The Progress of Research on Cucumoviruses in India

9

S.K. Raj, S. Kumar, K.K. Gautam, C. Kaur, A. Samad, M. Zaim, V. Hallan, and R. Singh

Abstract

The genus *Cucumovirus* of the family *Bromoviridae* contains only three virus species of which *Cucumber mosaic virus* (CMV) and *Tomato aspermy virus* (TAV) are known to occur in India. The research work on CMV and TAV causing numerous diseases in vegetables, pulses, ornamentals, medicinal and aromatic plants and weeds, which were reported in India during last 65 years (1951–2015) have been summarized in this chapter. Biological, biochemical, serological, and molecular characterization of CMV and TAV, the mode of spread of the diseases in nature through insect vectors, search of alternate hosts/reservoirs and diagnostics methods for sensitive detection of the virus/es at an early stage of infection in plants and in propagating materials are discussed.

Keywords

Cucumber mosaic virus • Important crops of India • Virus transmission • Particle morphology • Serological properties • Biochemical characterization and molecular identification

S.K. Raj (✉) • S. Kumar • K.K. Gautam • C. Kaur
Plant Molecular Virology Laboratory, CSIR-National Botanical Research Institute, Lucknow 226 001, India
e-mail: skraj2@rediffmail.com

A. Samad • M. Zaim
Crop Protection Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India

V. Hallan
CSIR-Institute of Himalayan Bioresource Technology, Post Box No. 6, Palampur 176061, Himachal Pradesh, India

R. Singh
Amity Institute of Biotechnology, Amity University, Lucknow campus, Gomti Nagar extension, Lucknow, Uttar Pradesh, India

© Springer Nature Singapore Pte Ltd. 2017
B. Mandal et al. (eds.), *A Century of Plant Virology in India*,
https://doi.org/10.1007/978-981-10-5672-7_9

9.1 Introduction

Cucumber mosaic virus (CMV) has been considered as a very important plant virus due to its capability to infect and cause huge losses in a number of plants in India. CMV was first reported by Doolittle (1916) as a causal agent of mosaic disease in cucumber in (country name?). CMV is also reported to causes mosaic, fern leaf, systemic necrosis, blight, ringspot, stunting, infectious chlorosis, heart rot and dwarfing in cucumber and other cucurbits, tomato, melon, spinach, pepper, clover, lupins, Lucerne, soybean, banana and many other species grown all over the world (Palukaitis 1992; Pratap et al. 2008; Raj et al. 2008a). CMV belongs to the genus *Cucumovirus* of the family *Bromoviridae* which contains ~28 nm (in diameter) isometric particles. It is transmissible by aphids in a non-persistent manner, and through the seed in some host plants. It is also transmissible easily by mechanical inoculations.

CMV has a tripartite plus sense RNA genome consisting of four RNA species designated as RNA1, RNA2, RNA3 and a subgenomic RNA4 responsible for the expression of CP. All the four RNAs contain a 7-methyl guanosine cap at 5′ ends. For infectivity, only the three largest RNAs are required. Occasionally a fifth RNA, known as the satellite RNA, about 330–386 nucleotides in length may be present (Palukaitis and Garcia-Arenal 2004). RNA 1 encodes one open reading frame, whereas RNA 2 and 3 each encodes two open reading frames. RNA 1 encodes 1a protein while RNA 2 encodes 2a proteins which are essential for replication. RNA 2 also encodes for 2b protein that affects long distance virus movement and symptomatology of the virus. RNA 3 is dicistronic and encodes two proteins, the 5′ encodes for movement protein (MP) whereas 3′ encodes for coat protein (CP). MP is involved in cell-to-cell movement of the virus while role for CP has been demonstrated in virus encapsidation, systemic movement, host range determination and aphid transmission (Palukaitis and Garcia-Arenal 2004).

Based on serology, peptide mapping of the CP, nucleic acid hybridization, RFLP of RT-PCR amplicon and sequence identity, isolates of CMV are divided into subgroup I and II (Palukaitis 1992; Roossinck 2002). Subgroup I is further splitted into IA and IB based on 5′ non-translated region of RNA 3 and CP gene (Roossinck 2002). Most of the Indian CMV strains have been clustered in IB subgroup (Srivastava and Raj 2004).

The occurrence of CMV was reported first time on chilli in India by Bhargava (1951a, b). The existence of CMV has been reported in India in early years from 1951 to 1965. There were only four reports of natural occurrence of CMV on chilli (Bhargava 1951a), properties of four strains of CMV infecting French bean, sugar beet and *Phytolacca* sp. (Bhargava 1951b) and a mosaic disease of tomato (Das and Raychaudhary 1953; Joshi and Bhargava 1965) are available in literature. These studies were based on symptomatology and transmission of the causal pathogen by sap and by aphids; responses of virus inoculations on various host species and

biophysical properties of the virus: dilution end point (DEP), thermal inactivation point (TIP) and longevity in vitro (LIV) of the sap of the infected plant. The similar work has also been reported on CMV from 1966 to 1980 by Indian workers (Anjaneyulu and Apparao 1967; Khatri and Chenulu 1967; Seth and Raychaudhuri 1973; Dhingra et al. 1979; Mali and Rajegore 1980). The detailed characterization of CMV based on host assay, electron microscopy, DEP/TIP/LIV, vector transmission, and serology has also been attempted by Chalam (1982) in India.

In 1983, the comparison of genome sequences of *Brome mosaic virus* (Murthy 1983), and virus particle stability and structural transition of *Tomato aspermy virus* (TAV) with CMV (Savithri et al. 1983) has been done for the first time in India. Further, a CMV isolated from chickpea was identified based on the sedimentation coefficient and a buoyant density of purified virus, and molecular weight of coat protein (25 kDa) by SDS-PAGE. PAGE of CMV nucleic acid revealed the presence of four RNA species (Chalam et al. 1986). The characterization and establishment of relationship of CMV infecting *Petuni*a using ELISA (Srivastava et al. 1991), and identification of CMV infecting banana using immuno/nucleic acid probes (Srivastava et al. 1995) has been done for the first time in India. Haq et al. 1996 characterized a CMV-banana isolate at molecular level using CP gene sequence which shared ~90% identity with CMV subgroup I strains. The potential role of satellite RNA in lethal necrosis of tobacco was also depicted for the time in India by Raj et al. (2000).

Srivastava and Raj (2004) investigated the high sequence similarity in three Indian isolates of CMV (Datura, Amaranthus and Henbane) and suggested their common origin based on RT-PCR, RFLP and sequence analysis of CP gene and observed that Indian isolates into subgroup IB. Vishnoi et al. (2013) identified a CMV-banana isolate as a member of CMV subgroup IB by sequence analysis of three RNA genomes. A CMV strain infecting Gladiolus was also identified as a member of subgroup IA based on distinct phylogenetic relationships with Indian isolate of IB (Dubey et al. 2010). The identification of CMV subgroup II isolate causing severe mosaic in cucumber was reported by Kumari et al. (2013) based on its complete genome for the first time in India.

Tomato aspermy virus (TAV; synonym Chrysanthemum aspermy virus), is another species of the genus *Cucumovirus* of the family *Boromoviridae* which causes rolling and twisting of corolla and reduction in size of flowers (Prentice 1952); slight dwarfing of the blooms together with waving and curling of ray florets including severe colour break, dwarfing and distortion of flowers (Hollings 1955; Brierley 1955, 1958; Marani 1969; Chuyan and Krylov 1979; Gupta and Singh 1981) and chlorotic ring mosaic on leaves (Raj et al. 1991) in chrysanthemums (*Chrysanthemum morifolium*). It causes serious losses in chrysanthemum crops and the symptoms include severe "flower-break" or "colour-break" in flowers, distortion of flowers and dwarfing of the chrysanthemum plant. TAV is also transferred from

plant to plant by aphids. The yield and flower quality of various cultivars of chrysanthemum has been affected frequently in India by infection of TAV (Gupta and Singh 1981; Raj et al. 1991, 2007a, b, c, 2009; Verma et al. 2007a, b). The natural occurrence of TAV on chrysanthemum has been reported earlier on the basis of biophysical properties (Sastry 1964), aphid transmission, Ouchterlony gel double diffusion tests (Gupta and Singh 1981), double antibody sandwiched-enzyme linked immunosorbent assay (DAS-ELISA; Raj et al. 1991) and reverse transcription-polymerase chain reaction (RT-PCR) of the capsid gene (Raj et al. 2007a, b, c; Verma et al. 2007a, b). Raj et al. (2009) reported biological studies, nucleotide sequence of the complete RNA 3 genome, sequence identities and phylogenetic relationships of three TAV isolates collected from three locations in India.

TAV contains a tripartite genome of messenger plus-sense single-stranded RNAs designated RNA 1, 2 and 3 (in decreasing molecular mass), which are encapsidated in 28 nm isometric particles. RNA 1 and 2 encode 1a and 2a proteins, respectively, responsible for virus replication (Palukaitis and Garicia-Arenal 2004). The overlapping small 2b protein expressed from subgenomic RNA 4A (Ding et al. 1994) influences virulence, and facilitates long distance movement and functions as a suppresser of plant–host mediated gene silencing (Ding et al. 1995, 1996). RNA 3 is dicistronic, and encodes the 3a movement protein (MP), responsible for cell–cell movement and a capsid protein (CP) that is expressed from subgenomic RNA 4 (Palukaitis et al. 1992), the structural protein. CP is multifunctional in addition to having a role in encapsidation; it affects virus movement in plants (Suzuki et al. 1991; Kaplan et al. 1998), aphid transmission, symptom expression and host range. Two novel subgenomic RNAs RNA 3b and RNA 5, putatively the derivatives of RNA 3, have also been recorded (Shi et al. 1997).

Furthermore, the natural occurrence of TAV has also been reported in gladiolus plants the basis of reverse transcription-polymerase chain reaction (RT-PCR) of the capsid gene (Raj et al. 2011). The sequence analysis of cloned PCR product revealed the presence of 657 nucleotide-containing single open reading frames of the coat protein gene of TAV coding for 218 amino acids. The analysis of sequence data showed 98–99% identities and close phylogenetic relationships with several strains of TAV, hence the virus isolates were identified as two isolates of TAV. This was the first molecular detection and identification of TAV naturally occurring on gladiolus in India (Raj et al. 2011).

Although, many of the researchers have worked out the identification and characterization of several strains of CMV and TAV infecting various economically important plants grown in India for the last 65 years (1951–2015), however, the data generated by them has not been compiled at one place either as a review or as a book chapter. Therefore, in this chapter, we described the general features, natural incidence, virus transmission, host range studies, particle morphology, serological relationships, biochemical characterization and molecular identification of CMV and TAV isolates/strains causing diseases and economic losses on various economically important plants cultivated in India and for their possible disease management.

9.2 Cucumber Mosaic Virus

9.2.1 Characterization of CMV Infecting Vegetable Crops

9.2.1.1 Tomato (*Solanum lycopersicumum*)

CMV is one of the important viruses infecting tomato and cause foliar mosaic, shoestring/fern leaf, yellowing and, stunting and malformation of entire plants. A report of CMV tomato strain causing mosaic in tomato was published from India by Das and Raychaudhary (1953) who identified the CMV by symptomatology and virus transmission. The diseased tomato plants appear stunted; the young leaves are small crinkled, deformed pale yellow and brittle. Sometimes small necrotic areas are seen on the leaves, while in a few cases the leaf margins become brown. Occasionally leaves also show 'shoestring' effect due to infection with cucumber mosaic. Transmission of the virus by sap and aphids revealed that CMV infects tomato was sap transmissible and has many important aphid vectors like: *Aphis gossypii*, *Myzus persicae* and *A. craccivora.*

CMV was detected from tomato showing symptoms such as: mosaic mottling of leaves, smaller than normal and have uneven margin, the virus had specific properties such as: TIP between 60 and 65 °C for 10 min and LIV between 3 and 4 days at 20–22 °C. The virus was easily transmitted by *M. persicae* and *A. gossypii* in nonpersistent manner and showed positive reaction with antiserum of CMV (Joshi and Bhargava 1965).

There is a report from India on molecular detection of a virus isolate causing shoestring disease in tomato plants, attempted by RT-PCR using CMV-specific primers by Pratap et al. (2008). The ~650 bp products obtained by PCR were cloned and sequenced. The obtained nucleotide sequence data was corrected as 657 bases and translated into 218 amino acid residues. The sequence data was deposited in GenBank database (accession no: DQ141675). BLASTn analysis of sequence data revealed 99% (655/657) identities with coat protein gene of CMV isolate of Amaranth. These results indicated that the virus isolate causing shoe string in tomato possesses the close relationship with Amaranth isolate of CMV and/or tomato may be considered a new host of the CMV Amaranth isolates. Screening of number of tomato plants was performed using CMV-tomato clone as positive probe and the disease incidence was measured. The infection was found more than 60–70% in some cultivars (Pratap et al. 2008).

9.2.1.2 Bell Pepper (*Capsicum annuum* var. grossum)

CMV was also isolated from bell pepper (*Capsicum annuum* var. grossum) plants in two Karnataka districts (Nagaraju and Reddy 1982). George et al. in 1993 did isolation and identification of two major viruses infecting bell pepper in Karnataka. The symptomatology, host assay, particle morphology and serology suggested that the two viruses infecting bell pepper (*Capsicum annuum* L.) were PVY and CMV (George et al. 1993). The reaction of this virus was positive only when tested against CMV antiserum. Taking into account of all these findings, this virus was identified as CMV belonging to cucumovirus group (George et al. 1993).

Sharma et al. 1993 studied the properties of the viruses associated with disease complex of bell pepper. Based on host range, symptomatology, transmission and electron microscopy, at least two different potyviruses and a cucumovirus associated with mosaic disease complex of bell pepper were characterized. Host range of these viruses was restricted. Symptoms induced in bell pepper included mosaic, mottling, vein banding, shoe-stringing, stunting and fruit deformities. All the virus isolates were found to be the sap and aphid transmissible. Virus particles of the potyviruses isolates were flexuous filaments measuring 741–785 × 12 nm while of the particles of cucumovirus were isometric and of 27 nm (in dia). Isolates of potyviruses induced cytoplasmic cylindrical inclusions and that of cucumovirus induced vesicles along the tonoplast. The cucumovirus isolate as serologically related to CMV -K; the isolates of potyviruses were not related to PVY (Sharma et al. 1993).

Kapoor investigated the viruses infecting bell pepper (*Capsicum annuum* L.) in India. The disease incidence in bell pepper growing districts of Himachal Pradesh was observed during 2003–2004 cropping seasons and the yield losses calculated ranged from 1% to 100%. The symptoms observed were leaf deformation, stunting and yellowing in fields. The two representative isolates of CMV (C-I and C-II) were characterized based on host range assay, biophysical properties, ISEM and DAS-ELISA using CMV antisera. Both the isolates were confirmed by RT-PCR by using cucumovirus primers and identified to be the strains of CMV.

