Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease

By

Chinju E. A.

(2017-11-030)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680656
KERALA, INDIA

2019

DECLARATION

I, hereby declare that the thesis entitled **"Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease"** is a bonafide record of research done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara **Chinju E. A.**

Date: **2017-11-030**

CERTIFICATE

Certified that this thesis entitled **"Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease"** is a record of research work done independently by **Ms. Chinju E. A. (2017-11-030)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara
Date:

Dr. Anita Cherian K.
(Major Advisor)
Professor and Head
Dept. of Plant Pathology
College of Horticulture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Chinju E. A.**, a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Pathology,** agree that the thesis entitled **"Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease"** may be submitted by **Ms. Chinju E. A.** in partial fulfilment of the requirement for the degree.

Dr. Anita Cherian K.
(Chairman, Advisory committee)
Professor and Head
Department of Plant Pathology
College of Horticulture
Vellanikkara

Dr. Vimi Louis
(Member, Advisory committee)
Professor
Plant Pathology
Banana Research Station
Kannara

Dr. Sainamole Kurian P.
(Member, Advisory committee)
Professor
Department of Plant Pathology
College of Horticulture
Vellanikkara

Dr. Deepu Mathew
(Member, Advisory committee)
Assistant Professor
Plant Biotechnology
College of Horticulture
Vellanikkara

ACKNOWLEDGEMENT

I humbly bow my head before the ***God Almighty****, who blessed me with strength, knowledge, will power and opportunity to undertake this research study and to complete this endeavor successfully.*

With immense pleasure I avail this opportunity to express my deep sense of whole hearted gratitude and indebtedness to my major advisor ***Dr. Anita Cherian K.,*** *Professor and Head, Dept. of Plant Pathology, College of Horticulture, Vellanikkara and major advisor of my advisory committee for her expert advice, inspiring guidance, practical suggestions, constant patience, untiring help, friendly approach and kind advice at various stages of my research work and thesis preparation and will be remembered forever.*

I sincerely thank ***Dr.Vimi Louis****, Professor, Plant Pathology, Banana Research Station, Kannara, member of my advisory committee for her unwavering encouragement, timely support throughout the study.*

I am extremely thankful to ***Dr. Sainamol Kurian P.,*** *Professor, AICVIP, College of Horticulture, Vellanikkara, and member of my advisory committee for her constant encouragement, timely support and critical examination of the manuscript that has helped me a lot for the improvement and preparation of the thesis.*

I would like to express my extreme indebtedness and obligation to ***Dr. Deepu Mathew****, Assistant Professor, CPBMB, college of horticulture for his meticulous help, expert advice, forbearance, critical evaluation, constant encouragement and support throughout my course of study.*

I express my heartiest gratitude to ***Dr. Reshmi Vigayaraghavan, Dr. Reshmi C. R. and Dr. Gleena Mary C. F.*** *for their ever willing help, valuable guidance and creative suggestions throughout the period of my study.*

I am thankful to ***Dr. M. T. Thomas****, Professor, Department of Botany, St. Thomas College, Thrissur for immense help and most precious suggestions for conducting the histopathological studies.*

The financial assistance provided by ***Kerala Agricultural University*** *for the conduct of the research work is deeply acknowledged.*

True words of thanks to all my friends, more personally I would like to express my sincere gratitude to my dearest and intimate classmates, ***Anjali, Resham, Seleena, Alan and Suhaib.*** *I have infinite pleasure to express my love and gratitude to my dear friends; Jayalakshmi Prakash and Reshmi S. I am also thankful to all my seniors, especially* ***Lishma chechi, Milsha chechi, Darsana chechi and Atheena itha*** *for their guidance and emotional support. I am extremely thankful to all the Research Associates,* ***Liji chechi*** *and* ***Lidiya chechi,*** *Teaching Assistants and labours especially* ***Josy chechi*** *and* ***Prasanna chechi*** *for their support during the conduct of research. I thankfully remember the services rendered by all the staff members of Student's computer club, College Library, Office of COH and Central library, KAU. I am thankful to Kerala Agricultural University for the technical and financial assistance for persuasion of my study and research work.*

I can barely find words to express all the wisdom, love and support given me for that I am eternally grateful to my beloved parents, ***Anilkumar*** *and* ***Yamuna*** *and my beloved brother* ***Abhilash,*** *for their unconditional love, fidelity, endurance and encouragement. They have been selfless in giving me the best of everything and I express my deep gratitude for their love, personal sacrifice and constant prayers without which this work would not have been completed.*

A word of apology to those I have not mentioned in person and a note of thanks to everyone who helped for the successful completion of this endeavor.

Chinju E. A.

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-26
3	MATERIALS AND METHODS	27-44
4	RESULTS	45-82
5	DISCUSSION	83-94
6	SUMMARY	95-98
	REFERENCES	i-xix
	APPENDICES	xx-xxi
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
2.1	List of begomoviruses infecting bhindi worldwide	5
3.1	Yellow vein mosaic disease grading chart	28
3.2	List of plants used for host range studies	33
3.3	List of naturally infected weed plants with symptoms collected from fields	33
3.4	Details of reported primers used	37
3.5	Range of annealing temperature tested	38
3.6	Composition of PCR reaction mix used for each primer set	38
3.7	Program for PCR reaction of reported primers	39
3.8	Details of begomovirus isolates used for phylogenetic analysis	42
3.9	Details of field treatments	44
4.1	Incidence and severity of bhindi yellow vein mosaic disease in different locations of Thrissur district	46
4.2	Reduction of leaf area due to infection	49
4.3	Histopathological changes caused by virus infection	51
4.4	Transmission of virus through seeds	53
4.5	Transmission of virus through grafting	53
4.6	Transmission of virus through vector	53
4.7	Host range of virus	54
4.8	Begomovirus infection in weed plants collected from fields	56
4.9	Details of local isolates	57
4.10	Quantity and quality of isolated DNA	58
4.11	Homology analysis of virus isolates	63
4.12	DNA barcodes of BYVMV and OELCuV	71-74
4.13	Effect of treatments on percent disease incidence (PDI)	75
4.14	Effect of treatments on percent disease severity (PDS)	77
4.15	Effect of treatments on vegetative characters	78
4.16	Effect of treatments on whitefly population	79
4.17	Effect of treatments on yield characters	82

LIST OF FIGURES

Figure No.	Title	After page No.
3.1	Locations of purposive sampling survey	27
3.2	General view of experimental field	43
4.1	BLASTn analysis of *Synedrella nodiflora* showing vein clearing symptom	56
4.2a- 4.2c	Nucleotide sequences of local isolates	60
4.3a	Open reading frames of local isolate VKA1	63
4.3b	Amino acid sequences of local isolate VKA1	63
4.4a	Open reading frames of local isolate CLR	63
4.4b	Amino acid sequences of local isolate CLR	63
4.5a	BLASTn analysis of local isolate VKA1	63
4.5b	BLASTp analysis of local isolate VKA1	63
4.6a	BLASTn analysis of local isolate CLR	63
4.6b	BLASTp analysis of local isolate CLR	63
4.7a	Open reading frames of local isolate VYR1	64
4.7b	Amino acid sequences of local isolate VYR1	64
4.8a	Open reading frames of local isolate VYR2	64
4.8b	Amino acid sequences of local isolate VYR2	64
4.9a	BLASTn analysis of local isolate VYR1	64
4.9b	BLASTp analysis of local isolate VYR1	64
4.10a	BLASTn analysis of local isolate VYR2	64
4.10b	BLASTp analysis of local isolate VYR2	64
4.11a	Open reading frames of local isolate ATR1	64

Fig. No.	Title	After page no.
4.11b	Amino acid sequences of local isolate ATR1	64
4.12a	Open reading frames of local isolate ATR2	64
4.12b	Amino acid sequences of local isolate ATR2	64
4.13a	BLASTn analysis of local isolate ATR1	64
4.13b	BLASTp analysis of local isolate ATR1	64
4.14a	BLASTn analysis of local isolate ATR2	64
4.14b	BLASTp analysis of local isolate ATR2	64
4.15a	Open reading frames of local isolate MVA	65
4.15b	Amino acid sequences of local isolate MVA	65
4.16a	Open reading frames of local isolate PND	65
4.16b	Amino acid sequences of local isolate PND	65
4.17a	BLASTn analysis of local isolate MVA	65
4.17b	BLASTp analysis of local isolate MVA	65
4.18a	BLASTn analysis of local isolate PND	66
4.18b	BLASTp analysis of local isolate PND	66
4.19a	Open reading frames of local isolate PNR	66
4.19b	Amino acid sequences of local isolate PNR	66
4.20a	Open reading frames of local isolate VNY	66
4.20b	Amino acid sequences of local isolate VNY	66
4.21a	BLASTn analysis of local isolate PNR	66
4.21b	BLASTp analysis of local isolate PNR	66
4.22a	BLASTn analysis of local isolate VNY	67
4.22b	BLASTp analysis of local isolate VNY	67
4.23a	Open reading frames of local isolate KRM	68

Fig. No.	Title	After page no.
4.23b	Amino acid sequences of local isolate KRM	68
4.24a	BLASTn analysis of local isolate KRM	68
4.24b	BLASTp analysis of local isolate KRM	68
4.25	Pairwise nucleotide sequence identities of the core CP sequences of begamoviruses using SDT analysis	70
4.26	Multiple sequence alignment in Clustal W	70
4.27	Neighbour-joining tree representing phylogenetic relationship of OELCuV coat protein sequences of collected isolates and other begomoviruses infecting bhindi.	74
4.28	Effect of different treatments on BYVMD incidence as per cent disease incidence	77
4.29	Effect of different treatments on BYVMD severity as per cent disease severity	77
4.30	Effect of different treatments on whitefly population in field	82
4.31	Effect of different treatments on yield as per cent increase in yield over control	82

LIST OF PLATES

Plate No.	Title	After page no.
3.1	Graft transmission of the virus	30
3.2	Materials used for vector transmission	31
4.1a-4.1c	Symptoms under natural conditions	48
4.1d-4.1f	Symptoms under natural conditions	49
4.1g	Symptoms under natural conditions	50
4.2	Symptoms under artificial conditions	50
4.3a-4.3b	Histopathological changes	51
4.4	Symptoms developed after graft transmission	53
4.5	Symptoms developed after vector transmission	53
4.6	Life cycle of *Bemisia tabaci*	53
4.7	Host range of virus	55
4.8a-4.8b	Symptoms on weed plants collected from fields	55
4.9	Molecular detection of host range of virus	56
4.10	Molecular detection of virus in collected weed plants	56
4.11	Gel profile of total genomic isolated DNA	57
4.12	Standardization of annealing temperature of begomovirus specific primer (AV494/AC1048)	58
4.13	Standardization of annealing temperature of BYVMV CP gene specific primer	58
4.14	Standardization of annealing temperature of OYVMV CP gene specific primer	59
4.15	Standardization of annealing temperature of OELCuV CP gene specific primer	59
4.16	PCR amplification of local isolates	59
4.17	Molecular detection of virus inside whiteflies	59
4.18	Molecular detection of seed infection	59

LIST OF APPENDICES

Appendix No.	Title	Page No.
I	Composition of reagents used for DNA isolation and agarose gel electrophoresis	xx
II	List of laboratory equipments used for the studies	xxi

ABBREVIATIONS

mg	: Milligram
kcal	: Kilocalorie
g	: Gram
µg	: Microgram
%	: Per cent
DNA	: Deoxyribo nucleic acid
ORF	: Open reading frame
min.	: Minute
h.	: Hour
kb	: Kilo bases
bp	: Base pairs
ELISA	: Enzyme linked immunosorbent assay
PCR	: Polymerase chain raction
DAC-ELISA	: Direct antigen coating enzyme linked immunosorbent assay
NaCl	: Sodium chloride
CTAB	: Cetyltrimethyl ammonium bromide
PEG	: Polyethylene glycol
PVP	: Polyvinylpyrrolidone
RAPD	: Random amplification of polymorphic DNA
ICTV	: International Committee on Taxonomy of Viruses
CP	: Coat protein
MP	: Movement protein
ml	: Millilitre
l	: Litre
a.i.	: Active ingredient
ha	: Hectare
kg	: Kilogram

PDI	: Per cent disease incidence
PDS	: Per cent disease severity
cm	: Centimetre
DAI	: Days after inoculation
AAP	: Acquisition access period
IAP	: Inoculation access period
BLAST	: Basic local alignment sequence tool
ng	: Nanogram
µl	: Microlitre
OD	: Optical denisty
DAS	: Days after sowing
PGPR	: Plant growth promoting rizhobacteria
m	: Meter

Introduction

INTRODUCTION

Bhindi (*Abelmoschus esculentus* L), also known as okra or ladies finger is a commercially grown vegetable crop which belongs to the family Malvaceae which is being cultivated across the globe *viz.* tropical, subtropical and warm temperate regions. It is often considered as a major component of balanced food due to its rich content of dietary fibres and amino acids like tryptophan and lysine (Hughes, 2008). Unripe bhindi fruits (100 g) contain high fibre (3.2 g), protein (1.93 g) vitamins (Vitamin A- 36 µg, Vitamin C- 23 mg and Vitamin K- 31.3 µg) and minerals (magnesium- 57 mg, potassium- 299 mg, and calcium- 82 mg) and low in carbohydrate (7.45 g), energy (33 kcal) and fat (0.1g) (USDA, 2019). It is also known for its high content of antioxidants and medicinal properties against high cholesterol, diabetes mellitus and cancer (Jenkins *et al*., 2005).

Internationally, India ranks first in production and contributes 61.87 per cent towards the total world production of bhindi. In India, it is cultivated in about 528.37million ha. with an average productivity of 11.63 million tonnes (INDIAAGRISTAT, 2017). Major bhindi producing states in India are Uttar Pradesh, Bihar, Odisha, West Bengal, Andhra Pradesh, Madhya Pradesh and Assam. Even though India stands first in production, its cultivation is challenged by many biotic stress such as pests and diseases. The crop is infected by a number of pathogenic fungi, bacteria, viruses and nematodes among which viral diseases are the most threatening hurdle to its cultivation. The major viral diseases in bhindi include yellow vein mosaic (Capoor and Varma, 1950), enation mosaic, enation leaf curl (Singh, 1986), okra leaf curl, okra mosaic (Lana and Bozarth, 1975), okra yellow mottle (Torre and Monsalvao-Reyes, 2003) and fruit distortion mosaic (Krishna Reddy *et al.,*2003). Among these, bhindi yellow vein mosaic disease (BYVMD) is the most devastating one. The first report of this disease in the world was from Bombay, Maharashtra (Kulkarni, 1924) indicating that it might have originated from India. Okra enation leaf curl disease (OELCuD) caused by okra enation leaf curl virus (OELCuV) which produce almost similar symptoms of BYVMD is now becoming an emerging viral disease in bhindi and reported to cause

a significant reduction in yield ranging from 30 to 100 per cent (Singh, 1996). Currently, these viral diseases are reported to occur in all bhindi growing states of India in endemic and as epidemic level causing threat to the cultivation of bhindi.

The major viruses infecting bhindi belong to the genus *Begomovirus* and family *Geminiviridae,* which is the second largest family of plant viruses (Fauquet and Stanley, 2003). The symptoms of yellow vein mosaic disease consist of vein clearing followed by yellowing of veins, vein thickening, downward curling of leaf and stunting (Capoor and Varma, 1950). The fruits produced on diseased plants are malformed having bleached appearance (Raychaudhuri and Nariani, 1977). The yellow vein mosaic disease of bhindi has been reported to cause huge loss in yield from different regions of the country. The loss in yield was recorded up to 50-94 per cent depending up on the stage of crop growth at which the infection occurs (Sastry and Singh 1974; Pun and Doraiswamy, 1999). In the case of OELCuD, the characteristic symptoms comprised of vein thickening, upward curling of leaves and reduction in leaf area. The infected plants also became severely stunted bearing small, malformed fruits which are unmarketable (Sanwal *et al*., 2014).

In the recent past, frequent break down of resistance against BYVMV have been reported in varieties like Arka Anamika, Parbhani Kranti, Punjab 7 and Arka Abhay all over the country. This might be due to the evolution of new viral strains or due to recombination between the viral strains (Sanwal *et al*., 2014). Padidam *et al.* (1999) suggested that recombination is a significant contributor to evolution of geminiviruses. Evidences of different strains of the virus from different parts of the country would help to develop the strain specific diagnostics for early detection and sustainable management of the disease. Hence, molecular characterization of the local isolates of virus associated with yellow vein mosaic disease in bhindi was proposed in this project.

Due to the occurrence of the polyphagous ‘B’ biotype, *Bemisia tabaci* expanded its host range which resulted in infection of geminiviruses in formerly unaffected crops (Chowda-Reddy *et al*., 2012). Weeds and other host plants are the

integral part of the virus transmission and could act as the reservoir of plant viruses. Therefore, the study on host range of the virus is essential for the effective management of the disease and was taken up in this project.

Many varieties resistant to YVMD have been developed. Though initially these were found to be resistant, most of the varieties later showed breakdown of resistance (Jambhale and Nerkar, 1986). The vector control using chemical insecticides is usually adopted for the management of viral diseases. However, extensive use of chemical pesticides poses great threat to the environment as well as human beings.

Considering all these aspects, the present study was undertaken with the objectives to undertake molecular characterization of the virus, host range studies and management of bhindi yellow vein mosaic disease using plant extracts, botanicals, insecticides and microbial formulations.

Review of literature

REVIEW OF LITERATURE

Bhindi or okra (*Abelmoschus esculentus* (L.) Moench.), is one of the most widely cultivated vegetable crop species in the family Malvaceae, which is mostly grown in the tropical and subtropical regions of the world. This crop is one of the potential foreign exchange earners of India (Singh *et al*., 2014).

The profitable cultivation of bhindi is threatened by the incidence of pathogens such as fungi, bacteria, viruses, nematodes and also insect pests which results in decrease in yield. Among the diseases, okra is often challenged by the infection of geminiviruses causing important diseases such as yellow vein mosaic disease (YVMD), okra enation leaf curl disease (OELCD) and okra leaf curl disease (OLCD). These viruses belong to the genus *Begomovirus* and family *Geminiviridae.* The plants infected with these diseases are generally associated with heavy infestation of the insect vector, whitefly (*Bemisia tabaci*), which transmits the virus in a persistent circulative manner (Varma, 1952). The list of begomoviruses infecting bhindi across the world is summarized in Table 2.1.

2.1. OCCURRENCE

Kulkarni (1924) reported the occurrence of YVMD from Bombay which was the first report of the disease in the world and named it as yellow mosaic of okra. Uppal *et al.* (1940) recognized the viral origin of the disease based on morphological symptoms expressed on plant and the disease was named as yellow vein mosaic (YVM). Capoor and Varma (1950) proved the viral nature of the pathogen causing the disease. The morphology of the virus and its serological relation with African cassava mosaic virus proved that it to be a geminivirus (Harrison *et al*., 1991). Since then, the disease was reported in many other countries.

OELCuD was first reported from Karnataka (Bangalore) in 1986 (Singh, 1986) and later reported from Pakistan and Africa (Mishra *et al.*, 2017).

Table 2.1: List of begomoviruses infecting bhindi worldwide

Sl. No	Begomovirus	Country	Remarks	Reference
1	Bhindi yellow vein mosaic virus	India	Monopartite	Kulkarni, 1924
2	Okra enation leaf curl virus	Nigeria	Monopartite	Atiri, 1984
3	Okra leaf curl virus	West Africa	Monopartite	Swanson and Harrison, 1993
4	Okra enation leaf curl virus	India	Monopartite	Singh, 1996
5	Okra yellow vein mosaic virus	Pakistan	Monopartite	Zhou *et al.*, 1998
6	Bhindi yellow vein mosaic virus	Pakistan	Monopartite	Zhou *et al.*, 1998
7	Okra leaf curl virus	Saudi Arabia	Monopartite	Ghanem, 2003
8	Bhindi yellow vein mosaic virus	India	Monopartite	Jose and Usha, 2003
9	Okra yellow crinkle virus	Bamako, Mali	Monopartite	Shih *et al.*, 2007
10	Okra yellow mosaic Mexico virus	Mexico	Monopartite	Hernandez-Zepeda *et al*., 2010
11	Bhindi yellow vein Maharashtra virus	India	Monopartite	Brown *et al*., 2012
12	Bhindi yellow vein Delhi virus	India	Monopartite	Venkataravanappa *et al*., 2012a
13	Bhindi yellow vein Haryana virus	India	Monopartite	Brown *et al*., 2012
14	Cotton leaf curl Alabad virus	India	Monopartite	Venkataravanappa *et al.*, 2012b, 2013a
15	Cotton leaf curl Bangaluru virus	India	Monopartite	Venkataravanappa *et al*., 2013a
16	Bhindi yellow vein Bhubhaneswar virus	India	Monopartite	Venkataravanappa *et al*., 2013b
17	Okra enation leaf curl virus	India	Monopartite	Singh *et al.*, 2013
18	Okra enation leaf curl virus	India	Monopartite	Sanwal *et al*., 2014
19	Bhindi yellow vein mosaic virus	India	Monopartite	Venkataravanappa and Reddy, 2013
20	Okra enation leaf curl virus	Pakistan	Monopartite	Hameed *et al.*, 2014
21	Bhindi yellow vein Madurai virus	India	Monopartite	Venkataravanappa *et al.*, 2014
22	Okra enation leaf curl virus	India	Monopartite	Kumar *et al*., 2019

Occasionally, BYVMV and OELCuV occur together and cause severe loss to okra cultivation in India. Mostly, in southern parts of India, BYVMV and OELCuV diseases produced either yellow vein mosaic or enation leaf curl symptoms (Sohrab *et al.,* 2013). Mishra *et al.* (2017) reported that under North Indian conditions, both YVMV and OELCuV occur on the same plant and which could be due to the evolution of new viral strains through recombination or pseudo-recombination. Malathi *et al.* (2017) reported that the symptom expression is also based on host genotype. In some cultivars, enation leaf curl symptom and yellow vein disease symptoms appeared separately while in some cultivars, both symptoms appeared together.

Venkataravanappa *et al*. (2014), reported that of the virus species such as OYVMV, OELCuV, BYVMV and BYVMaV are predominant in okra. BYVMV is present in all bhindi growing areas in India whereas, BYVMaV occurs only in few locations in Andra Pradesh, Tamilnadu and Maharashtra. Although the occurrence of OELCuV reported throughout the country, it is less distributed when compared to BYVMV.

The disease outbreak was found in epidemic and endemic forms in okra cultivating areas in India. The incidence ranged from 50 to 100 per cent and varied among different locations and seasons (Chelliah *et al*., 1975)

2.2. ECONOMIC IMPORTANCE AND IMPACT ON YIELD

The yellow vein mosaic disease of okra causes enormous losses both in terms of quality and quantity.

2.2.1. Quantitative losses

The yellow vein mosaic disease of bhindi was reported to cause huge loss in yield. Late incidence of the disease led to reduction in yield by 25 per cent while early infection caused 100 per cent loss (Capoor and Varma, 1950). The yield loss of okra due to virus infection was depended on the stage of the crop. Bhindi plants infected at 35 days after germination recorded yield loss of 93.80 per cent and when

crop was infected at 50 and 65 days after germination, the yield reduced to 83.63 and 49.36 per cent (Sastry and Singh, 1974).

Retardation of growth with few leaves and fruits were observed if the plants were affected within 20 days after germination and loss might be about 94 per cent. The damage was declined with delay of infection of the plants. A loss of 84 and 49 per cent was recorded when plants were infected at 50 and 65 days after germination respectively (Nath and Sakia, 1992). Pun and Doraiswamy (1999) reported that younger the age of plant at the time of infection, greater will be the damage incurred in terms of yield. They reported that yield loss of 95.70 per cent was recorded when plants inoculated one week after planting and 17.40 per cent when the plants were infected seven weeks after planting.

A survey on begomoviruses associated with bhindi in India revealed that the occurrence of YVMD incidence varied among different states *viz.,* Karnataka (23.00 to 67.67 %), Andhra Pradesh (45.89 to 56.78 %), Tamil Nadu (23 to 75.64 %), Kerala (42.45 to 75.64), Maharashtra (23 to 85.64 %), Haryana (24.85 to 65.78 %), Uttar Pradesh (35.76 to 57.00 %), Delhi (45.45 %), Chandigarh (67.78 %) and Rajasthan (45.89 to 66.78 %) (Venkataravanappa, 2008).

The OLCV infection caused yield loss up to 80 per cent (Basu, 1995). OELCuD resulted in yield loss of 30 to 100 per cent, depending upon the growth stage of the plant at the time of infection (Singh, 1996). He also reported that plants infected at 20, 35 and 50 days after germination caused 93.80, 83.60 and 49.30 per cent loss in yield respectively. Okra yellow vein mosaic disease along with okra leaf curl disease and OELCuD caused severe loss to okra cultivation in India.

2.2.2. Qualitative losses

The disease also affected the fruit quality which in turn affected the market value of the fruits. The fruits of the diseased plants were smaller in size with bleached appearance compared to fruits from healthy plants (Nariani and Seth, 1958).

Due to infection, there was reduction in plant height (24 %), number of fruits (32 %), fruit length (31 %), stem girth (16 %), root length (50 %) and the fruits. The infected plant also showed malformation. (Sheikh *et al*., 2013). Khaskheli *et al*. (2017) reported that the virus showed significant increase in number of leaves but reduced plant height, flowers, fruits and yield.

2.3. SYMPTOMATOLOGY

The YVMD infected plants showed vein clearing as initial symptom (Kulkarni, 1924). Vein clearing started from minor veins and prolonged to the major ones (Uppal *et al.*, 1940). This was followed by vein clearing and minute enations on the under surface of the leaves. The fruits produced in diseased plants were deformed with bleached appearance (Fernando and Uduravan, 1942).

The leaves with vein clearing symptom also showed thickening of veins and veinlets. Vein clearing was followed by veinal chlorosis. However, under severe infection, the complete leaves showed yellowing (Raychaudhuri and Nariani, 1977). The diseased plants remained stunted and produced few fruits which were hard and smaller in size (Mandahar and Singh, 1972).

The yellow vein mosaic disease was characterized by vein clearing symptom with interveinal green tissues. Primarily, leaves in infected plant exhibited only vein clearing while in the later stages, the whole leaf turned yellow. In severe cases, the infected leaf completely turned cream coloured. The infection at early stage of the plant led to stunted appearance. The fruits produced in the infected plants showed malformation with pale yellow colour (Singh 1990).

The characteristics symptoms of the yellow vein mosaic disease of okra were clearing of the veins and veinlets which got thickened under severe condition. Sometimes, the chlorosis extended to the interveinal area and the whole leaf became yellow during severe infection. The fruits remained yellowish green in colour, dwarf and malformed that might reduce the marketability of the produce (Gupta and Thind, 2006).

Based on the development stage at which infection occurred, Venkataravanappa *et al.* (2012a) distinguished three type symptoms under natural field conditions. In the first type, plants affected early in the season had completely yellow leaves and later became brown and dry up. The second type of symptom expressed when the plants were infected after flowering stage. The leaves in the upper part of the plant as well as flowering parts exhibited vein clearing. Such plants also produced fruits with bleached appearance. In the third type, infection occurred at the end of the season showed vein clearing on young shoots arise from the base of the stem. However, there was not much reduction in yield.

Okra leaf curl virus (OLCV) infected plants produced symptoms such as curling, yellowing and distortion of leaves along with stunting of the plant and yield loss (Sohrab *et al.,* 2013). OELCuD primarily caused pin-head enations on the abaxial surface of leaves and upward curling of leaves with warty and rough texture. Affected plants also showed bending of the stem and lateral branches with thick and leathery leaves. Plants also showed severe stunting with small and deformed fruit which were unfit for marketing (Singh, 1996).

Serfraz *et al*. (2014) observed rough texture with pin head leaf structure on the abaxial surface of the leaf with prominent vein thickening. Kumar *et al*. (2019) reported upward curling, vein thickening and green patches in OELCuV infected plants during the survey conducted in major okra cultivated parts of India *viz*., Bhubaneswar (Odisha), Hyderabad (Telangana), Vijayawada (Andhra Pradesh), Varanasi (UP) and Sonipat (Haryana).

2.4. HISTOPATHOLOGY

Ahmed *et al.* (2005) reported that appearance of morphological symptoms caused by virus infection was due to the disturbances at cellular level. Kunkalikar *et al.* (2007) reported that papaya ring spot virus (PRSV) caused histological and histo-chemical modifications in papaya. Palisade cells and spongy cells in the infected leaves were malformed with abnormal shape and complete disintegration.

Mohamed (2011) studied the effect of beet mosaic virus (BtMV) on the infected leaves and reported that BtMV reduced leaf dimentions such as mid-vein thickness, blade thickness, palisade tissue thickness, spongy tissue thickness and vascular bundles length.

Mohamed *et al*. (2012) reported the histopathological changes induced on the anatomy of different organelles such as leaf petiole, leaf blade and stem by squash leaf curl virus (SqLCV) through light microscopy. The infection led to a reduction in stem diameter by 10.90 per cent and leaf petiole by 24.40 per cent. They also reported that the viral infection increased the dimension of mid-vein due to increase in mid-vein vascular bundle dimensions. Severe damage in thylakoids and uneven thickening of cell walls resulted in curling symptoms in squash leaves.

Infection on bhindi plants induced hypertrophy and increase in inter-cellular spaces in the phloem cells of diseased mid-vein in comparison to healthy one. Inclusion bodies with the diseased mid-vein phloem cells were clearly visible in the nucleus in comparison to healthy mid-vein phloem cells of okra plants (Markam, 2014).

Harish (2018) reported that PRSV infection in papaya led to disorganized parenchyma cells and chloroplasts. The palisade cells and spongy mesophyll cells were also deformed.

2.5. THE VIRUS

Bhindi has been reported to host 12 begomoviral species including BYVMV, bhindi yellow vein Haryana virus (BYVHV), okra yellow vein mosaic virus (OYVMV), mesta yellow vein mosaic virus (MeYVMV), hollyhock yellow vein mosaic virus (HoYVMV), cotton leaf curl Alabad virus (CLCuAV), bhindi yellow vein Bhubhaneswar virus (BYVBhV), cotton leaf curl Bangalore virus (CLCuBaV), tomato leaf curl New Delhi virus (ToLCNDV), radish leaf curl virus (RLCuV), OLCV and OELCuV (Malathi *et al*., 2017). The yellow vein mosaic in bhindi is associated with bhindi yellow vein mosaic virus (BYVMV). The virus belongs to the genus *Begomovirus* and family *Geminiviridae.* Symptoms similar to

BYVMV was also reported in plants infected by OELCuV and OLCV causing OELCuD and okra leaf curl disease respectively. These viruses also belong to the genus *Begomovirus* and family *Geminiviridae.* Venkataravanappa *et al.* (2014) reported that the OELCuV had evolved through recombination of other malvaceous begomoviruses such as BYVMV, CLCuBaV, and MeYVMV and the BYVMV acts as the major contributor for the sequences make up of the virus.

Harrison *et al.* (1991) identified geminivirus particles by immunosorbent electron microscopy in extracts of BYVMV infected leaves in India. Geminiviruses include a diverse group of plant viruses with a circular, single stranded (ss) DNA genome of size (2.5-3.0 kb) and are encapsulated within twinned isometric particles (15-18 × 30 nm) (Stanley, 1983). Based on their genome organization, vector and host range, the geminiviruses are grouped into four different genera; *Begomovirus, Curtovirus, Mastrevirus,* and *Topocuvirus* (Fauquet *et al*., 2008). Zerbini *et al.* (2017) further classified family *Geminiviridae* into nine genera, *Capulavirus, Curtovirus, Becurtovirus, Eragrovirus, Begomovirus, Grablovirus, Topocuvirus, Mastrevirus* and *Turncurtovirus*. Harrison and Robinson (1999) reported that begomoviruses belonging to the family *Geminiviridae* have either monopartite (DNA A) or bipartite (DNA A and DNA B) genome.

Majority of begomoviruses consist of characteristic two circular ssDNA components, DNA A and DNA B (Varma and Malathi, 2003) each of about 2.6-2.8 kb genome size. DNA A contains genes which encodes encapsidation and viral replication protein and DNA B for intra and intercellular movement function (Harrison *et al.*, 1977). Monopartite begomoviruses consist of one or more small circular satellite DNA molecules of ~1.3 kb size, designated as DNA β instead of DNA B (Briddon *et al*., 2002a).

2.6. GENOME ORGANIZATION

Based on the coat protein and replication initiation protein sequences, begomovirus are differentiated into ‘old world’ and ‘new world’ begomoviruses which corresponds to the geographical origin of the virus. Old world viruses include

those from Asia, Africa, Meditterranean region and Australia and new world viruses belong to America. Old world begomoviruses could be either monopartite or bipartite whereas new world begomoviruses are exclusively bipartite.

The genome organization of BYVMV and OELCuV is mostly comparable with Old world begomoviruses with monopartite genome which include two virion (V1, V2) and five complementary sense open reading frames (ORFs). The interon sequences are incomplete direct repeats of GGTGT. The monopartite genome consists of DNA A component only (Harrison and Robinson, 1999; Varma and Malathi, 2003). It was reported that monopartite genomes have an association with a small circular ssDNA molecules of ~ 1.3 kb length which was designated as satellite DNA molecule (DNA β) (Saunders *et al*., 2000 and Zhou *et al*., 2003).

The DNA A component consists of six genes of which two genes were present in virion-sense strand (AV) and four were present in the complementary sense strand (AC). DNA B encodes two proteins, one on the virion-sense strand (BV) and one on the complementary strand (BC). The ORF AV1 and AV2 encoded the coat protein and cell-to-cell movement protein. The ORF AC1, AC2 and AC3encoded the rep protein, transcriptional activator protein (TrAP) and replication enhancer protein (REn) respzxectively. The function of AC4 has been implicated in cell cycle progression (Latham *et al.,* 1997; Krake *et al.,* 1998; Gutierrez, 2002).

The genome of BYVMV had only DNA A, while DNA B equivalent component was not detected in the infected bhindi. However, a beta satellite was identified in the BYVMV genome. The combined action of DNA A and betasatellite were necessary to cause infection in bhindi (Jose and Usha, 2003). Briddon and Stanley (2006) stated that beta satellites were essential for the symptom development, determination of host range and also for dominating the host defences.

2.7. TRANSMISSION

2.7.1. Vector transmission

The virus is transmitted by whitefly (*Bemisia tabaci* Genn.) in persistent manner by both nymph and adults (Varma, 1952). Muniyappa and Veeresh (1984) reported whiteflies to be the vectors of yellow mosaic, yellow vein mosaic and leaf curl diseases of economically important crops. Venkataravanappa *et al.* (2013a:2014) reported that the OELCuV and cotton leaf curl Bangalore virus (CLCuBV) infecting okra were transmitted by whiteflies. Singh *et al.* (2016) studied the transmission of BYVMV and reported that it was readily transmitted by whiteflies (*Bemisia tabaci*) and not through aphids (*Aphis gossypii*, *Aphis craccivora* and *Myzus persicae*).

Begomoviruses emerged as a major threat to different crops in different countries due to the incredible increase in population density of the vector, *B. tabaci* during 1970's (Bird and Maramorosch, 1978) and due to development of B-biotype of *B. tabaci* (Brown and Bird, 1992). The B-biotype created disastrous results by altering the epidemiology of many begomoviral diseases in most of the crops and also by introducing begomoviruses into crop plants which were earlier reported only in the weed hosts. Both nymph and adults transmitted the virus in a persistent manner (Mukhopadhyay *et al*., 1994).

When comparing the transmission efficiency of both male and female whiteflies, it was observed that the females were more efficient than males (Varma, 1952). Rathi and Nene (1974) also reported that, mung bean yellow mosaic virus was efficiently transmitted by female whiteflies.

2.7.1.1. Vector population required for virus transmission

Muniyappa and Reddy (1976) reported that single whitefly could be able to transmit horsegram yellow mosaic virus. Mandal (1989) stated that minimum of 15 whiteflies was required to get 100 per cent transmission in case of croton yellow vein mosaic virus. Nateshan (1992) reported that single whitefly was enough to

transmit cotton leaf curl virus (CLCuV). While, transmission efficiency was increased when 10-15 whiteflies used for transmission (Chenulu *et al*., 1979). Jayashree *et al.* (1999) also reported that 21.67 per cent infection of pumpkin yellow mosaic virus in pumpkin were caused by single viruliferous whitefly however, 15 whiteflies per plant were necessary for 100 per cent infection.

Venkataravanappa (2008) reported that 30 and 20 per cent incidence of BYVMD was obtained with single B biotype and two indigenous *B. tabaci* respectively. Ten adult whiteflies were sufficient to cause 100 per cent transmission of yellow mosaic disease in ridge gourd (Hurakadli *et al*., 2019).

2.7.1.2. Acquisition access period (AAP) and Inoculation access period (IAP)

Varma (1952) reported minimum acquisition access period (AAP) of 12 to 24 h for transmission of yellow vein mosaic disease by whiteflies. However, initial fasting evidently improved the efficiency of transmission of virus by vectors.

Costa and Benett (1950) described that a minimum of 30 min. AAP for whiteflies to transmit euphorbia mosaic virus. James (1965) reported that an AAP of one hour AAP was required for the transmission for BYVMV. Minimum of 30 min. of AAP and IAP was required for tomato yellow leaf curl virus (Cohen and Nitzany, 1966) and squash leaf curl virus (Cohen *et al*., 1983).

Jayashree *et al.* (1999) reported that minimum of 6 h. AAP and 3 h. of IAP was required by whiteflies to transmit pumpkin yellow vein mosaic virus to healthy plants. They also reported that a pre-acquisition starvation period of 3 h. resulted in 100 per cent transmission, but post-acquisition starvation was found to decrease the transmission efficiency. Chatterge *et al.* (2008) reported that the minimum AAP of whitefly was 12 h. for transmitting yellow vein mosaic disease in *Hibiscus cannabinus* and *Hibiscus sabdariffa.*

IAP of 24 h. resulted in high percentage of BYVMV infection (Murugesan and Chellaiah, 1977). Minimum of 15 min. AAP and IAP were found to be sufficient for B-biotype and 20 min. for indigenous whiteflies (Venkataravanappa, 2008).

Venkataravanappa *et al*. (2013b), reported that 24 h. of IAP and AAP was adequate for transmission of BYVBhV. The minimum of 1 h. AAP and 30 min. IAP was sufficient for OELCuV transmission by *B. tabaci* and the transmission rate showed a correlation with the AAP and IAP (Venkataravanappa *et al*., 2014).

2.7.1.3. Detection of virus in vector

Deng *et al.* (1994) reported that cowpea golden mosaic virus (CGMV), okra leaf curl virus (OLCV) and tomato yellow leaf curl virus (TYLCV) were detected in whiteflies using a pair of degenerate oligonucleotide primer. Polymerase chain reaction (PCR) based detection method was highly sensitive to detect TYLCV and tomato mottle virus (ToMoV) in viruliferous whiteflies *(*Mehta *et al.*, 1994).

Rosell *et al*. (1998) used PCR based molecular technique to track the pathway of squash leaf curl virus (SLCV) DNA in full body extracts and in saliva, hemolymph and honeydew of its whitefly vector *B. tabaci.* Khan (1999) detected the presence of tomato leaf curl virus (TLCV) in its vector *B. tabaci* by PCR based approach using two set of geminivirus specific degenerate primers *viz.*, PALv1978/PARc496 and AV494/AC1048 amplified DNA fragment of 1.1 kb and 0.5 kb respectively.

Venkataravanappa (2008) reported that the detection of BYVMV in both indigenous and B-biotype whiteflies by PCR amplification with primer specific to complete coat protein (CP) gene of DNA-A. He also revealed that, it could be able to detect the virus from single viruliferous whitefly.

2.7.2. Graft transmission

Capoor and Varma (1950) transmitted BYVMV through grafting. Cleft grafting efficiently transmitted yellow vein mosaic disease in *Hibiscus sabdariffa* and *Hibiscus cannabinus* (Chatterjee *et al*., 2008).

Venkataravanappa *et al.* (2012a) transmitted the cotton leaf curl Alabad virus associated with okra yellow vein mosaic disease in North India through grafting with a transmission efficiency of 65 per cent and the inoculated plants produced yellow vein mosaic symptoms 25–30 days after inoculation.

Venkataravanappa *et al*. (2013a) successfully transmitted cotton leaf curl Bangalore virus (CLCuBV) associated with okra yellow vein and leaf curl disease through side veneer grafting and obtained a transmission efficiency of 65 per cent and the grafted okra plants showed yellow vein mosaic symptoms within 20–25 days after grafting. Singh *et al*. (2016) reported that 9-10 days after inoculation, disease was transmitted through graft union and 100 per cent transmission was obtained. Ghevariya and Mahatma (2017) used both approach grafting and side veneer grafting for the transmission of BYVMV to healthy plants.

2.7.3. Seed transmission

Capoor and Varma (1950) identified that yellow vein mosaic of bhindi is not transmitted through sap or seed. Chatterjee *et al*. (2008) investigated the transmission of BYVMV through seeds and reported that the virus was not seed-borne. Venkataravanappa *et al.* (2014) reported that none of the seeds collected from okra plants infected with OELCuV developed any symptoms and negative results were obtained for ELISA and PCR diagnostic techniques.

2.8. HOST RANGE

Both virus and vector have been reported to have very wide host range that included crops, weeds and ornamentals. The natural hosts were essential for the survival of both virus and vector as they helped the virus to propagate and vector to

harbour and multiply. Further, these hosts act as reservoirs of the virus and play a role as a source of primary inoculum. Hence, these natural hosts are very significant in disseminating the virus inoculum.

Capoor and Varma (1950) reported that *Abelmoschus manihot, A. moschatus* and *Althaea rosea* were susceptible to yellow vein mosaic disease. Yellow vein net symptoms were observed in *Croton bonplandianum* when artificially inoculated with BYVMV (Handa and Gupta, 1993).

Pun *et al*. (1999) reported the infection of OYVMV in five weed plant species, *viz., Althea rosea* (L.) Cav., *Acalypha indica*, *Croton bonplandianum*, *Parthenium hysterophorus* L. and *Hibiscus rosa-sinesis* L. using direct antigen coating enzyme linked immuno-sorbent assay (DAC-ELISA) with polyclonal antibodies raised against Indian cassava mosaic virus (ICMV) and African cassava mosaic virus (ACMV). Gupta and Thind (2006) reported that BYVMV had a wide host range which included *Abelmoschus manihot, Althaea rosea, Croton sparsiflora, Hibiscus tetraphyllus, Malvastrum tricuspidatum* and *Ageratum* sp.

Venkataravanappa *et al*. (2013a) reported that few plants in Malvaceae and Solanaceae families; *viz., Althaea rosea, Datura stramonium* and *Nicotiana benthamiana* were susceptible to BYVMV.

Venkataravanappa *et al*. (2014) investigated the host range of OELCuV with 59 plant species. They reported that two species belonging to family Malvaceae (*Althaea rosea* Cav. and *Abelmoschus esculentus* (L.) Moench) and six in the family Solanaceae (*Datura stramonium, N. benthamiana, Nicotiana glutinosa, N. occidentalis, N. clevlandii* and *N. tabacum*) produced symptoms such as leaf curling and enation by whitefly transmission and proved to be susceptible to OELCuV.

Roy *et al.* (2015) first reported the occurrence of BYVMV on *Litsea* spp. in India. The infected *Litsea apetala* and *L. sebifera* (Lauraceae) showed symptoms such as vein clearing, mosaic, leaf curling, leaf yellowing, deformation and stunted growth.

Roy *et al.* (2017) reported the evidence of BYVMV infecting *Tabernaemontana divaricata* (Apocynaceae) and *Albizia saman* (Fabaceae) in West Bengal (India) and the symptoms include leaf yellowing, leaf curling and stunted growth.

2.9. MOLECULAR CHARACTERIZATION

Presently, molecular techniques such as polymerase chain reaction (PCR) is one of the most important techniques for the quick detection of plant viruses. PCR based detection has been now used very widely due to its high accuracy and sensitivity for the detection of plant virus infection even from the small amount of samples. In PCR technique, the genetic material of the diseased samples was extracted, purified and amplified with virus nucleic acid specific primers.

2.9.1. Isolation of genomic DNA

Jose and Usha (2000) described a protocol for the extraction of geminiviral DNA from BYVMV infected *Abelmoschus esculentus,* a host plant with high amounts of mucilage. This method comprises extraction with a buffer with sodium citrate (pH 6.0) and PEG precipitation of the virus followed by alkali lysis.

Rouhibakhsh *et al.* (2008) described a protocol for the detection of begomoviruses in legume plants. The method involved DNA extraction with a modified CTAB buffer with β-mercaptoethanol (5 %) and sodium chloride (1.4 to 2.0 M)

Singh and Kumar (2012) reported a modified protocol of Doyle and Doyle (1987) for DNA extraction from bhindi plants. Modification involved increased volume of DNA extracting buffer (1.5 ml/sample), decrease sample volume (50-60 mg), higher salt concentration (5 M) and use of polyvinylpyrrolidone (PVP) yielded a high quality DNA and was found to be suitable for PCR and RAPD analysis.

Senevirathna *et al*. (2016) isolated the genomic DNA from okra leaf tissues using modified Kochko *et al.* (1990) method *i.e.,* immature leaf samples and increased the time of incubation of ground samples by 10 min. Roy and Sherpa (2017) developed a modified CTAB method for the extraction of high quality DNA from mucilaginous plants, suitable for the detection of geminiviruses. They increased the concentration of PVP and β-mercaptoethanol and NaCl. Polyphenols were removed with the help of high concentration of PVP and β-mercaptoethanol and high levels of polysaccharides was removed by higher concentration of NaCl.

2.9.2. Polymerase Chain Reaction (PCR)

The coat protein (CP) gene is the conserved region in the genome of plant viruses. Differentiation of strains, species, and taxonomic lineages of begomoviruses using CP gene sequence has been accepted by International committee on taxonomy of viruses (ICTV) (Rybicki, 1994).

Wyatt and Brown (1996) designed a degenerate primer that amplify the core region of the coat protein of subgroup III geminivirus and produced the amplicons at ~550 bp. Briddon *et al*. (2002b) amplified full-length DNA β component using PCR with designed primers from the DNA isolated from infected plants.

Sharma *et al.* (2005) used thermal cycling to determine the complete genome of cotton leaf curl geminivirus (CLCuV-HS2) with primers specific to CP gene. The product size of the amplicon of CLCuV DNA fragment was ~750 bp.

Acharyya *et al*. (2008) amplified the CP gene and beta satellite of BYVMV infecting *Urena lobata* yielded DNA fragments of ~ 0.77 kb and ~ 1.0 Kb respectively. Ghosh *et al*. (2008) carried out the PCR for the amplification of CP gene and beta satellite of BYVMV of eastern India and produced an amplicon size of 0.77 kb and 1.3 kb respectively.

Venkataravanappa *et al*. (2014) identified and characterized okra enation leaf curl virus in okra from Haryana consisting of symptoms such as leaf curl with enations using PCR-mediated amplification.

Senevirathna *et al*. (2016) characterized symptomatic samples of okra collected from different locations of Sri Lanka showed positive results for OYVMV. Hamsa *et al*. (2016) characterized pumpkin yellow vein mosaic disease in Kerala by PCR amplification of CP gene and movement protein (MP) gene using specific primers

2.10. DIVERSITY AND EVOLUTION OF BEGOMOVIRUSES

Phylogenetic analysis of different begomoviruses showed that the relationship was close among viruses from the similar parts of the world (Hong and Harrison, 1995; Padidam *et al*., 1999). Recombination and pseudo-recombination are very common phenomena occurring within as well as between species and genera of geminiviruses and are major reasons for begomovirus evolution (Polston and Anderson, 1997). Comparison of CP gene sequences revealed that the phylogenetic tree of begomoviruses consisted of two main branches of old world and new world viruses, respectively and clusters were also developed within the branches depending on the region where these occur (Harrison and Robinson, 1999).

The evolution of new viral strains through recombination could be harmful on the host population and might increase the ability to infect new hosts (Padidam *et al.,* 1999). The high diversity between begomovirus species coupled with mixed infection might help in recombination and pseudo-recombination leading to the frequent development of new begomoviruses (Zhou *et al*., 1998; Padidam *et al.*, 1999; Sanz *et al*., 2000; Berrie *et al.*, 2001; Jeske *et al*., 2001). Begomoviruses in the Indian subcontinent showed evolutionary divergence from begomoviruses in other parts of the world (Lefeuvre *et al*., 2007).

