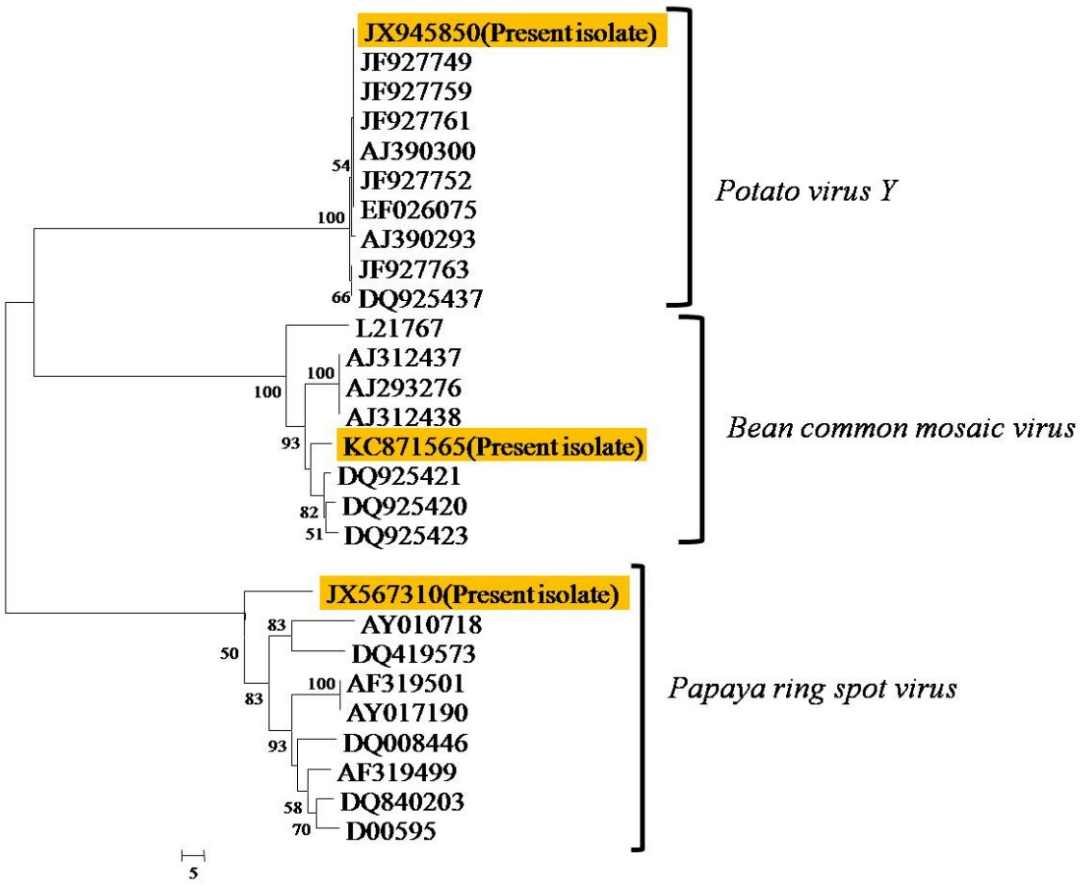

Fig. 21: The most parsimonious tree showing the relationship of partial coat protein genes (JX567310, JX945850, KC871565) of isolates of Potyvirus with published related *Potyvirus* sequences from GenBank using MEGA version 4.0 program. Numbers at nodes indicate the bootstrap percentage scores out of 1000 replicates.

Table 25: The sequence of CP genes of other potyviruses used in the present study for sequence analysis of the present virus isolates

Serial Number	Name of the virus	Source country	Source crop	Acc. No.
1	PRSV	Cote d'Ivore	*Carica papaya*	DQ840203
2	PRSV	U.S.A (Washington)	*Carica papaya*	DO0595
3	PRSV	Mexico (Yucanta)	*Carica papaya*	AF319499
4	PRSV	Thailand	*Carica papaya*	AY010718
5	PRSV	Mexico(Veracruz)	*Carica papaya*	DQ008446
6	PRSV	Mexico (Chiapa)	*Carica papaya*	AF319501
7	PRSV	Mexico	*Carica papaya*	AY017190
8	PRSV	China (Hainan)	*Carica papaya*	DQ419573
9	PVY	Poland	*Nicotiana tabacum*	JF927761
10	PVY	Poland	*Nicotiana tabacum*	JF927759
11	PVY	Poland	*Nicotiana tabacum*	JF927752
12	PVY	USA	Potato	EF026075
13	PVY	Hungary	Potato	AJ390300
14	PVY	Poland	*Nicotiana tabacum*	JF927763
15	PVY	Croatia	*Nicotiana tabacum*	JF927743
16	PVY	Viet Nam(Hanoi)	Potato	DQ925437
17	PVY	Slovenia	Potato	AJ390293
18	BCMV	Viet Nam (Hue)	Read bean	DQ925420
19	BCMV	Viet Nam (Hoabinh)	Black bean	DQ925423
20	BCMV	Viet Nam (Hue)	Red bean	DQ925421
21	BCMV	China (Zheziang)	Cowpea	AJ312437
22	BCMV	China (Zheziang)	Cowpea	AJ312438
23	BCMV	China (Zheziang)	Cowpea	AJ293276
24	BCMV	Puerto Rico	Cowpea	L21767

It seems that potyviruses and specifically aphid-transmitted viruses of the genus *Potyvirus* are one of the most successful groups of plant pathogens in the world. The genus has been claimed to be almost as ancient as flowering plants and has a worldwide distribution throughout higher plants. Many members of the family are important pathogen on plants (Rybicki, 1999). *Papaya ring spot virus* (PRSV) has been considered the most damaging virus infecting papaya worldwide. *Turnip mosaic virus* (TuMV) is ranked second most important virus infecting field grown vegetables. *Papaya ring spot virus* infecting papaya, *Bean common mosaic virus* infecting beans and potato infected by *Potato virus Y* are found to distribute in many parts of India. PRSV is grouped into two types: Type P (PRSV-p) infects cucurbits and papaya, whereas type W (PRSV-w) infects cucurbits but not papaya. The latter type was previously referred to as WMV-1, although both types are serologically closely related (Bateson, 1994). *Bean common mosaic virus* (BCMV) is a widely distributed destructive pathogen of bean. The virus is transmitted by seed and can cause 98% yield losses (Puttaraju *et al.*, 1999; Schmidt 1992; Varma 1988; Hampton *et al.*, 1982). On the other hand, *Potato virus Y* is most important potato virus in India and causes heavy yield losses which may extend up to 80% depending on the potato variety, environment and virus strain (Khurana *et al.*, 1987; Khurana and Singh, 1988; Singh *et al.*, 1982). Mukherjee *et al.* (2004) cloned and sequenced the Indian strain of *Potato virus Y* ordinary strain (PVY^0) and reported that PVY^0 strain fell into the European Union subgroup and introduced in China and India due to same geographical location. The detection of PVY by using polymerase chain reaction is well documented (Singh *et al.,* 1988b; Singh *et al.,* 1999; Fakhrabad *et al.,* 2012). Ghosh and Bapat (2006) used RT-PCR method to detect PVY in tobacco and potato using CP gene specific primers and reported that RT-PCR is more efficient method of virus detection. Volkov *et al.* (2009) detected PVY in potato and wild plants in the Far East of Russia by RT-PCR technique.

The occurrence of BCMV on common bean has been reported from India by several authors (Muniyapa 1976; Gupta and Chowla, 1990; Kapil, 2011). Saqib *et al.* (2005) reported the presence of BCMV in Western

Australia by using universal potyvirus specific primers. Sharma *et al.* (2011) reported that coat protein region is unable to delineate the strain of the same viruses; however, it clearly distinguishes different viruses of the family *Potyviridae*.

On the basis of electron microscopic study and PCR amplification of viral genome from the infected papaya, bean and potato plants, it was evident that the virus was a *Potyvirus* as reported by Anjaiah *et al.* (1989) and Singh *et al.* (2007) respectively. The isolated virus from papaya produced characteristic symptoms of vein clearing, chlorotic spots and chlorotic rings and later varied types of symptoms including leaf reduction to shoe string, leaf distortion, puckering, mosaic patterns on leaves and stunted growth of papaya plants as reported by Reddy *et al.* (2007) and the virus also infected members of Cucurbitaceae family. From the above discussion it was evident that the virus was *Papaya ring spot virus* (PRSV) and the type of the virus was PRSV-P infecting papaya.

5. General Discussion

Geminiviruses are a major constraint for the successful cultivation of tomato in tropic and subtropics (Czosnek, 1988; Hong and Harrison, 1995, Kumar *et al.,* 2012). In India several species of *Begomovirus* (family *Geminiviridae*) infecting tomatoes have been characterized (Kumari *et al.,* 2011; Chakraborty *et al.,* 2003; Kumar *et al.,* 2008; Tiwari *et al.,* 2010,13). Chakraborty *et al.* (2003) described *Tomato leaf curl virus* (ToLCV) as a threatening virus of tomato.

In the present study the occurrence and distribution of begomoviruses in tomato plants have been studied in sub-Himalayan West Bengal and in Brahmaputra valley of Assam. As the areas are of similar agro-climatic conditions and as it differ from rest of India, it was thought to study the different disease symptoms in the area. The disease incidence was found to vary from location to location and region to region. About 18-83% disease incidence was found in sub-Himalayan West Bengal and it was 14-71% in the Brahmaputra valley of Assam. High incidences of begomoviruses are associated with high populations of whiteflies and serious losses in several crops in the Americas and the Caribbean Basin have been reported (Brown and Bird, 1992; Morales and Anderson, 2001; Polston and Anderson, 1997).

Tomato leaf curl diseases (TLCDs) occur in many tomato producing regions of the world. TLCD is caused by *Begomovirus* and is transmitted by whitefly (Chandel *et al.,* 2010, Jyothsna *et al.,* 2013).

In the present case also TLCD was detected by PCR using *Begomovirus* specific primers. Thirty virus affected samples from the different locations of the area were tested. All the samples showed positive PCR reaction amplifying desired size, 530 nt sequence fragment of CP gene of begomoviruses. The results of the study confirmed the presence of TLCD (caused by *Begomovirus*) in the area. TLCD of all the samples (of five locations, three from West Bengal and two from Assam) could be transmitted to healthy tomato (var. Pusa Ruby) seedlings by whitefly with variable transmission efficiency depending on the samples. Mechanical sap

transmission of the *Begomovirus* (ToLCV) was also successful in the present study following the method of Chakraborty *et al.* (2003). They were successful in transmitting begomovirus (ToLCV) in tomato. Mechanical sap transmission of geminivirus (SqLCV) was also shown by El-Dougdoug *et al.* (2009). Thus our results are in agreement with the previous workers.

Electron microscopic observations of crude preparation of infected tomato leaves revealed the presence of isometric, pentagonal and 'paired Geminivirus' structures. The structures were similar with that of shown by Czosnek *et al.* (1988).

Host range of whitefly transmitted geminiviruses is vast which include Kenaf (Paul *et al.*, 2006), *Dimorphotheca sinuta* (Raj *et al.*, 2007), *Urena lobata* (Chatterjee *et al.*, 2007), *Mimosa invasa* (Koravieh *et al.*, 2008), Bitter gourd (Rajinimala and Rabindran, 2007), *Datura stramonium* (Ding *et al.*, 2007), Papaya (Wu and Zhou, 2006), *Duranta repens* (Tahir *et al.*, 2006), Chilli (Shih *et al*, 2006), Methi (Raj *et al.*, 2001), common bean (Papayiannis *et al.*, 2007), *Ageratum conyzoides* (Wong *et al.*, 1993), potato (Garg *et al.*, 2001), *Capsicum annum* (Strenger *et al.*, 1990) of India and other parts of the world.

In host range studies out of the 10 different plants tested 5 plants showed symptoms and contained detectable DNA of ToLCV when amplified by using DengA/DengB primers. Plants mechanically inoculated with sap of infected plants produced leaf curl symptoms on young leaves. In *Cucumis sativus*, *Lycopersicon esculentum* (cv. Pusa Ruby), *Capsicum annum* (cv. Akasmoni) and *Phaseolus vulgaris* (cv. Improved Tender Green) disease symptoms appeared as leaf curl. In *Nicotiana rustica* (cv. Hookah) local lesion was developed after inoculation. Thus, the alternative hosts of TLCD in the present study area were identified.

In the present study four pairs of DNA-A specific begomovirus primers were used to amplify the DNA-A genome. Only the isolate GUW-2 could be amplified using all the four primer pairs and a complete genome of 2763 nucleotide of DNA-A was submitted in the GenBank (Accession no. JN676053). DNA-B was successfully amplified (using BF518 / BR16141 primer pair) from isolates COB-2 and SILIGURI-2. But DNA-β could not be

amplified from any of the nine virus isolates of the present study, using Beta 01/Beta 02 primer pair.

Seven TLCD samples, designated as isolates GUW-1, GUW-2, COB-2, SILIGURI-2, COB-5, COB-6 and RAI-1 from seven locations were randomly taken for further molecular characterization of the viruses. On the basis of CP gene sequences isolate GUW-1 was identical with GUW-2 and COB-5 with COB-6 (100% nt identity). Therefore, one GUW-1 isolate and one COB-5 isolate was selected for further analysis along with the other three isolates. The phylogenetic analysis showed overall two genogroups among all the isolates including present isolates. The present isolates COB-2, RAI-1 and SILIGURI-2 fell into one group (GR-1) and COB-5 and GUW-1 into another group (GR-2).

The sequence analysis determined that COB2, RAI-1 and SILIGURI-2 are similar with begomoviruses like *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Tomato leaf curl Pakisthan virus* (ToLCPkV). The isolate COB-5 is related to *Tobacco curly shoot virus* (TbCSV) of China and Uttakhand and isolate GUW-1 to *Tomato leaf curl Ranchi virus* (ToLCRaV) and *Tomato leaf curl Bangladesh virus (*ToLCBnV). Thus, it may be considered that the TLCD in these regions are caused by ToLCNDV, ToLCPkV, ToLCRaV, ToLCBnV and TbCSV or mixture of the begomoviruses mentioned.

Universal DNA-A specific primers and universal DNA-B specific primers successfully amplified the partial DNA-A and DNA-B genomes from the isolates under study. But DNA-β specific primer pair was unable to amplify the DNA-β genome from the present isolates. Under the circumstances, Rolling circle amplification (RCA) technique was adopted to amplify full length genomes of the isolates where DNA-A were present but DNA-B were absent. This was done to detect the possibility of presence of DNA-β along with DNA-A. Monopartite begomoviruses have small circular single-stranded DNA satellites, named DNA β. These depend on begomoviruses for their proliferation and, in turn, they affect the accumulation and symptom expression of *Begomovirus* (Mansoor *et al.,* 2003). BamH1 could digest the amplicon by a single nick and was able to

separate DNA-A and DNA-β in agarose gel. The isolates COB-5 and GUW-2 showed positive response on RCA and RFLP as two distinct bands of ~2.7kb and ~1.5kb was appeared after restriction digestion and confirmed the presence of DNA A and DNA β. Thus it was proved that two different groups of geminiviruses are present in the region. One group (monopartite) contains DNA A and DNA β the other group (bipartite) contains DNA A and DNA B. Thus both monopartite and bipartite Virus genomes are prevalent in the region. Briddon *et al.* (2008) stated that Indian ToLCV isolates are mostly monopartite (DNA-A) in nature with few isolates possessing bipartite (DNA-A and DNA-B) genome organization such as tomato leaf curl New Delhi virus (ToLCNDV) and tomato leaf curl Palampur virus (ToLCPalV). Thus our studies significantly support the report of Briddon *et al.* (2008). DNA β satellites associated with some host plants have also been reported by some other workers (Saunders *et al.,* 2000, Jose and Usha, 2003, Zhou *et al.,* 2003). Our work also supports them about the presence of DNA β satellites in some of the monopartite begomoviruses of the region.

Insecticide resistance of the vectors or reduced susceptibility of crops has been reported in many cases. Frequent use of insecticides disrupts the ecology as well. Hence, judicious use of chemical in agriculture is necessary. Studies indicate that aqueous extracts of several plant extracts showed absolute control of vectors (Windham *et al.,* 1998, Rampersad, 2003).

For management of ToLCV (especially whitefly) diseases Leaf extracts of ten different plants (*Eucalyptus tereticornis.*, *Clerodendron viscosum*, *Camellia sinensis*, *Azadirachta indica*, *Datura stramonium*, *Xanthium strumarium*, *Polyalthia longifolia*, *Crotalaria pallida.*, *Mitracarpus hirtus.* and *Piper betle* were used. The results of the experiment revealed that the Neem leaf extract and *Clerodendron viscosum* leaf extract could reduce disease incidence in tomato plants. Neem extract could reduce disease incidence up to 90% where as *C. viscosum* leaf extract could reduce disease incidence up to 80%. Singh *et al.* (2011) used *Azadirachta indica* and

Clerodendron aculeatum leaf extracts and reduced *Mungbean yellow mosaic virus* significantly. Thus our study on control of some of the viral diseases by application of botanicals is in conformity with that of the previous scientists and the present study specifically screened five plant extracts which may be applied in the crop fields for control of the virus diseases.

Apical leaf curl disease causing viruses of potato were isolated and characterized from the fields of sub-Himalayan West Bengal. In potato fields of Coochbehar (West Bengal, India) the incidence of the disease was about 40%. The characteristic symptoms observed on naturally infected potato plants were apical leaf curl associated with crinkled leaves and mosaic. It was also observed that the size of the potato tubers of the infected plants were significantly smaller than that of healthy plants of the same fields. Although Leaf curl disease of potato has been reported from India and Pakistan (Usharani *et al.*, 2004; Mubin *et al.*, 2009, Garg *et al.*, 2001) but the present study area (sub-Himalayan Teesta-Brahmaputra plains) of India was not included in the previously reported studies. Hence, this is a first observation of begomovirus causing a severe disease of potato in sub-Himalayan Teesta-Brahmaputra plains. The disease was successfully transmitted to healthy potato plants using both whitefly vector *B. tabaci* (Mahesh *et al.*, 2010) and mechanical sap inoculation (using sap of infected potato leaves). The symptoms resulted both from artificial insect inoculations and mechanical sap inoculations were of similar nature.

Three different types of primers for DNA-A and one primer for DNA-B were used for the purpose of molecular characterization of the virus. The accession numbers for these submissions are HQ597033 for DNA-A and JN390432 for DNA-B. Basic local alignment search tool (BLAST) analysis of the virus showed highest sequence identity with *Tomato leaf curl New Delhi virus* (ToLCNDV) isolates. In phylogenetic analysis also the virus sequences clustered with the ToLCNDV isolates. *Tomato leaf curl new Delhi virus* from potato apical leaf curl diseased samples was detected by Gawande *et al.* (2007) from the Indo- Gangetic plains. Thus our results are in agreement with that of Gawande *et al.* (2007)

Several reports indicated that weeds act as reservoirs facilitating recombination and generation of new viral genomes (Frischmuth *et al.,* 1997, Jovel *et al.,* 2007, Morales and Anderson, 2001). Some uncultivated plants have been studied with an objective to test the plants whether they act as reservoirs of viruses or viral genomes. Molecular characterizations of the viruses were also performed using universal *Begomovirus* primers for partial DNA-A genomes. All together nine places of sub-Himalayan West Bengal were surveyed and about 20% to 89% disease incidence was observed with symptoms like leaf curl (in *Datura stramonium*), mosaic and dwarfing of plants (in *Jatropha curcas*) and yellow mosaic (in *Croton bonplandianus*, *Acalypha indica* and *Ageratum conyzoides*). Our studies are in agreement with those of several other workers of India and abroad (Snehi *et al.,* 2011; Zaffalon *et al.,* 2012; Paul *et al.,* 2012, Pramesh *et al.,* 2013). But it is adding new information (*i.e.* presence of begomoviruses in the weeds of sub Himalayan West Bengal) to our present knowledge. The study also shows that begomoviruses present in the weeds act as reservoirs of viruses which in turn damage economically important crops of the region. From the above informations it may be suggested that eradication of the virus affected weeds, plants or plant parts of the present study area are required for management of the viral diseases of crops of the present study area.

Three sets of universal degenerate primers were used for amplification and confirmation of the partial coat protein, coat protein and core coat protein genes of viruses from the infected *J. curcas, D. stramonium, C. bonplandianus*, *A. indica* and *A. conyzoides* plants. The amplified products were cloned and sequenced. The viral gene isolate from *J. curcas* contained a nucleotide sequence of 495bp (Accession number HQ597029). It showed highest 95% nucleotide sequence identity with *Jatropha mosaic India virus*-[Lucknow, strain SK-2, segment DNA-A (Accession no. HM230683)]. Virus from *D. stramonium* having a nucleotide sequence of 771bp (Accession no. JN676054). It showed highest 99% nucleotide sequence identity with *Tomato leaf curl New Delhi virus* DNA-A complete genome (Accession no. AM850115).

Three infected plant samples (*C. bonplandianus*, *A. indica* and *A. conyzoides*) gave positive virus gene amplification results when primer pair 'AV494 and AC1048' was used. The core CP gene of *Acalypha* yellow vein disease-associated complex showed highest (98%) nucleotide sequence identity (nsi) with *Jatropha mosaic India virus* [isolate Katarniaghat segment DNA-A (accession no. JN135236)] and clustered with the same in the phylogenetic tree.