Kapoor in 2012 again reported a CMV isolate in bell pepper crop from Himachal Pradesh. The CMV isolate was found to be mechanical as well as aphid transmissible. The identity of the isolate was established based on symptom expression on indicator plant species, biophysical properties, host range, serology, morphological properties of the virus particles by electron microscopy and RT-PCR. In ELISA, the virus isolate gave positive reaction with CMV specific antibodies. Seventeen weed species were tested as an alternate host of CMV and *Bidens pilosa* was added as a new reservoir host. RT-PCR assays with CMV specific primers and total nucleic acid extracted from symptomatic bell pepper leaves yielded the expected ~550 bp amplicon (Kapoor 2012).

9.2.1.3 Brinjal (*Solanum melongena*)

The studies on a new mosaic disease of brinjal (*Solanum melongena* L.) caused by CMV isolate was carried in Andhra Pradesh by Seth and Raychaudhuri (1973). The purified virus particles were observed to be spherical in shape and measured 35–36 mμ in diameter. The virus was identified as a new strain of CMV. It was successfully transmitted by sap inoculation to *Solanum gilo*, *S. indicum*, *S. intergrifolium*r, *S. khasianum*, *S. sisymbrifolium*, *Coccinia cordifolia*, *Cucumis anguria*, *C. melo*, *Cucurbita maxima*, *Lagenaria siceeraria*l, *Spinacia oleracea*, *Calendule officinalis*, *Carthamus tinctorius*, *Zinnia elegans*, *Ocimum sanctum*, *Salvia officinlis* and *Tropaeolum majus*. Virus purification was done by two procedures and the pellet in both cases was obtained after two cycles of high-speed (30,000–40,000 rpm) centrifugation of 2-h duration each. The virus particles were spherical and measured 35–36 mμ in diameter which was similar to reported for CMV–l (Seth and Raychaudhuri 1973).

Kiranmai et al. in 1997 characterized CMV isolates infecting three vegetable crops in Andhra Pradesh. In this study viruses isolated from commercial tomato, chilli and brinjal crops in Chittoor district, Andhra Pradesh were identified as strains of CMV based on host range, transmission, serology and physicochemical properties. *Dolichos lablab* for CMV-tomato isolate, *Cucumis sativus* for CMV-brinjal isolate and *Datura metel* for CMV-chilli isolate were identified as differential diagnostic host plants. The LIV was 4–6 days, TIP was 50–65 °C and DEP 10.3–10″ for the three isolates. The titres of polyclonal antisera produced against three isolates ranged from 1:5,000 to 1:23,000 in DAC-ELISA. CMV-brinjal and CMV-chilli isolates were serologically different but related to CMV-Tomato isolate. The Mr of coat protein of three isolates ranged from 24.5 to 25.7 × 1Q3 d. The Mr of three genomic RNA s and one subgenomic RNA of three isolates was 1.24 to 1.26; 1.05 to 1.12; 0.78 to 0.86 and 0.34 to 0.39 × 106 d. The dsRNA isolated from infected chilli, tomato and brinjal was resolved into three species with Mr ranged from 2.48 to 2.52; 2.10 to 2.26 and 1.56 to 1.72 × 106 d. The tomato, chilli and brinjal isolates of CMV were identified as CMV-To, CMV-Ch and CMV-Br strains, respectively (Kiranmai et al. 1997).

In another study by Bharti et al. (1997), one isolate was found to be the most virulent, sap transmissible and recorded 100% infection with an incubation period of 16 days. Based on the symptomatology in brinjal and reaction of indicator plants like *C. amaranticolor*, *C. sativus, Gomphrena globosa*, *N. glutinosa*, *N. tabacum*, *Physalis floridana*, *Vigna unguiculata* and *Zinnia elegans,* the virus isolate was identified as CMV. Of the 19 weeds, only 3 weeds *viz.*, *Amaranthus viridis*, *Digera arvensis* and *Physalis minima* were infected by this CMV isolate. When ten brinjal genotypes were tested against this virus, Pusa Purple Cluster had 10% infection. Selection of virulent isolate of CMV infecting brinjal was done based on the symptoms (mosaic, vein clearing, puckering and leaf distortion), incubation period and percentage of infection. Based on similarity of symptoms on brinjal and reaction on indicator plants, the present virus has been identified as a stain of CMV infecting brinjal (Bharti et al. 1997).

Further, the incidence and progress of CMV were recorded by Kiranmai et al. (1998c) in commercial brinjal, chilli and tomato crops around Tirupati (A.P.) during 1992–1994 Kharif and Rabi seasons which ranged to 9–21% in the young crops (10–15 days after transplanting) and progressed up to 72–86% as the crop aged. Several other crop and weed growing in and around the above crops found infected with CMV could probably act as alternate hosts. The average virus incidence at 10–15 days after transplanting ranged from 13% to 21% in brinjal, 9–16% in chilli and from 13% to 19% in tomato. It increased during 7 weeks period up to 76–85% in brinjal, 74–84% in chilli and 75–86% in tomato in both kharif and rabi seasons. There appears to be no significant difference in the incidence and progress of the virus infection during both seasons in all the 3 years. This indicates that cultural practices followed around Tirupati might have favoured the high level of initial infection as well as subsequent progress (Kiranmai et al. 1998c). The field collected crop plants that were positive for CMV in DAC-ELISA also produced chlorotic and/or necrotic local lesions an assay plants. Crops like *Dolichos lablab, Lagenaria*

siceraria, *Luffa acutangula*, *Momordica charantia*. *Sesamum indicum* and *Trichosanthes anguina*, collected in and around the three vegetable crops were found infected with CMV and they probably acted as alternative hosts. Based on the present studies, it is suggested that rouging of alternate virus source plants initially infected crop plants may help in decreasing the primary and secondary spread of Virus (Kiranmai et al. 1998c).

Kumar et al. in 2014 detected the association of CMV with severe mosaic disease of eggplant growing in Lucknow and Kanpur, India by host reaction and serological assay, and confirmed by RT-PCR using CMV specific primers. Furthermore, the complete RNA3 genome was cloned and sequenced which shared 97–99 % identities and close phylogenetic relationships with CMV subgroup IB members therefore identified as isolates of CMV subgroup IB (Kumar et al. 2014).

9.2.1.4 Carrot, French Bean, Sugar Beet and *Phytolacca* sp

Bhargava (1951) described properties of four CMV strains isolated from French bean, sugar beet and *Phytolacca* sp. for the first time in India. The CMV strains differed in their host range, symptoms, and transmissibility by aphids, DEP and TIP (Bhargava 1951).

Afreen et al. (2009) observed severe chlorotic mottle disease of carrot (*Daucus carota*) in fields of in northern Uttar Pradesh, India. The causal pathogen was transmitted through sap inoculations and by aphid to a number of indicator plants. The association of CMV was detected with the disease by RT-PCR an using CMV specific primer which was further identified by sequence analysis of cloned RNA3 genome. Analysis of complete RNA3 sequence revealed 97% identities and close phylogenetic relationship with various CMV strains of subgroup II available in database. The association of CMV of subgroup II with chlorotic mottle disease of *D. carota* was the only report (Afreen et al. 2009).

9.2.2 Characterization of CMV Infecting Pulse Crops

9.2.2.1 Sweet Pea (*Pisum sativum*)

The CMV strain causing mosaic disease of pea (*Pisum sativum*) is reported by Rao et al. (1995). During this study, survey was conducted in the year 1983, around Gorakhpur (India) and widespread occurrence of an apparently unrecorded mosaic disease of pea was observed. The symptoms in infected plants were severe mosaic, mottling, puckering of leaves and stunting. It was systemic in *Arachis hypogea, Crotolaria juncea*, *C. sericea*, *Cyamopsis tetragonoloba*, *Dolichos biflorus*, *D. lablab*, *Pisum sativum*, *Vigna mungo*, *V. radiata*, *V. sinensis*, *Vicia faba*, and *Cucumis sativus* and was localized in *Chenopodium amaranticolor* and *C. ambrisoides*. The symptoms in different host varied from mild mosaic, mottling, chlorotic spots, necrotic spots, vein clearing and vein banding to reduction of entire plant. The virus was serologically related to CMV and the particles were isomeric having an average diameter of ~32 nm. *Aphis craccivora*, *Acyrthosiphon pisum* and *Myzus persicae* were able to transmit the virus. Of these *A. pisum* was found to be most efficient

vector. The plants grown from the seeds of disease plants, only 5.5% showed mosaic symptoms suggesting a positive seed borne nature of the virus. The nucleic acid content in the virus particles was approximately 19.8%. The virus was identified as strain of CMV and is designated as CMV-pea (Rao et al. 1995).

9.2.2.2 Chickpea (*Cicer arientinum*)

Chickpea (*Cicer arientinum* L.) in India is known to be affected by two viral diseases causing tip necrosis and stunting. Dhingra et al. (1979) observed symptoms of slightly chlorotic, very small, narrow and deeply dentate leaves of chickpea BG-2 and G-113 cultivars. The plants remain stunted giving bushy appearance and phloem of roots and collar region of stem developed necrosis. Diseased plants bore lesser flowers and pods as compared to healthy plants. A sap transmissible virus was isolated and identified as CMV (Dhingra et al. 1979).

Chalam in 1982 identified and characterized the CMV along with a BYMV isolate infecting chickpea in India. CMV is reported to produce twisting of terminal bud initially and with progression of disease cause wilting and death of plants or proliferation and bushiness of branches bearing very small green leaves and very small pods. The virus was transmissible in non-persistent manner by aphids. The shape and size of CMV particles was spherical and measured about 30 nm in diameter, respectively. The CMV WAS revealed the presence of four RNA species in PAGE and was relatively closer to CMV-C l, followed by CMV-Ix and M-CMV (Chalam 1982; Chalam et al. 1986).

Sporadic occurrence of the disease of chickpea causing stunting of the plants and excessive axillary proliferation having smaller leaflets was noticed by Singh et al. (1996) in several parts of Himachal Pradesh. The virus induced necrotic lesions on *C. amaranticolor* and mosaic on *C. sativus*, *G. max*, and *N. glutinosa* while no symptoms were observed on *G. globosa*, *A. hypogea*, *C. annum*, *C. cajan*, *P. sativum* and *V. faba*. DEP of virus ranged between 1:5,000 and 1:10,000; TIP between 65 and 70 °C and LIV for 2 weeks in desiccated leaves stored at 5–7 °C. The virus isolate in the present study was identified as CMV on the basis of symptoms, reaction on diagnostic hosts and serological tests. Authors suggested that though the frequency of occurrence of CMV on chickpea is less than 1% but due to cropping pattern in India aid in the building up of aphid vector, this virus has the potential of becoming widespread on chickpea in future (Singh et al. 1996).

Singh et al. 2002 studied viral diseases of chickpea in Himachal Pradesh. In this study **e**xtensive survey of the chickpea fields in Himachal Pradesh, India was done to determine the prevalence of viral disease which revealed four different types of symptoms. Leaves from symptomatic plants were used to conduct the pathogenicity tests using chickpea cv. HPG-17. Based on transmission tests, host range, physical properties and serological tests, association of four viruses, namely, CMV, BYMV and chickpea stunt caused by bean leaf roll virus and chickpea chlorotic dwarf virus was observed in the infected chickpea plants. The incidence of CMV and BYMV was about 1% in the cv. HPG-17 (bold seeded *desi* type) whereas the incidence of chickpea stunt varied from 3% to 5% (Singh et al. 2002).

9.2.2.3 Cowpea (*Vigna unguiculata*)

To date, there is the only a report of Nagaraju and Murthy (1994) describing the association of CMV with mosaic disease of cowpea (*Vigna unguiculata*). CMV was found to be sap, seed and aphid transmissible. CMV had a DEP of 1:1,000–1:5,000, TIP between 60 and 65 °C and LIV of 24–48 h at room temperature (23–25 °C) and 7 days at 14 °C. It produced chlorotic spot and thickening along veins in *Cucurbita moschata* and *Luffa acutangula*, mosaic in *Cajanus cajan, Cyamopsis tetragonaloba, Phaseolus aureus* and *Phaseolus vulgaris*, chlorotic local lesions on *Chenopdoium amaranticolor* and *C. quinoa* and systemic necrosis on *Dolichos biflorus*.

9.2.3 Characterization of CMV Infecting Fruit Crops

9.2.3.1 Banana (*Musa paradisiaca*)

The production and quality of banana are greatly affected due to several diseases caused by various viral pathogens, and among viruses CMV, banana bunchy top virus (BBTV), banana streak virus and banana bract mosaic virus commonly infect. These diseases of banana are vertically transmitted through planting suckers. Although conventional control measures such as quarantine eradication and certified virus free stocks confirmed by ELISA has been used to reduce crop losses caused by these viruses, but they are not effective for controlling CMV and BBTV. Mali and Rajegore (1980) described a disease of banana caused by CMV in India. Banana heart rot in the Deccan was found to be a syndrome of banana mosaic. The virus isolated, identified as CMV, was transmissible mechanically and by aphid in stylet borne manner. It was inactivated as 65–70 °C and had a dilution end point of 104. It was viable up to 92 h at 27–30 °C. This was the first record of the virus on banana in India (Mali and Rajegore 1980).

Patel and Mali (1986) did comparative studies of three isolates of cucumber mosaic virus from banana. Isolates CMV-1, CMV-2 and CMV-3 were indistinguishable serologically, in particle size and morphologically and transmission characteristics, but differed in reactions of 13 hosts and physical properties, on the basis of which they are regarded as distinct structures of the virus (Patel and Mali 1986).

CMV causing infectious chlorosis in banana plants growing under natural conditions in Andhra Pradesh was observed. The virus culture was established on cow pea and single local lesion was subsequently was maintained on tobacco by sap inoculations and back inoculation in banana by injecting purified virus particles (Kiranmai 1989). Infection of CMV causing mosaic disease of banana was identified by immuno/nucleic acid probe using polyclonal antibodies of CMV-T and slot blot hybridization tests with nucleic acid probe of CMV-P genome by Srivastava et al. (1995).

The comparative CMV detection efficiency of three different tests in banana was done by Kiranmai et al. (1996). Direct ELISA like double antibody sandwich (DAS) ELISA, simplified rapid direct antigen coating (SRDAC)-ELISA and indirect form of DAC-ELISA for detection of CMV in leaf extracts and pseudostem sap exudates and purified virus diluted with antigen buffer or healthy banana leaf sap were

compared. The sensitivity levels of three ELISA tests were similar and the virus was detected up to 10–7 dilution with leaf extract, 1 μl/well with sap exudates, 20 ng/well with purified CMV and 100 ng/well with purified virus diluted with healthy banana leaf extract. Of three forms of ELISA compared DAC-ELISA was further evaluated with detection of infectious virus by local lesion bioassay on cowpea (*Vigna unguiculata*) and by double stranded RNA (dsRNA) analysis. The banana leaf and pseudostem sap exudate samples positive in DAC-ELISA were also positive by other two tests. Collection of pseudostem sap exudates by pin pricking from test plant and detection of virus by DAC-ELISA in them appears ideal for mass screening of banana plants (Kiranmai et al. 1996).

