Technical Bulletin No. 75

VIRUSES OF VEGETABLE CROPS: SYMPTOMATOLOGY, DIAGNOSTICS AND MANAGEMENT

ICAR - Indian Institute of Vegetable Research
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VIRUSES OF VEGETABLE CROPS: SYMPTOMATOLOGY, DIAGNOSTICS AND MANAGEMENT

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PREFACE

Vegetables become the part of human diet due its nutritional and nutraceutical benefits. Area under vegetable cultivation is about 9.4 million ha with a production of 162.90 million tons. The contribution of vegetables remains highest (59–61%) in horticulture crop productions. Area on vegetable cultivation is progressively increasing due to its high demand and shorter crop duration. As the production is increasing, threats posed by biotic and abiotic factors are also increasing every day. Among the biotic factors such as pests and diseases, viral diseases are emerging as a major constraint in the changing climatic scenario. At several instances, viral diseases are causing severe yield penalty upto 100%. There always remains confusion in differentiating the viral disease from other abnormalities inorder to choose correct management strategy for the virus free crop production.

In this endeavor, information generated at Indian Institute of Vegetable Research, Varanasi and other national level institutions are scattered in various formats and are not available under one head. In this view, efforts has been made to compile available information and presented in the form of bulletin entitled "**Viruses of Vegetable Crops: Symptomatology, Diagnostics and Management**". The information on major viruses infecting vegetable crops in India are presented under different subheadings like symptoms with figures, host range, mode of transmission and diagnostics. Management strategies to be followed for the viral disease on vegetable crops and crop specific modules are also presented. We hope that this bulletin will be highly useful to the farmers, extension workers, state officials, academicians, students and researchers. The help rendered by the scientists of crop protection division in the preparation of this manuscript is thankfully acknowledged.

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Background

Vegetable crops are cultivated worldwide for its nutritional benefits and neutraceutical properties. It is the only source to attain the goal of nutritional security. Among the plant pathogens, viruses are causing serious damage to agriculture and horticultural crops in reducing both quality and quantity of the produce. Viruses are ultramicroscopic pathogenic propagules which multiply in living cells using host components. They have either single stranded (ss) or double stranded (ds) RNA or DNA but not both, enclosed in a protein coat. Particles may be rigid rod, flexuous, filamentous or icosahedral particles. Annual losses due to virus diseases may vary, but under the pathogen favourable conditions, virus diseases lead to disasterous consequences on farmers. Since there is no cure to virus-infected plants, management options emphasizes on exclusion of virus, minimizing virus spread and development of host plant resistance. This important task critically depends on the comprehensive diagnosis of the virus/viruses involved in the disease etiology. In this bulletin importance of major viral diseases of vegetable crops, their diagnostics tools and management are described in detail for the benefit of vegetable growers in India.

About 130 vegetable crops used by man (excluding potato, sweet potato, cassava, and legumes) are infected with nearly 200 viruses. Of these, more than 90 viruses have been found in the most cultivated vegetables (tomato, watermelon, cabbage, onion, cucumber and brinjal). Based on a survey, top ten plant infecting viruses are Tobacco mosaic virus (tobamovirus, TMV), Tomato spotted wilt virus (orthotospovirus, TSWV), Tomato yellow leaf curl virus (begomovirus, TYLCV), Cucumber mosaic virus (cucumovirus, CMV), Potato virus Y (potyvirus, PVY), Cauliflower mosaic virus (caulimovirus, CaMV), African cassava mosaic virus (begomovirus, ACMV), Plum pox virus (potyvirus, PPV), Brome mosaic virus (bromovirus, BMV) and Potato virus X (potexvirus, PVX). Among these viruses, majority are infecting vegetables. Important viruses infecting vegetables crops in India are belongs to the genera *Begomovirus*, Orthotospovirus (*Tospovirus*), *Cucumovirus*, *Potyvirus* and *Tobamovirus*. In addition, other viruses such as *Polerovirus, Potexvirus, Ilarvirus,* etc are also emerging in recent days. Among them Begomoviruses and orthotospoviruses are of much significance to vegetable crops. Infections of different viruses cause an average yield penalty of 70 - 80 per cent to vegetable production.

Economic losses on different vegetable crops by virus infection

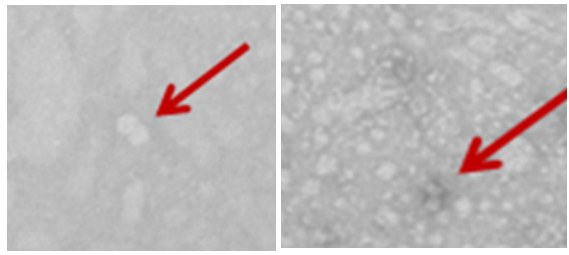
S. No.	Crop	Disease	Virus	Yield loss (%)
1.	Tomato	Leaf curl virus	*Begomovirus*	>70
2.	Chilli	Leaf curl virus	*Begomovirus*	>80

3.	Okra	yellow vein mosaic	*Begomovirus*	>50-9
4.	Okra	Enation leaf curl	*Begomovirus*	30-100
5.	Chayote	Mosaic	*Begomovirus*	>60
6.	Vegetables	Bud necrosis	*Orthotospovirus*	29-100
7.	Tomato	Bud necrosis	*Orthotospovirus*	80-100
8.	Watermelon	Bud necrosis	*Orthotospovirus*	60-100
9.	Cucurbits	Green mottle mosaic	*Tobamovirus*	10-15
10.	Watermelon	Green mottle mosaic	*Tobamovirus*	11.4-47.6
11.	Cucurbits	Yellow mosaic	*Potyvirus*	upto 95
12.	Cowpea	Mosaic	*Cucumovirus*	14
13.	Vegetables	Mosaic	*Cucumovirus*	10-20
14.	Tomato	Mosaic	*Cucumovirus*	25
15.	Cucurbits	Mosaic	*Cucumovirus*	upto 100
16.	Chilli	Mosaic	*Cucumovirus*	28-40

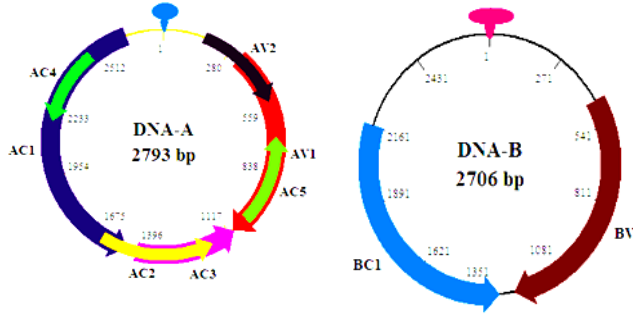
1. MAJOR VIRUS GENERA INFECTING VEGETABLE CROPS IN INDIA

1.1. BEGOMOVIRUS

Begomoviruses are circular single stranded DNA viruses and their genomes encapsidated inside small twinned icosahedral particles, belongs to the family Geminiviridae. These are major limiting factor in the production of vegetables and other crops worldwide. They are mono- or bipartite single-stranded DNA plant viruses and transmitted by whiteflies in circulative, non-propagative manner. The important diseases caused by begomviruses are leaf curl in solanaceous vegetables, yellow vein mosaic in okra, yellow mosaic in grain legumes, and yellow mottle in cucurbits. The disease incidence in all the cases ranges from 90-100 per cent during the dry season and losses may exceed 60 per cent.



Electron microscopic view of twinned icosahedral begomovirus particle



Genome organization of begomovirus (DNA A and DNA B) causing yellow vein mosaic disease on pumpkin

1.1.1. Symptoms

1.1.1.1. Leaf curl diseases:

Tomato: Tomato leaf curl disease caused by mono and bi-partite single-stranded DNA begomoviruses (Tomato leaf curl New Delhi virus, Tomato leaf curl Gujarat virus, Tomato leaf curl Palampur virus and Tomato leaf curl Bangalore virus) and is transmitted by whitefly in circulative persistent manner. The virus produces diverse symptoms as leaf curling, leaf puckering, mottle and leaf distortion. The disease incidence in all the cases ranges from 90-100 per cent during the dry season and losses may exceed 70 per cent.

Leaf curl disease on tomato

Yellowing and curling of leave

Chilli: Chilli leaf curl disease caused by mono or bi-partite single-stranded DNA begomoviruses (chill leaf curl virus, pepper leaf curl Bangladesh virus and tomato leaf curl Joydebpur virus). The virus produces different type of symptoms like curling, mottling and distortion of leaves, flowers, fruits and affected plants are shorter than the healthy plants.

Symptom variations of chilli leaf curl disease on chilli caused by begomovirus

Clearing of veinlets on begomovirus infected chilli plants

Severe stunting of plant

Severe curling of leaf

Brinjal: Leaf curl disease on brinjal caused by monopartite begomovirus Tomato leaf curl Joydebpur virus. Infected plants exhibiting symptoms, predominantly of leaf curl, little leaf and mosaic. Sometimes it is being associated with the betasatellite particles. The incidence of leaf curl disease varied between fields at different locations and ranged between 10% and 20%.