The core CP gene of the begomovirus associated with *Croton* yellow vein disease in the present study area (JQ796374) showed its close relationship (97% nsi) with *Croton yellow vein mosaic virus* (FN645898) which infects *Acalypha* plants in India (Zaffalon *et al.,* 2012) and *Croton* plants in India (accession no. JN817516). But in phylogenetic analysis it clustered with begomoviruses infecting papaya, tobacco, mesta and kenaf. The core CP gene of the begomovirus associated with *Ageratum* yellow vein disease of the present study area (JQ843097) showed its close relationship (99% nsi) with both *Tobacco curly shoot virus* infecting *Ageratum* plants in China (AJ971266, FN401522) and with *Tobacco curly shoot virus* of infected pepper (GU001879) plants (Qing *et al.,* 2010). The same core CP gene showed 98% nsi with *Ageratum enation virus* (JF728866) infecting *Ageratum* plants in India and clustering with both the begomoviruses.

Reports of potyvirus is available from southern part of West Bengal but reports of availability of potyviruses are scanty from sub-Himalayan West Bengal, although, symptoms of potyviruses were experienced during survey of the fields of the present study area. The presence of the symptoms of potyviruses during survey, prompted us to detect and analyze the viruses present in the three plants (Papaya, Bean and Potato) of the present study area. Different kinds of disease symptoms like severe mosaic, ring spot symptom on the fruit, severe leaf deformation and stunted plant growth were observed in the field conditions. Samples which showed distinct visible symptoms were subjected to RT-PCR and a positive reaction in PCR amplification confirmed the presence of potyviruses.

Transmission electron microscopic examination of crude preparation of papaya, potato and bean samples revealed the presence of flexuous rods.

Papaya ring spot virus disease was successfully transmitted from infected papaya plant to healthy papaya and pumpkin plants.

The 3'- terminal regions of papaya (677 nt long amplicon, Accession no. JX567310), of potato (801 nt long amplicon, Accession no. JX945850) and of bean (576 nt long amplicon, Accession no. KC871565) were cloned and sequenced. The sequenced regions contained a single open reading frame (ORF) of 450 and 453 nucleotides that could potentially encode partial coat protein of 150 and 151 amino acids of PRSV and PVY isolates respectively. The ORF was followed by an UTR of 206 and 328 nucleotides excluding the poly (A) tail in PRSV and PVY isolates. On the other hand the bean isolate contained a single open reading frame of 449 nucleotides and encode a product similar to polyprotein and the isolate also contains an UTR of 126 nucleotides. When the nucleotide sequences of the isolates were compared with those of other potyviruses, there was 92% nucleotide identity with Papaya ring spot virus when query coverage was 98% and 91% when query coverage was 100% in case of papaya isolate. Similarly, potato isolates showed 99% nucleotide identity with *Potato virus* Y when query coverage was 98% and it was 100% when query coverage was 97%. On the other hand bean isolate showed 98% nucleotide sequence with Bean common mosaic virus. The isolates were also found to cluster with the same viruses in the phylogenetic tree.

From the above discussions it may be concluded that the potyviruses found in the present study confirm that papaya, bean and potato might contribute an avenue of potyviruses in the sub-Himalayan West Bengal, which may quickly spread throughout the crop growing areas. These results also highlighted the possible need for more sensitive way of virus detection of the important cultivated crops.

All the investigations presented in the present thesis have confirmed and extended some of the findings of the earlier workers. During course of the study, certain new facts of fundamental importance have also been revealed. A study of the important plant viruses of our crops and of some uncultivated plants and weeds of sub-Himalayan West Bengal have been presented in the thesis. The virus genomes have been isolated partly and in

some cases fully. The genomes were duly sequenced, annotated and finally submitted to GenBank. The phylogenetic trees were constructed and compared. Possible, recombinations of some viruses have been indicated. Management of begomovirus caused diseases of tomato was done using botanicals. Eradication of the virus affected uncultivated plants and weeds growing near the crop fields have also been shown to be important as they act as reservoirs of the viruses. Thus the present study identifies the viruses of the region, compares with others and it also suggests some management strategies. Lastly it may be said that the viral diseases of our crops of the area should be taken care, to avoid epidemic viral diseases.

6. References

Abdel-Salam A. M. (1990) Mechanical transmission of two Egyptian isolate of Beet curly top and Tomato yellow leaf curl viruses. *Bulletin Faculty of Agriculture, University of Cairo* **41:** 825-842.

Adams M. J., Antoniw J. F. and Fauquet C. M. (2005) Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology* **150:** 459-479.

Agrios G. N. (1987) Plant Pathology. Academic Press, New York.

Agrios G. N. (1997) Plant Pathology. Fourth edition. Academic Press, San Diego, California. pp 635.

Ahmed M., Ren S. X., Mandour N., Maruthi M., Naveed M. and Qiu B. L. (2010) Phylogenetic analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations from cotton plants in Pakistan, China, and Egypt. *Journal of Pest Science* **83:** 135-141.

Alexandre M. A. V., Noronha A. B. and Vicente M. (1989) Occurrence and some properties of a virus inhibitor from *Silene schatta. Microbios letters* **42:** 35-42.

Allam E. K., Abo El-Nasar M. A., Othman B. A. and Thabeet S. A. (1994) A new method for mechanical transmission of Tomato yellow leaf curl virus. *Egyptian Phytopathol. Soc.* **7:** 91.

Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W. and Lipman D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389-3402.

Amin I., Mansoor S., Iram S., Khan M. A., Hussain M., Zafar Y., Bull S. E., Briddon R. W. and Markham P. G. (2002) Association of monopartite Begomovirus producing subgenomic DNA and a distinct DNA β on *Croton bonplandianus* showing yellow vein symptoms in Pakistan. *Plant Dis.* **86:** 444.

Anandhan S., Sivalingam P. N., Satheesh V., Saritha R. K. and Parameshwari B. (2011) Leaf curl disease of tomato in Haldwani (Uttarakhand), India region is caused by a begomovirus with satellite molecule DNA ß. *Archives of Phytopathology and Plant protection* **44:** 1840-1851.

Andou T., Yamaguchi A., Kawano S., Kawabe K., Ueda S. and Onuki M. (2010) *Ageratum yellow vein virus* isolated from tomato plants with leaf curl on Ishigaki Island, Okinawa, Japan. *Journal of General Plant Pathology* **76:** 287-291.

Anjaiah V., Reddy D. V. R., Manohar S. K., Naidu R. A, Nene Y. L. and Ratna S. A. (1989) Isolation and characterization of a potyvirus associated with bushy dwarf symptom in chickpea, *Cicer arietinum*, in India. *Plant Pathology* **38:** 520-526.

Assuncao I. P., Listik A. F., Barros M. C. S., Amorim E. P. R., Silva S. J. C, Izael O. S., Ramalho-Neto C. E. and Lima G. S. A. (2006) Genetic diversity of begomovirus infecting weeds in Northeastern Brazil. *Planta Daninha* **24:** 239-244.

Atreya C. D., Atreya P. L., Thornbury D. W. and Pirone T. P. (1992) Site-direct mutations in the potyvirus HC-Pro gene affect helper component activity, virus accumulation, and symptom expression in infected tobacco plants. *Virology* **191:** 106-111.

AVRDC (2012) The great pyramid: By stacking multiple genes for resistance, AVRDC plant breeders develop tomatoes that are a monument to better pest control. The World Vegetable Centre. http://avrdc.org1?P=51.

Banks G. K., Colvin J., Reddy R. V. C., Maruthi M. N. and Muniyappa V. (2001) First report of the *Bemisia tabaci* B-biotype in India and an associated tomato leaf curl virus disease epidemic. *Plant Dis.* **85:** 231-231.

Bansal R. D., Khatri H. L., Sharma O. P. and Singh, I. P. (1984) Epidemiological studies on viral diseases of mung and mash in Punjab. *Journal of Research* **21:** 54-58.

Bansal S. K. and Trehan S. P. (2011) Effect of potassium on yield and processing quality attributes of potato. *Karnataka J. Agric. Sci.* **24:** 48-54.

Barakat A. (1988) Studies on plant virus inhibitors from certain species of the sinaiflora. *Microbios Letters* **38:** 123-129.

Baranwala V. K., Nasim A. and Ahmad N. (1997) Effect of *Clerodendron aculeatum* leaf extract on tomato leaf curl virus. *Indian Phytopathology* **50:** 297-299.

Bateson M. F., Henderson J., Chaleeprom W., Gibbs A. J. and Dale J. L. (1994) Papaya ringspot potyvirus, isolate variability and the origin of PRSV-P (Aust). *Journal of General Virology* **75:** 3547-3553.

Bedford I. D., Briddon R. W., Brown J. K., Rosell R. C. and Markham P. G. (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology* **125:** 311-325.

Bellows T. S., Perring T. M. Jr., Gill R. J. and Headrick D. H. (1994) Description of a species of *Bemisia* (*Homoptera*: *Aleyrodidae*). *Ann. Entom. Soc. Am.* **87:** 195-206.

Berger P. H. and Pirone T. P. (1986) The effect of helper component on the uptake and localization of potyviruses in *Myzus persicae*. *Virology* **153:** 256-261.

Berger P. H., Wyatt S. D., Shiel P. J., Silbernagel M. J., Druffel K. and Mink G. I. (1997) Phylogenetic analysis of *Potyviridae* with emphasis on legume-infecting potyviruses. *Arch. Virol.* **142:** 1979-1999.

Bhandari M. M. (1952) Abnormal papaya fruits. *Biology and Medicine* pp 43.

Bhat J. A., Bhat Z. A., Dar W. A. and Rashid R. (2010) Economically significant bacterial and viral diseases of potato and their management. *International Journal of Current Research* **2:** 73-77.

Bird J. (1957) A whitefly transmitted mosaic of *Jatropha gossypifolia*. Technical paper 22. Agricultural Experimental station, University of Puerto Rico.

Borah B. K. and Dasgupta I. (2012) Begomovirus research in India: A critical appraisal and the way ahead. *J. Biosci.* **37:** 791-806.

Bosco D., Mason G. and Accotto G. P. (2004) TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). *Virology* **323:** 276-283.

Brandes J. and Wetter C. (1959) Classification of elongated plant viruses on the basis of particle morphology. *Virology* **8:** 99-115.

Briddon R. W. and Markham P. G. (2000) Cotton leaf curl virus disease. *Virus Research* **71:** 151-159.

Briddon R. W. and Stanley J. (2006) Sub-viral agents associated with plant single-stranded DNA viruses. *Virology* **344:** 198-210.

Briddon R. W., Brown J. K., Moriones E., Stanley J., Zerbini M., Zhou X. and Fauquet C. M. (2008) Recombination for the classification and nomenclature of the DNA-beta satellites of begomoviruses. *Arch. Virol.* **153:** 763-781.

Briddon R. W., Bull S. E. and Amin I. (2003) Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virology* **312:** 106-121.

Briddon R., Bebford I., Tsai J. H. and Markham P. (1996) Analysis of the nucleotide sequence of the treehopper-transmitted Geminivirus, Tomato pseudo-curly top virus, suggests a recombinant origin. *Virology* **219:** 387-394.

Brown J. K. (1994) The status of *Bemisia tabaci* (Genn.) as a pest and vector in world agroecosystems. *FAO Plant Prot. Bull.* **42:** 3-32.

Brown J. K. and Bird J. (1992) Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. *Plant Dis.* **76:** 220-225.

Brown J. K., Coats S. A., Bedford I. D., Markham P. G., Bird J. and Frohlich D. R. (1995) Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.)(Homoptera:Aleyrodidae). *Biochemical Genetics* **33:** 205-214.

Brown J. K., Idris A. M., Torres-Jerez I., Banks G. K. and Wyatt S. D. (2001) The core region of the coat protein gene is highly useful for establishing the provisional identification of begomoviruses. *Arch. Virol.* **146:** 1581-1598.

Byadgi A. S., Anahosur K. H. and Kulkarni M. S. (1995) Ring spot virus in papaya. *The Hindu* **118:** 28.

Capoor S. P. and Verma P. M. (1948) A mosaic disease on *Carica papaya* L. in the Bombay province. *Current Science* **17:** 265-266.

Carrington J. C. and Freed D. D. (1990) Cap-independent enhancement of translation of a plant potyvirus 5'-non translated region. *Journal of Virology* **64:** 1590-1597.

Carrington J. C., Jensen P. E., Schaad M. C. (1998) Genetic evidence for an essential role for potyvirus CI protein in cell-to-cell movement. *Plant J.* **14:** 393-400.

Castillo-Urquiza G. P., Becerra J. E. A., Bruckner F. P., Lima A. T. M. and Varsani A. (2008) Six novel begomoviruses infecting tomato and associated weeds in southeastern Brazil. *Archives of Virology* **153:** 1985-1989.

Chakraborty S., Pandey P. K., Banerjee M. K. and Fauquet C. M. (2003) Tomato leaf curl Gujarat virus, a new begomovirus species causing a severe leaf curl disease of tomato in Varanasi, India. *Phytopathology* **93:** 1485-1495.

Chamberlain E. E. (1954) Plant Virus Diseases in New Zealand, Vol. 108. R. E. Owen, Government Printer, Wellington.

Chandel R. S., Banyal D. K., Singh B. P., Malik K. and Lakra B. S. (2010) Integrated management of whitefly, *Bemisia tabaci* (Gennadius) and *Potato leaf curl virus* in India. *Potato Research* **53:** 129-139.

Chatchawankanphanich O. and Maxwell D. P. (2002) *Tomato leaf curl Karnataka virus* from Bangalore, India, appears to be a recombinant begomovirus. *Phytopathology* **92:** 637-645.

Chatterjee A., Roy A. and Ghosh S. K. (2007) First report of begomovirus Associated with yellow vein mosaic disease of *Urena lobata* in India. *Aus. Plant Dis. Notes* **2:** 27-28.

Cheema S. S. and Reddy R. S. (1885) Studies on the transmission of Papaya mosaic virus by *Rhopalosiphum maidis*. *Indian Journal of Virology* **1:** 49-53.

Cockbain A. J., Bowen R. and Vorra-Urai S. (1976) Seed transmission of broad bean stain virus and Echtes Ackerbohnenmosaik-Virus in field beans (*Vicia faba). The Annals of Applied Biology* **84:** 321.

Cohen S., Duffus J. E. and Liu H. Y. (1992) A new *Bemisia tabaci* (Gennadius) biotype in South Western United States and its role in silverleaf of squash and tansmission of *Lettuce infectious yellow virus*. *Phytopath.* **82:** 86-90.

Colariccio A., Souza-Dias J. A. C., Chagas C. M., Sawazaki H. E., Chaves A. L. R. and Eiras M. (2001) Novo surto de geminivirus em *Lycopersicon esculentum* na região de Campinas, SP. *Summa Phytopathologica* **27:** 105.

Conover, R.A. (1962) Virus diseases of Florida. *Phytopathology* **52:** 6.

Costa A. S. (1955) Studies of abutilon mosaic in Brazil. *Phytopathologische Zeitschrift* **24:** 97-112.

CRSP (2012) Feeding more people with healthier crops. Proceeding of virology symposium. June 12, 2012, IPM, CRSP, India.

Cui X. F., Tao X. R., Xie Y., Fauquet C. M. and Zhou X. P. (2004) A DNA β associated with Tomato yellow leaf curl China virus is required for symptom induction in hosts. *Virology* **78:** 13966-13974.

Czosnek H., Ber R., Antignus Y., Cohen S., Navot N. and Zamir D. (1988) Isolation of tomato leaf curl virus, a geminivirus. *Phytopathology* **78:** 508-512.

Czosnek H., Ghanim M. and Ghanim M. (2002) The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci*-insights from studies with *Tomato yellow leaf curl virus*. *Annals of Applied Biology* **140:** 215-231.

Dahal C., Lecoq, H. and Alberchtsen S. E. (1997) Occurrence of Papaya ringspot potyvirus and cucurbit viruses in Nepal. *Annals of Applied Biology* **130:** 491 - 502.

Dasgupta I., Malathi V. G. and Mukherjee S. K. (2003) Genetic engineering for virus resistance. *Curr. Sci.* **84:** 341-354.

Dellaporta S. L., Wood J. and Hicks J. B. (1983) A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep.* **1:** 19-21.

Ding M., Luo Y. Q., Dong J. H., Fang Q. and Zhang Z. K. (2007) First report of Tomato yellow leaf curl China virus with DNA β infecting *Datura stramonium* in China. *Aust. Plant Dis. Notes* **2:** 63.

Dolja V. V., Haldeman R., Robertson N. L., Dougherty W. G. and Carrington J. C. (1994) Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. *EMBO J.* **13:** 1482-1491.

Dolja V. V., Haldeman-Cahill R., Montgomery A. E., Vandenbosch K. A. and Carrington J. C. (1995) Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. *Virology* **206:** 1007-1016.

Dombrovsky A., Huet H., Chejanovsky N. and Raccah B. (2005) Aphid transmission of a potyvirus depends on suitability of the helper

component and the N terminus of the coat protein. *Archives of Virology* **150:** 287-298.

Domier L. L., Shaw J. G. and Rhoads R. E. (1987) Potyviral proteins share amino acid sequence homology with picorna-como- and caullimovirus proteins. *Virology* **158:** 20-27.

Drifhout E. (1978) Genetic interactions between *Phaseolus vulgaris* L. and *Bean common mosaic virus* and its strains. Agricultural Research Report Pudoc (Wageningen).

Dry B., Rigden J. E., Krake L. R., Mullineaux P. M. and Rezaiana M. A. (1993) Nucleotide sequence and genome organization of tomato leaf curl geminivirus. *Journal of General Virology* **74:** 147-151.

Dunoyer P., Thomas C., Harrison S., Revers F. and Maule A. (2004) A cysteine-rich plant protein potentiates Potyvirus movement through an interaction with the virus genome-linked protein VPg. *J. Virol.* **78:** 2301-2309.

Edwardson J. R., Christie R. G., Purcifull D. E. and Petersen M. A. (1993) Inclusions in diagnosis plant virus diseases. CRC Press, Boca Raton.

Eiras M., Colariccio A., Chaves A. L. R., Tavares C. A. M., Harakava R. (2002) Levantamento preliminar de geminivírus em tomateiros no Estado de São Paulo. *Summa Phytopathologica* **28:** 97.

El-Dougdoug K. A., El-Kader H. S. A., Hamad I. A., Ahmed E. A. and El-Monem A. F. A. (2009) Identification of *Squash leaf curl virus* (Egyptian isolate). *Australian Journal of Basic and Applied Sciences* **3:** 3470-3478.

El-Dougdoug K. A., Gomaa H. H. A. and El-Maaty S. A. (2006) The impact and interference between Tomato yellow leaf curl and Tomato mosaic viruses on tomato plants. *Journal of Applied Science Research* **2:** 1151-1155.

Essam El-Din K. M., Abo El-Abbas F. M., Aref N. A. and Abdallah N. A. (2004) A new whitefly transmitted *Geminivirus* infecting tomato plants in Egypt. *Egyptian J. Virol.* **1:** 283-300.

Fakhrabad F. Z., Ahmadikhah A. and Nasrollahnejad S. (2012) Identification and detection of Potato virus Y strains by molecular methods in tobacco fields of North Iran. *International Research Journal of Applied and Basic Sciences* **3:** 1422-1428.

Faria J. C., Souza-Dias J. A. C., Slack S. and Maxwell D. P. (1997) A new geminivirus associated with tomato in the State of Sao Paulo, Brazil. *Plant Disease* **81:** 423.

Fauquet C. M. and Stanley J. (2003) Geminivirus classification and nomenclature; progress and problems. *Annals of Applied Biology* **142:** 165-189.

Fauquet C. M., Bisaro D. M., Briddon R. W., Brown J. K., Harrison B. D., Rybicki E. P., Stenger D. C. and Stanley J. (2003) Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. *Archives of Virology* **148:** 405-421.

Fauquet C. M., Briddon R. W., Brown J. K., Moriones E., Stanley J., Zerbini M. and Zhou X. (2008) *Geminivirus* strain demarcation and nomenclature. *Arch. Virol.* **153:** 783-821.

Fauquet C. M., Mayo M. A., Maniloff J., Desselberger U. and Ball L. A. (2005) Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, California, USA.

Fauquet C., Maxwell D., Gronenborn B. and Stanley J. (2000) Revised proposal for naming geminiviruses. *Archives of Virology* **145:** 1743-1761.

Fazeli R., Heydarnejad J., Massumi H., Shaabanian M. and Varsani A. (2009) Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. *Virus Genes* **38:** 311-319.

Fernandes J. J., Carvalho M. G., Andrade E. C., Brommonschenkel S. H., Fontes E. P. B. and Zerbini F. M. (2006) Biological and molecular properties of *Tomato rugose mosaic virus* (ToRMV), a new tomato-infecting begomovirus from Brazil. *Plant Pathology* **55:** 513-522.

Fiallo-Olive E., Chirinos D. T., Geraud-Pouey F., Moriones E. and Navas-Castillo J. (2013) Complete genome sequences of two begomoviruses infecting weeds in Venezuela. *Archives of Virology* **158:** 277-280.