RT-PCR/PCR based detection of CMV was attempted by Vishnoi et al. (2006) in severely infecting banana plantations using their specific primers. Cloning and sequencing of CMV-CP gene was done and sequence data were submitted to GenBank (DQ152254, CMV CP-Banana). The work aims to developing a simple and accurate means of rapid detection system in banana and to search of virus-free propagating material of banana to be used for mass propagation or large-scale cultivation (Vishnoi et al. 2006).

Khan et al. (2011) attempted the CP gene based characterization of CMV isolates infecting banana in India. Banana plants exhibiting stripes on leaves in addition to leaf distortion and stunting of plant were collected from Karnataka (KAR), Maharashtra (MH) and Uttar Pradesh (UP) in India. The virus was identified as CMV based on TEM and RT-PCR analysis. CP gene of all isolates was amplified using gene specific primers, cloned and sequenced. Complete CP gene contains 657 nucleotides coding for 219 amino acids. Sequence analysis of CP gene showed 93–98% (at nucleotide) and 94–99% (at amino acid) sequence identity between all three Indian isolates. On comparing CP gene sequences of CMV isolates of KAR, MH and UP with P isolate; 94%, 99% and 96% identity respectively was obtained. High degree identity at nucleotide level between these isolates of banana and *Physalis minima* (a weed) suggest that *Physalis minima* could be an alternate host of CMV banana. Phylogeny of nucleotide along with amino acid sequence of CP gene showed that all our isolates belong to IB subgroup (Khan et al. 2011a). Further, Vishnoi et al. (2013) worked out molecular characterization of complete genome of CMV infecting Banana in three banana farms of Uttar Pradesh, India where it cause severe mosaic accompanied by leaf and fruit deformation with 18–25% disease incidence. The RNA 1a, RNA 2b and RNA 3 genomic fragments were amplified and sequenced for molecular identification of virus. Sequence analyses of these fragments revealed its highest identities and close relationships with Indian strains of CMV of subgroup IB; therefore, identified as an isolate of CMV of subgroup IB (Vishnoi et al. 2013).

Recently, Khan (2015) attempted the development of sensitive diagnostic for the detection of CMV in banana. The 300 diseased samples from 21 banana orchards in 3 bananas producing states: Karnataka, Maharashtra, and Uttar Pradesh were collected, of which 13 were positive for CMV tested by DAS-ELISA. One representative isolate from each state was sequenced and designated as CMV-KAR, CMV-MR, and CMV-UP. Comparison of these isolates with 29 CMV isolates reported from

various plants around the world showed that they were most closely related to subgroup-I isolates, sharing up to 95.81% and 96.84% sequence identity at nucleotide and amino acid, respectively and clustered with the CMV subgroup-IB strains. Study suggested that CP gene based RT-PCR assay may be a more sensitive, reliable, and convenient molecular tool for detection of the CMV, and can be used in quarantine, eradication, and tissue culture certification programs (Khan 2015).

9.2.3.2 Papaya (*Carica papaya*)

The infection of CMV in papaya was first reported by Kiranmai et al. (1998a) in India. A virus disease with mosaic, leaf distortion and filiform symptoms on commercially growing papaya (*Carica papaya* L.) around Kovvur, West Godavari district, Andhra Pradesh state was noticed during 1995. The sap transmissible virus from papaya leaves was successfully purified and its yield ranged from 7 to 9 mg/100 g leaf tissue. The purified virus was infectious to papaya. In DAC- ELISA, the virus positively reacted with three CMV antisera. PAGE revealed the molecular weight of CP subunit about 26.2 kDa. The isolated genomic nucleic acid from purified in agarose gel was resolved into three genomic and one sub genomic RNAs with Mr of 1.23, 1.12, 0.77 and 0.3×10^6 Daltons, respectively. The double stranded RNA isolated from infected papaya leaves was resolved in agarose into three species with *Mr* of 2.46, 2.24 and 1.54×10^6 Daltons. Based on these studies, virus causing leaf distortion in papaya was identified as CMV which was antigenically identical to CMV-banana and CMV-Chilli isolates previously reported from Andhra Pradesh.

9.2.3.3 Cape Goose Berry (*Physalis peruviana*)

A severe mosaic disease of cape goose berry (*Physalis peruviana*) showing severe puckering and blistering of leaf lamina was studied in Kanpur, U.P. The host reactions, physical properties, transmission and serological reactions confirmed it to be caused by a virulent strain (CMVSS) of CMV (Gupta and Singh 1997).

9.2.4 Characterization of CMV Infecting Spice Crops

9.2.4.1 Chillis (*Capsicum annuum*)

Bhargava in 1951 reported the occurrence of CMV on chilli for the first time. Then after, Anjaneyulu and Apparao (1967) reported the occurrence of CMV on chilli. Here, a collection of virus infected chilli plants obtained around Hyderabad and Warangal in Andhara Pradesh revealed that Chilli is affected by a number of viruses. One of these, as judged by the symptoms, appeared to be not described from India and a detailed investigation has been made to characterize the virus. The symptoms in the field consisted of mosaic mottling and various types of leaf distortions mainly exhibited by filiform leaf tip. Infected plants exhibited marked stunting in growth and severe reduction in size of the leaves and fruits. The virus was found to have a DEP of 1:250–1:500, TIP of 55–60 °C and LIV of 12–18 h at room temperature (26 °C) and 36 h at low temperature (10 °C). A comparison of the characters like transmission, host range and physical properties with CMV identified the virus under study as CMV (Anjaneyulu and Apparao 1967).

Prasada Rao (1976) and Bidari and Reddy (1983) identified the occurrence of CMV in chilli based on electron microscopy of purified preparations showing isometric particles with a diameter of 29 nm. Gowda and Reddy (1985) described the distribution of chilli mosaic virus in some parts of Kolar district. A survey in this area of Karnataka revealed five viruses causing mosaic of *Capsicum annuum*: CMV, PVY, pepper vein banding virus, pepper veinal mottle virus and TMV (Gowda and Reddy 1985).

Narayan and Dhawan (1989) characterized and identified viruses causing mosaic disease of *Capsicum*. *Capsicum* plants in Haryana, India, were found infected by various types of mosaic symptoms. The CMV, PVX, PVY and TMV were identified, associated singly or in combination with other types of mosaic diseases. CMV was found to be the most prevalent virus (Narayan and Dhawan 1989).

Singh et al. (1990) attempted identification of virus(es) associated with chilli mosaic syndrome. During the study, four isolates were obtained from naturally infected *Capsicum* plants showing different symptoms in the field at Ludhiana. Host range, transmission and serological tests established that they were all closely related to cucumber mosaic cucumovirus. Determination of physical properties confirmed the predominance of this virus as a cause of mosaic in *Capsicum,* no other virus being detected (Singh et al. 1990). Singh and Shukla (1990) described the properties of a new strain of cucumber mosaic virus from chilli. The properties of a virus causing necrosis and mosaic in *Capsicum* around Kanpur, Uttar Pradesh, India are described. The virus was transmitted mechanically in seeds and by 2 aphids, *Aphis gossypii* and *Myzus persicae,* and could infect 44 plant species, inducing a range of symptoms. EM of the purified virus showed spherical particles of *c.* 30 nm diameter and serological tests produced a positive reaction to cucumber mosaic cucumovirus antiserum. It is concluded that the virus is a new strain of cucumber mosaic cucumovirus (Singh and Shukla 1990).

Biswas et al. (2013) evidenced the CMV subgroup II infection in *C. annuum* L. in Himachal Pradesh of India associated with virus-like symptoms in the foliage of chilli plants. DAS-ELISA, host assay and RT-PCR suggest the occurrence of CMV in these samples. The CP gene was amplified form several samples, cloned and sequenced. Analysis revealed high (95–99%) homologies at nucleotide level with the sequences of CMV subgroup II and phylogram could assigned the clustering of CMV isolate under study with the other analyzed isolates belonging to subgroup II. These findings helped to understand the role of CMV subgroup II as a major virus-causing disease infecting chilli in western Himalayan region of India (Biswas et al. 2013).

9.2.4.2 Black Pepper (*Piper nigrum*)

The CMV associated with stunt disease of black pepper was recorded in Kerala, South India by Sarma et al. (2001). CMV was found transmissible through grafting. Virus was also sap transmissible from infected black pepper to healthy black pepper and to some assay plants. Virus produced local chlorotic/necrotic lesions on *C. amaranticolor*, *C. quinoa*, *V. unguiculata*, *V. radiata* and *V. mungo* while systemic mosaic on *C. sativus* plants. Sap as well as purified virus preparation reacted positively with polyclonal antisera of CMV-Banana (India), CMV-Brinjal (India),

CMV-Chilli (India), CMV-Tomato (India), CMV-L (USA) and CMV-A (China) in DAC-ELISA. In agreement to other CMV isolates, it also showed non-enveloped isometric virions, 26.1 kDa CP subunits and presence of four species with *M*r 1.21, 1.10, 0.81 and 0.37 × 106 Da. Hence, the virus associated with stunt disease of black pepper was identified as an isolate of CMV (Sarma et al. 2001).

Bhat et al. (2003) reported viral disease – a new threat to black pepper cultivation in India. The symptoms, causal agents [CMV (Cucumovirus) and an uncharacterized virus (Badnavirus)], and transmission and spread of viral disease infecting black pepper in India, particularly in Karnataka, Kerala, and Tamil Nadu are discussed. The different methods (i.e. virus-free vegetative planting material, rouging and eradiation of infected plants and chemical control of insect vectors) for effective viral disease management are also discussed (Bhat et al. 2003).

Bhat et al. (2004a) also characterized a CMV strain infecting black pepper. CMV strain was transmissible to *N. benthamiana* and *N. glutinosa*. The virus was purified by differential and sucrose density gradient centrifugation which showed the presence of isometric cored particles of ~28 nm diameter. Polyclonal antiserum against the virus was raised and DAS-ELISA method was standardized for detection of CMV in diseased black pepper samples collected from different regions of Karnataka, Kerala and Tamil Nadu. The CMV was also detected in other *P. chaba*, *P. colubrinum* and *P. longum* and weeds such as *Ageratum conyzoides*, *Colacasia esculanta*, *Synedrella nodiflora*, *Cynodon dactylon* and *Sonchus oleraceus* found in and around black pepper gardens (Bhat et al. 2004a). Further Bhat et al. in 2005 reported the association of CMV with stunted disease of black pepper based on sequence analysis of CP gene containing 657 nucleotides encoding a protein of 218 amino acids. CP gene showed 92–99% sequence identity with members of CMV subgroup I and close relationship with CMV-H strain isolated from Egyptian henbane in India. The study suggested that virus infecting black pepper is a strain of CMV belonging to subgroup I (Bhat et al. 2005).

Bhat and coworkers in 2013 reported quick and sensitive detection of CMV along with Piper yellow mottle virus (PYMV) in black pepper by loop-mediated isothermal amplification (LAMP) method. The LAMP and RT-LAMP assay for PYMV and CMV, respectively were done using five primer pairs targeting the conserved sequences in the viral genome. The assay detected presence of both the viruses. The detection limit for both LAMP and RT-LAMP assays was up to 100 times as compared to PCR and real-time PCR (Bhat et al. 2013).

9.2.4.3 Betelvine (*Piper betle*) and Long Pepper (*Piper longum*)

Raj and coworkers in 2003 detected the association of CMV and BYMV with mosaic disease of betelvine grown at Mahoba and Banthara (in India) by ELISA and RT-PCR methods using their specific antisera and universal primers. DAC-ELISA could detect only CMV. However, RT-PCR detected both CMV and BYMV infections in several betelvine samples. The association of CMV with betelvine was reported for the first time (Raj et al. 2003).

Bhat et al. (2004b) reported occurrence and identification of a CMV isolate infecting Indian long pepper (*Piper longum*). The virus causing mosaic mottling in

long pepper was identified as CMV based on biological, physicochemical and serological properties. The virus isolate was easily sap transmissible to *C. album*, *N. benthamiana*, *N. glutinosa*, *N. tobacum* cv. white burley, *P. floridana* indicator hosts and a few other cultivated plants species including black pepper. The purified virus preparations revealed ~28 nm isometric particles when observed under TEM. The PAGE showed presence of 25.6 kDa capsid protein subunits. The leaf extract from diseased *Piper longum* and virus preparations showed positive reaction with polyclonal antiserum of CMV both in DAC-ELISA and electro-blot immunoassay (EBIA). Based on particle morphology, antigenic relationship and molecular weight of CP subunits suggest that the virus associated with Indian long pepper was an isolate of CMV (Bhat et al. 2004b).

In another study, characterized a CMV strain infecting Indian long pepper (*Piper longum* L.) and betel vine (*Piper betle* L.). The infection of CMV in was detected by RT-PCR using CMV-CP gene specific primers. For virus identification, the resulting amplicons were cloned and sequenced. Analysis showed that both of them consisted of 657 nucleotides and encoding for a protein of 218 amino acids. The sequence comparisons revealed 100% identity with each other suggesting a common origin. Both of them also showed that CMV infecting banana and black pepper were close to CMV isolated from long pepper and betel vine. Based on sequence identities Indian long pepper and betel vine isolates were identified as members of CMV-subgroup I for the first time.

9.2.4.4 Vanilla (*Vanilla planifolia)*

Madhubala et al. (2005) recorded occurrence of CMV causing mosaic, leaf distortion and stunting of vanilla (*Vanilla planifolia* Andrews) for the first from India. The virus was characterized on the basis of biological and coat protein properties. The virus was purified from inoculated *N. benthamiana* plants and purified preparations showed isometric particles of ~28 nm. The viral coat protein subunits were of 25.0 kDa. DAS-ELISA method was also standardized for the detection of CMV infection in vanilla plants. The coat protein gene region of the virus was amplified by RT-PCR, cloned and sequenced. Sequence analysis revealed presence of a single open reading frame of 657 nucleotides coding for 218 amino acids. The isolate of vanilla showed 99% identity and close relationship with black pepper isolate of CMV belongs to subgroup IB (Madhubala et al. 2005).

9.2.5 Characterization of CMV Infecting Ornamental Plants

9.2.5.1 Phlox

Khatri and chenulu (1967) did studies on mosaic disease of phlox. In this study, a virus that caused mosaic disease of phlox had been studied with regard to symptomatology, mode of transmission, host range and biophysical properties. The virus was readily transmitted by juice inoculation and by *Aphis gossypii* Glover. It has been mechanically transmitted to and produce mosaic symptoms on *Phlox drummondii*, *Nicotiana glutinosa*, *N. tabacum* white burley and Harrisons special, *Capsicum*

annuum, *Solanum nigrum*, *Datura stramonium* var. tatula, *Lycopersicon esculentum*, *Cucurbita pepo*, *Cucumis melo* and *C. sativus*, *C. amaranticolor* induced local necrotic lesions. The virus was inactivated at 60 °C, at a dilution of 1:50 and after storage for 16 h at room temp. Freezing the virus-infected leaves for 80 h resulted in loss of infection. A comparison of the virus studied with several other viruses reported to cause mosaic symptoms on phlox either naturally or under artificial conditions has shown that the viruses resembles Cumis virus 1, smith orits strain. These studies confirmed an earlier report by Faan and Johnson that phlox serves as an over wintering host of cucumber mosaic virus (Khatri and Chenulu 1967).