Leaf curl disease on brinjal

Mosaic and curling of leaves

Radish: Infected radish plants show typical virus infection symptoms such as stunted and distorted growth and leaf curl. In case of severe infection, enations can be seen on the lower side of the leaves. Causal agent has been identified as Radish leaf curl virus and Croton yellow vein mosaic virus. Incidence ranging between 10 and 40% depending on the cultivars.

Leaf curl disease on radish

Twisting and curling of leaves with enation on lower side

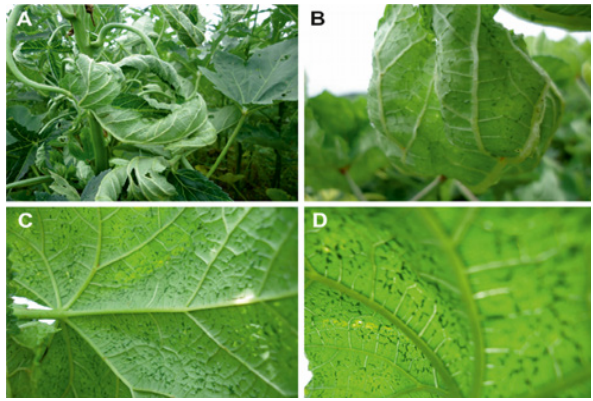
Bhendi yellow vein mosaic disease: It is caused by mono and bi-partite single-stranded DNA begomoviruses (Bhendi yellow vein mosaic virus, Bhendi yellow vein Delhi virus). The affected plant shows bleaching of vein and veinlets on leaves whereas interveinal areas remains green. Upon advancement of disease, entire plants become white. Infected plants remain stunted by producing bleached fruits of unmarketable quality. The disease incidence in all the cases range from 90-100 per cent during the dry season and losses may exceed 50 per cent.

Yellow vein disease on okra

Clearing of veins and veinlets

Okra enation leaf curl disease: It is caused by monopartite single-stranded DNA begomovirus (Okra enation leaf curl virus) and is transmitted by whitefly causing upto 90% crop loss. The disease initially causes small pinhead enations on the under surface of the leaves. This is followed by a warty and rough texture of leaves, later leaves curl upwards. Affected plants show a twisting of the stem, petioles and lateral branches with leaves becoming thick and leathery. In severely infected plants, fruit setting drastically reduced and seeds may be aborted.

Enation leaf curl disease on okra



Twisting and curling of leaves (A); Cupping of leaves (B); Enation on lower side of leaves (C-D)

Golden mosaic disease: Golden mosaic disease caused by bi- or mono- partite single-stranded DNA begomovirus (Mungbean yellow mosaic virus, Mungbean yellow mosaic India virus and Dolichos yellow mosaic virus) and is transmitted by whitefly in circulative persistent manner. Virus produces golden yellow mosaic patches on leaves of infected plant. Infected plants show stunted plant growth.

Yellow mosaic on cowpea

Yellow mosaic on dolichos bean

Yellow mosaic on French bean

Mosaic disease on cucurbits: It is mainly caused by two viruses namely, Tomato leaf curl New Delhi virus and Squash leaf curl China virus. These diseases produce characteristics symptoms such as mosaic mottling of leaves, reduced leaf size, crinkling of leaves, distortion of leaves, yellow vein, enation on lower side of leaves, stunted growth of plant, yellow mosaic patches on leaves, etc. Incidence in several instances recorded upto 100%.

Mosaic disease on pumpkin

Yellow mosaic on leaves

Rosetting and reduced size of leaves

Mosaic disease on bitter gourd

Mosaic disease on ridge gourd

Yellow mosaic on leaves

Stem and leaf deformation

Mosaic disease on ash gourd

Mosaic disease on squash

Mosaic disease on chayote

Severe mosaic on leaves and fruit malformation

Enation on lower side of leaves

Curly shoot disease on French bean: Infected plants of common bean exhibit stunting, stem twisting, curly shoot, thickening of lower leaf surface veins and galling with dark green colour. It is found to be caused by monopartite begomovirus Tobacco curly shoot virus (TbCSV).

Curly shoot disease on French bean

1.1.2. Host Range

Tomato leaf curl viruses have a wide host range and infect tomato, eggplant, cucurbits, cotton, chilli, weeds, etc. ToLCV virus has been reported on various cucurbitaceous crops *viz*., pumpkin, cucumber and melon, sponge gourd, chayote, squash, bottle gourd, cucumber, muskmelon, etc. Squash leaf curl virus (SLCV) has been reported on pumpkin, wax gourd, squash and summer squash.

1.1.3. Transmission

All members of the begomoviruses are transmitted by the whitefly (*Bemisia tabaci*) in circulative and persistent manner. The minimum acquisition access period (AAP) and inoculation access period (IAP) required by *B. tabaci* to transmit ToLCV is found to be 30 minutes each. After acquisition the vector required 6 h to become viruliferous. Single whitefly is able to transmit the virus, the whiteflies remained infective throughout their life span and the virus is not transmitted to the progeny of the whitefly. These viruses are also transmitted through grafting with 100% efficicency but not through mechanical, pollen, seeds and sap.

1.1.4. Diagnostics

Due to non availability of specific antiserum for vegetable infecting begomoviruses, polyclonal antibodies of Squash leaf curl virus (SLCV), Indian cassava mosaic virus (ICMV) and African cassava mosaic virus (ACMV) can be used in DAS-ELISA for the detection of begomoviruses infecting vegetable crops.

1.1.4.1. Molecular detection

1.1.4.1.1. Isolation of genomic DNA

Total DNA was extracted from symptomatic young leaves by CTAB method using 2 per cent β-mercapto ethanol. 100mg of leaves were ground to fine powder using liquid nitrogen. Prewarmed DNA extraction buffer (N-cetyl-N,N,N trimethyl ammonium bromide (2 %), 100 mM Tris Hcl (pH: 8.0), 1.4 M NaCl, 20 mM EDTA (pH: 8.0) and β-mercaptoethanol (2%) was added to the ground leaves and incubated at 65°C for 30 min followed by the addition of 0.7-0.8 vol. of chloroform and isoamylalcohol (24:1 v/v). The contents were gently mixed by inverting the tube for 10 min and then centrifuged at 10,000 rpm for 10 min. The upper aqueous phase containing DNA was transferred to a new 1.5 ml micro centrifuge tube and added with equal volume of isopropanol and mixed well by inverting the tube to precipitate the nucleic acid and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and the DNA pellet was washed with 70% ethanol and air dried. The DNA pellet was resuspended in 50 µl of sterile double distilled water. The genomic DNA was checked by 0.8% agarose gel electrophoresis and stored at -20°C for further use.

1.1.4.1.2. Enrichment of viral DNA through rolling circle amplification

In order to characterize the genomic components of the begomovirus, 70ng of total nucleic acid extracted were subjected to rolling circle amplification (RCA) using Ø29 DNA polymerase. The RCA utilizes the DNA polymerase of the *Bacillus subtilis* bacteriophage Ø29, which possesses polymerase and strand-displacement activity, allowing circular templates to be amplified preferentially. The reaction mixture consisted of

Genomic DNA template	:	0.7 µl (70 ng)
Exo-resistant Random hexamer primer (preferably 3' protected)	:	2.0 µl (50 µM)
dNTPs	:	2.0 µl (1 mM)
10X Ø29 DNA polymerase buffer	:	2.0 µl (1X)
Sterile distilled water (to make up the volume to 20µl)	:	8.6 µl

- The reaction mixture was incubated at 94ºC for 3 min to denature the template DNA.
- Cooled it to room temperature slowly to facilitate the annealing of primers and then added the enzyme.

Ø29 Polymerase (10 units/µl)	:	0.7 µl
Pyrophosphotase Inorganic (from yeast) (0.1 unit/µl)	:	4.0 µl

- The whole mixture was incubated at 30°C for 18-20 h in a water bath.
- Reaction was stopped by inactivating at 65ºC for 10 min in a dry bath incubator.

The products can be used for detection of begomoviruses through PCR assay with the following set of universal and specific primer pairs.