Fondong V. N., Pita J. S., Rey M. E. C., de Kochko A., Beachy R. N., Fauquet C. M. (2000) Evidence of synergism between Africans cassava mosaic virus and a new double recombinant geminivirus infecting cassava in Cameroon. *J. Gen. Virol.* **81:** 287-297.

Francki R. I. B., Van Regenmortel H. V. and Fraenkel-Conrat H. (1996) The Plant Viruses. Plenum Press, New York.

Frischmuth T., Engel M., Lauster S. and Jeske H. (1997) Nucleotide sequence evidence of the occurrence of three distinct whitefly-transmitted, Sida-infecting bipartite geminiviruses in Central America. *Journal of General Virology* **78:** 2675-2682.

Gafni Y. and Epel B. L. (2002) The role of host and viral proteins in intra- and inter-cellular trafficking of geminiviruses. *Phyl. Mol. Plant Pathol.* **60:** 231-241.

Gao S.Q., Qu J., Chua N. H. and Ye J. (2010) A new strain of Indian cassava mosaic virus causes a mosaic disease in the biodiesel crop *Jatropha curcas*. *Arch. Virol.* **155:** 607-612.

Garcia-Neria M. A. and Rivera-Bustamante R. F. (2011) Characterization of Geminivirus resistance in an accession of *Capsicum chinense* Jacq. *Mol. Plant Microbe Interactions* **24:** 172-182.

Garg I. D., Khurana S. M. P., Kumar S. and Lakra B. S. (2001) Association of a geminivirus with potato apical leaf curl in India and its immune-electron microscopic detection. *Potato Journal* **28:** 227-232.

Garga R. P. (1963) Studies on virus diseases of plants in Madhya Pradesh, a serious virus disease of papaya. *Indian Phytopathology* **16:** 31-33.

Gawande S. J., Kaundal P., Kaushal N. and Garg I. D. (2007) Print capture PCR-A simple technique for the detection of tomato leaf curl New Delhi

virus- causal agent of potato apical leaf curl disease in India. *Potato Journal* **34:** 87-88.

Ghanem M., Morin S. and Czosnek H. (2001) Rate of tomato yellow leaf curl virus pathway at its vector, the whitefly *Bemisia tabaci*. *Phytopathology* **91:** 188-196.

Ghosh B. P. and Bapat V. A. (2006) Development of RT-PCR based method for detection of *Potato virus* Y in tobacco and potato. *Indian Journal of Biotechnology* **5:** 232-235.

Gibbs A. J., Mackenzie A. M. and Gibbs M. J. (2003) The 'potyvirid primers' will probably provide phylogenetically informative DNA fragments from all species of potyviridae. *Journal of Virological Methods* **12:** 41-44.

Govindappa M. R., Shankergoud I., Shankarappa K. S., Wickramaarachchi W. A. R. T., Reddy B. A. and Rangaswamy K. T. (2011). Molecular detection and partial characterization of begomovirus associated with leaf curl disease of sunflower (*Helianthus annus*) in South India. *Plant pathology Journal* **10:** 29-35.

Gupta Y. and Chowla S. C. (1990) Screening of French bean germplasm for resistance to bean common mosaic virus. *Indian Phytopathol*. **43:** 434-436.

Gutierrez C. (1999) Geminivirus DNA replication. *Cellular and Molecular Life Sciences* **56:** 313-329.

Gutierrez C. (2000) DNA replication and cell cycle in plants: learning from geminiviruses. *EMBO Journal* **19:** 792-799.

Gutierrez C., Ramirez-Parra E., Mar-Castellano M., Sanz-Burgos A. P., Luque A. and Missich R. (2004) Geminivirus DNA replication and cell cycle interactions. *Veterinary Microbiology* **98:** 111-119.

Haible D., Kober S. and Jeske H. (2006) Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *J. Virol. Methods* **135:** 9-16.

Haider, M. S. Tahir M., Evans A. A. F. and Markham P. G. (2007) Coat protein gene sequence analysis of three begomovirus isolates from Pakistan and their affinities with other begomoviruses. *Pakistan Journal of Zoology* **39:** 165-170.

Hampton R., Waterworth H., Goodman R. M. and Lee R. (1982) Importance of seed-borne viruses in crop germplasm. *Plant Dis.* **66:** 977-978.

Hanley-Bowdoin L., Settlage S. B. and Robertson D. (2004) Reprogramming plant gene expression: a prerequisite to Geminivirus DNA replication. *Molecular plant pathology* **5:** 149-156.

Hanley-Bowdoin L., Settlage S. B., Orozco B. M., Nagar S. and Robertson D. (2000) Geminiviruses: model for plant DNA replication, transcription and cell cycle regulation. *Critical Review in Biochemistry and Molecular Biology* **35:** 105-140.

Hanley-Bowdoin L., Settlage S. B., Orozco B. M., Nagar S. and Robertson D. (1999) Geminiviruses: model for plant DNA replication, transcription and cell cycle regulation. *Critical Review in Plant Sciences* **18:** 71-106.

Harrison A. D., Muniyappa V., Swanson M. M. and Roberts D. J. (1991) Recognition and differentiation of seven whitefly transmitted Geminiviruses from India and their relationship to African cassava mosaic and Thailand mungbean yellow mosaic viruses. *Annals of Applied Biology* **118:** 299-308.

Harrison B. D. (1985) Advances in geminivirus research. *Annual Review of Phytopathology* **23:** 55-82.

Harrison B. D. and Robinson D. J. (1999) Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). *Annual Review of Phytopathology* **37:** 369-398.

Hayes R. J., MacDonald H., Coutts R. H. A. and Buck K. W. (1988) Priming of complementary DNA synthesis *in vitro* by small DNA molecules tightly bound to virion DNA of *Wheat dwarf virus*. *J. gen. Virol.* **69:** 1345-1350.

Haywood V., Kragler F. and Lucas W. J. (2002) Plasmodesmata: pathways for protein and ribonucleoprotein signaling. *Plant Cell* **14:** 303-325.

Hegde P. (1998) Further studies on *Papaya ring spot virus*. *M. Sc.* (*Agri) Thesis*,University of Agricultural Sciences, Dharwad, p. 87.

Hehnle S., Wege C. and Jeske H. (2004) Interaction of DNA with the movement proteins of geminiviruses revisited. *J. Virol.* **78:** 7698-7706.

Heinlein M., Epel B. L., Padgett H. S. and Beachy R. N. (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **270:** 1983-1985.

Heinlein M., Padgett H. S., Gens J. S., Pickard B. G., Casper S. J., Epelc B. L. and Beachya R. N. (1998) Changing patterns of localization of the tobacco mosaic virus movement protein and replicase to the endoplasmic reticulum and microtubules during infection. *Plant Cell* **10:** 1107-1120.

Hofer P., Engel M., Jeske H. and Frischmuth T. (1997) Nucleotide sequence of a new bipartite geminivirus isolated from the common weed *Sida rhombifolia* in Costa Rica. *Journal of General Virology* **78:** 1785-1790.

Hofius D., Maier A. T., Dietrich C., Jungkunz I., Bornke F., Maiss E and Sonnewald U. (2007) Capsid protein-mediated recruitment of host DnaJ-like proteins is required for Potato virus Y infection in tobacco plants. *J. Virol.* **81:** 11870-11880.

Hong Y. G. and Harrison B. D. (1995) Nucleotide sequences from tomato leaf curl viruses from different countries- evidence for 3 geographically separate branches in evolution of the coat protein of whitefly-transmitted geminiviruses. *Journal of General virology* **76:** 2043-2049.

Hooker W. J., Salazar L. F. and Brown C. R. (1985) Field infection of potato by the *Solanum apical leaf curling virus* (SALCV). *American Journal of Potato Research* **62:** 263-272.

Hull R. (2002) Matthews' Plant Virology. Fourth edition. Academic Press, New York.

Hunter W. B., Hiebert E., Webb S. E., Tsai J. H. and Polston J. E. (1998) Location of geminiviruses in the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Plant Disease* **82:** 1147-1151.

Hussain K., Hussain M., Mansoor S. and Briddon R. W. (2011) Complete nucleotide sequence of a begomovirus and associated betasatellite infecting croton (*Croton bonplandianus*) in Pakistan. *Arch. Virol.* **156:** 1101-1105.

ICTV (2012) Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Ed: King A.M.Q., Adams M.J., Carstens E.B. and Lefkowitz E.J. San Diego: Elsevier Academic Press.

Idris A. M. and Brown J. K. (2000) Identification of a new, monopartite begomovirus associated with leaf curl disease of cotton in Gezira, Sudan. *Plant Disease* **84:** 809.

Jain R. K., Sharma J., Sivakumar A. S., Sharma P. K., Byadgi A. S. and Verma A. K. and Verma A. (2004) Variability in the coat protein gene of papaya ringspot virus isolates from multiple locations in India. *Archives of Virology* **149:** 2435-2442.

Jiu M., Zhou X. P. and Liu S. S. (2006) Acquisition and transmission of two begomoviruses by the B and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *Journal of Phytopathology* **154:** 587-591.

Jiu M., Zhou X., Tong L., Xu J., Yang X., Wan F. and Liu S. (2007) Vector virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE* 2: e182. doi:10.1371/journal.pone.0000182

John P., Sivalingam P. N., Kumar N., Mishra A., Ahlawat Y. S. and Malathi V. G. (2006) A new begomovirus associated with yellow mosaic disease of Clerodendron inerme. *Plant Pathology* **55:** 291.

Johne R., Muller H. Rector A., Ranst M. and Stevens H. (2009) Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends in Microbiology* **17:** 205–211.

Jose J. and Usha R. (2003) Bhendi yellow vein mosaic disease in India is caused by association of a DNA [beta] satellite with a Begomovirus. *Virology* **305:** 310-317.

Jovel J., Prei W. and Jeske H. (2007) Characterization of DNA intermediates of an arising geminivirus. *Virus Research* **130:** 63-70.

Jyothsna P., Haq Q. M. I., Singh P., Sumiya K. V., Praveen S., Rawat R., Briddon R. W. and Malathi V. G. (2013) Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellite. *Appl. Microbiol. Biotechol.* DOI 10.1007/s00253-012-4685-9.

Kapil R., Sharma P., Sharma S. K., Sharma O. P., Sharma O. P., Dhar J. B. and Sharma P. N. (2011) Pathogenic and molecular variability in bean common mosaic virus infecting common bean in India. *Archives of Phytopathology and Plant Protection* **44:** 1081-1092.

Kataria R. and Kumar D. (2012) Occurrence and infestation level of sucking pests: Aphids on various host plants in agricultural fields of Vadodara, Gujarat (India). *Int. J. Scientific Res. Publicat.* **2:** 1-6.

Khan J. A. and Dijkstra J. (2002) Plant viruses as Molecular pathogens. Food Products Press, New York.

Khan J. A., Lohuis D., Goldbach R. and Dijkstra J. (1993) Sequence data to settle the taxonomic position of *Bean common mosaic virus* and *Blackeye cowpea mosaic virus* isolates. *J. Gen. Virol.* **74:** 2243-2249.

Kheyr-Pour A., Bendahmane M., Matzeit V., Accota G. P., Crepsi S. and Gronenborn B. (1991) The tomato yellow leaf curl virus from Sardinia (TYLCV-S) has a single genomic component. *Nucleic Acids Research* **19**: 6763-6769.

Khurana S. M. P. and Bhargava K. S. (1970) Induced apocarpy and "double papaya fruit" formation in papaya with distortion ringspot virus infection. *Plant Disease Reporter* **54:** 181-183.

Khurana S. M. P., Garg I. D., Singh B. P. and Gadewar A. V. (1998) Major diseases of potato and their management. *In*: Upadhyay R. K. *et al* (ed) Integrated Pest and Disease Management, APH Publ. Corpn., New Delhi, pp.11-64.

Khurana S. M. P., Singh M. N. and Kumar S. (1987) Easy schedule for *Potato virus Y* purification and production of its antiserum. *Current Science* **56:** 420-422.

Khurana S. P. M and Singh M. N. (1988) Yieldloss potential of *Potato virus X* and Y in Indian potatoes. *J. Indian Potato Assoc.* **15:** 27-29.

Kirthi N., Maiya S. P., Murthy M. R. N. and Savitri H. (2002) Evidence of recombination among the tomato leaf curl virus strains/species from Bangalore, India. *Archives of virology* **147:** 225-272.

Kirthi N., Priyadarshini C. G. P., Sharma P., Maiya S. P., Hemalatha V., Sivaraman, Dhawan P., Rishi P. N. and Savithri H. S. (2004) Genetic variability of begomoviruses associated with cotton leaf curl disease originating from India. *Arch. Virol.* **149:** 2047-2057.

Koraveih M. M., Roff M. N. M. and Othman R. Y. (2008) First report of whitefly-transmitted geminivirus infecting *Mimosa invasa* in Malaysia. *Aust. Plant Dis. Notes* **3:** 25-26.

Kumar S. P., Patel S. K., Kapopara R. G., Jasrai Y. T. and Pandya H. A. (2012) Evolutionary and molecular aspects of Indian Tomato leaf curl virus coat protein. *International Journal of Plant Genomics* DOI: 10. 1155/2012/417935.

Kumar Y., Hallan V. and Zaidi A. A. (2008) Molecular characterization of a distinct begomovirus species infecting tomato in India. *Virus Genes* **37:** 425-431.

Kumari P., Chattopadhyay B., Singh A. K. and Chakraborty S. (2009) A New Begomovirus Species Causing Tomato Leaf Curl Disease in Patna, India. *Plant Dis.* **93:** 545.

Kumari P., Singh A. K., Chattopadhyay B. and Chakraborty S. (2011) A new begomovirus species and betasatellite causing severe tomato leaf curl disease in Ranchi, India. *New Disease Reports* **23:** 11.

Kumari S. G., Najar A., Attar N., Loh M. H. and Vetten H. J.(2010) First report of Beet mosaic virus infecting chickpea (*Cicer arietinum* L.) in Tunisia. *Plant disease* **49:** 1068.

Lakra B. S. (2002) Leaf curl: a threat to potato crop in Haryana. *J. Mycol. Plant Pathol.* **32:** 367.

Larsen R. C. and Kim K. S. (1985) Ultra structure of *Diodia virginiana* infected with a whitefly transmitted virus-like disease agent. *Phytopath.* **82:** 86-90.

Laufs J., Jupin I., David C., Schumacher S., Heyraud-Nitschke F. and Gronenborn B. (1995) Geminivirus replication: Genetic and biochemical characterization of Rep protein function, a review. *Biochimie* **77:** 765-773.

Lazarowitz S. G. (1992) Geminiviruses: Genome and structure and gene function. *Rev. Plant Sci.* **11:** 327-349.

Lefeuvre P., Delatte H., Naze F., Dogley W., Reynaud B. and Lett J. M. (2006) A new tomato leaf curl virus from the Seychelles archipelago. *New Disease reports* **13:** 26.

Legg J. P. (1996) Host-associated strains within Ugandan populations of the whitefly *Bemisi tabaci* (Genn.), (*Homoptera*, *Aleyrodidae*). *J. Appl. Entom.* **120:** 523-527.

Legg J. P. and Fauquet C. M. (2004) Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* **56:** 585-599.

Li Z. H., Zhou X. P., Zhang X. and Xie Y. (2004) Molecular characterization of tomato-infecting begomoviruses in Yunnan, China. *Archives of Virology* **149:** 1721-1732.

Li Z., Xie Y. and Zhou X. (2005) Tobacco curly shoot virus DNAβ is not necessary for identification but intensifies symptoms in a host-dependent manner. *Phytopathology* **95:** 902-908.

Lima J. M. A. and Gomes M. N. S. (1975) Identification of papaw ring spot virus in Fortaleza, Ceara. *Fitossanidade* **1:** 56-69.

Linder R. C., Jenson D. D. and Ikeda W. (1945) Ring spot new papaya plunderer. *Hawaii Farm and Home* **8:** 10-12.

Lucas W. J. (2006) Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology* **344:** 169-184.

Lucas W. J. and Lee J. Y. (2004) Plasmodesmata as a supracellular control network in plants. *Nat. Rev. Mol. Cell Biol.* **5:** 712-726.

Luria S. E., Darnell J. E., Bultimore D. and Cambell A. (1978) General Virology, Third edition. John Wiley and Sons, New York.

Mahesh Y. S., Shankarappa K.S., Rangaswamy K.T., Prameela H.A. and Narayana D.S.A. (2010) Detection and characterization of a begomovirus associated with leaf curl disease of ornamental croton (*Condiaeum variegatum*). *J. Hort. Sci. Biotechnol.* **85:** 101-105.

Maki-Valkama T. K. (2000) Resistance to *Potato virus Y* (PVY) in potato cv. Pito transformed with the P1 gene of PVY. University of Helsinki, Department of plant Production, Section of Crop Husbandry, Publication No. 57.

Malik P. S., Kumar V., Bagewadi B. and Mukherjee S. K. (2005) Interaction between coat protein and replication initiation protein of Mung bean yellow mosaic India virus might lead to control of viral DNA replication. *Virology* **337:** 273-283.

Maloy O. C. (1993) Plant Disease Control: Principles and Practice. John Wiley and Sons, New York.

Mandal B., Varma A. and Malathi V. G. (1997) Systemic infection of *Vigna mungo* using the cloned DNAs of the blackgram isolate of Mungbean yellow mosaic geminivirus through agroinoculation and transmission of the progeny virus by whiteflies. *J. Phytopathol.* **145:** 505-510.

Mansoor S., Briddon R. W., Zafar Y., and Stanley J. (2003) Geminivirus disease complex: an emerging threat. *Trends Plant Sci.* **8:** 128-134.

Mansoor S., Khan S. H., Bashir A., Saeed M., Zafar Y., Malik K. A., Briddon R.W., Stanley J. and Markham P. G. (1999) Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology* **259:** 190-199.

Maruthi M. N., Alam S. N., Kader K. A., Rekha A. R., Cork A. and Colvin J. (2005) Nucleotide sequence, whitefly transmission and screening tomato for resistance against two newly described begomoviruses in Bangladesh. *Phytopathology* **95:** 1472-1481.

Maruthi M. N., Colvin J., Seal S., Gibson G. and Cooper J. (2002) Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Research* **86:** 71-85.

Maruthi M. N., Manjunatha B., Rekha A. R., Govindappa M. R., Colvin J. and Muniyappa V. (2006) Dolichos yellow mosaic virus belongs to a distinct lineage of Old World begomoviruses; its biological and molecular properties. *Ann. Appl. Biol.* **149:** 187-195.

Maruthi M. N., Rekha A. R., Mirza S. H., Alam S. N. and Colvin J. (2007) PCR-based detection and partial genome sequencing indicate high genetic diversity in Bangladeshi begomoviruses and their whitefly vector, *Bemisia tabaci*. *Virus Genes* **34:** 373-385.

Maruthi M. N., Seal S., Colvin J., Briddon R. W. and Bull S. E. (2004) *East African cassava mosaic Zanzibar virus*- a recombinant begomovirus species with a mild phenotype. *Arch. Virol.* **149:** 2365-2377.

Marwal A., Sahu A., Prajapat R., Choudhary D. K., and Gaur R. K. (2012) First Report of Association of a Begomovirus with the Leaf Curl Disease of a Common Weed, *Datura inoxia*. *Indian Journal of Virology* **23:** 83-84.

Mathioudakis M. M., Veiga R., Ghita M., Tsikou D., Medina V. and Canto T. (2012) *Pepino mosaic virus* capsid protein interacts with a tomato heat shock protein cognate 70. *Virus Res.* **163:** 28-39.

Matthews R. E. F. (1991) Plant Virology. Third edition. Academic Press, San Diego.

Mayo M. A., Leibowitz M. J., Palukaitis P., Scholth K. B. G., Simon A. E., Stanley J. and Taliansky M. (2005) Satellites. *In*: Faquet, C. M., Mayo M. A., Maniloff, J., Desselberger, U., Ball, L. A. (*eds*), Eighth Report of the International Committee on Taxonomy of Viruses. Virus Taxonomy, Elsevier/Academic Press, London, pp.1163-1169.

McGrath P. F. and Harrison B. D. (1995) Transmission of tomato leaf curl Geminiviruses by *Bemisia tabaci*: Effects of virus isolate and vactor biotype. *Annals of Applied Biology* **126:** 307-316.

McLaughlin P. D., McLaughlin W. A., Maxwell D. P. and Roye M. E. (2008) Identification of begomoviruses infecting crops and weeds in Belize. *Plant Viruses* **2:** 58-63.

McLean B. G., Zupan J. and Zambryski P. C. (1995) Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* **7:** 2101-2114.

Meetei P. A., Singh P., Nongdam P., Prabhu N. P., Rathore R. S. and Vindal V. (2012) NeMedPlant: A database of therapeutic applications and chemical constituents of medicinal plants from North-East region of India. *Bioinformation* **8:** 209-211.

Mendez-Lozano J., Torres-Pacheco I., Fauquet C. M. and Rivera-Bustamante R. F. (2002) Interaction between geminiviruses in a naturally occurring mixture: *Pepper huasteco virus* and *Pepper golden mosaic virus*. *Phytopathology* **93:** 270-277.