9.2.5.2 Amaranths (*Amaranthus* spp.)

Sharma and Chowfla (1986), described a mosaic disease of amaranths (*Amaranthus caudatus* L.) in Himachal Pradesh. They described the serological and biophysical properties of the virus isolates, its host range and transmission indicated that the severe mosaic disease of this green vegetable crop was caused by CMV (Sharma and Chowfla 1986).

A number of *Amaranthus* spp. viz. *A. tricolor*, *A. hypochondriacus*, *A. deflexus*, *A. lividus* and *A. retroflexus* are known as natural hosts of CMV, however, there was no record of CMV infection from India until 1997. The disease was important due to reduction of seed yield in *A. hypochondriacus* and the total loss of ornamental qualities of *A. tricolor* plants. About 10–12% disease incidences were recorded in experimental fields at CSIR-NBRI, Lucknow, in 1992 and 1993, respectively by Raj et al. (1997a, b). The virus was easily transmissible by sap inoculations on a number of test hosts that developed local lesions or systemic symptoms. The virus isolate was purified and observed by TEM which revealed the presence of polyhedral virus particles of ~28.0 nm. SDS-PAGE and Western immunoblot analysis revealed the presence of one band of 26 kDa coat protein in naturally infected *A. tricolor*. The nucleic acid isolated from purified virus particles remained infectious when treated with DNAase. During electrophoresis of the extracted nucleic acid, RNA species separated as three distinct bands which were identified as RNA 1 and RNA 2 altogether, RNA 3 and RNA 4, similar to that of usually observed for CMV. The isolate infecting *A. tricolor* and *A. hypochondriacus* was more closely related to CMV-C than to CMV-D (Raj et al. 1997).

Further, molecular characterization was also done using CP and MP ORFs located in its RNA 3 genome. The complete coat protein gene (657 bp) of CMV-A was cloned and sequenced and phylogenetic status of the Indian strains of CMV vis-à-vis the global CMV was analyzed. The studies based on CP analysis indicated that the Indian CMV strains are more close to the subgroup IB rather than to subgroup IA or II according to the currently accepted phylogeny. However, a high degree of homology depicted between CMV-A and other Indian strains may be reflective of the adaptation of the virus to Indian location or the Indian hosts it infects (Srivastava 2003). The complete movement protein gene (840 bp) of CMV-A was cloned, sequenced and analyzed. The phylogenetic relationship based on MP sequence data also placed the Indian strain into subgroup IB rather than IA. Within subgroup IB, maximum evolutionary relationship was revealed to IA-3a strain, a

subgroup IB member from Indonesia. The phylogenetic analysis based on MP had also showed maximum homology of the Indian strain under study with the MP sequence of Indonesian strain except CMV-D, H and Phym, the other Indian strains (Srivastava 2003).

9.2.5.3 Gladiolus (*Gladiolus* spp.)

Several gladiolus cultivars maintained in CSIR-NBRI, Lucknow gardens were found exhibiting the mosaic, stripe on leaves and flower colour-breaking symptoms. The association of CMV was identified as on the basis of aphid transmission, presence of 28–29 nm isometric particles, 26 kDa coat protein subunits, four species of RNA and serological relationship with CMV strains. SDS-PAGE of partially purified preparations from infected gladiolus and tobacco plants revealed one major band of viral coat protein subunits of 26 kDa molecular weight and a minor 52 kDa band (dimer was also observed) as seen in case of CMV strain obtained earlier from *Dianthus barbatus*. Result of Western immunoblot analysis using CMV-T antibodies also confirm the presence of 26 kDa band in infected Gladiolus samples which indicated that the gladiolus isolate of CMV is serologically related to CMV-T (Raj et al. 2002). In order to co-relate these findings at nucleic acid level, total RNA was extracted from infected and healthy gladiolus leaves. These RNA preparations (50–200 µl) corresponding to leaf tissue (25–100 mg) were transferred to a zeta-probe membrane (Bio-Rad) under vacuum using a slot blot apparatus. cDNA clone of CMV-P containing part of CP gene and MP genes was used for preparation of α-^{32}P labeled probe by random primer extension method. Positive signals in all infected gladiolus leaf samples indicated the presence of CMV; however, no signals were obtained with healthy gladiolus leaves. On the basis of slot blot hybridization results the gladiolus strain of CMV seems to carry certain homology with CMV-P strain (Raj et al. 2002).

Dubey et al. in 2010 reported molecular characterization of CMV infecting gladiolus by sequence analysis of cloned 657 bp coat protein region amplified by RT-PCR. The phylogenetic relationships were determined by the comparison of coat protein gene nucleotide (DQ295914) and its amino acid sequences with other CMV isolates reported from India and worldwide. The comparisons of nucleotide and amino acid sequences and phylogenetic tree revealed a close resemblance of the virus isolated from gladiolus with the Fny strain, which was an unusual in the Asian continent (Dubey et al. 2010).

9.2.5.4 Petunia (*Petunia hybrid)*

Petunia plant exhibiting severe mosaic, necrosis and stunting symptoms were observed in various gardens in Lucknow. The association of a strain of CMV was identified on the basis of non-persistent transmission by aphids, molecular weight of protein sub units as 24,500 Da, spherical particles of 29 nm diameter and serological relationship with several CMV isolates as established by enzyme immunoassay. Naturally infected *P. hybrida* plants exhibited necrosis of leaves and petioles and apical growth (Fig. 9.1). The affected plants gave a stunted appearance and did not bear flowers. The virus was easily transmitted by mechanical means from *P.*

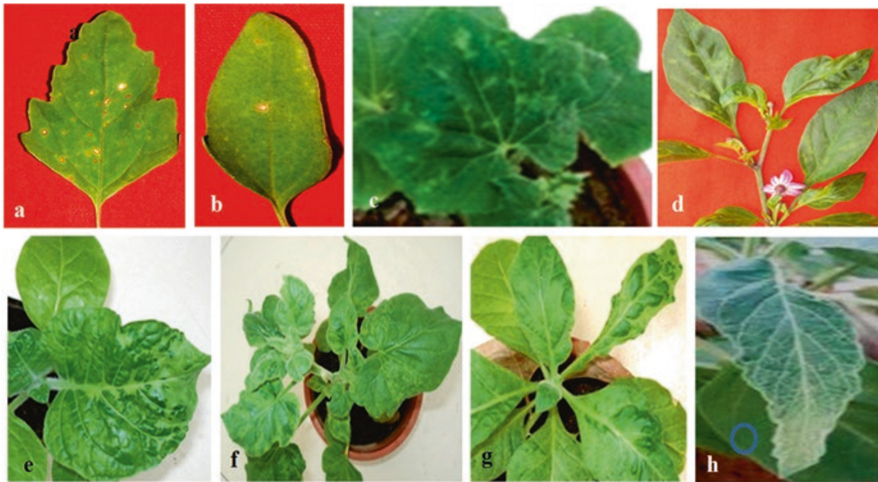

Fig. 9.1 Necrotic local lesions and systemic mosaic, blistering, leaf deformations and puckering induced by CMV-Petunia virus isolate (Gautam et al. 2014)

hybrida to *P. hybrida* and by aphids to an extent of 60% (6/10) and 80% (8/10), respectively. Results on DAC-ELISA indicated that the virus isolate from petunia is related to CMV-C, CMV-D, CMV-T, CMV-L and CMV-S. Chrysanthemum aspermy (PVAS-24) and Tomato aspermy virus-N (TAV-N) however, showed a very distant relationship with the isolate (Srivastava et al. 1991).

Praveen et al. (2001) differentiated the biologically distinct CMV isolates of *N. tabacum*, *Commellina* and *Petunia* by double stranded RNA profiling. In this study, four isolates of CMV from *N. tabacum*, *Commellina* and *Petunia*, designated as CMV-T, C, P1 and P2, respectively, differed in their host reaction and temperature sensitivity, at the molecular level. Serologically related isolates of CMV *viz.*, CMV-P1 and P2C and T were analyzed on the basis of their symptomatology, temperature tolerance and double stranded RNA (ds-RNA) pattern. The ds-RNA pattern of these CMV isolates representing biological diversity were examined, Two distinct patterns differing by the number and or/position of the ds-RNA bands were obtained for the four isolates. The ds-RNA pattern cannot be necessarily correlated with the symptom severity nor with any specific biological characteristics, however, it can be utilized in distinguishing isolates (Praveen et al. 2001).

Gautam et al. (2012) identified a CMV strain associated with mosaic disease on petunia. The association of CMV was detected based on presence of cored virus particles of ~28 nm and positive amplification of 650 bp during RT-PCR using CMV -CP gene specific primers. Further, the complete RNA 3 genomic fragment of 2.2 Kb of virus isolate was amplified by RT-PCR using RNA 3 specific primers and obtained amplicon was cloned and sequenced. The analysis of sequence data of RNA 3 showed 96% with several CMV strains possessed the closest phylogenetic relationships with banana strain of CMV of subgroup IB reported from India. Therefore, virus isolate of petunia was identified as a new member of CMV subgroup IB which was the first report from India (Gautam et al. 2012).

9.2.5.5 Chrysanthemums (*Chrysanthemum morifolium*)

Naturally infected *Chrysanthemum morifolium* showing diffused chlorosis, chlorotic dots near veins and necrosis of leaves were seen in gardens in CSIR-NBRI, Lucknow. Based on the obvious symptoms observed it may be concluded that the disease was prevalent in about 20% of the chrysanthemum grown in the gardens of NBRI at Lucknow. Naturally infected plant showed conspicuous ring mosaic symptoms of leaves, stunting of the plants and distortion of the florets. The virus was sap transmissible to *N. tabacum*, *C. amaranticolor* and *C. murale*. *C. amaranticolor* and *C. murale* developed necrotic local lesions 7 dpi. *M. persicae* and *A. gossypii* could transmit the virus from chrysanthemum to chrysanthemum and from chrysanthemum to *N. tabacum* var. Samsun NN to an extent of 80% (8/10) and 60% (6/10), respectively, when tested in non persistent manner. In Ouchterlony double diffusion tests, the isolate form precipitin lines with antisera to tomato aspermy virus-N and *Chrysanthemum aspermy virus* (PVAS-24 ATCC). However, the isolate did not react in identical tests carried out using antisera of few strains of CMV viz. CMV-D, CMV-L, CMV-T and CMV-S. The molecular weight of the coat protein sub units was calculated as 24,500 + 500 Da by linear regression based on log of the molecular standard vs. their mobility in SDS-PAGE.

In another study, chrysanthemum plants were observed showing browning of major veins, leading to downward cupping and, finally abscission symptoms. The virus was transmitted by mechanical means to several hosts. The virus in sap of *C. morifolium* remained infectious after it was diluted to 10–3, heated at 55 °C for 10 min, and stored for 2 days at ambient temperature 28–30 °C. The gradient purified virus preparations produced symptoms on *C. morifolium* identical to those observed in nature. Purified virus gave a characteristic peak of nucleoprotein at $A_{max/min}$ at $A_{260/240nm}$ with a ratio of 1.58. Spherical particles averaging 29 nm were observed in preparations stained with uranyl acetate. Molecular weight of viral CP subunits was approximately 24 kDa as determined by SDS-PAGE (Srivastava et al. 1992).

Viral RBA isolated from virus particles was infectious in White Burley tobacco without any further treatment or when treated with DNase. Infectivity was however lost after treatment with RNase and S1 nuclease, which showed ssRNA as the infectious entity. Electrophoresis of nucleic acid preparation revealed five major bands that moved along with two CMV strains isolated from *Physalis minima* L. *and Petunia hybrida* Hort. Vilm-Andr. In ODDT, crude antigen and purified virus preparations were tested which reacted strongly with antisera to CMV-L, CMV-C, CMV-T and CMV-S. No reaction was observed with these antigens and antisera to either TAV-N or CAV (PVAS-24). Chrysanthemum isolates as well as three other CMV strains did react with antisera to CMV-T, but under similar conditions CAV did not react with CMV-T antiserum. Results of ELISA confirm the findings of ODDT. CMV-D antiserum, which was not included in ODDT, showed strong reaction with the chrysanthemum isolates (Srivastava et al. 1992).

Further, Kumar and coworkers in 2005 attempted to develop a molecular tool based sensitive diagnostic method for reliable detection of CMV infection in chrysanthemum cultivars. RT-PCR with CMV-CP gene specific primers resulted in

amplification of ~650 bp band in most of the samples. The identities of amplicons were confirmed by Southern hybridization using the α32P radiolabelled probes prepared from the cloned CP gene of CMV-A strains. Positive signal of hybridization with PCR product in CMV and CMV probe proved the identity of PCR amplicons as a fragment derived from the CMV genome in infected chrysanthemum samples (Kumar et al. 2005).

Verma et al. (2007) screened several chrysanthemum cultivars of Northern India for presence of Chrysanthemum virus B (CVB), CMV, TAV, tospo- and potyviruses by ELISA, northern hybridization and RT-PCR methods. During screening of 80 cultivars, 48, 42.5, 26.2, 12.5 and 6.25% cultivars were found infected by CVB, CMV, TAV, tospo- and potyviruses, respectively.

9.2.5.6 Carnation (*Dianthus barbatus*)

The association of CMV with crinkle and stunting disease of carnation (*Dianthus barbatus*) was reported for the first time in India by Raj et al. (1993). The virus isolate was sap transmissible to *D. barbatus* and *Vaccaria pyramidata* which was further confirmed by Western immunoblotting assay. RNA extracted from purified CMV-CR isolate showed four distinct bands of RNA 1, 2, 3, and 4 during electrophoresis. cDNA synthesis of RNA4 of both the strains and their cloning into pBS-KS II (+) vector resulted in several clones carrying insert of about 1 kb when digested with *Pst* I/*BamH* I or *Pvu* II enzymes. Northern hybridization using the *Pst* I/*BamH* I (1 kb), *Sal* I (700 bp) and *BamH* I/*Sal* I (200 bp) fragments as probes confirmed the presence of cDNA of CMV RNA3 and 4 relating to coat protein gene in our clones. Northern hybridization using radiolabelled RNA transcripts produced *in-vitro* in each of the directions from T3 and T7 promoters located on opposite site of the inserts in recombinant clones, gave (+) ve signals with RNA3 and RNA4 by T3 transcripts and (−) ve signals by T7 transcripts. This confirmed that CP gene was located in sense orientation with respect to β-galactosidase gene in recombinant clones (Raj et al. 1993). Clones containing the inserts of CP gene of strains CMV-CR and CMVP were partially sequenced and compared with other CMV strains. Comparison of 249 nucleotide sequences of 5′ coding region of coat protein gene of both clones showed 88–90% similarity with C, O, Y and Fny strains of CMV subgroup I but only 60–64% homology with CMV-Q and CMV-WL strains of CMV subgroup II. Indian isolates CMV-CR and CMV-P also possess 97% similarity with each other in nucleotide sequences of above strains, they showed 91–95% similarity with CMV-C, CMV-O, Fny, CMV-Y and 61–67% homology with CMV-WL and CMV-Q strains respectively. Dendogram based on alignment of coat protein gene of eight CMV strains revealed that both the strains CMV-CR and CMV-P belong to subgroup I to which CMV Fny belong but not to CMV-Q of subgroup II (Raj et al. 1993).