Recombination of OYVMV and other geminiviruses probably involved in the evolution of cotton leaf curl virus (CLCV) which led to epidemics of cotton leaf curl disease in Pakistan (Zhou *et al*., 1998). In tropical and subtropical regions mixed infection of different geminiviruses was very common (Ribeiro and Al, 1998). Interaction between co-infecting viruses might play a significant role which

led to the development of complex diseases (Monci *et al*., 2002). Mixed infection might result in the synergestic, additive or antagonistic interactions in plants. Priyavathi *et al*. (2016) confirmed such mixed infection of OELCuV and BYVMV DNA-A, alpha and beta satellite associated with yellow vein mosaic disease in bhindi by sequencing, Southern hybridization and qPCR and suggested that this might be lead to the recombination among the co-infecting viruses and evolution of new viral species.

Serfraz *et al*. (2014) suggested that inter-malvaceous recombination between okra and cotton infecting begomoviruses resulted in the evolution of OELCuV in Pakistan. Venkataravanappa *et al*. (2012a) reported the association of cotton leaf curl Alabad virus (CLCuAV) with yellow vein mosaic disease in bhindi. Phylogenetic and recombination analysis of these virus showed that the major genome component was derived from different begomoviruses (CLCuAV, BYVMV and cotton leaf curl Multan virus (CLCuMuV)) which act as the parents for evolution of the virus.

Recombination has also been reported between distinct *Geminivirus* species co-existing and infecting tomato in Spain. Genome analysis of the natural recombinant of tomato yellow leaf curl virus (TYLCV) and tomato yellow leaf curl Sardinia virus (TYLCSV) revealed that the intergenic region contain recombination sites (Monci *et al*., 2002). AV1, AV2, AC1 and intergenic regions of the viral genome are the potential sites of recombination in ToLCV (Kirthi *et al*., 2002).

Venkataravanappa *et al.* (2014) discovered recombination in OELCuV and identified BYVMV, BYVBhV and MeYVMV as the major parents of recombination. They also reported that the origin of replication of OELCuV isolates originated from BYVMV.

The phylogenetic analysis revealed that BYVDV and BYVHV along with BYVMV formed a major cluster however, these were occupied as a separate group. The OELCuV and tomato leaf curl New Delhi virus (ToLCNDV) clusters were different and separated from BYVMV and BYVMaV. They also conducted

neighbour net analysis using split tree programme and confirmed the distinct nature of OELCuV and BYVBhV (Venkataravanappa *et al.*, 2014). Lima *et al*. (2017) reported that diversification among begomovirus population was mostly driven by mutational dynamics than recombination.

2.11. MANAGEMENT OF YELLOW VEIN MOSAIC DISEASE OF BHINDI

Application of various biocontrol agents, plant extracts and insecticides through spraying or soil application or seed dressing have been evaluated for managing the whitefly vector in order to prevent the spread of yellow vein mosaic disease of bhindi.

2.11.1. Effect of insecticides

Nath and Debnath (2002) reported that spraying of metasystox at 15 days interval after sowing were found to produce the highest yield with comparatively lower disease incidence. Ali *et al*. (2005b) evaluated the efficacy of pesticide/bio-pesticide against insect vector *Bemisia tabaci* Genn. and reported that imidacloprid significantly reduced the whitefly population followed by neem extract and effective microbes.

Rameshwar (2010) evaluated different management studies for controlling yellow vein mosaic disease in bhindi and reported that spraying of Admire + neem oil followed by acephate + neem oil were the most successful practice for reducing the disease incidence. Spraying with acephate + neem oil, imidaclopride + neem oil, Admire + neem oil and Hostothion + neem oil one by one at 10 days interval, significantly increased the yield followed by the sprays of Admire + Neem oil. While highest benefit over the control was recorded in the treatment with spray of Hostothion + neem oil followed by Admire + neem oil.

Vethanayagam and Rajendran (2010) investigated the efficacy of neem insecticidal soap (NIS) to reduce the yellow vein mosaic disease incidence in bhindi. Maximum fruit yield was recorded from the field applied with NIS only with 17 per cent disease incidence.

Mohanasundaram and Sharma (2011) reported that thiamethoxam (20 g a.i/ha) and fipronil (50 g a.i/ha) were effective for reducing the population of sucking pests *viz.*, leafhopper, whiteflies and red spider mites. Ali *et al*. (2012) reported the effectiveness of three insecticides; Mycotel, Tracer and imidacloprid to manage the whitefly population and among which, imidacloprid was the most effective one.

Birah *et al.* (2012) described that biointensive approaches such as neem cake application, sowing of maize as barrier crop, clipping of diseased shoots and fruits at weekly intervals, pheromone traps, foliar spray of neem seed kernel extract, aqueous leaf extracts of cloves (*Syzygium aromaticum*), karanj oil effective for controlling the BYVMD. The results revealed that integrated and bio-intensive practices significantly reduced jassid and whitefly population than untreated control in okra.

Ali *et al*. (2014) reported that NPK fertilizer solution and neem extract were effective among nutrients and plant extracts respectively. Nutrient application increased the plants ability to fight against the diseases. Plant extracts repel the whiteflies and thus decreased the OYVMV infection. Sale *et al.* (2015) reported that combined application of neem and cowdung manure was more effective to increase okra growth and yield of okra plant and significantly reduced the number of whitefly and leaf defoliation. Hafeez-ur-Rehman *et al*. (2015) reported that lambda cyhalothrin and neem oil at 4 and 5 per cent concentration were equally effective in controlling jassid and whiteflies and showed the same impact on yield of bhindi plant.

Pagire *et al*. (2017) conducted an experiment to test the effect of insecticides against whiteflies checking the incidence of BYVMV. The results revealed that thiomethoxam at 0.005 per cent was the most effective treatment which is followed by acetamiprid (0.005 %) and imidacloprid (0.01 %) against whiteflies checking BYVMV incidence.

Kumar and Kumar (2017) used resistant variety along with seed treatment using thiomethoxam 70WS at 3.0 g/kg seed, soil application of carbofuran at 15 kg a.i./ha during sowing, roguing of diseased plants at early stage, two to three spray of systemic insecticide before flowering for reducing yellow vein mosaic disease.

2.11.2. Effect of biocontrol agents

Application of plant growth-promoting rhizobacteria (PGPR) as a combined seed-powder treatment significantly reduced the disease severity of tomato mottle virus and increased the fruit yield in tomato (Murphy *et al*., 2000).

Patil *et al*. (2011) reported the efficacy of rhizobacterial isolates against yellow vein mosaic disease in bhindi. Bioformulations of rhizobacreria applied to seed, soil and foliage considerably decreased the infection of BYVMV with increase in plant growth and fruit yield under glasshouse conditions.

El-Dougdoug *et al.* (2012) identified five *Streptomyces* spp. which were able to produce an antiviral component, and effective for the control of local as well as systemic infection of cucumber mosaic virus (CMV).

Mishra *et al*. (2014) described that the use of *Pseudomonas* sp. along with chitosan resulted in 80.33-90.33 per cent reduction in the severity of ToLCV. The plants also recorded maximum plant height, total biomass, chlorophyll content, fruit number and yield over the diseased control.

Lee and Ryu (2016) reported that leaf-colonizing *Bacillus amyloliquefaciens* protected *Nicotiana benthamiana* and pepper plants from CMV by increasing salicylic acid and jasmonic acid signalling under field conditions. *Paenibacillus lentimorbus* B-30488, a potential biocontrol agent isolated from cow's milk effective against economically important CMV in *Nicotiana tabacum*. Soil application of B-30488 improved the plant vigour and significantly reduced the virulence and accumulation of the virus in tobacco plants (Kumar *et al*., 2016).

Abdalla *et al*. (2017) reported that soil drenching with mixtures of PGPR strains resulted in highest reduction in disease severity of both papaya ringspot virus

(PRSV-W) and tomato chlorotic spot virus (TCSV) followed by root dipping and seed coating treatments.

Beris *et al*. (2018) tested the antiviral activity of *Bacillus amyloliquefaciens* strain MBI600 and found that drenching, foliar or soil amendment applications reduced the incidence of tomato spotted wilt virus up to 80 per cent under two different sets of environmental conditions and also delayed potato virus Y systemic accumulation in tomato.

Cherian (1998) reported the efficiency of *Lecanicillium lecani* against whitefly *B. tabaci*, which is the vector of tomato yellow leaf curl virus (TYLCV). Chandrashekharaiah *et al*. (2013) evaluated the efficacy of entomopathogenic fungus, *L. lecanii* against assorted populations of *B. tabaci* nymphs in field conditions and reported that *L. lecanii* at 2.50 kg/ha with three foliar applications at 12 days intervals after the first at 25 DAS were the best treatments. The reduction in whitefly populations resulted in increased yields *i.e.* 11.75 and 10.98 per cent, respectively.

2.11.3. Effect of plant extracts

Balasaraswati *et al.* (1998) identified bougainvillea antiviral protein I (BAP I), an antiviral protein extracted from root tissues of *B. spectabilis* and are effective for the control of tomato spotted wilt virus. Madhusudhan *et al.* (2011) identified that *B. spectabilis* extract was found to be the most effective for the management of tobamoviruses.

Elsharkawy and El-Sawy (2015) investigated the efficacy of different plant extracts against bean common mosaic virus (BCMV) and observed that mixing of *Plectranthus tenuiflorus* extracts with BCMV inoculum recorded the highest reduction in BCMV infection (92 %). Spray treatments of *P. tenuiflorus* and *Mirabilis jalapa* reduced disease incidence up to 12 and 17 per cent under greenhouse conditions, whereas 17 and 23 per cent reduction was obtained under field conditions.

Chaudhary *et al.* (2016) evaluated various plants extract to manage OYVMV and its vector and observed that, *Azadiracta indica* (5 %) was effective in reducing the whitefly population and disease incidence under field condition.

Elbeshehy (2017) conducted a study to evaluate the effect of five medicinal plant extracts against zucchini yellow mosaic virus (ZYMV) infecting watermelon plants and proposed that the bougainvillea and neem extracts might played an important role in the reduction of disease incidence by producing pathogenesis-related proteins which might produce resistance against ZYMV.

Hamidson *et al*. (2017) compared the effect of application of medicinal plant extracts such as *A. indica, Piper bitle, Cymbopogon citrates, Curcuma domestica, Averroa bilimbi, Datura stramonium*, and *Annona muricata* on the incidence of CMV on the chilli (*Capsicum annuum* L.). He reported that extracts of *A. muricata* and *D. stramonium* were most effective in suppressing disease severity caused by the virus.

Seed treatment followed by six foliar sprays with root extract of *Boerhaavia diffusa* was found to be the most effective treatment against viral diseases in watermelon and also showed maximum vine length, number of fruits plant per plant, fruit diameter, fruit weight and fruit yield followed by leaf extract of *A. indica* and *Clerodendrum aculeatum* and bark extract of *Terminalia arjuna* (Sharma *et al*., 2017)

El-Sawy *et al*. (2018) reported that treatment of tomato plants with extracts of ginger (*Zingiber officinale*) and horsemint along with silica nanoparticles (*Mentha longifolia*) showed less and delayed symptoms of tomato yellow leaf curl virus (TYLCV).

Materials and Methods

3. MATERIALS AND METHODS

The experiments pertaining to the present investigation on "Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease" was conducted during 2017-2019. The laboratory experiments were carried out at the molecular laboratory, Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur and field experiment was laid out in the farm of Department of Plant Pathology, College of Horticulture. The materials used and methods followed during the study are described in detail in this chapter.

3.1. SURVEY FOR DISEASE INCIDENCE

A purposive sampling survey was carried out during November 2018 – May 2019 at different vegetable growing areas of Thrissur district of Kerala (Fig. 3.1.). Per cent disease incidence (PDI), per cent disease severity (PDS) and the symptoms expressed on different plant parts were recorded during the survey. The PDI was assessed by recording the number of plants infected out of the total number of plants in the field by the following formula.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Based on the severity symptoms, the PDS (in terms of vulnerability index) was also calculated. The scoring was done as per the 0-5 scale disease grading chart developed by Ali *et al.* (2005a) (Table 3.1).

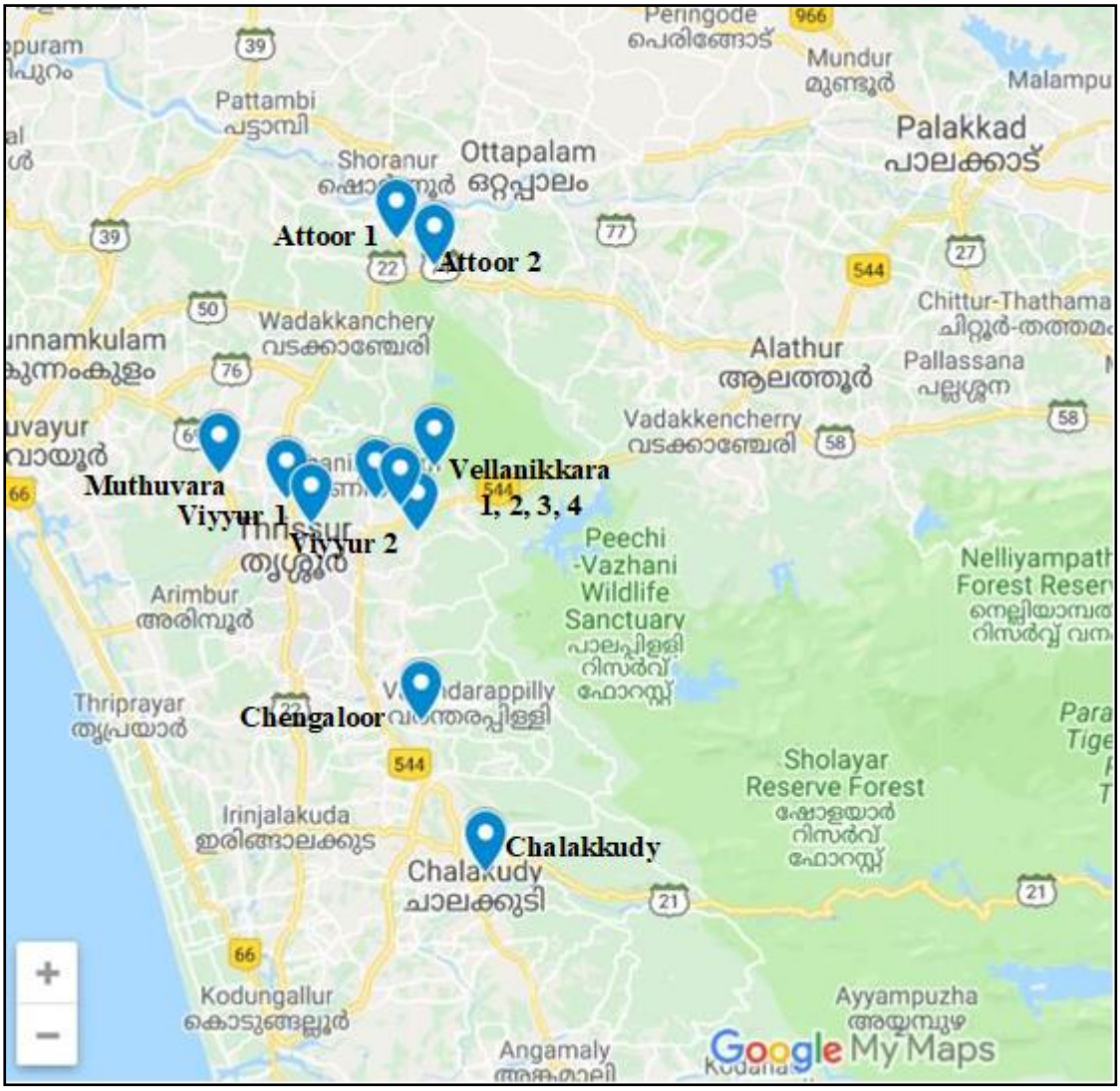


Fig. 3.1: Locations of purposive sampling survey

Table 3.1.: Yellow vein mosaic disease grading chart

Description of symptoms	Severity scale	Severity range (%)
Absence of symptoms	0	0
Very mild symptoms with initial vein clearing	1	1-20
Complete yellowing of vein with inter-veinal regions remain green or normal	2	20-40
Curly leaves and whole leaf get yellow colour	3	40-60
Whole leaf yellow coloured. Leaf margin start drying	4	60-80
Yellowish and deformed pods with all the leaves turned yellow coloured.	5	80-100

Based on the scoring, PDS in terms of vulnerability index (V) was calculated using the equation,

$$\text{Per cent disease severity} = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t\,(n_c-1)}$$

Where, n_0, n_1,...n_5 = number of plants in disease category 0 to 5 respectively

n_t = total number of plants

n_c = total number of categories

3.2. COLLECTION OF SAMPLES

Fresh leaves of bhindi showing yellow vein mosaic symptoms were collected freshly in polythene bags during the survey from different fields of Thrissur district and brought to the laboratory. The infected samples were also collected from other districts like Thriruvananthapuram, Kottayam, Kannur and Kasaragod. These were stored in deep freezer (-20 °C) for further identification and characterization of the virus associated with the disease.

3.3. MAINTENANCE OF VIRUS CULTURE

Infected bhindi plants served as the initial source of virus inoculum. The virus culture was maintained in the net house through grafting to healthy bhindi plants as mentioned in section 3.5.1.2. These plants served as virus inoculum for subsequent transmission and host range studies.

3.4. SYMPTOMATOLOGY

3.4.1. Symptoms under natural field conditions

During the survey, symptoms expressed on different parts of the infected plant *viz*., leaves, petioles, flower buds and fruits were documented under natural field conditions. In order to assess the reduction in leaf area due to virus infection, the leaf area of ten leaves both from healthy and infected plants of three bhindi varieties *viz.,* Arka Anamika, Salkeerthi and a farmer's local variety were recorded and per cent reduction was calculated.

3.4.2. Symptoms under artificial conditions

The development of symptoms under artificial conditions was studied by artificially inoculating the virus on healthy bhindi plants through grafting and vector transmission as mentioned in section 3.5.1.2. and 3.5.1.3. respectively. Inoculated plants were kept under insect proof cages and monitored for symptom development.

3.4.3. Histopathology

Thin sections of healthy and infected leaves showing yellow vein mosaic symptoms were taken using sharp razor and observed under the light microscope at 100 X magnification in order to study the internal symptoms and anatomical changes in different tissues *viz.*, parenchyma cells, palisade, spongy mesophyll cells and vascular tissues due to the virus infection.

3.5. BIOLOGICAL CHARACTERIZATION OF THE VIRUS

3.5.1. Transmission studies

3.5.1.1. Seed transmission

Ten pods were collected from both healthy and infected bhindi plants for seed transmission studies. 100 seeds each from healthy and infected pods were sown in pro trays and kept in insect proof cage. The germination percentage was calculated and the seedlings were monitored regularly for the development of symptoms up to 30 days after sowing. The DNA isolation and PCR amplification was also carried out to confirm the results as described in section 3.6.1 and 3.6.3. respectively.

3.5.1.2. Graft transmission

Graft transmission of yellow vein mosaic disease was done through cleft grafting. The rootstocks were raised in polythene bags under insect proof condition. The scions for grafting were collected from the infected plant during the survey and this was immediately grafted on to one month old healthy bhindi plants which served as the rootstock. The grafted plants were maintained under insect proof cages in the glass house of Department of Plant Pathology.

The rootstock was beheaded at the stem portion where there is active growth followed by a downward cut (cleft) through the centre of the stem. The scion was prepared with a slanting cut at both sides at the base so that the base is wedge or 'V' shaped. The base portion of the scion inserted into the cleft of the prepared rootstock and tied using polythene tape. The grafted plants were covered with moistened polythene cover to maintain high humidity and were kept inside the net house for symptom development (Plate 3.1.). The newly formed leaves on rootstock were monitored for symptom development.

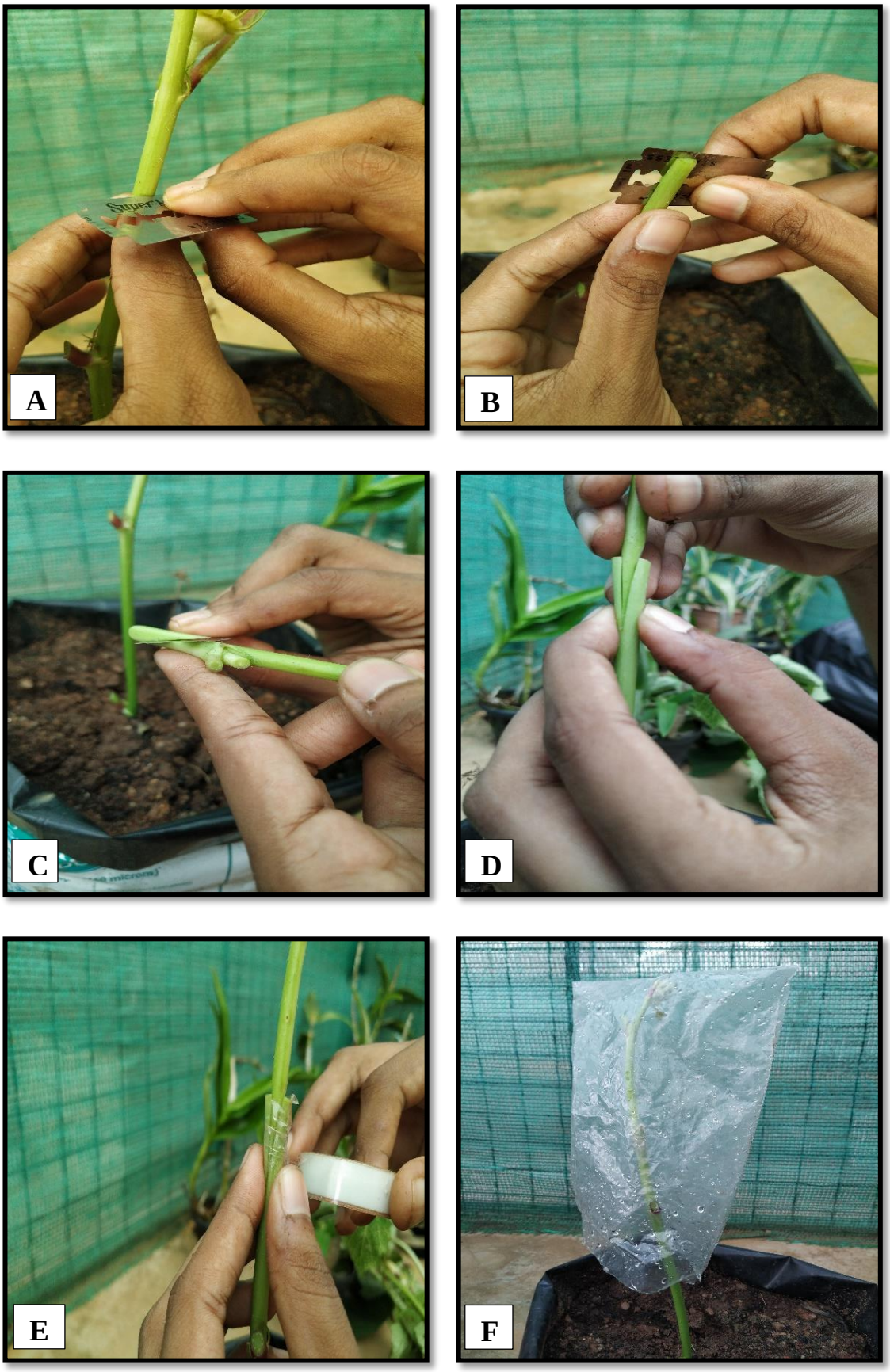


Plate 3.1: Graft transmission of the virus (A) and (B) Preparation of rootstock (C) Preparation of scion (D) Insertion of scion into rootstock (E) Tying of scion with rootstock (F) Covering the graft with polythene cover

3.5.1.3. Vector transmission

The vector transmission studies were conducted using pure colonies of insect vector, whiteflies (*Bemisia tabaci* Genn.) maintained in insect proof cages in the glass house of Department of Plant Pathology.

3.5.1.3.1. Maintenance of vector

Seedlings of brinjal, one of the most preferred host for rearing the colony of whiteflies were raised in polybags filled with potting mixture and the pots were kept inside insect proof cages (65 x 52 x 52 cm) designed with insect proof net (Plate 3.2).

Adults whiteflies were collected from the field and released on healthy brinjal plants kept inside the insect proof cages. The second generation whiteflies released after 30 days were transferred to new healthy brinjal seedlings. Subsequent generations were used for transmission studies. The whiteflies were transferred to fresh brinjal seedlings monthly to maintain the population (Plate 3.2.) The different stages of whiteflies such as egg, nymph, pupa and adult were also documented using stereomicroscope.

3.5.1.3.2. Cages for acquisition of virus from source plants

The cylindrical plastic bottles of 15 cm diameter and 20 cm depth were taken and cut at both ends with a knife and one end was covered with white coloured muslin cloth and a hole was cut out on the cylindrical side of the bottle 15 cm from the bottom of the bottle for releasing the whiteflies and hole was closed with cello tape to avoid escaping of the whiteflies from the plant (Plate 3.2.).

3.5.1.3.3. Cages for inoculation of virus on bhindi seedlings

Inoculation cages were prepared using plastic bottles of 6 cm diameter and 20 cm length so as to accommodate test seedlings of bhindi. The bottles were cut at both ends, one end was covered with a piece of white muslin cloth and a small hole was cut out on the side of the bottle and closed with a cello tape (Plate 3.2.)

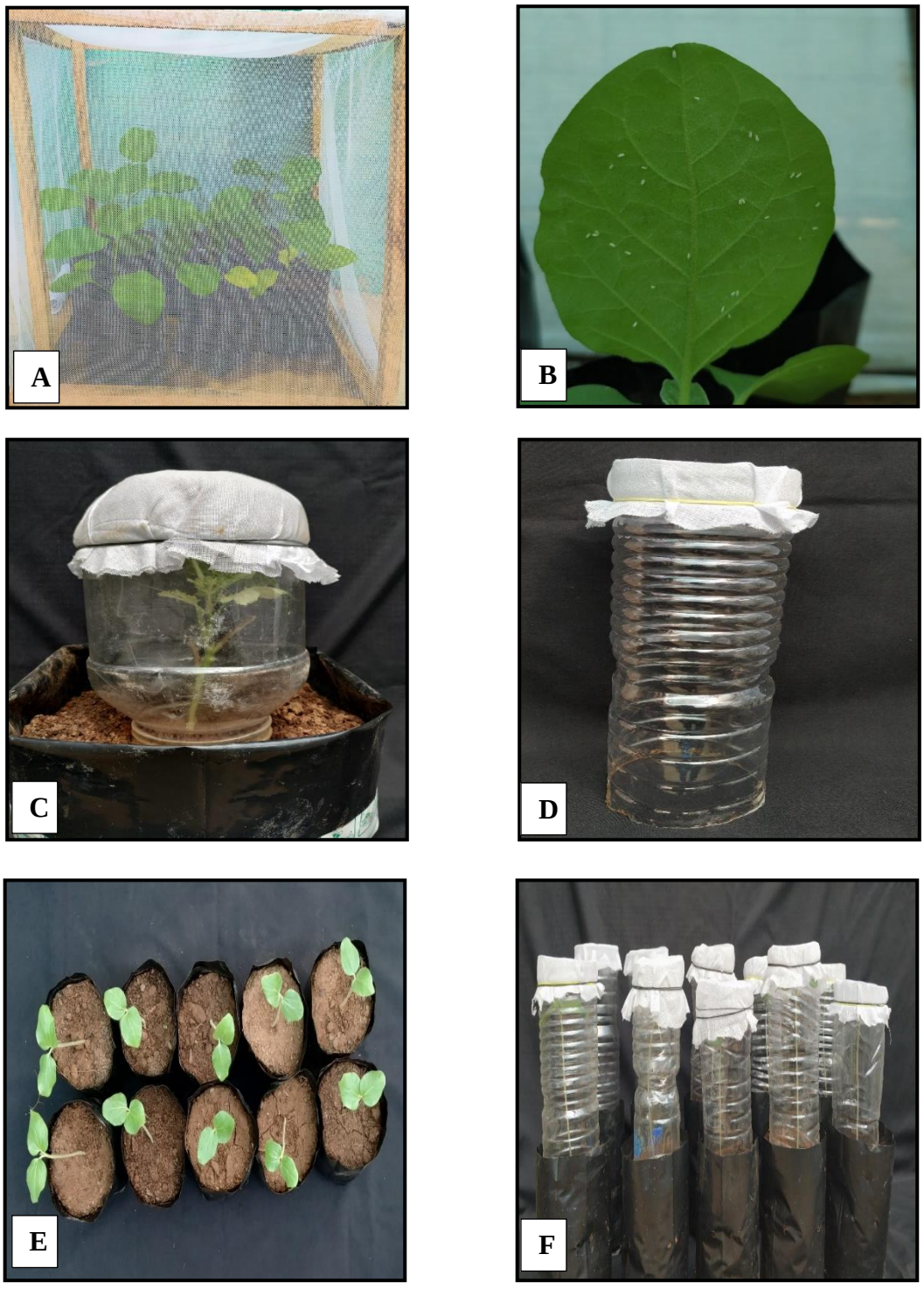


Plate 3.2: Materials used for vector transmission: A-whitefly rearing cage, B- whitefly population on the under surface of brinjal leaves, C- cage for acquisition of virus, D- cage for inoculation of virus, E- ten days old bhindi seedlings, F- seedlings inoculated with viruliferous whiteflies

3.5.1.3.4. Acquisition and inoculation of virus on bhindi seedlings

Healthy whiteflies were collected from the brinjal plants maintained in insect proof cages and allowed to starve for 2 h. These were then released on to infected bhindi plants inside the acquisition cage and allowed to feed for 24 h so as to acquire the virus. Ten viruliferous whiteflies were released to 10 days old test seedlings of bhindi and kept inside the inoculation cage for 24 h (Plate 3.2.). After inoculation access period, whiteflies were killed by spraying imidacloprid (0.03 %). Plants without inoculation served as control. These seedlings were kept inside the insect proof cage and observed for the development of symptoms. The presence of virus inside whiteflies were also carried out as described in section 3.6.4.

3.5.2. Host range studies

The host range of the virus was studied by vector transmission of the virus to plant species belonging to different families which included crops and weeds seen in and around the vegetable fields (Table 3.2.). The plants were raised in polybags containing soil and vermicompost in 1:1 ratio. Virus infected bhindi plants maintained in insect proof net house were used as source of inoculum. Five plants of each species were inoculated with the virus through viruliferous whiteflies as described in section 3.5.1.3.4 and monitored for symptom expression. Uninoculated plants were also maintained which served as control.

The plants showing symptoms after artificial inoculation were brought to the laboratory. DNA isolation and PCR amplification was carried out using primer specific to the core CP gene of begomoviruses (AV494/AC1048) in order to confirm the virus infection as mentioned in section 3.6.1 and 3.6.2

Weeds seen around the vegetable fields showing viral symptoms like mosaic and vein clearing were also collected and brought to the laboratory to study whether such weeds are infected with begomoviruses (Table 3.3.). Healthy plants of the same weed species was also collected. Total genomic DNA was isolated from the symptomatic leaves and apparently healthy leaves using the protocol described

in section 3.6.1. To confirm the presence or absence of virus, PCR amplification was carried out using universal *Begomovirus* primer (AV494/AC1048) specific to the core CP gene of as mentioned in section 3.6.2. and the PCR products were sequenced to identify the virus associated with the weed species.

Table 3.2: List of plants used for host range studies

Sl. No.	Type of plants	Host	Scientific name	Family
1	Crops	Red amaranthus	*Amaranthus polygamous*	Amaranthaceae
2		Cucumber	*Cucumis sativus*	Cucurbitaceae
3		Pumpkin	*Cucurbita moschata*	
4		Tomato	*Solanum lycopersicum*	Solanaceae
5		Chilli	*Capsicum annuum*	
6		Brinjal	*Solanum melongena*	
7		Cowpea	*Vigna unguiculata*	Fabaceae
8	Weeds	Cinedrella weed	*Synedrella nodiflora*	Asteraceae
9		Goat weed	*Ageratum conyzoides*	
10		Joyweed	*Alternanthera ficoidea*	Amaranthaceae
11		Wireweed	*Sida acuta*	Malvaceae

Table 3.3: List of naturally infected weed plants with symptoms collected from fields

Sl. No.	Host	Scientific name	Family
1	Common wire weed	*Sida acuta*	Malvaceae
2	Node weed	*Synedrella nodiflora*	Asteraceae
3	Lilac tassel flower	*Emilia sonchifolia*	
4	Goat weed	*Ageratum conyzoides*	
5	Little iron weed	*Cyanthilium cinereum*	
6	Indian Sarasaparilla	*Hemidesmus indicus*	Apocynaceae
7	Indian stinging nettle	*Tragia involucrate*	Euphorbiaceae
8	Asthma herb	*Euphorbia hirta*	
9	Water primerose	*Ludwigia hyssopifolia*	onagraceae

3.6. MOLECULAR DETECTION AND CHARACTERIZATION OF VIRUS

The molecular detection of the virus was carried out in the Molecular laboratory, Dept. of Plant Pathology, College of Horticulture, Vellanikkara. The leaves showing typical yellow vein symptoms were collected from eight different bhindi fields of Thrissur district and one each from Thiruvananthapuram (Vellayani), Kottayam (Kumarakom), Kannur (Panniyur) and Kasaragod (Padannakkad). The collected leaves were brought to the laboratory and stored at -20 ^{o}C for further studies.

3.6.1. Isolation of total genomic DNA

Total genomic DNA was extracted from the leaf samples collected during survey using CTAB method (Doyle and Doyle, 1987) with slight modification. The quantity of CTAB buffer during grinding was increased from one ml to three ml and the quantity of leaf sample was reduced to 50 mg.

3.6.1.1. Protocol for DNA isolation

1. 50 mg of the fresh leaf samples stored at -20 °C were ground with a pinch of polyvinylpyrrolidone and 50 µl of β-mercaptoethanol (2 %) in a pre-heated sterile mortar and pestle.
2. 3 ml of pre-warmed (at 65 °C for 20-30 min. in hot water bath) extraction buffer was added into ground samples and again ground.
3. The whole content taken in 2 ml eppendorf tube were incubated at 65 °C in hot water bath for 30 min. with occasional gentle swirling.
4. After incubation, equal volume of chloroform:isoamyl alcohol (24:1 v/v) was added to the tube, mixed by gentle inversion and centrifuged at 12000 rpm for 20 min. at room temperature.
5. The top aqueous layer was transferred into fresh 1.5 ml effendorf tube, equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed by gentle inversion and again centrifuged at 12000 rpm for 20 min.
6. The aqueous phase was transferred into new centrifuge tube and added 0.6 volume of chilled isopropanol and mixed by inversion till the DNA got

precipitated. The tubes were incubated overnight at -20 °C for the complete precipitation of the DNA.

7. Next day, the tubes were centrifuged at 12000 rpm for 15 min at 4 °C and the supernatant was gently poured off.
8. The DNA pellet was washed twice with 70 per cent and 100 per cent ethyl alcohol and centrifuged at 10000 rpm for 5 min at 4 °C.
9. Excess alcohol was poured off and pellets were air dried at room temperature.
10. Finally pellets were suspended in 50 µl sterile distilled water and stored at -20 °C in eppendorf tubes.

3.6.1.2. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out using 0.8 per cent agarose gel in electrophoresis unit (Tarson) to check the quality of the genomic DNA. An aliquot of DNA sample (5 µl) mixed with 2 µl of gel loading dye was loaded in each of the wells. The electrophoresis was carried out at 85 V till the dye moved two third length of the gel. The gel was then visualized and the image was documented using BIORAD Molecular Imager (Gel Doc^{TM} XR+).

3.6.1.3. Quantification and purity analysis of genomic DNA

The quality and quantity of the isolated DNA samples were measured using NanoDrop 1000 spectrophotometer (Thermoscientific, USA). The spectrophotometer was calibrated to blank (zero absorbance) with 1 µl of nuclease free water. Then the concentration of DNA samples and the absorbance at 260 nm and 280 nm wavelength were recorded. The absorbance was recorded at 260 and 280 nm wavelength and purity was indicated by the ratio $A_{260/280.}$

3.6.2. Molecular detection of virus associated with infected plants

Polymerase chain reaction (PCR) was used to detect the virus associated with yellow vein mosaic symptom in bhindi. Four sets of already reported primers were used for the PCR assay and are mentioned in Table 3.4.

3.6.2.1. Standardization of annealing temperature

The optimum annealing temperature for PCR reaction was standardized through gradient PCR in Eppendorf master cycler. The range of temperatures used for each set of primer are given in Table 3.5. The temperature at which good amplicons obtained was selected as the optimum annealing temperature for each primer set.

3.6.2.2. Protocol

1. Autoclaved PCR tubes of 0.2 ml volume were taken, labeled and kept on ice crystals in ice box
2. Master mixture was prepared by adding all reagents except the template DNA. The composition of master mix for each primer is given in Table 3.6.
3. Required quantity of DNA template as given in Table 3.6. was separately added into the PCR tubes and then the master mix was pipetted out into each PCR tubes. The contents of the tubes were given a short spin.
4. The tubes were kept in the wells of the thermocycler (eppendorf Mastercycler gradient). Then the PCR was run with the specific programs. The details of the PCR thermal profile adopted is mentioned in Table 3.7.
5. After completion of the PCR program, the tubes were taken out from the thermocycler and kept at 4 °C for further analysis of the PCR product.

Table 3.4: Details of reported primers used

Sl. No.	Primer	Sequence 5'-3'	Amplified gene	PCR product size (bp)	Reference
1	AV494 - FP	GCCYATRTAYAGRAAGCCMAG	Core coat protein gene of *Begomovirus*	550	Wyatt and Brown (1996)
	AC1048 - RP	GGRTTDGARGCATGHGTACATG			
2	OE34F	AAGAATTATGTCGAAGCGTCCTGCTT	Complete coat protein gene of OELCuV	770	Kumar *et al*. (2019)
	OE35R	AAGAATCGTAGAAGTAACTCCTAACTT			
3	OYVMV - FP	ATGTCGAAGCGAGCTGCCG	Complete coat protein gene of OYVMV	800	Senevirathna *et al.* (2016)
	OYVMV - RP	TCAATTCGTTACAGAGTCATAAA			
4	BYVMV - FP	AAGCTTATGTCGAAGCGAGCTGCCG	Complete coat protein gene of BYVMV	770	Jose and Usha (2000)
	BYVMV - RP	TCAATTCGTTACAGAGTC			

Table 3.5: Range of annealing temperature tested

Sl. No.	Primer	Temperature range (°C)
1	AV494/AC1048	54.7-57.7
2	BYVMV FP/RP	52.0-58.0
3	OYVMV FP/RP	47.9-56.1
4	OE34F/OE35R	52.2-58.0

Table 3.6: Composition of PCR reaction mix used for each primer set

Sl. No.	Components	Volume in µl/tube			
		AV494/ AC1048	OELCuV	BYVMV	OYVMV
1	Sterile distilled water	14.5	11.3	9.3	11
2	Taq buffer (10x)	2.5	2	2	2
3	$MgCl_2$ (25mM)	1.5	1.2	1.2	1.5
4	dNTP (2mM)	2	2	2	2
5	Primers Forward(5pM) Reverse (5pM)	 1 1	 1 1	 2 2	 1 1
6	DNA template	2	1	1	1
7	Taq DNA polymerase(3U/µl)	0.5	0.5	0.5	0.5
	Total	**25**	**20**	**20**	**20**

Table 3.7: Program for PCR reaction of reported primers

Process	Primer							
	AV494/ AC1048		OELCuV		BYVMV		OYVMV	
	Temp (°C)	Time (min.)	Temp (°C)	Time (min.)	Temp (°C)	Time (min.)	Temp (°C)	Time (min.)
Initial denaturation	95	5	94	4	94	4	95	5
Denaturation	95	1	94	1	94	1	95	1
Annealing	*		*		*		*	
Extension	72	1	72	1	70	1	72	1
No. of cycles	35 cycles		35 cycles		30 cycles		39 cycles	
Final extension	72	10	72	10	70	10	72	7
Hold	4		4		4		4	

* Annealing temperature standardized as described under section 3.6.2.1 using gradient PCR

3.6.3. Molecular detection of seed transmission

The seedlings raised from seeds of infected pods were tested for the presence of virus using PCR amplification. The DNA was isolated from the seedlings and PCR amplification was done using universal begomovirus primer (AV494/AC1048). The DNA isolated from symptomatic plants maintained through artificial inoculation was used as a positive control.

3.6.4. Molecular detection of virus in *Bemisia tabaci*

For the molecular detection of virus in viruliferous whiteflies, adults were collected from the cage and released to yellow vein mosaic infected bhindi plant for 24 h. Then the DNA was extracted from viruliferous as well as non viruliferous whiteflies using Qiagen blood and tissue kit following manufacturer's protocol. The core coat protein gene of the virus was amplified using a universal *Begomovirus* primer (AV494/AC1048). The DNA isolated from symptomatic plants maintained through artificial inoculation was used as a positive control.

3.6.5. Analysis of PCR amplicons by agarose gel electrophoresis

10 µl of each amplicon were resolved in 1.5 per cent agarose gel prepared in 1X TAE buffer containing ethidium bromide. Electrophoresis was performed at 85V for one hour using 1 X TAE buffer as electrophoresis buffer and the amplification was viewed under UV light in the gel documentation unit (BIORAD Molecular Imager (Gel Doc^{TM} XR+)).

3.6.6. Sequencing

The amplified PCR products of 11 isolates obtained using universal primer for begomovirus (AV494/AC1048), three isolates obtained using primer specific to BYVMV and one isolate obtained using OELCuV were sequenced. The sequencing of the PCR products was done at AgriGenome Labs Pvt. Ltd., Kochi, Kerala, India.

3.6.7. Sequence deposition

Nucleotide sequences of the 11 local isolates were deposited in the NCBI database through NCBI Bankit program.

3.6.8. *In silico* analysis

The sequences obtained were compared with the accessions available in the National Centre of Biotechnology Information (NCBI) database using BLAST tool. Nucleotide BLAST (BLASTn) was carried out for the viral sequences to find out the best aligned sequences available in NCBI (http://ncbi.nlm.n hm.gov./blast.cgi). The amino acid sequences were deduced using ExPASy translate tool. The amino acid sequences were subjected to protein blast in order to assess the identity percentage with reported sequences of begomovirus isolates available on NCBI database.

3.6.9. Sequence comparison using species demarcation tool (SDT)

The nucleotide sequences of collected isolates were compared with the top hits downloaded from NCBI database using the species demarcation tool (SDT).

The pairwise nucleotide sequence comparison was carried out using Muscle algorithm available in SDT (Muhire *et al*., 2014).

3.6.10. DNA barcoding

Multiple sequence alignement of the sequences of local isolates and sequences of BYVMV and OELCuV isolates retrieved from NCBI database was employed using ClustalW tool provided by MEGA-X software. The aligned sequences of CP gene were manually analyzed for the presence of barcode gaps *i.e.,* those regions with absence of '*' symbol was considered as the barcode gaps. The region with identical nucleotides within the species and differ between species were identified as barcodes specific to BYVMV and OELCuV.

3.6.11. Phylogenetic analysis

The nucleotide sequences of the virus isolates were aligned with top hit sequences specific to the coat protein gene of other begomoviruses of infecting bhindi from different geographical regions in India and elsewhere retrieved from NCBI database (Table 3.8.). The alignment of the sequences was done using ClustalW program available in MEGA X software. The phylogenetic tree of the aligned sequences was constructed in MEGA X software by using Neighbor-joining algorithm with bootstrap value of 1000 (Saitou and Nei, 1987).

3.7. MANAGEMENT OF BHINDI YELLOW VEIN MOSAIC DISEASE

A field trial was undertaken to develop a recommendation for the management of bhindi yellow vein mosaic disease. The effect of insect proof net and trap, different chemicals, plant extracts, micronutrients, and microbial formulations against yellow vein mosaic disease in bhindi was evaluated under field conditions. The experiment was conducted in the field of Department of Plant

Table 3.8: Details of begomovirus isolates used for phylogenetic analysis

Sl. No.	Name of the virus	Virus isolate	Accession No.
1	*Bhindi yellow vein mosaic virus* (BYVMV)	Bhindi yellow vein mosaic virus isolate BhYVMV-CKTD	KY083753.1
2		Bhindi yellow vein mosaic virus isolate Barrackpore	EF417918.1
3		Bhindi yellow vein mosaic virus [India:Coimbator:OYCO4:2005]	GU112080.1
4		Bhindi yellow vein mosaic virus [India:Bangalore:OY34:2005]	GU112064.1
5		Bhindi yellow vein mosaic virus [India:Phalaghat:OY07:2005]	GU112062.1
6	*Okra enation leaf curl virus* (OELCuV)	Okra enation leaf curl virus isolate Hyderabad	KT898975.1
7		Okra enation leaf curl virus isolate Hyderabad	MH052569.1
8		Okra enation leaf curl virus isolate OK92-HR	KT390310.1
9		Okra enation leaf curl virus isolate Vijayawada	KT935487.1
10		Okra enation leaf curl virus isolate India:Ludhiana	KP208672.1
11		Okra enation leaf curl virus isolate Surat	KC342220.1
12		Okra enation leaf curl virus clone 6.2, Maharashtra	MK084768.1
13		Okra enation leaf curl virus clone SZ157 Pakistan	KX710156.1
14	*Mesta yellow vein mosaic virus* (MeYVMV)	Mesta yellow vein mosaic virus isolate Var1	KJ462083.1
15		Mesta yellow vein mosaic virus isolate Jalg1	KJ462074.1
16		Mesta yellow vein mosaic virus segment A, clone 10c-RCAAI-F	FN645922.1
17		Mesta yellow vein mosaic virus isolate Okra:Ludhiana	JX181786.1
18		Mesta yellow vein mosaic virus isolate Okra:Tirupati	JX242520.1
19	*Cotton leaf curl virus* (CLCV)	Cotton leaf curl Multan virus	NC004607.1
20		Cotton leaf curl Alabad virus	NC004582.1

Pathology from March 2019 to June 2019. The susceptible variety Salkeerthi was selected to test the efficacy of different treatments in this experiment (Fig. 3.2). The experimental details were as follows:

Design	: Randomized block design
No. of treatments	: Seven
No. of replications	: Three
Plot size	: 1.5 X 3.0 m^2
Variety	: Salkeerthi
No. of plants / treatment	: 54

Land preparation was done by ploughing and pulverizing the soil. The field again ploughed after the addition of lime and cowdung and was laid out as 21 plots of 4.5 m^2 size. A plant to plant spacing of 30 cm and row to row spacing of 60 cm was maintained in each plot. Bhindi seeds soaked in water for 24 h were sown in the field. The method of application of treatments were seed treatment and foliar spray. The treatment details of the field experiment are given in Table 3.9.

The observations on different types of symptoms, per cent disease incidence (PDI), per cent disease severity (PDS) and whitefly count were taken at ten days intervals. Per cent disease incidence (PDI) was recorded by counting the number of plants infected out of total number of plants in each plot. PDS was calculated based on 0-5 scale disease grading chart developed by Ali *et al*. (2005a) as mentioned in section 3.1. Whitefly count was taken from each plot which was randomly done by visual counting of the number of whiteflies on underside of the upper five leaves. The vegetative characters such as plant height at 30, 45, 60 days after sowing (DAS) and at last harvest, number of days taken for first flowering, number of days taken for 50 per cent flowering were also recorded from the field. Yield parameters such as total yield from each plot (kgm^{-2}) and number of fruits per plant were recorded.

Fig. 3.2 (A) and (B) General view of exprerimental field

3.7.2 Statistical analysis

The statistical analysis *i.e.*, analysis of variance (ANOVA) was performed for the data obtained from the field experiment using Web Agri Stat Page (WASP 2.0).

Table 3.9: Details of field treatments

Treatment no.	Treatment details
T1	Biopriming of seeds with PGPR mix II (2 %) + protecting the seedlings with 40 mesh net upto 20 days + foliar spray of micronutrient formulation Sampoorna (0.5 %) – 3 times at 30, 45, and 60 DAS
T2	Biopriming of seeds with PGPR mix II (2 %) + Alternate foliar spray of PGPR mix II (2 %) and *Bougainvillea spectabilis* (10 % aqueous leaf extract) 4 sprays at 15-20 days interval
T3	Foliar spray of neem based insecticide (1 %) thrice at 20 days interval
T4	Hanging yellow sticky traps + foliar spray of *Lecanicillium lecanii* (2 %) 4 sprays at 20 days interval
T5	IDM: Biopriming of seeds with PGPR mix II (2 %) + protecting the seedlings with 40 mesh net upto 20 days + hanging yellow sticky traps + foliar spray of PGPR mix II (2 %), *Bougainvillea spectabilis* (10 % aqueous leaf extract) + foliar spray of neem based insecticide (azadiractin 300 ppm)
T6	Seed treatment and foliar spray with insecticide imidacloprid (3 ml/10 L) twice at monthly intervals
T7	Control

Results

4. RESULTS

The results of the project on "Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease" carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2017-2019 are presented in this chapter.