Merits A., Guo D. and Saarma M. (1998) VPg, coat protein and five non structural proteins of *Potato A potyvirus* bind RNA in a sequence-unspecific manner. *Journal of General Virology* **79:** 3123-3127.

Mink G. I., Vetten H. J., Ward C. W., Berger P. H., Morales F. J., Myers J. M., Silbernagel M. J. and Barnett O. W. (1994) Taxonomy and classification of legume-infecting potyviruses. A proposal from the *Potyviridae* study group of plant virus sub-committee of ICTV. *Arch. Virol.* **139:** 231-235.

Mishra and Jha A. (1955) Mosaic of papaya (*Carica papaya* L.) in Bihar. *Proceedings of Bihar Academy of Agricultural Science* **4:** 102-103.

Miyoshi H., Suehiro N., Tomoo K., Muto S., Takahashi T., Tsukamoto T., Ohmori, T. and Natsuaki T. (2006) Binding analyses for the interaction between plant virus genome-linked protein (VPg) and plant translational initiation factors. *Biochimie*. **88:** 329-340.

Moffat A. S. (1999) Geminiviruses emerge as serious crop threat. *Science* **286:** 1835.

Morales F. J. and Anderson P. (2001) The emergence and distribution of whitefly-transmitted geminiviruses in Latin America. *Arch. Virol.* **146:** 415-441.

Morinaga T., Ikegami M. and Miura K. I. (1993) The nucleotide sequence and genome structure of mungbean yellow mosaic geminivirus. *Microbiol. Immunol.* **37:** 471-476.

Mubin M., Briddon R. W. and Mansoor S. (2009) Complete nucleotide sequence of chili leaf curl virus and its associated satellites naturally infecting potato in Pakistan. *Arch. Virol.* **154:** 365-368.

Mubin M., Shahid M. S., Tahir M. N., Briddon R. W. and Mansoor S. (2010) Characterization of begomovirus components from a weed suggests that begomoviruses may associate with multiple distinct DNA satellites .*Virus Genes* **40:** 452-457.

Mukherjee K., Verma Y., Chakrabarti S. K. and Khurana S. M. P. (2004) Phylogenetic Analysis of 5' UTR and P1 Protein of Indian Common Strain of Potato Virus Y Reveals its Possible Introduction in India. *Virus Genes* **29(2):** 229-237.

Muniyappa V. (1976) Studies on a mosaic disease of french bean (*Phaseolus vulgaris*). *Madras Agricult. J.* **63:** 69-70.

Muniyappa V. and Saikia A. K. (1983) Prevention of the spread of tomato leaf curl diseases. *Indian Phytopathology* **36:** 183.

Muniyappa V., Venkatesh H. M., Ramappa H. K., Kulkarni R. S., Zeidan M., Tarba C. Y., Ghanim M. and Czosnek H. (2000) Tomato leaf curl virus from Bangalore (ToLCV-Ban4): sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Arch. Virol.* **145:** 1583-1598.

Murphy F. A., Fauquet C. M., Bishop D. H. L., Ghabrial S. A., Jarvis A. W., Martelli G. P., Mayo M. A. and Summers M. D. (1995) Virus Taxonomy-Classification and Nomenclature of Viruses. Springer-Verlag, Vienna.

Nagaraju N., Reddy H. R. and Ravi K. C. (1997) Effect of exogenously applied plant products on pepper vein banding virus transmission, multiplication and symptom production in bell pepper (*Capsicum annum* L.) *Indian Journal of Virology* **13:** 161-163.

Nagy P. D., Wang R. Y., Pogany J., Hafren A. and Makinen K. (2011) Emerging picture of host chaperons and cyclophilin roles in RNA virus replication. *Virology* **411:** 374-382.

Narayana D. S. A., Rangaswamy K. T., Shankarappa K. S., Maruthi M. N. and Reddy C. N. L. (2007) Distinct begomovirus closely related to cassava mosaic viruses cause Indian jatropha mosaic disease. *Int. J. Virol.* **3:** 1-11.

Narayana D. S. A., Shankarappa K. S., Govindappa M. R., Prameela H. A., Rao M. R. G. and Rangaswamy K. T. (2006) Natural occurrence of *Jatropha mosaic virus* disease in India. *Current Science* **91:** 584-585.

Navas-Castillo J., Díaz J. A., Sánchez-Campos S. and Moriones E. (1998) Improvement of the print-capture polymerase chain reaction procedure for efficient amplification of DNA virus genomes from plants and insect vectors. *J. Virol.* **75:** 195-198.

Navot N., Pichersky E., Zeiden M., Zamir D. and Czosnek H. (1991) Tomato yellow leaf curl virus- a whitefly-transmitted geminivirus with a single genomic component. *Virology* **185:** 151-161.

Noordam D. (1973) Identification of Plant Viruses. Centre for Agricultural Publishing and Documentation, Wagningen, Netherlands.

Oku H. (1994) Plant Pathogenesis and Disease Control. Lewis publisher, Tokyo.

Oliveira M. R. V., Henneberry T. J. and Anderson P. (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection* **20:** 709-723.

Otim-Nape G. W., Thresh J. M. and Shaw M. W. (1997) The effects of cassava mosaic virus disease on yield and compensation in mixed stands of healthy and infected cassava. *Annals of Applied Biology* **130:** 503-521.

Packialakshmi R. M., Srivastava N., Girish K. R. and Usha R. (2010) Molecular characterization of a distinct begomovirus species from *Vernonia cinerea* and its associated DNA-β using the bacteriophage Φ29 DNA polymerase. *Virus Genes* **41:** 135-143.

Padidam M., Beachy R. N. and Fauquet C. M. (1995) Classification and identification of new geminiviruses using sequence comparisons. *Journal of General Virology* **76:** 249-263.

Padidam M., Beachy R. N. and Fauquet C. M. (1996) The role of AV2 (precoat) and coat protein in viral replication and movement in tomato leaf curl geminivirus. *Virology* **224:** 390-404.

Padidam M., Sawyer S. and Fauquet C. M. (1999) Possible emergence of new geminiviruses by frequent recombination. *Virology* **265:** 218-225.

Pandey P., Mukhopadhya S., Naqvi A. R., Mukherjee S. K., Shekhawat G. S. and Choudhury N. R. (2010) Molecular characterization of two distinct monopartite begomoviruses infecting tomato in India. *Virology Journal* **7:** 337.

Papayiannis L. C., Paraskevopoulos A., Katis N. I. (2007) First report of Tomato leaf curl virus infecting common bean (*Phaseolus vulgaris*) in Greece. *Plant Disease* **91:** 465.

Paul S., Ghosh R., Roy A. and Ghosh S. K. (2012) Analysis of coat protein gene sequences of begomoviruses associated with different weed species in India. *Phytoparasitica* **40:** 95-100.

Paul S., Ghosh R., Roy A., Mir J. I. and Ghosh S. K. (2006) Occurrence of a DNA β-containing begomovirus associated with leaf curl disease of kenaf (*Hibiscus cannabinus* L.) in India. *Aus. Plant Dis. Notes* **1:** 29-30.

Paximadis M., Muniyappa V. and Rey M. E. C. (2001) A mixture of begomoviruses in leaf curl-affected tobacco in Karnataka, South India. *Annals Applied Biology* **139:** 101-109.

Perring T. M., Cooper A. D., Rodriguez R. J., Farrar C. A. and Bellows T. S. (1993) Identification of a whitefly species by genomic and behavioral studies. *Science* **259:** 74-77.

Pita J. S., Fondong V. N., Sangare A., Otim-Nape G. W., Ogwal S. and Fauquet C. M. (2001) Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *J. Gen. Virol.* **82:** 655-665.

Piven N., de Uzcategui R. C. and Infante D. (1995) Resistance to *Tomato yellow mosaic virus* in different species of *Lycopersicon*. *Plant Dis.* **79:** 590-594.

Polston J. E. and Anderson P. K. (1997) The emergence of whitefly-transmitted geminiviruses in tomato in the Western hemisphere. *Plant Dis.* **81:** 1358-1369.

Polston J. E., Bois D., Ano G., Poliakoff F. and Urbino C. (1997) Occurrence of a strain of potato yellow mosaic geminivirus infecting tomato in the eastern Caribbean. *Plant Disease* **82:** 126.

Poutaraud A., Desbiez C., Lemaire O., Lecoq H. and Herrbach E. (2004) Characterisation of a new potyvirus species infecting meadow saffron (*Colchicum autumnale*). *Archives of Virology* **149:** 1267-1277.

Pramesh D., Mandal B., Phaneendra C. and Muniyappa V. (2013) Host range and genetic diversity of croton yellow vein mosaic virus, a weed-infecting monopartite begomovirus causing lef curl disease in tomato. *Archives of Virology* **158:** 531-542.

Preiss W. and Jeske H. (2003) Multitasking in replication is common among geminiviruses. *Journal of Virology* **77:** 2972-2980.

Prowidentii R. (1996) A Taiwan strain of papaya ring spot virus causing prominent symptoms on cultivated cucurbits. Report-Cucurbit-Genetics Co-operative No. 19, pp.83-84.

Pun K. B. and Doraiswamy S. (1999) Effect of age of okra plants on susceptibility to Okra yellow vein mosaic virus. *Indian J. Virol.* **15:** 57-58.

Puttaraju H. R., Prakash H. S., Albrechtsen S. E., Shetty H. S. and Mathur S. B. (1999) Detection of bean common mosaic potyvirus in French bean seed samples from Karnataka. *Indian J Virol* **15:** 27-29.

Qazi J., Ilyas M., Mansoor S. and Briddon R. W. (2007) Legume yellow mosaic viruses genetically isolated begomoviruses. *Mol. Plant Pathol.* **8:** 343-348.

Qing L., Xiong Y., Sun X. C., Yang S. Y. and Zhou C. Y. (2010) First Report of Tobacco curly shoot virus Infecting Pepper in China. *Plant Disease* **94:** 637.

Raccah B. (1986) Nonpersistent viruses: epidemiology and control. *Advances in Virus Research* **31:** 387-429.

Raj S. K., Khan M. S., Snehi S. K. and Roy R. K. (2007) Yellow vein netting of bimili jute (*Hibiscus cannabinus* L.) in India caused by a strain of Tomato yellow leaf curl new Delhi virus DNA β. *Aus. Plant Dis. Notes* **2:** 45-47.

Raj S. K., Pandey S. K., Chandra G., Singh B. P. and Gupta R. K. (2001) First report of geminivirus causing leaf curl disease of *Trigonella corniculata* evidenced by PCR using degenerate primers. *OEPP/EPPO Bulletin* **31:** 111-117.

Raj S. K., Snehi S. K., Kumar S., Khan M. S. and Pathre U. (2008) First molecular identification of a begomovirus in India that is closely related to *Cassava mosaic virus* and causes mosaic and stunting of *Jatropha curcas* L. *Australian Plant Disease Notes* **3:** 69-72.

Rajinimala N. and Rabindran R. (2007) First report of Indian cassava mosaic virus on bittergourd (*Momordica charantia*) in Tamilnadu, India. *Aus. Plant Dis. Notes* **2:** 81-82.

Ramakrishna R. P. (1988) Studies on Papaya Ring Spot Virus infecting *Carica papaya*. Ph. D. Thesis, Marathwada Agricultural University, Parbhani, p. 89.

Ramappa H. K., Muniyappa V. and Colvin J. (1998) The contribution of tomato and alternative host plants in leaf curl virus inoculums pressure in different areas of South India. *Annals of Applied Biology* **133:** 187-198.

Ramkat P. C., Calari A., Maghuly F. and Laimer M. (2011) Biotechnological approaches to determine the impact of viruses in the energy crop plant *Jatropha curcas*. *Virology* **8:** 386.

Rampersad S. N. (2003) Proposed strategies for begomovirus disease management in tomato in Trinidad. Online. Plant Health Progress doi:10.1094/PHP-2003-1006-01-HM.

Rangaswamy K. T., Raghavendra N., Shankarappa K. S., Narayana D. S. A., Prameela H. A., Govindappa M. R. and Rao M. R. G. (2005) Natural occurrence of a mosaic virus disease on Jatropha. *In:* Proceedings of the National Symposium on Crop Disease Management in Dry Land Agriculture and 57th IPS, January 12-14, 2005, New Delhi, India, p.156 (Abstract).

Rataul H. S. and Brar J. S. (1989) Status of *Tomato leaf curl virus* research in India. *Trop. Sci.* **29:** 111-118.

Reddy B. A., Patil M. S. and Rajasekharam T. (2010) Effect of plant extracts in the incidence of tomato leaf curl virus and whitefly population under field condition. *Karnataka J. Agric. Sci.* **23:** 807-808.

Reddy L., Nagaraju C. N., Kumar M. K. P. and Venkatarvanappa V. (2007) Transmission and Host Range of the Papaya Ring spot virus (PRSV). *J.Pl. Dis. Sci.* **2:** 9-13.

Reddy R. V. C., Colvin J., Muniyappa U. and Seal S. (2005) Diversity and distribution of *Begomoviruses* infecting tomato in India. *Archives of Virology* **150:** 845-867.

Reddy B. A. (2006) molecular characterization, epidemiology and management of *Tomato leaf curl virus* (TOLCV) in northern Karnataka. PhD-thesis, University of Agricultural Sciences, Dharwad.

Revers F., Le Gall O., Andresse T. and Maule A. J. (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. *Molecular Plant-Microbe Interactions* **12:** 367-376.

Ribeiro S. G., Ambrozevicius L. P., Ávila A. C., Bezerra I. C., Calegario R. F., Fernandes J. J., Lima M. F., Mello R. N., Rocha H. and Zerbini F. M. (2003) Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. *Archives of Virology* **148:** 281-295.

Riechmann J. L., Lain S. and Garcia J. A. (1992) Review articles: Highlights and prospects of potyvirus molecular biology. *Journal of General Virology* **73:** 1-16.

Rigden J. E., Dry I. B., Mullineaux P. M. and Rezaian M. A. (1993) Mutagenesis of the virion-sense open reading frames of tomato leaf curl geminivirus. *Virology* **193:** 1001-1005.

Riley D., Nava-Camberos U. and Allen J. (1995) Population dynamics of *Bemisia* in Agricultural systems. *In*: Gerling, D. and Mayer, R.T. (eds) *Bemisia* (1995) Taxonomy, Biology, Damage, Control and Management. Intercept Ltd, Hants, Andover. pp. 93-109.

Roberts I. M., Wang D., Findlay K. and Maule A. J. (1998) Ultrastructural and temporal observations of the potyvirus cylindrical inclusions (Cls) show that the Cl protein acts transiently in aiding virus movement. *Virology* **245:** 173-181.

Rocha C. S., Xavier C. A. D., Lima A. T. M., Silva F. N., Zerbini F. M. (2012) Molecular analysis of the DNA-A of the begomovirus *Tomato mottle leaf curl virus* (ToMoLCV). *Virus Reviews and Research.* http://www.sbv.org.br/vrr/volume16nr1,2/molecular_analysis.pdf

Rochester D. E., Kositratan W. and Beachy R. N. (1990) Systemic movement and symptom production following agroinoculation with a single DNA of tomato yellow leaf curl geminivirus (Thailand). *Virology* **178:** 520-526.

Rodriguez-Cerezo E. and Shaw J. G. (1991) Two newly detected non-structural proteins in potyvirus infected cells. *Virology* **185:** 572-579.

Rodriguez-Cerezo E., Findlay K., Shaw J. G., Lomonossoff G. P., Qiu S. G., Linstead P., Shanks M. and Risco C. (1997) The coat and cylindrical inclusion proteins of a potyvirus are associated with connections between plant cells. *Virology* **236:** 296-306.

Rodriguez-Pardina P. C., Zerbini F. M. and Ducasse D. (2006) Genetic diversity of begomoviruses infecting soybean, bean and associated weeds in Northwestern Argentina. *Fitopatologia Brasileira* **31:** 342-348.

Rojas A., Kvarheden A., Valkonen J. P. T. (2000) Geminiviruses affecting tomato crops in Nicaragua. *Plant Disease* **84:** 843-846.

Rojas M. R., Gilbertson R. L., Russell D. R. and Maxwell D. P. (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Dis.* **77:** 340-347.

Rojas M. R., Noueiry A. M., Lucas W. J. and Gilbertson R. L. (1998) Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form and size specific manner. *Cell* **95:** 105-113.

Rojas M. R., Zerbini F. M., Allison R. F., Gilbertson R. L., Lucas W. J. (1997) Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. *Virology* **237:** 283-295.

Roossinck M. J. (2011) The good viruses: viral mutualistic symbioses. *Nat. Rev. Microbiol.* **9:** 99-108.

Roy G., Jain R. K., Bhat A. I. and Varma A. (1999) Comparative host range and serological studies of papaya ring spot potyviruses isolates. *Indian Phytopathology* **52:** 14-17.

Roye M. E., McLaughlin W. A., Nakhla M. K. and Maxwell D. P. (1997) Genetic diversity among geminiviruses associated with weed specied *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima* from Jamaica. *Plant Disease* **81:** 1251-1258.

Roye M., Collins A. and Maxwell D. P. (2005) The first report of a begomovirus associated with the common weed *Jatropha gossypifolia* in Jamaica. *New Disease Reports* **11:** 46.

Roye M., Collins S. and Maxwell D. P. (2006) The first report of a begomovirus associated with the common weed *Jatropha gossypifolia* in Jamaica. *Plant Pathology* **55:** 286.

Rybicki E. P. and Pietersen G. (1999) Plant virus disease problems in the developing world. *Advances in Virus Research* **53:** 127-175.

Saikia A. K. and Muniyappa V. (1989) Epediology and control of tomato leaf curl virus in Southern India. *Tropical Agriculture* **66:** 350-354.

Saitou N. and Nei M. (1997) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4:** 406-425

Salati R., Nahkla M. K., Rojas M. R., Guzman P., Jaquez J., Maxwell D. and Gilbertson R. L. (2002) *Tomato yellow leaf curl virus* in the Dominican Republic: characterization of an infectious clone, virus monitoring in whiteflies and identification of reservoir host. *Phytopathology* **92:** 487-496.

Samad A., Gupta M. K., Shasany A. K., Ajayakumar P. V. and Alam M. (2009) Begomovirus related to Tomato leaf curl Pakistan virus newly reported in Mentha crops in India. *Plant Pathology* **58:** 404.

Sambrook J. and Russel D. W. (2001) *A laboratory manual*, Second edition, Cold Spring Harbour, Laboratory Press, USA.

Santoso T. J., Hidayat S. H., Duriat A. S., Herman M. and Sudarsono (2008) Identity and Sequence Diversity of Begomovirus Associated with Yellow Leaf Curl Disease of Tomato in Indonesia. *Microbiology* **2:** 1-7.

Sanz A. I., Fraile A., Garcia-Arenal F., Zhou X., Robinson D. J., Khalid S., Butt T. and Harrison B. D. (2000) Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. *Virology* **81:** 1839-1849.

Saqib M., Jones R. A. C., Cayford B. and Jones M. G. K. (2005) First report of *Bean common mosaic virus* in Western Australia. *Plant Pathology* **54:** 563.

Sastry S. and Singh S. J. (1973) Assessment of losses in tomato by Tomato leaf curl virus. *Ind. J. Mycol. Plant Pathol.* **3:** 50-54.

Saunders K., Bedford I. D., Briddon R. W., Markham P. G., Wong S. M. and Stanley J. (2000) A unique virus complex causes Ageratum yellow vein disease. *Proceeding of National Academy of Sciences USA* **97:** 6890-6895.

Saunders K., Bedford I. D., Yahara T. and Stanley J. (2003) The earliest recorded plant virus disease. *Nature* **422:** 831.

Saunders K., Norman A., Gucciardo S. and Stanley J. (2004) The DNA β satellite component associated with Ageratum yellow vein disease encodes an essential pathogenicity protein (bC1). *Virology* **324:** 37-47.

Schaad M. C., Jensen P. E. and Carrington J. C. (1997) Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. *The EMBO Journal* **16:** 4049-4059.

Schmidt H. E. (1992) Bean mosaics. *In:* Plant Diseases of International Importance. Vol. II, Chaube HS, Singh US, Mukhopadhya AN, Kumar J(Eds.), Prentice Hall, New Jersey, pp 40-73.

Scholthof H. B. (2005) Plant virus transport: motions of functional equivalence. *Trends Plant Sci.* **10:** 376-382.

Scholthof H. B., Alvarado V. Y., Vega-Arreguin J. C., Ciomperlik J., Odokonyero D., Brosseau C., Jaubert M., Zamora A. and Moffett P. (2011) Identification of an argonaute for antiviral RNA silencing in *Nicotiana benthamiana*. *Plant Physiol.* **156:** 1548-1555

Settlage S. B., See R. G. and Hanley-Bowdoin L. (2005) Geminivirus C3 protein: replication enhancement and protein interactions. *Journal of Virology* **79:** 9885-9895.

Shahabuddin M., Shaw J. G. and Rhoads R. E. (1988) Mapping of the *Tobacco vein mottling virus* VPg cistron. *Virology* **163:** 635-637.

Shaikh B. (1996) Studies on Papaya ringspot virus, M. Sc. (Agri) Thesis, University of Agriultuarl Sciesnces, Dharwad, pp. 71.