1. Begomovirus universal primer (Deng *et al.*, 1994)

Deng 540	5'TAATATTACCKG WKGVCSC3'
Deng 541	5'TGGACYTTRCAWGGBCCTTCACA3'

Amplification size: ~510bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	56	1 m	
Extension	72	2 m	
Final Extension	72	30 m	

2. Begomovirus Universal primer (Rojas *et al*., 1994)

PALIc1960	5'ACNGGNAA RACNATGTGGGC3'
PALIr772	5'GGNAARTHTGGATGGA3'

Amplification size: ~1200bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	30 cycles
Annealing	55	2 m	
Extension	72	3 m	
Final Extension	72	10 m	

3. Begomovirus coat protein specific primer (Nagendran *et al*., 2014)

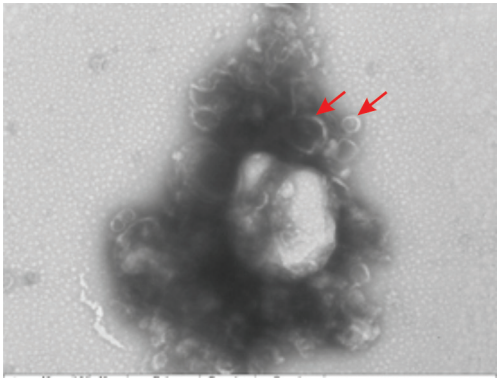
GK ToLCV F	5'ATGKYGAAGCGACCAGCMGA3'
GK ToLCV R	5'CGCCCKCMGAYTGGG MTTTTCTT3'

Amplification size: ~950bp

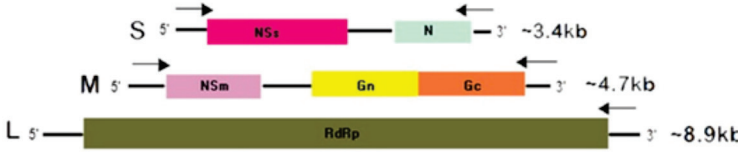
Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30s	35 cycles
Annealing	57	30s	
Extension	72	90s	
Final Extension	72	10 m	

1.2. ORTHOTOSPOVIRUS

Orthotospovirus (*Tospovirus*) is the only plant infecting virus genera belongs to Tospoviridae family under the order Bunyavirales, transmitted by thrips (Thysanoptera: Thripidae). Virion particles are quasi-spherical shaped with a size ranging from 80-120 nm in diameter surrounded by a host derived membrane envelope on which two glycoprotein's (Gc and Gn) are embedded. Genome comprises tripartite negative sense RNA genome packed inside the nucleoprotein (N). Based on the size of RNA, they were named as large (L RNA), medium (M RNA) and small (S RNA). Dispersal and survival of orthotospovirus is mainly depends thrips vector which are effective in transmitting orthotospovirus from infected plants to healthy ones under natural conditions. Thrips feed on infected plants and transmit the orthotospoviruses in persistent and propagative manner. Larvae of 1st and 2nd instars can acquire the virus and adults can transmit the virus after a latent period. Orthotospoviruses acquired by adult thrips can not be transmitted. In recent years, orthotospoviruses are becoming most serious threats to vegetable cultivation in India. Infection of orthotospovirus on vegetable crops causes 29 to 100% yield loss and also deteriorates the fruit quality. Among 23 orthotospovirus species reported globally, *Groundnut bud necrosis virus, Watermelon bud necrosis virus, Capsicum chlorosis virus, Iris yellow spot virus* and *Peanut yellow spot virus* have been reported on different vegetable crops in India.



Electron microscopic view of quasi-spherical shaped orthotospovirus particle



Genome organization of orthotospovirus

1.2.1. Symptoms

Symptoms of orthotospovirus differ among crops and also varieties of symptoms are associated with single virus on single host species. Stunting of infected plant is common symptoms of orthotospovirus infecting vegetable crops. Chlorotic or necrotic rings are seen on the leaves of many infected plants and sometimes on fruits of some hosts. Necrosis may develop on the foliage and stem of some hosts and make the plants to dry from tip downwards and is commonly called as "bud necrosis". Sometimes it causes chlorosis on leaves of infected plants. Advancement of disease may cause death of infected plant.

Symptom variations of PBNV infection on tomato

Circular necrotic spots on the leaves with green center

Necrotic lesions on leaves

Necrotic lesions on stem

Circular concentric ring spots on fruit surface

Symptom variations of PBNV on brinjal

Chorotic concentric rings on leaves

Circular necrotic lesions with green centre

Symptom variations of CaCV on chilli

Chorotic lesions with concentric rings on leaves

Circular concentric necrotic ringspots with green center

Necrotic lesions on the stems

Symptom variations of WBNV on watermelon

Necrosis of growing tips followed by drying from tip downwards

Necrotic spots on leaves

Marginal necrosis of leaves

Chlorosis of leaves in the infected plants

Necrotic lesions on young fruit lead to drying

Circular chlorotic spots on the infected fruit surface

Reduced fruit size with chlorotic lesions on its surface

Symptom variations of orthotospovirus infection on bittergourd

Chlorosis and yellowing of bitter gourd leaves

Necrosis of growing tips followed by drying from tip downwards

Symptom variations of PBNV infection on cowpea

Chlorotic circular lesions with concentric rings on leaves and necrosis on vein and veinlets


Yellowing of young infected leaves with concentric circular chlorotic spots

Symptoms of orthotospovirus infection on weed host (Reservoir plant)

Chlorotic spots on young shoot and stunted plant growth of Solanum nigrum

1.2.2. Host range

PBNV is predominant in Leguminous and Solanaceous hosts, while WBNV is largely confined to curbitaceous hosts and CaCV reported only on solanaceous crops.

PBNV: Tomato, brinjal, chilli, watermelon, bitter gourd, cowpea, peas, potato, greengram, groundnut, sunflower, etc,

WBNV: Watermelon, ridge gourd, cucumber, muskmelon, bitter gourd, pumpkin, chilli, tomato

CaCV: Tomato, chilli

1.2.3. Transmission

Dispersal and survival of orthotospovirus mainly depends thrips vector. They are transmitting it from infected plants to healthy ones under natural conditions. Thrips feed on infected plants and specifically transmit the orthotospoviruses in persistent and propagative manner. Larvae of 2nd instars can acquire the virus and adults can transmit the virus after a latent period. Orthotospoviruses acquired by adult thrips cannot be transmitted. Thrips species transmitting orthotospovirus infecting vegetables in India are listed (Table 1).

Table 1: Different thrips species acting as vector for orthotospovirus infecting vegetables in India

Species	Acronym	Vector
Groundnut bud necrosis virus / Peanut bud necrosis virus	GBNV/ PBNV	*Frankliniella occidentalis, Thrips palmi*
Capsicum chlorosis virus (Gloxinia tospovirus)	CaCV	*T. palmi*
Iris yellow spot virus	IYSV	*T. tabaci*
Watermelon bud necrosis virus	WBNV	*T. palmi*

1.2.4. Diagnostics

Diagnotics of orthotospoviruses is possible through biological, serological and molecular methods.

1.2.4.1. Biological detection:

PBNV infected cowpea samples showing their characteristic chlorotic/necrotic spots on leaves, and veinal necrosis upon mechanical inoculation on cowpea cv Pusa Komal produce typical chlorotic local lesion symptoms within 4-6 days of inoculation. The virus extract prepared by macerating infected plant tissue with 0.1M sodium phosphate buffer pH 7.0 containing 0.1% β-mercaptoethanol, under ice cold conditions. Inoculation carried out by gentle rubbing with inoculum using broad end of the pestle on the cotyledonary leaves of six day old cowpea plants, which were previously dusted with 600 mesh carborundum powder. After few min, the excess inoculum washed with a jet of sterile distilled water using wash bottle. Similarly, tomato plants showing bud necrosis, chlorotic and necrotic circular spots on leaves and necrotic lesions on the stem infected with PBNV also produces similar symptoms

on cowpea cv. C152 upon mechanical inoculation in 0.1M sodium phosphate buffer pH 7.0 containing 0.1% β-mercaptoethanol.

Circular chlorotic spots on cowpea cotyledonary leaves upon mechanical inoculation of PBNV from tomato

1.2.4.2. Serological detection:

Among the different serogroups of orthotospoviruses, watermelon silver mottle virus group (serogroup IV) is commonly prevalent in India. All the tospoviruses so for recorded in India on vegetable belong to the serogroup IV. Hence antiserum developed against any serogroup IV orthotospoviruses can detect the other viruses belonging to this group. Through DAS-ELISA, Dot Immuno Binding Assay (DIBA) and Tissue Immuno Binding Assay (TIBA) with the PBNV antiserum, viruses infecting vegetables such as WBNV, PBNV and CaCV can be detected.

1.2.4.2.1 Direct antigen coating ELISA (DAC-ELISA)

The virus infected plant samples showing characteristic symptoms are subjected to direct antigen coating- Enzyme linked immuno sorbant assay (DAC- ELISA). The polystyrene plates are coated with 200 µl of antigen extract by grinding 1g of plant sample in 10ml of 0.05M carbonate buffer pH 9.6 (Na_2CO_3 -1.59g; $NaHCO_3$ - 2.93g; distilled water- 1l) with different dilutions *viz*., raw, 1:10, 1:25, 1:50 and 1:100 along with the healthy sap and incubated at 37°C for 3 h. The plates are washed three times in PBS-Tween (Na_2PO_4 – 1.45g; K_2HPO4-0.2g; KCl- 0.2g; NaCl- 8g; Dissolved in 1 litre of distilled water; pH 7.4 and add 0.5ml of Tween 20) with an interval of 3 min for each washing. Blocking solution (1g bovine serum albumin in 100ml carbonate buffer) at a rate of 200 µl is added to each well and incubated at 37°C for 1hr. The plates were washed once with PBS-T. The polyclonal antibody of *Groundnut bud necrosis virus* (GBNV) diluted in carbonate buffer to 1:5000 were added to each

well @ 200 µl and incubated at 4°C for overnight. The plates are washed three times with PBS-T with an interval of 3 min for each washing. The secondary antibody (IgG) conjugate linked with alkaline phosphatase diluted in carbonate buffer to 1:5000 are added to each well @ 200 µl and incubated at 37°C for 3 h and were washed with PBS-T for three times with an interval of 3 min for each washing. The substrate, para-nitrophenyl phosphate (pNPP; 1mg ml^{-1} in substrate buffer) is added to each well @ 100 µl per well and incubated for 30 min at room temperature in dark for colour development. Yellow colour development indicates the strong to weak reaction and the results are recorded in an ELISA reader @ 405nm.The test sample values at least two or three times higher than the respective healthy controls are considered as positive.