4.1. SURVEY

Purposive sampling survey was carried out during the period from November 2018 to June 2019 in 11 locations of Thrissur district *viz.*, Vellanikkara, Chengaloor, Viyyur, Chalakkudy, Attoor and Muthuvara. During the survey, various parameters like growth stage of the crop, total number of plants, number of plants infected and scoring of the infected plants were recorded and these data was used to assess, per cent disease incidence (PDI) and per cent disease severity (PDS). The results are given in Table 4.1. The distinct symptoms observed on different parts of infected plant were also documented from each location. Symptomatic leaves were collected in cool box from infected plants from all locations and brought to the laboratory for further analysis. Apart from the surveyed locations, symptomatic samples were also collected from infected fields of other districts like Thiruvananthapuram, Kottayam, Kannur and Kasaragod.

4.1.1. Assessment of disease incidence and disease severity

During the survey, it was observed that the disease incidence ranged from 61.20 to 98.16 per cent. Among the various fields surveyed, the highest disease incidence was recorded in the fields of Vellanikkara *viz.*, field 1, 2 and 3 with a PDI of 93.55, 98.16 and 94.00 per cent respectively. This was followed by Attoor (91.66 %) and Chalakkudi (89.33 %). The PDI of 81.11 and 84.00 per cent was recorded from Viyyur field 1 and Viyyur field 2 respectively. The lowest PDI of 61.20 per cent was recorded in Chengaloor.

Table 4.1: Incidence and severity of bhindi yellow vein mosaic disease in different locations of Thrissur district

Sl. No.	Location	Month of survey	Variety	Crop growth stage	PDI*	PDS**
1	Vellanikkara field 1	April 2019	Salkeerthi	Flowering and fruiting	93.55	90.00
2	Vellanikkara field 2	August 2018	Arka Anamika	Flowering and fruiting	98.16	89.00
3	Vellanikkara field 3	June 2019	Arka Anamika	Flowering and fruiting	65.00	50.00
4	Vellanikkara field 4	June 2019	Aruna	Flowering	94.00	69.00
5	Chengaloor	October 2018	Local	Flowering	61.20	48.00
6	Viyyur field 1	February 2019	Arka Anamika	Flowering and fruiting	81.11	68.00
7	Viyyur field 2	February 2019	Local	Flowering and fruiting	84.00	86.00
8	Chalakkudi	June 2019	Arka Anamika	Flowering	89.33	56.00
9	Muthuvara	June 2019	Aruna	Flowering	80.00	52.00
10	Attoor field 1	May 2019	Local	Flowering and fruiting	91.66	74.00
11	Attoor field 2	May 2019	Local	Flowering and fruiting	78.25	79.00

*PDI- Per cent disease incidence

**PDS- Per cent disease severity

The per cent disease severity was expressed by calculating the vulnerability index which was worked out in accordance to the disease grading chart with 0-5 scale developed by Ali *et al.* (2005a) as mentioned in section 3.1. PDS in different fields varied from 48.00 to 90.00 per cent. Among the fields, the highest PDS was recorded in Vellanikkara field 1 (90 %) and Vellanikkara field 2 (89 %). This was followed by Viyyur field 2 (86 %), Attoor field 2 (79 %) and Attoor field 1 (74 %). The lowest PDS was recorded in Chengaloor (48 %).

Among the different varieties cultivated by the farmers, the variety Arka Anamika, once released as a resistant variety from Indian Institute of Horticultural Research (IIHR), Bangalore showed highest PDI (98.16 %) followed by Salkeerthi (93.55 %). In the case of PDS, maximum was recorded in Salkeerthi variety (90 %) followed by Arka Anamika (89 %).

4.2. MAINTENANCE OF VIRUS CULTURE

The virus culture was maintained through grafting of infected scion on healthy rootstock. The scions were collected from the infected plant during the survey and brought to the laboratory. This was carefully grafted on one month old healthy bhindi plants maintained in insect proof plant virology glass house of Department of Plant Pathology through cleft grafting. The grafted plants produced with symptoms on newly emerged leaves of rootstock and this was used as a source of virus inoculum for further studies. This was maintained by repeated transmission through grafting and also through insect vector, whiteflies.

4.3. SYMPTOMATOLOGY

The symptoms of the disease expressed on different parts of the plant *viz*., leaf lamina, veins, veinlets, petiole, flower buds and fruits under natural conditions were documented during the survey. The symptoms produced under artificial conditions were also studied by inoculating the plants through graft transmission and vector transmission. The internal symptoms produced in the plant due to virus infection was documented by studying the histopathology of infected leaves as compared to the healthy plants.

4.3.1. Symptoms under natural conditions

The predominant symptoms recorded under natural conditions were yellowing of the veins and veinlets, interveinal green islands, upward curling and discolouration of leaves, wavy margins of leaf lamina, leaf enation, clustering of flower buds with linear chlorotic striations on the calyx and stunting of plant. In case of fruits, linear chlorotic striations on fruit surface, reduction in size and malformation were observed.

4.3.1.1. Symptoms on leaves

The most prominent symptom on leaf lamina was yellowing of veins and veinlets. The infected leaves showed typical vein clearing symptom with interveinal green islands. The network of the veins and veinlets also showed thickening and small pin head like enations were visible on veins of abaxial leaf surface as compared to the healthy leaves (Plate 4.1a.). The different stages of development of yellow vein symptom were also documented (Plate 4.1a). This kind of symptom were more prominent on middle leaves compared to younger leaves. When these symptoms intensify, the leaves turned completely chlorotic and yellow coloured. Another type of symptom observed was puckering. In this case, interveinal area of the leaf lamina consisted of raised area with bulging of tissues (Plate 4.1b.). The margins of the infected leaf lamina showed wavy appearance (Plate 4.1b.). The under surface of the infected leaf showed vein thickening and produced small pin head like enation (Plate 4.1b.). In some cases, the leaf margins showed wavy appearance. Upward curling of the leaves was also observed in the field (Plate 4.1c.). The leaves were rough and brittle in texture. In case of severe infection, the entire leaf lamina became light yellow or cream in colour and necrosis of the leaves were also observed (Plate 4.1c.).

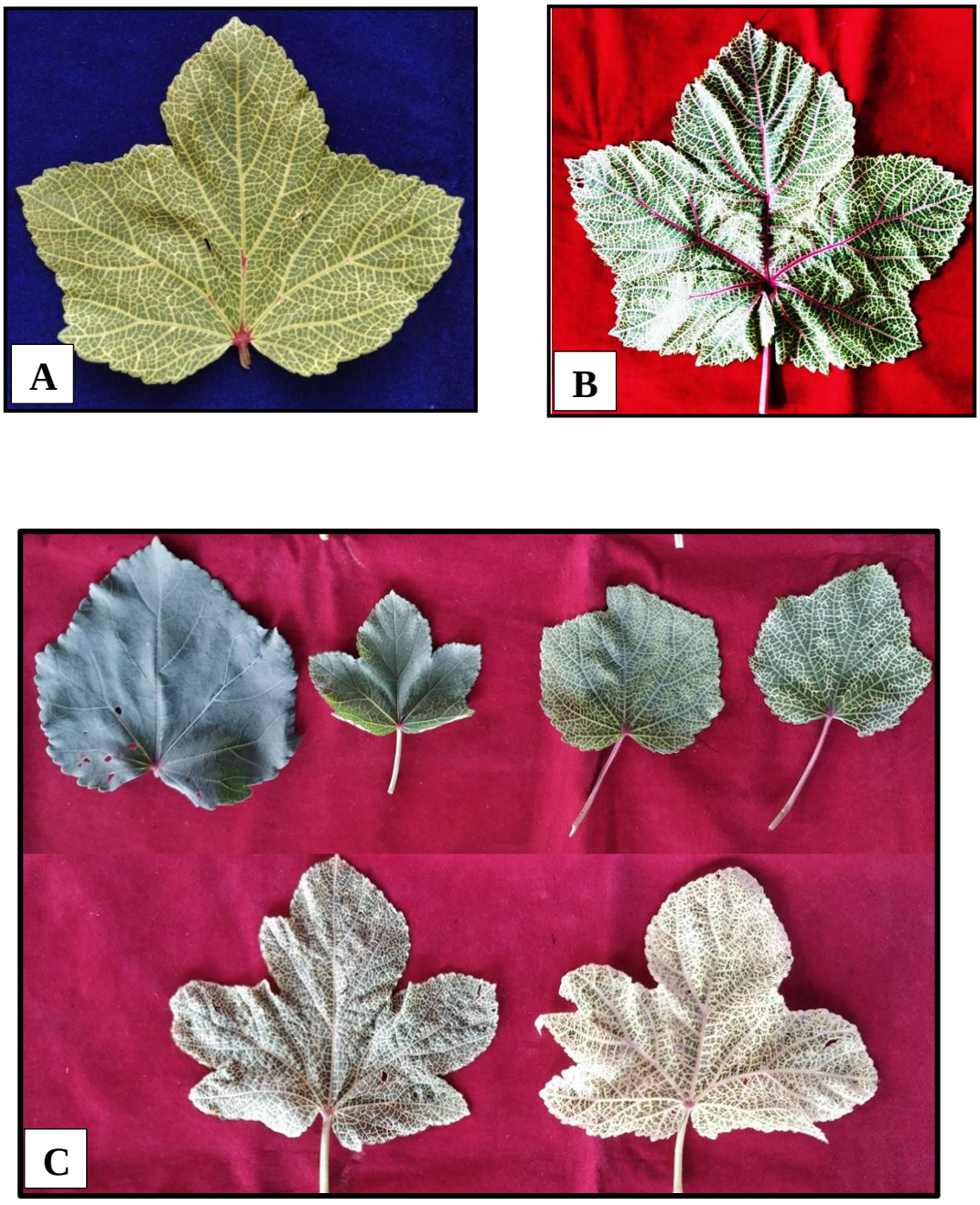


Plate 4.1a: Symptoms under natural conditions: (A) Vein clearing in var. Arka Anamika, (B) Vein clearing in var. Aruna, (C) Stages of development of vein clearing symptom

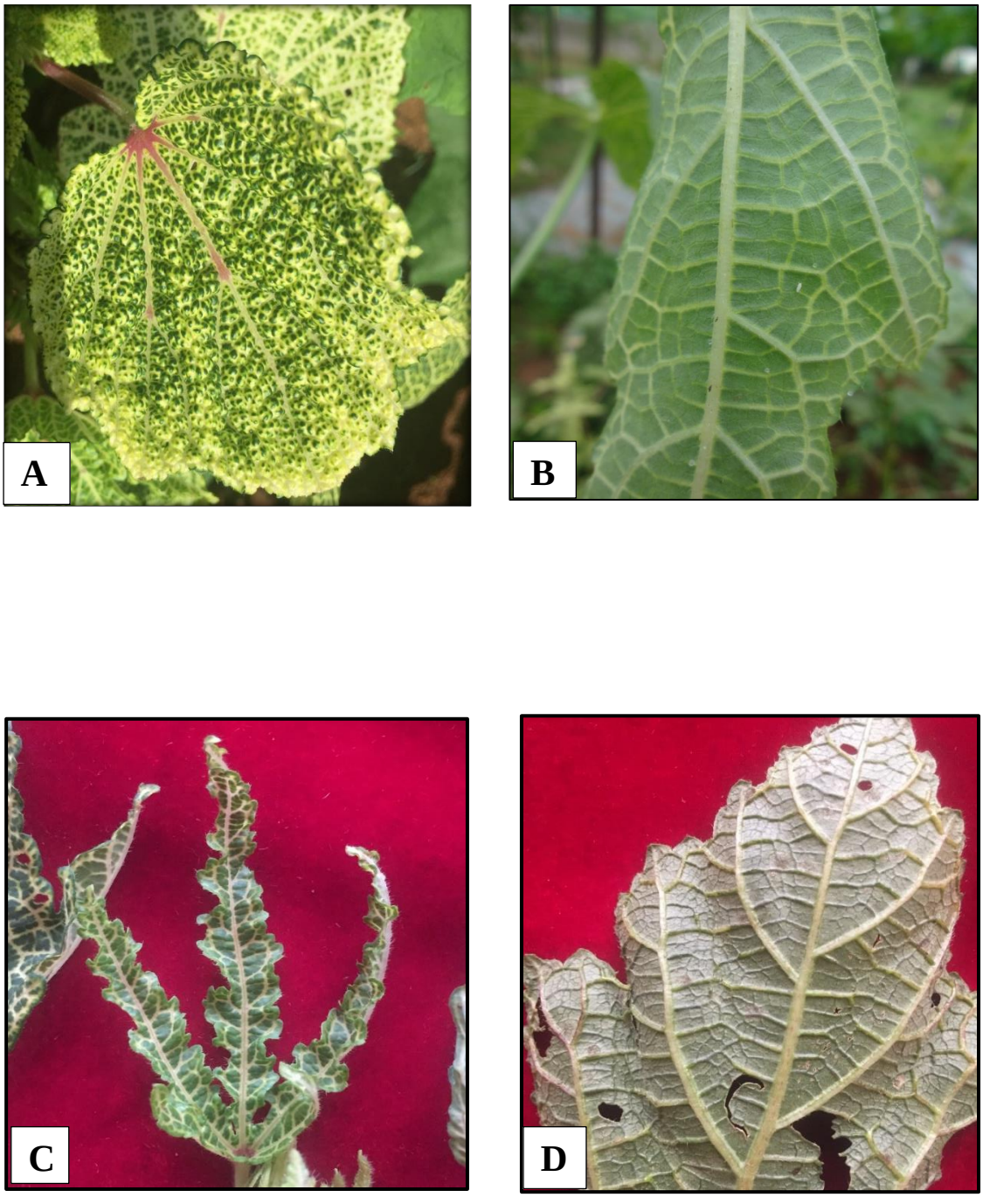


Plate 4.1b: Symptoms under natural conditions: (A) Puckering (B) Vein thickening on the lower surface of leaf (C) Wavy appearance of leaf margin, (D) pin head enations on lower surface of leaf

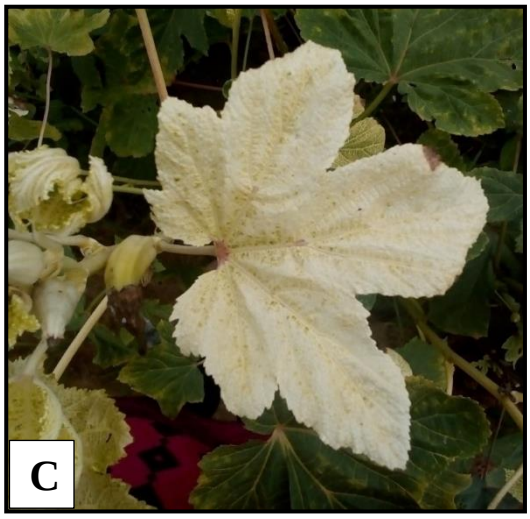


Plate 4.1c: Symptoms under natural conditions: (A) Reduction in leaf area, (B) upward curling of leaf lamina, (C) Bleached appearance of leaf lamina, (D) Marginal necrosis of leaf lamina

4.3.1.2. Impact of disease on leaf area

The area of leaf was drastically reduced due to infection (Plate 4.1c). the extent of leaf area reduction in three different varieties *viz.*, Arka Anamika, Salkeerthi and local variety due to infection was assessed. The per cent of reduction of leaf lamina compared to healthy leaves ranged from 71.16 to 76.18 per cent (Table 4.2.).

Table 4.2: Reduction of leaf area due to infection

Sl. No.	Variety	Leaf area (cm^2)		Percent reduction of leaf area (%)
		Healthy leaf	Infected leaf	
1	Arka Anamika	105.80	45.20	76.18
2	Salkeerthy	147.60	38.40	73.98
3	Local	118.60	54.20	71.16

Other than these, a different kind of symptom was observed in two surveyed fields *i.e.*, Vellanikkara and Viyyur. The leaves showed reduction in size with severe puckering on both sodes of the veins. The leaf lamina also showed upward curling, reduction in size, yellowing and marginal necrosis. Petiole bending and stunting was also observed in such plants (Plate 4.1d.). These symptoms were not reported earlier.

4.3.1.3. Symptoms on flowers and fruits

The flower buds were clustered in case of severely infected plants and the calyx of the infected flower bud showed linear chlorotic striations (Plate 4.1e.). The affected fruits initially showed linear chlorotic striations and in the advanced stage, these fruits appeared bleached in appearance. The infected fruits also showed malformation and reduction in size (Plate 4.1f).

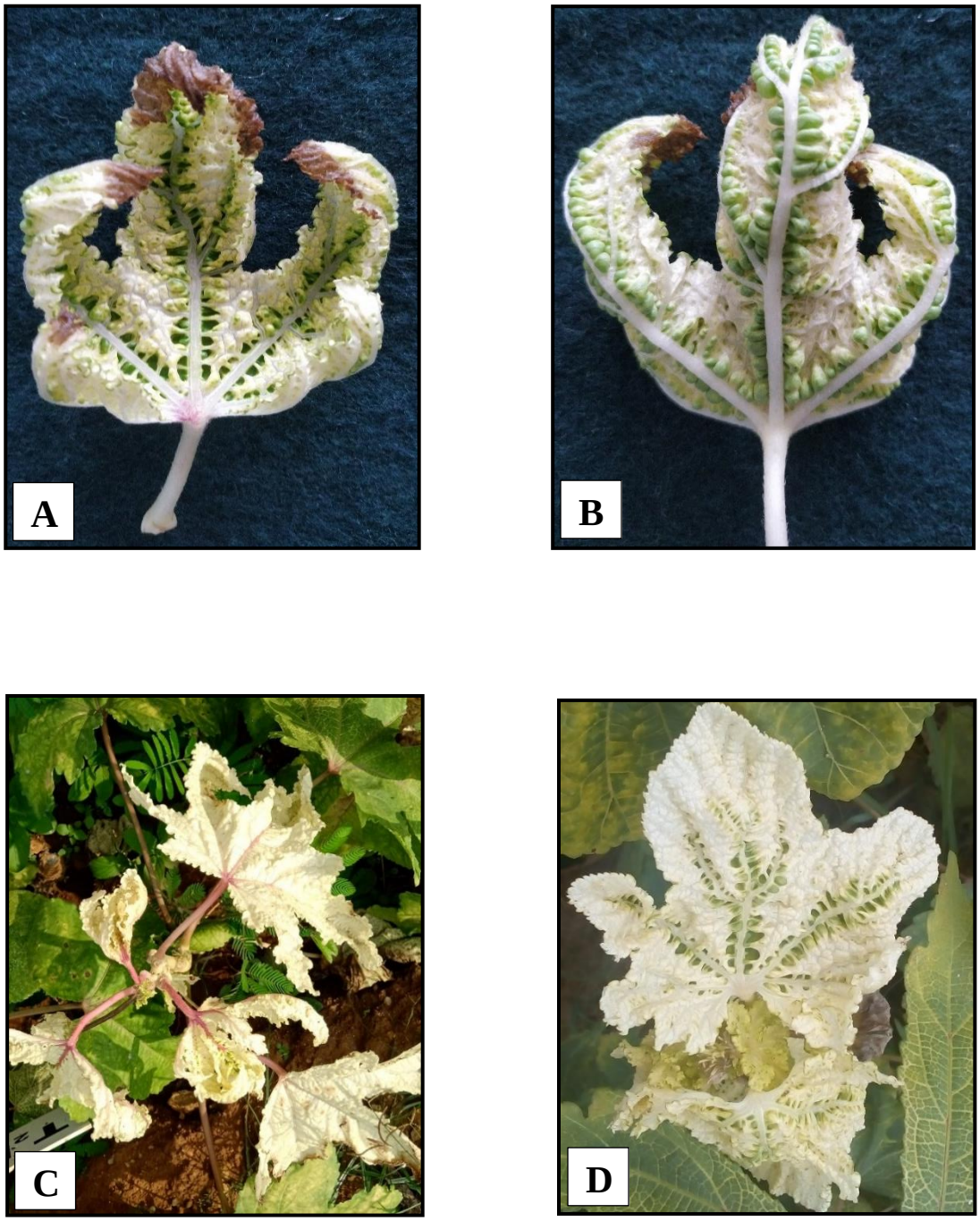


Plate 4.1d: Symptoms under natural conditions: (A) upper surface of infected leaf with extreme puckering (B) lower surface of infected leaf with extreme puckering (C) Petiole bending (D) stunting of plant

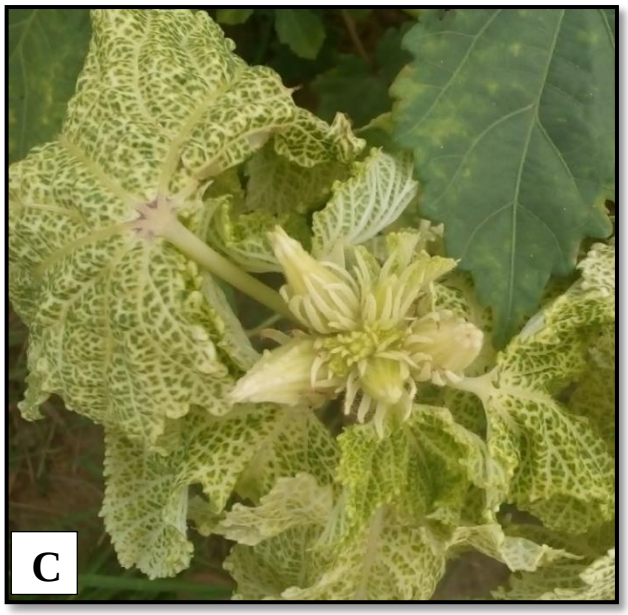


Plate 4.1e: Symptoms under natural conditions: On flower buds - (A) Healthy flower buds, (B) Linear chlorotic striations on infected flower buds, (C) Clustering of flower buds in infected plant

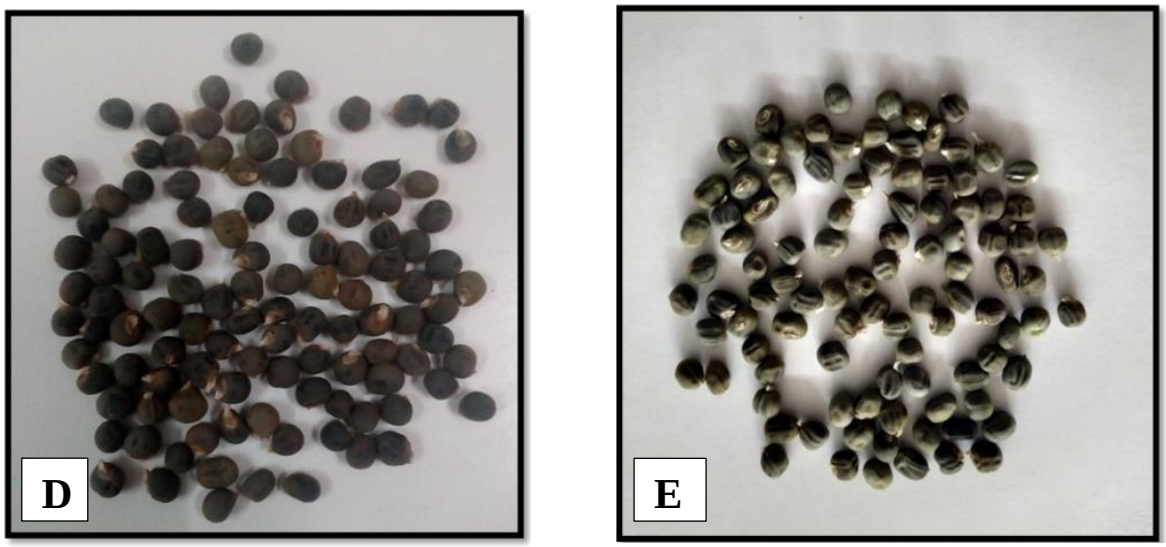


Plate 4.1f: Symptoms under natural conditions: on fruits- (A) Healthy fruits, (B) Linear chlorotic striations on infected fruits, (C) Bleached appearance of infected fruits, On seeds- (D) Seeds from healthy plant, (E) Seeds from infected plant

4.3.1.4. Symptoms on seeds

The seeds collected from infected pods were shrunken and deformed compared to healthy pods (Plate 4.1f.). There was also reduction in the number of seeds in the infected pods when compared with healthy one.

The whole plant showed stunted appearance due to the infection in the early growth stage (Plate 4.1g.).

4.3.2. Symptoms under artificial condition

The development of symptoms were also documented by artificially inoculation of the virus into healthy plants through vector and graft transmission. In case of vector transmission, the symptoms were expressed 12-15 DAI. Initially, the leaves showed mild puckering like symptom (Plate 4.2). Later, yellowing of veinlets started from margin and gradually spread to all veins and veinlets of the leaf. The veins also showed thickening like symptom which was more visible on the under surface of the leaves.

In case of graft transmission, the development of symptoms on the newly emerging leaves from the rootstock was monitored daily. The symptom expression started 9-14 days after grafting. Initially the veinlets showed yellowing followed by thickening of vein and veinlets on the whole leaf lamina. The newly emerged leaves also showed puckering, yellowing and vein thickening symptoms (Plate 4.2).

4.4. HISTOPATHOLOGICAL CHANGES

In order to study the histopathological or anatomical changes due to infection, thin sections of the leaves were taken with a sharp razor and observed under microscope. The histopathological changes due to the infection on different leaf tissues were analysed. Thin sections of healthy leaves was also taken and compared with those taken from infected leaves. The results are given in Table 4.3.

It was observed that anatomical changes was brought about due to infection especially in various tissues like parenchyma, palisade and spongy mesophyll cells

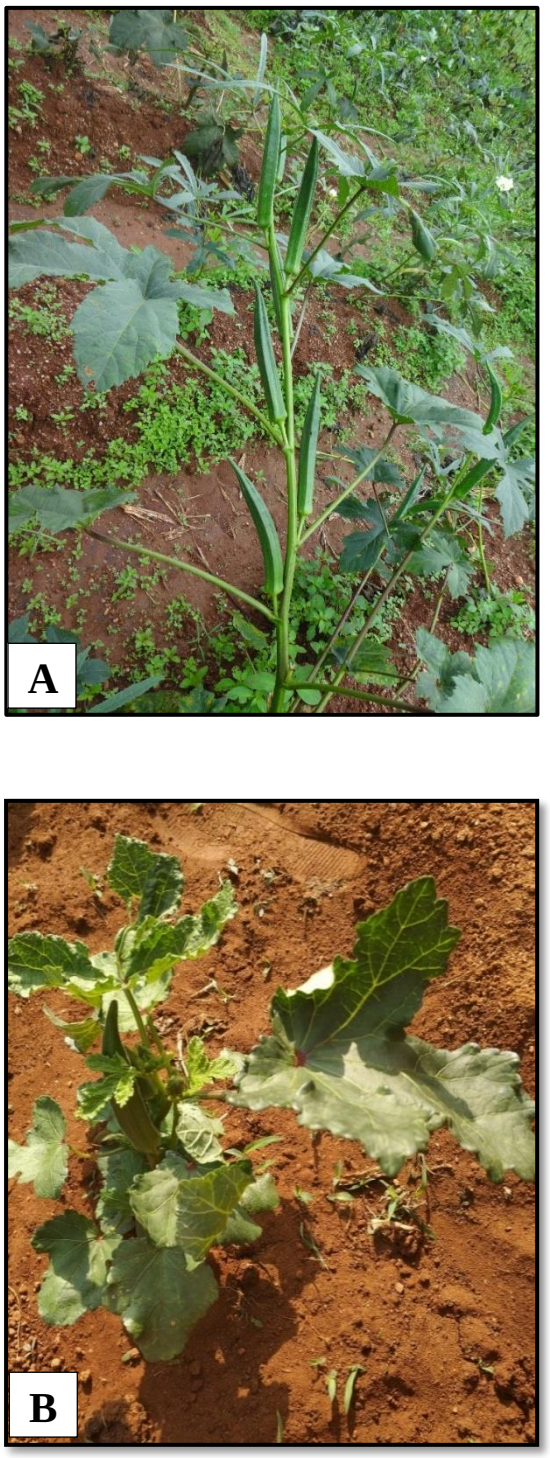


Plate 4.1g: Symptoms under natural conditions: on plant- (A) Healthy plant, (B) Stunted appearance of infected plant

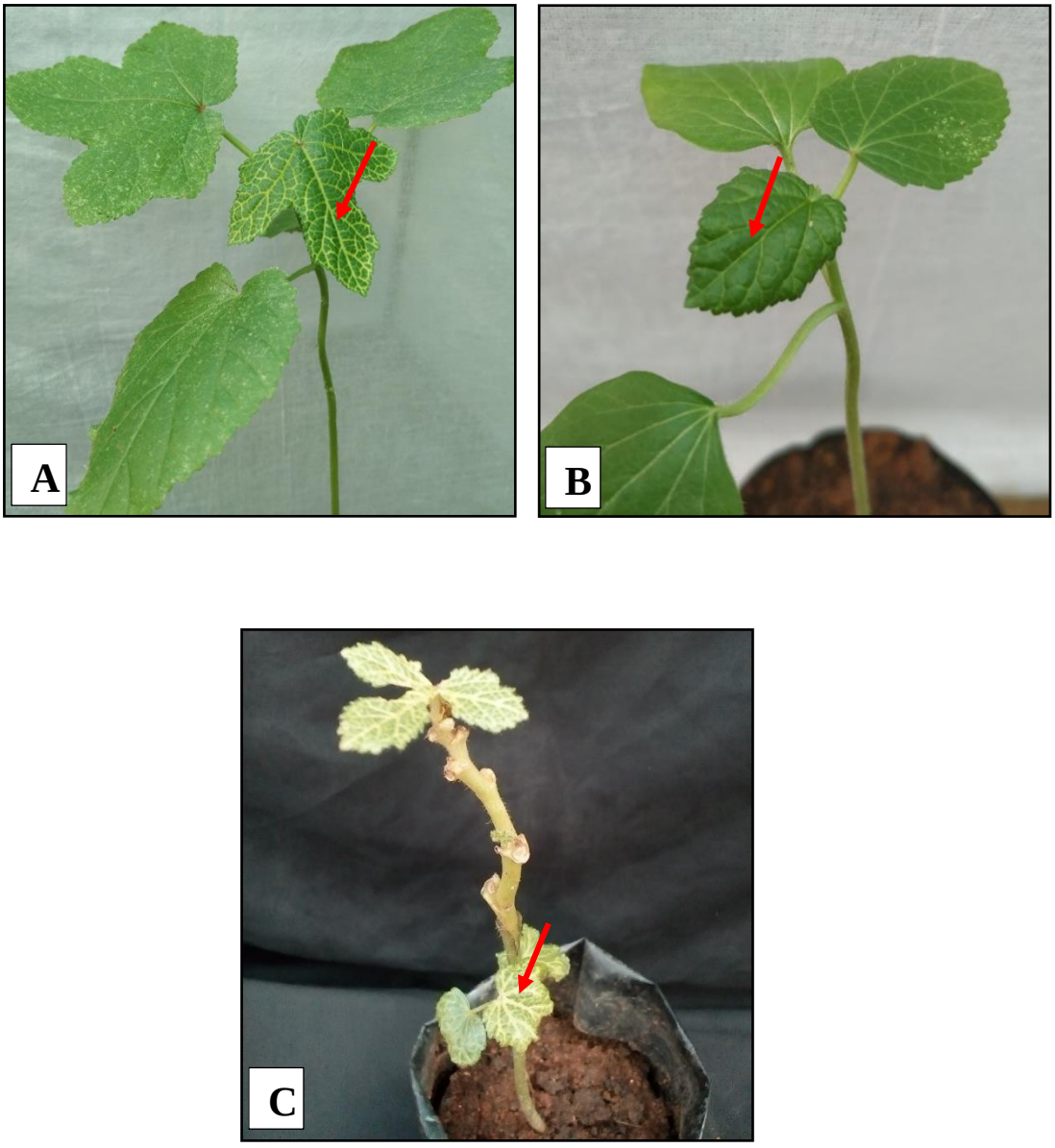


Plate 4.2: Symptoms under artificial conditions: (A) vein clearing and (B) puckering after vector transmission. (C) vein clearing after graft transmission

and vascular bundles. In the leaves showing yellow vein symptoms, the parenchyma cells in upper and lower epidermis were irregular in shape and arrangement in contrast to the well-organized parenchyma cells of healthy leaf. The mesophyll palisade tissues were irregular and crushed with very less quantity of chloroplast in cytoplasm (Plate 4.3a.). The parenchyma cells in the veins of healthy leaves were regular with compact arrangement whereas, the parenchyma cells in the veins of infected leaf had air spaces. Phloem cells were abnormal in size and shape. The number of protoxylem and metaxylem vessels were comparatively less in infected leaves and showed variation in shape compared to the healthy leaves (Plate 4.3b.).

Table 4.3: Histopathological changes caused by virus infection

Leaf tissues	Healthy	Diseased
Upper epidermis	Thick cuticle, single layered parenchyma with regular arrangement	Highly thick cuticle, single layered parenchyma, tightly packed and were irregularly arranged
Mesophyll-Palisade tissue	Single layered, tightly packed, parallel to long axis, plenty of chloroplast suspended in peripheral cytoplasm	Single layered, tightly packed, irregular and crushed, parallel to long axis, very less amount of chloroplast in cytoplasm
Mesophyll-Spongy tissue	5-6 layered parenchyma, irregular shape, loosely arranged, starch grains present, chloroplast abundant in the 4-5 layers of the spongy tissue, Lowermost layer almost without chloroplast	5-6 layered parenchyma, irregular shape, loosely arranged, crushed appearance, chloroplast scarce
Veins	Parenchyma cells regular, compactly arranged	Parenchyma cells with air spaces seen
Veins-Vascular tissue	Well-developed phloem, large number of protoxylem and metaxylem vessels were clearly seen, vessels were circular or oval, sclereids were present	Phloem cells were abnormal in size and shape, comparatively less number of protoxylem and metaxylem vessels were clearly seen, and vessels were rounded or polygonal.
Lower epidermis	Single layered parenchyma, tubular shape, stomata with kidney shaped guard cells	Single layered parenchyma, tubular shape, irregular shape and arrangement

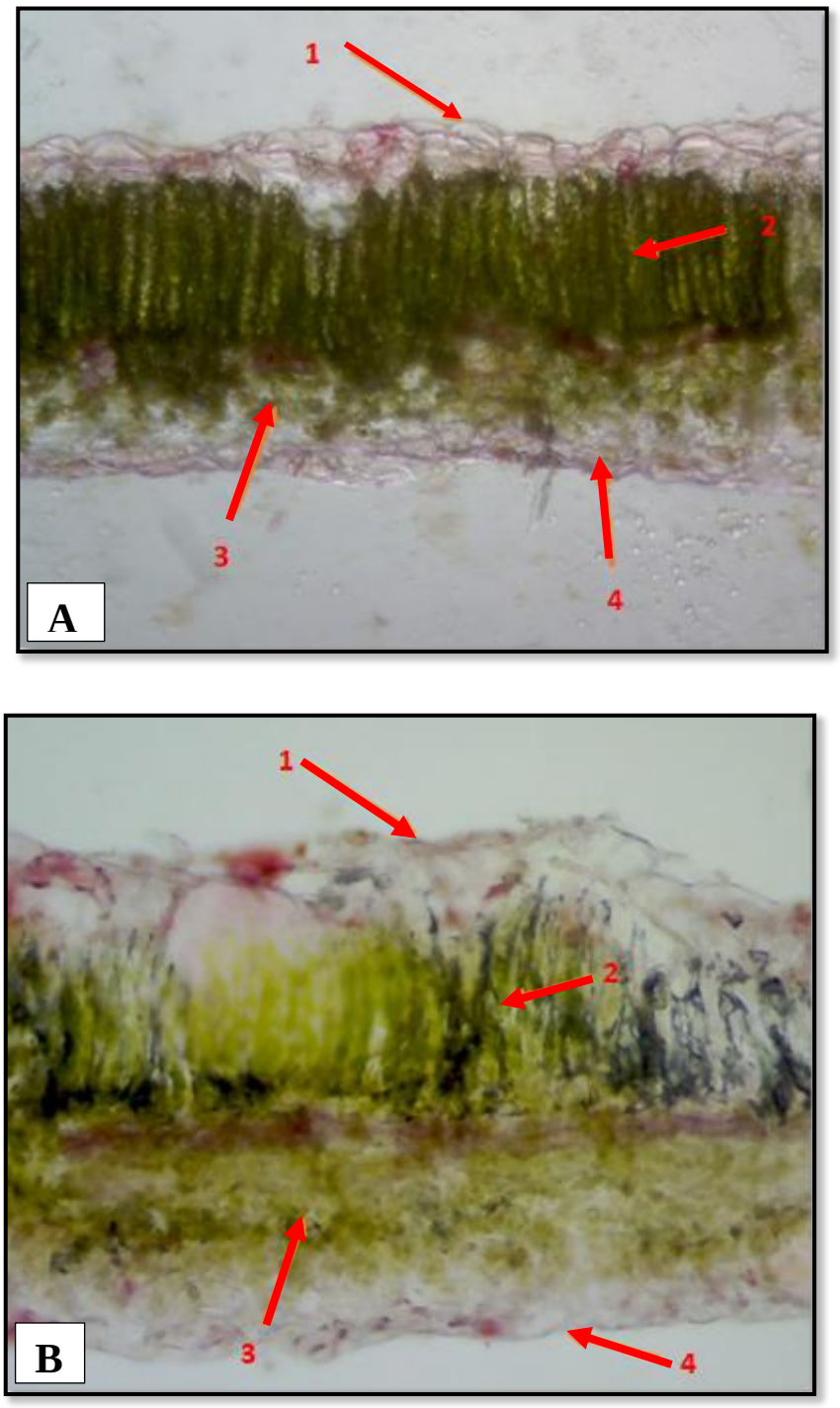
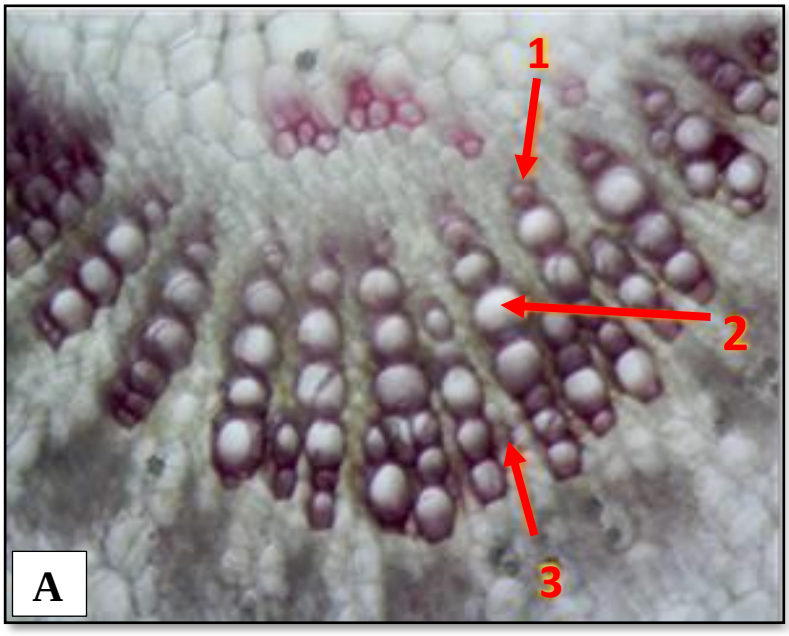


Plate 4.3a: Histopathological changes: (A) healthy leaf, (B) infected leaf 1-upper epidermis, 2-mesophyll palisade, 3- mesophyll spongy cells, 4- lower epidermis



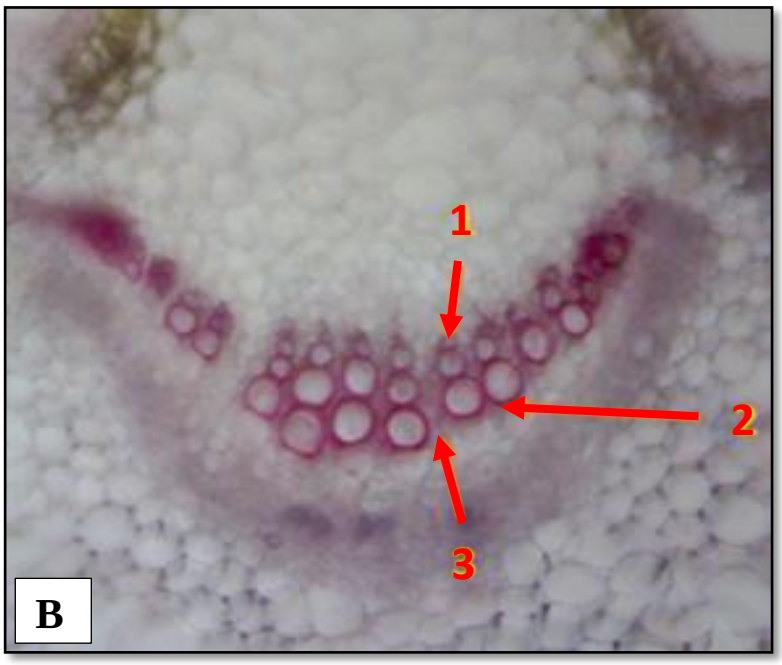


Plate 4.3b: Histopathological changes: A-healthy leaf, B- infected leaf 1-protoxylem, 2-metaxylem, 3-phloem

4.5. BIOLOGICAL CHARACTERIZATION OF THE VIRUS

The biological characterization of the virus was done by conducting studies on different means of transmission and host range of the virus.

4.5.1. Transmission studies

4.5.1.1. Seed transmission

The pods were collected from healthy and infected plants and the extracted seeds were used for seed transmission studies. The infected seeds showed only 84 per cent germination as compared to 98 per cent in healthy seeds. There was 16.32 per cent reduction in germination of the seeds collected from infected plants (Table 4.4.). None of the seedlings raised from seeds of infected pods expressed any symptoms. This result was confirmed through DNA isolation from the seedlings raised from the infected seeds followed by PCR amplification and the details were given under section 4.6.3.3.

4.5.1.2. Graft transmission

Cleft grafting was performed to transmit the virus to healthy plants. The newly emerged leaves from the healthy rootstock showed characteristic vein clearing symptoms 11-14 days after grafting. The symptomatic leaves also showed puckering and vein thickening. The rate of transmission was 100 per cent while, the success percentage of the grafting was very low. The results are given in Table 4.5. and the symptoms developed after graft transmission are depicted in Plate 4.4.

4.5.1.3. Vector transmission

The experiment was conducted on seedlings of susceptible okra var. Salkeerthi at primary leaf stage raised under insect proof conditions as mentioned in section 3.5.1.1. Ten number of whiteflies exposed with 24 h AAP and 24 h IAP gave 100 per cent transmission in healthy plants. The symptoms developed following vector transmission were vein clearing, vein thickening and puckering of leaves (Plate 4.5). In order to study the life stages of whiteflies the colonies in brinjal plants were examined under stereomicroscope and observed the different

Table 4.4: Transmission of virus through seeds

Sl. No.	Test plant	No. of seeds		Germination percentage	No. of plants expressing symptoms	Per cent transmission	Confirmatory test (PCR amplification)
		Sown	Germinated				
1	Salkeerthi (diseased)	100	84	84	0	0	_
2	Salkeerthi (apparently healthy)	100	98	98	0	0	_

Table 4.5: Transmission of virus through grafting

Test plant	No. of plants		Type of symptoms	Per cent transmission	Incubation period of virus	Confirmatory test (PCR amplification)
	Inoculated	Infected				
Bhindi var. Salkeerthi	10	6	Vein clearing, vein thickening and puckering	60	9-14 days	+

Table 4.6: Transmission of virus through vector

Test plant	No. of plants		Type of symptoms	Per cent transmission	Incubation period of virus	Confirmatory test (PCR amplification)
	Inoculated	Infected				
Bhindi (Salkeerthi)	10	10	Vein clearing, vein thickening and puckering	100	12-15 days	+

(-) – Negative amplification (+) – Positive amplification

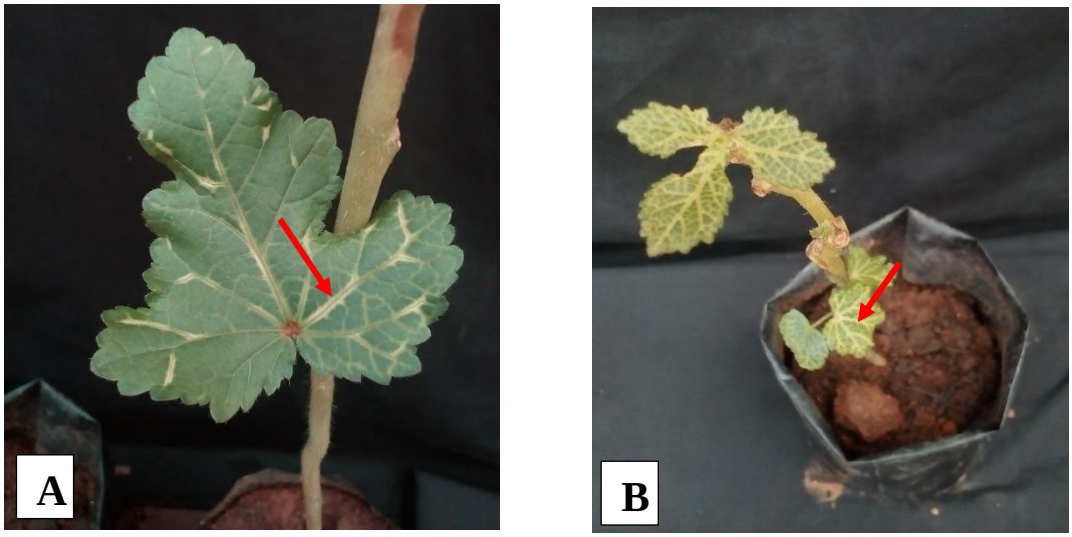


Plate 4.4: Symptoms developed after graft transmission: (A) beginning of vein clearing and (B) vein clearing with puckering

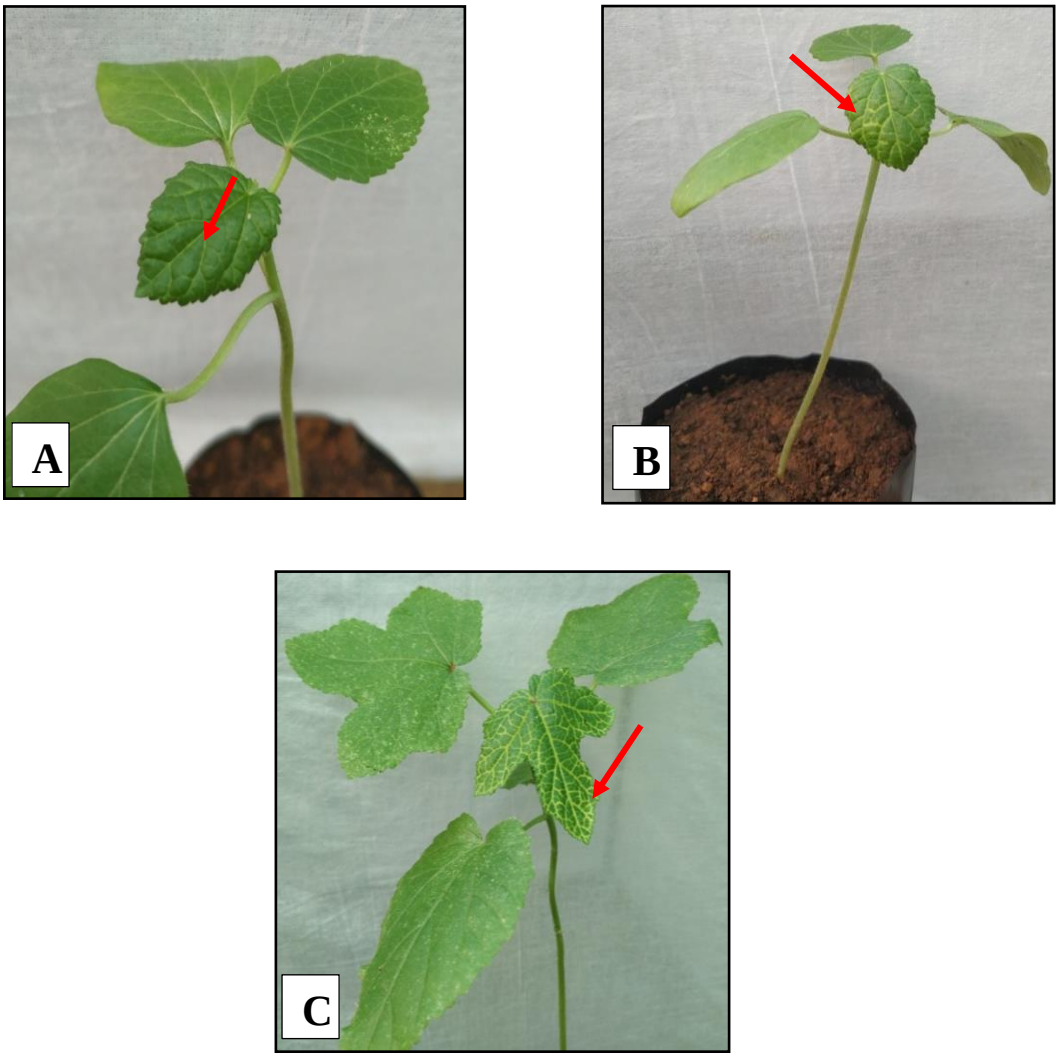


Plate 4.5: Symptoms developed after vector transmission: (A) puckering, (B) beginning of vein clearing (C) leaf with complete vein clearing

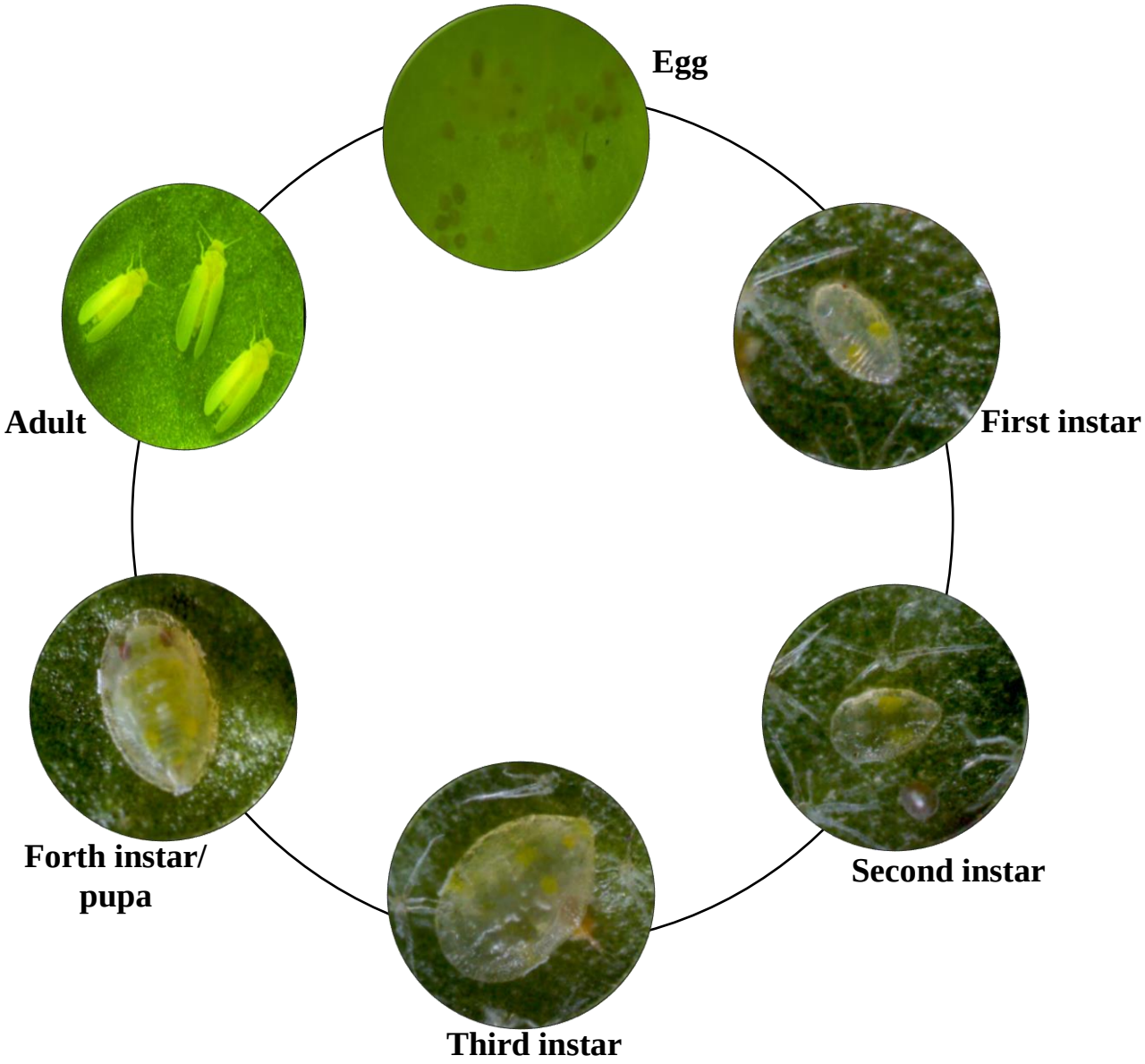


Plate 4.6: Life stages of *B. tabaci*

stages such as egg, first, second and third instar of nymph, red eyed pupa and the adults (Plate 4.6.). The details of the number of plants infected and transmission percentage are given in Table 4.6.. The presence of virus inside the whiteflies were also confirmed through DNA isolation from the viruliferous insects followed by PCR amplification and the details were given in section 4.6.3.2.