Sharma P., Sharma P. N., Kapil R., Sharma S. K. and Sharma O. P. (2011) Analysis of 3'-terminal region of *Bean common mosaic virus* strains infecting common bean in India. *Indian J. Virol.* **22:** 37-43.

Sharma R., Mahila H. R., Mohapatra T., Bhargava S. C. and Sharma M. M. (2003) Isolating plant genomic DNA without liquid nitrogen. *Plant molecular biology Reporter* **21:** 43-50.

Shen W., Yan P., Gao L., Pan X., Wu J. and Zhou P. (2010) Helper component-proteinase (HC-Pro) protein of Papaya ringspot virus interacts with papaya calreticulin. *Mol. Plant Pathol.* **11:** 335-346.

Shih S. L., Tsai W. S., Green S. K. and Sing D. (2006) First report of Tomato leaf curl Joydebpur virus infecting chilli, India. *New Disease Reports* 13. http://www.bspp.org.uk/ndr/jan2007/2006-65.asp

Shukla D. D., Colin W. W. and Brunt A. A. (1994) *The Potyviridae*, Cambridge University Press, Cambridge, UK.

Singh A. K., Sing M. and Singh A. K. (1988a) Antiviral activity and physical properties of the extracts of *Azadirachta indica* L. *Indian Journal of virology* **4(2):** 76-81.

Singh M. K., Sherpa A. R., Hallan V., Zaidi A. A. (2007) A potyvirus in Cymbidium spp. in northern India. *Australasian Plant Disease Notes* **2:** 11-13.

Singh M. N., Khurana S. M. P., Nagaich B. B. and Agarwal H. O. (1988b) Environmental factors influencing aphid transmission of PVY and *Potato leaf roll virus*. *Potato research* **31:** 501-509.

Singh M.N., Khurana S. M. P., Nagaich B.B. and Agrawal H.O. (1982) Efficiency of *Aphis gossypii* and *Acyrthosiphan pisun* in transmitting potato viruses leafroll and Y. *In:* Potato in developing countries (B.B Nagaich *et al.* Eds.) IPA, Simla pp. 89-305.

Singh R. K., Singh D. and Singh J. (2003) Incidence, distribution and detection of a virus infecting papaya (*Carica papaya* L.) in Eastern Uttar Pradesh. *Indian Phytopathology* **21:** 51-56.

Singh R. P. (1998) Reverse transcription polymerase chain reaction for the detection of viruses from plants and aphids. *J. Virol. Methods* **74:** 125-138.

Singh R. P. (1999) Development of the molecular methods for potato virus and viroid detection and prevention. *Genome* **42:** 592-604.

Singh R. P., Valkonen J. P., Gray S. M., Boonham N., Jones R. A., Kerlan C. and Schubert J. (2008) The naming of Potato virus Y strains infecting potato. *Archives of Virology* **153:** 1-13.

Singh S. and Awasthi L. P. (2009) Plant products for the management of yellow mosaic disease of mungbean and urdbean. *The Journal of Plant Protection Sciences* **1:** 87-91.

Singh S. K., Awasthi L. P., Singh S. and Sharma N. K. (2011) Protection of mungbean and urdbean crops against vector borne *Mungbean yellow mosaic virus* through botanicals. *Curr. Bot.* **2:** 8-11.

Singh U. C, Singh R. and Nagaich K. N. (1999) Evaluation of tomato varieties against jassid (*Empoasca devastans*), whitefly (*Bemisia tabaci*) and leaf curl. *Indian Journal of Entomology* **61(2):** 173-176.

Sivalingam P. N., Malathi V. G. and Varma A. (2010) Molecular diversity of DNA-β associated with tomato leaf curl disease in India. *Arch. Virol.* **155:** 757-764.

Sivalingam P. N., Padmalatha K. V., Mandal B., Monga, D., Ajmera B. D. and Malathi V. G. (2007) Detection of begomoviruses by PCR in weeds and crop plants in and around cotton field infected with cotton leaf curl disease. *Indian Phytopathology* **60:** 356-361

Snehi S. K., Khan M. S., Raj S. K. and Prasad V. (2011) Complete nucleotide sequence of *Croton yellow vein mosaic virus* and DNA-β associated with yellow vein mosaic disease of *Jatropha gossypifolia* in India. *Virus Genes* **43:** 93-101.

Sohrab S. S., Mirza Z., Karim S., Rana D., Abuzenedah A. M., Choudhary A. G. Bhattacharya P. S. (2013) detection of Begomovirus associated with Okra leaf curl disease. *Archives of Phytopathology and Plant Protection* **46:** 1047-1053.

Somashekhara Y. M., Nateshan H. M. and Muniyappa V. (1997) Evaluation of neem products and insecticides against whitefly (*Bemisia tabaci*), a vector of tomato leaf curl virus. *Current Science* **44:** 716.

Somowiyarjo S. (1993) Detection and identification of cucurbit viruses in yogyakarta. *Iimu Pertanian* **5:** 657-663.

Srivastava K. M., Hallan V., Raizada R. K., Chandra G., Singh B. P. and Sane P. V. (1995) Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. *Journal of Virological Method* **51:** 297-304

Ssekyewa C. (2006) Incidence, distribution and characteristic of major tomato leaf curl and mosaic virus diseases in Uganda. PhD-thesis, Faculty of bioscience engineering, Ghent University, Ghent, Belgium.

Stanley J., Boulton M. I. and Davies W. J. (2001) Geminiviridae Encyclopedia of Life Sciences (www.els.org).

Stanley J., Frischmuth T. and Ellwood S. (1990) Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants. *Proc. Natl. Acad. Sci. USA* **87:** 6291-6295.

Stonor J., Hart P., Gunther M., DeBarro P. and Rezaian M. A. (2003) Tomato leaf curl geminivirus in Australia: occurrence, detection, sequence diversity and host range. *Plant Pathology* **52:** 379-388.

Strange R. N. (1993) Plant Diseases Control: towards environmentally acceptable methods. Champman and Hall, London.

Strenger D. C., Duffus J. E. and Villalon B. (1990) Biological and genomic properties of a geminivirus isolated from pepper. *Phytopathology* **80:** 704-709.

Surekha S. K., Mathur K., Shukla D. D. (1977) Virus disease of papaya (*Carica papaya)* in Udaipur. *Indian Journal of Mycology and Plant Pathology* **7:** 115-121.

Susan John S. K. (1985) Studies on mosaic disease of papaya (*Carica papya*), M. Sc. (Agri) Thesis, Acharaya N.G Ranga Agricultural University, Hyderabad, pp. 43.

Syller J. (2006) The roles and mechanisms of helper component proteins encoded by potyviruses and caulimoviruses. *Physiol. Mol. Plant Pathol.* **67:** 119-130.

Tahir M., Haider M. S., Shah A. H., Rashid N. and Saleem F. (2006) First report of bipartite begomovirus associated with leaf curl disease of *Duranta repens* in Pakistan. *J. Plant Pathol.* **88:** 339.

Tamura K., Dudley J., Nei M., Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24:** 1596-1599

Tamura K., Nei M. and Kumar S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceeding of the National Academy of Science (USA)* **101:** 11030-11035.

Tatineni S., Ziems A. D., Wegulo S. N. and French R. (2009) Triticum mosaic virus: A distinct member of the family Potyviridae with an unusually long leader sequence. *Phytopathology* **99:** 943-950.

Tewari J. P., Dwivedi H. D., Pathak M. and Srivastava S. K. (2007) Incidence of a mosaic disease in *Jatropha curcas* L. from eastern Uttar Pradesh. *Current science* **93:** 1048-1049.

Teycheney P. Y., Aaziz R., Dinant S., Salanki K., Tourneur C., Balaz E., Jacquemond M. and Tepfer M. (2000) Synthesis of (-) strand RNA from the 3' untranslated region of plant viral genomes expressed in transgenic plants upon infected with related viruses. *J. Gen. Virol.* **81:** 1121-1126.

Thomason I. J. and McKenry M. (1975) Chemical control of nematode vectors of plant viruses. *In:* Lamberti F, Taylor CE and Seinhorst JW (eds) Nematode Vectors of Plant Viruses, pp. 423–439. London: Plenum Press.

Thompson J. D., Higgins D. G. and T. J. Gibson (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673-4680.

Thresh J. M. (1980) The origins and epidemiology of some important plant virus diseases. *Applied Biology* **5:** 1-65.

Thresh J. M. (1986) The control of cocoa swollen shoot disease in Ghana: an evaluation of eradication procedures. *Crop Protection* **5:** 41-52.

Thresh J. M. and Cooter R. J. (2005) Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology* **54:** 587-614.

Thresh J. M., Otim-Nape G. W., Thankappan M. and Muniyappa V. (1998) The mosaic diseases of cassava in Africa and India caused by whitefly-borne geminiviruses. *Review of Plant Pathology* **77:** 935-945.

Tiwari N., Padmalatha K. V., Singh V. B., Haq Q. M. I. and Malathi V. G. (2010) Tomato leaf curl Bangalore virus (ToLCBV): infectivity and enhanced pathogenicity with diverse betasatellite. *Arch. Virol.* **155:** 1343-1347.

Tiwari N., Singh V. B., Sharma P. K. and Malathi V. G. (2013) Tomato leaf curl Joydebpur virus: a monopartite begomovirus causing severe leaf curl in tomato West Bengal. *Arch. Virol.* **158:** 1-10.

Torrance L. and Mayo M. A. (1997) Proposed re-classification of furoviruses. *Archives of Virology* **142:** 435-439.

Trigiano T. N., Windham M. T. and Windham A. S. (2003) Plant Pathology: Concepts and Laboratory Exercises. CRC Press, Florida.

Tzfira T., Rhee Y., Chen M. H., Kunik T. and Citovsky V. (2000) Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. *Annu. Rev. Microbiol.* **54:** 187-219.

Umaharan P., Padidam M., Phelps R. H., Beachy R. N. and Fauquet C. M. (1998) Distribution and diversity of Geminiviruses in Trinidad and Tobago. *Phytopathology* **88:** 1262-1268.

Urcuqui-Inchima S., Haenni A. L. and Bernardi F. (2001) Potyvirus proteins: a wealth of functions. *Virus Research* **74:** 157-175.

Usharani K. S., Surendranath B., Khurana S. M. P., Garg I. D. and Malathi V. G. (2003) Potato leaf curl - a new disease of potato in northern India caused by a strain of *Tomato leaf curl New Delhi virus*. *New Disease Reports* **8:** 2.

Usharani K. S., Surendranath B., Paul-Khurana S. M., Garg I. D. and Malathi V. G. (2004) Potato leaf curl - a new disease of potato in northern India caused by a strain of *Tomato leaf curl New Delhi virus*. *Plant Pathology* **53:** 235.

Van-Regenmortel H. V. (1990) Virus species, a much overlooked but essential concept in virus classification. *Intervirology* **31:** 241-254.

Varma A. (1988) The economic impact of fi lamentous viruses. In: *The Plant Viruses: The Filamentous Plant Viruses*. Chapter 10, Milne RG (Ed.), Plenum Press, New York and London, pp 331–407.

Varma A. (1990) Changing pattern of plant diseases caused by whitefly transmitted geminiviruses. Abstracts of the 8th International Congress of Virology, Berlin, pp. 31.

Varma A. and Malathi V. G. (2003) Emerging geminivirus problems: a serious threat to crop production. *Ann. Appl. Biol.***142:** 145-164.

Varma A., Dhar A. K., Mandal B. (1992) MYMV transmission and control in India. *Mungbean Yellow Mosaic Disease: Proceedings of an International Workshop*, Asian Vegetable Research and Development Center, Taipei, pp. 8-27.

Varma P. M. (1963) Transmission of plant viruses by whiteflies. *Bulletin NISI,* National Institute of Science in India, pp. 11-33.

Vasudeva R. S. and Samraj J. (1948) A leaf curl disease of tomato. *Phytopathology* **38:** 364-369.

Venkataravanappa V., Swarnalatha P., Lakshminarayana R. C. N., Mahesh B., Rai A. B. and Krishna R. M. (2012) Molecular evidence for association of Tobacco curly shoot virus and a betasatellite with curly shoot disease of common bean (*Phaseolus vulgaris* L.) from India. *Journal of Plant Pathology and Microbiology* **3:** 148.

Venkatasalam E. P., Singh S., Sivalingam P. N., Malathi V. G. and Garg I. D. (2011) Polymerase chain reaction and nucleic acid spot hybridization detection of begomovirus (es) associated with apical leaf curl disease of potato. *Archives of Phytopathology and Plant Protection* **44:** 987-992.

Venkatasalam E. P., Singh S., Verma Y., Bhatt M. N., Garg I. D., Khurana S. M. P. and Malathi V. G. (2005) Detection of Geminivirus causing potato apical leaf curl by ELISA and NASH. *Indian Journal of Virology* **16:** 53.

Verma A. (1993) Integrated management of viral diseases. *In:* Ciba Foundation Symposium 177, Crop Protection and Sustainable Agriculture Book Series: Novartis Foundation Symposia.

Verma A. and Verma H. N. (1993) Management of viral disease of mungbean by Clerodendrum leaf extract. *Indian J. Plant Pathology* **11:** 63-65.

Verma H. N., khan M. M. A. A. and Dwivedi S. D. (1985) Biophysical properties of highly antiviral agents presente in *Pseuderunthamum* and *Bougainvillea spectabilis* extracts. *Indian Journal of Plant Pathology* **3(1):** 13-20.

Vetten H. J., Lasemann D. E. and Maiss E. (1992) Serotype A and B strains of Bean common mosaic virus are two distinct potyviruses. *Arch. Virol.* **5:** 415-431.

Volkov Y. G., Kakareka N. N., Balabanova L. A., Kozlovskaya Z. N. and Sapotsky M. V. (2009) Characterization of a novel far-eastern *Potato virus Y* isolates. *Plant Pathology Journal* **8:** 62-67.

Wang J., Bing X. L., Li M., Ye G. Y. and Liu S. S. (2012) Infection of tobacco plants by a Begomovirus improves nutritional assimilation by a whitefly. *Entomol. Exper. Applicate.* **144:** 191-201.

Whitham S. A. and Wang Y. (2004) Roles for host factors in plant viral pathogenicity. *Current Opinion in Plant Biology* **7:** 365-371.

Windham A. S., Hale F. S. and Yanes J. (1998) Impatiens necrotic spot virus-a serious pathogen of floral crops. *In*: Agricultural Extension Service. The University of Tennessee.

Wong S. M., Swanson M. M. and Harrisn B. D. (1993) A geminivirus causing vein yellowing of *Ageratum conyzoides* in Singapore. *Plant Pathology* **42:** 137-139.

Wu C. Z. and Zhou X. P. (2006) First report of *Malvastrus leaf curl virus* infecting papaya. *J. Plant Pathol.* **88:** 342.

Wutscher H. K. (1977) Citrus tree virus and virus-like diseases. *Horticultural Science* **12:** 478-484.

Wyatt S. D. and Brown J. K. (1996) Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology* **86:** 1288-1293.

Xiong Q., Fan S., Wu J. and Zhou X. (2007) Ageratum yellow vein China virus Is a Distinct Begomovirus Species Associated with a DNAβ Molecule. *Virology* **97:** 405-411.

Yemewar S. I. and Mali V. R. (1980) On the identity of a sap transmissible virus of papaya in Marathawada. *Indian Journal of Mycology and Plant Pathology* **10:** 155-160.

Zaffalon V., Mukherjee S. K., Reddy V. S., Thompson J. R. and Tepfer M. (2012) A survey of geminiviruses and associated satellite DNAs in the cotton-growing areas of northwestern India. *Archives of Virology* **157:** 483-495.

Zaitlin M. and Palukaitis P. (2000) Advances in understanding plant viruses and virus diseases. *Annual Review of Phytopathology* **38:** 117-143.

Zhou X. P., Xie Y., Tao X. R., Zhang Z. K., Li Z. H. and Fauquet C. M. (2003) Characterization of DNA beta associated with Begomoviruses in China and evidence for co-evolution with their cognate viral DNA A. *Journal of General Virology* **84:** 237-247.

7. Appendix

Appendix I: GenBank submissions

Sl No.	Accession Number	Title	Host	Status
1	HM245383	Tomato leaf curl New Delhi virus isolate TLCV-Cob-02 coat protein gene, complete cds	*Lycopersicon esculentum*	Published on 28-Sep-2010
2	HM856624	Tobacco leaf curl virus isolate COB1 pre-coat protein (AV2) gene, complete cds; and coat protein gene (AV1) gene, partial cds	*Lycopersicon esculentum*	Published on 08-Nov-2010
3	HM856625	Tomato leaf curl New Delhi virus isolate COB-2 pre-coat protein (AV2), coat protein (AV1), AC5 (AC5), AC3 (AC3), and AC2 (AC2) genes, complete cds	*Lycopersicon esculentum*	Published on 08-Nov-2010
4	HM856626	Tobacco curly shoot virus isolate SILIGURI-1 coat protein (AV1) gene, partial cds; replication enhancer protein (AC3) gene, complete cds; and transcription activator protein (AC2) gene, partial cds	*Lycopersicon esculentum*	Published on 09-NOV-2010
5	HM856627	Tobacco curly shoot virus isolate HALDI-1 AV1 (AV1), AC3 protein (AC3) AC2 (AC2) gene, partial cds	*Lycopersicon esculentum*	Publushed on 08-Nov-2010
6	HM856628	Tomato leaf curl New Delhi virus isolate SILIGURI-2 coat protein (AV1) gene, partial cds	*Lycopersicon esculentum*	Published on 09-NOV-2010
7	HQ597029	Jatropha mosaic India virus isolate SILIGU-1 pre-coat protein (AV2) gene, complete cds; and coat protein (AV1) gene, partial cds	*Jatropha curcas*	Published on 25-May-2011
8	HQ597030	Croton yellow vein virus isolate SILIGURI pre-coat protein (AV2) gene, complete cds; and coat protein (AV1) gene, partial cds	*Croton bonplandianus*	Published on 25-May-2011
9	HQ597031	Tobacco curly shoot virus isolate SILIGURI-1 pre-coat protein (AV2) gene, complete cds; and coat protein (AV1) gene, partial cds	*Lycopersicon esculentum*	Published on 25-May-2011
10	HQ597032	Tomato leaf curl New Delhi virus isolate SILIGURI-2 AV3 (AV3), pre-coat protein (AV2), coat protein (AV1), AC5 (AC5), AC3 (AC3), and AC2 (AC2) genes, complete cds; and AC1 (AC1) gene, partial cds	*Solanum tuberosum*	Published on 25-May-2011
11	HQ597033	Tomato leaf curl New Delhi virus isolate COB-3 pre-coat protein (AV2), coat protein (AV1), AC5 (AC5), AC3 (AC3), and AC2 (AC2) genes, complete cds; and AC1 (AC1) gene, partial cds	*Lycopersicon esculentum*	Published on 25-May-2011
12	JF461062	Tobacco curly shoot virus isolate Cob4 coat protein (AV1) gene, complete cds	*Lycopersicon esculentum*	Published on 24-Aug-2011
13	JF509102	Tobacco curly shoot virus isolate COB5 coat protein (AV1) gene, complete cds	*Lycopersicon esculentum*	Published on 4-SEP-2011
14	JF825865	Tomato leaf curl New Delhi virus isolate RAI-1 coat protein (AV1) gene, complete cds	*Lycopersicon esculentum*	Published on 2-Oct-2011
15	JF825866	Tomato leaf curl Bangladesh virus isolate GUW-1 coat protein (AV1) gene, complete cds	*Lycopersicon esculentum*	Published on 2-Oct-2011

Contd....

GenBank submissions (Contd...)