1.2.4.2.2 Dot immuno binding assay (DIBA)

Dot immuno binding assay is performed for detection of virus by following the method described by Dijkstra and de Jager (1998). The infected plant tissues are extracted (1:10 w/v) in antigen extraction buffer [TBS (0.02M Tris-2.42g; 0.5M NaCl-29.24g; distilled water- 1litre; pH 7.5) + 50mM DIECA] and filtered through double layer of cheese cloth. From the supernatant 800µl is taken and added with 500 µl of chloroform, mixed and centrifuged at 12000 rpm for 2 min. 200 µl of clarified sap (upper aqueous layer) is taken and added with 800 µl antigen extraction buffer and vortexed. A desired size piece of nitrocellulose membrane (NCM) is taken and drew a lattice of squares of 1 1 cm each with a soft lead pencil. Always used forceps for handling the membrane. The NCM is wet by floating it on TBS and then air dried. The WBNV infected and healthy plant samples collected from insect proof glasshouse are spotted (5-10µl) on NCM separately and air dried. The membrane is immersed in blocking solution (5g Skimmed milk powder in 100ml TBS) with gentle oscillation for overnight at 4°C. The membrane is then washed for one time in TBS for 10 min and the membrane is incubated for 1 h at room temperature in polyclonal antibody of GBNV diluted in blocking solution to 1:5000. The membrane is washed three times in TBS with an interval of 10 min for each washing. The membrane is incubated in secondary antibody (IgG) conjugate linked with alkaline phosphatase diluted in blocking solution to 1:5000 for 1 h at room temperature. The membrane is washed three times in TBS with an interval of 10 min for each washing. Then membrane is immersed in substrate buffer (0.1M Tris- 12.114g; 0.1M NaCl- 5.848g; 5mM $MgCl_2$, distilled water- 1l; pH 9.5) and the readymade substrate BCIP-NBT (2ml in 100ml of SB) is added and incubated for 15-30 min in dark at room temperature for purple colour development. After the colour development, the membrane is immersed in the fixing solution (10mM Tris; 1mM EDTA; distilled water-1l; pH 7.5) for 10 min and air dried.

1.2.4.2.3 Tissue immuno binding assay (TIBA)

Tissue immuno binding assay is performed by following the method described by Dijkstra and de Jager (1998). The leaves collected are rolled up tightly and cut firm and smoothly with a new razor blade. The freshly cut surfaces of the infected and the healthy samples are stamped on NCM separately. The membrane is immersed in blocking solution (5g skimmed milk powder in 100ml TBS) with gentle oscillation for overnight at 4°C. The membrane is washed for one time in TBS for 10 min. Then the membrane is incubated for 1 h at room temperature in polyclonal antibody of GBNV diluted in blocking solution to 1:5000. The membrane is washed three times in TBS with an interval of 10 min for each washing. The membrane is incubated in secondary antibody (IgG) conjugate linked with alkaline phosphatase diluted in blocking solution to 1:5000 for 1 h at room temperature. The membrane is washed three times in TBS with an interval of 10 min for each washing. Then the membrane is immersed in substrate buffer (0.1M Tris- 12.114g; 0.1M NaCl- 5.848g; 5mM $MgCl_2$, distilled water- 1l; pH 9.5) and the readymade substrate BCIP-NBT (2ml in 100ml of SB) is added and incubated in the dark at room temperature for colour development. Purple colour development is observed after 15 to 30 min. The membrane is immersed in the fixing solution (10mM Tris; 1mM EDTA; distilled water-1l; pH 7.5) for 10 min and air dried.

1.2.4.3. Molecular detection:

1.2.4.3.1. Isolation of RNA and conversion of cDNA

Grind 100-200mg sample in liquid Nitrogen & Add 1 ml of Trisol solution

↓

Mix well & transfer to the eppendorf tube & incubate it @ room temperature for 5 min

↓

Centrifuge @ 10000 rpm for 15 min @ 4ºC

↓

Take supernatant & add 250µl of chloroform

↓

Invert the tube & then centrifuge @ 12000 rpm @ 4ºC for 5 min

↓

Take the aqueous phase & add 250 µl of isoproponal + 250 µl of 2 M NaCl

↓

Mix well & incubate in ice for 10 min

↓

Centrifuge @ 12000 rpm for 15 min & discard the supernatant

↓

Wash the pellet in 75% ethanol (1 ml) & centrifuge 7500 rpm for 5 min

↓

Air dry the pellet

↓

Dissolve in DEPC water (35 µl) and store at -80ºC

First strand cDNA synthesis carried out using cDNA synthesis kit (Thermo Scientific Revert Aid First Strand cDNA synthesis kit, USA) as per manufacturer's instruction. The reaction was performed at 42ºC for 60 min followed by incubation at 70ºC for 5 min.

1.2.4.3.2. Universal and specific primers for the orthotospovirus diagnosis

Several molecular based markers are standardized for the orthotospovirus detection. Universal primers and species specific primers used for the detection are listed below:

1. Universal orthotospovirus primer (Chu *et al*., 2001)

gL3637	5′CCTTTAACAGTDGAAACAT3′
gL 4435c	5′CATDGCRCAAGARTGRTARACAGA3′

Amplification size: ~800bp

Step	**˚C**	**Time**	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	50	1 m	
Extension	72	1 m	
Final Extension	72	10 m	

2. Peanut bud necrosis virus coat protein specific primer (Nagendran *et al*., 2017)

GK PBNV CP F	5′ATGTCTAACGTYAAGCAGCTC3′
GK PBNV CP R	5′TTACAATTCCAGCGAAGGAC3′

Amplification size: 830bp

Step	**˚C**	**Time**	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	55	1 m	
Extension	72	1 m	
Final Extension	72	10 m	

3. Watermelon bud necrosis virus coat protein specific primer

GK WBNV CP F	5′AATAAACTAATGACACACACAAA3′
GK WBNV CP R	5′ACGTTTCCAKAGTAAACACCAT3′

Amplification size: 947 bp

Step	**˚C**	**Time**	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	51	1 m	
Extension	72	1 m	
Final Extension	72	10 m	

4. Capsicum chlorosis virus coat protein specific primer (Haokip *et al*., 2016)

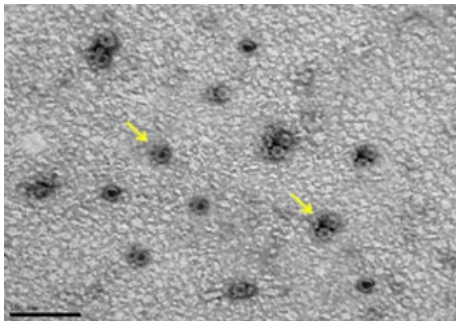
GKCaCV CP F	5′AACCAATAGTTTGCCTCCG3′
GKCaCV CP R	5′AGAGCAATCGAGGCACTA3′

Amplification size: ~1200bp

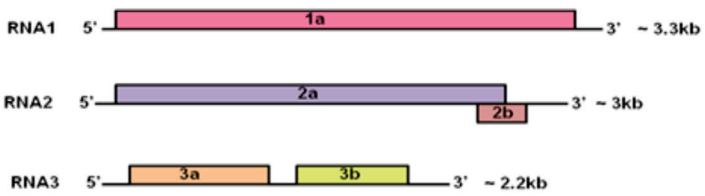
Step	**˚C**	**Time**	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	60	1 m	
Extension	72	1 m	
Final Extension	72	10 m	

1.3. CUCUMOVIRUS

Cucumoviruses infect important vegetable and other crops in temperate, tropic and subtropic regions worldwide. Among this group of viruses, the genus *Cucumovirus* under family *Bromoviridae*, contains several distinct virus species with cucumber mosaic virus (CMV) as the type member. CMV is a small isometric virus with a particle size of 28 nm in diameter, which is very efficiently transmitted by more than 60 aphid species in a nonpersistent manner. The genome consists of three single-stranded RNAs and each existing in a separate but identical particle. A fourth RNA, which codes for the coat protein of the virus, is generated from the smallest of the three RNAs. On an average, losses of 10-20% are common, and in some instances the crop may still be harvested, but is of poorer quality and appearance. CMV in yam caused the average yield loss of around 30% by significantly reducing the mass of yam tubers.



Transmission electron microscopic photograph of cucumber mosaic virus from snake gourd sample showing 29nm isometric particle



Genome organizaton of Cucumber mosaic virus

1.3.1. Symptoms

Symptoms of cucumber mosaic can vary greatly depending on the crop infected and the age of the plant when infection occurs. Almost all cucurbitaceous crops are susceptible to CMV, with symptoms varying in severity. Infected cucurbits plants show leaf mosaic, leaf distortion, fruit mosaic, stunting, mottling and yellowing.