4.5.2 Host range studies

The host range study of the virus was conducted to identify the plants susceptible to the virus infection. Eleven plant species belonging to six families *viz.*, Leguminaceae, Solanaceae, Amaranthaceae, Cucurbitaceae, Asteraceae, Malvaceae were selected These plants were inoculated with the insect vector *B. tabaci* as described under section 3.5.1.3.4. The plants were maintained in the insect proof cage for symptom expression. The results are presented in Table 4.7.

Among the crop plants tested, only brinjal showed mild yellowing of veins (Plate 4.7.) while it doesn't produced positive result during the PCR amplification of the infected plant sample with primer specific to the core CP gene of begomoviruses. Among the weed species, mild vein clearing symptoms were expressed on *Synedrella nodiflora* (Plate 4.7.). Initially, the inoculated plants exhibited crinkling of leaf lamina 19-20 DAI. The size of the leaves was also got reduced compared to the control plants and the vein clearing symptoms on leaf lamina were observed 27-28 DAI. Positive result was also obtained during PCR amplification of the infected plant sample with primer specific to the core CP gene of begomoviruses which confirmed that it is a host of the virus (Plate 4.9).

Reduced leaf size and small puckering was noticed in *Sida* sp. 20 DAI and mild vein clearing symptom was observed 25 DAI. *Ageratum conyzoides* inoculated with the virus showed crinkling of leaf lamina and puckering symptoms 21 DAI (Plate 4.7.). However, these plants gave negative results in PCR amplification.

The DNA isolated from the symptomatic weeds collected from the vegetable field showed mosaic and vein clearing symptoms (Plate 4.8a and 4.8b.).

Table 4.7: Host range of virus

Sl. No.	Host	Family	No. of plants		Symptoms produced (+/-)	Type of symptom	Confirmatory test (PCR amplification)
			Inoculated	Symptoms expressed			
1	*Amaranthus polygamous*	Amaranthaceae	5	0	-	Nil	-
2	*Cucumis sativus*	Cucurbitaceae	5	0	-	Nil	-
3	*Cucurbita moschata*		5	0	-	Nil	-
4	*Solanum lycopersicum*	Solanaceae	5	0	-	Nil	-
5	*Capsicum annum*		5	0	-	Nil	-
6	*Solanum melongena*		5	1	+	Mild yellowing of veins	-
7	*Vigna unguiculata*	Fabaceae	5	0	-	Nil	-
8	*Synedrella nodiflora*	Asteraceae	5	2	+	Crinkling of leaf lamina, reduced leaf area, yellowing of veins	+
9	*Ageratum conyzoides*		5	2	+	Crinkling of leaf lamina, puckering	-
10	*Alternanthera ficoidea*	Amaranthaceae	5	0	-	Nil	-
11	*Sida acuta*	Malvaceae	5	1	+	Puckering, mild yellowing of veins	-

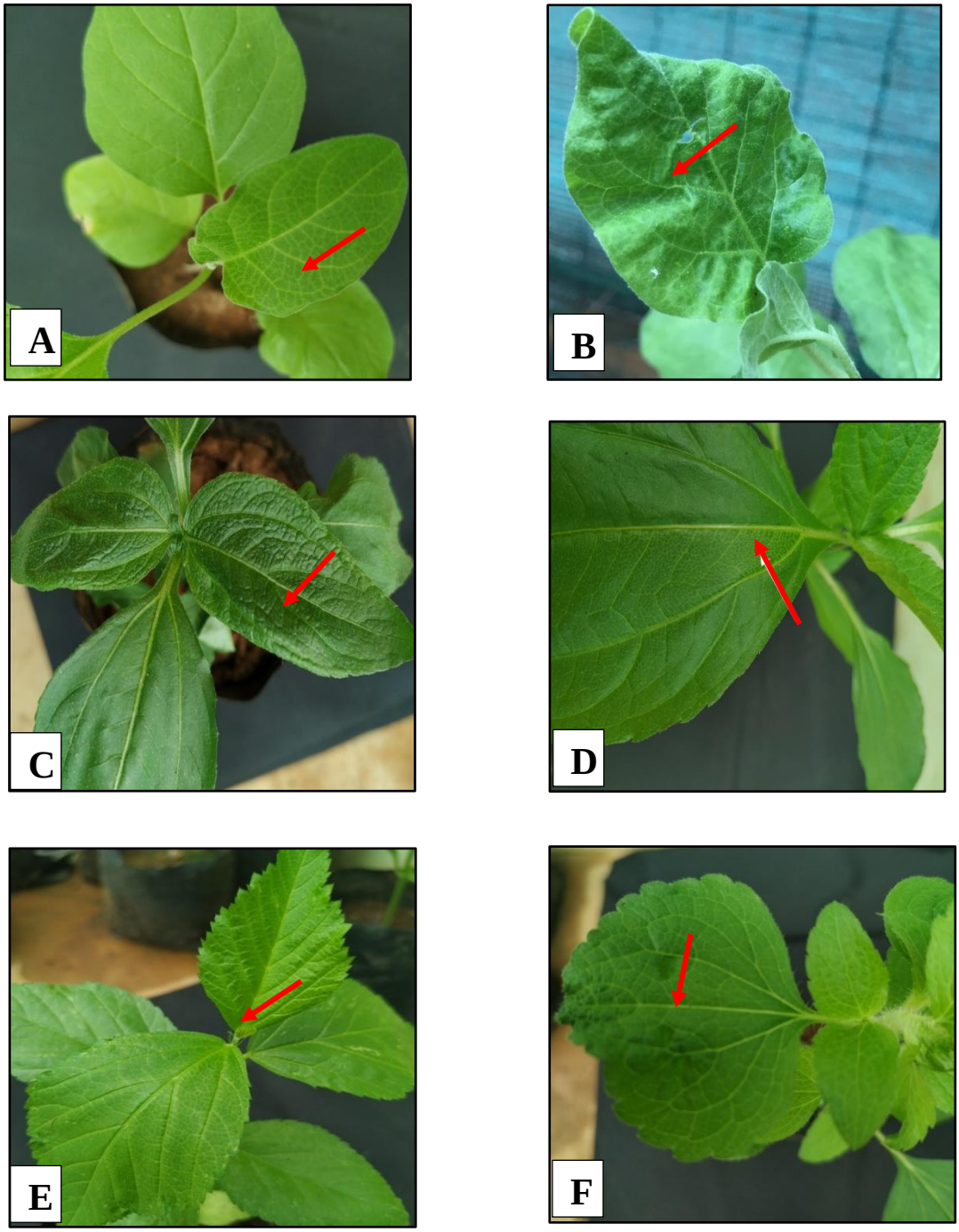


Plate 4.7: Host range of virus: (A) vein clearing and (B) puckering on brinjal, (C) leaf crinkling and (D) vein clearing on *Synedrella nodiflora,* (E) mild vein clearing on *Sida* sp. (F) puckering on *Ageratum conyzoides*

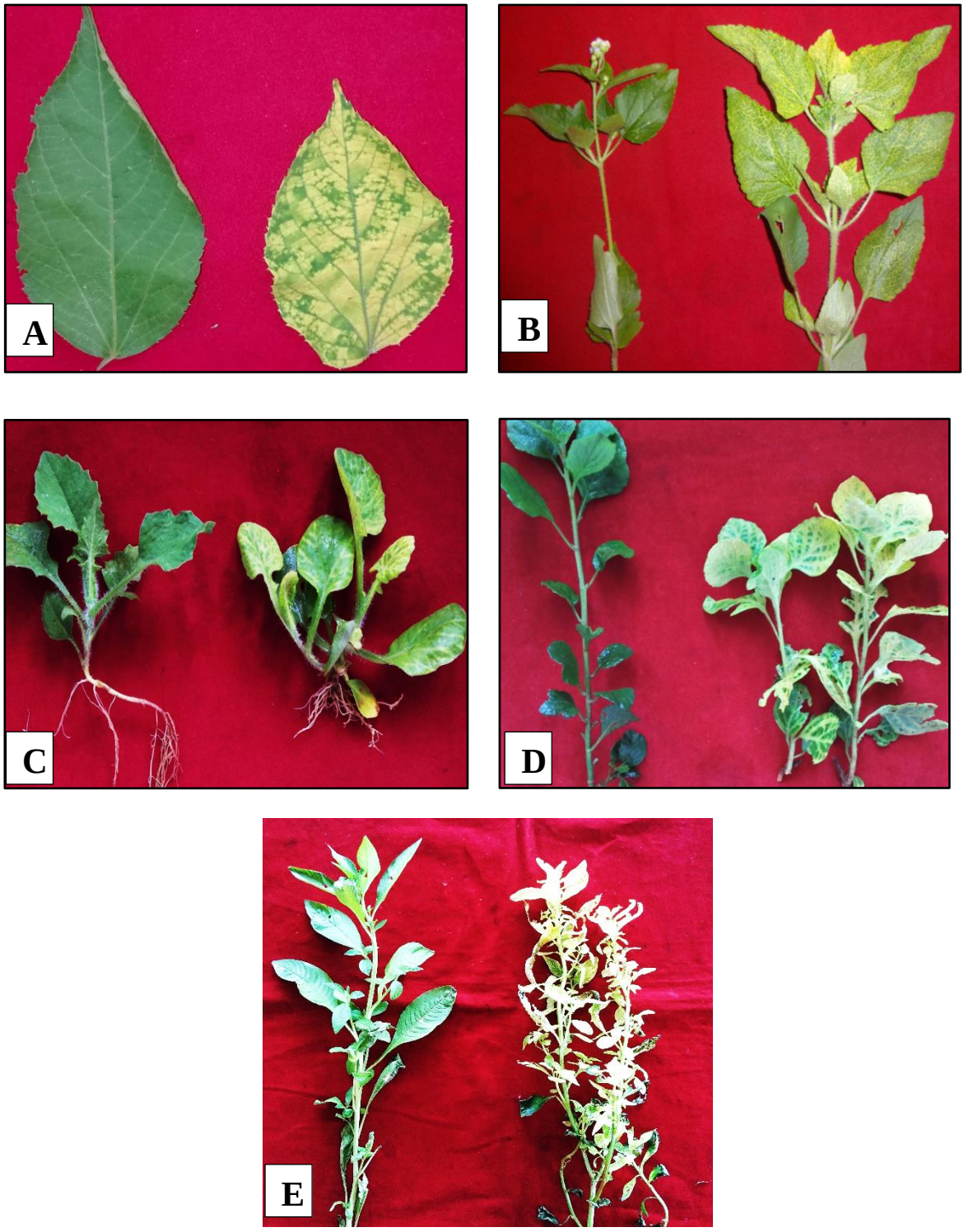


Plate 4.8a: Symptoms on weed plants collected from fields: (A) yellowing on *Tragia involucrate* (B) vein clearing on *Ageratum conyzoides* (C) vein clearing on *Emilia sonchifolia* (D) vein clearing on *Cyanthilium cinereum* (E) yellowing on *Ludwigia hyssopifolia*

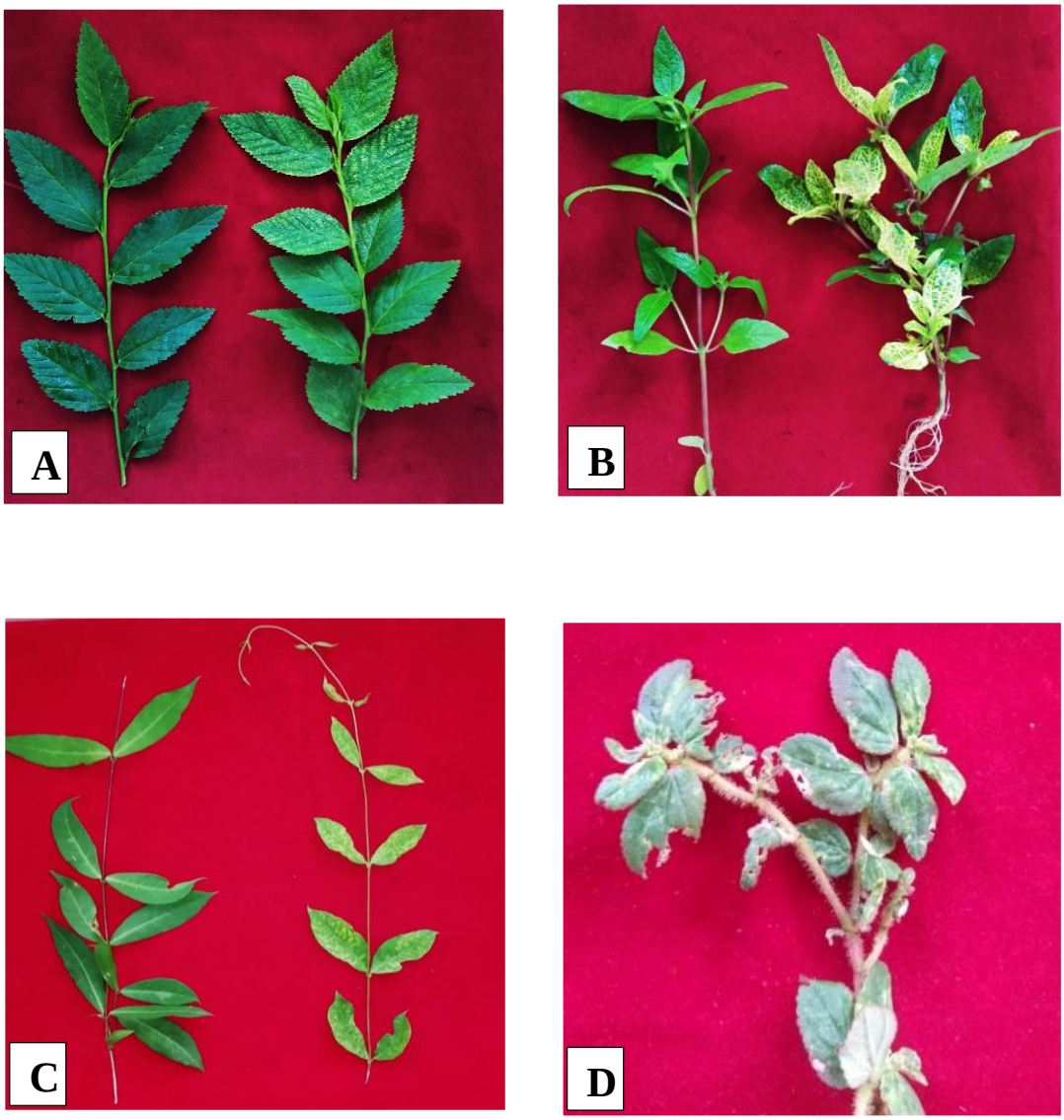


Plate 4.8b: Symptoms on weed plants collected from fields: (A) vein clearing on *Sida acuta* (B) vein clearing and leaf curling on puckering on *Synedrella nodiflora*, (C) mosaic on *Hemidesmus indicus* (D) yellowing on *Euphorbia hirta*

The DNA isolated from these plants were subjected to PCR amplification with universal begomovirus primer (AV494/AC1048) specific to the core CP region of the virus. The results revealed that positive amplification was obtained at expected band size of 550 bp only in two species *i.e.*, *Synedrella nodiflora* and *Hemidesmus indicus* showing vein clearing and mosaic symptoms respectively and confirmed the presence of begomovirus (Plate 4.10). The sequencing of the PCR product obtained from *S. nodiflora* was also carried out. The BLASTn analysis of the sequence showed maximum similarity with tomato leaf curl virus in Kerala (Fig. 4.1). The sequences showed only less than 85 per cent similarity with BYVMV. The results of the experiment are given in Table 4.8.

Table 4.8: Host range of *Begomovirus in* weed plants with symptoms collected from fields

Sl. No.	Host	Family	Symptom	Confirmatory test (PCR amplification)
1	*Sida* sp.	Malvaceae	Vein clearing	-
2	*Synedrella nodiflora*	Asteraceae	Vein clearing, leaf curling	+
3	*Emilia sonchifolia*		Vein clearing	-
4	*Ageratum conyzoides*		Vein clearing	-
5	*Cyanthilium cinereum*		Vein clearing	-
6	*Hemidesmus indicus*	Apocynaceae	Yellow mosaic	+
7	*Tragia involucrate*	Euphorbiaceae	Yellow mosaic	-
8	*Euphorbia hirta*		Yellow mosaic	-
9	*Ludwigia hyssopifolia*	Onagraceae	Yellowing of leaves	-

4.6. MOLECULAR DETECTION AND CHARACTERIZATION

Molecular detection and characterization of local isolates from Thrissur district and one isolate each from Thiruvananthapuram, Kottayam, Palakkad, Kannur and Kasaragod was undertaken. The isolates were named according to the location from which the samples were collected (Table 4.9.).

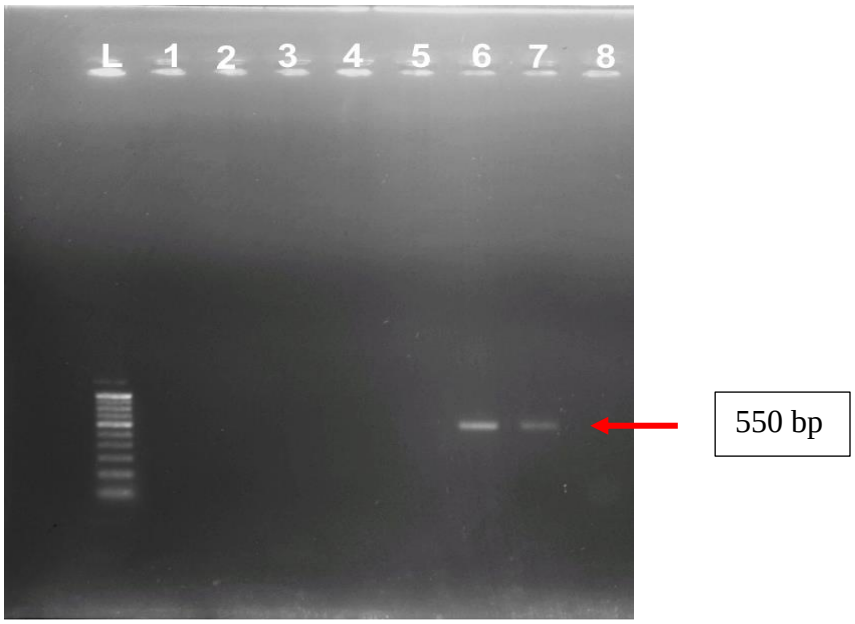


Plate 4.9: Molecular detection of host range of virus: lane L : 100 bp ladder, lane 1- chilli, 2- brinjal, 3- tomato, 4- *Sida acuta*., 5- *Ageratum conyzoides,* 6- *Synedrella nodiflora,* 7- Positive control, 8- blank

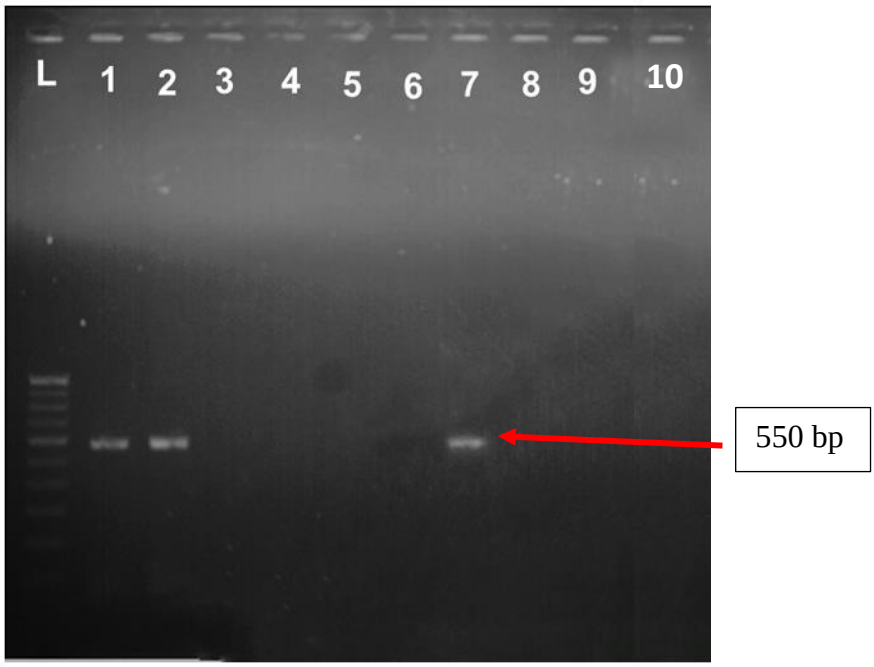
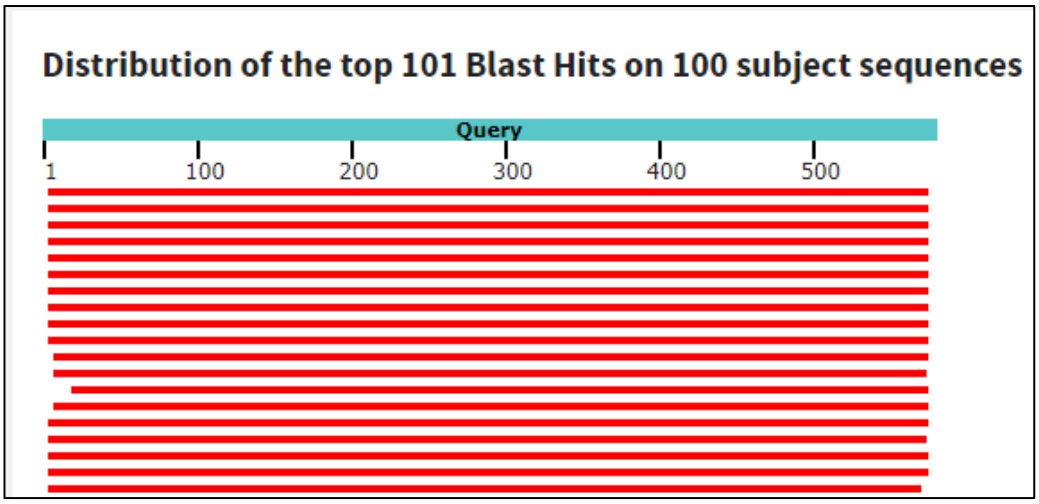


Plate 4.10: Molecular detection of virus in collected weed plants: lane L : 100 bp ladder, lane 1- positive control, 2- *S. nodiflora*, 3- *E. sonchifolia*, 4- *Sida acuta*., 5- *Ageratum conyzoides,* 6- *Cyanthilium cinereum*, 7- *Hemidesmus indicus,* 8- *Tragia involucrate* 9- *Euphorbia hirta*, 10- *Ludwigia hyssopifolia*



Graphical output of BLASTn analysis

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Tomato leaf curl Kerala virus isolate RM425, complete genome	937	937	98%	0.0	96.32%	KY926894.1
☑	Tomato leaf curl Kerala virus isolate RM424, complete genome	937	937	98%	0.0	96.32%	KY926893.1
☑	Tomato leaf curl Kerala virus isolate RM430, complete genome	931	931	98%	0.0	96.14%	KY926898.1
☑	Tomato leaf curl Kerala virus isolate RM427, complete genome	931	931	98%	0.0	96.14%	KY926896.1
☑	Tomato leaf curl Kerala virus isolate RM426, complete genome	931	931	98%	0.0	96.14%	KY926895.1
☑	Tomato leaf curl Kerala virus strain IH-34, complete genome	931	931	98%	0.0	96.14%	KX665543.1
☑	Tomato leaf curl Kerala Virus complete genome, clone 5NA3-2RS2	931	931	98%	0.0	96.14%	LN886521.1
☑	Tomato leaf curl Kerala virus strain IH-48, complete genome	931	931	98%	0.0	96.14%	KX671963.1
☑	Tomato leaf curl Kerala virus isolate begomovirus genome assembly, segment: I	931	931	98%	0.0	96.14%	LT556075.1
☑	Tomato leaf curl Kerala virus isolate TC343 segment DNA-A, complete sequence	931	931	98%	0.0	96.14%	KF551575.1

Text output of BLASTn analysis

Fig 4.1: BLASTn analysis of *Synedrella nodiflora* with yellow vein symptom

4.6.1. Isolation of total genomic DNA

Isolation of total genomic DNA was standardized from the leaf samples of infected and healthy bhindi plants using Doyle and Doyle (1987) method with modifications as mentioned in section 3.6.1.

Table 4.9: Details of local isolates

Sl. No.	Location	District	Code assigned
1	Vellanikkara field 1	Thrissur	VKA 1
2	Vellanikkara field 2	Thrissur	VKA 2
3	Vellanikkara field 3	Thrissur	VKA 3
4	Vellanikkara field 4	Thrissur	VKA 4
5	Chengaloor	Thrissur	CLR
6	Muthuvara	Thrissur	MTV
7	Attoor field 1	Thrissur	ATR 1
8	Attoor field 2	Thrissur	ATR 2
9	Viyyur field 1	Thrissur	VYR 1
10	Viyyur field 2	Thrissur	VYR 2
11	Vellayani	Thiruvananthapuram	VNY
12	Kumarakom	Kottayam	KMR
13	Panniyur	Kannur	PNR
14	Padannakkad	Kasaragod	PND

4.6.1.1. Assessment of quality and quantity of isolated genomic DNA

The total DNA obtained was quantified using Nanodrop spectrophotometer (Nanodrop ND-1000). The concentration and the absorbance ratios of the DNA are given in Table 4.10. The concentration of isolated DNA ranged from 265.48 to 752.42. The DNA with an absorbance value ($A_{260/280}$) between 1.8 and 2.0 was considered as good quality DNA. The absorbance value of isolated DNA ranged from 1.82 to 2.14 which indicated that the isolated DNA have good quality with less contamination of RNA. Gel documentation of total DNA isolated was done under BIORAD Molecular Imager (Gel Doc^{TM} XR+) (Plate 4.11.).

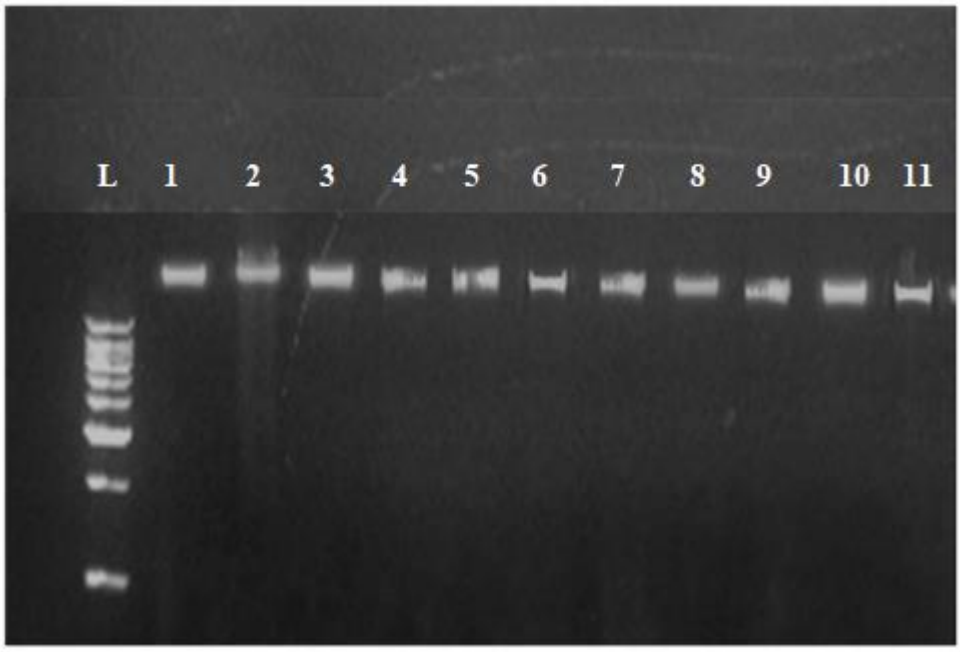


Plate 4.11: Gel profile of total genomic DNA isolated from symptomatic leaf samples: lane L: 1 kb ladder, lane 1-11: local isolates

Table 4.10: Quantity and quality of isolated DNA

Sl. No.	Isolate	DNA yield (ng/µl)	OD value ($A_{260/280}$)
1	VKA 1	498.65	1.98
2	VKA 2	621.35	1.99
3	VKA 3	572.14	2.01
4	VKA 4	752.42	2.14
5	CLR	265.48	2.13
6	MTV	610.84	1.89
7	ATR 1	378.12	2.08
8	ATR 2	308.45	2.06
9	VYR 1	351.60	1.99
10	VYR 2	655.80	2.03
11	VNY	434.81	2.16
12	KMR	452.36	2.15
13	PNR	443.51	2.11
14	PND	346.09	1.82

4.6.2. Standardization of annealing temperature

PCR amplification was carried out using different set of primers and the details are given in the Table 3.4. Initially, annealing temperature was standardized for each primer by performing gradient PCR.

4.6.2.1. Annealing temperature for primer specific to core coat protein of Begomovirus (AV494/AC1048)

Among the range of temperature (54.7-57.7 ^{o}C) used good quality band of amplicons were produced at 56.8 ^{o}C. So this temperature was taken as optimum annealing temperature for further PCR amplification using the primer set AV494/AC1048 (Plate 4.12).

4.6.2.2. Annealing temperature for BYVMV coat protein gene specific primers

In case of BYVMV coat protein gene specific primer, a range of temperature (52.0-58.0 ^{o}C) were tested and good quality amplicons were produced at 55.4^{o}C (Plate 4.13.). Hence, 55.4 ^{o}C was the optimum temperature for further reactions using this primers.

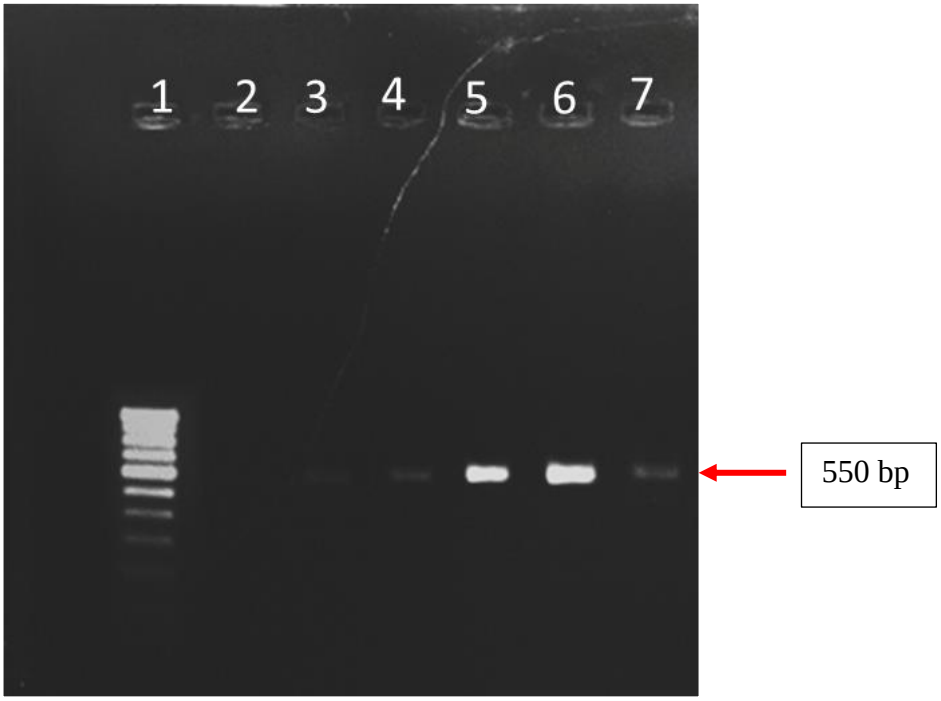


Plate 4.12: Standardization of annealing temperature of AV494/AC1048 primer: lane L : 100 bp ladder, lane 1- 53.2 ºC, lane 2- 54.7 ºC, lane 3- 55.2 ºC, lane 4- 55.8 ºC, lane 56.2 ºC, lane 6- 57.3 ºC, lane 7- 58.0 ºC.

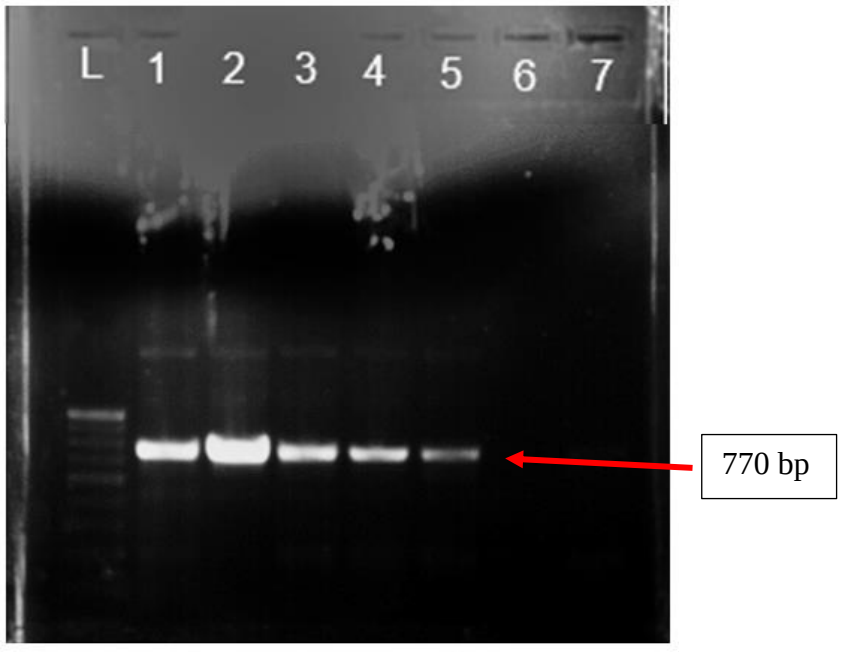


Plate 4.13: Standardization of annealing temperature of BYVMV CP gene specific primer: lane L : 100 bp ladder, lane 1- 539 ºC, lane 2- 54.6 ºC, lane 3- 55.4 ºC, lane 4- 56.9 ºC, lane 57.7 ºC, lane 6- 58.0 ºC, lane 7- 58.6 ºC.

4.6.2.3. Annealing temperature for OYVMV coat protein specific primer

In case of OYVMV CP gene specific primer, multiple bands were produced for all the temperature tested (Plate 4.14.). Therefore, the OYVMV CP gene specific primer was not used for further PCR detection.

4.6.2.4. Annealing temperature for OELCuV coat protein gene specific primer

In case of OELCuV coat protein specific primer, a range of temperature (52.2-58.0 ^{o}C) were tested and produced good quality bands at 56.9 ^{o}C which was selected as optimum annealing temperature for amplification (Plate 4.15.).

4.6.3. Molecular detection of the virus

4.6.3.1. Molecular detection of the virus in infected plant sample

The three set of primers *i.e.,* AV494/AC1048, BYVMVF/R and OELCuVF/R produced amplicons at expected size of 550, 770 and 770 bp respectively (Plate 4.16.). Good quality bands were visualized and documented in infected samples which indicated the presence of the virus infection.

4.6.3.2. Molecular detection of virus inside Bemisia tabaci

The presence of virus inside viruliferous vector was confirmed by standardizing isolation of DNA from the insect followed by PCR amplification. The DNA isolated from whiteflies were subjected to PCR amplification using the universal begomovirus primer (AV494/AC1048) specific to the core CP gene of the virus. The amplification was obtained in the case of DNA isolated from viruliferous whiteflies and no amplification was observed in non viruliferous whiteflies (Plate 4.17.). This confirmed that *B. tabaci* is the vector of the virus causing yellow vein mosaic symptoms.

4.6.3.3. Molecular detection of seed infection

The seeds collected from infected plants were raised in pro trays. The DNA isolated from the seedlings were subjected to PCR amplification using universal begomovirus primer (AV494/AC1048) and no amplification was obtained in any

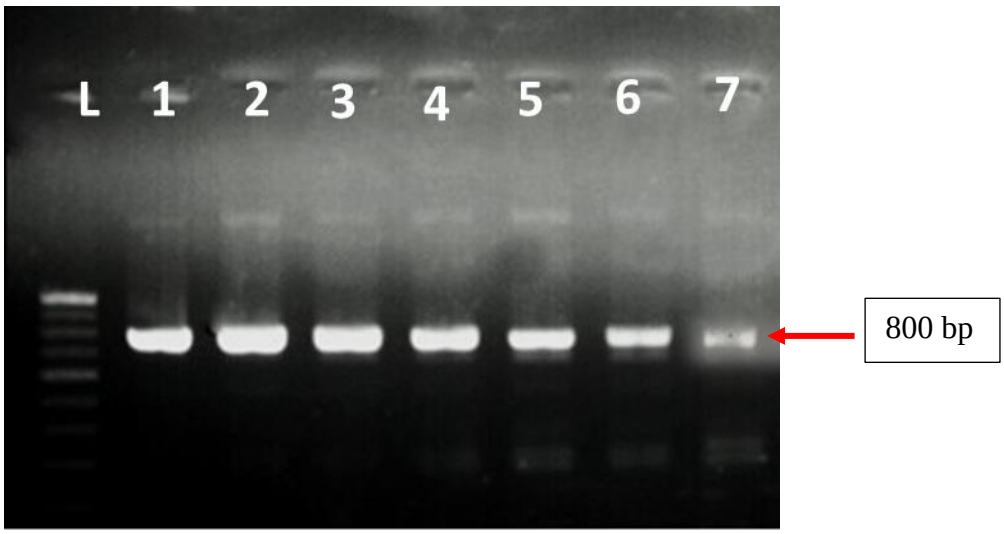


Plate 4.14: Standardization of annealing temperature of OYVMV CP gene specific primer: lane L : 100 bp ladder, lane 1- 47.9 ºC, lane 2- 48.8 ºC, lane 3- 51.4 ºC, lane 4- 52.6 ºC, lane 5- 53.9 ºC, lane 6- 55.2 ºC, lane 7- 56.1 ºC.

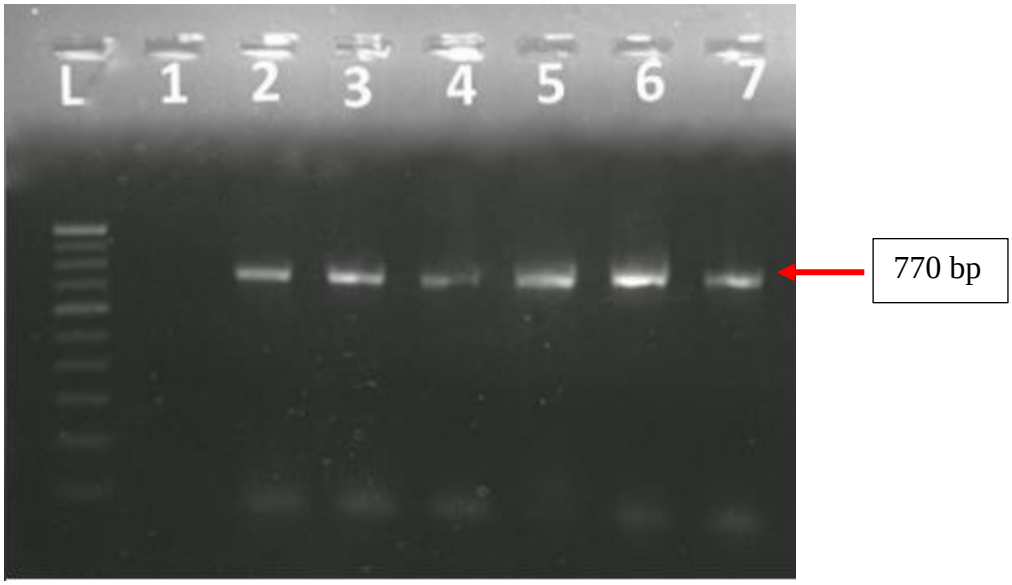


Plate 4.15: Standardization of annealing temperature of OELCuV CP gene specific primer: lane L : 100 bp ladder, lane 1- 52.2 ºC, lane 2- 53.4 ºC, lane 3- 54.6 ºC, lane 4- 55.2 ºC, lane 56.4 ºC, lane 6- 57.3 ºC, lane 7- 58.1 ºC.