Sl No.	Accession Number	Title	Host	Status
16	JF825867	Tobacco curly shoot virus isolate COB-6 pre-coat protein (AV2), coat protein (AV1), replication enhancer protein (AC3) and transcription activator protein (AC2) genes, complete cds and replication associated protein (AC1) gene, partial cds	*Lycopersicon esculentum*	Published on 2-Oct-2011
17	JF922010	Tobacco curly shoot virus isolate SILIGU-3 pre-coat protein (AV2) gene, complete cds; coat protein gene (AV1) partial cds	*Ageratum conyzoides*	Published on 16-Oct-2011
18	JF922011	Tobacco curly shoot virus isolate SILIGU-3 coat protein gene (AV1) partial cds; replication enhancer protein (AC3) gene, complete cds; transcription activator protein (AC2) gene, complete cds; replication associated protein (AC1) gene, partial cds	*Ageratum conyzoides*	Published on 16-Oct-2011
19	JN390432	Tomato leaf curl New Delhi virus isolate COB-3 nuclear shuttle protein (BV1), partial cds and movement protein (BC1), partial cds	*Solanum tuberosum*	Published on 13-Dec-2011
20	JN390433	Tomato leaf curl New Delhi virus isolate COB-2 nuclear shuttle protein (BV1), partial cds and movement protein (BC1), partial cds	*Lycopersicon esculentum*	Published on 13-Dec-2011
21	JN676052	Acalypha yellow mosaic Siliguri virus SILIGU-1 AV1 (AV1) gene, partial cds; AC3 (AC3), AC2 (AC2) gene, complete cds; AC1 (AC1) gene, partial cds	*Acalypha indica*	Published on 21-Feb-2012
22	JN676053	Tomato leaf curl Ranchi virus isolate GUW-2, complete genome	*Lycopersicon esculentum*	Published on 21-Feb-2011
23	JN676054	Tomato leaf curl New Delhi virus isolate SILIGU-4 coat protein (AV1) gene, complete cds	*Datura stramonium*	Published on 21-Feb-2012
24	JN676055	Tobacco curly shoot virus isolate SILIGURI-1 AV1 (AV1) gene, partial cds; AC3 (AC3) gene; AC2 (AC2) gene, complete cds; AC1 (AC1) gene, partial cds	*Lycopersicon esculentum*	Published on 21-Feb-2012
25	JQ796374	Croton yellow vein mosaic virus isolate SILIGURI capsid protein (AV1) gene, partial cds.	*Croton bonplandianus*	Published on 11-Jun-2012
26	JQ811770	Jatropha mosaic India virus isolate SILIGURI-2 capsid protein (AV1) gene, partial cds.	*Acalypha indica*	Published on 13-Jun-2012
27	JQ843097	Tobacco curly shoot virus isolate SILIGU-3 capsid protein (AV1) gene, partial cds.	*Ageratum conyzoides*	Published on 18-Jun-2012
28	JX945850	Potato virus Y isolate SLGPVY1 coat protein gene, partial cds	*Solanum tuberosum*	Published on 25-Dec-2012
29	JX567310	Papaya ringspot virus P isolate SLGPRSV1 coat protein gene, partial cds.	*Carica papaya*	Published on 11-Nov-2012
30	KC871565	Bean common mosaic virus isolate Coochbehar polyprotein gene, partial cds	*Phaseolus vulgaris* L.	Published on 30-Jul-2013

Apppendix II: Nucleotide sequences of isolated viral genome

1. Isolate: TLCV-Cob-02 [Tomato leaf curl New Delhi virus]

Accession Number- HM245383

TCCACCTACAAGGGTTTTGTTGGGGGGGGCCCAATTTATAGGCGCCCCCCCCCCAGGTTCAATTCACAAA
TATGGGGGATCCATTATTGCGCGAATTTCCAGAAAGCGTTCATGGTGTCAGGTGCATGCTAGCTGTAAAA
TATCTCCAAGAGATAGAAAAGAACTATTCCCCAGACACAGTCGGATACGATCTTGTCCGAGATCTAATTC
TTGTTCTTCGAGCAAAGAACTATGGTGAAGCGACCAGCAGATATCATCTTTTCAACGCCCGCATCGAAAG
TACGCCGACGTCTCAACTTCGACAGCCCCTTTGGAGCTCGTGCAGTTGTCCCCATTGTCCGCGTCACAAA
AGCAAAGGCCTGGACCAACAGGCCGATGAACAGAAAACCCAGAATATACAGAATGTATAGAAGTCCCGAC
GTGCCAAGGGGATGCGAAGGCCCTTGTAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCATATTG
GCAAAGTCATGTGTGTTAGTGATGTTACCCGAGGATCTGGACTCACCCATCGCGTAGGGAAGCGATTTTG
TGTGAAATCTGTTTATGTGCTGGGGAAGATATGGATGGATGAAAACATCAAGACAAAAAACCATACTAAC
AGCGTCATGTTTTTTTTGGTCCGTGACCGTCGTCCTACAGGATCTCCACAAGATTTTGGGGAAGTTTTTA
ACATGTTTGACAATGAACCGAGCACAGCAACGGTGAAGAACATGCATCGTGATCGTTATCAAGTCTTACG
GAAGTGGCATGCTACTGTGACGGGAGGAACATATGCATCTAAGGAGCAAGCATTAGTTAGCAAGTTTGTT
AGAGTTAATAATTATGTTGTGTATAACCAACAAGAGGCCGGCAAGTATGAGAATCATACTGAAAACGCAT
TAATGTTGTATATGGCCTGTACTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTT
CTATGATTCGGTAACAAATTAATAAATATCGAGTTTTATATCATATGAAGTACATACATCAATCGTTTGT
TCCATTACATTATCCAATACATGATAAACTGCTCTTATTACATTATAAATTCCTATTACACCAACCATAT
CCAGGTACCTAAGGACCTGTGTTTTGAAGACTCTCAAGAAAATCCCAATCTGAGGGCGTAAGCCCGTCCA
GATTTTGAAAGTTAGAAAACACTTGTGAAGTCCCAGGGCTTTCCGCAGGTTGTGGTTGAATTGTATTTGA
ATATTGATGATGTCGTGCTGTCTTAGGAAGGGTCTGCTGTTGTGTTTCAAAATTTTGAAATACAGGGGAT
TTCGAATTTCCCAGGTATATACGCCACTCACTGCTCGATCCGCAGTGATGGACTCCCCTGTGCGTGAATC
CGTGATCATGGCAGTTGATCGATATGTAATACGTACACCCCAACTGCAGATCACTCCGCCTCCTGCGAAT
GCTCTCCTTCGTCTCCTGTGGGATCGATGTTTTCGCGACCGGATTAGAGTGGTTCTTCGAGTGTGATAAA
GACTGCATCGTGATCGTTATCAAGTCTTACGGAAGTGGCATGCTACTGTGACGGGAGGAACATATGCATC
TAAGGAGCAAGCATTAGTTAGCAAGTTTGTTAGAGTTAATAATTATGTTGTGTATAACCAACAAGAGGCC
GGCA

2. Isolate: COB1 [Tobacco leaf curl virus]

Accession Number- HM856624

GGGTCGTGATAATATTACAAAGTGGGCCCCTTGATGTGATATGTCATCCAATCAGAACGCTCCCCCAAAG
CTTAATTGTCTTGTGGTCCCTTATTTAAACTTGCTCACCAAGTAGTGCACTCCGCACTATGTGGGATCCA
TTAGTAAACGAGTTTCCCGAAACCGTTCACGGCTTTAGGTGTATGTTAGCAGTTAAATATCTGCAGTTAG
TAGAGAAGACTTATTCGCCTGACACATTAGGGCACGATTTAATTAGGGATTTAATTTCAGTTATTAGGGC
TAGAAATTATGTCGAAGCGACCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCT
CAACTTCGACAGCCCATATGTGATCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAATCAAGAGCATGG
GCGAACAGGCCCATGAACAGAAAGCCGAAGATGTACAGGATGTACAGAAGTCCAGATGTCCCTAGAGGA

3. Isolate: COB-2 [Tomato leaf curl New Delhi virus]

Accession Number- HM856625

TCCACCTACAAGGGTTTTGTTGGGGGGGGCCCAATTTATAGGCGCCCCCCCCCCAGGTTCAATTCACAAA
TATGGGGGATCCATTATTGCGCGAATTTCCAGAAAGCGTTCATGGTGTCAGGTGCATGCTAGCTGTAAAA
TATCTCCAAGAGATAGAAAAGAACTATTCCCCAGACACAGTCGGATACGATCTTGTCCGAGATCTAATTC
TTGTTCTTCGAGCAAAGAACTATGGTGAAGCGACCAGCAGATATCATCTTTTCAACGCCCGCATCGAAAG

TACGCCGACGTCTCAACTTCGACAGCCCCTTTGGAGCTCGTGCAGTTGTCCCCATTGTCCGCGTCACAAA
AGCAAAGGCCTGGACCAACAGGCCGATGAACAGAAAACCCAGAATATACAGAATGTATAGAAGTCCCGAC
GTGCCAAGGGGATGCGAAGGCCCTTGTAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCATATTG
GCAAAGTCATGTGTGTTAGTGATGTTACCCGAGGATCTGGACTCACCCATCGCGTAGGGAAGCGATTTTG
TGTGAAATCTGTTTATGTGCTGGGGAAGATATGGATGGATGAAAACATCAAGACAAAAAACCATACTAAC
AGCGTCATGTTTTTTTTGGTCCGTGACCGTCGTCCTACAGGATCTCCACAAGATTTTGGGGAAGTTTTTA
ACATGTTTGACAATGAACCGAGCACAGCAACGGTGAAGAACATGCATCGTGATCGTTATCAAGTCTTACG
GAAGTGGCATGCTACTGTGACGGGAGGAACATATGCATCTAAGGAGCAAGCATTAGTTAGCAAGTTTGTT
AGAGTTAATAATTATGTTGTGTATAACCAACAAGAGGCCGGCAAGTATGAGAATCATACTGAAAACGCAT
TAATGTTGTATATGGCCTGTACTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTT
CTATGATTCGGTAACAAATTAATAAATATCGAGTTTTATATCATATGAAGTACATACATCAATCGTTTGT
TCCATTACATTATCCAATACATGATAAACTGCTCTTATTACATTATAAATTCCTATTACACCAACCATAT
CCAGGTACCTAAGGACCTGTGTTTTGAAGACTCTCAAGAAAATCCCAATCTGAGGGCGTAAGCCCGTCCA
GATTTTGAAAGTTAGAAAACACTTGTGAAGTCCCAGGGCTTTCCGCAGGTTGTGGTTGAATTGTATTTGA
ATATTGATGATGTCGTGCTGTCTTAGGAAGGGTCTGCTGTTGTGTTTCAAAATTTTGAAATACAGGGGAT
TTCGAATTTCCCAGGTATATACGCCACTCACTGCTCGATCCGCAGTGATGGACTCCCCTGTGCGTGAATC
CGTGATCATGGCAGTTGATCGATATGTAATACGTACACCCCAACTGCAGATCACTCCGCCTCCTGCGAAT
GCTCTCCTTCGTCTCCTGTGGGATCGATGTTTTCGCGACCGGATTAGAGTGGTTCTTCGAGTGTGATAAA
GACTGCATCGTGATCGTTATCAAGTCTTACGGAAGTGGCATGCTACTGTGACGGGAGGAACATATGCATC
TAAGGAGCAAGCATTAGTTAGCAAGTTTGTTAGAGTTAATAATTATGTTGTGTATAACCAACAAGAGGCC
GGCA

4. Isolate: SILIGURI-1 [Tobacco curly shoot virus]

Accession Number - HM856626

AACAGTGTTATGTTTTTTTTAGTTAGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTT
TTAACATGTTTGATAATGAGCCCAGTACGGCTACTGTGAAGAATGTTCATCGTGATAGGTATCAAGTGCT
TCGCAAATGGCATGCAACTGTTACGGGTGGACAATATGCGTCGAAGGAACAAGCCCTTGTGAAGAAATTT
GTTAGGGTTAATAATTATGTTGTGTATAACCAGCAAGAAGCTGGCAAGTATGAAAATCATTCTGAGAATG
CGTTAATGTTGTATATGGCATGTACTCATGCATCTAACCCAGTGTATGCTACTTTGAAGATACGGATCTA
TTTCTATGATTCCGTAACAAATTAATAAATATTAAATTTTATTGAATAAGATTGGTCTACATATACAATG
TGTTCTAATACATCCCATAATACACGATCAACTGCACGAATTACATTATTAATACTGATAATTCCTAAAT
TATTTAAATATTTAAAAACTTGAGTCTTAAAGACCCTTAAGAAACGACCAGTCTGAGGCTGTGAAGTCAT
CCAGATTCGGTAGACTAGAAAACACTTGTGCACTCCCAGAGCTTTCCGCAGGTTGTAGTTGAACTGTATT
TGGATTGTGATTATGTCTTCTTTCATGAGAAATGGACGGTTCTGGTGCTCTGTTATCTTGAAATAAAGGG
GATTTTGAATCTCCCAGATATACACGCCATTCTCTGCTTGAGCTGCAGTGATGAGTTCCCCTGTGCGTGA
ATCCATGATCGTGACAGGCTAGTGCTATGAAATATGAACATCCACAAGGGAGATCAACACG

5. Isolate: HALDI-1 [Tobacco curly shoot virus]

Accession Number - HM856627

AGTGTTATGTTTTTTTTAGTTAGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTTTTA
ACATGTTTGATAATGAGCCCAGTACGGCTACTGTGAAGAATGTTCATCGTGATAGGTATCAAGTGCTTCG
GAAATGGCATGCAACTGTTACGGGTGGACAATATGCGTCAAAGGAACAAGCTCTTGTGAAGAAATTTGTT
AGGGTTAATAATTATGTTGTGTATAATCAGCAAGAAGCTGGCAAGTATGAGAATCATTCTGAGAATGCGT
TAATGTTGTATATGGCATGTACTCATGCCTCTAATCCAGTGTATGCTACGTTGAAGATACGGATCTATTT
CTATGATTCCGTAACAAATTAATAAATGTTAAATTTTATTGAATAAGATTGGTCTACATATACAATGTGT
TGTAATCTATTCCATAATACATGATCAACTGCTCTAATTACATTATTCAAACTAATTACACCCAAATTAT
TAAGAAACTTCAAAACTTGAGTCTTAAAGACCCTTAAGAAACGACCAGTCTGAGGCTGTGAAGTCATCCA
GATTCGGTAGACTAGAAAACACTTGTGCACTCCCAGAGCTTTCCTCAGGTTGTAGTTGAACTGTATTTGT
ATCGTGATTATGTCTTCCTTCATTAGAAATGGACGGTTCTGGTGCTCTGTGATCTTGAAATAAAGGGGAT
TTTGAATCTCCCAGATATACACGCCATTCTCTGCTTGAGCT

6. Isolate: SILIGURI-2 [Tomato leaf curl New Delhi virus]

Accession Number - HM856628

ATGGCGAAGCGACCAGCAGATATCATCATTTCAACGCCCGCATCGAAGGTACGCCGACGTCTCAACTTCG
ACAGCCCCTATGGAGCTCGTGCAGTTGTCCCCATTGCCCGCGTCACAAAAGCAAAGGCCTGGACCAACAG
GCCGATGAACAGAAAACCCACAATGTACAGAATGTATAGAAGTCCCCACGTGCCAAGGGGCTGTGAAGGC
CCTTGCAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCACATTGGCAAAGTCATGTGCGTTAGTG
ATGTTACTCGAGGAACTGGACTCACACATCGCGTAGGGAAGCGATTTTGTGTCAAATCTGTCTATCTGCT
GGGCACTTGTACATATAATGAAAACATCAAGACAAAAAACCATACTAACAGTGTCATGTTTTTTTTGGTC
CGTGACCGTCGTCCTACAGGATCCCCTCAAGATTTTGGGGAAGTTTTTAACATGTTTGACAATGA

7. Isolate: SILIGU-1 [Jatropha mosaic India virus]

Accession Number - HQ597029

TGTGGTCCCTCACCCCACGTGGAGTTGTCGCCCACTCAGAACGCGCACTCAACGCTTGAATAATTGTGGT
CCCCGTTATAAGTACTTCGTGAGCAAGTTAATACTTGCAACATGTGGGATCCACTACTTAACGAGTTTCC
AGACTCCGTTCACGGTTTCCGTTGTATGCTAGCGCTGAAGTATCTTCAGCTTGTCGAAAGTACTTATTCC
CCTGATACGCTCGGGTACGATCTAATCCGGGACCTGATTTTGGTTATCAGGGCCAGAAGCTATGCCGAAG
CGACCAGGAGATATCATCATTTCAACTCCCGCCTCGAAGGTACGTCGCCGTCTGATCTTCGACAGCCCGT
ACAGCAACCGTGCTTCTGTCTTCACTGTCCGCGTCACCAAAAGACAAGCCTGGACAAACAGGCCCATGAA
TCGGAAGCCCAGATGGTACAGGATGTTCAGAAGCCCAGATGTTCCTAGGGGATGTGAAGGCCCATGTAAG
GTCCA

8. Isolate: SILIGURI [Croton yellow vein virus]

Accession Number - HQ597030

TAATATTACCTGATGGCCGCGAAAAAAAAAGTGGTGGGCCCCACGGCGCACTAACTGATAAAGACATATC
CACCAATGAAAAGAGCTCCTCAGAGCTTAATTGTTTTGTGGTCCCCTATTTAAACTTCGCCACCAAGTAG
TGCATTGCGCATTATGTGGGATCCATTAGTAAACGAGTTTCCCGAAACCGTTCATGGGTTTAGATGTATG
TTAGCAGTTAAATATCTGCAGTTAGTAGAGAAAACTTATGCTCCTGACACATTGGGTCACGATTTAATTA
GGGATTTAATTTCAGTAATTAGGGCTAGAAATTATGTCGAAGCGTCCAGCAGATATAATCATTTCCACGC
CCGCTTCGAAGGTACGCCGTCGTCTCAACTTCGACAGCCCATATGTGAACCGTGCTGCTGCCCCCATTGT
CCGCGTCACCAAAGCAAAGGCATGGGCGAACAGACCCATGTACCGGAAGCCCAGGATGTACAGGATGTAC
AGAAGCCCAGATGTCCCTAAGGGATGTGAAGGGCCATGCAAAGTCC

9. Isolate: SILIGURI-1 [Tobacco curly shoot virus]

Accession Number - HQ597031

TGATTTTTTTTAAAGTGGGCCCCTTGATGTGATATGTCATTCAATCAGAACGCTCCCCTAAAGCTTAATT
GTTTTGTGGTCCCTTATTTATACTTGCTCTGCAAGTAGTGCACTCCGCACTATGTGGGATCCATTAGTAA
ACGAGTTTCCCGAAACCGTTCACGGCTTTAGGTGTATGTTAGCAGTTAAATATCTGCAGTTAGTAGAGAA
AACGTATTCTCCCGACACATTAGGGCACGATTTAATTAGGGATTTAATTTCAGTTATTAGGGCTAGAAAT
TATGTCGAAGCGTCCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTC
GACAGCCCATATGTGAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAAGCAAGAGCATGGGCGAACA
GGCCCATGAACAGAAAACCCAGGATGTACAGGATGTACAGAAGTCCTGATGTCCCTAGAGGATGTGAAGG
GCCATGCAAAGTCCAAC

10. Isolate: SILIGURI-2 [Tomato leaf curl New Delhi virus]

Accession Number - HQ597032

ATTTTTCCTGATGGCCGCGCAAATTTATAAGTGGGCCCTCAACCAATGAAATTCACGCTACATGGCCTAT
TTAGTGCGTGGGGATCAATAAATAGACTTGCTCACCAAGTTTGGATCCACAAACATGTGGGATCCATTAT
TGCACGAATTTCCAGAAAGCGTTCATGGTCTAAGGTGCATGCTAGCTGTAAAATATCTCCAAGAGATAGA
AAAGAACTATTCCCCAGACACAGTCGGCTACGATCTTGTCCGAGATCTCATTCTTGTTCTCCGAGCAAAG
AACTATGGCGAAGCGACCAGCAGATATCATCATTTCAACGCCCGCATCGAAGGTACGCCGACGTCTCAAC
TTCGACAGCCCCTATGGAGCTCGTGCAGTTGTCCCCATTGCCCGCGTCACAAAAGCAAAGGCCTGGACCA
ACAGGCCGATGAACAGAAAACCCACAATGTACAGAATGTATAGAAGTCCCCACGTGCCAAGGGGCTGTGA
AGGCCCTTGCAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCACATTGGCAAAGTCATGTGCGTT
AGTGATGTTACTCGAGGAACTGGACTCACACATCGCGTAGGGAAGCGATTTTGTGTCAAATCTGTCTATC
TGCTGGGCACTTGTACATATAATGAAAACATCAAGACAAAAAACCATACTAACAGTGTCATGTTTTTTTT
GGTCCGTGACCGTCGTCCTACAGGATCCCCTCAAGATTTTGGGGAAGTTTTTAACATGTTTGACAATGAA
CCGAGCACAGCAACGGTGAAGAACATGCATCGTGATCGTTATCAAGTCTTACGTAAATGGCATGCGACTG
TGACGGGAGGAACTTATGCATCTAAGGAGCAAGCATTAGTTAGGAAGTTTGTTAGGGTTAATAATTATGT
AGTTTACAATCAACAAGAGGCCGGCAAGTATGAGAATCATACTGAAAATGCATTAATGTTGTATATGGCC
TGTACTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTTCTATGATTCGGTAACAA
ATTAATAAATATCCAGCTTTATATCATATGAAGTCTGTACATCAATCGTCTGTTCCATTACATTATCCAA
AACATGATAAACTGCTCTTATTACATTATAAATTCCTATTACACCAAGCATATCCAGGTACCTAAGGACC
TGGGTTTTGAAGACTCTCAAGAAAATCCCAGTCTGAGGGCGTAAGCCCGTCCAGATTTTGAAAGTTAGAA
AACACTTGTGAAGTCCCAGGGCTTTCCGCAGGTTGTGGTTGAATTGTATTTGAATCTTGATGATGTCGTG
CTGTGTTAGGAAGGGCCTGCTGTCGTGCTTCAAAAGTTTGAGATACAGGGGATTTCGAATTTCCCAGGTA
TATACGCCACTCTCTGCTCGTTCCGCAGTGATGTACTCCCCTGTGCGTGAATCCGTGATCATGGCAGTTG
ATCGATATATAATACGTACACCCACACGGCAGATCAACTCGCCTCTTGGGAATGCTCTTCTTCTTCTTCT
GTGGGAGGGAGGTCTTCGCGACCGGAATAGAGTGCTTCCCCGAGTGTGTTGAAGTTTGCATTCTGGATTG
CCCACTGGTTCCGTGATTCATTTTTCTCTTCGTCTAAATATTCTTTATGATGGCCATGTGGACCTTTATT
GCACAGGAAGATAGTGGGAATTCCACCGTTAACACTTACTGGCTTACCGTGGCTGGGCTTGCTTTTCCAG
CCTCGCTGGGCCCCCATGAATTCTTTAAATTGCTTTAGATATTGGGGATCAACGTCATCAATGACGTTGT
ACCAGGCATCATTGCTATACACCTTTGGGCTCAGATCAAGATGTCCACACAAGTAATTGTGGGGTCCTAA
GCATCGAGC