9.2.5.7 Alstroemeria Hybrids

Verma et al. in 2005 reported the CMV strain of subgroup 1 on *Alstroemeria hybrids* for the first time in India based on host range, transmission by aphids, ELISA, electron microscopy, RT-PCR using CMV-CP gene specific primers and dot-blot hybridization. In this study, CMV was detected in nine hybrids and 61% of plants (Verma et al. 2005a, b).

9.2.5.8 Lily

The screening for presence of viruses in lilies (*Lilium longiflorum* and *L. tigrinum*) grown in Himachal Pradesh, India was carried out by Sharma et al. (2005). These viruses were detected by testing bulbs and leaves by ELISA and RT-PCR. During the study, CMV, Lily symptomless virus (LSV) and Lily mottle virus (LMoV) were detected in Asiatic and Oriental hybrids of lily. The viruses were identified by sequence analysis of cloned amplicons. Sequence analysis of CMV infecting lily revealed 96–98% nucleotide and 93–97% amino acid homology with CMV subgroup II sequences confirming presence of CMV of subgroup II in lilies (Sharma et al. 2005).

9.2.5.9 Salvia Splendens

A virus disease of garden sage (*Salvia splendens* Ker-Gawl.) was observed in gardens of Aligarh, UP, India by Ali et al. (2012). Sage plants showed symptoms of severe mosaic, mottling and distortion of leaves and retarded growth. By sap and aphid inoculations, the virus was transmitted to *Salvia* spp. and other hosts. Partially purified virus preparations revealed presence of ~28 nm particles. Crude sap also reacted positively with CMV specific polyclonal anti-serum (PVAS 242a, ATCC, USA) during DAC-ELISA. For molecular detection, RT-PCR was performed using total RNA and CMV-CP gene specific primers which resulted in 650 bp expected size fragments only in the infected samples. Further sequence analysis suggested that virus was a strain of CMV (Ali et al. 2012).

9.2.5.10 Calendula and Marigold

A disease on calendula plants was observed where plants exhibit mosaic disease. From leaf samples virus was purified using 0.05 M phosphate buffer (pH 7.5) and chloroform followed by differential centrifugation. Purified preparations were infectious and gave spectrum typical of nucleoprotein. Virus had a spherical particle about 35 nm diameters, infectious RNA isolated from purified virus preparations. It is concluded that calendula yellow net is a strain of CMV (Naqvi and Samad 1985).

A strain of CMV casing marigold mosaic was partially characterized by Singh et al. (1999). The virus was isolated from diseased marigold (*Tagetes erecta* L.) plant growing at Aligarh. Diseased plants showed mosaic, mottling and stunting Singh et al. (1999).

9.2.5.11 Ornithogalum

Ornithogalum thyrsoides is a cut flower ornamental crop and have long vase. Ornithogalum plants were found exhibiting severe mosaic on the leaves growing in a floriculture field at the Palampur, India. The virus was transmissible by sap and cause local lesions on *C. amaranticolor* and *C. album* whereas mosaic on *N. glutinosa*, *N. clevelandii*, *N. megalosiphon* and *N. tabacum* cv. White Burley. Virus reacted positively with CMV-specific antiserum in ELISA and has particle size of about 29 nm isometric particles (Verma et al. 2005). RT-PCR was performed using total RNA and CPTALL primer pair which resulted in expected size ~950 bp amplicons. Sequencing and analysis of the amplicon (accession no. AJ745092) revealed

highest 97% and 93–95% nucleotide and amino acid sequence identity, respectively with the available sequences of CMV subgroup II. These studies confirmed the association of CMV with severe mosaic disease of ornithogalum (Verma et al. 2005).

9.2.5.12 Gerbera

The occurrence of CMV in *Gerbera jamesonii* growing in floriculture fields at the IHBT, Palampur and nearby nurseries was reported by Verma et al. (2004). The infected gerbera plants showed color break symptoms on the petals, asymmetrical ray florets, and deformed flowers. The gerbera virus isolate induced chlorotic local lesions on *C. amaranticolor*, *Chenopodium album* and *C. quinoa* while mosaic on *C. sativus*, *N. clevelandii*, *N. benthamiana*, *N. glutinosa*, and *N. tabacum* cv. Samsun plants. As well the virus could be transmitted in non-persistent manner by aphids. Occurrence of CMV strains was expected as crude sap from infected plants reacted positively with CMV-specific antibodies and size was consistent with the reported CMV strains. Molecular detection by RT-PCR with CMV-specific primers resulted in amplification of predicted size of approximately 540 bp. Sequence alignment of 533 bp amplicon (accession no. AJ634532) revealed 91–99% sequence identity with partial intercistronic region and CP gene of CMV in subgroup I. This was the first report of CMV on gerbera (Verma et al. 2004).

Gautum et al. (2014) also detected the occurrence of CMV in *Gerbera jamesonii* where leaf samples exhibit severe chlorotic mosaic and flower deformation symptoms. The complete RNA 3 genome was amplified by RT-PCR from the three infected gerbera leaf samples, cloned sequenced, and deposited in GenBank under accessions JN692495, JX913531 (from cv. Zingaro) and JX888093 (from cv. Silvester). The RNA3 sequences shared 98–99 % identities to each other and with a strain of CMV-Banana reported from India. Phylogenetic analysis revealed their closest affinity with CMV-Banana strain, and close relationships with several other strains of CMV of subgroup IB (Gautum et al. 2014).

9.2.5.13 Zinnia

CMV isolates of *Zinnia elegans* (CMV-Z), Petunia (CMV-Pet) and Chrysanthemum (CMV-CI) were identified as CMV strains based on aphid transmission, electron microscopy and protein analysis and serological studies. They also showed some biological, serological and satellite RNA based differentiation with other CMV strains isolate earlier from chrysanthemum, petunia and tobacco (Raj et al. 1997). The virus isolates could be transmitted by mechanical inoculation to various plant species. The total viral RNA isolated from the partially purified virus preparations when electrophoressed in agarose gels (1.2%) revealed the four RNA species separated as four distinct bands which were identified as RNA 1, RNA 2, RNA 3 and RNA 4.

In an independent study, the virus isolated from *Z. elegans* and *O. sanctum* were identified as CMV strain based on aphid transmission, presence of isometric cored particle (28 nm), 26 kDa CP subunits and close serological relationships with CMV-C and CMV-D strains. RNA extracted from purified virus particles was ssRNA and infectious, and revealed presence of RNA 1, RNA 2, RNA 3 and RNA 4 but no evidence of Sat-RNA in its genome (Raj et al. 1997).

9.2.6 Characterization of CMV Infecting Medicinal and Aromatic Plants

9.2.6.1 Egyptian Henbane (*Hyoscyamus* sp.)

Zaim and Khan in 1988 reported the occurrence of CMV in *Hyoscyamus niger* and *Rauvolfia serpentine* plants exhibiting a severe green mosaic symptoms. The virus was isometric cored and sized ~28 nm in diameter and was transmitted mechanically by aphid. Based on the mode of transmission, host range, biophysical properties and serological relationship revealed that the causal entity in both the cases was an isolate of cucumber mosaic virus (Zaim and Khan 1988).

Later Samad et al. (2000) also observed the mottle crinkle and severe mosaic symptoms in Egyptian henbane (*H. muticus* L.) in Northern India. The virus associated with the disease was characterized as CMV based on aphid transmission, presence of ~28 nm isometric cored virus particles, 26 kDa CP and single-stranded tripartite RNA genome with a subgenomic RNA (RNA 4) lacking satellite RNA. The CMV henbane isolate showed strong serological relationship with CMV-S and CMV-A strains in ODDT test and therefore identified as an isolate CMV and designated as CMV-H strain (Samad et al. 2000).

A molecular relationship of CMV-A, CMV-D and CMV-H strains isolated from *A. tricolor*, *D. innoxia* and *H. muticus*, respectively was investigated by RT-PCR RFLP and sequence analysis of CP gene by Srivastava and Raj in 2004. The RFLP analysis of CP gene with *Hind*III, *Sal*I, *Alu*I and *Rsa*I restriction enzymes indicated their placement into CMV subgroup I. Sequence analysis and phylogenetic trees of nucleotide and amino acid sequences grouped them with Indian isolates and into subgroup IB. CMV strains revealed high molecular similarity among themselves and appeared as a distinct cluster within subgroup IB, indicating their common origin in relation to other members of the subgroup (Srivastava and Raj 2004).

9.2.6.2 Basil (Ocimum sanctum)

Raj et al. (1997) isolated two CMV isolates from *Ocimum sanctum* and *Zinnia elegans* and identified them on the basis of aphid transmission, 28 nm cored spherical virus particles, molecular weight of 26 kDa of CP subunits and serological relationships with CMV. Besides this, the virus strains showed a few biological, serological and satellite RNA based differentiation with other CMV strains isolated earlier from chrysanthemum, petunia and tobacco (Raj et al. 1997) and suggested that some Indian strains of CMV lacking satellite RNA.

After this, Khan et al. in 2011 also found the occurrence of mosaic disease on *O. sanctum* L.) in Aligarh, India. The associated disease was sap transmissible from infected *O. sanctum* to healthy *O. sanctum* and to some assay species. In RT-PCR using CP gene specific primers a fragment of 650 bp in infected samples was amplified, cloned and sequenced (Accession no. EU600216). CP gene sequence analysis revealed high 97–99% nucleotide and amino acid identities with CMV subgroup II strains and identification as new isolate of CMV subgroup II and designated as CMV-Basil (Khan et al. 2011).

9.2.6.3 Winged Bean (*Psophocarpus tetragonolobus*)

Singh in 1990 recorded a virus causing mosaic symptoms in winged bean (*Psophocarpus tetragonolobus*) for the first time in India. The virus was sap and aphid transmissible and had a TIP of 60–65 °C, DEP of 1:1,000–1:10,000, and LIV for 36 h at room temp. (25–30 °C). During EM studies of leaf-dip preparations of infected leaves showed the presence of ~26 nm isometric cored virus particles. The virus was serologically related to CMV and identified as a strain of CMV (Singh 1990).

9.2.6.4 Datura (*Datura innoxia*)

Severe mosaic, leaf deformation and shoestring symptoms in *D. innoxia* plants were observed in 1999 by Raj et al. (1999). The associated virus with the disease was identified as CMV based on RT-PCR using CMV-CP gene specific primers and authenticity of amplicons was evidenced by Southern hybridization tests with a probe derived from cloned CP cDNA of CMV. This was the first report of CMV in *D. innoxia*.

9.2.6.5 Geranium (*Pelargonium* spp.)

Verma et al. (2006) noticed a viral disease on geraniums plants grown in a greenhouse at IHBT, Palampur, where plants were exhibiting mild mottling and stunting symptoms. The associated virus was transmissible to indicator hosts of CMV by sap and aphid inoculations. The virus reacted positively with CMV-specific antiserum in ELISA and its genome hybridized positively with probe during DNA-RNA hybridization. A CP gene using degenerate primers was amplified by RT-PCR and sequenced which showed 97–98% and 96–99% sequence identity at nucleotide and amino acid residues, respectively and close phylogenetic relationships with the sequences of CMV subgroup II. Based on sequence analysis, the virus infecting geraniums was identified as CMV subgroup II strain (Verma et al. 2006).

9.2.6.6 Lemongrass (*Cymbopogon citratus*)

Raj et al. in 2007 reported the natural occurrence of CMV on lemongrass (*Cymbopogon citratus*). The occurrence of CMV was detected by ELISA using CMV-CP specific antiserum and RT-PCR using CMV-CP gene specific primers. The amplified CP gene sequence was cloned and analyzed. The sequence analysis revealed 93–97% nucleotide sequence identities and close phylogenetic relationship with CMV isolates of subgroup Ib (Raj et al. 2007). This was the first record of CMV on lemongrass.

9.2.6.7 Sarpagandha (*Rauvolfia serpentine*)

Sarpagandha (*Rauvolfia serpentine*), family *Apocynaceae* is cultivated for roots which are used in several herbal formulations. The severe mosaic and stunting symptoms were observed on *R. serpentina* plants growing in cultivated plots of CVSIR-NBRI, Lucknow. The causal agent was transmissible by sap inoculation on to a few tobacco species (*N. tabacum* cv. White Burley, *N. rustica*, and *N. glutinosa*) and indicator plant (*C. sativus*) for CMV which produced mosaic symptoms. The virus reacted positively with antiserum of CMV) (PVAS 242a, ATCC, USA) in gel ODDT tests, suggesting the presence of CMV. RT-PCR with CMV-CP specific

primers (AM180922/AM180923) resulted in amplification of ~650 bp band in infected samples which was cloned sequenced (Accession No. DQ914877). BLASTn analysis of the virus sequnece showed highest 99% nucleotide sequence identity with CMV isolates (DQ640743, AF350450, X89652, and AF281864). The virus isolate under study shared close phylogenetic relationships with CMV subgroup IB strains reported from India and identified as CMV-R strain. This was the first record of occurrence of CMV on *R. serpentine* (Raj et al. 2007).

9.2.6.8 Castor Bean (*Ricinus communis*)

A blister and leaf distortion disease of castor bean (*Ricinus communis* L.) was observed by Raj et al. (2010). The association of CMV with castor bean was detected by ODDT tests, WIBA tests using antiserum specific to CMV and confirmed by RT-PCR using CMV-CP gene primers. RT-PCR resulted in expected size band of 650 bp which was sequenced. Sequence analysis of amplicon revealed 96–98% nucleotide sequence identities and close relationships with CMV subgroup IB isolates. Based on these studies, the virus was identified as an isolate of CMV of subgroup IB. This was the first record of the natural occurrence of CMV on *R. communis* (Raj et al. 2010).

9.2.6.9 Periwinkle (*Catharanthus roseus*)

Samad and co-workers in 2008 observed the mosaic, leaf distortion and stunting disease in periwinkle (*Catharanthus roseus*). The virus isolate of periwinkle showed all the characters consistent with the CMV properties as similar host range, biophysical properties, isometric cored particles of ~28 nm, ~26 kDa CP subunit, affection to CMV-S during ODDT and transmission by aphids and sap to indicator assay hosts. The CP gene of ~657 bp was amplified from infected samples by RT-PCR using CMV-CP primers, cloned and sequenced. Sequence analysis of amplicon revealed high 98% nucleotide sequence identity and phylogenetic relationships with CMV-R strains of subgroup IB isolated from *R. serpentine* (Samad et al. 2008).