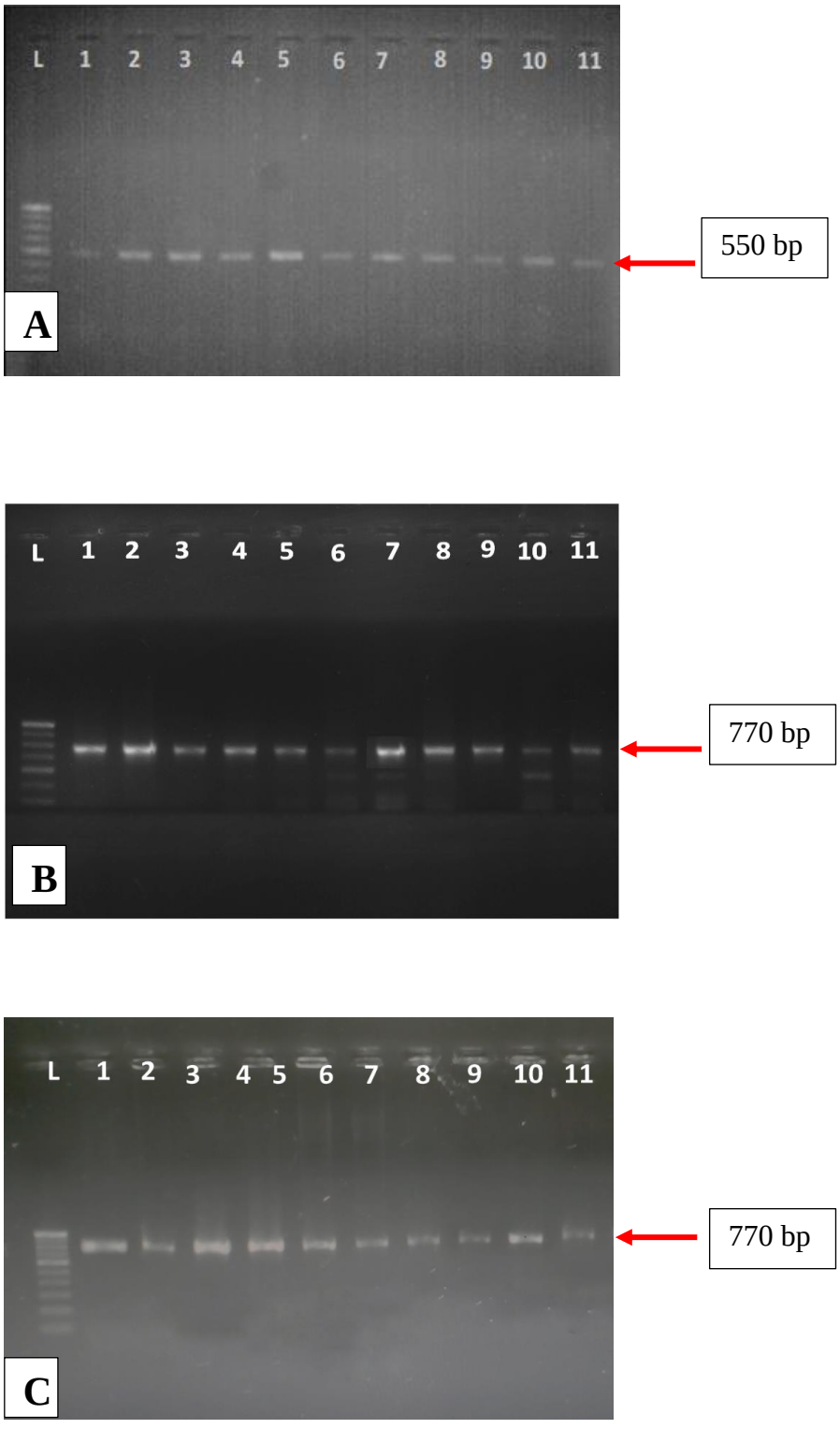


Plate 4.16: PCR amplification of local isolates (A) AV494/AC1048 primer, (B) BYVMV specific primer, (C) OELCuV specific primer: lane L- ladder (100 bp), lane 1-12-local isolates

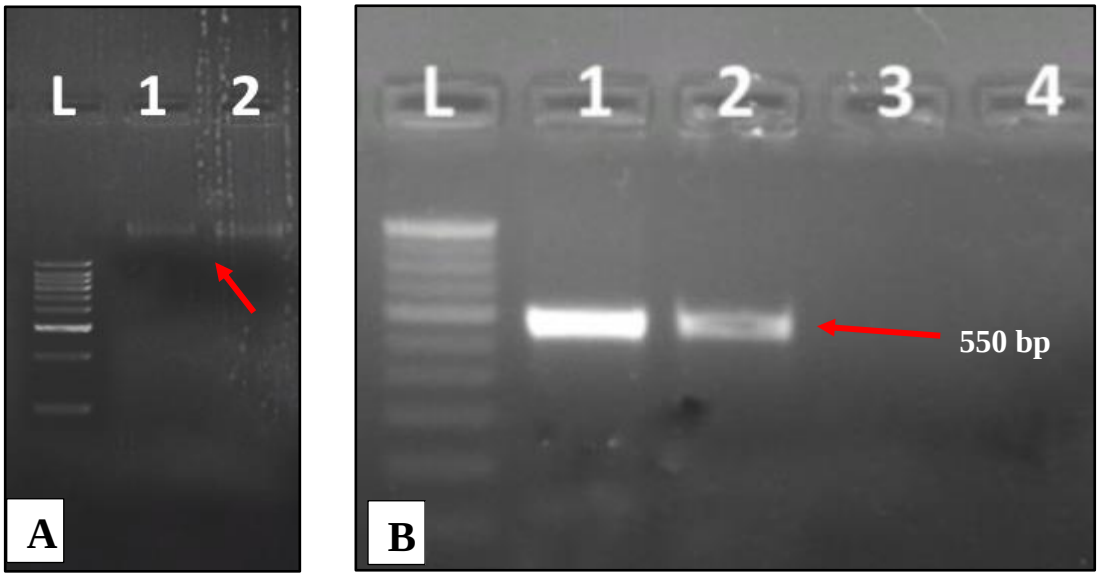


Plate 4.17: Molecular detection of virus inside whiteflies (A) DNA isolated from whiteflies: lane L: 1 kb ladder, lane 1- viruliferous, 2- non viruliferous (B) PCR amplification: lane L: 100 bp ladder, lane 1- viruliferous whiteflies, 2- positive control, 3- non viruliferous whiteflies and 4- blank

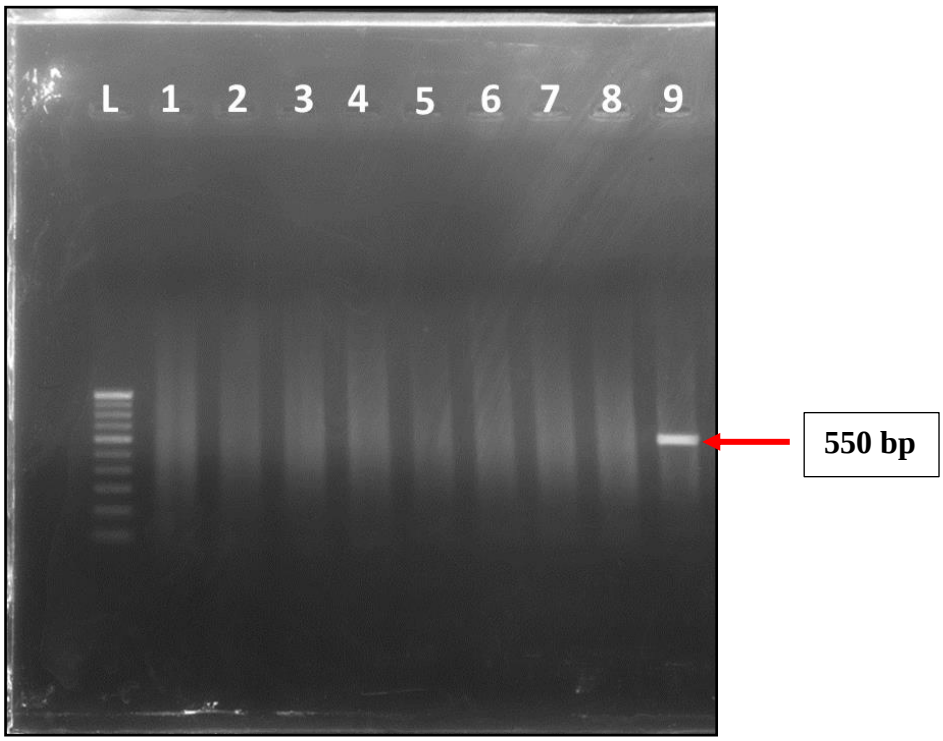


Plate 4.18: Molecular detection of seed infection: lane L- 100 bp ladder, lane 1-8: seedlings raised from infected seeds, lane 9- positive control

of the sample (Plate 4.18.). This confirmed that the virus of yellow vein mosaic disease is not seed-borne.

4.6.4. Sequencing

The PCR products amplified using three sets of primers *viz.,* AV494/AC1048 (11 isolates), CP gene specific primer of BYVMV (three isolates) and CP gene specific primer of OELCuV (one isolate) coat protein specific primer were sequenced. The PCR amplicons obtained were sent to Agrigenome, Kochi for purification and sequencing. The details of nucleotide sequences obtained from all the isolates were given in Fig.4.2a to Fig. 4.2c.

4.6.5. *In silico* analysis

The nucleotide sequences of core CP gene of virus isolates obtained were analysed using BLASTn programme (http://www.ncbi.nlm.nih.gov/BLAST/). In general, the sequence identity of above 89 per cent is considered as species demarcation threshold which was set by the International Committee on Taxonomy of Viruses (ICTV; Fauquet *et al.,* 2008). In the present study, all the 11 local isolates showed similarity of more than 89 per cent with the begomoviruses infecting bhindi *viz*., BYVMV, OELCuV, mesta yellow vein mosaic virus (MeYVMV) and hollyhock yellow vein mosaic virus (HoYVMV).

However, among the different begomoviruses, the local isolates showed maximum sequence identity of 93.35 to 100 per cent with okra enation leaf curl virus (OELCuV) followed by 91.61 to 97.83 per cent sequence with BYVMV, 90.35 to 99.82 per cent with MeYVMV and 89.84 to 95.53 with HoYVMV available in the NCBI database (Table 4.11).

The nucleotide sequences of three local isolates (VKA 2, CLR and VYR 1) amplified with BYVMV coat protein specific primer were analysed using BLASTn program and the results revealed that the local isolates showed similarity with begomoviruses of bhindi *viz*., BYVMV, OELCuV, MeYVMV. Among these, the local isolates showed maximum sequence identity of 99.73 per cent with OELCuV

Isolate VKA1

5’GAAGCCCAGAATGTACAGGATGTACAGAAGCCCTGATGTTCCTAGAGGATGTG
AAGGCCCATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGG
TAAGGTTATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAG
GGAAACGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAG
AATATTAAGACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACA
GGCGTCCTACAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGACAAC
GAGCCTTCTACGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGA
GGAGGTTTCAGGCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCC
AATTAGGAAATTCTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCT
GGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACA 3’

Isolate CLR

5’CCCAGAATGTACAGGATGTACAGAAGCCCTGATGTTCCTAGAGGATGTGAAGGC
CCATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGG
TTATTTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAGGGAAA
CGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATAT
TAAGACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCGT
CCTACAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGACAACGAGCC
TTCTACGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAGG
TTTCAGGCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCAATTA
GGAAATTCTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGGAA
GTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATG 3’

Isolate MVA

5’CCCAGAATGTACAGGATGTACAGAAGCCCTGATGTTCCTAGAGGATGTGAAGGC
CCATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGG
TTATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAGGGAA
ACGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATA
TTAAGACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCG
TCCTACAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGACAACGAGC
CTTCTACGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAG
GTTTCAGGCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCAATT
AGGAAATTCTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGGA
AGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTC 3’

Isolate VYR1

5’GTTCCTAGAGGATGTGAAGGCCCATGTAAGGTGCAGTCTTTTGATGCGAAGAAC
GATATTGGTCACATGGGTAAGGTTATCTGTCTATCTGATGTTACTAGGGGTATGGG
GCTGACCCATCGAGTAGGGAAACGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCA
AGATATGGATGGATGAGAATATTAAGACTAAGAACCATACGAACACCGTTATGTT
TTGGATCGTGAGAGACAGGCGTCCTACAGGCACCCCCTACGATTTCCAGCAAGTG
TTCAATGTTTATGACAACGAGCCTTCTACGGCTACTGTAAAGAACGACCAGCGTG
ATCGATTCCAGGTTTTGAGGAGGTTTCAGGCGACAGTTACAGGAGGACAGTATGC
TTGTAAGGAACAAGTTCCAATTAGGAAATTCTATCGTGTTAACAATTACGTGGTGT
ATAATCACCAGGAAGCTGGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGTT
GTATATGGCATGTACA 3’

Fig. 4.2a: Nucleotide sequences of local isolates

Isolate VYR2

5’ATTGCCCATGTACAGGAAGCCCAGAATGTACAGGATGTACAGAAGCCCTGATGTT
CCTAGAGGATGTGAAGGCCCATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATAT
TGGTCACATGGGTAAGGTTATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGA
CCCATCGAGTAGGGAAACGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATAT
GGATGGATGAGAATATTAAGACTAAGAACCATACGAACACCGTTATGTTTTGGATC
GTGAGAGACAGGCGTCCTACAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGT
TTATGACAACGAGCCTTCTACGGCTACTGTAAAGAACGACCAGCGTGATCGATTCC
AGGTTTTGAGGAGGTTTCAGGCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAA
CAAGTTCCAATTAGGAAATTCTATCGTGTTAACAATTACGTGGTGTATAATCACCA
GGAAGCTGGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCAT
GTACTCATGCCTTCCAACACCA 3’

Isolate ATR1

5’CCAGAATGTACCGGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAAGGCC
CATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGGTT
ATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAGGGAAACG
TTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATATTAA
GACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCGTCCTA
CAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGACAACGAGCCTTCTA
CGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAGGTTTCAG
GCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCAATTAGGAAATT
CTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGGAAGTATGAAA
ATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCCCTCC 3’

Isolate ATR2

5’CCAGAATGTACCGGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAAGGCC
CATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGGTT
ATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAGGGAAACG
TTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATATTAA
GACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCGTCCTA
CAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGACAACGAGCCTTCTA
CGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAGGTTTCAG
GCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCAATTAGGAAATT
CTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGGAAGTATGAAA
ATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCC 3’

Isolate VNY

5’AATGTACCGGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAAGGCCCATG
TAAGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGGTTATCT
GTCTATCTGATGTTACTAGGGGTATGGGGCTGACCCATCGAGTAGGGAAACGTTTT
TGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATATTAAGAC
TAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCGTCCTACAG
GCACCCCCTACGATTTCCAGCAAGTGTTTAATGTTTATGACAACGAGCCTTCTACGG
CTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAGGTTTCAGGCG
ACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCAATTAGGAAATTCTA
TCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGGAAGTATGAAAATC
ACACTGAGAATGCTTTGTTGTTGTATATGGCATGTA 3’

Fig. 4.2b: Nucleotide sequences of local isolates

Isolate KRM

5’CTTTGCCTATGTACAGGAAGCCCAGAATGTACCGGATGTACAGAAGTCCGGATGTT
CCAAGGGGATGTGAAGGCCCATGTAAGGTGCAGTCTTTTGATGCGAAGAACGATATT
GGTCACATGGGTAAGGTTATCTGTCTATCTGATGTTACTAGGGGTATGGGGCTGACCC
ATCGAGTAGGGAAACGTTTTTGCGTGAAGTCATTGTATTTTGTTGGCAAGATATGGAT
GGATGAGAATATTAAGACTAAGAACCATACGAACACCGTTATGTTTTGGATCGTGAG
AGACAGGCGTCCTACAGGCACCCCCTACGATTTCCAGCAAGTGTTCAATGTTTATGAC
AACGAGCCTTCTACGGCTACTGTAAAGAACGACCAGCGTGATCGATTCCAGGTTTTG
AGGAGGTTTCAGGCGACAGTTACAGGAGGACAGTATGCTTGTAAGGAACAAGTTCCA
ATTAGGAAATTCTATCGTGTTAACAATTACGTGGTGTATAATCACCAGGAAGCTGGG
AAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACACATGCCT
CCAACCCA 3’

Isolate PNR

5’CAGGATGTACAGAAGCCCTGATGTTCCTAGAGGATGTGAAGGCCCATGTAAGGTGC
AGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGGTTATCTGTCTATCTGA
TGTTACTAGGGGTATGGGTCTGACCCATCGAGTAGGGAAACGTTTTTGCGTGAAGTCA
TTGTATTTTGTTGGCAAGATATGGATGGATGAGAATATTAAGACTAAGAACCATACG
AACACCGTTATGTTTTGGATCGTGAGAGACAGGCGTCCTACAGGCACCCCCTACGATT
TCCAGCAAGTGTTCAATGTTTATGACAACGAGCCTTCTACGGCTACTGTAAAGAACGA
CCAGCGTGATCGATTCCAGGTTTTGAGGAGGTTTCAGGCGACAGTTACAGGAGGACA
GTATGCTTGTAAGGAACAAGTTCCAATTAGAAATTCTATCGTGTTAACAATTACGTGG
TGTATAATCACCAGGAAGCTGGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGT
TGTATATGC 3’

Isolate PND

5’AATGTACAGGATGTACAGAAGCCCTGATGTTCCTAGAGGATGTGAAGGCCCATGTA
AGGTGCAGTCTTTTGATGCGAAGAACGATATTGGTCACATGGGTAAGGTTATCTGTCT
ATCTGATGTTACTAGGGGTATGGGTCTGACCCATCGAGTAGGGAAACGTTTTTGCGTG
AAGTCATTGTATTTTGTTGGCAAGATATGGATGGATGAGAATATTAAGACTAAGAAC
CATACGAACACCGTTATGTTTTGGATCGTGAGAGACAGGCGTCCTACAGGCACCCCCT
ACGATTTCCAGCAAGTGTTCAATGTTTATGACAACGAGCCTTCTACGGCTACTGTAAA
GAACGACCAGCGTGATCGATTCCAGGTTTTGAGGAGGTTTCAGGCGACAGTTACAGG
AGGACAGTATGCTTGTAAGGAACAAGTTCCAATTAGGAAATTCTATCGTGTTAACAA
TTACGTGGTGTATAATCACCAGGAAGCTGGGAAGTATGAAAATCACACTGAGAATGC
TTTGTTGTTGTATATGC 3’

Fig. 4.2c: Nucleotide sequences of local isolates

followed by 99.60 with MeYVMV, 95.48 per cent with HoYVMV and 93.74 per cent with BYVMV.

Similarly, the nucleotide sequences of one local isolate *i.e.,* VKA which was amplified with OELCuV coat protein specific primer showed similarity with four begomoviruses of bhindi *viz*., BYVMV, OELCuV, MeYVMV and HoYVMV. Among these, the local isolates showed maximum sequence identity of 99.73 per cent with OELCuV followed by 99.46 per cent with MeYVMV, 95.40 per cent with HoYVMV and 93.50 per cent with BYVMV.

The amino acid sequences were deduced using the ExPASy translate tool and the homology of protein were analysed using protein BLAST. The homology analysis of nucleotide sequences and CP amino acid sequences of each isolate was done separately and detailed below.

4.6.5.1. Homology analysis of isolate VKA 1

The sequences of CP gene of local isolate VKA 1 which was collected from Vellanikkara in Thrissur district showed maximum sequence similarity of 100 per cent with accessions of OELCuV isolates from India available in the NCBI database *viz.*, isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and Gandhinagar (KC019308.1) (Fig. 4.5a). This isolate also showed 100 per cent sequence similarity with OELCuV isolates from other countries including Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1). It also showed sequence similarity of 99.82 per cent with two isolates of OELCuV from Hyderabad (MH052569.1and KT898975.1) and 99.63 per cent with Vijayawada isolate (KT898976.1). Apart from these, the isolate also showed sequence similarity of 90.35 to 99.82 per cent with mesta yellow vein mosaic virus (MeYVMV), 91.45 to 96.73 per cent with BYVMV and 90.35 to 95.83 per cent with hollyhock yellow vein mosaic virus (HoYVMV).

On translating the nucleotide sequences of this isolate to amino acids using the ExPASy translate tool, six possible open reading frames (ORFs) (Fig. 4.3a) and

the longest frame *i.e.,* 5’3’ frame 2 was used to deduce the amino acid sequences. It comprised of 180 amino acids (Fig. 4.3b). The protein BLAST analysis of this deduced amino acid sequences showed 100 per cent identity with the coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.5b).

4.6.5.2. Homology analysis of isolate CLR

The sequences of CP gene of local isolate CLR which was collected from Chengaloor in Thrissur district showed maximum sequence similarity of 99.82 per cent similarity to OELCuV isolates from India available in the NCBI database *viz.*, isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and Gandhinagar (KC019308.1) (Fig. 4.6a). It also showed 99.82 per cent sequence similarity with OELCuV isolates from Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1). It also showed 99.63 per cent similarity with the coat protein gene of a south Indian isolate (KT898975.1) from Hyderabad and 99.45 per cent sequence similarity with Vijayawada isolate (KT898976.1) and Ludhiana isolate (KP208672.1) with minimum E value. The isolate also showed sequence similarity of 90.54 to 99.63 per cent with MeYVMV, 91.53 to 96.69 per cent with BYVMV and 90.54 to 95.77 per cent with HoYVMV.

Translation of nucleotide sequence to amino acid sequences using the ExPASy translate tool gave six possible ORFs and the longest frame *i.e.*, 5’3’ frame 1 was used to deduce the amino acid sequences (Fig. 4.4a). It was comprised of 178 amino acids (Fig. 4.4b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with the coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.6b).

Table 4.11: Homology analysis of local isolates

Sl. No.	Isolate	GeneBank Accession number	Query coverage (%)	E value	Identity (%)	Most similar isolate
1	VKA1	MN524912	83-99	0.0	93.81-100	Okra enation leaf curl virus (MK084768.1)
2	CLR	MN524913	100	0.0	93.54-99.82	Okra enation leaf curl virus (MK084768.1)
3	MVA	MN524914	100	0.0	93.78-100	Okra enation leaf curl virus (MK084768.1)
4	VYR1	MN524915	98-99	0.0	93.55-100	Okra enation leaf curl virus (MK084768.1)
5	VYR2	MN524916	99-100	0.0	93.65-100	Okra enation leaf curl virus (MK084768.1)
6	ATR1	MN524917	99	0.0	93.84-99.82	Okra enation leaf curl virus (KT935487.1)
7	ATR2	MN524918	84-100	0.0	93.84-99.82	Okra enation leaf curl virus (KT935487.1)
8	VNY	MN524919	93-100	0.0	93.52-99.63	Okra enation leaf curl virus (KT935487.1)
9	KRM	MN524922	97-100	0.0	93.82-99.85	Okra enation leaf curl virus (KT935487.1)
10	PNR	MN524920	99	0.0	93.35-99.81	Okra enation leaf curl virus (MK084768.1)
11	PND	MN524921	99	0.0	93.42-99.81	Okra enation leaf curl virus (MK084768.1)

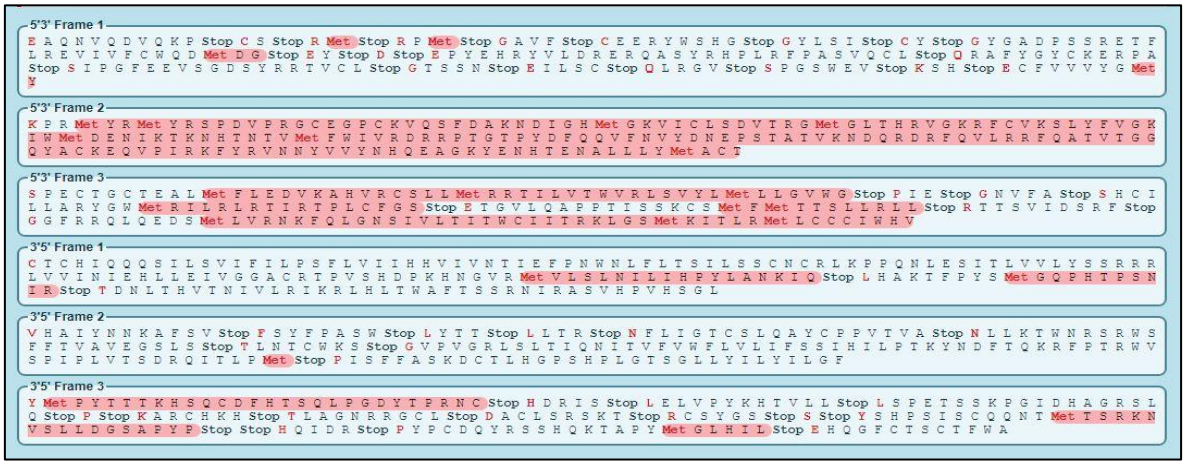


Fig. 4.3a: Open reading frames of local isolate VKA1

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVG
KRFCVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNV
YDNEPSTATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVV
YNHQEAGKYENHTENALLLYMACT

Fig. 4.3b: Amino acid sequences of local isolate VKA1

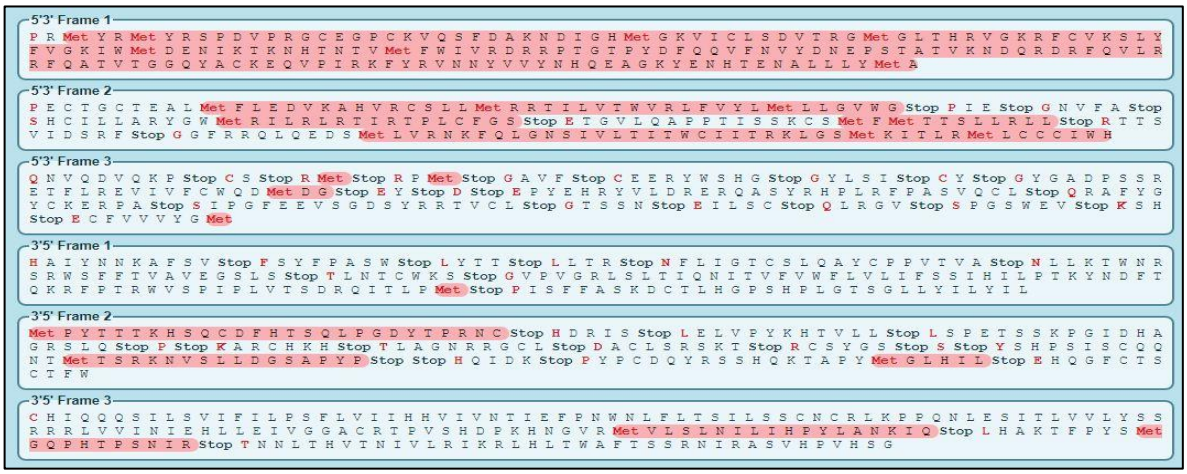
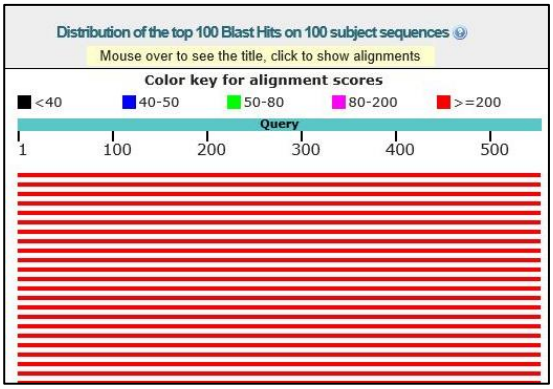


Fig. 4.4a: Open reading frames of local isolate CLR

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRV
GKRFCVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFN
VYDNEPSTATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYV
VYNHQEAGKYENHTENALLLYMA

Fig. 4.4b: Amino acid sequences of local isolate CLR



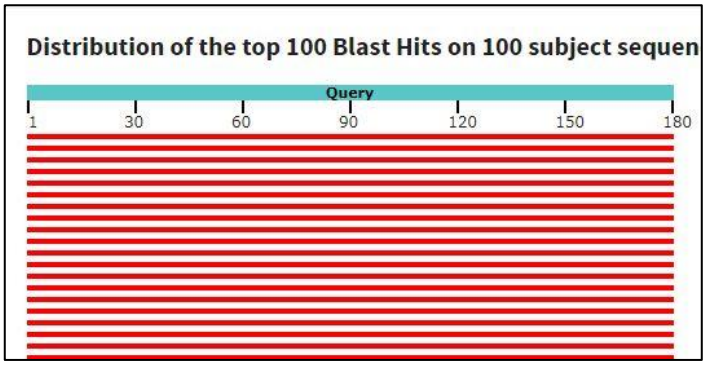
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	1014	1014	99%	0.0	100.00%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	1014	1014	99%	0.0	100.00%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	1014	1014	99%	0.0	100.00%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	1014	1014	99%	0.0	100.00%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activat	1014	1014	99%	0.0	100.00%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	1014	1014	99%	0.0	100.00%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	1014	1014	99%	0.0	100.00%	KX698091.1

Text output of BLASTn analysis

Fig 4.5a: BLASTn analysis of local isolate VKA1



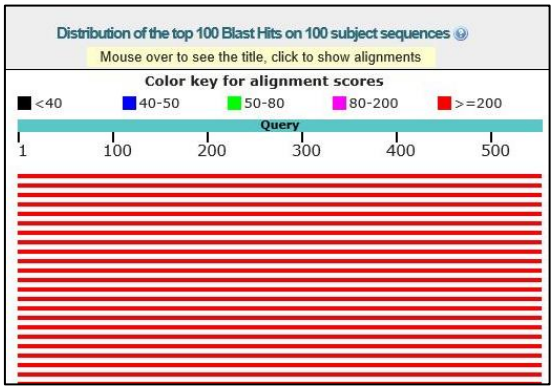
Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	385	385	100%	1e-134	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	385	385	100%	1e-134	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	384	384	100%	4e-134	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	384	384	100%	5e-134	99.44%	CDI30248.2
coat protein [Okra enation leaf curl virus]	384	384	100%	5e-134	99.44%	AGF33920.1

Text output of BLASTp analysis

Fig 4.5b: BLASTp analysis of local isolate VKA1



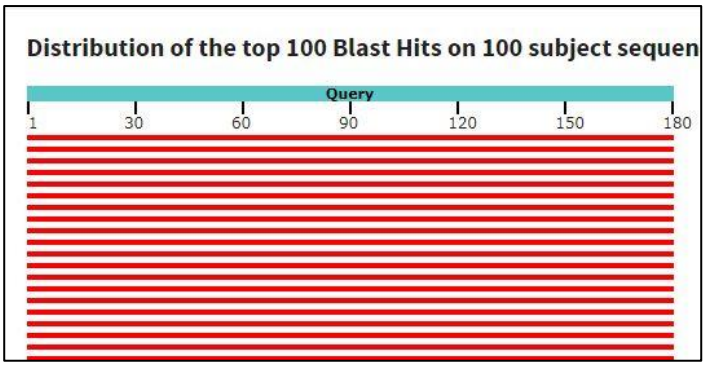
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	996	996	100%	0.0	99.82%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	996	996	100%	0.0	99.82%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	996	996	100%	0.0	99.82%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	996	996	100%	0.0	99.82%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	996	996	100%	0.0	99.82%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	996	996	100%	0.0	99.82%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	996	996	100%	0.0	99.82%	KX698091.1
Okra enation leaf curl virus isolate Trincomalee segment DNA-A, complete sequence	996	996	100%	0.0	99.82%	NC_031754.1
Okra enation leaf curl virus isolate Surat AV2, coat protein, AC5, Ren, TrAP, Rep, and AC4 genes, complete cds	996	996	100%	0.0	99.82%	KC342220.1

Text output of BLASTn analysis

Fig 4.6a: BLASTn analysis of local isolate CLR



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	381	381	100%	9e-133	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	380	380	100%	1e-132	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	380	380	100%	1e-132	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	380	380	100%	2e-132	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	380	380	100%	2e-132	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	380	380	100%	3e-132	99.44%	CDI30248.2
coat protein [Okra enation leaf curl virus]	379	379	100%	3e-132	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	379	379	100%	4e-132	99.44%	AGF33920.1
coat protein [Okra enation leaf curl virus]	379	379	100%	5e-132	99.44%	ALB26005.1

Text output of BLASTp analysis

Fig 4.6b: BLASTp analysis of local isolate CLR

4.6.5.3. Homology analysis of isolate VYR 1 and VYR 2

The isolate VYR 1 and VYR 2 were collected from two different fields from Viyyur in Thrissur district. The BLASTn analysis was carried out for the isolate VYR1 and VYR2 revealed that this isolate showed maximum similarity of 99.81 per cent with the accessions of OELCuV available in the NCBI database *viz.*, isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and Gandhinagar (KC019308.1) (Fig. 4.9a and Fig. 4.10a). This isolate also showed 99.81 per cent sequence similarity with OELCuV isolates from other countries including Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1). It also showed sequence similarity of 99.62 per cent with two south Indian isolates of OELCuV from Hyderabad (MH052569.1and KT898975.1) and 99.63 per cent with Vijayawada isolate (KT898976.1). The isolate also exhibited sequence similarity of 89.45 to 99.80 per cent with MeYVMV, 91.42 to 97.27 per cent with BYVMV and 89.84 to 95.53 per cent with HoYVMV.

On translating the nucleotide sequence to amino acid sequences using the ExPASy translate tool, six ORFs were obtained and the longest frame *i.e.*, 5'3'frame 2 was used to deduce the amino acid sequences (Fig. 4.7a and Fig. 4.8a). It was comprised of 191 amino acids (Fig.4.7b and Fig. 4.8b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with the coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.9b and 4.10b).

4.6.5.4. Homology analysis of isolate ATR 1 and ATR 2

The isolate ATR 1 and ATR 2 were collected from two different locations of Attoor in Thrissur district. BLASTn analysis of nucleotide sequences of isolate ATR1 and ATR2 showed 99.82 per cent similarity to coat protein gene of OELCuV isolates from Vijayawada (KT935487.1) (Fig. 4.13a and Fig. 4.14a). It also showed 99.46 per cent sequence similarity to isolate OK143-PUNJ (KT 390317.1) from Ludhiana, Punjab. This isolate also showed 99.09 per cent sequence identity with

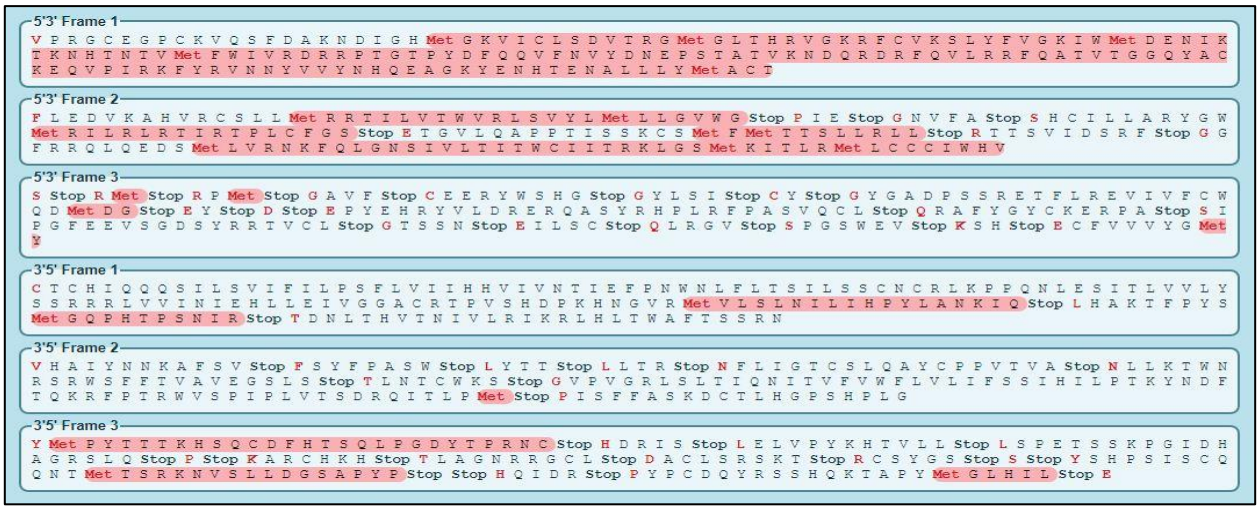


Fig. 4.7a: Open reading frames of local isolate VYR1

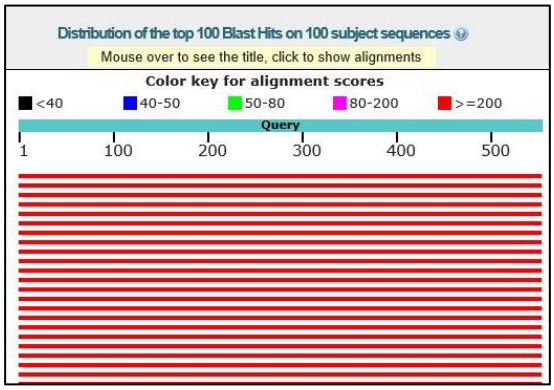
MGKVICLSDVTRGMGLTHRVGKRFCVKSLYFVGKIWMDENIKTKNHTNTVMFWIV
RDRRPTGTPYDFQQVFNVYDNEPSTATVKNDQRDRFQVLRRFQATVTGGQYACKE
QVPIRKFYRVNNYVVYNHQEAGKYENHTENALLLYMACT

Fig. 4.7b: Amino acid sequences of local isolate VYR1

Fig. 4.8a: Open reading frames of local isolate VYR2

MYRKPRMYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLT
HRVGKRFCVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFN
VYDNEPSTATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYN
HQEAGKYENHTENALLLYMACTHAFQH

Fig. 4.8b: Amino acid sequences of local isolate VYR2



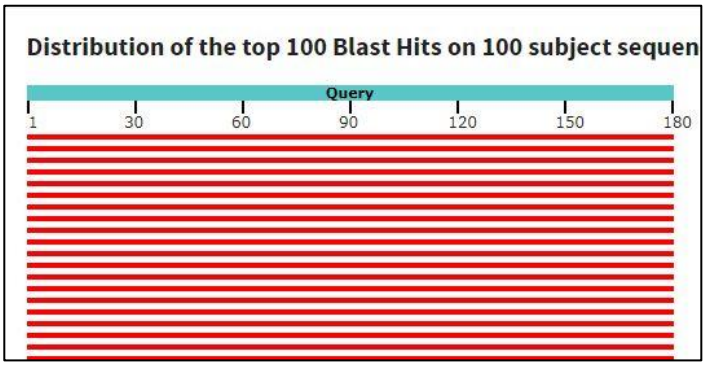
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	946	946	99%	0.0	100.00%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	946	946	99%	0.0	100.00%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	946	946	99%	0.0	100.00%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	946	946	99%	0.0	100.00%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activat	946	946	99%	0.0	100.00%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	946	946	99%	0.0	100.00%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	946	946	99%	0.0	100.00%	KX698091.1
Okra enation leaf curl virus isolate Trincomalee segment DNA-A, complete sequence	946	946	99%	0.0	100.00%	NC_031754.1
Okra enation leaf curl virus isolate Surat AV2, coat protein, AC5, Ren, TrAP, Rep, and AC4 genes, complete cds	946	946	99%	0.0	100.00%	KC342220.1

Text output of BLASTn analysis

Fig 4.9a: BLASTn analysis of local isolate VYR 1



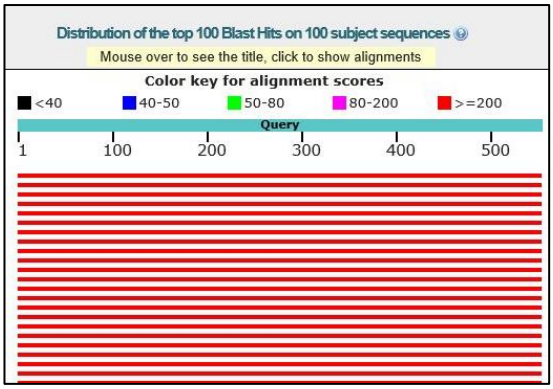
Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	318	318	100%	2e-108	100.00%	ALN96436.1
coat protein [Okra enation leaf curl virus]	318	318	100%	3e-108	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	318	318	100%	3e-108	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	317	317	100%	3e-108	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	317	317	100%	3e-108	100.00%	AGF33914.1
AV1 coat protein [Okra enation leaf curl virus]	314	314	100%	3e-108	99.33%	ATL75711.1
coat protein [Okra enation leaf curl virus]	317	317	100%	4e-108	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	317	317	100%	6e-108	99.33%	CDI30248.2

Text output of BLASTp analysis

Fig 4.9b: BLASTp analysis of local isolate VYR 1



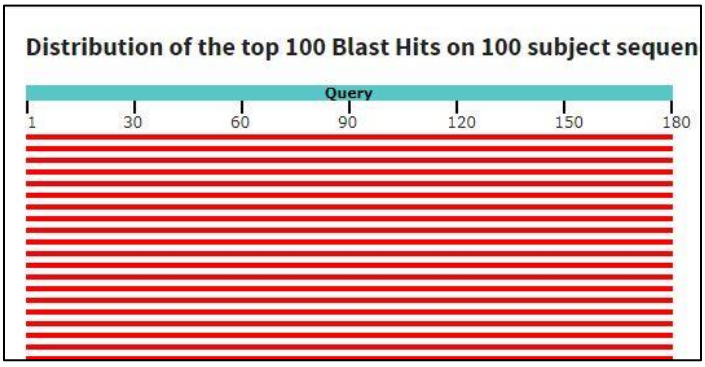
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	1044	1044	97%	0.0	99.82%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	1044	1044	97%	0.0	99.82%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	1044	1044	97%	0.0	99.82%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	1044	1044	97%	0.0	99.82%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	1044	1044	97%	0.0	99.82%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	1044	1044	97%	0.0	99.82%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	1044	1044	97%	0.0	99.82%	KX698091.1
Okra enation leaf curl virus isolate Trincomalee segment DNA-A, complete sequence	1044	1044	97%	0.0	99.82%	NC_031754.1
Okra enation leaf curl virus isolate Surat AV2, coat protein, AC5, Ren, TrAP, Rep, and AC4 genes, complete cds	1044	1044	97%	0.0	99.82%	KC342220.1

Text output of BLASTn analysis

Fig 4.10a: BLASTn analysis of local isolate VYR 2



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	402	402	98%	4e-141	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	402	402	98%	4e-141	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	402	402	98%	5e-141	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	402	402	98%	6e-141	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	402	402	98%	9e-141	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	400	400	98%	1e-140	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	401	401	98%	2e-140	99.47%	CDI30248.2
coat protein [Okra enation leaf curl virus]	401	401	98%	2e-140	99.47%	AGF33920.1

Text output of BLASTp analysis

Fig 4.10b: BLASTp analysis of local isolate VYR 2

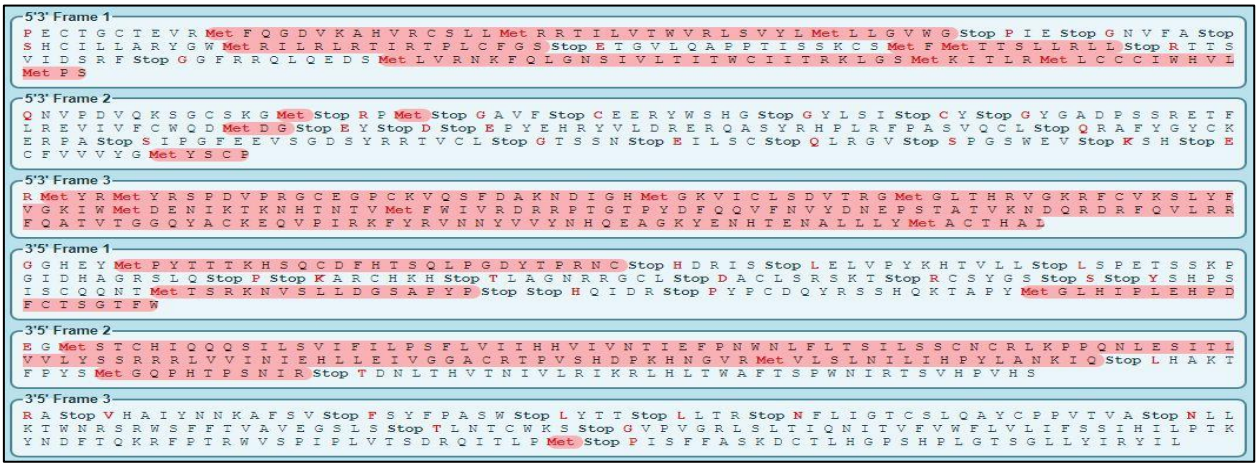


Fig. 4.11a: Open reading frames of local isolate ATR1

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRF
CVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPS
TATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGK
YENHTENALLLYMACTHAL

Fig. 4.11b: Amino acid sequences of local isolate ATR1

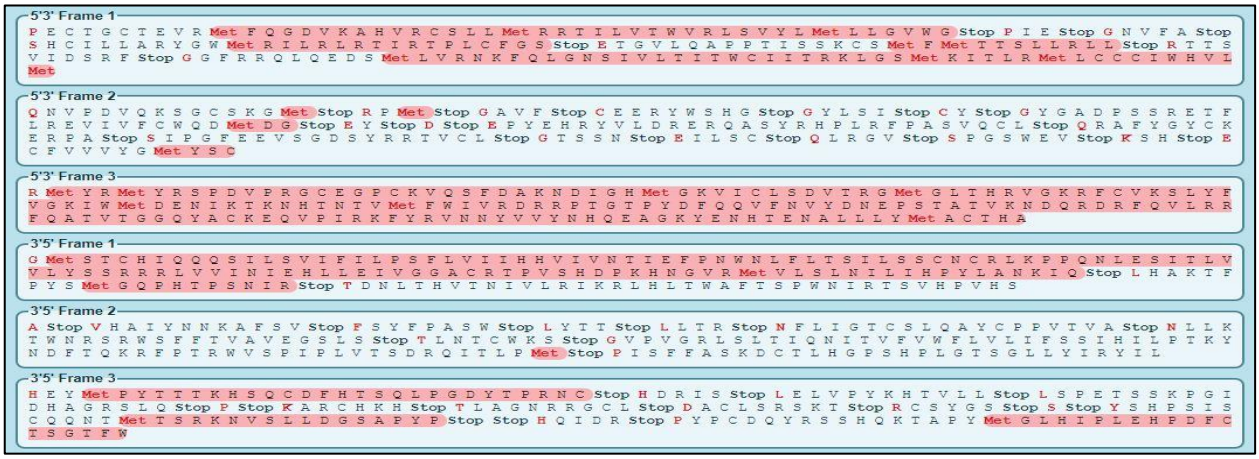
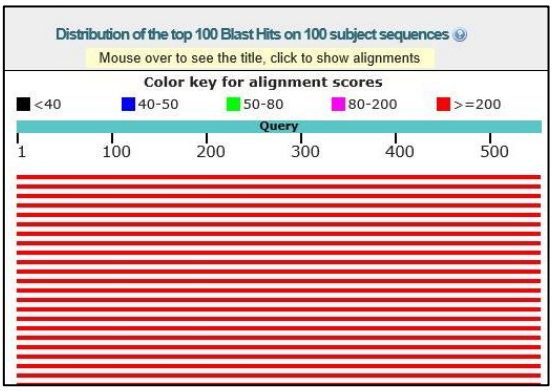


Fig. 4.12a: Open reading frames of local isolate ATR2

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRF
CVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPS
TATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGK
YENHTENALLLYMACTHA

Fig. 4.12b: Amino acid sequences of local isolate ATR2



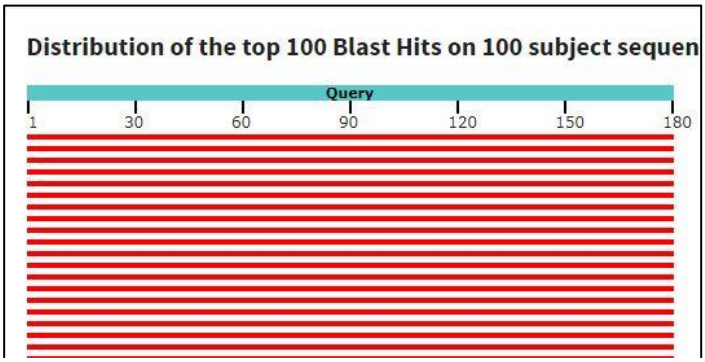
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus isolate Vijayawada coat protein (AV1) gene, complete cds	1013	1013	100%	0.0	99.82%	KT935487.1
Okra enation leaf curl virus isolate OK143-PUNJ segment DNA A, complete sequence	1002	1002	100%	0.0	99.46%	KT390317.1
Okra enation leaf curl virus isolate OK92-HR segment DNA A, complete sequence	1002	1002	100%	0.0	99.46%	KT390310.1
Okra enation leaf curl virus clone 6.2, complete genome	990	990	100%	0.0	99.09%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	990	990	100%	0.0	99.09%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	990	990	100%	0.0	99.09%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	990	990	100%	0.0	99.09%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	990	990	100%	0.0	99.09%	KX710156.1

Text output of BLASTn analysis

Fig 4.13a: BLASTn analysis of local isolate ATR 1



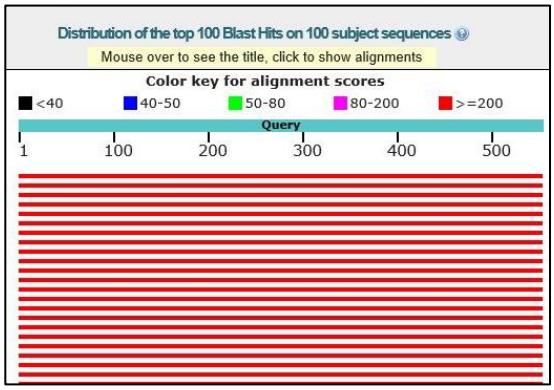
Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	391	391	99%	1e-136	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	390	390	99%	2e-136	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	390	390	99%	2e-136	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	390	390	99%	2e-136	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	390	390	99%	2e-136	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	389	389	99%	5e-136	99.45%	AGF33920.1
coat protein [Okra enation leaf curl virus]	389	389	99%	5e-136	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	389	389	99%	7e-136	98.90%	ALB26345.1

Text output of BLASTp analysis

Fig 4.13b: BLASTp analysis of local isolate ATR 1



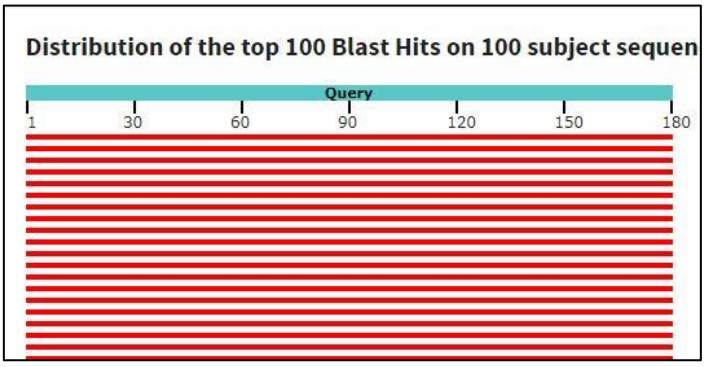
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus isolate Vijayawada coat protein (AV1) gene, complete cds	1013	1013	99%	0.0	99.82%	KT935487.1
Okra enation leaf curl virus isolate OK143-PUNJ segment DNA A, complete sequence	1002	1002	99%	0.0	99.46%	KT390317.1
Okra enation leaf curl virus isolate OK92-HR segment DNA A, complete sequence	1002	1002	99%	0.0	99.46%	KT390310.1
Okra enation leaf curl virus clone 6.2, complete genome	990	990	99%	0.0	99.09%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	990	990	99%	0.0	99.09%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	990	990	99%	0.0	99.09%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	990	990	99%	0.0	99.09%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	990	990	99%	0.0	99.09%	KX710156.1

Text output of BLASTn analysis

Fig 4.14a: BLASTn analysis of local isolate ATR 2



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	390	390	100%	2e-136	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	390	390	100%	2e-136	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	390	390	100%	2e-136	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	390	390	100%	2e-136	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	390	390	100%	4e-136	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	389	389	100%	6e-136	99.45%	AGF33920.1
coat protein [Okra enation leaf curl virus]	388	388	100%	6e-136	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	389	389	100%	7e-136	99.45%	CDI30248.2

Text output of BLASTp analysis

Fig 4.14b: BLASTp analysis of local isolate ATR 2

other accessions including from Mattara (KX698092.1), Puttalam (KX698091.1), Trincomalee (NC031754.1), Surat (KC342220.1) and Gandhinagar (KC019308.1). The isolate also exhibited 98.91 per cent sequence similarity with coat protein gene of two south Indian isolate of OELCuV from Hyderabad (MH052569.1and KT898975.1). The isolate also exhibited sequence similarity of 89.96 to 98.91per cent with MeYVMV, 92.21 to 97.83 per cent with BYVMV and 89.93 to 95.12per cent with HoYVMV.