11. Isolate: COB-3 [Tomato leaf curl New Delhi virus]

Accession Number - HQ597033

TTTGCTGCGGGGGGACCAATAAATAGACTTACTCACCCTGTTTGGATCCACAAACATGTGGGATCCATTA
TTGCACGAATTTCCAGAAAGCGTTCATGGTTTAAGGTGCATGCTAGCTGTAAAATATCTCCAAGAGATAG
AAAAGAACTATTCCCCAGACACAGTCGGATACGATCTTGTCCGAGATCTCATTCTTGTTCTTCGAGCAAA
GAACTATGGTGAAGCGACCAGCAGATATCATCTTTTCAACGCCCGTATCGAAAGTACGCCGACGTCTCAA
CTTCGACAGCCCCTTTGGAGCTCGTGCAGTTGTCCCCATTGCCCGCGTCACAAAAGCAAAGGCCTGGACC
AACAGGCCGATGAACAGAAAACCCAGAATGTACAGAATGTATAGAAGTCCCGACGTGCCAAGGGGCTGTG
AAGGCCCTTGTAAGGTCAATTCCTTTGAATCTAGGCACGATGTTTCTCATATTGGCAAAGTCATGTGTGT
TAGTGATGTTACCCGAGGATCTGGACTCACCCATCGGGTAGGGAAGCGATTTTGTGTGAAATCTGTCTAT
GTGCTGGGGAAGATATGGATGGATGAAAACATCAAGACAAAAAACCATACTAACAGTGTCATGTTTTTTT
TGGTCCGTGACCGTCGTCCTACAGGATCTCCACAAGATTTTGGAGAAGTTTTTAACATGTTTGACAATGA
ACCGAGCACAGCAACGGTGAAGAACATGCATCGTGATCGTTATCAAGTCTTACGGAAGTGGCATGCTACT
GTGACGGGAGGAACATATGCATCTAAGGAGCAAGCATTAGTTAGGAAGTTTGTTAGGGTTAATAATTATG
TTGTGTATAACCAACAAGAGGCCGGCAAGTATGAGAATCATACTGAGAACGCATTAATGTTGTATATGGC
CTGTACTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTTCTATGATTCGGTAACA
AATTAATAAATATCGAGTTTTATATCATATGAAGTCCATACATCAATCGTTTGTTCCATTACATTATCCA
AAACATGATAAACTGCTCTTATTACATTATAAATTCCTATTACACCAAGCCTATCCAGGTACCTAAGGAC
CTGTGTTTTGAAGACTCTCAAGAAAATCCCAATCTGAGGGCGTAAGCCCGTCCAGATTTTGAAAGTTAGA
AAACACTTGTGAAGTCCCAGGGCTTTCCGCAGGTTGTGGTTGAATTGTATTTGAATATTGATGATGTCGT

GCTGTCTTAGGAAGGGTCTGCTGTTGTGTTTCAAAATTTTGAAATACAGGGGATTTCGAATTTCCCAGGT
ATAAACGCCACTCTCTGCTCGATCCGCAGTGATGGACTCCCCTGTGCGTGAATCCGGGATCATGGAAGTG
GATCGATATGTAAAACGTACACCCACACTGCAAATCAATTCGCCTCCTGCGAATGCTCTTCTTCTTCTTC
TGTGGGAGCGATGTTTTCGCGACCGGAATAGAGTGGTTCTTCGAGTGTGATAAAGACTGCATTCTTGAT

12. Isolate: Cob4 [Tobacco curly shoot virus]

Accession Number - JF461062

ATGTCGAAGCGACCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTCG
ACAGCCCATATGTGAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAAGCAAGAGCATGGGCGAACAG
GCCCATGAACAGAAAGCCGAAGATGTACAGGATGTACAGAAGTCCTGATGTCCCTAGAGGATGTGAAGGC
CCATGTAAGGTCCAGTCGTTTGAGTCTAGACATGACATTCAGCATATAGGTAAAGTTATGTGTATCAGTG
ATGTTACGCGTGGAACTGGGCTGACCCATCGAGTGGGTAAAAGGTTTTGTGTTAAATCCGTTTATGTCTT
GGGTAAGATCTGGATGGATGAAAATATTAAGACCAAGAACCACACTAACAGTGTTATGTTTTTTTTAGTT
AGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTTTTAACATGTTTGATAATGAGCCCA
GTACGGCTACTGTGAAGAATGTTCATCCTGATAGGTATCAAGTGCTTCGGAAATGGCTTGCAACTGTTAC
GGGTGGACAATATGCGTCAAAGGAACAAGCTCTTGTGAAGAAATTTGTTAGGGTTAATAATTATGTTGTG
TATAACCAGCAAGAAGCTGGCAAGTATGAGAATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTA
CTCATGCCTCTAACCCAGTGTATGCTACGTTGAAGATACGGATCTATTTCTATGATTCCGTAACAAATTA
A

13. Isolate: COB5 [Tobacco curly shoot virus]

Accession Number - JF509102

ATGTCGAAGCGACCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTCG
ACAGCCCATATGTGATCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAATCAAGAGCATGGGCGAACAG
GCCCATGAACAGAAAGCCGAAGATGTACAGGATGTACAGAAGTCCTGATGTCCCTAGAGGATGTGAAGGC
CCATGTAAGGTCCAGTCGTTTGAGTCTAGACATGACATTCAGCATATAGGTAAAGTTATGTGTATCAGTG
ATGTTACGCGTGGAACTGGGCTGACCCATCGAGTGGGTAAAAGGTTTTGTGTTAAATCCGTTTATGTCTT
GGGTAAGATCTGGATGGATGAAAATATTAAGACCAAGAATCACACTAACAGTGTTATGTTTTTTTTAGTT
AGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTTTTAACATGTTTGATAATGAGCCCA
GTACGGCTACTGTGAAGAATGTTCATCGTGATAGGTATCAAGTGCTTCGCAAATGGCATGCAACTGTTAC
GGGTGGACAATATGCGTCAAAGGAACAAGCCCTTGTGAAGAAATTTGTTAGGGTTAATAATTATGTTGTG
TATAACCAGCAAGAAGCTGGCAAGTATGAAAATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTA
CTCATGCCTCTAACCCAGTGTATGCTACTTTGAAGATACGGATCTATTTCTATGATTCCGTAACAAATTA
A

14. Isolate: RAI-1 [Tomato leaf curl New Delhi virus]

Accession Number - JF825865

ATGGTGAAGCGACCAGCAGATATCATCTTTTCAACGCCCGCATCGAAAGTACGCCGACGTCTCAACTTCG
ACAGCCCCTATGGAGCTCGTGCAGTTGTCCCCATTGCCCGCGTCACAAAAGCAAAGGCCTGGACCAACAG
GCCGATGAACAGAAAACCCAGAATATACAGAATGTATAGAAGTCCCGACGTGCCAAGGGGATGTGAAGGC
CCTTGTAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCATATTGGCAAAGTCATGTGTGTTAGTG
ATGTTACCCGAGGATCTGGACTCACCCATCGCGTAGGGAAGCGATTTTGTGTGAAATCTGTCTATGTGCT
GGGGAAGATATGGATGGATGAAAACATCAAGACAAAAAACCATACTAACAGTGTCATGTTTTTTTTGGTC
CGTGACCGTCGTCCTACAGGATCTCCACAAGATTTTGGGGAAGTTTTTAACATGTTTGACAATGAACCGA
GCACAGCAACGGTGAAAAACATGCATCGTGATCGTTATCAAGTCTTATTGAAGTGGCATGCTTCTGTGAC
GGGAGGGACATATGCATCTAAGGAGCAAGCATTAGTTAGCAAGTTTGTTAGGGTTAATAATTATGTTGTG
TATAACCAACAAGAAGCCGGCAAGTATGAGAATCATACTGAAAACGCATTAATGTTGTATATGGCCTGTA
CTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTTCTATGATTCGGTAACAAATTA
A

15. Isolate: GUW-1 [Tomato leaf curl Bangladesh virus]

Accession Number - JF825866

ATGTCGAAGCGACCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTCG
ACAGCCCATATGCGAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAAGCAAGAGCATGGGCGAACAG
GCCCATGAACAGAAAGCCCAGGATGTACAGGATGTACAGAAGTCCAGATGTGCCTAGAGGCTGTGAAGGC
CCATGTAAGGTGCAGTCTTTTGAGTCTAGACATGACATTCAGCATATAGGTAAAGTCATGTGTGTCAGTG
ATGTTACTCGTGGGACTGGGCTGACCCATCGAGTGGGTAAAAGGTTTTGTGTTAAGTCTGTGTATGTTCT
CGGAAAGATATGGATGGACGAAAATATTAAGACGAAGAATCACACCAATAGTGTGATTTTTTTTCTTGTT
AGAGATCGTAGGCCTGTTGATAAACCTCAAGATTTTGGAGAAGTGTTTAACATGTTTGATAATGAGCCCA
GTACGGCTACTGTGAAGAATGTTCATCGTGACAGGTATCAAGTGCTTCGGAAATGGCATGCAACCGTTAC
TGGTGGACAGTATGCGTCTAAGGAACAAGCTCTCGTGAAGAAGTTTGTTAGGGTTAATAATTATGTTGTG
TATAACCAGCAAGAAGCTGGCAAGTATGAGAATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTA
CACATGCCTCTAACCCAGTGTATGCTACTTTGAAGATACGGATCTATTTCTATGATTCCGTAACAAATTA
A

16. Isolate: COB-6 [Tobacco curly shoot virus]

Accession Number - JF825867

AAAGTGGGCCCCTTGATGTGATATGTCATCCAATCAGAACGCTCCCCCAAAGCTTAATTGTCTTGTGGTC
CCTTATTTAAACTTGCTCACCAAGTAGTGCACTCCGCACTATGTGGGATCCATTAGTAAACGAGTTTCCC
GAAACCGTTCACGGCTTTAGGTGTATGTTAGCAGTTAAATATCTGCAGTTAGTAGAGAAGACTTATTCGC
CTGACACATTAGGGCACGATTTAATTAGGGATTTAATTTCAGTTATTAGGGCTAGAAATTATGTCGAAGC
GACCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGTACGCCGCCGTCTCAACTTCGACAGCCCATA
TGTGATCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAATCAAGAGCATGGGCGAACAGGCCCATGAAC
AGAAAGCCGAAGATGTACAGGATGTACAGAAGTCCTGATGTCCCTAGAGGATGTGAAGGCCCATGTAAGG
TCCAGTCGTTTGAGTCTAGACATGACATTCAGCATATAGGTAAAGTTATGTGTATCAGTGATGTTACGCG
TGGAACTGGGCTGACCCATCGAGTGGGTAAAAGGTTTTGTGTTAAATCCGTTTATGTCTTGGGTAAGATC
TGGATGGATGAAAATATTAAGACCAAGAATCACACTAACAGTGTTATGTTTTTTTTAGTTAGGGATCGTA
GGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTTTTAACATGTTTGATAATGAGCCCAGTACGGCTAC
TGTGAAGAATGTTCATCGTGATAGGTATCAAGTGCTTCGCAAATGGCATGCAACTGTTACGGGTGGACAA
TATGCGTCAAAGGAACAAGCCCTTGTGAAGAAATTTGTTAGGGTTAATAATTATGTTGTGTATAACCAGC
AAGAAGCTGGCAAGTATGAAAATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTACTCATGCCTC
TAACCCAGTGTATGCTACTTTGAAGATACGGATCTATTTCTATGATTCCGTAACAAATTAATAAATGTTA
AATTTTATTGAATAAGATTGGTCTACATATACAATGTGTTGCAATCTATTCCATAATACATGATCAACTG
CTCGAATTGCATTATTCATACTAATTACACCCAAATTATTAAAAAACTTCAAAACTTGAGTCTTAAAGAC
CCTTAAGAAACGACCAGTCTGATGCTGTGAAGTCATCCACATTCCGTATACTACAAGACACTTGTGCACT
CCCTGAGCTTTCCTCATGTTGTATTTGAACTGTATTTGACTCGTGATTATGTCTTCCTTCATTACAAATG
GACGGTTCTGGTGCTCTATTATCTTGACATAAAAGGGATTTTGAATCTCCCATATATACACGCCATTCAC
CGCTTGAGCTGCAGTGATGAGTTCCCCTGTGCGTGAATCCATGATCGTGACATGCTAGTGCTATGAAATA
TGAACATCCACAAGGGAGATCAACACGACGACGTCTGTTCCCCTTCTTGGCTAGCCTGTGCTTCACTTTG
ATTGGAACCTGAGTAGAGTGGGCTTTCGAGGGTGACGAAGATCGCATTCTTTAAAGCCCAATTTTTAAGT
GCTGTATTTTTCTCTTC

17. Isolate: SILIGU-3 [Tobacco curly shoot virus]

Accession Number - JF922010

TAATATTACCTGATGGCCGCGATTTTTTTTAAAGTGGGCCCCTTGATGTGATATGTCATTCAATCAGAAC
GCTCCCCCGAAGCTTAATTGTTTTGTGGTCCCTTATTTATACATACTCATCAAGTAGCGCACTCCACACT
ATGTGGGATCCATTAGTAAACGATTTTCCCGAAACCGTTCACGGCTTTAGGTGTATGTTAGCAGTTAAAT
ATCTGCAGTTAGTAGAGAAGACGTATTCTCCCGACACATTAGGGCACGATTTAATTAGGGATTTAATTTC
GGTTATTAGGGCTAGAAATTATGTCGAAGCGTCCAGCAGATATAATCATTTCCACGCCCGCTTCGAAGGT
ACGCCGCCGTCTCAACTTCGACCGCCCATATGTGAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACCAAA

GCAAGAGCATGGGCGAACAGGCCCATGAACAGAAAACCCAGGATGTACAGGATGTACAGAAGTCCTGATG
TCCCTAGAGGATGTGAAGGGCCATGCAAAGTCCAG

18. Isolate: SILIGU-3 [Tobacco curly shoot virus]

Accession Number - JF922011

ACCAAGAACCAAACTAACAGTGTTATGTTTTTTTTAGTTAGGGATCGTAGGCCTGTGGATAAACCTCAAG
ATTTTGGAGAGGTTTTTAACATGTTTGATAATGAGCCCAGTACGGCTACTGTGAAGAATGTTCATCGTGA
TAGGTATCAAGTGCTTCGCAAATGGCATGCAACTGTTACGGGTGGACAATATGCGTCCAAGGAACAAGCC
CTTGTGAAGAAATTTGTTAGGGTTAATAATTATGTTGTGTATAACCAGCAAGAAGCTGGCAAGTATGAAA
ATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTACTCATGCCTCTAACCCAGTGTATGCTACTTT
GAAGATACGGATCTATTTCTATGATTCCGTAACAAATTAATAAATATTAAATTTTATTGAATAAGATTGG
TCTACATATACAATGTGTTCTAATACATCCCATAATACATGATCAACTGCACGAATTACATTATTAATAC
TGATAATTCCTAAATTATTTAAATATTTAAAAACTTGAGTCTTAAAGACCCTTAAGAAACGACCAGTCTG
AGGCTGTGAAGTCATCCAGATTCGGTAGACTAGAAAACACTTGTGCACTCCCAGAGCTTTCCTCATGTTG
TATTTGAACTGTATTTGGATGGTGATTATGTCTTCTTTCATGACAAATGGACGGTTCTGGTGCTCTATTA
TCTTGAAATAAAAGGGATTTTGAATCTCCCATATATACACGCCATTCTCTGCTTGAGCTGCAGTGATGAG
TTCCCCTGTGCGTGAATCCATGATCGTGACATGCTAGTGCTATGAAATATGAACATCCACAAGGGAGATC
AACACGACGACGTCTGTTCCCCTTCTTGGCTAGCCTGTGCTTCACTTTGATTGGAACCTGAGTAGAGTGG
GCTTTCGAGGGTGACGAAGATCGCATTCTTTAAAGCCCAATTTTTAAGTGCTGTATTTTTCTCTTC

19. Isolate: COB-3 [Tomato leaf curl New Delhi virus]

Accession Number - JN390432

AGTACTCATTCCGTCCCACGTTCTCCAACTGAACTGTTTGGCGATCCAATCTCCAAACAATATACGCGTA
AGGAAATCTGTGAAACACAGGAGGGATCGGAGTATCTGCTGCACAACAATCGTTACATGACGTCATATGT
CACGTATCCAGCTAAAACAAGAACTGGAACGAACAACCGCGTTCGTTCCTATATCAAGCTAAAGAGTCTG
AACATATCTGGCACATTTGCTGTTCGTAAATCTGATTTGATGACCGAAGTGGTGCAAACTAATGGACTAT
ACGGAGTGATGTCTGTAGTTGTAGTCCGCGATAAATCTCCAAAGATTTATTCAACGACCCAACCCTTAAT
ACCGTTTGTTGAGCTATTTGGATCTGTTAATGCTTGCAGGGGAAGTTTGAAAGTTGCAGAACGCCACCAT
GACCGTTTCGTACTGCTGAATCAAACATCCATCGTCGTCAATACCCCATATTCTAACGCTATCAAGAAAT
TCTGCATTCGTAACTGCATCCCAAGAACTTACACAACTTGGGTAACGTTTAAGGACGAAGAAGAAGATAG
CTGTACTGGACTATATTCAAACACCCTTAGAAATGCAATTATATTATATTATGTATGGTTAAGCGATGTA
TCCTCACAAGTCGATCTCTATAGCAATGTAATCCTCAATTACATTGGTTGATCTAAGAAAATGCAATACT
AACATTTTATTTATGAATAAATTCGGCTTAAAATTTATTACACGCCCTTTGATATTGGAGCATTTACATT
AGATTTTATACATTGCTCTACAGTCTTCTTAATTATATCGGCAATGTCATCTCTCGTAATACTCCCCGCT
TGTGATGCCGATGGCCCTGGATCGATTGCCGAATCGTCCAATCCGTTCAGGTGTTTATAGGGTCTGCTGG
TGACGGACGAATGTCCGATCTCCGATCTGCTTGCCCATGATTCGTTCGGACCTATTGCCAAATATGGTAC
TCGTAACGATCTTGAACTATG

20. Isolate: COB-2 [Tomato leaf curl New Delhi virus]

Accession Number - JN390433

CATTCCGTCCCACGTTCTCCAACTGAACTGTTTGGCGATCCAATCTCCAAACAATATACGCGTAAGGAAA
TCTGTGAAACACAGGAGGGATCGGAGTATCTGCTGCACAACAATCGTTACATGACGTCATATGTCACGTA
TCCAGCTAAAACAAGAACTGGAACGAACAACCGCGTTCGTTCCTATATCAAGCTAAAGAGTCTGAACATA
TCTGGCACATTTGCTGTTCGTAAATCTGATTTGATGACCGAAGTGGTGCAAACTAATGGACTATACGGAG
TGATGTCTGTAGTTGTAGTCCGCGATAAATCTCCAAAGATTTATTCAACGACCCAACCCTTAATACCGTT
TGTTGAGCTATTTGGATCTGTTAATGCTTGCAGGGGAAGTTTGAAAGTTGCAGAACGCCATCATGACCGT
TTCGTACTGCTGAATCAAACATCCATCGTCGTCAATACCCCATATTCTAACGCTATCAAGAAATTCTGCA
TTCGTAACTGCATCCCAAGAACTTACACAACTTGGGTAACGTTTAAGGACGAAGAAGAAGATAGCTGTAC

TGGACTATATTCAAACACCCTTAGAAATGCAATTATATTATATTATGTATGGTTAAGCGATGTATCCTCA
CAAGTCGATCTCTATAGCAATGTAATCCTCAATTACATTGGTTGATCTAAGAAAATGCAATACTAACATT
TTATTTATGAATAAATTCGGCTTAGAATTTATTACACGCCCTTTGATATTGGAGCATTTACATTAGATTT
TATACATTGCTCTACAGTCTTCTTAATTATATCGGCAATGTCATCTCTCGTAATACTCCCCGCTTGTGAT
GCCGATGGCCCTGGATCGATTGCCGAATCGTCCAATCCGTTCAGGTGTTTATAGGGTCTGCTGGTGACGG
ACGAATGTCCGATCTCCGATCTGCTTGCCCATGATTCGTTCGGACCTATTGCCAAATATGGTACTCGTAA
CGATCTTGAACTATGTCC