Afreen et al. in 2011 studied a virus causing mosaic and leaf deformation on *C. roseus* observed in the adjoining areas of Aligarh. The diseased plants showed mosaic, leaf deformation and reduced flower size. The virus was transmitted through sap inoculation and aphids, and showed symptoms on different plant species belonging to families: *Cucurbitaceae*, *Chenopodiaceae*, *Solanaceae* and *Leguminosae*. The size of virus ~28 nm diameter was in agreement with the size of CMV and also showed positive reaction with CMV specific antiserum in DAS-ELISA. Based on these analyses, the presence of CMV on *C. roseus* was confirmed (Afreen et al. 2011).

9.2.6.10 *Acorus calamus* and *Typhonium trilobatum*

Being CMV such a high economically importance virus, the screening for CMV in medicinal plants of *Acorus calamus, Typhonium trilobatum, Hippeastrum hybridum*, *Dahlia pinnata* and *Hemerocallis fulva*, plants exhibiting severe leaf mosaic symptoms was done by Kumar et al. (2015). The CP gene of ~650 bp was amplified from these samples using CMV-CP gene specific primers, cloned and sequenced and isolates were designated as HH (KP698590), AC (KP698588), DP (JF682239),

HF (KP698589), and TT (JX570732). The sequence data was analyzed for sequence and genetic diversity by BLASTn. The CMV-HH, −AC, −DP, −HF and –TT isolates revealed 82–99% nucleotide sequence identity among them and close relationships with CMV subgroup IB isolates. During alignment analysis of amino acid sequences HH and AC isolates showed fifteen and twelve unique substitutions, suggesting high genetic diversity as compared to HF, DP and TT isolates (Kumar et al. 2015).

9.2.7 Characterization of CMV Infecting Oil Crops

9.2.7.1 Safflower (*Carthamus tinctorius*)

Sangar and Rai (1988) identified a mosaic disease of safflower (*C. tinctorius* L.) from Satpura region of India in 1984. The virus isolate of safflower was identified as CMV based on biological, serological and physical properties similar to as CMV.

9.2.7.2 Jatropha (*Jatropha curcas*)

Raj et al. in 2008b observed the *Jatropha curcas* plants exhibiting severe mosaic disease accompanied by yellow spots in 15% plants in plots of CSIR-NBRI, India in 2006. Sap inoculations from infected to healthy Jatropha seedlings induced mosaic symptoms as were in infected plants. OODT tests suggested the presence of CMV as sap from infected plants reacted only with CMV (PVAS-242a, ATCC, USA). RT-PCR with CMV-CP gene primers resulted in expected size ~650-bp amplicon which was cloned and sequenced (accession no. EF153739). BLASTn analysis showed 98–99% nucleotide sequence identity with several CMV accessions DQ914877, DQ640743, AF350450, AF281864, X89652, AF198622, DQ152254, DQ141675, and DQ028777. Phylogeny revealed its close relationships with Indian isolates of CMV of subgroup Ib. This was the first report of CMV on *J. curcas* (Raj et al. 2008b) besides a report of Jatropha mosaic virus on *J. gossypiifolia* in Puerto Rico (Raj et al. 2008b).

9.2.8 Characterization of CMV Infecting Weed Plants

Trainthema monogyna and *Boerhavia diffusa* were reported as alternate hosts of CMV in 1970 by Khurana at Gorakhpur University and the virus was readily transmitted to eggplant from the weeds (Khurana 1970). In another study by Joshi and Dubey (1975), five weed plants *viz.*,: *A. viridis* L., *Nicotiana plumbginifolia* Vir., *Physalis minima* L., *Salvia plebeian* R. Br. and *Solanum nigrum* L. were found affected by CMV.

The incidence and progress of CMV were recorded by Kiranmai et al. (1998a, b, c) in commercial brinjal, chilli and tomato crops around Tirupati (A.P.) during 1992–1994 Kharif and Rabi seasons. The field collected crop plants that were positive for CMV in DAC-ELISA also produced chlorotic and/or necrotic local lesions an assay plants. The weed plants like *Acalypha indica*, *Amaranthus spinosis*, *A. viridis*,

Commelina bengalensis, *Digeria arvensis*, *Phyllanthus niruri* and *Physalis minima* collected in and around the three vegetable crops were found infected with CMV and they probably acted as alternative hosts. Based on the present studies, it is suggested that rouging of alternate virus source plants initially infected crop plants may help in decreasing the primary and secondary spread of Virus (Kiranmai et al. 1998a, b, c).

CMV was also detected from the naturally infected weed hosts around tarai region in Uttar Pradesh (Gupta et al. 2004). The weeds plants were collected and screened for CMV by using biological indicator host technique. Thirty one out of thirty five tested weeds species of twenty families were found CMV positive such as: *Achyranthes aspera*, *Amaranthus spinosus*, *Amaranthus viridis*, *Boerhavia diffusa*, *Croton bonplandianus*, *Euphorbia hirta* and *Solanum nigrum*. The only six weeds species remained free from CMV infection were: *Ageratum conyzoides*, *Ruellia prostrata*, *Rumex dentatus*, *Salvia officinalis*, *Sonchus oleraceus* and *Xanthium strumarium* (Gupta et al. 2004).

9.3 CMV Strains Reported from India

The reported CMV strains have been clustered into two major subgroups: I and II based on serological properties and nucleotide sequence homology (Palukaitis 1992). Furthermore, the subgroup I has been further been split into subgroup IA and IB by phylogenetic analysis of all reported CMV sequences (Roossinck et al. 1999; Palukaitis and Garcia-Arenal 2004). Most of the Indian strains of CMV have been included in 1B subgroup; however, some members of 1A and II subgroups of CMV have also been reported from India (Table 9.1).

Most of the Indian CMV strains detected from various plants: henbane (Samad et al. 2000), Amaranths (Srivastava et al. 2004), chrysanthemums (Kumar et al. 2005), Jatropha (Raj et al. 2008a, b), caster bean (Raj et al. 2010), tomato (Pratap et al. 2012a, b), banana (Vishnoi et al. 2013), eggplants (Kumar et al. 2014) have been identified as the members of subgroup IB. While other Indian CMV strains reported from *Ornithogalum* (Verma et al. 2005a, b); palargonium (Verma et al. 2006); gladiolus (Dubey et al. 2010); carrot (Afreen et al. 2009); basil (Khan et al. 2011a, b); cucumber (Kumari et al. 2013); and oxalis (Sheikh et al. 2013) were identified as members of subgroup 1A or II.

9.4 Genetic Diversity of CMV and TAV in India

To observe genetic diversity among strains of CMV in India, the phylogenetic analysis of CMV subgroup IA, IB and II strains was done by the aligning the nucleotide sequences of CP gene and phylogenetic tree was constructed by MEGA tool using neighbor joining method (Tamura et al. 2004). The CMV strains: Fny, Tfn and Trk7 strains were selected as reference sequences for subgroup IA, IB and II, respectively and a strain of *Peanut stunt virus* was taken as out-group for rooting the

Table 9.1 CMV strains of subgroup 1 (A and B) and II reported from last decade in India

Sub group	Natural host	Family	References
Subgroup-IB			
CMV-IB	*Hyoscyamus muticus*	*Solanaceae*	Samad et al. (2000)
CMV-IB	*Amaranthus tricolor*	*Amaranthaceae*	Srivastava et al. (2004)
CMV-IB	*Datura innoxia*	*Solanaceae*	Srivastava and Raj (2004)
CMV-IB	*Gerbera jamesonii*	*Asteraceae*	Verma et al. (2004)
CMV-IB	*Piper nigrum*	*Piperaceae*	Bhat et al. (2005)
CMV-IB	*Vanilla planifolia*	*Orchidaceae*	Madhubala et al. (2005)
CMV-IB	*Alstroemeria*	*Alstroemeriaceae*	Verma et al. (2005a, b)
CMV-IB	*Chrysanthemum morifrolium*	*Asteraceae*	Kumar et al. (2005)
CMV-IB	*Piper betle*	*Piperaceae*	Hareesh et al. (2006)
CMV-IB	*Cymbopogon citratus*	*Poaceae*	Raj et al. 2007a, b, c
CMV-IB	*Rauvolfia serpentina*	*Apocynaceae*	Raj et al. (2007a, b, c)
CMV-IB	*Solanum lycopersicon*	*Solanaceae*	Pratap et al. (2008)
CMV-IB	*Jatropha curcus*	*Euphorbiaceae*	Raj et al. (2008a, b)
CMV-IB	*Catharanthus roseus*	*Apocynaceae*	Samad et al. 2008
CMV-IB	*Ricinus communisis*	*Euphorbiaceae*	Raj et al. (2010)
CMV-IB	*Musa paradisiaca*	*Musaceae*	Khan et al. (2011a, b)
CMV-IB	*Petunia hybrida*	*Solanaceae*	Gautam et al. (2012)
CMV-IB	*Solanum lycopersicum*	*Solanacece*	Pratap et al. (2012a, b)
CMV-IB	*Musa paradisiaca*	*Musaceae*	Vishnoi et al. (2013)
CMV-IB	*Oxalis corymbosa*	*Oxalidaceae*	Sheikh et al. (2013)
CMV-IB	*Solanum melongena*	*Solanaceae*	Kumar et al. (2014)
CMV-IB	*Gerbera jamesonii*	*Asteraceae*	Gautam et al. (2014)
CMV-IB	*Musa paradisiaca*	*Musaceae*	Khan (2015)
CMV-IB	*Acorus calamus*	*Acoraceae*	Kumar et al. (2015)
Subgroup-IA			
CMV-IA	*Ornithogalum umbellatum*	*Asparagaceae*	Verma et al. (2005a, b)
CMV-IA	*Pelargonium graveolens*	*Geraniaceae*	Verma et al. (2006)
CMV-IA	*Gladiolus* sp	*Iridaceae*	Dubey et al. (2010)
Subgroup-II			
CMV-II	*Lilium longiflorum*	*Liliaceae*	Sharma et al. (2005)
CMV-II	*Pelargonium* sp	*Geraniaceae*	Verma et al. (2006)
CMV-II	*Daucus carota*	*Apiaceae*	Afreen et al. (2009)
CMV-II	*Ocimum sanctum*	*Lamiaceae*	Khan et al. (2011a, b)
CMV-II	*Solanum lycopersicum*	*Solanacece*	
CMV-II	*Cucumis sativus*	*Cucurbitaceae*	Kumari et al. (2013)
CMV-II	*Capsicum annum*	*Solanaceae*	Biswas et al. (2013)

phylogenetic tree. The generated phylogenetic tree reflected that the CMV strains clustered in subgroups: IA, IB and II (Fig. 9.2). Majority of CMV strains (15 out of 33 taken for the study) from India (DQ141675, AF198622, AF350450, AF281864, EF593026, JQ779842, AM158321, JN642676, HM484375, GU253913, AY545924, AY754359, DQ285569, JF279609 and AY125575) were found to lie in subgroup IB

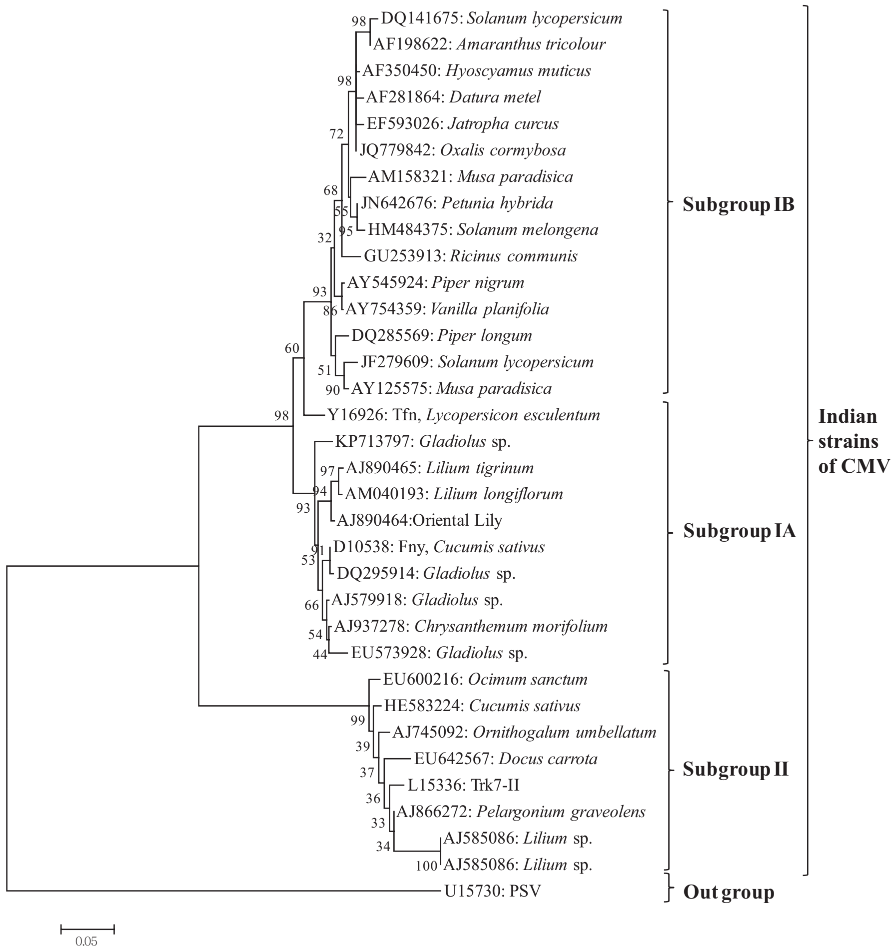

Fig. 9.2 Phylogenetic analysis of different CMV strains of subgroups: *IA*, *IB* and *II*, reported from various parts of India. Fny, Tfn and Trk7 were taken as reference sequences for subgroup *IA*, *IB* and *II*, respectively; whereas, PSV was taken as out-group for rooting

along with Tfn (acc. Y16926) taken as reference. Eight strains of CMV reported from India were clustered in subgroup IA (KP713797, AJ890465, AM040193, AJ890464, DQ295914, AJ937278 and EU573928) along with the reference strain: Fny (acc. D10538). While the six strains of CMV reported from India (acc. EU600216, HE583224, AJ745092, EU642567, AJ866272 and AJ585086) (out of total 33 strains retrieved from NCBI database for study) clustered with the strain of CMV subgroup-II (Trk7, acc. L15336) taken as reference.