Additionally, fruits are malformed and unmarketable because of pronounced rugosity (roughness) on the fruit surface. Sometimes color breaking on the fruit leading the fruit to show green blotchy patterns are seen. CMV is one of the important viral pathogen of tomato where it induces various symptoms *viz*., necrosis, mottling, mosaic, narrowing or shoe-string of leaves and stunting of plants. Various symptoms like yellowing, mottling, twisting, shoe-string of leaves, leaf distortion and fern-like appearance of the leaves on tomato infected with CMV.

CMV on cucumber

Mosaic on cucumber leaves

CMV on chilli

Mosaic on chilli leaves

1.3.2. Host range

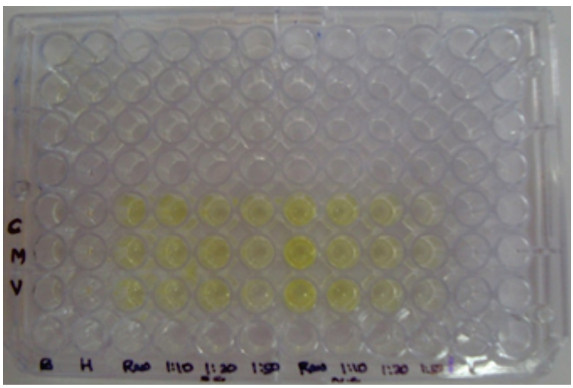
CMV found to infect over 1200 species belonging to 100 families of monocots and dicots, including many vegetable crops. In addition to cucurbits (chayote, cucumber, loofah, melon, pumpkin, summer and winter squash, watermelon), host range of CMV includes many important vegetable crops including artichoke, beans (broad, lima, snap), sugar beet, carrot, celery, parsley, lettuce, pea, pepper, potato, sweet potato, spinach, brinjal and tomato. Other emerging vegetable crops affected by CMV include chickpea lentil, soybean, and yams (*Dioscorea* spp).

1.3.3. Mode of Transmission

Mechanical transmission	:	Yes
Insect transmission	:	Yes (Aphids sp.)
Seed transmission	:	Yes
Other modes	:	Dodder

1.3.4. Diagnostics

CMV strains are divided into two subgroups, designated subgroups I and II that are distinguished by serological relationships and sequence analysis. Subgroup I strains are further divided into IA and IB, based on differences in pathogenicity in cowpea (*Vigna unguiculata*), whereby IA strains induce systemic mosaic symptoms and IB strains induce necrotic local lesions on inoculated leaves, is restricted to Asia. Some CMV strains are host specific, infecting certain hosts in the same family like the legume strain of CMV. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with a CMV polyclonal antibody and triple antibody sandwich (TAS)-ELSIA with specific monoclonal antibody can be employed for the CMV detection.



Detection of CMV in cucumber samples using DAS-ELISA

1.3.4.1. Isolation of RNA and conversion of cDNA

As described in the section **1.2.4.3.1**

1.3.4.2. Primers used in the detection of CMV

For molecular detection and characterization, following primer pairs can be used.

1. Coat protein gene specific primer for CMV (Nagendra *et al*., 2017)

GK CMV F	5′GAGTTCTTCCGCGTCCCGCT3′
GK CMV R	5′AAACCTAGG AGATGGTTTCA3′

Amplification size: 1216bp

Step	°C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	54	1 m	
Extension	72	90 s	
Final Extension	72	10 m	

2. Movement protein gene specific primer for CMV (Unpublished)

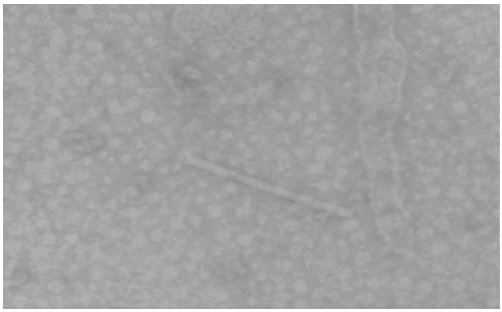
GKCMV MP F	5′GTAATCTTACCACTGTGTGTG3′
GKCMV MP R	5′ATGTGCTYTCTTCTCAACAC3′

Amplification size: 1090bp

Step	°C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 s	35 cycles
Annealing	55	30 s	
Extension	72	60 s	
Final Extension	72	10 m	

1.4. TOBAMOVIRUS

Genus *Tobamovirus* belonging to family *Virgaviridae* infect several crop plants, including vegetable crops. Currently there are 37 species under this genus with tobacco mosaic virus (TMV) as type member. TMV, tomato mosaic virus (ToMV), cucumber green mottle mosaic virus (CGMMV) and pepper mild mottle virus (PMMoV) are the common vegetable infecting tobamoviruses. These are highly stable in the environment and can survive on implements, trellis wires, stakes, containers and contaminated clothing for many months in the absence of any plant material. The viruses can also survive in crop (leaves, stems) and root debris on the soil surface for several months and can infect a new crop planted into a contaminated site. The genome consists of one positive single-stranded RNA [(+) ssRNA] of approximately 6,400 nucleotides (6.4 kb). These are rigid rod shaped viruses with a particle dimension of around 18 × 300 nm size. Infection of CGMMV can cause yield loss of 10-15% under protected condition.



Rigid rod shaped CGMMV particle from infected bottle gourd

Genome organization of Cucumber green mottle mosaic virus

1.4.1. Symptoms

Upon infection, symptoms vary with the virus and crop plants. Green mosaic on leaves is commonly seen on the infected plants. In addition, systemic mosaic mottling, stunting of plant growth, chlorosis, curling, distortion and dwarfing of leaves are also seen. In some plants, necrotic areas develop on the leaves and leaflets may become long and pointed and sometimes shoe-string like.

CGMMV on cucumber

Mosaic mottling of cucumber leaves

CGMMV on bottle gourd

Mosaic mottling of bottle gourd leaves

CGMMV on pumpkin

Mosaic mottling of bottle gourd leaves

Tomato mosaic virus on Tomato

Mosaic mottling of tomato leaves

1.4.2. Host range

Tobamoviruses infect vegetables of cucurbits, solanaceous, malvaceous and brassicas plants.

TMV: Tomato, chilli, *Solanum nigrum*

ToMV: Including Tomato and chilli, it infects many solanaceous plants. Also most species tested in the families of Aizoaceae, Amaranthaceae, Chenopodiaceae and Scrophulariaceae are also susceptible.

CGMMV: Watermelon, oriental melon, bottle gourd, muskmelon, cucumber, pumpkin, squash, and snake gourd, *Amaranthus blitoides, A. retroflexus, Chenopodium album, Heliotropium europium, Portulacea oleracea* and *Solanum nigrum.*

1.4.3. Transmission

The viruses are transmitted mechanically and in nature they are spread by incidental contact and wounding. The viruses can be carried on seed. The viruses survive in crop debris, including roots in soil and on contaminated equipment and clothing. They do not seem to be transmitted by any vectors.

1.4.4. Diagnostics

Through DAS-ELISA, specific viruses can be easily detected with their antiserum. Also under transmission electron microscope it is possible to visualize the rigid rod shaped virion particles from the infected plant extract upon counter staining with the 2% uranyl acetate.

1.4.4.1. Isolation of RNA and conversion of cDNA

As described in the section **1.2.4.3.1**

1.4.4.2. Primers used in the detection of tobamoviruses

Many species specific and universal primers are also available for their detection.

1. Universal primer for tobamovirus (Pappu and Druffel, 2007)

Tob Uni1	5'ATTTAAGTGGAGGGAAAACCACT3'
Tob Uni2	5'GTYGTTGATGAGTTCGTGGA3'

Amplification size: 686bp

Step	˚C	Time	
Initial Denaturation	94˚C	5min	
Denaturation	94	60s	25 cycles
Annealing	60	45s	
Extension	72	60s	
Final Extension	72	5 min	

2. Cucumber green mottle mosaic virus (Nagendran et al., 2015)

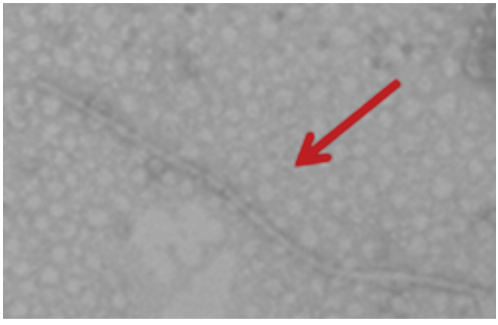
GK CGMMV F	5'TAAG CGGCATTCTAAACCTCCA3'
GK CGMMV R	5'CACTATGCACTTTG GTGTGC3'

Amplification size: 604bp

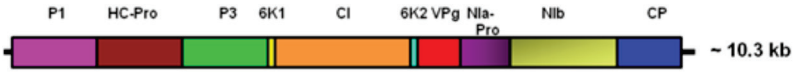
Step	˚C	Time	
Initial Denaturation	94	2min	
Denaturation	94	30s	35 cycles
Annealing	48	30s	
Extension	72	60s	
Final Extension	72	10min	

1.5. POTYVIRUS

Potyviruses are the largest plant infecting virus genera accounting for ~30% of the currently known plant viruses. It belongs to the family potyviridae and they transmitted by different aphids species either by persisitent or non-persistent manner. There are about 158 species included in this genus with the type member Potato virus Y. The genus is named after the type virus (**Pot**ato virus **Y**). Similar to begomoviruses, members of this genus cause significant losses in agricultural, horticultural and ornamental crops. Potyviruses are considered as one among the top 10 plant infecting viruses. Genome of this virus made of single stranded RNA (positive sense) molecule of ~9600bp in single fragment. Under electron microscope, particles are visualized as flexuos rod shaped with a dimension of 11 - 20 × 680 - 900 nm. Important vegetable infecting potyvirus species are papaya ring spot virus (PRSV), zucchini yellow mosaic virus (ZYMV), chilli veinal mottle virus (ChiVMV), bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), Pepper mottle virus (PepMV) and watermelon mosaic virus (WMV1 and WMV2). The typical feature of all potyviruses is the induction of characteristic pinwheel or scroll-shaped inclusion bodies in the cytoplasm of the infected cells.