The nucleotide sequence was translated to six possible ORFs using the ExPASy translate tool and the longest frame *i.e.,* 5'3' frame 3, out of the six ORFs was used to deduce the amino acid sequences (Fig. 4.11a and 4.12a). It was comprised of 183 amino acids (4.11b and 4.12b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with the coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.13b and Fig.4.14b).

4.6.5.5. Homology analysis of isolate MVA

The isolate MVA was collected from Muthuvara in Thrissur district showed 100 per cent sequence similarity with accessions of OELCuV in the NCBI database which include isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and also isolates from other countries like Pakistan (KX710156.1) (Fig. 4.17a). It also showed 99.82 per cent sequence similarity with two south Indian isolates of OELCuV from Hyderabad (MH052569.1and KT898975.1) and 99.63 per cent sequence similarity with Vijayawada isolate (KT898976.1). The MVA isolate also exhibited sequence similarity of 90.35 to 99.82 per cent with MeYVMV, 91.61 to 96.90 per cent with BYVMV and 90.31 to 95.81per cent with HoYVMV.

On translating the nucleotide sequence to amino acid sequences using the ExPASy translate tool, the longest frame *i.e.,* 5'3' Frame 1, out of the six ORFs was used to deduce the amino acid sequences (Fig. 4.15a). It was comprised of 180 amino acids (Fig. 4.15b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with the coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.17b).

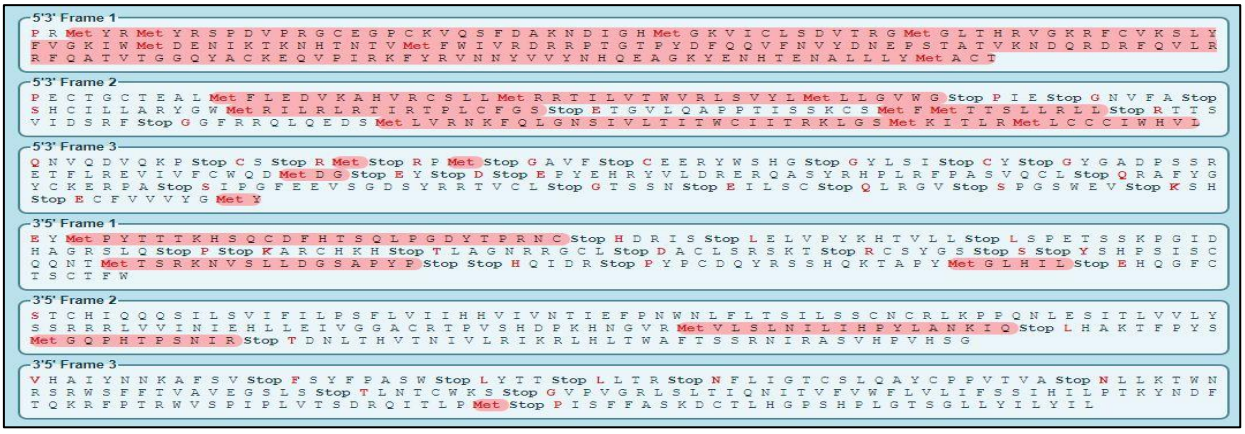


Fig. 4.15a: Open reading frames of local isolate MVA

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRF
CVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPS
TATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGK
YENHTENALLLYMACT

Fig. 4.15b: Amino acid sequences of local isolate MVA

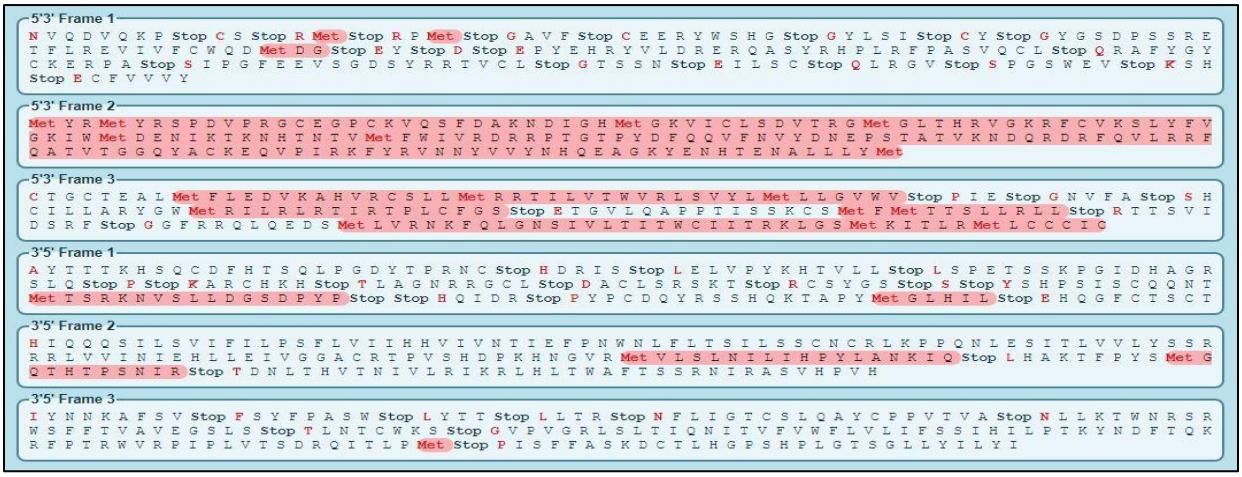
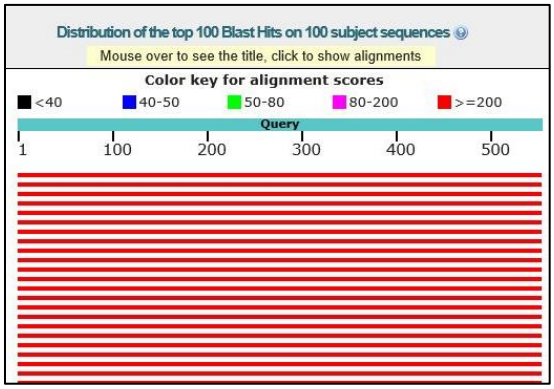


Fig. 4.16a: Open reading frames of local isolate PND

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRF
CVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPS
TATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGK
YENHTENALLLYM

Fig. 4.16b: Amino acid sequences of local isolate PND



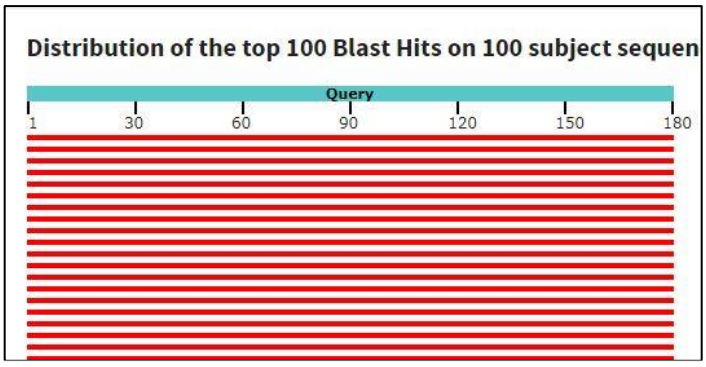
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	1011	1011	100%	0.0	100.00%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	1011	1011	100%	0.0	100.00%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	1011	1011	100%	0.0	100.00%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	1011	1011	100%	0.0	100.00%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activat	1011	1011	100%	0.0	100.00%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	1011	1011	100%	0.0	100.00%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	1011	1011	100%	0.0	100.00%	KX698091.1
Okra enation leaf curl virus isolate Trincomalee segment DNA-A, complete sequence	1011	1011	100%	0.0	100.00%	NC_031754.1
Okra enation leaf curl virus isolate Surat AV2, coat protein, AC5, Ren, TrAP, Rep, and AC4 genes, complete cds	1011	1011	100%	0.0	100.00%	KC342220.1

Text output of BLASTn analysis

Fig 4.17a: BLASTn analysis of local isolate MVA



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignmer

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	385	385	100%	1e-134	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	385	385	100%	1e-134	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	385	385	100%	2e-134	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	384	384	100%	4e-134	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	384	384	100%	5e-134	99.44%	CDI30248.2
coat protein [Okra enation leaf curl virus]	384	384	100%	5e-134	99.44%	AGF33920.1

Text output of BLASTp analysis

Fig 4.17b: BLASTp analysis of local isolate MVA

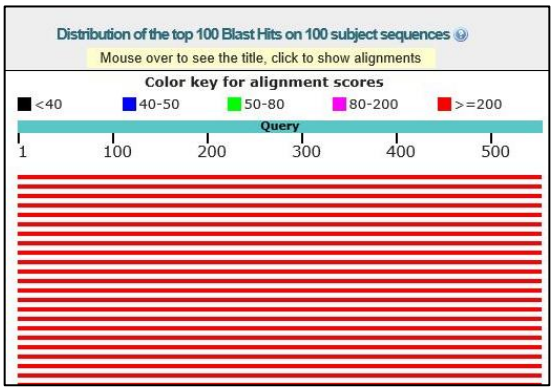
4.6.5.6. Homology analysis of isolate PND

The isolate PND was collected from Padannakkad in Kasaragod district showed maximum similarity of 99.81 per cent with accessions of OELCuV in the NCBI database *viz.,* isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1), Gandhinagar (KC019308.1) and also isolates reported from other countries like Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1) (Fig. 4.18a). It also showed 99.62 per cent sequence similarity with two south Indian isolates of OELCuV from Hyderabad (MH052569.1and KT898975.1) and 99.63 per cent sequence similarity with Vijayawada isolate (KT898976.1). This isolate also exhibited sequence similarity of 90.33 to 99.62 per cent with MeYVMV, 91.56 to 96.62 per cent with BYVMV and 90.23 to 95.51 per cent with HoYVMV.

The nucleotide sequence was translated to six possible ORFs using the ExPASy translate tool and the longest frame *i.e.,* 5'3' frame 2, out of the six possible ORFs was used to deduce the amino acid sequences (Fig. 4.16a). It was comprising of 177 amino acids (Fig. 4.16b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.18b).

4.6.5.7. Homology analysis of isolate PNR

The isolate PNR was collected from Panniyur in Kannur district showed maximum similarity of 99.81 per cent to ten OELCuV isolates in NCBI database *viz.,* isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and Gandhinagar (KC019308.1) and isolates from other countries like Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1), Trincomalee (NC031754.1) (Fig 4.21a). It also showed 99.62 per cent sequence similarity with south Indian isolate of OELCuV from Hyderabad (KT898975.1) and 99.43 per cent sequence similarity with Vijayawada isolate (KT898976.1). This isolate also exhibited sequence similarity of 89.54 to 99.62 per



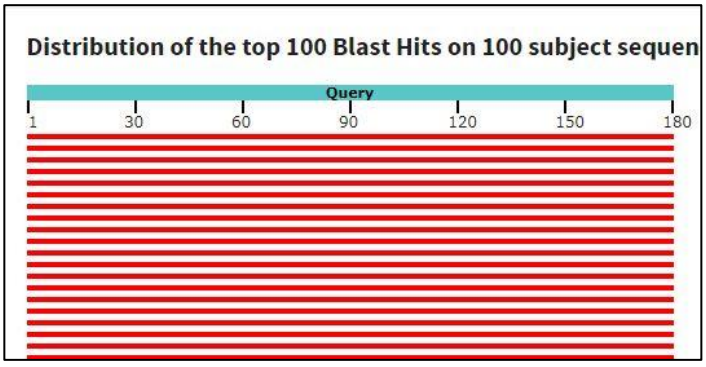
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	977	977	99%	0.0	99.81%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	977	977	99%	0.0	99.81%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	977	977	99%	0.0	99.81%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	977	977	99%	0.0	99.81%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	977	977	99%	0.0	99.81%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	977	977	99%	0.0	99.81%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	977	977	99%	0.0	99.81%	KX698091.1
Okra enation leaf curl virus isolate Trincomalee segment DNA-A, complete sequence	977	977	99%	0.0	99.81%	NC_031754.1
Okra enation leaf curl virus isolate Surat AV2, coat protein, AC5, Ren, TrAP, Rep, and AC4 genes, complete cds	977	977	99%	0.0	99.81%	KC342220.1

Text output of BLASTn analysis

Fig 4.18a: BLASTn analysis of local isolate PND



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	379	379	100%	5e-132	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	379	379	100%	6e-132	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	379	379	100%	7e-132	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	379	379	100%	8e-132	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	378	378	100%	1e-131	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	377	377	100%	2e-131	99.44%	AGF33920.1
coat protein [Okra enation leaf curl virus]	377	377	100%	2e-131	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	377	377	100%	2e-131	99.44%	CDI30248.2

Text output of BLASTp analysis

Fig 4.18b: BLASTp analysis of local isolate PND

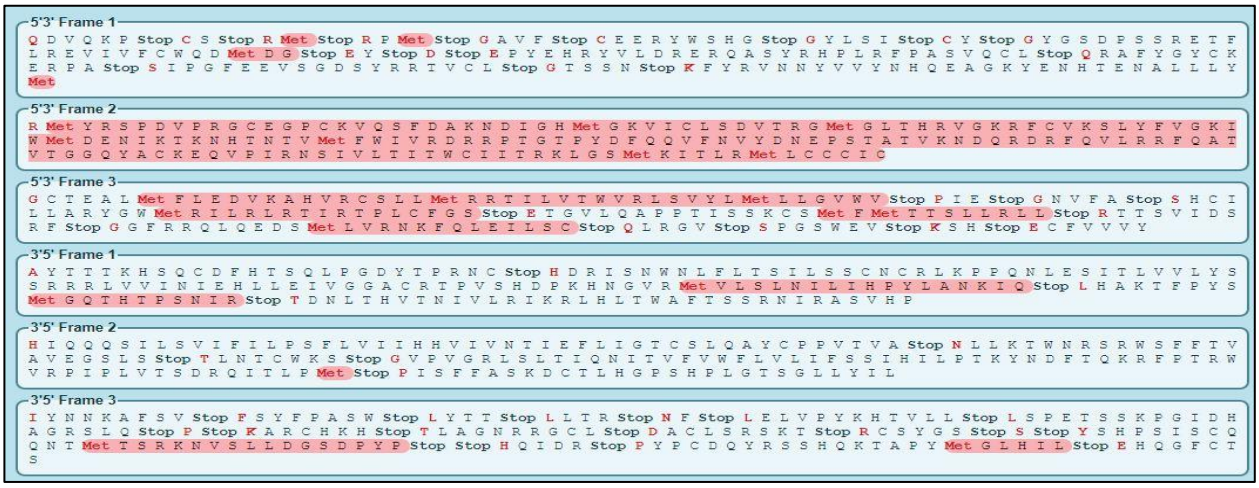


Fig. 4.19a: Open reading frames of local isolate PNR

MYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRFCVK
SLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPSTAT
VKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGKYEN
HTENALLLYM

Fig. 4.19b: Amino acid sequences of local isolate PNR

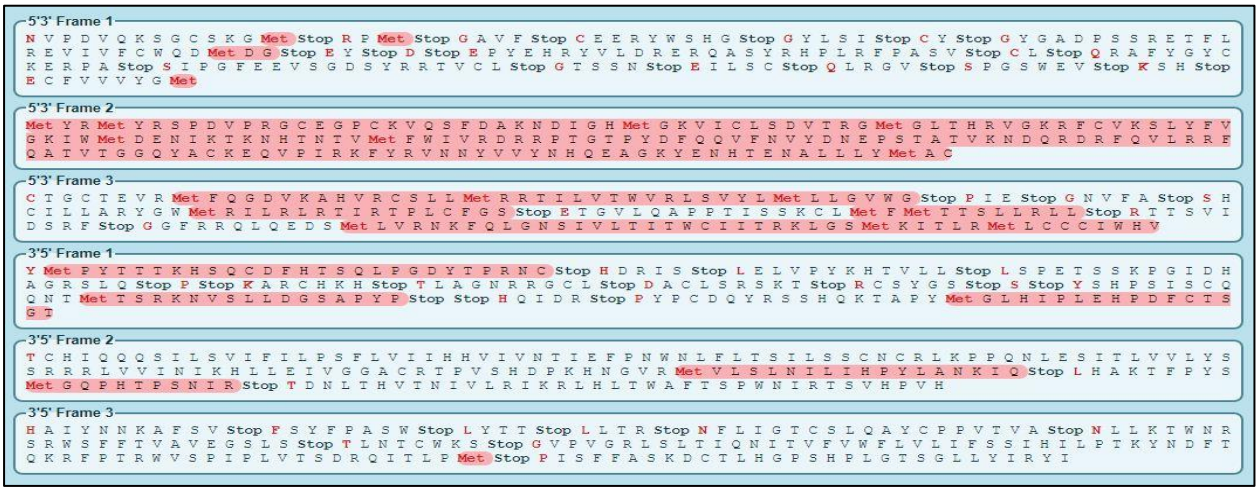
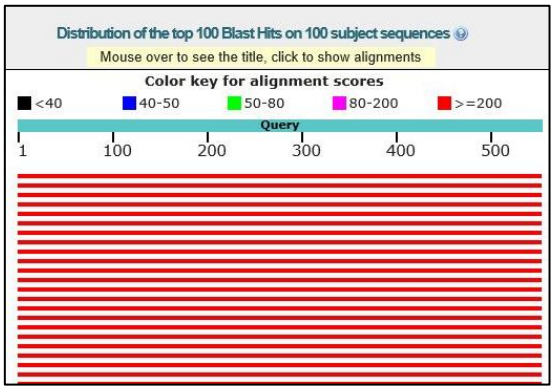


Fig. 4.20a: Open reading frames of local isolate VNY

MYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLTHRVGKRF
CVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFNVYDNEPS
TATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYNHQEAGK
YENHTENALLLYMAC

Fig. 4.20b: Amino acid sequences of local isolate VNY



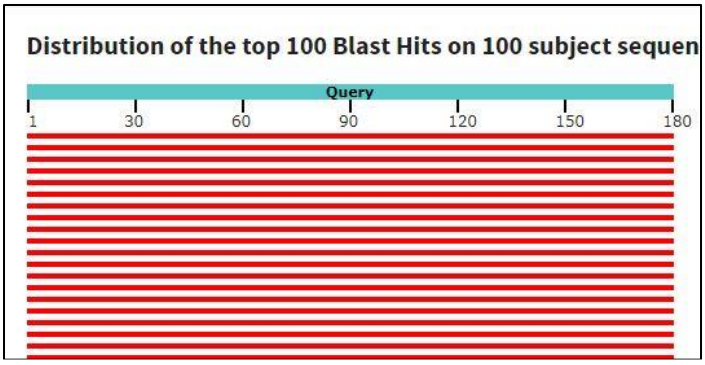
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus clone 6.2, complete genome	959	959	99%	0.0	99.62%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	959	959	99%	0.0	99.62%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	959	959	99%	0.0	99.62%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	959	959	99%	0.0	99.62%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	959	959	99%	0.0	99.62%	KX710156.1
Okra enation leaf curl virus isolate Matara segment DNA-A, complete sequence	959	959	99%	0.0	99.62%	KX698092.1
Okra enation leaf curl virus isolate Puttalam segment DNA-A, complete sequence	959	959	99%	0.0	99.62%	KX698091.1

Text output of BLASTn analysis

Fig 4.21a: BLASTn analysis of local isolate PNR



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	372	372	100%	2e-129	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	372	372	100%	2e-129	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	372	372	100%	3e-129	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	372	372	100%	3e-129	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	372	372	100%	3e-129	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	371	371	100%	5e-129	99.43%	CDI30248.2
coat protein [Okra enation leaf curl virus]	371	371	100%	7e-129	99.43%	AGF33920.1

Text output of BLASTp analysis

Fig 4.21b: BLASTp analysis of local isolate PNR

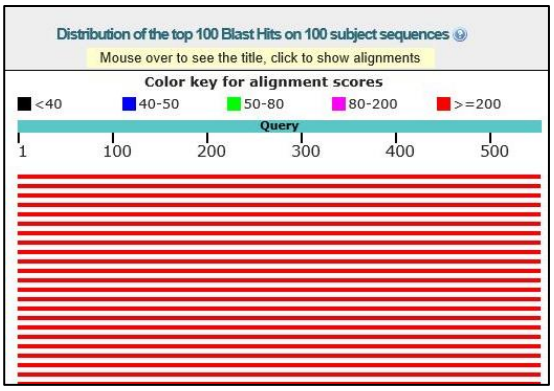
cent with MeYVMV, 91.56 to 96.62 per cent with BYVMV and 90.11 to 95.45per cent with HoYVMV.

On translating the nucleotide sequence to amino acid sequences using the ExPASy translate tool, six open reading frames were obtained and the longest frame *i.e.*, 5'3' frame 2 was used to deduce the amino acid sequences (Fig. 19a). It was comprised of 174 amino acids (Fig. 4.19b). The protein BLAST analysis of this deduced amino acid sequence showed 100 per cent identity with coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.21b).

4.6.5.8. Homology analysis of isolate VNY

The isolate VNY was collected from Vellayani in Thiruvananthapuram district showed 99.63 per cent similarity to coat protein gene of OELCuV isolates from Vijayawada (KT935487.1). It also showed 99.26 per cent sequence similarity to isolate OK143-PUNJ (KT 390317.1) from Ludhiana, Punjab and isolate OK92-HR (KT 390310.1) from Karnal, Haryana. This isolate also showed 98.89 per cent sequence similarity with ten other accessions *viz.*, Maharashtra (MK084768.1, MK084767.1, MK069435.1), Surat (KC342220.1) and Gandhinagar (KC019308.1), Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1) (Fig. 4.22a). This isolate also exhibited sequence similarity of 89.77 to 98.70 per cent with MeYVMV, 91.85 to 97.59 per cent with BYVMVand 89.80 to 94.82 per cent with HoYVMV.

The nucleotide sequence was translated to six possible ORFs using the ExPASy translate tool and the longest frame *i.e.*, 5'3' frame 2, out of six possible ORFs was used to deduce the amino acid sequences (Fig. 4.20a). It was comprised of 179 amino acids (Fig. 4.20b). The protein BLAST analysis of this deduced amino acid sequences exhibited 100 per cent identity with coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.22b).



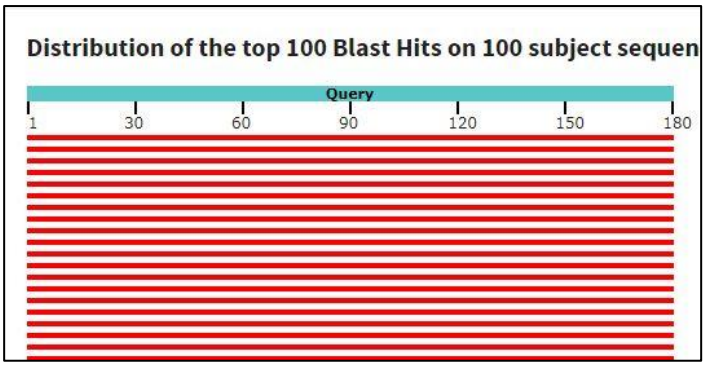
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus isolate Vijayawada coat protein (AV1) gene, complete cds	985	985	100%	0.0	99.63%	KT935487.1
Okra enation leaf curl virus isolate OK143-PUNJ segment DNA A, complete sequence	974	974	100%	0.0	99.26%	KT390317.1
Okra enation leaf curl virus isolate OK92-HR segment DNA A, complete sequence	974	974	100%	0.0	99.26%	KT390310.1
Okra enation leaf curl virus clone 6.2, complete genome	963	963	100%	0.0	98.89%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	963	963	100%	0.0	98.89%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	963	963	100%	0.0	98.89%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	963	963	100%	0.0	98.89%	MK069433.1
Okra enation leaf curl virus clone SZ157 pre-coat protein (V2), coat protein (V1), replication enhancer protein (V3), and transcriptional activato	963	963	100%	0.0	98.89%	KX710156.1

Text output of BLASTn analysis

Fig 4.22a: BLASTn analysis of local isolate VNY



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	384	384	100%	6e-134	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	384	384	100%	8e-134	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	383	383	100%	1e-133	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	383	383	100%	1e-133	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	383	383	100%	2e-133	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	382	382	100%	2e-133	99.44%	CDI30248.2
coat protein [Okra enation leaf curl virus]	382	382	100%	3e-133	99.44%	AGF33920.1
coat protein [Okra enation leaf curl virus]	382	382	100%	3e-133	100.00%	AZL41043.1

Text output of BLASTp analysis

Fig 4.22b: BLASTp analysis of local isolate VNY

4.6.5.9. Homology analysis of isolate KRM

The isolate KRM was collected from Kumarakom in Thrissur district. BLASTn analysis of nucleotide sequences of isolate KRM exhibited 99.13 per cent similarity to coat protein gene of OELCuV isolates from Vijayawada (KT935487.1). It also showed 98.96 per cent sequence similarity to isolate OK143-PUNJ (KT 390317.1) from Ludhiana, Punjab and isolate OK92-HR (KT 390310.1) from Karnal, Haryana (Fig 4.24a). This isolate also exhibited 98.43 per cent sequence identity with other accessions including isolates from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Mattara (KX698092.1), Puttalam (KX698091.1), Trincomalee (NC031754.1), Surat (KC342220.1) and Gandhinagar (KC019308.1). The isolate also exhibited 98.91 per cent sequence similarity with coat protein gene of two south Indian isolate of OELCuV from Hyderabad (MH052569.1and KT898975.1). The isolate also exhibited sequence similarity of 89.96 to 98.91per cent with MeYVMV, 91.56 to 96.62 per cent with BYVMV and 89.93 to 95.12 per cent with HoYVMV.

On translating the nucleotide sequence to amino acid sequences, using the ExPASy translate tool, the longest frame *i.e.,* 5'3' frame 3 was used to deduce the amino acid sequences from the 6 possible open reading frames (ORF) obtained (Fig. 4.23a). It was comprised of 191 amino acids (Fig. 4.23b). The protein BLAST analysis of this deduced amino acid sequences exhibited 100 per cent identity with coat protein of OELCuV Hyderabad isolate (ALN96435.1) (Fig. 4.24b).

4.6.5.10. Identification of virus associated with the disease

The *in silico* analysis of all the local isolates collected from different locations of Thrissur, Thiruvananthapuram, Kottayam, Kannur and Kasaragod revealed that these isolates were identified as okra enation leaf curl virus (OELCuV) belonging to the genus *Begomovirus* and family *Geminiviridae.*

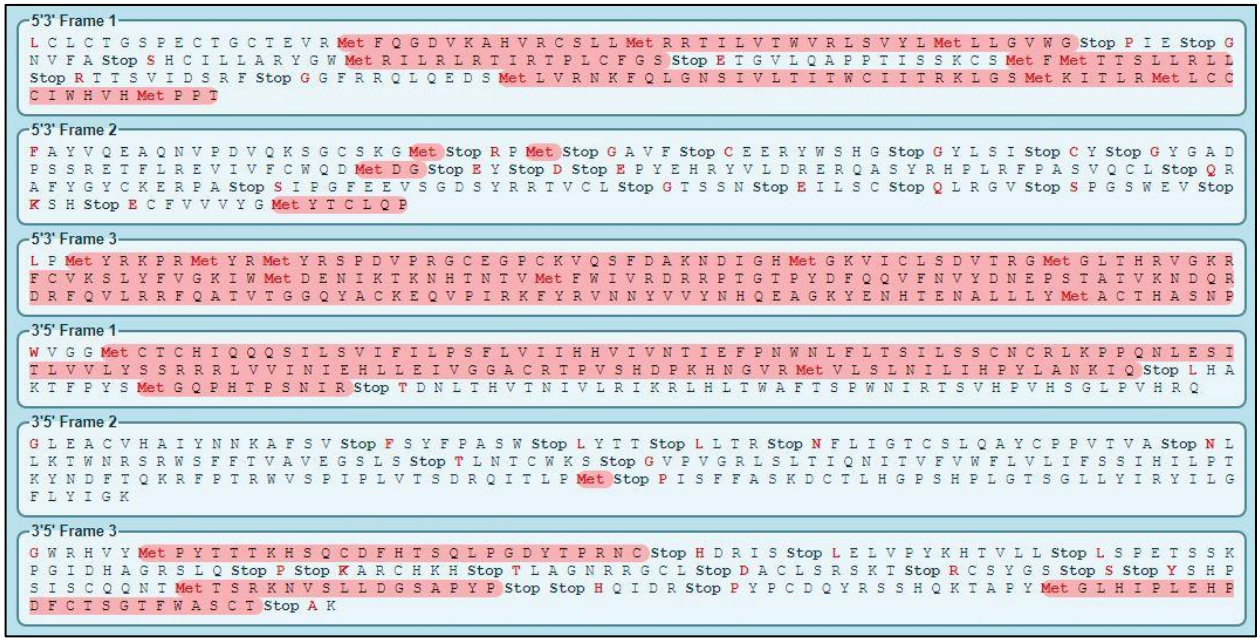
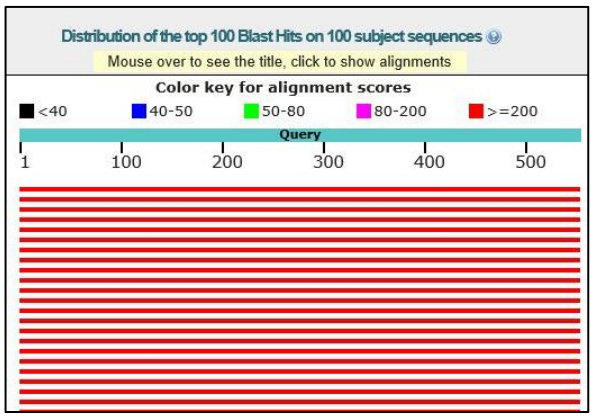


Fig. 4.23a: Open reading frames of local isolate KRM

MYRKPRMYRMYRSPDVPRGCEGPCKVQSFDAKNDIGHMGKVICLSDVTRGMGLT
HRVGKRFCVKSLYFVGKIWMDENIKTKNHTNTVMFWIVRDRRPTGTPYDFQQVFN
VYDNEPSTATVKNDQRDRFQVLRRFQATVTGGQYACKEQVPIRKFYRVNNYVVYN
HQEAGKYENHTENALLLYMACTHASNP

Fig. 4.23b: Amino acid sequences of local isolate KRM



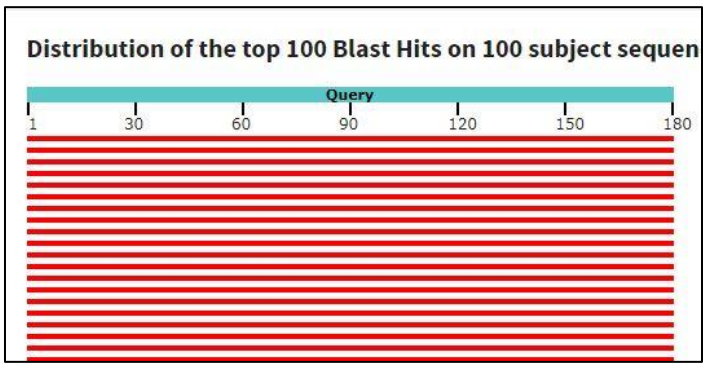
Graphical output of BLASTn analysis

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Okra enation leaf curl virus isolate Vijayawada coat protein (AV1) gene, complete cds	1037	1037	99%	0.0	99.13%	KT935487.1
Okra enation leaf curl virus isolate OK143-PUNJ segment DNA A, complete sequence	1031	1031	99%	0.0	98.96%	KT390317.1
Okra enation leaf curl virus isolate OK92-HR segment DNA A, complete sequence	1031	1031	99%	0.0	98.96%	KT390310.1
Mesta yellow vein mosaic virus isolate Okra:Tirupati:2010 pre-coat protein (V1) gene, partial cds	1018	1018	99%	0.0	98.44%	JX242520.1
Okra enation leaf curl virus clone 6.2, complete genome	1013	1013	98%	0.0	98.43%	MK084768.1
Okra enation leaf curl virus clone 6.1, complete genome	1013	1013	98%	0.0	98.43%	MK084767.1
Okra enation leaf curl virus clone S9C4, complete genome	1013	1013	98%	0.0	98.43%	MK069435.1
Okra enation leaf curl virus clone 1(1), complete genome	1013	1013	98%	0.0	98.43%	MK069433.1

Text output of BLASTn analysis

Fig 4.24a: BLASTn analysis of local isolate KRM



Graphical output of BLASTp analysis

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
coat protein [Okra enation leaf curl virus]	409	409	100%	9e-144	100.00%	ALN96435.1
coat protein [Okra enation leaf curl virus]	409	409	100%	2e-143	100.00%	ALN96434.1
coat protein [Okra enation leaf curl virus]	409	409	100%	2e-143	100.00%	ALB26312.1
coat protein [Okra enation leaf curl virus]	409	409	100%	2e-143	100.00%	AGF33914.1
coat protein [Okra enation leaf curl virus]	408	408	100%	3e-143	100.00%	YP_009315913.1
coat protein [Okra enation leaf curl virus]	407	407	100%	3e-143	100.00%	AZL41043.1
coat protein [Okra enation leaf curl virus]	407	407	100%	5e-143	99.48%	AGF33920.1
coat protein [Okra enation leaf curl virus]	407	407	100%	6e-143	99.48%	CDI30248.2

Text output of BLASTn analysis

Fig 4.24b: BLASTp analysis of local isolate KRM

4.6.6. Confirmation of virus identification using Species Demarcation Tool (SDT)

The identity of the virus associated with yellow vein mosaic disease in bhindi was further confirmed through Species demarcation tool (SDT) which is a tool used for the classification of viruses based on pairwise sequence identity. The top hits downloaded from NCBI database were used to compare the sequences ofocal isolates. SDT analysis revealed that the core CP sequences of all the 11 local isolates were 100 per cent similar to each other with high nucleotide sequence similarity of 99.00 to 100 per cent with OELCuV in okra from India and Pakistan (Fig 4.25). Hence, it confirmed the identity of virus associated with bhindi yellow vein mosaic disease in Kerala as OELCuV belongs to the genus *Begomovirus* and family *Geminiviridae.*

4. 6.7. DNA barcoding

DNA barcoding was also done to confirm the identity of the virus species. The sequences of local isolates were multiple aligned along with that of BYVMV and OELCuV isolates retrieved from NCBI database using Clustal W tool provided by MEGA-X software (Fig. 4.26). The maximum aligned length of coat protein gene of OELCuV were 771 bp and the barcode gaps were identified at 110 alignment positions (Table 4.12). The barcode analysis also confirmed that all the virus isolates in the present study were OELCuV. This barcodes which was developed for BYVMV and OELCuV could be specifically used for the identification of these viruses in future.

4.6.8. Phylogenetic analysis

The top hits of CP gene sequences of different begomovirus infecting bhindi from India and elsewhere were downloaded from NCBI database and were aligned along with the local isolates using ClustalW program in MEGA-X software. The phylogenetic tree was constructed using the aligned sequences in MEGA-X software in Neighbour-joining algorithm with a bootstrap value of 1000. The phylogenetic tree constructed shown in Fig 4.27.

The phylogenetic tree produced two major clades. The first clade included all the local isolates along with already reported OELCuV and MeYVMV isolates. The second clade formed by clustering the isolates of BYVMV and cotton leaf curl virus (CLCV). The analysis of phylogenetic tree revealed similarity between the local isolates and a common ancestor for the local isolates and already reported begomoviruses (BYVMV, CLCV, MeYVMV and OELCuV).

The isolates VYR 1, VYR 2, VKA and MVA collected from Thrissur district formed a single cluster and are closely related with each other. These four isolates showed maximum similarity to OELCuV isolate from Surat. The isolate CLR formed a separate cluster and exhibited maximum similarity with OELCuV isolate from Maharashtra.

The isolate PNR and PND are closely related with each other than the remaining isolates in the present study and showed maximum similarity with OELCuV isolate from Hyderabad. Isolates ATR 1, ATR 2 and KRM clustered into a single group whereas, the VNY isolate formed a separate cluster from the common node indicating that this might be dissimilar from all other isolates.

4.8. MANAGEMENT OF BHINDI YELLOW VEIN MOSAIC DISEASE

A field experiment was conducted to evaluate the effect of insect proof net, insect trap, insecticides, plant extracts, micronutrients and microbial formulations for the management of BYVMD. The natural incidence of disease appeared 25-30 days after sowing (DAS) under open field condition. The vulnerability index indicating the severity of the disease was assessed 10 days interval from the first appearance of symptom using 0-5 scale developed by Ali *et al*. (2005a) as described under section 3.1.

The inference of the experiment was drawn by statistical analysis of the data on per cent disease incidence (PDI), per cent disease severity (PDS), whitefly count, vegetative and yield parameters.

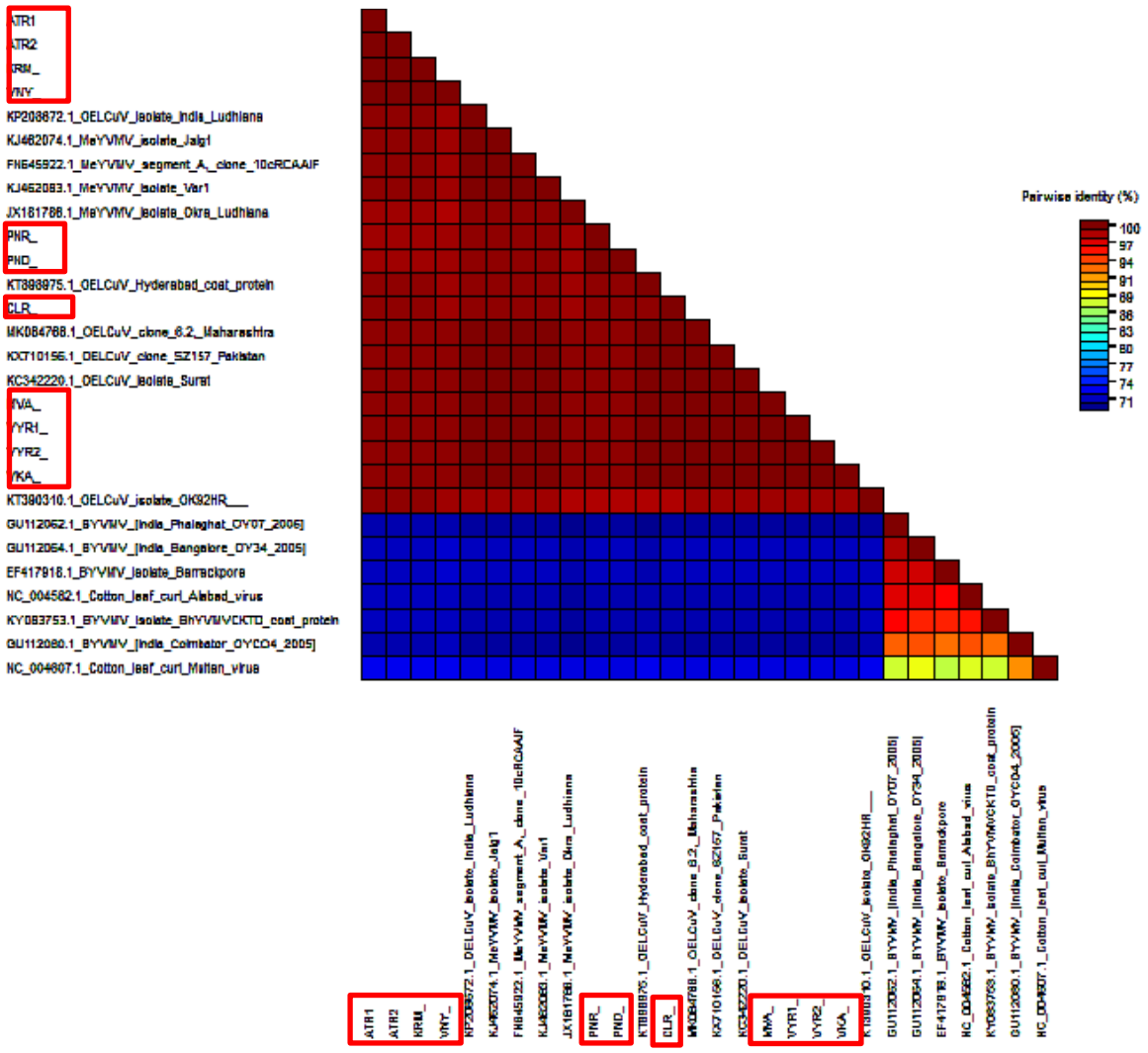


Fig. 4.25: Pairwise nucleotide sequence identities of the core CP sequences of begamoviruses using SDT analysis. The virus isolates identified in this study have been highlighted in boxes.

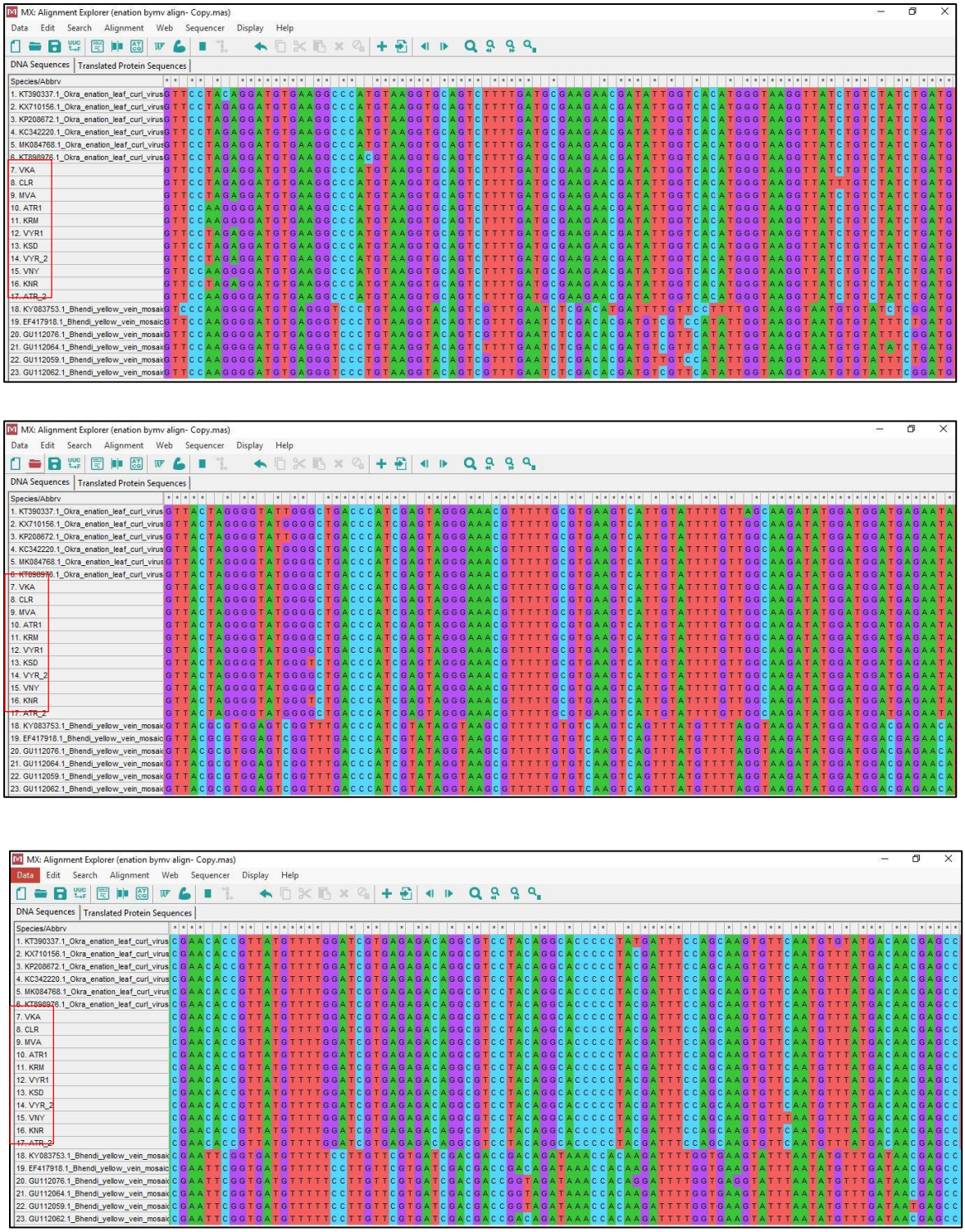


Fig. 4.26.: Multiple sequence alignment in Clustal W. Local isolates were highlighted in red boxes

Table 4.12: DNA barcodes of BYVMV and OELCuV

Virus	Isolate	210	213	222	235	237	239	240	241	251	255	258	267	270	274	288	289	291	294	295	301	312	313	318	321	330	333	340	342
BYVMV	KY083753.1	T	C	A	T	T	G	A	C	T	T	T	A	G	A	G	C	T	A	G	T	T	A	T	G	T	G	G	T
	EF417918.1	T	C	A	T	T	G	A	C	T	T	T	A	G	A	G	C	T	A	G	T	T	A	T	G	T	G	G	T
	GU112080.1	T	C	A	T	T	G	A	C	T	T	T	A	G	A	G	C	T	A	G	T	T	A	T	G	T	G	G	T
	GU112076.1	T	C	A	T	T	G	A	C	T	T	T	A	G	A	G	C	T	A	G	T	T	A	T	G	T	G	G	T
	GU112064.1	T	C	A	T	T	G	A	C	T	T	T	A	G	A	G	C	T	A	G	T	T	A	T	G	T	G	G	T
OELCuV	KT390337.1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	KX710156.1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	KP208672.1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	KC342220.1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	MK084768.1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
Collected isolates	VKA	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	CLR	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	MVA	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	ATR1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	ATR2	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	VYR1	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	VYR2	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	VNY	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	KRM	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	PNR	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G
	PND	C	A	G	G	G	A	G	A	G	C	G	T	C	C	T	A	G	T	A	C	A	G	G	A	C	C	T	G

Table 4.12: contd.

Virus	Isolate	346	349	351	354	369	375	384	399	400	402	405	413	414	415	417	420	421	423	426	427	429	432	435	441	443	444	447	448
BYVMV	KY083753.1	G	T	T	T	C	C	C	T	T	G	G	T	C	C	T	T	C	T	T	C	A	A	G	T	A	A	A	C
	EF417918.1	G	T	T	T	C	C	C	T	T	G	G	T	C	C	T	T	C	T	T	C	A	A	G	T	A	A	A	C
	GU112080.1	G	T	T	T	C	C	C	T	T	G	G	T	C	C	T	T	C	T	T	C	A	A	G	T	A	A	A	C
	GU112076.1	G	T	T	T	C	C	C	T	T	G	G	T	C	C	T	T	C	T	T	C	A	A	G	T	A	A	A	C
	GU112064.1	G	T	T	T	C	C	C	T	T	G	G	T	C	C	T	T	C	T	T	C	A	A	G	T	A	A	A	C
OELCuV	KT390337.1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	KX710156.1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	KP208672.1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	KC342220.1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	MK084768.1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
Collected isolates	VKA	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	CLR	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	MVA	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	ATR1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	ATR2	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	VYR1	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	VYR2	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	VNY	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	KRM	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	PNR	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T
	PND	T	G	A	C	T	T	T	C	A	C	T	G	G	A	C	G	A	A	C	A	G	T	T	C	C	C	C	T

Table 4.12: contd.