These findings suggested that genetic diversity exists among Indian strains of CMV and all CMV strains reported from India belong to subgroups of CMV (IB, IA and II) and the majority of Indian strains belong to subgroup IB. For finding the possibility of recombination, identification of likely parents and localization of

possible breakpoints in CMV strains, recombination analysis was performed by a RDP v4 (Martin et al. 2010) using seven methods (RDP, GENECONV, BOOTSCAN, Maximum Chi Square, CHIMEARA, SISCAN and 3Seq). The results eliminated the possibility of recombination in 29 Indian strains of CMV among the 33 sequences retrieved from NCBI database and suggest that the Indian CMV strains may have evolved independently as the subgroups IA, IB and II members.

The genetic diversity of TAV causing severe mosaic, chlorotic ringspots and flower deformation in chrysanthemums (*Chrysanthemum morifolium*) in Lucknow (UP), Dhanbad (MP) and Kolkata (WB), India has also been studied for the first time in India by Raj et al. (2009). TAV was detected by ELISA and by RT-PCR using TAV specific primers. The complete RNA 3 of each TAV isolate was cloned and sequenced and determined to be 2,386 nucleotides (nt) long, and to encode two open reading frames (ORFs): the movement protein (MP) of 741 nt and the coat protein (CP) of 657 nt translating in to 246 and 218 amino acid (aa), respectively. Indian isolates shared 98–99% identities among themselves and with the KC, V, P, B, I and C strains of TAV when aligned with seven other strains of TAV occurring worldwide. During phylogenetic analysis, Lucknow and Kolkata isolates of TAV showed a close relationship with KC-TAV strain of South Korea, while Dhanbad isolate showed closeness with V-TAV strains of Spain and Australia. Recombination events were also observed in the CP region of the Dhanbad isolate, supporting its diverse behavior (Raj et al. 2009). The nucleotide and amino acid sequences of the ORFs of Indian TAV isolates revealed high conservation despite their different geographical origin, and indicated that there are lesser radical radiations of the TAV populations in the natural environment. The nt and aa sequences of the ORFs of Indian TAV isolates revealed high conservation despite their different geographical origin, and indicated that there are lesser radical radiations of the TAV populations in the natural environment. These observations has been strengthened by the finding of Choi et al. (2002) and Moriones et al. (1991) emphasizing lesser or no quasi species of TAV were generated through fitness and recombination mechanisms in host plants, contrary to the other members of Cucumoviruses, as CMV which had a part of an evolutionary lines.

9.5 Concluding Remarks

In view of the literature available on CMV and TAV from India until now, it seems that an ample of research work has been done on biological, biochemical, serological, and molecular characterization of CMV and TAV for its proper identification. For management of the disease caused by CMV and TAV, the significant progress has also been made by control of the vector by conventional method as well as by non-conventional methods. The elimination of virus by culturing of shoot meristem tip, chemotherapy, thermotherapy and electrotherapy of the virus infected explants by many researchers in chrysanthemum (Singh and Gupta 1978; Kumar et al. 2009), gladiolus, carnation, Asiatic lily and gerbera (Gautam 2014). The mixed infection of CMV and TAV was eliminated in *Chrysanthemum morifolium* Ramat. cv. Pooja

(Kumar et al. 2009) by in vitro culturing 0.3 mm long shoot meristem of infected plants on MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA. The regenerated plants were indexed by DAC-ELISA and confirmed by RT-PCR. A total of 78.1% CMV and TAV-free shootlets were obtained from the regenerated shoot meristem as indexed by DAC-ELISA, of which only 65.6% were found truly virus-free when confirmed by RT-PCR. Virus-free shootlets were rooted on half MS medium and acclamatized under glasshouse. These plants showed better growth and quality of blooms as compared to diseased ones (Kumar et al. 2009).

The significant progress has been achieved in the country for developing virus resistant transgenic plants by expression of antisense RNA, satellite RNA and coat protein that resulted in considerable degree of resistance/tolerance against CMV infection in tobacco (Srivastava and Raj 2008), tomato (Pratap et al. 2012a, b), brinjal (Pratap et al. 2011), chrysanthemum (Kumar et al. 2012) crops.

It is also suggested by many workers that a holistic approach needs to be taken up for designing the disease control/management practices. These include judicious use of integrated management practices such as use of healthy and certified propagating materials, control of vector by use of insecticides, regular surveillance and use of sensitive diagnostic procedures, avoiding alternate hosts and eradication of weeds, inter-cropping with barrier crops, breeding resistant cultivars, cross-protection using mild strains and use of virus resistant transgenic plants using CP mediated resistance.

Acknowledgements Authors are thankful to the Director, CSIR-National Botanical research institute (CSIR-NBRI), Lucknow, India for permission for summarizing the data generated by the researchers of Plant Virus Laboratory and its inclusion as a chapter in this book.

References

Afreen B, Khan AA, Naqvi QA, Kumar S, Pratap D, Snehi SK, Raj SK (2009) Molecular identification of a Cucumber mosaic virus subgroup II isolate from carrot (*Daucus carota*) based on RNA3 genome sequence analyses. J Plant Dis Prot 123:89–94

Afreen B, Baghel G, Khan AA, Naqvi QA (2011) Studies of a virus causing mosaic and leaf deformation on *Catharanthus roseus*. Nat Acad Sci Lett India 80:514–518

Ali S, Akhtar M, Singh KS, Naqvi QA (2012) RT-PCR and CP gene based molecular characterization of a cucumber mosaic cucumovirus from Aligarh, U.P., India. Agric Sci 3:971–978

Anjaneyulu A, Apparao A (1967) Natural occurrence of cucumber mosaic virus on chilli in India. Indian Phytopathol 20:380–381

Bhargava KS (1951a) Some properties of four strains of cucumber mosaic virus. Ann Appl Biol 38:377–388

Bhargava KS (1951b) Natural occurrence of cucumber mosaic virus on chilli in India. Indian Phytopathol 20:380–381

Bharti M, Anandam RJ, Roa AS (1997) Effect of *Cucumber mosaic virus* on indicator plants, non-host crops and brinjal genotypes. Indian J Virol 13:147–151

Bhat AI, Devasahayam S, Anandaraj M (2003) Viral disease – a new threat to black pepper cultivation in india. Indian J Areca Spices Med Plants 5:46–48

Bhat AI, Venugopal MN, Pant RP, Bhai RS (2004a) Occurrence and distribution of viral diseases on vanilla in India. J Spices Aromat Crop 13:143–148

Bhat AI, Faisal TH, Madhubala R, Hareesh PS, Pant RP (2004b) Purification, production of antiserum and development of enzyme linked immunosorbent assay-based diagnosis for Cucumber mosaic virus infecting black pepper (*Piper nigrum* L.) J Spices Aromat Crop 13:16–21

Bhat AI, Hareesh PS, Madhubala R (2005) Sequencing of coat protein gene of an isolate of cucumber mosaic virus infecting black pepper (*Piper nigrum* L) in India. J Plant Biochem Biotechnol 14:37–40

Bhat AI, Siljo A, Deeshma KP (2013) Rapid detection of Piper yellow mottle virus and cucumber mosaic virus infecting black pepper (*Piper nigrum*) by loop-mediated isothermal amplification (LAMP). J Virol Methods 44:901

Bidari VB, Reddy HR (1983) Prevalence of chilli viruses in Dharwad district. Plant Pathol Newslett 1:11–12

Biswas K, Hallan V, Zaidi AA, Pandey PK (2013) Molecular evidence of cucumber mosaic virus subgroup II infecting *Capsicum annuum* L. in the western region of India. Curr Discov 2:97–105

Brierley P (1955) Symptoms induced in chrysanthemums on inoculation with virus of mosaic aspermy and flower distortion. Phytopathology 54:2–7

Brierley P (1958) Tomato aspermy in chrysanthemum from Asia and Europe. Plant Dis Rep 42:61–62

Chalam TV (1982). Identification and characterization of cucumber mosaic and bean yellow mosaic viruses affecting chickpea (*Cicer arietinum* L.) in India. Thesis submitted to the Andhra Pradesh Agricultural University

Chalam TV, Reddy MV, Nene YL, Beniwal SPS, Subbayya J (1986) Some properties of a strain of cucumber mosaic virus isolated from chickpea in India. Plant Dis 70:128–130

Choi SK, Choi JK, Ryn KH, Park WM (2002) Generation of infectious clones of a Korean strain of tomato aspermy virus. Mol Cell 13:52–60

Chuyan AKH, Krylov AV (1979) Properties of tomato aspermy virus from chrysanthemum and its host range in the Priomore region. Byull Glam Bot Kogo Soda 114:84–92

Das CR, Raychaudhary SP (1953) A probable strain of tomato aucuba mosaic virus. Indian Phytopathol 6:116–122

Dhingra KL, Chenulu VV, Verma MDA (1979) A leaf reduction disease of *Cicer arientinum* in India caused by cucumovirus. Curr Sci 48:486–488

Ding SW, Anderson BJ, Haase HR, Symons RH (1994) New overlapping gene encoded by the cucumber mosaic virus genome. Virology 198:593–601

Ding S, Li W, Symons RH (1995) A novel naturally occurring hybrid gene encoded by a plant RNA virus facilitates long distance virus movement. EMBO J 14:5762–5772

Ding S, Shi B, Li W, Symons R (1996) An interspecies hybrid RNA virus is significantly more virulent than either parental virus. Proc Natl Acad Sci U S A 93:7470–7474

Doolittle SP (1916) A new infectious mosaic disease of cucumber. Phytopathology 6:145–147

Dubey VK, Aminuddin, Singh VP (2010) Molecular characterization of cucumber mosaic virus infecting gladiolus, revealing its phylogeny distinct from the Indian isolate and alike the Fny strain of CMV. Virus Genes 41:126–134

Gautam KK (2014). Molecular detection and characterization of strains of cucumber mosaic virus infecting petunia and gerbera and their disease management. PhD thesis, Lucknow University, Lucknow

Gautam KK, Kumar S, Raj SK (2012) Molecular characterization of Cucumber mosaic virus strain causing severe mosaic disease on petunia in India. Phytoparasitica 138:597–640

Gautum KK, Raj R, Kumar S, Raj SK, Roy RK, Katiyar R (2014) Complete sequence of RNA3 of Cucumber mosaic virus isolates infecting *Gerbera jamesonii* suggests its grouping under IB subgroup. Virus Dis 25:398–401

George TE, Anand N, Singh SJ (1993) Isolation and identification of two major viruses infecting bell pepper in Karnataka. Ind Phytopathol 46:24–28

Gowda KTP, Reddy HR (1985) Distribution of chilli mosaic virus in some parts of Kolar district. Curr Res 14:16–17

Gupta RP, Singh BP (1981) Studies on chrysanthemum aspermy virus on chrysanthemum in India. J Mycol Plant Pathol 11:158–160

Gupta SP, Singh BR (1997) Severe mosaic of cape gooseberry due to cucumber mosaic virus. Indian J Virol 12:155–156

Gupta PK, Ansari NA, Gautam A, Pandey A, Tewari JP (2004) Incidence of some cucurbit virus isolates naturally infected weed host around devipatan mandal in tarai region. J Liv World 11:1–6

Haq QMR, Singh BP, Srivastava KM (1996) Biological, serological and molecular characterization of a cucumber mosaic virus isolate from India. Plant Pathol 45:823–828

Hollings M (1955) Investigation of chrysanthemum viruses I. Aspermy flower deformation. Ann Appl Biol 43:83–102

Joshi RD, Bhargava KS (1965) A mosaic disease of tomato (*Cyphomandra betacea* Sendt.) Indian Phytopathol 18:313

Joshi RD, Dubey LN (1975) Some weed reservoirs of cucumber mosaic virus in Gorakhpur and distant area. Indian Phytopathol 28:568

Kaplan IB, Zhang L, Palukaitis P (1998) Characterization of cucumber mosaic virus. V. Cell-to-cell movement requires capsid protein but not virions. Virology 246:221–231

Kapoor P (2012) Detection of cucumber mosaic virus in bell pepper crop from himachal Pradesh. Plant Dis Res 27:71–80

Khan S (2015) Partial characterization and development of sensitive and reliable diagnostic for the detection of cucumber mosaic virus. Turk J Agric For 8:4539–4541

Khan S, Jan AT, Aquil B, Haq QMR (2011a) Coat protein gene based characterization of cucumber mosaic virus isolates infecting banana in India. J Phytol 32:94–101

Khan AA, Sharma R, Afreen B, Naqvi QA, Kumar S, Snehi SK, Raj SK (2011b) Molecular identification of a new isolate of cucumber mosaic virus subgroup II from basil (*Ocimum sanctum*) in India. Phytoparasitica 82:941–945

Khatri HL, Chenulu VV (1967) Studies on mosaic disease of phlox. Plant Dis Rep 51:768–771

Khurana SMP (1970) *Trainthema monogyna* and *Boerhavia diffusa*: unrecorded hosts of brinjal mosaic disease. Plant Dis 54:437–438

Kiranmai GM (1989) M Sc dissertation, S. V. University, Tirupati

Kiranmai G, Sreenivasulu P, Nayudu MV (1996) Comparison of here different tests for detection of *Cucumber mosaic virus* in banana (*Musa paradsiiaca*). Curr Sci 71:764–767

Kiranmai G, Sreenivasulu P, Nayadu MV (1997) Characterization of cucumber mosaic cucumovirus isolates naturally infecting three solanaceous vegetable crops in Andhra Pradesh. Indian Phytopathol 50:416–425

Kiranmai G, Hema M, Sreenivasulu P (1998a) Identification of a virus causing leaf distortion disease of papaya in Andhra Pradesh as cucumber mosaic cucumovirus. Indian Phytopathol 51:130–135

Kiranmai G, Sreenivasulu P, Nayadu MV (1998b) Epidemiology of cucumber mosaic cucumovirus isolates naturally infecting three solanaceous vegetables crops around Tirupati. Indian Phytopathol 51:315–318

Kiranmai G, Sreenivasulu V, Nayudu MV (1998c) Characterisation of cucumber mosaic cucumovirus isolation naturally infecting three solanaceous vegetable crops in Andhra Pradesh. Indian Phytopathol 50:416–425

Kumar S, Srivastava A, Raj SK (2005) Molecular detection of cucumber mosaic virus in chrysanthemum cultivars. Acta Phytopathol Entomol Hung 40:205–211

Kumar S, Khan MS, Raj SK, Sharma AK (2009) Elimination of mixed infection of *Cucumber mosaic* and *Tomato aspermy virus* from *Chrysanthemum morifolium* Ramat. cv. Pooja by shoot meristem culture. Sci Hortic 119:108–112

Kumar S, Raj SK, Sharma AK, Varma HN (2012) Genetic transformation and development of *Cucumber mosaic virus* resistant transgenic plants of *Chrysanthemum morifolium* cv. Kundan. Sci Hortic 134:40–45

Kumar S, Gautam KK, Raj SK (2014) Molecular identification of cucumber mosaic virus isolates of subgroup IB associated with mosaic disease of eggplant in India. Virus Dis 25:129–131