Electron microscopic view of flexuous rod shaped potyvirus particle from infected bottle gourd



Genome organization of Papaya ringspot virus

1.5.1. Symptoms

General symptoms of potyvirses are alternating light and dark green pattern (mosaic) on leaves, stunting of plants, leaf curling, blistering puckering of leaves and fruit distortion.

PRSV: Symptoms on cucurbits of PRSV-P strain include mottling, ringspots and

distortion of leaves, rings and spots on fruit and streaks with a greasy or water-soaked appearance on stems and petioles while PRSV-W induces mottling and distortion of leaves and fruits. Additionally mosaic, blistering, vein thickening, vein clearing, blisters and narrow leaf blades and leaf distortion are also observed on associated with cucurbits affected by PRSV.

Symptoms variation of PRSV on cucurbits

Blistering of leaves on Pumpkin

Blistering of leaves on ridge gourd

Blistering of leaves on cucumber

Blistering of leaves on snake gourd

Vein banding on snake gourd leaves

Ring spots on leaves of bottle gourd

ZYMV: Plants exhibits yellowing, leaf deformation and stunting, severe mosaic, blisters, enations and filiformism of leaves with a diversity of symptoms on the fruits (mottle and hardening of the flesh, cracks on the fruits). Squash and pumpkin fruits develop knobby areas, which cause prominent deformation. Melon and watermelon fruits are also malformed and often develop deep longitudinal and radial cracks. Seed production is drastically reduced, and seeds are frequently deformed.

Symptom variation of ZYMV infection on cucurbits

Mosaic mottling of leaves on pumpkin

Chlorosis and yellow spots on leaves of snake gourd

Reduction in size and malformation on fruits of snake gourd

Yellowing and mosaic on leaves of bitter gourd

Yellow mosaic mottling of cucumber leaves

ChiVMV: Mottling of leaves and dark green vein-banding are the most characteristic symptoms. Additionally infected plants become stunted and produce smaller and distorted leaves and have dark-green streaks on their stems and branches. Also flowers drop are common and such plants produce mottled and distorted fruit.

ChiVMV on chilli

BCMV: Infected plant shows systemic green to bluish-green mottled or mosaic pattern on leaves which usually exhibit a downward curling or cupping. Mosaic symptom often follows the leaf veins, which become darker than the interveinal area.

1.5.2. Host range

PRSV: PRSV has two different strains (W and P) that can be differentiated biologically by their ability to infect different hosts, but not serologically and morphologically. PRSV-W infects cucurbits but is unable to infect papaya. PRSV-P primarily infects papaya but can also infect cucurbits. PRSV-W infects 40 plant species with 38 of them being in the cucurbit family and two of them in the goosefoot family (Chenopodiaceae). PRSV-P recorded on *Momordica charantia* and *C. moschata.* Host range of PRSV-P includes 15 species in three families (Caricaceae, Chenopodiaceae, Cucurbitaceae), while PRSV-W infects 38 species of 11 genera in two families (Cucurbitaceae, Chenopodiaceae).

ZYMV: It is a major pathogen of members of the family Cucurbitaceae. The main cultivated host species include zucchini, squash, muskmelon, pumpkin, watermelon, yellow squash, *Cayaponia tibiricae*, *Siraitia grosvenorii*, *Fevillea trilobata*, bottle gourd, gherkins, snake gourd and bitter gourd. Non-cucurbitaceous natural hosts on are hollycock (*Althaea rosea*) and *Begonia semeperflorens*.

1.5.3. Transmission

Mechanical: Potyviruses are transmitted easily by mechanical means.

Insect: More than 200 species of aphids spread potyviruses and most are belongs to the genera *Myzus, Aphis* and *Macrosiphum*.

- **PRSV**: Aphids transmit the virus to papaya and cucurbits in a nonpersistent manner; in other words, the virus is acquired and transmitted by its vector

in short period of time that are measured in seconds to a minute. The virus does not replicate in the vector. PRSV-P isolates are transmitted by 21 aphid species in 11 genera with *Myzus persicae* and *Aphis gossypii* the most important natural vectors and PRSV-W isolates are transmitted by 24 aphid species in 15 genera with *Myzus persicae, Acyrthosiphum solani, Aphis craccivora* and *Macrosiphum euphorbiae* as natural vectors. *M. persicae* (56%) and *A. gossypii* (53%) are significantly more efficient in transmitting PRSV than *A. craccivora* (38%). PRSV transmission efficiency was 100% with a group of five aphids per plant with AAP and IAP of 5mins.

- **ZYMV:** Transmitted in a non-persistent manner by *A. gossypii* and *M. persicae*. Maximum transmission of the virus (40%) was obtained by *A. gossypii*. Also transmisson of ZYMV by other aphid species *viz., A. citricola*, *A. middletrii, A. craccivora, Achyrthosiphon pisum, Lipahis erysimi* and *Uroleucon* spp. has also been reported.
- **BCMV:** Transmitted by aphids in non-persistent manner.

Seed transmission: Both ZYMV and BCMV are transmitted through contaminated seed. In case of PRSV and ChiVMV, there was no seed transmission.

Other modes: BCMV can be transmitted mechanically by plant-to-plant abrasion, pollen and by movement of contaminated equipment between fields. ChiVMV can also be transmitted by grafting.

1.5.4. Diagnostics

As the potyviruses are transmitted mechanically, they can be characterized based on the biological assays, as well. Upon inoculation on the indicator hosts such as *Chenopodium amaranticolor, Nicotiana glutinosa, N. bentamiana, N. tabaccum,* etc., these potyviruses produces the local or systemic symptoms on these host plants. Similarly lot of serological based ELISA kits and Immunostrips are available commercially for the detection of these viruses.

1.5.4.1. Isolation of RNA and conversion of cDNA

As described in the section **3.4.3.1.**

1.5.4.2. Primers used in the detection of potyviruses

For molecular detection and characterization some of the commonly used primer pairs provided here are:

1. Universal potyvirus primer (Hsu *et al*., 2005)

PNIbF1	5’GGBAAYAATAGTGGNCAACC3’
PCPR1	5’GGGGAGGTGCCGTTCTCDATRCACCA3’

Amplification size: ~1100bp

Step	°C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	1 m	35 cycles
Annealing	55	1 m	
Extension	72	2 m	
Final Extension	72	30 m	

2. Universal potyvirus primer (Zheng *et al*., 2008)

NIb2F	5′GTITGYGTIGAYGAYTTYAAYAA3′
NIb3R	5′TCIACIACIGTIGAIGGYTGNCC3′

Amplification size: ~350bp

Step	°C	Time	
Initial Denaturation	94	3 m	
Denaturation	94	45 s	40 cycles
Annealing	45	30 s	
Extension	72	60 s	
Final Extension	72	7	

3. Papaya ringspot virus coat protein specific primer (Nagendran *et al*., 2017)

GK PRSV F	5′GCAATGATAGARTC ATGGGG3′
GK PRSV R	5′AAGCGGTGGCGCAGCCACACT3′

Amplification size: 1264bp

Step	°C	Time	
Initial Denaturation	94	2m	
Denaturation	94	60s	35 cycles
Annealing	55	60s	
Extension	72	90s	
Final Extension	72	10m	

4. Zucchini yellow mosaic virus coat protein specific primer (Nagendran *et al*., 2017)

GK ZYMV F	5′ATAGCTGAGACA GCACT3′
GK ZYMV R2	5′CGGCAGCRAAACGATAACCT3′

Amplification size: 1004bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 s	35 cycles
Annealing	52	30 s	
Extension	72	60 s	
Final Extension	72	10 m	

2. PROFILING OF VEGETABLE VIRUSES

Twenty nine samples showing symptoms as described (Table 2) were collected from different vegetable crops in the IIVR farm during April, 2016. Virus disease incidence on different crops were also recorded which ranged from 3 to 70% (Table 2). Samples were tested through Double Antibody Sandwich-Enzyme Linked Immunosorbant Assay (DAS-ELISA) with 15 polyclonal antiserum obtained from DSMZ, Germany belongs to different genera such as *Tobamovirus* (Cucumber green mottle mosaic virus – CGMMV; Tobacco mosaic virus – TMV; Tomato mosaic virus - ToMV), *Tospovirus* (Capsicum chlorosis virus – CaCV; Watermelon silver mottle virus – WSMoV; Iris yellow spot virus - IYSV), *Potyvirus* (Watermelon mosaic virus – WMV; Zucchini yellow mosaic virus – ZYMV; Chilli veinal motlle virus - ChiVMV), *Cucmovirus* (Cucumber mosaic virus - CMV), *Tombusvirus* (Cucumber necrosis virus - CNV), *Crinivirus* (Cucurbits yellow stunting disorder virus – CYSDV; Tomato chlorosis virus - ToCV), *Carmovirus* (Melon necrotic spot virus - MNSV) and *Potexvirus* (Pepino mosaic virus - PepMV).