Virus	Isolate	456	457	458	459	460	465	468	472	476	480	489	490	491	511	512	516	517	519	527	542	543	545	546	549	552	555	561	564
BYVMV	KY083753.1	T	G	G	T	G	A	T	A	T	T	C	A	G	A	T	T	A	G	A	A	A	G	G	T	A	C	T	T
	EF417918.1	T	G	G	T	G	A	T	A	T	T	C	A	G	A	T	T	A	G	A	A	A	G	G	T	A	C	T	T
	GU112080.1	T	G	G	T	G	A	T	A	T	T	C	A	G	A	T	T	A	G	A	A	A	G	G	T	A	C	T	T
	GU112076.1	T	G	G	T	G	A	T	A	T	T	C	A	G	A	T	T	A	G	A	A	A	G	G	T	A	C	T	T
	GU112064.1	T	G	G	T	G	A	T	A	T	T	C	A	G	A	T	T	A	G	A	A	A	G	G	T	A	C	T	T
OELCuV	KT390337.1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	KX710156.1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	KP208672.1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	KC342220.1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	MK084768.1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
Collected isolates	VKA	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	CLR	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	MVA	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	ATR1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	ATR2	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	VYR1	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	VYR2	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	VNY	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	KRM	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	PNR	C	C	A	G	C	G	C	G	A	C	T	T	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A
	PND	C	C	A	G	C	G	C	G	A	C	C	A	C	G	A	G	C	T	T	G	G	T	T	G	G	A	A	A

Table 4.12: contd.

Virus	Isolate	570	588	590	591	592	593	594	595	599	606	608	610	612	624	627	630	633	636	648	651	654	657	660	682	685	687
BYVMV	KY083753.1	A	G	C	G	T	T	G	G	A	T	T	A	G	T	T	T	C	C	A	A	A	C	G	A	C	T
	EF417918.1	A	G	C	G	T	T	G	G	A	T	T	A	G	T	T	T	C	C	A	A	A	C	G	A	C	T
	GU112080.1	A	G	C	G	T	T	G	G	A	T	T	A	G	T	T	T	C	C	A	A	A	C	G	A	C	T
	GU112076.1	A	G	C	G	T	T	G	G	A	T	T	A	G	T	T	T	C	C	A	A	A	C	G	A	C	T
	GU112064.1	A	G	C	G	T	T	G	G	A	T	T	A	G	T	T	T	C	C	A	A	A	C	G	A	C	T
OELCuV	KT390337.1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	KX710156.1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	KP208672.1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	KC342220.1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	MK084768.1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
Collected isolates	VKA	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	CLR	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	MVA	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	ATR1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	ATR2	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	VYR1	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	VYR2	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	VNY	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	KRM	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	PNR	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G
	PND	G	A	T	T	C	C	A	A	G	C	A	C	T	C	G	G	T	T	T	G	G	T	A	T	T	G

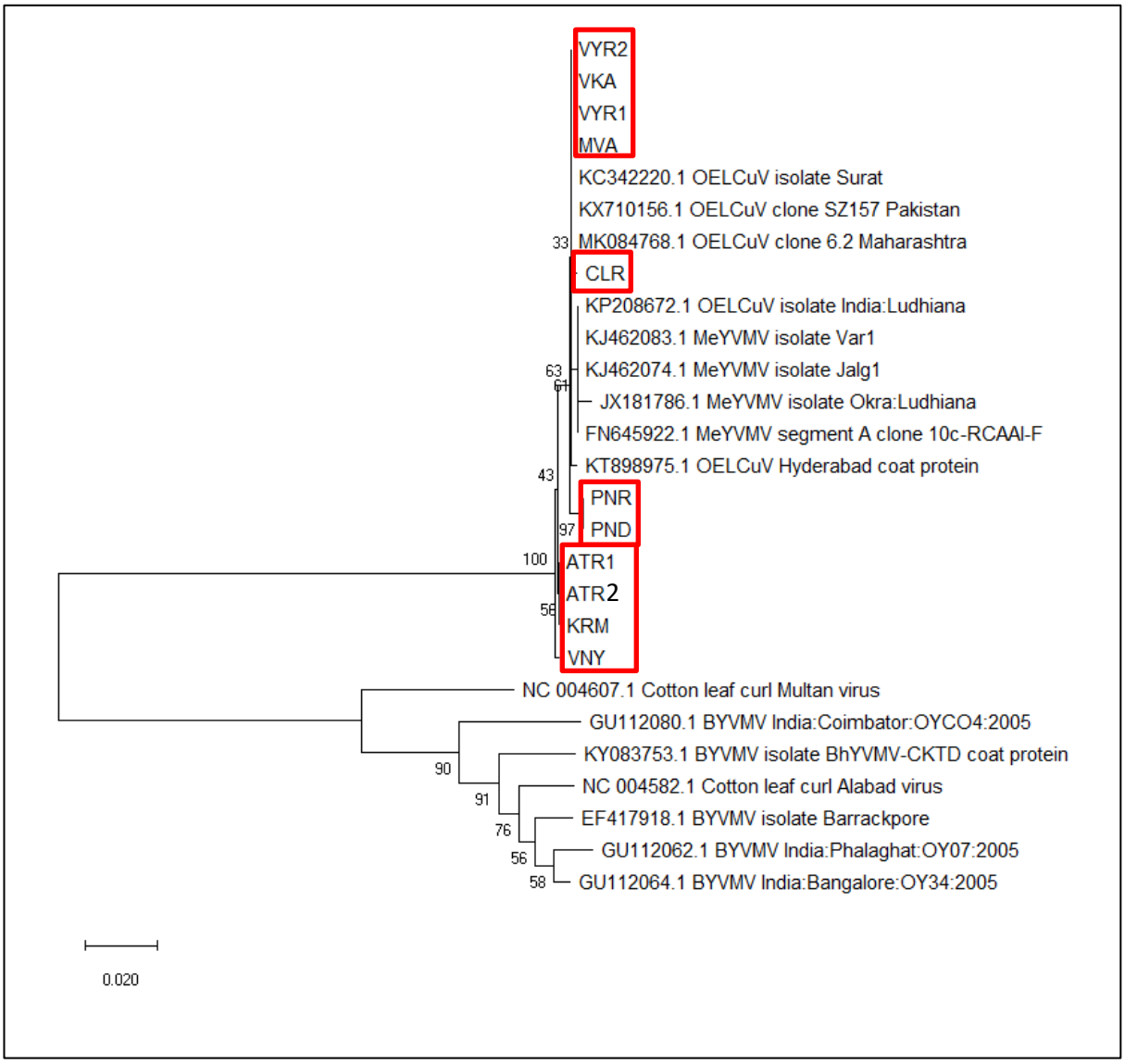


Fig. 4.27.: Neighbour-joining tree representing phylogenetic relationship of OELCuV coat protein sequences of collected isolates and other begomoviruses infecting bhindi. Numbers are per cent support of branching based on bootstrap analysis (1000 replications). The scale bar represents nucleotide substitution per site

4.7.1. Effect of treatments on per cent disease incidence

The statistical analysis of data on PDI at 30, 45, and 60 DAS revealed that all the treatments were significantly superior when compared with the unsprayed control, but the efficacy varied between the treatments. The results are given in Table 4.13 and Fig. 4.28.

At 30 DAS, there was no incidence of the disease in any of the plants in treatment T1 (insect proof net + PGPR mix II + sampoorna), T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii)*, T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) while PDI of 0.62, 3.7 and 1.85 per cent were recorded in plants in T3 (azadiractin), T6 (imidacloprid) and T7 (control) respectively. The highest PDI of 1.85 per cent was recorded in plants with unsprayed control at 30 DAS.

Table 4.13: Effect of treatments on percent disease incidence (PDI)

Treatment details	Per cent disease incidence				Per cent disease reduction over control
	30 DAS	45 DAS	60 DAS	Mean	
T1	0	38.88 ^{a}	68.51 ^{b}	35.80	9.38
T2	0	22.22 ^{cd}	59.26 ^{c}	27.16	31.25
T3	0.62	33.33 ^{ab}	66.66 ^{bc}	33.54	15.10
T4	0	27.77 ^{bcd}	66.66 ^{bc}	31.48	20.31
T5	0	20.37 ^{d}	49.98 ^{d}	23.45	40.62
T6	3.7	29.62 ^{bc}	66.66 ^{bc}	33.33	15.63
T7	1.85	38.88 ^{a}	77.77 ^{a}	39.50	-
CD (0.05)	NA	9.10	8.18	-	-

At 45 DAS, it was observed that the PDI was increased in all the treatments. However, the lowest PDI of 20.37 per cent was recorded in plants in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*)

and T6 (imidacloprid) with PDI of 22.22, 27.77 and 29.62 per cent respectively. While the PDI was highest (38.88 %) in untreated control plants.

At 60 DAS also, the lowest PDI (49.98 %) was recorded in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) followed by T2 (PGPR mix II + *B. spectabilis)* with a PDI of 59.26 per cent. PDI of 66.66 per cent was recorded in treatments T3 (azadiractin), T4 (yellow sticky trap + *L. lecanii*) and T6 (imidachloprid) and are statistically on par. While the highest PDI of 77.77 per cent was recorded in the untreated control plants.

Comparing the mean values of PDI revealed that the treatment T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) recorded lowest PDI (23.45 %) followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*) and T6 (imidachloprid) with PDI of 27.16, 31.48 and 33.33 per cent respectively. The highest PDI of 39.50 per cent was recorded in untreated control plants.

When per cent disease reduction of disease over control was assessed, the maximum per cent reduction of disease was recorded in T5 (40.62 %) followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*), T6 (imidachloprid), T3 (azadiractin) and T1 (insect proof net + PGPR mix II + sampoorna) with a per cent disease reduction of 31.25, 20.13, 15.63, 15.10 and 9.38 per cent respectively.

4.7.2. Effect of treatments on per cent disease severity

The statistical analysis of data on PDS at 30, 45, and 60 DAS showed significant difference among treatments compared with the unsprayed control plants (Table 4.14. and Fig. 4.29).

Table 4.14: Effect of treatments on percent disease severity (PDS)

Treatment details	Per cent disease severity				Per cent reduction over control
	30 DAS	45 DAS	60 DAS	Mean	
T1	0	12.22 [b]	41.48 [b]	17.90	25.25
T2	0	8.14 [b]	31.85 [c]	13.33	44.33
T3	0.74	11.11 [b]	46.29 [b]	19.38	19.07
T4	0	8.88 [ab]	40.73 [b]	16.54	30.94
T5	0	6.29 [ab]	31.11 [c]	12.47	47.94
T6	2.22	12.22 [ab]	47.41 [ab]	20.62	13.91
T7	0.74	17.03 [a]	54.07 [a]	23.95	-
CD (0.05)	NS	6.02	7.544	-	-

At 30 DAS, disease severity was not recorded in plants in T1 (insect proof net + PGPR mix II + sampoorna), T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*), T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) while PDS of 0.74, 2.22 and 0.74 per cent was recorded in plants in T3 (azadiractin), T6 (imidachloprid) and untreated control (T7) respectively.

At 45 DAS, the lowest PDS of 6.29 per cent was recorded in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*) and T3 (azadiractin), with PDS of 8.14, 8.88 and 11.11 per cent respectively. While the highest PDS of 17.03 per cent was recorded in untreated control plants.

During 60 DAS also, the lowest PDS of 31.11 per cent was recorded in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin). This was followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*) and T1 (insect proof net + PGPR mix II + sampoorna) with a PDS of 31.85, 40.73 and 41.48 per cent respectively. While the highest PDS of 54.07 per cent was recorded in the untreated control plants. Comparing the mean values of

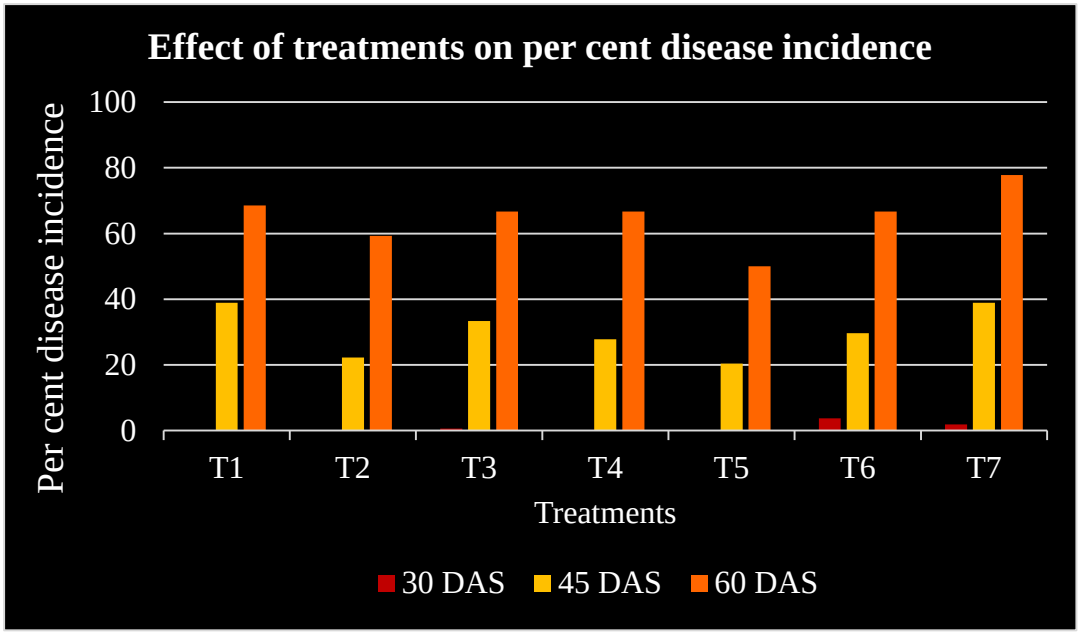


Fig. 4.28: Effect of different treatments on BYVMD incidence

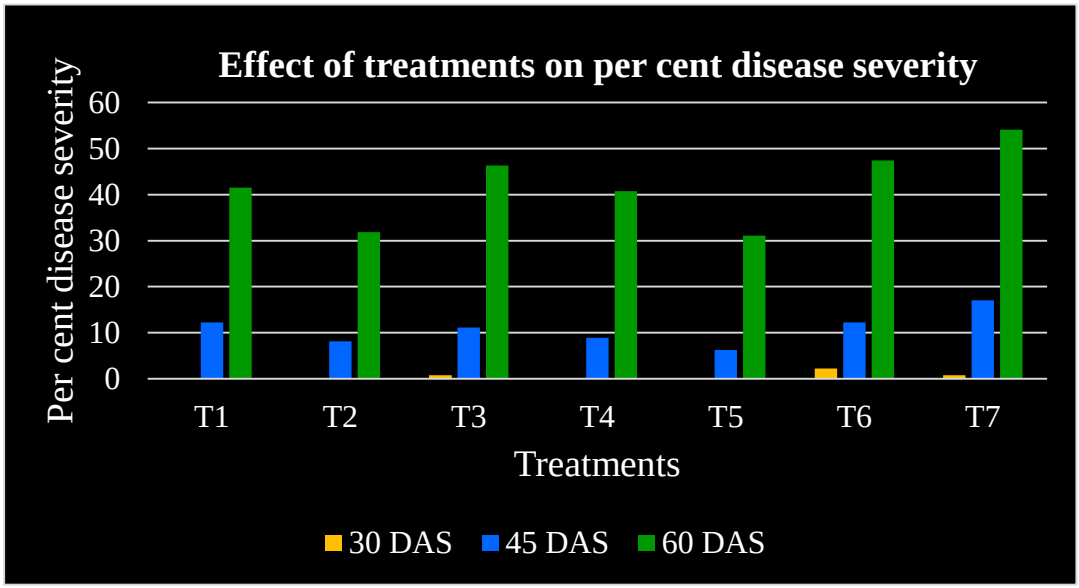


Fig. 4.29: Effect of different treatments on BYVMD severity

PDS revealed that the treatment T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) recorded lowest PDS (13.33%) followed by T2 (PGPR mix II + *B. spectabilis*), T4 (yellow sticky trap + *L. lecanii*) and T1 (insect proof net + PGPR mix II + sampoorna) with PDS of 13.33, 16.54 and 17.90 per cent respectively. The highest PDS of 23.95 per cent was recorded in untreated control plants.

When per cent disease reduction of disease over control was calculated, the maximum of 47.94 per cent reduction of disease severity was recorded in T5 followed by T2, T4, T1, T3 and T6 with reduction of 44.33, 30.94, 25.25, 19.07 and 13.91 per cent respectively.

4.7.3. Effect of treatments on vegetative characters

The effect of different treatments on vegetative characters such as plant height, number of days taken for first flowering and 50 per cent flowering was recorded and the results are presented in Table 4.15. Statistically no significant difference in plant height, number of days taken for first flowering and 50 per cent flowering was noticed among the treatments.

Table 4.15: Effect of treatments on vegetative characters

Treatment details	Plant height (cm)	No. of days taken for first flowering	No. of days taken for 50 per cent flowering
T1	57.06	43.33	54.33
T2	57.24	43.66	54.33
T3	55.91	43.33	54.33
T4	56.44	42.66	54.00
T5	56.90	40.33	51.00
T6	56.10	41.66	51.66
T7	55.53	44.33	55.33
CD (0.05)	NS	NS	NS

4.7.3.1. Plant height

The highest plant height (57.24 cm) was recorded in plants treated with T2 (PGPR mix II + *B. spectabilis*) which was followed by T1 (insect proof net + PGPR mix II + sampoorna) and T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) having plant height of 57.06 cm and 56.90 cm respectively. While the lowest plant height (55.53 cm) was recorded in plants without any treatment (T7).

4.7.3.2. Number of days taken for first flowering

The plants in treatment T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) took minimum of 40.33 number of days for flowering followed by T6 (yellow sticky trap + *L. lecanii*), T4 (yellow sticky trap + *L. lecanii*), T3 (azadiractin) and T1 (insect proof net + PGPR mix II + sampoorna) with 41.66, 42.66, and 43.33 days respectively. While the plants in untreated control (T7) took maximum of 44.33 number of days for first flowering compared to all other treatments.

4.7.3.3. Number of days taken for 50 per cent flowering

The plants in treatment T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) took minimum of 51.00 number of days for 50 per cent flowering. This treatment was followed by T6 (imidachloprid) and T4 (yellow sticky trap + *L. lecanii*) with 51.66 and 54.00 days respectively while treatments T1 (insect proof net + PGPR mix II + sampoorna), T2 (PGPR mix II + *B. spectabilis*) and T3 (azadiractin) took 54.33 days for reaching 50 per cent flowering. Maximum of 55.33 number of days were taken for attaining 50 per cent flowering in the untreated control (T7).

4.7.4. Effect of treatments on vector population

The vector population (whitefly count) from different treatments were recorded and the data is presented in Table 4.16 and Fig. 4.30.

Table 4.16: Effect of treatments on whitefly population

Treatment details	Whitefly count				Per cent reduction over control
	30 DAS	45 DAS	60 DAS	Mean	
T1	6.47 ^{bc}	6.67 ^{bc}	6.60 ^{bc}	6.58	22.50
T2	7.33^{b}	6.90^{b}	6.70^{b}	6.97	17.82
T3	6.60 ^{b}	6.80 ^{bc}	6.33 ^{c}	6.58	22.54
T4	5.60 ^{cd}	5.73 ^{c}	6.07 ^{c}	5.80	31.68
T5	5.20 ^{d}	5.60 ^{c}	5.93 ^{c}	5.58	34.31
T6	6.53 ^{b}	6.73 ^{bc}	6.53 ^{bc}	6.60	22.30
T7	8.40 ^{a}	8.67 ^{a}	8.40 ^{a}	8.49	-
CD (0.05)	0.90	1.20	0.80	-	-

At 30 DAS, the minimum number of whiteflies was observed on T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) with a whitefly population of 5.20. This was followed by T4 (yellow sticky trap + *L. lecanii*), T6 (imidachloprid) and T1 (insect proof net + PGPR mix II + sampoorna) with a whitefly population of 5.20, 6.53, and 6.47 respectively and are found to be statistically on par with each other. The maximum of 8.40 whitefly population was recorded in untreated control (T7).

During 45 DAS also the whitefly count was minimum in T5 (5.60) which was followed by T4 with a whitefly count of 5.73. While the other treatments, T1, T3 and T6 were found to be statistically on par with each other having whitefly population of 6.67, 6.80 and 6.73 respectively. While the maximum whitefly population (8.67) was recorded in untreated control (T7).

At 60 DAS also the minimum whitefly population of 5.93 was observed in T5 and statistically on par with T3 and T4 with a whitefly population of 6.33 and 6.07 respectively. These were followed by T1 and T6 with a whitefly population of 6.60 and 6.53 respectively. The maximum whitefly population (8.40) was recorded in T7 (control).

Comparing the mean values of whitefly population at 30, 45 and 60 DAS revealed that, the minimum population (5.58) was recorded in T5 followed T4 (5.80), T3 (6.58), T1 (6.58), and T6 (6.60). The maximum population (8.49) was recorded in T7 (untreated control).

When per cent disease reduction of whitefly population over control was calculated the maximum per cent reduction (34.31) was recorded in T5 followed T4 (31.68), T3 (22.54) and T1 (22.50).

4.7.5. Effect of treatments on yield characters

The data on effect of different treatments on yield characters is given in Table 4.17 and Fig. 4.31.

4.7.5.1. Fruit yield

The statistical analysis of data on fruit weight revealed that all the treatments were significantly superior to the untreated control. The yield in terms of the fruit yield was recorded highest in plants in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) with a fruit yield of 2.56 kg. This treatment was found to be statistically on par with T6 (imidachloprid 0.03%), T4 (yellow sticky trap + *L. lecanii*), T2 (PGPR mix II + *B. spectabilis*) and T1 (insect proof net + PGPR mix II + sampoorna) which recorded fruit yield of 2.41, 2.39, 2.38 and 2.33 kg respectively. The lowest fruit yield (1.73 kg) was recorded in T7 (untreated control).

The per cent increase in yield over control was calculated. The results revealed that the maximum increase in yield (47.98 %) was recorded in T5 (insect proof net + PGPR mix II + yellow sticky trap + *B. spectabilis* + azadiractin) which was followed by T6 (imidachloprid), T4 (yellow sticky trap + *L. lecanii*), T2 (PGPR mix II + *B. spectabilis*) and T1 (insect proof net + PGPR mix II + sampoorna) with a fruit yield of 39.31, 38.32, 37.40 and 34.51 per cent respectively.

Table 4.17: Effect of treatments on yield characters

Treatment details	No. of fruits per plant	Yield (kg/plot)	Yield (t/ha)	Per cent increase in yield over control
T1	9.80	2.33 abc	8.62	12.99
T2	11.80	2.37 ab	8.78	15.28
T3	9.20	2.29 bc	8.48	11.16
T4	11.60	2.40 ab	8.89	16.39
T5	11.40	2.56 a	9.48	24.15
T6	10.80	2.41 ab	8.92	16.87
T7	9.60	2.06 c	7.62	-
CD (0.05)	NS	0.273	-	-

4.7.5.2. Number of fruits per plant

Significantly no differences were recorded in number of fruits per plant among the treatments. The highest number of fruits per plant (11.80) was observed in plants in T2 (PGPR mix II + *B. spectabilis*) followed by T4 (yellow sticky trap + *L. lecanii*) with 11.40 fruits per plant. The lowest number of fruits per plant (9.30) was noticed in T7 (untreated control).

In general, T5 *i.e.*, integrated management with early seedling protection using insect proof net, yellow sticky trap, seed bio-priming and foliar spray of PGPR mix II (2 %), alternate foliar spray of *Bougainvillea spectabilis* (10 %) and azadiractin (300 ppm) was found to be most effective with lowest disease incidence and disease severity, least whitefly population and maximum yield. The plants in T4 (yellow sticky trap and Foliar spray of *L. lecanii*), T2 (bio-priming with PGPR mix II and foliar spray of *B. spectabilis*), T6 (seed treatment and foliar spray of imidachloprid-0.03 %) were also found to be effective for reducing the disease incidence, disease severity and whitefly population and for increasing the yield.

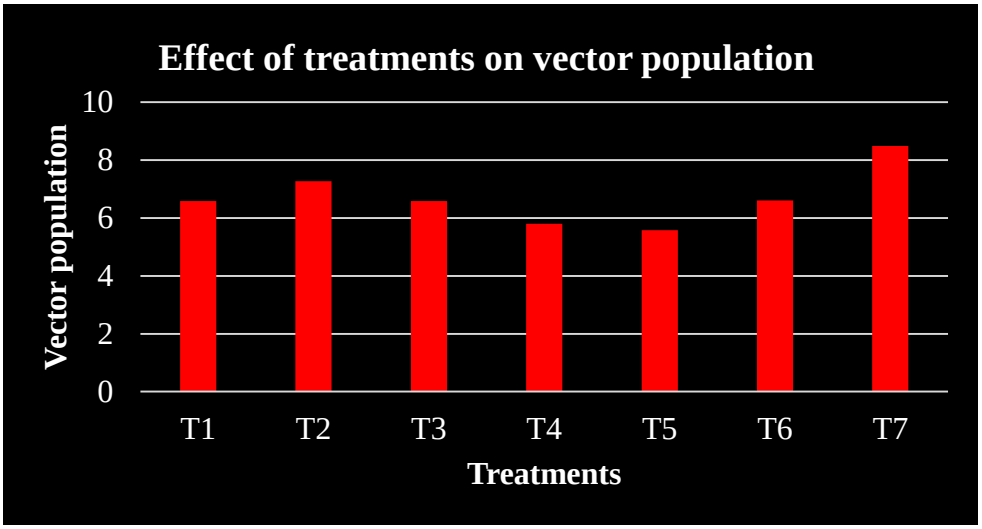


Fig. 4.30: Effect of different treatments on whitefly population in field

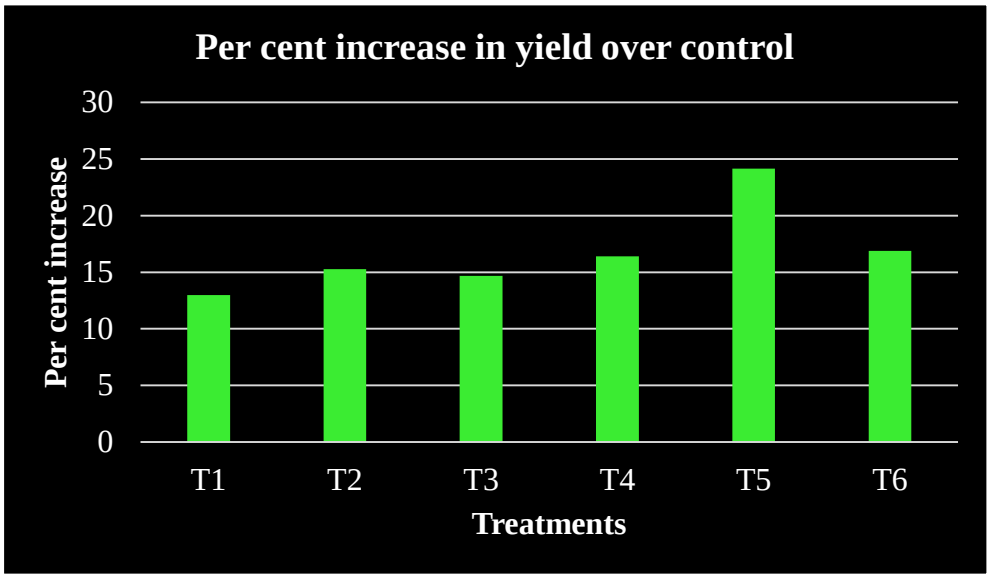


Fig. 4.31: Effect of different treatments on yield as per cent increase in yield over control

Discussion

DISCUSSION

Bhindi (*Abelmoschus esculentus* (L.) Moench.) also known as okra or ladies finger is an important vegetable crop widely grown throughout the world because of its high nutritional value. It is an important source of vitamins and minerals (IBPGR, 1990). Globally, India is the largest producer of okra with a share of 73.6 per cent (NHB, 2015). Also it has a major share in the revenue generation of the country through the export of fresh vegetable after onion (Sanwal *et al.,* 2014). Even though the production is high, crop cultivation is often challenged by the incidence of various pests and diseases. Among the diseases, yellow vein mosaic is the most serious one which leads to complete yield loss depending on the stage of growth at which infection occurs. Yellowing of veins and veinlets is the most conspicuous symptom associated with this disease. In many cases, the yellow vein mosaic disease is also characterized by enation and leaf curl symptoms. However, these symptoms are expressed by different begomoviruses associated with viral diseases of okra *viz.,* bhindi yellow vein mosaic virus (BYVMV), okra enation leaf curl virus (OELCuV), mesta yellow vein mosaic virus (MeYVMV) and hollyhock yellow vein mosaic virus (HoYVMV).

The begomoviruses which taxonomically belonging to the genus *Begomovirus* and family *Geminiviridae* are devastating plant viruses causing significant economic loss in the agricultural scenario especially in tropical and subtropical regions across the globe. The natural transmission of begomoviruses is through whitefly (*Bemisia tabaci* Genn.) in a semi persistent manner. The viruses which infect bhindi and express yellow vein mosaic symptoms was reported as early as 1942 by Kulkarni. Many varieties resistant to YVMD have been developed and used to manage the disease. However, in recent years, frequent breakdown of resistant were reported in those varieties which might be due to the evolution of new viral strains or due to recombination between the viral strains (Sanwal *et al*., 2014). Moreover, the emergence of the ‘B’ biotype, *B. tabaci* expanded its host range which resulted in infection of geminiviruses in formerly unaffected crops (Chowda-Reddy *et al*., 2012). The vector control using chemical insecticides poses

great threat to the environment as well as human beings. Therefore, the present study was undertaken on molecular characterization and host range of the devastating pathogen of BYVMD in Kerala. The evaluation of plant extracts, sticky traps, microbial formulations and various chemical against the disease were also undertaken to develop a possible recommendation for the management of the disease.

The project initiated with purposive sampling survey in 11 different vegetable growing areas in Thrissur district of Kerala during the period from November 2018 to June 2019. Four locations from Vellanikkara, two locations each from Viyyur and Attoor, one location each from Chengaloor, Muthuvara and Chalakkudy were surveyed and the observations such as total number of plants, number of infected plants and severity of disease were recoded. The varieties such as Arka anamika and farmer's local varieties were widely cultivated and observed to be highly susceptible to the disease. The disease incidence ranged from 61.20 to 98.16 per cent and thus varied from location to location. The maximum per cent disease incidence (98.16 %) was recorded at Vellanikkara field 1 followed by Vellanikkara field 4 (94.00 %) and Vellanikkara field (93.55 %). The per cent disease incidence of 81.11 and 84.00 per cent were recorded from two fields of Viyyur. The lowest PDI of 61.20 per cent was recorded in Chengaloor. Venkataravanappa (2008) reported incidence of BYVMD in Kerala ranging from 42.45 to 75.64 per cent. However, during the present study, the maximum incidence of 98.16 per cent was recorded. During the survey, moderate level of infestation of insects like whiteflies and leaf hoppers were also observed. The whitefly population and the climatic conditions prevailed at the time of survey might have favoured the high incidence of the disease in the fields. N'guessant *et al.* (1992) reported a direct correlation between disease incidence and vector population under natural field conditions.

During the survey, the per cent disease severity ranged from 48.00-90.00 per cent. The highest per cent disease severity of 90.00 and 89.00 per cent was recorded from two fields of Vellanikkara. The lowest per cent of disease severity

(48.00 %) was recorded in Chengaloor. The disease severity was very high in areas cultivated with susceptible varieties like Salkeerthi and local variety. The variety Arka Anamika released from Indian Institute of Horticultural Research, Bangalore was considered as a resistant variety at the time of release. However, during the survey, this variety also showed 81.11 to 98.16 per cent disease incidence and 56 to 68 per cent disease severity which confirms the breakdown of host resistance.

The development of symptoms of the disease was investigated in detail under natural field and artificial conditions. Under natural field conditions, the most common and predominant symptoms recorded on leaves included vein clearing and vein thickening. Symptoms such as puckering of leaves, reduction in leaf area, malformation, bleached appearance of fruits and general stunting of plant were also recorded. In severely affected plants the leaves became completely yellow or cream in colour with necrosis of leaf margins. At flowering stage, the flower buds were clustered and expressed linear chlorotic striations on the calyx of flower buds. Similar symptoms were described in the earlier reports of yellow vein mosaic disease of bhindi (Kulkarni, 1924; Fernando and Uduravan, 1942; Mandhar and Singh, 1972; Singh *et al*., 1977; Raychaudhuri and Nariani, 1977; Singh, 1990; Gupta and Thind, 2006 and Senevirathna *et al*., 2016).

Apart from these symptoms, a unique type of symptom was observed on infected bhindi plants in two locations *viz*., Vellanikkara and Viyyur. The leaves showed extreme puckering on both sides of the veins with reduction in size, upward curling and chlorosis. The severely affected leaves also showed necrosis from the leaf margin and petiole bending. The infected plants were stunted in appearance. These types of symptoms were not reported by earlier workers.

The symptoms produced under artificial condition, *i.e.*, by artificially inoculating the virus in to healthy plants through insect vector and grafting included yellowing of veins, vein thickening and puckering which is similar to the symptoms observed under natural field condition.

The histological and cytological changes in the foliar tissue of bhindi brought about by the virus were studied and documented. Parenchyma cells in the upper and lower epidermis were irregular in shape and arrangement when compared to healthy one. Similarly, the palisade and spongy mesophyll cells showed regular arrangement with abundant chloroplast in healthy leaf whereas it had a crushed appearance with less amount of chloroplast in the infected leaf. There was also a considerable reduction and change in the shape of protoxylem and metaxylem vessels. The abnormality was also noticed in size and shape of pholem cells. Kunkalikar *et al.* (2007) reported that palisade cells in the papaya ring spot virus (PRSV) infected leaves were highly distorted and have spongy cells with abnormal size and shape with complete disintegration. Similar results were also reported by Mohamed (2011) in beet mosaic virus (BtMV) infected leaves and Harish (2018) in PRSV infected leaves. Mohamed *et al*. (2012) reported that the damage in thylakoids with thickening of cell walls resulted in curling symptoms in squash leaves.

Seed transmission studies were conducted to confirm the possibility of seed transmission of the virus. None of the seedlings germinated from the seeds collected from the infected plant showed any symptoms. Therefore, from the present study it was confirmed that the virus is not transmitted through the seeds. Moreover, the PCR amplification of the DNA isolated from the seedlings with universal *Begomovirus* primer showed negative results which confirmed that the virus is not seed-borne. The earlier reports on seed transmission of BYVMD are comparable with the present findings (Capoor and Varma, 1950; Chatterjee *et al*., 2008). Venkataravanappa *et al.* (2014) reported that seeds collected from okra plants infected with OELCuV did not developed any kind of symptoms and negative results were obtained for ELISA and PCR diagnostic techniques.

In the present study, attempts were made to transmit the virus through grafting. Yellow vein mosaic infected plant used as scion and grafted on one month old healthy bhindi seedlings. The newly emerged leaves from rootstock showed vein clearing symptoms 9-14 days after grafting. Puckering and vein thickening

symptoms were also noticed in the later stage. Even though the success rate of grafts was less (60 %), 100 per cent transmission was obtained in successful grafts. In the same way, Chatterjee *et al*. (2008) reported transmissibility of yellow vein mosaic through cleft grafting was 80 per cent to *H. sabdariffa* and 60 per cent to *H. cannabinus*. Venkataravanappa *et al.* (2013a) transmitted CLCBV in okra through side veneer grafting using infected plant as scion and non-symptomatic plants as rootstock. Singh *et al*. (2016) reported that after 9-10 days of inoculation, disease was transmitted through graft union and 100 per cent success rate were recorded. Ghevariya and Mahatma (2017) reported that both approach and side veneer grafting is suitable for transmission of BYVMV to healthy plants.

The studies on transmission of the virus using the insect vector *i.e., B. tabaci* resulted in 100 per cent transmission. Ten days old healthy bhindi seedlings were used for the transmission studies. The whiteflies were subjected to an acquisition access period and inoculation access period of 24 h each. The time taken for the appearance or the incubation period was 12-15 days. The presence of virus was confirmed through PCR using universal Begomovirus primer (AV494/AC1048). The results were in accordance with the earlier findings (Varma, 1952; Murugesan and Chellaiah, 1977; Jayasree *et al.* 1999; Venkataravanappa, 2008; Venkataravanappa *et al*., 2012a:2013b; Hurakadli *et al*., 2019).

PCR detection of the virus in the insect vector, whitefly was also carried out. DNA from viruliferous whiteflies was extracted using Qiagen blood and tissue kit. The PCR amplification of the virus was done with universal *Begomovirus* primer (AV494/AC1048). Positive amplification was obtained which confirmed the presence of virus in viruliferous whiteflies. Correspondingly, Deng *et al.* (1994) detected cowpea golden mosaic virus (CGMV), okra leaf curl virus (OLCV) and tomato yellow leaf curl virus (TYLCV) in whiteflies using a pair of degenerate oligonucleotide primer. Khan (1999) detected the presence of tomato leaf curl virus (TLCV) in the vector *B. tabaci* using two set of geminivirus specific degenerate primers *viz.*, PALv1978/PARc496 and AV494/AC1048. Venkataravanappa (2008)

detected the presence of BYVMV associated with yellow vein mosaic in bhindi using primers specific to the coat protein gene of the virus.

In the present study, efforts were taken to ascertain the role of plants belonging to family other than Malvaceae in being the reservoir of the virus inoculum. Total 12 plant species belonging to six families were inoculated with viruliferous whiteflies after giving 24 h of AAP and IAP. Among the crop plants inoculated, only brinjal showed mild yellowing of veins and leaf curling symptom but didn't produced positive amplification during molecular detection. Among the weed species inoculated, *Synedrella nodiflora, Ageratum conyzoides* and *Sida* sp. exhibited mild vein clearing and crinkling of leaf lamina 28-31 DAI. All these weeds are earlier reported with the presence of begomovirus (Malathi *et al.,* 2017). However, positive amplification during molecular detection was obtained only in *S. nodiflora*. Among the weeds showing different symptoms collected from fiels condition, only *S. nodiflora* and *Hemidesmus indicus* showed positive amplification. Likewise, Venkataravanappa *et al*. (2013b) reported that the virus causing yellow vein mosaic in bhindi could be transmitted to plants only in family Malvaceae and Solanaceae. Plants belonging to the family Malvaceae (*Abelmoschus esculentus* (L.) Moench and *Althaea rosea* Cav.) and Solanaceae (*Datura stramonium, N. benthamiana, , N. occidentalis, N. glutinosa, N. clevlandii,* and *N. tabacum*) were susceptible to OELCuV (Venkataravanappa *et al*. 2014).

An attempt was made to carry out molecular characterization of virus causing BYVMD. PCR amplification of virus offers a sensitive method for detection of begomoviruses, even if it is present in a very low concentration inside the host. The extraction of the quality DNA from the infected samples is very important for the accurate detection of viruses. The main hindrance preventing the isolation and purification of DNA from bhindi leaves is the presence of highly acidic polysaccharides (mucilage) and polyphenols (Ahmed *et al.,* 2013). DNA that dissolves in the presence of polysaccharides prevents various biotechnological activities such as PCR and restriction digestion (Sahu *et al*., 2012).

In the present study, the good quality genomic DNA was extracted from the bhindi leaves collected from different locations using the protocol reported by Doyle and Doyle (1987) with slight modifications. During the standardization of the protocol, it was noticed that young leaves are more suitable than older leaves. The DNA pellet isolated from older leaves appeared brown in colour. This might be due to high amount of polysaccharides and phenols in the older leaves compared to younger leaves. Isolation with the modified method *i.e.*, increased volume of the DNA extraction buffer to 3 ml and decreased sample volume to 50 mg gave good quantity of DNA in the present study ranging from 247.70 ng/µl to 655.80 ng/µl as verified by spectrophotometer analysis. This method gave good quality DNA without much RNA and protein contamination with an $OD_{260/280}$ value in the range of 1.8 to 2.1. A similar method was developed by Singh and Kumar (2012) by modifying the standard protocol of Doyle and Doyle (1987) for DNA extraction from okra tissues. Their modifications included increase in the volume of DNA extracting buffer (1.5 ml/sample), decrease sample volume (50-60 mg), higher salt concentration (5 M) and use of polyvinylpolypyrrolidone. Likewise, Roy and Sherpa (2017) developed a modified CTAB method for the DNA from extraction of high quality DNA from mucilaginous plants, suitable for detection of geminiviruses and downstream applications. Rouhibakhsh *et al.* (2008) also developed a protocol using a modified CTAB buffer containing β-mercaptoethanol (5 %) and sodium chloride (1.4 to 2.0 M) for the detection of genomic components of whitefly-transmitted begomoviruses in symptomatic legumes.

PCR is considered as a reliable method for the detection of virus in the infected plants even at low concentration. In this study, the amplification of viral DNA was done using three set of reported primers specific to the coat protein gene of the virus. Standardization of the PCR conditions are very essential for the successful detection of viral DNA. The annealing temperature was optimized for each primer. The AV494/AC1048 primer showed amplification at 56.8 °C with an expected amplicon size of 550 bp. OYVMV and BYVMV complete coat protein specific primers showed good amplification at 51.4 °C and 54.5 °C respectively.

The primer specific to the coat protein gene of OELCuV produced good amplicons at 56.9 ^{o}C. These temperatures were used for the further amplification of collected viral isolates. PCR amplification of the virus isolates showed the presence of a begomovirus component equivalent to the core CP gene. The amplified PCR products were sequenced at Agrigenome Lab, Kochi, Kerala.

The nucleotide sequences of virus isolates obtained were analysed using nucleotide BLAST and the results revealed that the core CP gene of all the local isolates showed 99-100 per cent sequence homology to different okra enation leaf curl virus (OELCuV) isolates in NCBI database. The sequences of isolates such as VKA, CLR, VYR1, VYR2, MVA, PND and PNR showed 99-100 per cent similarity with OELCuV isolates reported from Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1) and Gandhinagar (KC019308.1) with 99-100 per cent query coverage and 0.0 E value. These isolates also showed 99-100 per cent sequence similarity with isolates reported from Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1). They also showed more than 99 per cent sequence similarity with coat protein gene of South Indian isolates of OELCuV from Hyderabad (MH052569.1and KT898975.1) and Vijayawada (KT898976.1).

The isolates VNY, ATR1 and ATR2 showed highest similarity with the coat protein gene of OELCuV isolate from Vijayawada (KT935487.1) followed by OK142-PUNJ (KT 390317.1) from Ludhiana, Punjab isolate OK92-HR (KT 390310.1) from Karnal, Haryana. They also showed more than 98 per cent similarity with other accessions *viz.,* Maharashtra (MK084768.1, MK084767.1, MK069435.1 and MK069433.1), Surat (KC342220.1), Gandhinagar (KC019308.1), Pakistan (KX710156.1), Mattara (KX698092.1), Puttalam (KX698091.1) and Trincomalee (NC031754.1). Analysis also revealed that all the collected isolates showed sequence similarity with other begomoviruses in bhindi such as BYVMV (91.53-97.83 %), mesta yellow vein mosaic virus (89.45-99.82 %) and hollyhock yellow vein mosaic virus (89.86-95.83 %).

The wide diversity among the *Begomovirus* is due to the evolution of new viral strains through recombination and pseudo-recombination events (Padidam *et al*., 1999). Recombination has played an important role in the evolution of geminiviruses including *Cotton leaf curl Bangalore virus* (CLCuBV) and OELCuV (Venkataravanappa 2013a:2014). Serfraz *et al*. (2014) conducted a comprehensive sequence analysis and suggested that intermalvaceous recombination between begomoviruses that infect okra and cotton resulted in the evolution of new species.

Venkataravanappa *et al.* (2013a) reported the presence of cotton leaf curl Bangalore virus (CLCuBV) associated with yellow vein mosaic and leaf curl disease of okra. This virus also shared highest sequence homology with other begomoviruses infecting cotton and okra *viz.*, BYVMV, OELCuV and CLCuMuV and suggests that, the begomovirus reported that it might have been derived through recombination of related begomoviruses. Venkataravanappa *et al.* (2014) discovered recombination in OELCuV and identified BYVMV, BYVBhV and MeYVMV as the major parents of recombination. They also reported that the origin of replication of OELCuV isolates originated from BYVMV.

Serfraz *et al.* (2014) reported that OELCuV isolates from Pakistan is a recombinant between south Indian isolates of BYVMV as a major parent and northwest Indian isolates of OELCuV as a minor parent. They also suggested that MeYVMV and cotton leaf curl Multan virus (CLCuMuV) recombined to form the complete region of rep protein gene in OELCuV.

The sequence demarcation tool (SDT) analysis of the virus isolates with other begomoviruses retrieved from NCBI database revealed that all the core CP sequences were 100 per cent similar to each other and have high nucleotide sequence identity of 99.00 to 100 per cent to OELCuV in okra from India and Pakistan. Similar to the present study, Alhudaib (2017) conducted molecular characterization of begomoviruses associated infecting okra in Saudi Arabia and performed SDT analysis of the virus isolates. The results revealed that all the CP gene sequences were 97.2-99.8 per cent similar to cotton leaf curl Gezira virus

(CLCuGeV) in cotton from Pakistan. The DNA barcoding analysis of the core CP sequences of virus isolates confirmed that the virus isolates in the present study are OELCuV with 110 barcodes.

The phylogenetic analysis showed two major cluster groups. The group 1 included all the collected isolates along with isolates of OELCuV and MeYVMV. The second group formed by clustering the isolates of BYVMV and CLCV. The analysis of phylogenetic tree revealed that the similarity within the collected isolates and presence of a common ancestor for the collected isolates and other begomoviruses (BYVMV, CLCV, MeYVMV and OELCuV). Hence, the virus associated with the yellow vein mosaic disease in Kerala were identified as okra enation leaf curl virus (OELCuV) belongs to the genus *Begomovirus* and family *Geminiviridae* and suggested that this might be evolved though the recombination of other begomoviruses infecting bhindi.

The phylogenetic analysis of local isolates with other begomoviruses showed that all the local isolates formed two major clusters with a common ancestor. The first one include all the local isolates along with OELCuV and MeYVMV isolates retrieved from NCBI database. The second cluster include BYVMV and CLCV. Serfraz *et al.* (2014) reported that pairwise sequence comparison of OELCuV-PK showed maximum identity (96.3–96.5 %) with the recently identified isolates of OELCuV from southern India. Venkataravanappa *et al*. (2014) conducted a complete analysis involving 130 sequences begomoviruses infecting bhindi and observed four main clusters, consisting of ToLCNDV, OELCuV, BYVMV and BYVMaV. The BYVHV, BYVDV and BYVKnV belonged to the main cluster of BYVMV while, occupied as a separate group. The ToLCNDV and OELCuV clusters were distinct and formed a separate cluster from BYVMV and BYVMaV. They also conducted neighbour net analysis using split tree programme and confirmed the distinct nature of OELCuV and BYVBhV.

Similarly, Kumar *et al*. (2019) reported that sequences obtained from the samples collected in Hyderabad and Vijayawada showed a maximum identity of

99.20 per cent with Surat (KC342220), and 99.70 per cent with Gandhinagar (KC019308) isolates identified from okra. Comparison of the CP gene sequences of Varanasi, Hyderabad and Vijayawada isolates showed highest level of sequence homology with Haryana and Gujarat isolates of OELCuV.

Management of yellow vein mosaic disease in bhindi was also conducted as a field experiment using a susceptible variety Salkeerthi. The effect of microbial formulations, plant extracts, micronutrient, insect proof net, sticky traps and different chemicals were evaluated for controlling the virus and vector population. The microbial formulation, plant extract, micronutrient and different chemicals were given as foliar spray at specific intervals.

The plants in the integrated treatment, T5 (insect proof net + PGPR mix II-2 % + yellow sticky trap + *B. spectabilis*-10 % + azadiractin 300 ppm) were found to be significantly superior with lowest disease incidence and severity, whitefly population when compared with all other treatments. The disease severity was also less in plants treated with T2 sprayed with Bougainvillea leaf extract and PGPR mix II. This clearly indicates that both PGPR mix II and *Bougainvillea* leaf extract and PGPR mix II are most effective for reducing the disease severity. Madhusudhan *et al.* (2011) reported that *B. spectabilis* extract was found to be most effective in reducing the number of local lesions formed by the challenge inoculation of tobamoviruses and reduced the concentration of viruses in the seedlings. Similarly, Balasaraswati *et al.* (1998) identified bougainvillea antiviral protein I (BAP I), an antiviral protein in the root tissues of *B. spectabilis* wild active against mechanical transmission of *Tomato spotted wilt virus*.