21. Isolate: SILIGU-1 [Acalypha yellow mosaic Siliguri virus]

Accession Number - JN676052

GCATCCAATACCCATAGTGTGATGTTTTTCCTCGTCAGGGATCGTATGCCCGTGGATACCCCCAAGGCTT
CGGGGAAGGTGCTTCACATGTTCGATAATGAGCCTATTACAGCAACGGTGAAAAACATGCATCGTGATCG
TTACCAAGTGCTCAAGAAGTGGAGTGCTACGGTGACTGGTGGTCAGTATGCGAGCAAGGAGCAGGCTTTG
GTTAGGCGTTTTTTTAGGGTTAATAATTATGTTATTTATAATCGGCAAGAGGCTGGCAAGTATGAAAACC
AAACGGAGAATGCTTTGATGCTCTACATGGCGTGTACTCACGCCTCTAACCCCTTGTATGCTACGCTTAA
GATACGGATCTATTTTTATGACTCTGTATCCAATTAATAAAGATTAAATTTTATTATATTGGCCTGCTCG
ATATGAGCAGTTTTTGTTAATACATTATACATTACATGTTCTACAGCATTTACAACGTTATTTATTGAAA
TAACCCCTACCATATCCAGGTATTTAAGTACTTGGTAATTAAATACTCTCAAGAAACGCCCAGTCTGAGG
GTGTAAGGCTGTCCAGATTTTGAAATCCATCCAGCACTGATGTAGTCCCAACGCTTTCCTCAGGTTGTGG
TTGAACCGTATCTGGACGGTGATTATGTCTAGGTGCATGCGGAACGGCAGGCTCTGGTGGTCTGTGATCT
TGAAATAGAGGGGATTTGGAACCTCCCAGATAAACACGCCATTCGTCGCCTGAGCTGCAGTGATGAGTTC
CCCTGTGCGTGAATCCATGGTTGTGGCAGTTGATATGGAGATAGTACGAACAGCCGCATTGGAGGTCTAC
CCTCGTGCGCCGGAAGGCCCTACTTTTAGCAGGCGTGTGTTGAATGTTGATTGGAACTTGAGTAGAGCGG
GTGGTGGACGGTGACGAAGGGTGCATTCTTTAGTGCCCAAGCCTTCAGTGCCGTATTCTTCGGCTCTTCA
AGGAACTCTTTATAGCTGGAATTGGGCCCAGGATTGCAGAGGAAGATAGTGGGTATCCCCCCAGTAATTT
GAACTGTCCTTCCGTAGTTGGTGTGGCTTTGCCAGTCTCTCTGGGCCCCCACGAACTCTTTAAAGGGCTT
TAGATAGTGCGGATTTAAGTCATCAATGAAGTTATACCAGGCATCATTGGTAAAGACCCTGGGAGTCAAG
TCCAGACGTCCACATAGATAGTCCTGCAGACT

22. Isolate: GUW-2 [Tomato leaf curl Ranchi virus]

Accession Number - JN676053

TAATATTACCGGATGGCCGCGATTTTTTTAAAGGGGTCCCCGCAGCACACTAATTGACAAAGACCTGTGG
ATCATTGAAAATCGTCCCTCAAAGCTTAATTGTTTCCTGGTCCCCTCTATAAACTTGGTCCCCAAGTTCC
CACATTACAAAATGTGGGATCCTTTAGTGAACGAGTTTCCCGAATCCGTTCACGGTTTTAGATGTATGTT
AGCAGTTAAATATCTGCAGTTAGTAGAGAAAACTTATTCTCCCGACACCTTAGGGTACGATTTAATTAGG
GATTTAATTTCAGTTATTAGGGCTAGAAATTATGTCGAAGCGACCAGCAGATATAATCATTTCCACGCCC
GCTTCGAAGGTACGCCGCCGTCTCAACTTCGACAGCCCATATGCGAGCCGTGCTGCTGCCCCCATTGTCC
GCGTCACCAAAGCAAGAGCATGGGCGAACAGGCCCATGAACAGAAAGCCCAGGATGTACAGGATGTACAG
AAGTCCAGATGTGCCTAGAGGCTGTGAAGGCCCATGTAAGGTGCAGTCTTTTGAGTCTAGACATGACATT
CAGCATATAGGTAAAGTCATGTGTGTCAGTGATGTTACTCGTGGGACTGGGCTGACCCATCGAGTGGGTA
AAAGGTTTTGTGTTAAGTCTGTGTATGTTCTCGGAAAGATATGGATGGACGAAAATATTAAGACGAAGAA
TCACACCAATAGTGTGATTTTTTTTCTTGTTAGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGA
GAAGTGTTTAACATGTTTGATAATGAGCCCAGTACGGCTACTGTGAAGAATGTTCATCGTGACAGGTATC
AAGTGCTTCGGAAATGGCATGCAACCGTTACTGGTGGACAGTATGCGTCTAAGGAACAAGCTCTCGTGAA
GAAGTTTGTTAGGGTTAATAATTATGTTGTGTATAACCAGCAAGAAGCTGGCAAGTATGAGAATCATTCT
GAGAATGCGTTAATGTTGTATATGGCATGTACTCATGCCTCTAACCCAGTGTATGCTACTTTGAAGATAC
GGATCTATTTCTATGATTCCGTAACAAATTAATAAATATTGAATTTTATTGAATAAGATTGGTCTACATA
TACAATATGTTCTAATCTATTCCATAATACATGATCAACTGCACGAATTACATTATTAATACTGATAATT
CCTAAATTATCTAAATATTTAAAAACTTGAGTCTTAAAGACCCTTAAGAATTGACCAGTCGGAGGCTGTG
AAGTCATCCAGATTCGGAAGACTAGAAAACACTTGTGCACTCCCAGAGCTTTCCGCAGGTTGTAATTGAA
CTGTATTCGGATTGTCATTATGTCCTCTTGCATCAGAAATGGACGGTTCTGGTGCTCCAAGATCTTGAAA

TAAAGGGGATTTTGAATCTCCCAGATATACACGCCATTCTCTGCTTGAGCTGCAGTGATGACTTCCCCTG
TGCGTGAATCCATGATCGTGACAGGCTAGTGCTATGAAATATGAACATCCACAAGGGAGATCAACACGAC
GACGTCTGTTCCCCTTCTTGGCTAGCCTGTGCTTCACTTTGATTGGAACCTGAGTAGAGTGGGCTTTCGA
GGGTGACGAAGATCGCATTCTTTAAAGCCCAATTTTTAAGTGCTGTATTTTTCTCTTCATCCAAGAATTC
TTTATAGCTAGAGTTGGGTCCTGGATTGCAGAGAAAGATAGTGGGTATTCCGCCTTTAATTTGAACTGGC
TTTCCGTACTTTGTATTGCTTTGCCAGTCTCTTTGGGCCCCCATGAATTCTTTAAAGTGCTTTAGGTAGT
GCGGATCTACGTCATCAATGACGTTATACCAAGCATCATTATTGTACACTTTAGGGCTTAGATCTAAATG
ACCACACAAGTAATTATGTGGTCCCAATGACCTGGCCCACATCGTCTTCCCTGTTCTACTGTCACCTTCA
ATTACTATACTTATGGGTCTTAACGGCCGCGCAGCGGCACTGACGACATTCTCGGCAGCCCACTCCTCAA
GTTCTTCTGGAACTTGATCAAAAGAAGAAGAAGAAAAAGGAGAGACATAAACCTCCATAGGAGGTGTAAA
AATTCGATCTAAATTTGAATTTAAATTATGAAATTGTAATACATAATCCTTAGGAGCTTTTTCCTTTAAT
ATATTGAGGGCCTCAGCTTTGGACCCTGAATTGATTGCCTCGGCATATGCGTCGTTGGCAGATTGGCAAC
CTCCTCTAGCTGATCTTCCATCGACTTGGAAAACTCCATGATCAAGCACGTCTCCGTCTTTCTCCATGTA
GGTTTTGACATCTGTAGAGCTCTTAGCTCCCTGAATGTTCGGATGGAAATGTGCTGACCTGGTTGGGGAT
GTGAGGTCGAAGAATCTATTGTTTCTGCACTGGAATTTACCTTCGAATTGGATGAGCACATGCAAGTGAG
GAGTCCCATCTTCGTGAAGCTCTCTGCAAATTCTAATAAATTTTTTGGAAGTGGGTGTTTGGATATTTAA
TAATTGGGAAAGTGCCTCTTCTTTAGCTAGAGAGCACTTGGGATAAGTGAGAAAATAATTTTTTGCATTT
ATTTTAAACCGATTGGGGGCTGCCATATTGACTTGGTCAATCGGTGTCTCTCAACTCTCTCTATGTATCG
GTGTATTGGAGTCCTATATATATGGAGACTCCAAATGGCATATTTGTAATTTTGAAAAGTTCTTTAATTC
AAAATTCAAAATTCCCAAAGCGGCCATCCGTA

23. Isolate: SILIGU-4 [Tomato leaf curl New Delhi virus]

Accession Number - JN676054

ATGGTGAAGCGACCAGCAGATATCATCTTTTCAACGCCCGTATCGAAAGTACGCCGTCGTCTCAACTTCG
ACAGCCCCTTTGGAGCTCGTGCAGTTGTCCCCATTGCCCGCGTCACAAAAGCAAAGGCCTGGACCAACAG
GCCGATGAACAGAAAACCCAGAATGTACAGAATGTATAGAAGTCCCGACGTGCCAAGGGGCTGTGAAGGC
CCTTGTAAGGTGCAGTCCTTTGAATCTAGGCACGATGTCTCTCATATTGGCAAAGTCATGTGTGTTAGTG
ATGTTACCCGAGGATCTGGACTCACACATCGCGTAGGGAAGCGATTTTGTGTGAAATCTGTCTATGTACT
GGGGAAGATATGGATGGATGAAAACATCAAGACAAAAAACCATACTAACAGTGTCATGTTTTTTTTGGTC
CGTGACCGTCGTCCTACAGGATCTCCACAAGATTTTGGGGAAGTTTTTAACATGTTTGACAATGAACCGA
GCACAGCAACGGTGAAGAACATGCATCGTGATCGTTATCAAGTCTTACGGAAGTGGCATGCTACTGTGAC
GGGAGGAACATATGCATCTAAGGAGCAAGCATTAGTTAGGAAGTTTGTTAGGGTTAATAATTATGTTGTC
TATAACCAACAAGAGTCCGGCAAGTATGAGAATCATACTGAGAACGCCTTAATGTTGTATATGGCCTGTA
CTCATGCATCAAATCCTGTGTATGCTACTTTGAAAATCCGGATCTATTTCTATGATTCGGTAACAAATTA
A

24. Isolate: SILIGURI- 1 [Tobacco curly shoot virus]

Accession Number - JN676055

AAGATCTGGATGGATGAAAATATTAAGACCAAGAATCACACTAACAGTGTTATGTTTTTTTTAGTTAGGG
ATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGGTTTTTAACATGTTTGATAATGAGCCCAGTAC
GGCTACTGTGAAGAATGTTCATCGTGATAGGTATCAAGTGCTTCGCAAATGGCATGCAACTGTTACGGGT
GGACAATATGCGTCGAAGGAACAAGCCCTTGTGAAGAAATTTGTTAGGGTTAATAATTATGTTGTGTATA
ACCAGCAAGAAGCTGGCAAGTATGAAAATCATTCTGAGAATGCGTTAATGTTGTATATGGCATGTACTCA
TGCATCTAACCCAGTGTATGCTACTTTGAAGATACGGATCTATTTCTATGATTCCGTAACAAATTAATAA
ATATTAAATTTTATTGAATAAGATTGGTCTACATATACAATGTGTTCTAATACATCCCATAATACACGAT
CAACTGCACGAATTACATTATTAATACTGATAATTCCTAAATTATTTAAATATTTAAAAACTTGAGTCTT
AAAGACCCTTAAGAAACGACCAGTCTGAGGCTGTGAAGTCATCCAGATTCGGTAGACTAGAAAACACTTG
TGCACTCCCAGAGCTTTCCGCAGGTTGTAGTTGAACTGTATTTGGATTGTGATTATGTCTTCTTTCATGA
GAAATGGACGGTTCTGGTGCTCTGTTATCTTGAAATAAAGGGGATTTTGAATCTCCCAGATATACACGCC
ATTCTCTGCTTGAGCTGCAGTGATGAGTTCCCCTGTGCGTGAATCCATGATCGTGACAGGCTAGTGCTAT
GAAATATGAACATCCACAAGGGAGATCAACACGACGACGTCTGTTCCCCTTTTTGGCTAGCCTGTGCTTC
ACTTTGATTGGAACCAGAGTAGAGTGGGCTTTCGAGGGTGACGAAGATCGCATTCTTTAAAGACCAATTT

TTAAGTGCGGTATTTTTCTCTTCATCCAAGAATTCTTTATAGCTAGAGTTGGGTCCTGGATTGCAGAGAA
AGATAGTGGGAATTCCGCCTTTAATTTGAACTGGCTTTCCGTACTTTGTATTTGATTGCCGGTCTTTTTG
GGCCCCCACGAATTCTTTAAAGAGCTTTAGGTAGTGCGGATTTACGTCATCAATGACGTTTAACCA

25. Isolate: SILIGURI [Croton yellow vein mosaic virus]

Accession Number - JQ796374

CCCAGGATGTACAGGATGTACAGAAGCCCAGATGTCCCTAAGGGATGTGAAGGCCCATGTAAGGTGCAAT
CTTTTGATGCTAAGAATGATATTGGTCACATGGGTAAGGTTATATGTTTGTCTGATGTCACGAGGGGAAT
GGGCCTGACCCATCGAGTAGGGAAACGTTTCTGCGTGAAGTCATTGTATTTTGTTGGCAAAATATGGATG
GACGAGAACATCAAGACTAAGAACCATACTAATACTGTTATGTTTTGGATCGTTAGAGATAGGCGTCCTT
CAGGAACCCCAAATGATTTCCAGCAAGTGTTCAATGTTTATGATAATGAGCCCTCTACAGCTACTGTGAA
GAACGACCAGCGTGATCGTTATCAGGTGTTGAGGAGGTTTCAAGCAACAGTCACAGGTGGTCAATATGCT
GCTAAGGAACAAGCTATAATTAGGAAATTCTATCGTGTTAATAATTATGTGGTGTATAATCACCAGGAAG
CTGGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCCTCT

26. Isolate: SILIGURI-2 [Jatropha mosaic India virus]

Accession Number - JQ811770

GGCTGTGAAGGTCCATGTAAGGTTCAGTCATTTGAGTCCAGGCACGCTGTCGTGCATATAGGTAAGGTCA
TGTGCATCTCCGATGTCACTCGAGGTACCGGTCTTACCCATCGCGTGGGTAAGAGGTTCTGTGTCAAATC
AGTCTACATTCTGGGCAAGATTTGGATGGATGAGAACATAAAGACTAAGAATCACACCAATAGTGTGATG
TTTTTCCTCGTCAGGGATCGTAGGCCCGTGGATAAGCCCCAGGACTTCGGGGAAGTCTTCAACATGTTCG
ATAATGAGCCTAGTACAGCAACTGTTAAAAACATGCATCGTGATCGCTACCAAGTGCTCAGGAAGTGGAG
TGCTACGGTGACTGGTGGTCAGTACGCAAGCAAGGAGCAGGCTTTAGTTAGGCGTTTTTTTAGGGTTAAT
AATTATGTTATTTACAATCAGCAAGAGGCTGGCAAATATGAGAATCACGCTGAGAATGCGTTGATGCTCT
ACATGGCGTGTACTCACGCCTCCAAC

27. Isolate: SILIGU-3 [Tobacco curly shoot virus]

Accession Number - JQ843097

CCTGATGTCCCTAGAGGATGTGAAGGGCCATGCAAAGTCCAGTCATTTGAGTCTAGACATGACATTCAGC
ATATAGGTAAAGTTATGTGTGTCAGTGATGTTACGCGTGGAACTGGGCTGACTCATCGAGTGGGTAAAAG
GTTTTGTGTTAAATCCGTTTATGTCTTGGGTAAGATCTGGATGGATGAAAATATTAAGACCAAGAACCAC
ACTAACAGTGTTATGTTTTTTTTAGTTAGGGATCGTAGGCCTGTGGATAAACCTCAAGATTTTGGAGAGG
TTTTTAACATGTTTGATAATGAGCCCAGTACGGCTACTGTGAAGAATGTTCATCGTGATAGGTATCAAGT
GCTTCGCAAATGGCATGCAACTGTTACGGGTGGACAATATGCGTCCAAGGAACAAGCCCTTGTGAAGAAA
TTTGTTAGGGTTAATAATTATGTTGTGTATAACCAGCAAGAAGCTGGCAAGTATGAAAATCATTCTGAGA
ATGCGTTAATGTTGTATATGGCATGTACTCAT

28. Isolate: SLGPVY1 [Potato virus Y]

Accession Number - JX945850

TGGTGCATTGAAAATGGAACCTCGCCAAACATCAACGGAGTTTGGGTTATGATGGATGGAGATGAACAAG
TCGAATACCCACTGAAACCAATCGTTGAGAATGCAAAACCAACACTTAGGCAAATCATGGCACATTTCTC
AGATGTTGCAGAAGCGTATATAGAAATGCGCAACAAAAAGGAACCATATATGCCACGATATGGTTTAGTT
CGTAATCTGCGCGATGGAAGTTTGGCTCGCTATGCTTTTGACTTTTATGAGGTCACATCACGAACACCAG
TGAGGGCTAGGGAAGCGCACATTCAAATGAAGGCCGCAGCATTGAAATCAGCCCAATCTCGACTTTTCGG
GTTGGACGGTGGCATCAGTACACAAGAGGAGAACACAGAGAGGCACACCACCGAGGATGTCTCTCCAAGT

ATGCATACTCTACTTGGAGTCAAGAACATGTGATGTAGTGTCTCTCCGGACGATATATAAGTATTTACAT
ATGCAGTAAGTATTTTGGCTTTTCCTGTACTACTTTTATCATAATTAATAATCAGTTTGAATATTACTAA
TAGATAGAGGTGGCAGGGTGATTTCGTCATTGTGGTGACTCTATCTTTTAATTCCGCATTATTAAGTCTT
AGATAAAAGTGCCGGGTTGTCGTTGTTGTGGATGATTCATCGATTAGGTGATGTTGCGATTCTGTCGTAG
CAGTGACTATGTCTGGATCTATCTGCTTGGGTGGTGTTGTGATTTCGTCATAACAGTGACTGTAAACTTC
AATCAGGAGACAAAAAAAAAAAAAAAAAAA

29. Isolate: SLGPRSV1 [Papaya ringspot virus P]

Accession Number - JX567310

TGGTGGATTGAGAATGGTACATCTCCAGACATATCTGGTGTCTGGGTAATGATGGATGGTGACACTCAAG
TTGACTATCCAATCAAGCCTTTGATTGAGCATGCAACTCCTTCATTTAGGCAGATTATGGCTCACTTCAG
TAACGCGGCAGAGGCATACATTGCAAAGAGAAACGCAACTGAAAAGTACATGCCACGATATGGAATCAAG
AGAAATTTGACTGACACTAGCCTTGCTAGATATGCTTTCGATTTCTATGAGGTGAATTCAAAAACACCTG
ATAGAGCTCGTGAAGCTCACATGCAGATGAAGGCTGCAGCGCTACGTAACGCTAATCGTAGAATGTTTGG
CATGGATGGTAGTGTCAGTACCAAGGAAGAAAACACGGAGAGACACACAGTGGACGATGTCAACAGAGAC
ATGCACTCTCTCCTGGGTATGCGTAACTAAATACCCGCACTTGTGTGTTCGTCGGGCCTGGCTCGACTCT
GTTTCACCTTATAGTACTATTTAAGCATTAAAATACAGTGTGGCTGCGCCACCGTTCCTATTTTATAGTG
AGGGTATCCCTCCGTGCTTTTAGTATTATTCGAGTTCTCTGAGTCTCCATACAGTGTGGGCGGCCCACGT
GATATTTGAGCCTCTTAGAATGAGAGAAAAAAAAAAAAAAAAAAA

30. Isolate: Coochbehar [Bean common mosaic virus]

Accession Number - KC871565

TTGAAAATGCCACTTCACCGGATGTGAATGGTACATGGGTGATGATGGATGGAGATGAGCAAGTTGAATA
CCCACTCAAACCAATGGTTGAGAATGCAAAACCAACACTCCGTCAAATCATGCACCATTTCTCAGATGCA
GCTGAAGCATACATTGAGATGAGAAATTCCGAGAAACCGTACATGCCTAGGTACGGACTACTTCGGAATT
TGAGGGATAAAAATCTAGCTCGCTACGCTTTTGATTTCTATGAGGTGACATCCAAAACATCGGATCGAGC
CAGAGAAGCAGTAGCACAGATGAAGGCAGCAGCCCTCAGCAACGTTAGCAGCAAGTTGTTTGGACTTGAC
GGTAATGTTGCAACAACCAGCGAGAATACTGAAAGGCACACTGCAAGGGACGTTAACCAAAACATGCACA
CCCTTCTTGGCATGGGTTCCCCGCAGTAAAGGTTGGGGAACCTGACCACAGTTAGCGTCTCGCGTCGCTG
AATAATTTCATATAGTAATCTTTTATGGTTCTCTTTAGTTTCAGTGTGGTTTTACCCCCTTTGGGGTTAC
TATTGGGATAGCGTGG