Among the 15 viruses tested across 29 different vegetable samples, only 6 viruses were detected among the tested samples. Prevalent viruses are CGMMV (cucurbits and tomato), TMV (tomato, musk melon and pumpkin), ToMV (bottle gourd), CaCV (tomato, bottle gourd, musk melon, pumpkin and capsicum), WSMoV (cucumber) and CMV (tomato, bottle gourd, musk melon and capsicum). Detected viruses were grouped under three families such as *Tobamovirus* (CGMMV, TMV and ToMV), *Tospovirus* (CaCV and WSMoV) and *Cucumovirus* (CMV). TMV and ToMV detected on cucurbits which might be due to the detection of CGMMV infection by the TMV and ToMV antiserum being closely related species. Similarly CaCV infecting solanaceous crops were also detected on cucurbits. Since CaCV, WSMoV, PBNV (Peanut bud necrosis virus) and WBNV (Watermelon bud necrosis virus) are closely related tospoviruses belongs to serogroup IV, infection of WBNV on cucurbits was detected by the CaCV antiserum.

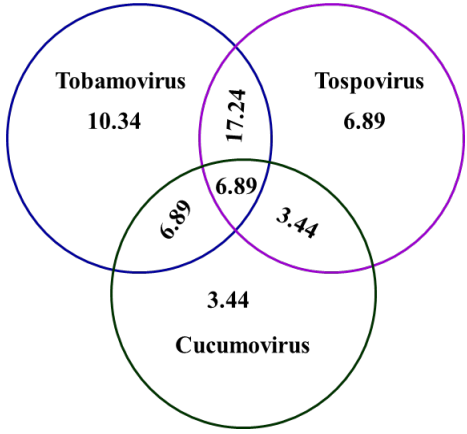
All these viruses detected are transmitted by aphids and thrips. Due to the polyphagous nature of these insect vectors and availability of host plants in field throughout the year, viruses are continuously present in the cropping vicinity. In future this may leads to expansion of their host range and evolution of new viruses. Interestingly 34.5% of samples were found to have mixed infection of more than one of viruses. Some symptomatic samples were also not detected with any of these 15 RNA viruses tested. These samples might be infected with begomoviruses which is emerging as a devastating pathogen to crop cultivation including both agricultural and horticultural crops. Among the virus infected samples, 89% samples were found associated with the seed borne viruses (tobamoviruses and Cucumber mosaic virus). Hence role of seeds in the transmission of virus disease also needs to be ascertained. This preliminary virus profiling will be useful in developing management strategies

of seed borne and vector borne virus in vegetable crops. The pattern of distribution of viruses among vegetable crops in IIVR farm is representing the country scenario.

Table 2: Serological detection of viruses infecting vegetable crops

No.	Crop	Symptoms	Percent disease incidence	Virus tested					
				CGMMV	TMV	ToMV	CaCV	WSMoV	CMV
1	Tomato	Necrotic spot with green centre on leaves	20	-	-	-	+	-	-
2	Tomato	Stem necrosis	14	+	-	-	+	-	+
3	Bottle gourd	Mosaic mottling	58	+	-	+	+	-	+
4	Tomato	Yellow specks on leaves	3	+	+	-	+	-	-
5	Tomato	Leaf curl	64	-	+	-	-	-	-
6	Bottle gourd	Mosaic	62	+	-	-	-	-	-
7	Bottle gourd	Mosaic	62	+	-	-	+	-	-
8	Bottle gourd	Mosaic	62	-	-	-	+	-	-
9	Bottle gourd	Mosaic	62	-	-	-	-	-	-
10	Cowpea	Necrotic spot on leaves	24	+	-	-	-	-	-
11	Musk melon	Mosaic	12	+	+	-	-	-	+
12	Musk melon	Mosaic and necrosis on leaves	8	+	-	-	+	-	
13	Musk melon	Mosaic	14	-	+	-	-	-	+
14	Cucumber	Marginal necrosis on leaves	7	+	-	-	-	+	-
15	Pumpkin	Yellowing of leaf with mosaic	45	-	-	-	-	-	-
16	Pumpkin	Mosaic	50	+	-	-	+	-	-
17	Pumpkin	Crinkling of leaves	15	-	-	-	-	-	-
18	Pumpkin	Marginal necrosis on leaves	8	-	-	-	-	-	-
19	French bean	Yellow mosaic on leaves	5	-	-	-	-	-	-
20	Capsicum	Leaf curl	70	-	-	-	-	-	+

21	Capsicum	Leaf curl	70	-	-	-	-	-	-
22	Musk melon	Mosaic	14	-	-	-	-	-	-
22	Capsicum	Leaf curl	70	-	-	-	-	-	-
23	Capsicum	Leaf curl	70	-	-	-	+	-	+
24	Cowpea	Mosaic mottling	15	-	-	-	-	-	-
25	Cowpea	Mosaic mottling	15	-	-	-	-	-	-
26	Water melon	Necrosis of leaf margin and stem	60	-	-	-	-	-	-
27	Water melon	Necrosis of leaf margin and stem	60	-	-	-	-	-	-
28	Water melon	Yellow vein	5	-	-	-	-	-	-
29	French bean	Leaf crinkling	5	-	-	-	-	-	-



Percentage of samples showing single and mixed infection of different virus genera

3. MANAGEMENT OF VIRUS DISEASES ON VEGETABLE CROPS

Developing management strategy for the management of insect transmitted virus diseases is found difficult due to following reasons: wide host range of both insect vectors and viruses; faster resistance development by the insect vectors; quicker evolution of viruses infecting vegetables; and availability of scanty resistance sources in crops plants. Hence integrated strategies involving host plant resistance, induced resistance, biological, cultural and chemical can be incorporated as viable components for management of viral diseases in vegetable crops with a view to reduce pesticide load in the environment.

3.1. HOST RESISTANCE

Use of tolerant/resistant cultivars is an important component in the integrated disease management strategy. Genes *viz*., *Sw*-5 and *Tsw* confereing resistance against orthotospovirus, should be exploited in the breeding programmes for the management of orthotospovirus disease in vegetable crops. Some of the varieties developed for management of viruses in different vegetable crops are listed below.

Table 3: List of resistant varieties identified against the viral diseases of vegetable crops

Crop	Disease	Resistant genotype
Tomato	Leaf curl	Kalianpur Angoorlata, Kashi Aman, H-24
Chilli	CMV, leaf curl	Punjab lal, Punjab Surkh, BS-35
Cowpea	Golden mosaic virus	Kashi Gauri, Kashi NidhiCowpea- 263, KLS-10, Arka Garima, Arka Samrudhi, Swarna Suphala, Kashi Kanchan, BC-244002
French Bean	Bean common mosaic	Pant Anupama
Indian Bean	Yellow mosaic virus	Wal Konkan-1
Squash	Cucumber mosaic virus	Punjab Chappan Kaddu-1
Ridge gourd	Mosaic	Deepthi
Okra	Yellow vein mosaic virus (YVMV)	Punjab-7, Varsha Uphar, Hisar Unnat, HBH-142, Hisar Naveen, CO-3, Arka Anamika, Kashi Lila, Arka Abhaya, NDO-10
	Enation leaf curl virus (ELCV)	Kashi Mangal
	YVMV and ELCV	Sheetla Upahar, Sheetla Jyoti, Kashi Pragati, Kashi Vibhuti, Kashi Bhairav, Kashi Mahima, Kashi Mohini

3.2. CULTURAL

Cultural practices followed for crop cultivation strongly influences build-up of insect vector population and virus incidence. Virus incidence can be reduced by raising the crop when the most sensitive stage is least invaded by insect vectors. In that context the following general aspects need to be followed for the virus disease management.

- Nursery should be maintained weed free, away from the cropping field and monitored periodically for insect vector population to ensure virus free seedling production.
- Insect proof net with a pore size of <0.88mm (25-40 mesh size) to exclude viruliferous vectors from nursery area will provide higher level of protection.
- To reduce the virus disease in the main field maintaining a buffer zone free of weeds in around field at least 25m from viral inoculum.
- Destruction of weeds before flowering is important as the insects are attracted towards the pollen for feeding. Also weeds should be cleared from cropping area.
- Removal of old infected crops infested by insects earlier, if young crops are to be planted nearby.
- Avoidance of overlapping susceptible crops side by side to minimize virus spread from one crop to the next.
- Sowing of crops in such a way that early stage (sensitive stage) of crop is least invaded by insect vector.
- Closer planting of crops can be adopted to compensate yield loss due to virus disease.
- Rouging of infected plants should be adopted soon after transplanting to avoid building up of virus inoculum.
- Intercropping vegetable crops with two rows of border crops such as pearl millet, maize and sorghum significantly reduces the disease incidence by limiting the movement of insect vectors, which ultimately delay the intial incidence upto 10-15 days.