Murphy *et al*. (2000) reported that application of plant growth-promoting rhizobacteria (PGPR) as a combined seed-powder treatment significantly reduced the disease severity of tomato mottle virus and increased the fruit yield in tomato. A similar experiment was conducted by Patil *et al*. (2011) where they applied rhizobacterial isolates to seed, soil and foliage against yellow vein mosaic disease in bhindi and reported that significant reduction of YVMD with a concomitant increase in plant growth and fruit yield under glasshouse conditions.

Yellow sticky trap and foliar application of *L. lecanii* (T4) also gave considerable reduction of per cent disease severity (30.94%) over the untreated control plants. The whitefly population was also low in this treatment compared to the control. Cherian (1998) and Chandrashekharaiah *et al*. (2013) revealed the possibility of utilization of *L. lecanii* as a potent bio-agent against *Bemisia tabaci.*

The foliar application of neem based insecticide (300 ppm) and imidachloprid (0.03 %) showed reduction in PDI and PDS compared to the plants in untreated control (T7). Earlier reports suggests that these two are potential for reducing the whitefly population (Ali *et al*., 2005b; Vethanayagam and Rajendran, 2010; Ali *et al*., 2012). Plants that covered for the first 20 DAS in treatment T1 along with foliar application of micronutrient formulation, Sampoorna gave 25.25 per cent reduction of disease severity over the control plants.

The results were also examined on the basis of biometric observations. There is no significant difference in plant height, number of days taken for first flowering and 50 per cent flowering. However, the plants in the treatment T5 took less number of days for first flowering and 50 per cent flowering compared to other treatments. The maximum plant height was recorded in plants sprayed with PGPR mix II and *Bougainvillae* leaf extract (T2). The analysis of yield data revealed that the treatment T5 gave higher yield (2.56 kg) compared to all other treatments.

The field experiment revealed that integrated treatment T5 which include early seedling protection using insect proof net, yellow sticky trap, seed bio-priming and foliar spray of PGPR mix II (2 %), alternate foliar spray of *Bougainvillea spectabilis* (10 %) and azadiractin (300 ppm) was found to be most effective with lowest disease incidence and disease severity, least whitefly population and maximum yield. The plants in T4 (yellow sticky trap and Foliar spray of *L. lecanii*), T2 (bio-priming with PGPR mix II and foliar spray of *B. spectabilis*), T6 (seed treatment and foliar spray of imidachloprid-0.03 %) were also found to be effective for reducing the disease incidence, disease severity and whitefly population.

Summary

SUMMARY

Bhindi or okra is an important vegetable crop widely grown in the tropical, subtropical and warm temperate regions. However, the biotic problems, especially diseases caused by viruses are a major hurdle for bhindi cultivation. Bhindi yellow vein mosaic disease has a devastating effects on the yield of the crop and may cause up to 100 per cent yield loss.

The study entitled "Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Kerala Agricultural University during 2017-2019 with the view to understand the symptomatology, mode of transmission, host range of the virus. The present study was also undertaken to characterize the coat protein gene of the virus at molecular level. The present research was envisaged to evaluate the effects of plant extracts, sticky traps, microbial formulations and various chemicals on bhindi yellow vein mosaic disease.

Survey on disease incidence revealed the presence of bhindi yellow vein mosaic disease in 11 locations and the disease incidence ranged from 61.20 to 98.16 per cent. The maximum disease incidence of 98.16 per cent was recorded in Vellanikkara on variety Arka Anamika followed by 91.66 per cent in Attoor on a local variety and 89.33 per cent in Chalakkudi on Arka Anamika. The varieties such as Arka anamika which once released as a resistant variety from IIHR and farmer's local varieties were widely cultivated and observed to be highly susceptible to the disease. The disease severity was ranged from 48 to 90 per cent among the survey locations.

The symptoms of bhindi yellow vein mosaic disease under natural conditions were documented during the survey in different locations. The most common and predominant symptoms recorded on leaves under field conditions include vein clearing and vein thickening. In fields recorded with high disease severity, symptoms such as puckering of leaves, reduction in leaf area, wavy

appearance of leaf margin, clustering and linear chlorotic striations on flower buds were recorded. In severe cases, complete bleached appearance of leaf laminawith marginal necrosis were observed in fields. The immature fruits showed linear chlorotic striations and in the advanced stage, the fruits showed reduction in size, malformation and bleached appearance. General stunting of plant was also observed under field conditions.

In artificial conditions, typical vein clearing symptoms were documented on healthy bhindi seedlings when they were inoculated through vectors and grafting. The incubation period was ranged from 12-15 days in case of vector transmission and 9-14 days in case of graft transmission. Studies on histopathological changes due to virus infection revealed that the infected leaf showed abnormalities in the parenchyma cells in the upper and lower epidermis, palisade and spongy mesophyll cells and vascular bundles such as xylem and phloem.

Cent per cent transmission was observed through grafting and vector transmission. However, the virus failed to produce symptoms on seedlings emerged from seeds collected from the infected pods confirming that bhindi yellow vein mosaic disease is not seed-borne. The presence of virus inside the insect vector was confirmed through PCR using universal *Begomovirus* primer (AV494/AC1048) specific to the core coat protein of the virus.

In the present study, host range studies were conducted on 11 plant species belonging to six different families indicated that the host range of the virus is limited to Malvaceae, Solanaceae and Asteraceae. *Solanum melongena* showed mild yellowing of veins and leaf curling symptoms on leaves however, doesn't produced positive amplification during PCR. *Synedrella nodiflora* initially showed crinkling of leaf lamina and later produced mild vein clearing symptoms on leaves.positive amplification was observed in PCR reactions. *Ageratum conyzoides* also showed crinkling of leaf lamina along with puckering symptom. *Sida acuta* that belongs to the Malvaceae family showed mild vein clearing as well as puckering symptom. However, thsese two were not produced positive amplification during PCR

reactions. The weed species such as *Hemidesmus indicus* and *Synedrella nodiflora* collected from vegetable field were also found to be act as the host of *Begomovirus*.

As a part of molecular characterization, the protocol for the molecular diagnosis of virus associated with bhindi yellow vein mosaic disease was standardized. The genomic DNA from the infected plant samples were standardized using CTAB method. PCR product of approximately 550 bp and 770 bp were amplified using the reported primers specific to the coat protein gene of *Begomovirus*, BYVMV and OELCuV. *In silico* analysis of the virus isolates revealed 99-100 per cent nucleotide sequence homology with OELCuV isolates. The isolates were also showed significant homology of amino acid identity (100 %) to the Hyderabad isolate of OELCuV. The identity of the virus was confirmed though DNA barcoding and 110 barcodes were identified for the identification of BYVMV and OELCuV isolates. The identity of the virus was also confirmed using species demarcation tool (SDT) analysis. The phylogenetic analysis of the virus isolates by the alignment of the CP gene sequences with 20 other *Begomovirus* isolates also revealed that the isolates under the present study were more similar to OELCuV.

A field experiment was conducted to evaluate the efficiency of different chemicals, plant extracts, microbial formulations, yellow sticky trap and insect proof net for reducing the severity of the disease. Among the seven treatments, T5 *i.e.,* integrated management with early seedling protection using insect proof net, yellow sticky trap, seed bio-priming and foliar spray of PGPR mix II (2 %), alternate foliar spray of *Bougainvillea spectabilis* (10 %) and azadiractin (300 ppm) was found to be most effective with lowest disease incidence and disease severity, least whitefly population and maximum yield.

The plants in T4 (yellow sticky trap and Foliar spray of *L. lecanii*), T2 (bio-priming with PGPR mix II and foliar spray of *B. spectabilis*), T6 (seed treatment and foliar spray of imidachloprid-0.03 %) were also found to be effective for reducing the disease incidence, disease severity and whitefly population and for increasing the yield. The biometric observations such as plant height, number of

days taken for first flowering, no. of days taken for 50 per cent flowering was found to be non-significant among the seven treatments.

In general, the field experiment revealed that the integrated treatment, T5 which include early seedling protection using insect proof net, yellow sticky trap, seed bio-priming and foliar spray of PGPR mix II (2 %), alternate foliar spray of *Bougainvillea spectabilis* (10 %) and azadiractin (300 ppm) was found to be most effective with lowest disease incidence and disease severity, least whitefly population and maximum yield. The plants in T4 (yellow sticky trap and Foliar spray of *L. lecanii*), T2 (bio-priming with PGPR mix II and foliar sprays of *B. spectabilis*), T6 (seed treatment and foliar spray of imidachloprid-0.03 %) were also found to be effective for reducing the disease incidence, disease severity and whitefly population.

References

REFERENCES

Abdalla, O. A., Bibi, S., and Zhang, S., 2017. Application of plant growth-promoting rhizobacteria to control papaya ringspot virus and tomato chlorotic spot virus. *Arch. Phytopathology Plant. Protect.* 50(12):584-597.

Acharyya, S., Paul, S., Ghosh, R., Das, S., Palit, P., Das, A., Mir, J.I., Ghosh, S. K., and Roy, A. 2008. Molecular evidence for natural occurrence of bhendi yellow vein mosaic virus in *Urena lobata* from eastern India. *Indian J. Virol*. 19(1):119.

Ahmed, N., Nawaz, S., Iqbal, A., Mubin, M., Butt, A., and Lightfoot, D. A. 2013. Extraction of high-quality intact DNA from okra leaves despite their high content of mucilaginous acidic polysaccharides. *Biosci. Methods,* 4:19-22.

Ahmed, Z., Patil, M. S., and Prabhakar, A. S. N. 2005. Histological changes induced by bhendi yellow vein mosaic virus on infected leaves and fruit rind of bhendi. *Karnataka J. Agrl. Sci.* 18 (1): 181-183.

Alhuaib, K. 2017. Molecular identification of Begomoviruses infecting okra in eastern province of Saudi Arabia Molecular identification of Begomoviruses infecting okra in eastern province of Saudi Arabia. *Indian J. Plant Prot.,* 45(3): 297-302.

Ali, M. I., Khan, M. A., Rashid, A., Javed, M. T., and Sajid, M. 2012. Epidemiology of okra yellow vein mosaic virus (OYVMV) and its management through tracer, mycotal and imidacloprid. *Am. J. Plant Sci.* 3(12):1741.

Ali, S., Khan, M. A., Habib, A., Rasheed, S., and Iftikhar, Y. 2005a. Correlation of environmental conditions with okra yellow vein mosaic virus and *Bemisia tabaci* population density. *Int. J. Agric. Biol.* 7:142–144.

Ali, S., Khan, M. A., Zeshan, M. A., and Usman, M., 2014. Eco-friendly approaches for the management of Okra yellow vein mosaic virus disease (OYVMVD) incidence. *Pakistan J. Phytopathol.*, 26(1):113-116.

Ali, S., Khan, M.A., Habib, A., Rasheed, S. and Iftikhar, Y., 2005b. Management of yellow vein mosaic disease of okra through pesticide/bio-pesticide and suitable cultivars. *Int. J. Agric. Biol.*

Atiri, G. I. 1984. The occurrence and importance of okra mosaic virus in Nigerian weeds. *Ann. Appl. Biol*. 104:261–265.

Balasaraswathi, R., Sadasivam, S., Ward, M., and Walker, J. M. 1998. An antiviral protein from *Bougainvillea spectabilis* roots, purification and characterization. *Phytochemistry*, 47:1561-1565

Basu, A. N. 1995. *Bemisia tabaci Genn. Crop pest and principal whitefly vector of plant virus.* Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, India. 183 pp.

Beris, D., Theologidis, I., Skandalis, N., and Vassilakos, N., 2018. *Bacillus amyloliquefaciens* strain MBI600 induces salicylic acid dependent resistance in tomato plants against tomato spotted wilt virus and potato virus Y. *Sci. rep.*, *8*(1):10320.

Berrie, L. C., Rybicki, E. P., and Rey, M. E. C. 2001. Complete nucleotide sequence and host range of south African cassava mosaic virus: further evidence for recombinations amongst begomoviruses. *J. Gen. Virol*. 82:53–58.

Birah, A., Srivastava, R. C., Kumar, K., Singh, P. K., and Bhagat, S. 2012. Efficacy of pest management practices against pest complex of okra (*Abelmoschus esculentus*) in Andaman. *Indian J. Agrl. Sci.* 82(5):470.

Bird, J. and Maramorosch, K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22: 55-110.

Briddon, R. W. and Stanley, J. 2006. Subviral agents associated with plant single-stranded DNA viruses. *Virology*, 344:198–210.

Briddon, R. W., Bull, S. E., Mansoor, S., Amin, I., and Markham, P.G. 2002a. Universal primers for the PCR-mediated amplification of DNA beta: a

molecule associated with some monopartite begomoviruses. *Mol. Biotechnol*. 20:315–318.

Briddon, R.W., Bull, S. E., Amin, I., Idris, A. M., Mansoor, S., Bedford, I. D., Dhawan, P., Rishi, N., Siwatch, S. S., Abdel-Salam, A. M. and Brown, J. K. 2002b. Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses. *Virology*, 312(1):106-121.

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., Fauquet, C. M., Briddon, R.W., Zerbini, M., Moriones, E., and Navas-Castillo, J. 2012. "*Geminiviridae*," in virus taxonomy- ninth report of the International Committee on Taxonomy of Viruses, eds A. M. Q. King, M. J.

Capoor, S. P. and Varma, P. M. 1950. Yellow vein-mosaic of *Hibiscus esculentus* L. *Indian J. Agrl. Sci.* 20:217-230.

Chandrashekharaiah, Gowda G. B., Kumar, A. D. N. T., and Chakravarthy, A. K. 2013. Bio-efficacy of *Verticillium lecanii* against whitefly on greengram. *Insect Environ*. 19(2): 83-86.

Chatterjee, A., Roy, A., and Ghosh, S. K., 2008. Acquisition, transmission and host range of a begomovirus associated with yellow vein mosaic disease of mesta (*Hibiscus cannabinus* and *Hibiscus sabdariffa*). *Australasian Plant Path*. 37(5):511-519.

Chaudhary, A., Khan, M., and Bilal, Y. 2016. Management of okra yellow vein mosaic virus and its vector through plant extracts. *J. Plant Pathol. Microbiol.* 8:1-3.

Chelliah, S., Murugesan, S., and Murugesan, M. 1975. Influence of weather factors on the incidence of bhendi. *Madras Agric. J*. 62(7): 412-419.

Chenulu, V. V., Venkateswarlu, V., and Rangaraju, R. 1979. Studies on yellow mosaic disease of mungbean. *Indian Phytopath*. 32: 230-235.

Cherian A. K. 1998. Management of tomato leaf curl virus in Tomato through the control of its whitefly vector, *Bemisia tabaci* Genn. by entomopathogenic fungi. Ph. D. thesis, University of Agricultural science, Bangalore, 226p.

Chowda-Reddy, R. V., Kirankumar, M., Seal, S. E., Muniyappa, V., Girish, B., Valand Govindappa, M. R., and Colvin, J. 2012. *Bemisia tabaci* phylogenetic groups in India and the relative transmission efficacy of tomato leaf curl Bangalore virus by an Indigenous and an exotic population. *J. Integrative Agricul*. 11(2): 235–248.

Cohen, S. and Nitzany, F. E. 1966. Transmission and host range of the tomato yellow leaf curl virus. *Phytopathology,* 56: 1127-1131.

Cohen, S., Duffus, J. E., Larsen, R. C., Lir, H.Y., and Flock, R. A. 1983. Purification, serology and virus vector relationship of squash leaf curl virus, a whitefly transmitted geminivirus. *Phytopathology,* 73: 1669-1673.

Costa, A. S. and Bennett, C. W. 1950. Whitefly transmitted mosaic of *Euphorbia prunifolia*. *Phytopathology,* 40: 266-283.

Deng, D., McGrath, P. F., Robinson, D. J., and Harrison, B. D. 1994. Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Ann. Appl. Biol.* 125(2): 327-336.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-15.

Elbeshehy, E. K. 2017. Inhibitor activity of different medicinal plants extracts from *Thuja orientalis, Nigella sativa* L., *Azadirachta indica* and *Bougainvillea spectabilis* against zucchini yellow mosaic virus (ZYMV) infecting *Citrullus lanatus. Biotechnol. Biotec. Eq.* 31(2): 270-279.

El-Dougdoug, K. A., Ghaly, M. F., and Taha, M. A. 2012. Biological control of cucumber mosaic virus by certain local *Streptomyces* isolates: inhibitory effects of selected five Egyptian isolates. Int. J.Virol. 8(2):151-164.

El-Sawy, M. M., Elsharkawy, M. M., Abass, J. M., and Hagag, E. S. 2018. Inhibition of tomato yellow leaf curl virus by *Zingiber officinale* and *Mentha longifolia* extracts and silica nanoparticles. *Int. J. Antivir. Antiretrovirology*, 1 (1):001-006.

Elsharkawy, M. M. and El-Sawy, M. M. 2015. Control of bean common mosaic virus by plant extracts in bean plants. *Int. J. Pest Manage.* 61(1):54-59.

Fauquet, C. M. and Stanley, J. 2003. *Geminivirus* classification and nomenclature; progress and problems. *Ann. Appl. Biol.* 142: 165-189.

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.

Fernando, M. and Udurawana, S.B., 1942. The nature of the mosaic disease of Banbakka (*Hibiscus esculentus* L.). *Trop. Agric*. 18(1):16-24.

Ghanem, G. A. M. 2003. *Okra leaf curl virus*: a monopartite begomovirus infecting okra crop in Saudi Arabia. *Arab. J. Biotechol*. 6:139–152.

Ghevariya, T. V. and Mahatma, L. 2017. Biological characterization of okra yellow vein mosaic virus (OYVMV) infecting okra in South Gujarat, India. *Int. J. Curr. Microbiol. App. Sci.* 6(7): 2017-2024

Ghosh, R., Paul, S., Palit, P., Das, S., Das, A., Acharyya, S., Mir, J. I., Ghosh, S. K., and Roy, A., 2008. Molecular characterization and computational analysis of coat protein gene of an East Indian isolate of bhendi yellow vein mosaic virus and associated DNA-beta satellite. *Indian J. Virol.* 19(2):173-182

Gupta, S. K. and Thind, T. S. 2006. *Disease problems in vegetable production*. Scientific Publisher, Jodhpur, 576p.

Gutierrez, C. 2002. Strategies for geminivirus DNA replication and cell cycle interference. *Phys. Mol. Plant Path.*, 60: 219-230.

Hafeez-ur Rehmana, Nadeema, M., Ayyazb, M., and Beguma, H. A. 2015. Comparative efficacy of neem oil and lambdacyhalothrin against whitefly (*Bemisia tabaci*) and Jassid (*Amrasca devastans* Dist.) in okra field. *Russian Agrl. Sci*. 41(23).

Hameed, U., Zia-Ur-Rehman, M., Herrmann, H.-W., Haider, M. S., and Brown, J. K. 2014. First report of okra enation leaf curl virus and associated cotton leaf curl Multan betasatellite and cotton leaf curl Multan alphasatellite infecting cotton in Pakistan: a new member of the cotton leaf curl disease complex. *Plant Dis.* 98:1447.

Hamidson, H., Damiri, N., and Angraini, E. 2017. Effect of medicinal plants extracts on the incidence of mosaic disease caused by cucumber mosaic virus and growth of chili. In *IOP Conference Series: Earth and Environmental Science* 102(1) 012062).

Hamsa, S., Girija, D., Nazeem, P. A., Mathew, S. K., Deepa, K., Balakrishnan, S., and Sumbula, V. 2016. Molecular characterization of *geminivirus* causing yellow vein mosaic in pumpkin. *Int. J. Sci. Environ. Technol*. 5 (1):133 – 147.

Handa, A. and Gupta, M. D. 1993. Management of bhendi yellow vein mosaic virus disease. *Indian Phytopath.* 46: 123-130.

Harish, A. 2018. Characterization, host range and management of papaya ringspot virus (PRSV). M.Sc. Ag thesis, Kerala Agricultural University, Thrissur, 109p.

Harrison, B. D. and Robinson, D. J. 1999. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (Begomoviruses). *Ann. Rev. Phytopathol*. 37: 369–398.

Harrison, B. D., Barker, H., Bock, K. R., Guthrie, E. J., Meredith, G., and Atkinson, M. 1977. Plant viruses with circular single-stranded DNA. *Nature*, 270: 760-762.

Harrison, B. D., Muniyappa, V., Swanson, M. M., Roberts, I. M. and Robinson, D. J., 1991. Recognition and differentiation of seven whitefly-transmitted geminiviruses from India, and their relationships to African cassava mosaic and Thailand mung bean yellow mosaic viruses. *Ann. Appl. Biol*. 118(2):299-308.

Hernandez-Zepeda, C., Isakeit, T., Scott, A., and Brown, J. K. 2010. First report of okra yellow mosaic Mexico virus in okra in the United States. *Plant Dis*. 94:924.

Hong, Y. G. and Harrison, B. D. 1995. Nucleotide sequences from tomato leaf curl virus from different countries evidence for three geographically separate branches in evolution of the coat protein of whitefly transmitted geminiviruses. *J. Gen. Virol.* 76:2043-2049.

Hughes, J. 2008. March. Just famine foods? What contributions can underutilized plants make to food security? In International Symposium on Underutilized Plants for Food Security, Nutrition, Income and Sustainable Development 806:39-48.

Hurakadli, M. S., Rangaswamy, K. T., and Kumari, S. 2019. Virus–vector relationships of yellow mosaic virus and whitefly (*Bemisia tabaci*) in Ridge Gourd. *J. Pure Appl. Microbiol.* 10(4):2883-2889.

IBPGR [International Bureau of Plant Genetic Resources]. 1990. International Crop Network Series: 5. Report International Workshop on Okra Genetic Resources,

held at NBPGR New Delhi, India from 08 to 12 October, 1990. International Board for Plant Genetic Resources, Rome. Available online at: http://pdf.usaid.gov/pdf_docs/PNABK682.pdf

INDIAAGRISTAT. 2017. Selected State-wise Area, Production and Productivity of Okra in India (2015-2016 and 2016-2017). [online] Available: https://www.indiaagristat.com/agriculture-data/2/horticulture/118/ladys-finger-okra/23059/stats.aspx [27 Dec 2018]

Jambhale, N. D. and Nerkar, Y. S. 1986. Performance of yellow vein mosaic resistant Parbhani Kranti variety of okra. *Indian J. Virol*. 56 (9):677-680.

James, E. D. 1965. Beet pseudo yellows virus, transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*). *Phytopathology,* 55: 450-453.

Jayshree, K., Pun, K. B., and Doraiswamy, S. 1999. Virus vector relationship of yellow vein mosaic virus and whitefly (*Bemisia tabaci*) in pumpkin. *Indian Phytopath*. 52(1): 10-13.

Jenkins, D. J. A., Kendall, C.W. C., Marchie, A., Faulkner, D. A., Wong, J. M. W., and de Souza, R. 2005. Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. *Am. J. Clin. Nutr.* 81: 380–387.

Jeske, H., Lutgemeier, M., and Preiss, W. 2001. DNA forms indicate rolling circle and recombination-dependent replication of abutilon mosaic virus. *Eur. Mol. Biol. J.* 20:6158–6167.

Jose, J. and Usha, R. 2000. Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). *Plant Mol. Biol. Rep*. 18:349-355.

Jose, J. and Usha, R., 2003. Bhendi yellow vein mosaic disease in India is caused by association of a DNA β satellite with a begomovirus. *Virology,* 305(2):310-317.

Khan, J. A. 1999. Detection of tomato leaf curl geminivirus in its vector *Bemisia tabaci. Indian J. Exp. Biol*. 38: 512-515.

Khaskheli, M. I., Jiskani, M. M., Goswami, S. P., Poussio, G. B., and Khaskheli, M. A. 2017. Effect of okra yellow vein mosaic virus (OYVMV) on plant growth and yield. *J. Basic Appl. Sci*. 13:1-7.

Kirthi, N. and Savithri, H. S. 2002. A conserved zinc finger motif in the coat protein of tomato leaf curl virus is responsible for binding to ssDNA. *Arch. Virol.* 148: 2369–2380.

Kochko, A. and Hamon, S. 1990. A Rapid and Efficient Method for the Isolation of Restrictable Table DNA from Plants of the Genus Abelmoschus, *Plant Mol. Bio. Repoter*, 8:3-7.

Krake, L. R., Rezaian, M. A., and Dry, I. B. 1998. Expression of the tomato leaf curl geminivirus C4 gene produces virus like symptoms in transgenic plants. *Mol. Plant Microbe In.* 11(5):413-417.

Krishnareddy. M., Jalali S., and Samul, D. K. 2003. Fruit distortion mosaic disease of okra in India. *Plant Dis*. 87(11): 122.

Kulkarni, G. S. 1924. Mosaic and other related diseases of crops in the Bombay Presidency. Poona Agricultural College Magazine. 6:12.

Kumar U. and Kumar S. 2017. IDM module for management of yellow vein mosaic disease in okra. *HortFlora Res. Spectrum*, 6(3): 215-217.

Kumar, R., Esakky, R., and Palicherla, S. R. 2019. The emergence of okra enation leaf curl virus-an important begomovirus, infecting okra in several states across India. *Arch. Phytopathol. Plant Prot.,* 10:1-5.

Kumar, S., Chauhan, P. S., Agrawal, L., Raj, R., Srivastava, A., Gupta, S., Mishra, S. K., Yadav, S., Singh, P. C., Raj, S. K. and Nautiyal, C. S. 2016. *Paenibacillus*

lentimorbus inoculation enhances tobacco growth and extenuates the virulence of cucumber mosaic virus. *PLoS One*, *11*(3):e0149980.

Kunkalikar, S., Byadgi, A. S., Kulkarni, V. R., Krishnareddy, M., and Prabhakar, A. S. N. 2007. Histopathology and histo-chemistry of papaya ring spot disease in papaya. *Indian J. Virol*. 18(1): 33-35.

Lana, A. O. and Bozarth, R. F. 1975. Studies on a virus induced mosaic disease of *Abelmoschus esculentus* in Nigeria. *J. Phytopathol*. 83(1):77-86.

Latham, J. R., Saunders, K., Pinner, M. S., and Stanley, J. 1997. Induction of plant cell division by beet curly top virus gene C4. The Plant J. 11(6):1273-1283.

Lee, G. H. and Ryu, C. M. 2016. Spraying of leaf-colonizing *Bacillus amyloliquefaciens* protects pepper from cucumber mosaic virus. *Plant Dis.* 100 (10):2099-2105.

Lefeuvre, P., Martin, D. P., Hoareau, M., Naze, F., Delatte, H., Thierry, M., Varsani, A., Becker, N., Reynaud, B., and Lett, J. M., 2007, Begomovirus melting pot in the south-west Indian Ocean islands: molecular diversity and evolution through recombination. *J. Gen. Virol*. 88: 3458 – 3468.

Lima, A., Silva, J. C., Silva, F. N., Castillo-Urquiza, G. P., Silva, F. F., Seah, Y. M., Mizubuti, E. S., Duffy, S. and Zerbini, F. M., 2017. The diversification of begomovirus populations is predominantly driven by mutational dynamics. *Virus Evol*. 3(1).

Madhusudhan, K. N., Vinayarani, G., Deepak, S. A., Niranjana, S. R., Prakash, H. S., Singh, G. P., Sinha, A. K., and Prasad, B. C. 2011. Antiviral activity of plant extracts and other inducers against tobamoviruses infection in bell pepper and tomato plants. *Int. J. Plant Path.* 2(1): 35-42.

Malathi, V. G., Renukadevi, P., Chakraborty, S., Biswas, K. K., Roy, A., Sivalingam, P. N., Venkataravanappa, V., and Mandal, B. 2017. Begomoviruses and their

satellites occurring in India: Distribution, diversity and pathogenesis. In *A century of plant virology in India* (pp. 75-177). Springer, Singapore.

Mandahar, C. L. and Singh, J. S. 1972. Destruction of chlorophylls a and b in virus infected leaves. *Sci. Cultivation*. 37:485487.

Mandal, B. 1989. Studies on croton yellow vein mosaic virus, M.Sc. (Agri.) Thesis, Univ. Agril. Sci., Bangalore, India, 132 pp.

Markam, K. V. 2014. Yellow vein mosaic of okra: histopathology, cytopathology and sources of resistance. M. Sc. (Ag.) thesis, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), 92p

Mehta, P., Wyman, J. A., Nakhla, M. K. and Maxwell, D. P. 1994. Polymerase chain reaction detection of viruliferous *Bemisia tabaci* (Homoptera: Aleyrodidae) with two tomato-infecting geminiviruses. *J. Econ. Entomol*. 87(5):1285-1290.

Mishra, G.P., Singh, B., Seth, T., Singh, A. K., Halder, J., Krishnan, N., Tiwari, S. K., and Singh, P. M. 2017. Biotechnological advancements and begomovirus management in okra (*Abelmoschus esculentus* L.): status and perspectives. *Front. Plant Sci.* 8:360.

Mishra, S., Jagadeesh, K. S., Krishnaraj, P. U., and Prem, S. 2014. Biocontrol of tomato leaf curl virus (ToLCV) in tomato with chitosan supplemented formulations of Pseudomonas sp. under field conditions. *Aust. J. Crop Sci*. 8(3):347.

Mohamed, E. F. 2011. Changes in protein, amino acids composition and leaf cells of beet plants (*Beta vulgaris* L.) due to beet mosaic virus infection. *J. Am. Sci.* 7(12): 845-854.

Mohamed, E. F., Farag, A. G., Osman, T. A. M., and Eman, A. A. 2012. Histo-pathological changes in leaves cells of squash plants infected with squash leaf curl begomovirus (SqLCV). Rep. Opinion, 4(5): 65-75.

Mohanasundaram, A. and Sharma, R. K. 2011. Effect of newer pesticide schedules on the population of sucking pests and predators on okra. *Pestic Res. J.* 23(1):55-63.

Monci, F., Sanchez-Campos, S., Navas-Castillo, J., and Moriones, E. 2002. A natural recombinant between the geminiviruses tomato yellow leaf curl Sardinia virus and tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Phytopathology,* 303: 317-326.

Muhire, B. M., Varsani, A., and Martin, D. P. 2014. SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE,* 9:1-4.

Mukhopadhyay, S., Nath, P. S., Das, S., Basu, D., and Verma, A. K. 1994. *Competitive epidemiology and cultural control of some whitefly transmitted viruses in West Bengal.* Virology in the tropics. Malhotra Publishing House, New Delhi, 308p.

Muniyappa, V. and Reddy, H. R. 1976. Studies on the yellow mosaic disease of horsegram (*Dolichos biflorus* L.) - Virus vector relationships. *Mysore J. Agric. Sci.* 10: 605-610.

Muniyappa, V. and Veeresh, G. K. 1984. Plant virus diseases transmitted by whiteflies in Karnataka. *Proceedings: Animal Sciences, 93*(4):397-406.

Murphy, J. F., Zehnder, G. W., Schuster, D. J., Sikora, E. J., Polston, J. E. and Kloepper, J. W. 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against tomato mottle virus. *Plant Dis.* 84(7):779-784.

Murugesan, S. and Chellaiah, S. 1977. Transmission of greengram yellow mosaic virus by the whitefly, *Bemisia tabaci*. *Madras Agric. J.* 64: 437-438.

N'guessant, K. P., Fargettet, D., Fauquet, C., and Thouvenel, J. C. 1992. Aspects of the epidemiology of okra leaf curl virus in Côte d'Ivoire. *Trop. Pest Manag*. 38: 122–126.

Nariani, T. K. and Seth, M. L. 1958. Reaction of *Abelmoschus* and *Hibiscus* species to yellow vein mosaic virus. Indian Phytopathol. 11:137-140.

Nateshan, H. M. 1992. Biological properties of cotton leaf curl geminivirus. M.Sc (Agri.) Thesis, University of Agricultural Science, Bangalore, India, 123 pp.

Nath, P. and Saikia, A. K. 1992. Relative resistance of okra cultivars to yellow vein mosaic virus. *New Agric*. 3:199-202.

Nath, P. S. and Debnath, S. 2002. Management of yellow vein mosaic disease of okra through insecticides, plant products and suitable varieties. *Ann. Plant Protect. Sci*. 10:340-342.

NHB [National Horticulture Board]. 2015. *Horticulture statistics at a glance 2015*. [online] Available: http://nhb.gov.in/area-pro/ horst-galance-2016.pdf [18 Nov 2015].

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

Pagire, K. S., Sonalkar, V.U., Dipali, P., Thakare, V.V., Kalpande, and Chavan, V. M. 2017. Effectiveness of insecticidal sprays against whitefly checking yellow vein mosaic virus (YVMV) incidence in okra (*Abelomoschus esculentus* L. Moench). *Int. J. Curr. Microbiol. App. Sci*. 6(2): 362-366.

Patil, N. M. 2011. Potential of Rhizobacteria to control bhendi yellow vein mosaic virus in bhindi. M. Sc. (Ag) Thesis, University of Agricultural Sciences, Dharwad, 86p.

Polston, J. E. and Anderson, P. L. 1997. The emergence of whitefly transmitted geminiviruses in tomato in the Western hemisphere. *Plant Dis*. 81:1358–1369.

Priyavathi, P., Kavitha, V., and Gopal, P. 2016. Complex nature of infection associated with yellow vein mosaic disease in Bhendi (*Abelmoschus esculentus*). *Curr. Sci*. 111:1511-1515.

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

Pun, K. B., Doraiswamy, S., and Jeyarajan, R. 1999. Effect of plant extracts on Pumpkin yellow vein mosaic virus transmission. *Indian Phytopathol.* 52: 357-361.

Rameshwar, B. D. 2010. Studies on management strategies of yellow vein mosaic disease of okra (*Abelmoschus esculentus* (L.) Moench). M. Sc. (Ag.) thesis. Indira Gandhi Krishi Vishwavidyalaya Raipur (C.G.), 152p.

Rathi, Y. P. S. and Nene, Y. L. 1974. Some aspects of the relationship between mungbean yellow mosaic virus and its vector *Bemisia tabaci*. *Indian Phytopathol*. 27: 459-462.

Raychaudhuri, S. P. and Nariani, T. K. 1977. *Viruses and Mycoplasma diseases of plants in India*. Oxford and IBH Publishing company, New Delhi, pp-160-167.

Ribeiro and Al. E. 1998. Widespread occurrence of tomato geminiviruses in Brazil, associated with the new biotype of the whitefly vector. *Plant Disease note,* 82: 830.

Rosell, R. C., Torres-Jerez, I., and Brown, J. K. 1998. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph, and honeydew. *Phytopathology*, 89:239-246.

Rouhibakhsh, A., Priya, J., Periasamy, M., Haq, Q. M. I., and Malathi, V. G. 2008. An improved DNA isolation method and PCR protocol for efficient detection of multicomponents of begomovirus in legumes. *J. Virol. Methods*, 147(1):37-42.

Roy and Sherpa. 2015. Available: https://www.ijsr.net/archive/v6i5/ART20173077.pdf. *Int.J. Sci and Res.* 2319-7064.

Roy, B., Chakraborty, B., Mitra, A., Sultana, S., and Sherpa, A. R. 2015. Natural occurrence of bhendi yellow vein mosaic virus on *Litsea* spp. in India. *New Dis. Rep. 31*:7-7.

Roy. B., Sultana, S., and Sherpa, A. R. 2017. First Report of bhendi yellow vein mosaic virus in *Tabernaemontana divaricata* and *Albizia saman* in India. *Austin J Plant Biol.* 3(1): 1017.

Rybicki, E.P. 1994. A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Arch. Virol.*, 139: 49-77.

Sahu, S. K., Thangaraj, M., and Kathiresan, K. 2012. DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. *Mol. Biol*. 1:1-6.

Saitou N. and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4:406-425.

Sale, F. A., Fadipe, T. F., and Adah, H. 2015. Effect of neem extract preparations and nutrient sources on performance of okra (*Abelmoschus esculentus*) plant. *Nigerian J. Agric. Food Environ.* 11(2):221-225.

Sanwal S. K, Singh M, Singh B, and Naik P. S. 2014. Resistance to yellow vein mosaic virus and okra enation leaf curl virus: challenges and future strategies. *Curr. Sci.* 106:470-1471.

Sanz, A. I., Fraile, A., GarciaArenal, F., Zhou, X. P., 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. *J. Gen. Viro*l. 81: 1839-1849.

Sastry, K. S. M. and Singh, S. J. 1974. Effect of yellow vein mosaic virus infection on growth and yield of okra crop. *Indian Phytopathol*. 27: 294–297.

Saunders, K., Bedford, I. D., Briddon, R. W., Markham, P.G., Wong, S.M., Stanley, J. 2000. A novel virus complex causes Ageratum yellow vein disease. *Proc. Natl. Acad. Sci.* 97:6890–6895.

Senevirathna, H. M. S. I., Wasala, S. K., Senanayake, D. M. J. B., Weerasekara, D., Wickamasinghe, H. A. M., and Deepal, P. K. G. A. 2016. Characterization and detection of yellow vein disease of okra (*Abelmoschus esculentus* (L.) Moench) in .Sri Lanka. *Trop. Agrl. Res*. 27(4):360-369.

Serfraz, S., Amin, I., Akhtar, K. P., and Mansoor, S. 2014. Recombination among begomoviruses on malvaceous plants leads to the evolution of okra enation leaf curl virus in Pakistan. *J. Phytopathol*, 163(9):764-776.

Sharma, N. K., Singh, S., and Awasthi, L. P. 2017. Prevention and control of viral diseases in watermelon through botanical biopesticides. *Virol Res Rev*. 1(3): 1-8

Sharma, P., Rishi, N., and Malathi, V. G. 2005. Molecular Cloning of Coat Protein Gene of an Indian cotton leaf curl virus (CLCuV-HS2) Isolate and its Phylogenetic Relationship with others Members of *Geminiviridae*. *Virus Gen*. 30(1):85–91

Sheikh, M. A., Safiuddin, Khan, Z., and Mahmood, I. 2013. Effect of bhendi yellow vein mosaic virus on yield components of okra plants. *J. Plant Pathol.* 12*:* 391-393.

Shih, S. L., Green, S. K., Tsai, W. S., Lee, L. M., and Levasseur, V. 2007. First report of a distinct begomovirus associated with okra yellow crinkle disease in Mali. *Plant Pathol.* 56:718.

Singh, B., Sanwal, S., Venkataravanappa, V., and Halder, J. 2013. "Breeding strategies for biotic stresses of okra: prospects and potential," in Abstract Book of National Symposium on Abiotic and Biotic Stress Management in Vegetable Crops, (Varanasi), 32–33.

Singh, B., Singh, P. M., Sanwal, S. K., and Pal, A. K. 2014. Standardization of cost effective hybridization technique for hybrid seed production in okra (*Abelmoschus esculentus*). *Indian J. Agr. Sci*. 84, 1111–1114

Singh, S. J. 1996. Assessment of losses in okra due to enation leaf curl virus. *Indian J. Virol.* 12:51–53.

Singh, S. J., Shastry, K. S., and Sastry, K. M. S. 1977. Yellow vein mosaic virus disease of bhendi. *Indian Hort.* 22(1): 14-15.

Singh, S. P., Singh, S. K. Awasthi, L. P., and Chaudhary, A. K. 2016. Studies on the transmission of yellow vein mosaic virus disease in okra. *Res. Environ. Life Sci.* 9(1):29-30.

Singh, S.J. 1986. Enation leaf curl of okra a new virus disease. *Indian J. Virol*., 2:114-117.

Singh, S. J. 1990. Etiology and epidemiology of whitefly transmitted virus diseases of okra in India. *Plant Dis. Res*., 5(1): 64-70.

Singh, V. and Kumar, V. 2012. An optimised method of DNA isolation from highly mucilage rich okra (*Abelmoschus esculentus* L.) for PCR analysis. *Adv. Appl. Sci. Res*, 3(3):1809-1813.

Sohrab, S. S., Mirza, Z., Karim, S., Rana, D., Abuzenadah, A. M., Chaudhary, A.G., and Bhattacharya, P. S. 2013. Detection of begomovirus associated with okra leaf curl disease. *Arch. Phytopathol Plant Protect*. 46(9):1047-1053.

Stanley, J. M. R. 1983. Nucleotide sequence of cassava latent virus DNA. *Nature*, 301:260-262.

Swanson, M. M. and Harrison, B. D. 1993. Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. *Biochimie* 75:707–711.

Torre- Almarz and Monsalvao- Reyes, A. C. 2003. First report of geminivirus including yellow mottle in okra (*Abelmoschus esculentus*) in Mexico. *Plant Dis*. 87:202.

Uppal, B. N., Varma, P. M., and Capoor, S. P. 1940. Yellow vein mosaic of bhendi. *Curr. Sci.* 9:227-228.

USDA [United States Department of Agriculture]. 2019. Nutrient composition of okra. [online] Available: https://ndb.nal.usda.gov/ndb/foods/show/11278? [20 Oct 2018]

Varma, A. and Malathi, V.G. 2003. Emerging geminivirus problems: a serious threat to crop production. *Ann. Appl. Biol.* 142(2):145-164.

Varma, P. M. 1952. Studies on the relationship of the bhendi yellow vein mosaic virus and vector, the whitefly (*Bemisia tabaci*). *Indian J. Agric. Sci*. 22:75-91.

Venkataravanappa, V. 2008. Molecular characterization of *bhendi yellow vein mosaic virus.* Ph. D. Thesis. University of Agricultural Sciences, Bangalore, 356p.

Venkataravanappa, V., and Reddy, M. K. 2013. Begomovirus characterization, and development of phenotypic and DNA-based diagnostics for screening of okra genotype resistance against bhendi yellow vein mosaic virus. *Biotech.* 3:461–470.

Venkataravanappa, V., Reddy, C. N. L., Devaraju, A., Jalali, S., and Reddy, M. K. 2013a. Association of a recombinant cotton leaf curl Bangalore virus with yellow vein and leaf curl disease of okra in India. *Indian J. Virol*. 24:188–198.

Venkataravanappa, V., Reddy, C. N. L., Jalali, S., and Reddy, M. K. 2012a. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Gen.* 44:522–535.

Venkataravanappa, V., Reddy, C. N. L., Jalali, S., and Reddy, M. K. 2013b. Molecular characterization of a new species of begomovirus associated with yellow vein

mosaic of bhendi (okra) in Bhubhaneswar, India. *Eur. J. Plant Pathol*. 136:811–822.

Venkataravanappa, V., Reddy, C. N. L., Jalali, S., Briddon, R. W., and Reddy, M. K. 2014. Molecular identification and biological characterization of a begomovirus associated with okra enation leaf curl disease in India. *Eur. J. Plant Pathol.* 141:217–235.

Venkataravanappa, V., Reddy, C. N. L., Swarnalatha, P., Devaraju, A., Jalali, S., and Reddy, M. K. 2012b. Molecular evidence for association of cotton leaf curl Alabad virus with yellow vein mosaic disease of okra in North India. *Arch. Phytopathol. Plant Prot*. 45:2095–2113.

Vethanayagam, S.M. and Rajendran, S.M., 2010. Bioefficacy of neem insecticidal soap (NIS) on the disease incidence of bhendi, *Abelmoschus esculentus* (L.) Moench under field conditions. *J. Biopesticides*, 3:246.

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: 1288–93.

Zerbini, F. M., Briddon, R.W., Idris, A., Martin, D. P., Moriones, E., Nacas-Castillo, J., Bustamante, R., Roumagnac, P., and Varsani, A. 2017. ICTV virus taxonomy profile: Geminiviridae. *J. Gen. Virol.* 98(2):131–133.

Zhou, X. P., Liu, Y., Calvert, L., Munoz, C., Otime-Nape, G. W. Robinson, D. J., and Harrison, B. D. 2003. Evidence that DNA-A of geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *J. Gen. Virol*. 78: 2101–2111.

Zhou, X., Liu, Y., Robinson, D. J., and Harrison, B. D. 1998. Four variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. *J. Gen. Virol*. 79: 915–923.

Appendices

APPENDIX I

Composition of reagents used for DNA isolation and agarose gel electrophoresis

1. **CTAB buffer (2X)**

   - 2 per cent CTAB (w/v)
   - 100mM Tris base (pH 8)
   - 20mM EDTA (pH 8)
   - 1.4 M NaCl
   - 1.0 per cent polyvinyl pyrrolidin
   - 0.2 per cent 2-β mercaptoethanol

2. **Chloroform:isoamyl alcohol (24:1 v/v)**

3. **TAE buffer (50X) for 1 litre**

   - Tris base -247g
   - glacial acetic acid -57.1 ml
   - 0.5 M EDTA (pH 8) -100 ml
   - Distilled water -1000 ml

4. **Loading dye (6X)**

   - 0.25 per cent Bromophenol blue
   - 0.25 per cent Xylene cyanol
   - 30 per cent Glycerol in water

APPENDIX II

List of laboratory equipments used for the studies

Refrigerated centrifuge	: REMI CPR 24 Plus
Electrophoresis unit	: Tarson
PCR machine	: Eppendorf Master Cycler
Gel documentation	: BIORAD Molecular Imager
Nanodrop	: Nanodrop 1000

Molecular characterization, host range and integrated management of bhindi yellow vein mosaic disease

By

Chinju E. A.

(2017-11-030)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2019

ABSTRACT

Bhindi (*Abelmoschus esculentus* (L.) Moench) is one of the most important vegetable crops cultivated across the globe. However, its cultivation is often hindered by biotic stresses like incidence of pests and diseases. Among the diseases, yellow vein mosaic disease is one of the major constraints in bhindi cultivation which leads to 100 per cent yield loss especially when infected at an early stage of the crop. In recent years, the evolution of new viral strains is a serious problem especially among *Begomoviruses* belonging to the family *Geminiviridae* which has an adverse effect on the host plant resistance. Considering these facts, the present study was undertaken to carry out the molecular characterization of the virus causing bhindi yellow vein mosaic disease (BYVMD), to study the host range and seed transmission of the virus and to develop a sustainable disease management strategy.

The project was initiated with purposive sampling survey conducted in elevan different locations of Thrissur district, Kerala. The disease incidence recorded during the survey ranged from 61.20 to 98.16 per cent while the disease severity ranged from 48 to 90 per cent. The predominant symptoms observed on the leaves of infected plants under natural conditions were vein clearing, vein thickening, reduction in leaf area, bleached appearance and marginal necrosis. A novel type of symptom observed during the survey was general yellowing of leaves with severe puckering along the veins and upward curling of leaves. Linear cholorotic striations were observed on the calyx of the flower buds. The immature fruits produced by the infected plants showed linear chlorotic striations, while the mature fruits were bleached in appearance along with reduction in fruit size. The plants infected during the early vegetative stage were extremely stunted. The major symptoms developed under artificial conditions were vein clearing, vein thickening and puckering of leaves.

Histopathological studies of the infected leaf revealed disruption of parenchymtous cells in the epidermis, disintegration of chloroplast, reduction in number of metaxylem and protoxylem along with abnormality of phloem vessels.

The studies on virus transmission confirmed that it is transmitted through grafting and insect vector, *Bemisia tabaci*. The presence of virus inside the insect body was also confirmed through polymerase chain reaction (PCR) based molecular technique. The studies on seed transmission revealed that BYVMD is not seed-borne. Host range studies revealed that weed species *Synedrella nodiflora* and *Hemidesmus indicus* were proved to be hosts of the begomovirus.

Molecular detection of the virus causing BYVMD was standardized through PCR, using universal primer specific to the core coat protein gene of *Begomovirus* which yielded amplicons at expected size of about 550 bp. The amplification was also carried out using primers specific to coat protein gene of bhindi yellow vein mosaic virus (BYVMV) and okra enation leaf curl virus (OELCuV) which yielded amplicons at expected band size of about 770 bp.

The molecular characterization of the elevan isolates was carried out through *in silico* analysis to identify the virus associated with BYVMD and for diversity analysis. The results revealed that all the isolates showed 99-100 per cent nucleotide homology to OELCuV. BLASTp analysis of the isolates also showed 100 per cent identity with coat protein of OELCuV and thus confirming that the virus causing yellow vein mosaic disease in bhindi in Kerala is okra enation leaf curl virus. The identity was further confirmed through DNA barcoding technique and species demarcation analysis.

A field experiment was also conducted to develop a disease management package against BYVMD. Among the seven treatments, T5 *i.e.,* integrated management with early seedling protection using insect proof net + yellow sticky trap + seed bio-priming and foliar spray of PGPR mix II + alternate foliar spray of *Bougainvillea spectabilis* and azadiractin was found to be most effective with

lowest disease incidence and severity, least whitefly population and maximum yield.

It is concluded that yellow vein mosaic disease affecting bhindi cultivation in Kerala is caused by okra enation leaf curl virus, an evolved strain of BYVMV. This virus is transmitted through grafting and the insect vector *Bemisia tabaci* and not though seeds. The outcome of the study would also facilitate early detection and elimination of sources of infection so as to reduce the spread of the disease. An integrated disease management package was also developed for the benefit of farming community.