Kumar S, Gautam KK, Raj SK (2015) Sequence analysis and genetic diversity of five new Indian isolates of cucumber mosaic virus. Acta Virol 59:398. (In press)

Kumari R, Bhardwaj P, Singh L, Zaidi AA, Hallan V (2013) Biological and molecular characterization of cucumber mosaic virus subgroup II isolate causing severe mosaic in cucumber. Indian J Virol 60:135–147

Madhubala R, Bhadramurthy V, Bhat AI, Hareesh PS, Retheesh ST, Bhai RS (2005) Occurrence of cucumber mosaic virus on vanilla (*Vanilla planifolia* Andrews) in India. J Biosci 30:339–350

Mali VR, Rajegore SB (1980) A cucumber mosaic virus disease of banana in India. Phytopathol Z 98:127–136

Marani F (1969) Chrysanthemum aspermy virus. Phytopathol Mediterr 8:142–149

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P (2010) RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26:2462–2463

Moriones E, Roossinck MJ, Gracia-Arenal F (1991) Nucleotide sequence of tomato aspermy virus RNA 2. J Gen Virol 72:779–783

Murthy MRN (1983) Comparison of the nucleotide sequences of cucumber mosaic virus and brome mosaic virus. J Mol Biol 168:469–475

Nagaraju N, Murthy KVK (1994) Studies on a sap transmissible virus associated with mosaic disease of cowpea. Ind Phytopathol 47:23–26

Nagaraju, Reddy HR (1982) Occurrence and distribution of bell pepper viruses around Bangalore. Curr Res 10:155

Naqvi QA, Samad A (1985) Purification and properties of calendula yellow net virus. Indian J Virol 1:143–146

Narayan R, Dhawan P (1989) Characterization and identification of viruses causing mosaic disease of *Capsicum* in nature. Indian Phytopathol 42:221–226

Palukaitis P (1992) *Cucumber mosaic virus*. Adv Virus Res 41:281–348

Palukaitis P, Garicia-Arenal F (2004) Cucumoviruses. Adv Virus Res 62:241–323

Patel KV, Mali VR (1986) Comparative studies of three isolates of cucumber mosaic virus from banana. Indian Phytopathol 36:443–447

Prasada Rao RDV (1976) Characterization and identification of some chilli mosaic viruses. PhD thesis University of Agricultural Sciences, Hebbal, Bangalore, Karnataka, India

Pratap D, Kumar S, Raj SK (2008) First molecular identification of a cucumber mosaic virus isolate associated with shoestring symptom on tomato in India. Aust Plant Dis Notes 3:57–58

Pratap D, Kumar S, Raj SK, Sharma AK (2011) *Agrobacterium*-mediated transformation of eggplant (*Solanum melongena* L.) using cotyledon explants and coat protein gene of *cucumber mosaic virus*. Indian J Biotechnol 10:19–24

Pratap D, Kumar S, Snehi SK, Raj SK (2012a) Biological and molecular characterization of cucumber mosaic virus isolate causing shoestring disease of tomato in India which has closer affinity to European or East Asian isolates of CMV. Indian J Virol 135:193–207

Pratap D, Raj SK, Kumar S, Snehi SK, Gautam KK, Sharma AK (2012b) Coat protein-mediated transgenic resistance in tomato against a IB subgroup *Cucumber mosaic virus* strain. Phytoparasitica 40:375–382

Praveen S, Tripathi S, Giri BK (2001) Differentiation of biologically distinct cucumber mosaic virus isolates by double stranded RNA profile. Indian Phytopathol 54:362–365

Prentice IW (1952) Chrysanthemum flower distortion survey. Plant Pathol 1:77–80

Raj SK, Srivastava KM, Raizada RK, Singh BP (1991) Chlorotic ring mosaic of chrysanthemum caused by a strain of chrysanthemum aspermy virus in India. J Plant Dis Prot 98:640–646

Raj SK, Aminuddin, Srivastava KM, Singh BP (1993) Natural infection of cucumber mosaic virus on *Dianthus barbatus* in India. Plant Pathol 42:811–813

Raj SK, Aminuddin, Singh BP, Pal M (1997a) Characterisation of a cucumber mosaic virus causing leaf crinkle and severe mosaic disease in Amaranthus in India. Can J Plant Pathol 19:97–100

Raj SK, Chandra G, Singh BP (1997b) Some Indian strains of cucumber mosaic virus (CMV) lacking satellite RNA. Indian J Exp Biol 35:1128–1131

Raj SK, Srivastava A, Chandra G, Singh BP (1999) Natural occurrence of cucumber mosaic cucumovirus in *Datura innoxia* in India. OEPP/EPPO Bull 29:455–457

Raj SK, Srivastava A, Chandra G, Singh BP (2000) Role of satellite RNA of and Indian isolate of cucumber mosaic virus in inducing lethal necrosis of tobacco plants. Indian J Exp Biol 38:613–616

Raj SK, Srivastava A, Chandra G, Singh BP (2002) Characterization of cucumber mosaic virus isolate infecting gladiolus cultivars and comparative evaluation of serological and molecular methods for sensitive diagnosis. Curr Sci 83:1132–1137

Raj SK, Srivastava A, Chowdhury MR, Johri JK (2003) Association of cucumber mosaic virus and a potyvirus with betelvine (*Piper betle* L.) as evidenced by ELISA and RT-PCR. Indian J Exp Biol 41:273–276

Raj SK, Kumar S, Choudhari S (2007a) Identification of tomato aspermy virus as the cause of yellow mosaic and flower deformation on chrysanthemums in India. Australas Plant Dis Notes 2:1–2

Raj SK, Kumar S, Pratap D, Vishnoi R, Choudhari S, Chandra S (2007c) Natural occurrence of cucumber mosaic virus on lemongrass (*Cymbopogon citratus*), a new record. Australas Plant Dis Notes 2:95–96

Raj SK, Kumar S, Pratap D, Vishnoi R, Snehi SK (2007b) Natural occurrence of cucumber mosaic virus on *Rauvolfia serpentina*, a new record. Plant Dis 91:322

Raj SK, Khan MS, Kumar S, Pratap D, Vishnoi R (2008a) Cucumber mosaic virus infecting vegetable and pulse crops in India. Characterization, diagnosis & management of plant viruses. In: Rao GP, Lava Kumar P, Holguin RJ, Pena (eds) Vegetable and pulse crops, vol 3. Studium Press LLC, Texas, pp 39–62

Raj SK, Kumar S, Snehi SK (2008b) First report of cucumber mosaic virus on *Jatropha curcas* in India. Plant Dis 92:171

Raj SK, Kumar S, Choudhari S, Verma DK (2009) Biological and molecular characterization of three isolates of tomato aspermy virus infecting chrysanthemums in India. J Phytopathol 157:117–125

Raj SK, Snehi SK, Gautam KK, Khan MS (2010) First report of association of cucumber mosaic virus with blister and leaf distortion of castor bean (*Ricinus communis*) in India. Phytoparasitica 160:106–111

Raj SK, Kumar S, Verma DK, Snehi SK (2011) First report on molecular detection and identification of tomato aspermy virus naturally occurring on gladiolus in India. Phytoparasitica 39:303–307

Rao GP, Singh M, Shukla K, Gupta SN (1995) A new strain of cucumber mosaic virus causing mosaic disease of pea (Pisum sativum L.) in India. Indian J Virol 11:43–48

Roossinck MJ (2002) Evolutionary history of *cucumber mosaic virus* deduced by phylogenetic analyses. J Virol 76:3382–3387

Roossinck MJ, Zhang L, Hellward K (1999) Rearrangements in the 5′ nontranslated region and phylogenetic analyses of *Cucumber mosaic virus* RNA3 indicate radial evolution of three subgroups. J Virol 73:6752–6758

Samad A, Raj SK, Srivastava A, Chandra G, Ajayakumar PV, Zaim M, Singh BP (2000) Characterization of an Indian isolate of cucumber mosaic virus infecting Egyptian henbane (*Hyoscyamus muticus* L.) Acta Virol 476:264–270

Samad A, Ajayakumar PV, Gupta MK, Shukla AK, Darokar MP, Somkuwar B, Alam M (2008) Natural infection of periwinkle (Catharanthus roseus) with cucumber mosaic virus, subgroup IB. Australas Plant Dis Notes 3:30–34

Sangar RBS, Rai MK (1988) A mosaic disease of safflower from Satpura region of India. Farm Sci J 3:185–187

Sarma YR, Kiranmai G, Sreenivasulu P, Anandaraj M, Hema M, Venkatramana M, Murthy AK, Reddy DVR (2001) Partial characterization and identification of a virus associated with stunt disease of black pepper (*Piper nigrum*) in South India. Curr Sci 22:595–605

Sastry KSM (1964) A new strain of CAV from India. Indian J Microbiol 4:37–40

Savithri HS, Devarajan S, Murthy MRN (1983) Stability and structural transition of tomato aspermy virus and cucumber mosaic virus. Virology 134:398–405

Seth ML, Raychaudhuri SP (1973) Further studies on a new mosaic disease of Brinjal. Proc Indian Natl Sci Acad 39:122–128

Sharma PN, Chowfla SC (1986) Studies on a mosaic disease of amaranthus (*Amaranthus caudatus* L.) in Himachal Pradesh. Indian J Mycol Plant Pathol 16:349–350

Sharma PN, Chowfla SC, Garg ID, Paul Khurana SM (1993) Properties of the viruses associated with disease complex of bell pepper. Ind Phytopathol 46:347–353

Sharma A, Mahinghara BK, Singh AK, Kulshrestha S, Raikhy G, Singh L, Verma N, Hallan V, Ram R, Zaidi AA (2005) Identification, detection and frequency of lily viruses in Northern India. Sci Hortic 106:213–227

Sheikh M, Naqvi QA, Mahmood I (2013) Molecular detection of an isolate of cucumber mosaic virus infecting *Oxalis corymbosa* in Aligarh, India. Arch Phytopathol Plant Protect 46:1816–1820

Shi BJ, Ding SW, Symons RH (1997) In vivo expression of an overlapping gene encoded by the cucumoviruses. J Gen Virol 78:237–241

Singh SJ (1990) Studies on virus causing mosaic disease in winged bean. Plant Dis Res 5:1–6

Singh BP, Gupta RP (1978) Control of chrysanthemum aspermy virus by heat therapy. Phytopathology 47:909–910

Singh BR, Shukla P (1990) Properties of a new strain of cucumber mosaic virus from chilli. Ind J Virol 6:58–63

Singh A, Cheema SS, Hundal JS (1990) Identification of virus(es) associated with chilli mosaic syndrome. J Res Punjab Agric Univ 27:75–83

Singh A, Bishnoi SS, Rishi N, Krishi HP (1996) A disease of chickpea caused by a cucumo virus in Himachal Pradesh. Ind J Virol 12:59–61

Singh D, Naqvi QA, Garg ID (1999) A strain of cucumber mosaic cucumovirus causing mosaic in marigold in India. Ind Phytopathol 52:114–117

Singh A, Bishnoi SS, Sirohi A, Rishi N (2002) Viral diseases of chickpea in Himachal Pradesh. Plant Dis Res 17:61–64

Srivastava A (2003) Molecular characterization of cucumber mosaic virus isolate infecting *Amaranthus* spp. and development of transgenic plants utilizing its coat protein gene for resistance against CMV. PhD thesis submitted to University of Lucknow, Lucknow, India

Srivastava A, Raj SK (2004) High molecular similarity among Indian isolates of cucumber mosaic virus suggests a common origin. Curr Sci 87:1126–1131

Srivastava A, Raj SK (2008) Coat protein mediated resistance against an Indian isolate of *Cucumber mosaic virus* of subgroup IB member in *Nicotiana benthamiana*. J Biosci 33:249–257

Srivastava A, Chandra G, Raizada RK, Srivastava KM, Singh BP (1991) Characterization of a virus causing necrosis in *Petuni*a *hybrida* and establishment of its relationship using ELISA. Indian J Exp Biol 29:591–593

Srivastava KM, Raj SK, Singh BP (1992) Properties of a cucumber mosaic virus strain naturally infecting *Chrysanthemum* in India. Plant Dis 76:474–477

Srivastava A, Raj SK, Haq QMR, Srivastava KM, Singh BP, Sane PV (1995) Association of a cucumber mosaic virus strain with mosaic disease of banana, (*Musa paradisiaca*) – an evidence using immuno/nucleic acid probes. Indian J Exp Biol 33:986–988

Srivastava A, Chandra G, Raj SK (2004) Molecular characterization of a strain of cucumber mosaic virus based on coat protein and movement protein genes. Acta Virol 48:229–239

Suzuki M, Kuwata S, Kataoka J, Masuta C, Nitta N, Takanami Y (1991) Functional analysis of deletion mutants of cucumber mosaic virus RNA 3 using an in vitro transcription system. Virology 183:106–113

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. PNAS (USA) 101:11030–11035

Verma N, Singh AK, Singh L, Kulshreshtha S (2004) Occurrence of cucumber mosaic virus in *Gerbera jamesonii* in India. Plant Dis 88:1161

Verma N, Singh L, Singh AK, Kulshrestha S, Raikhy G, Hallan V, Ram R, Zaidi AA (2005a) Ornithogalum: a new host of cucumber mosaic virus (CMV) from India. Plant Pathol 54:256

Verma N, Singh AK, Singh L, Raikhy G, Kulshrestha S, Singh MK, Hallan V, Ram R, Zaidi AA (2005b) Cucumber mosaic virus (CMV) infecting *Alstroemeria* hybrids in India. Austral Plant Pathol 34:119–120

Verma N, Mahinghara BK, Ram R, Zaidi AA (2006) Coat protein sequence shows that cucumber mosaic virus isolate from geraniums (*Pelargonium* spp.) belongs to subgroup II. J Biosci 51:3–10

Verma N, Kumar K, Kulshrestha S, Raikhy G, Hallan V, Ram R, Zaidi AA, Garg ID (2007a) Molecular studies on tomato aspermy isolates infecting chrysanthemum. Arch Phytopathol Plant Protect 40:1–13

Verma N, Mehra A, Singh L, Hallan V, Singh AK, Jabeen N, Singh MK, Ram R, Zaidi AA (2007b) Screening for viruses infecting chrysanthemum cultivars in India. Sci Hortic 86:89–97

Vishnoi R, Kumar S, Khan MS, Raj SK (2006) Molecular diagnosis of banana mosaic virus and banana bunchy top virus affecting banana cultivation India. XVI Annual convention and International symposium on management of vector borne viruses. 7–10 February, ICRISAT, Hyderabad; PP-8/05

Vishnoi R, Kumar S, Raj SK (2013) Molecular characterization of a cucumber mosaic virus isolate associated with mosaic disease of banana in India. Phytoparasitica 71:59–66

Zaim M, Khan MMAA (1988) Green mosaic of Hyoscyamus niger L. and Rauvolfia serpentine Benth. Ex Kurz. Indian J Plant Apathol 6:152–157