Border cropping with pearl millet and black silver mulching in okra crop

Untreated control plot of okra

- Mulching with black silver mulch significantly reduces the virus incidence and insect vector population under field conditions on vegetable crops.
- Use of blue sticky trap @ 25 nos. / ha reduces the thrips population (yellow sticky traps for other insects) in the field conditions thereby spread of virus will be checked

Installation of yellow sticky trap in the bittergourd field

3.3. BIOLOGICAL

- *Pseudomonas fluorescens*, a bacterial antagonist, when applied through seed treatment @ 300 CFU/ml for 24 h, seedling dip @ 300CFU/ml for 2 h, soil drenching with 25 ml @ 300CFU/ml per plant and foliar spray@300CFU/ml is found to induce systemic resistance on tomato plants against the orthotospovirus causing bud necrosis disease. This can be utilized in the management strategy.
- Eulophid parasitioid such as *Ceranisus* sp. and *Thripobius* sp. parasitize thrips vector on vegetable ecosystem can be utilized for its management.
- Some predatory anthacorid bugs *viz., Orius maxidentex* and *O. tantillus* were found feeding on thrips on tomato and capsicum crops will help in reducing the vector population.
- Release of predatory mites *viz*., *Amblyseius cucumeris, A. swirski* and *Stratiolaelaps scimitus* at the rate of 50-100 nos./m^2 are found necessary in reducing the thrips population.
- An entomopathogenic nematode, *Steinernema feltiae* is known to parasitize thrips.
- Entomopathogenic fungus *viz*., *Metarhizium anisopliae, Verticillium lecani* and *Beauveria bassiana* @ 1litre/ha are effective in controlling insect vectors such as thrips, whitefly, hopper, etc under field conditions.

3.4. CHEMICAL

Chemical control of insect vectors is important component which cannot be eliminated in the integrated pest management program. Use of chemical insecticides will eliminate the insects with immediate effect from the cropping canopy. Several systemic insecticides such as dimethoate (2ml/l), imidacloprid (0.5-0.75ml/l), thiamethoxam (500g/ha), acetamiprid (100g/ha), fipronil (1.5ml/l), azadirachtin (3l/ha) and neem oil (3ml/l) from crop emergence to fruit formation stage will effectively reduce insect vector population and minimize loss caused by virus. In addition, resistance inducing chemicals *viz*., Acibenzolar S methyl, salicylic acid were found reducing the virus concentration and symptom expression on diseased plants.

Table 4: List of insecticides for targeted vectors of viral diseases on vegetable crops

Chemical Name	Target pest	Active ingredient	Formulation (g/ml)	Dilution in water (l)
Tomato				
Azadirachtin 5%	Aphids, Whitefly	-	200	400
Carbofuran 3%G	Whitefly	1200	40000	-
Dimethoate 30% E C	Whitefly	300	990	500-1000
Imidacloprid 17.8% SL	Whitefly	30-35	150-175	500
Malathion 50% EC	Whitefly	750	1500	500-1000
Oxydemeton methyl 25% EC	Whitefly	250	1000	500-1000
Phorate 10% G	Whitefly	1500	15000	-
Thiamethoxam 25% WG	Whitefly	50	200	500
Brinjal				
Difenthiuron 50% WP	Whitefly	300	600	500-750
Fenpropathrin 30% EC	Whitefly	75-100	250-340	750-1000
Phorate 10% G	Aphids, Thrips	1500	15000	-
Phosphomidon 40% SL	Aphid, Whitefly	250-300	625-750	500
Thiamethoxam 25% WG	Whitefly	50	200	500
Thiameton 25% EC	Aphids	250	1000	750-1000
Chilli				
Acetamiprid 20% SC	Thrips	10-20	50-100	500-600
Carbofuran 3% G	Thrips	1000	33300	-
Carbosulfan 25% EC	Whitefly, Aphids	200-250	800-1000	500-1000
Emamectin benzoate 5% SG	Thrips	10	200	500
Endosulfan 35% EC	Aphids	140	400	500-1000

Ethion 50% EC	Thrips	750- 1000	1500-2000	500-1000
Fenpropathrin 30% EC	Thrips, Whitefly	75-100	250-340	750-1000
Fipronil 5% SC	Thrips, Aphids	40-50	800-100	500
Imidacloprid 70% WS	Aphids, Thrips	700- 1050 (per 100 kg seed)	500-1000	-
Imidacloprid 17.8% SL	Aphid, Thrips	20-25	125-250	500-700
Lambda Cyhalothrin 5% EC	Thrips	15	300	400-600
Methomyl 40% SP	Thrips	300-450	750-1125	500-1000
Oxydemeton methyl 25% EC	Aphids, Thrips	250-400	1000-1600	500-1000
Phorate 10% G	Aphids, Thrips	1000	10000	-
Phosalone 35% EC	Aphid, Thrips	700	2000	500-1000
Quinalphos 25%EC	Aphids	250	1000	500-1000
Thiacloprid 21.7% SC	Thrips	54-72	225-300	500
Indoxacarb 14.5% + Acetamiprid 7.7% SC	Thrips	88.8- 111	400-500	500
Okra				
Azadirachtin 0.03%	Whitefly	-	2500-5000	500-1000
Azadirachtin 5%	Whitefly, Aphids	-	200	400
Fenpropathrin 30% EC	Whitefly	75-100	250-340	750-1000
Oxydemeton methyl 25% EC	Whitefly	250	1000	500-1000
Thiamethoxam 25% WG	Whitefly	25	100	500-1000
Cucurbits				
Imidacloprid 70% WG	Aphids, Jassids	24.5	35	500
Crucifers				
Acetamiprid 20% SC	Aphids	15	75	500-600
Azadirachtin 0.03%	Aphids	-	2500-5000	500-100
Azadirachtin 5%	Aphids	-	200	400
Dimethoate 30% EC	Aphids	200	660	500-1000
Malathion 50% EC	Aphids	750	1500	500-1000
Phorate 10% G	Aphids	2000	20000	-
Phosalone 35% EC	Aphids	500	1428	500-1000
Quinalphos 25% EC	Aphids	250	1000	500-1000

3.5. IDM MODULE FOR THE VIRAL DISEASE MANAGEMENT ON VEGETABLES

3.5.1. Management of yellow vein mosaic disease on okra:

- Raising two rows of pearl millet as border crop 15 days before okra sowing
- Covering of soil with black silver reflective polythene mulch
- Seed treatment with virkon S @ 5g/l (as seed disinfectant) followed by imidacloprid @ 3g/kg seeds after 24 hrs
- Spray of salicylic acid 2mM at 15 DAS
- Installation of yellow sticky trap 1-2 traps/50 sq m at 15 DAS
- Spray of flonicamid 50 WG @ 0.3 g/l @ 17 DAS
- Spray of boron 0.2% + zinc 0.5% (or micronutrient mix @ 2ml/l) + soil drenching of humic acid 5ml/l at 20 DAS
- Spray of chlorantraniliprole 10 OD @ 1.8 ml/l at 27 DAS
- Spray of salicylic acid 2mM at 25 DAS
- Spray of flupyridifurone @ 2.5 ml/l at 35 DAS
- Spray of neem insecticide 3-5 ml/l at 45 DAS

This treatment was most effective in reducing the jassids, whitefly populations with highest marketable fruit yield. In integrated module the YVM disease was lowest as compared to untreated control.

3.5.2. Management of vector borne virus diseases in chilli

- Application of neem cake @ 1.0kg/m^2 in the seed bed
- Seed treatment with imidacloprid @ 8gm/kg
- Spraying of cyantraniliprole @ 1.8ml/liter 2-3 three days before transplanting
- Seedling dip of imidacloprid @ 0.5ml/L for 3o mins before transplanting
- Growing of two rows of maize/sorghum/pearl millet as border crop in the main field
- Covering of soil with black silver reflective polythene mulch
- Spray with acephate @ 1.5 g/L + Neem Oil @ 2.0ml/L at 15 DAT
- Spray with fipronil @ 1.0 ml/L + Neem Oil @ 2.0ml/L at 21 DAT
- Spray with imidacloprid @ 2 g/15L + Neem oil @ 2.0ml/L at 28 DAT
- Spray with cyantraniliprole @ 1.8ml/L at 35 DAT
- Repeat spraying of insecticide in above sequence at 7 days interval till fruit formation

This treatment has found to reduce the leaf curl disease and mosaic disease. Also reduced insect populations of whitefly, aphids and thrips were observed compared to control.

3.5.3. Management of mosaic disease on cucurbits

- Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seed
- Soil application of FYM @ 10kg/pit
- Soil application of *P. fluorescens* @ 2.5 kg/ha in planting pits along with neem cake @ 250 kg/ha
- Soil drenching with humic acid @ 0.2%
- Foliar spray with zinc sulphate @ 0.5% and boron @ 0.2%
- Installation of yellow sticky traps @25nos/ha
- Application of botanical pesticides (5% NSKE / 3% Neem oil)

IPM is found to reduce the incidence of mosaic disease on cucurbits with higher fruit yield compared to farmers practice.

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