Characterisation of Carlaviruses Occurring in India

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Abstract

Carlaviruses infects field, vegetable and ornamental crops in India. The genus *Carlavirus* (family *Betaflexiviridae*) has as many as 43 recognised virus species and 13 tentative members. Only five carlavirus species, *Cowpea mild mottle virus, Chrysanthemum virus B, Lily symptomless virus, Potato virus S* and *Garlic common latent virus* and one tentative member, football lily mosaic virus are known in India. In this chapter, characterisation of carlavirus occurring in India is presented.

Keywords

Carlaviruses in India • Carnation latent virus • Cowpea mild mottle virus • Chrysanthemum virus B • Lily symptomless virus • Potato virus S • Garlic common latent virus • Football lily mosaic virus

6.1 Introduction

The name 'carlavirus' has been derived from the type species, acquired from *Carnation latent virus* (CLV). Due to its particle morphology and RNA size, carlaviruses is grouped between potexviruses and potyviruses. The International Committee on Taxonomy of Viruses (ICTV) has categorized carlaviruses as a genus

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B. Mandal et al. (eds.), *A Century of Plant Virology in India*,
https://doi.org/10.1007/978-981-10-5672-7_6

of the family *Betaflexiviridae* derives from the subsequent subdivision of *Flexiviridae*. Currently, 52 virus species have been included in this genus.

Carlavirus has narrow host range and causes mild or no symptoms. In most of the cases they were discovered accidentally with other viruses from mixed infection. Caralviruses are easily spread by vegetative propagation and aphids in a semi-persistent mode. Transmission through seed and *Bemisia tabaci* also has been reported for cowpea mild mottle virus (CPMMV).

The virions of carlaviruses are non-enveloped, filamentous morphology with size of 610–700 × 12 nm (Wetter and Milne 1981). The virus particle contains linear, positive-sense ssRNA of 5.8–9 kb in size. *Potato virus S* (PVS) (Foster and Mills 1991b) and CLV (Meehan and Mills 1991) of carlavirus also contains subgenomic RNA. The RNA genome lacks viral protein-genome linked (VPg) at the 5′ of the genomic RNA (Monis and de Zoeten 1990) but has cap structure (Foster and Mills 1991a, 1991c). The cap structure is absent at the 5′ end of subgenomic RNA (Foster and Mills 1990).

The genome of carlaviruses has six open reading frames (ORF). RNA replicase with methyltransferase and helicase activity is coded from the ORF1. ORFs 2, 3 and 4 codes a Triple Gene Block (TGB) is responsible for virus transport and cell membrane modification. The coat protein (CP) is coded by ORF5 (CP) and the protein from ORF6 is a cysteine-rich protein. This protein of ORF6 contains a zinc finger motif binds to single- and double-stranded nucleic acid *in vitro* (Chen et al. 2002). Between ORF1 and ORF2 and ORF4 and ORF5, the genome of carlaviruses contains two small non-coding regions (Chen et al. 2002). There are as many as six carlaviruses known in India. A brief description of the work on carlaviruses carried out in India is presented here.

6.2 Cowpea Mild Mottle Virus (CPMMV)

CPMMV from cowpea plant was first reported in Ghana (Brunt and Kenten 1973). In India, the infection of CPMMV has been observed in cowpea (Jeyanandarajah and Brunt 1993), groundnut (Iizuka et al. 1984) and soybean (Bhargandi et al. 1987; Manoj et al. 2013). Jeyanandarajah and Brunt (1993) reported that CPMMV is serologically related to tomato pale chlorosis virus, Psophocarpus necrotic mosaic virus, Voandzeia mosaic virus and groundnut crinkle virus (Naidu et al. 1998). Based on the physicochemical properties, Brunt (1995) classified CPMMV as the definite member of genus Carlavirus.

Transmission electron microscopic analysis of CPMM from infected soybean has shown that CPMMV as the flexuous filamentous particles measuring 650 × 12 nm in size (Manoj et al. 2013). CPMMV contains ssRNA of 8127 nucleotides codes for six ORFs. In India, during 2011 and 2012, high disease incidence of CPMMV on soybean occurred from 25.1–38.3 % to 45.1–71.0 % respectively (Manoj et al. 2013). The different symptoms like mottling, mosaic, leaf distortion

and plant stunting were observed in soybean (Manoj et al. 2013). Non-persistent transmission of CPMMV is occurred in groundnut and soybean by the whitefly (Jeyanandarajah and Brunt 1993; Muniyappa and Reddy 1983; Mali et al. 1987; Badge et al. 1996; Manoj et al. 2013). The reports are available regarding the transmission of CPMMV in cowpea, soybean and French bean through seeds (Iizuka et al. 1984; Siva et al. 1990; Almeida et al. 2005; Tavasoli et al. 2009).

CPMMV showed mild mosaic mottling symptoms in peanuts grown in Mianpuri in Northern India designated as CPMMV-M and in Hyderabad (Southern India), it showed severe disease symptoms, designated as CPMMV-S (severe) (Naidu et al. 1998). The sequencing of 2.5-kb from 3′-terminal of these isolates (GenBank Accession number for CPMMV-S: AF024628 and for CPMMV-M: AF024629) showed 70 % sequence identity. The organization of ORFs and molecular weight of proteins coded from the respective ORFs are similar between these isolates (Naidu et al. 1998). The TGB proteins of CPMMV-M and CPMMV-S not only similar to TGB of other member of *Carlavirus* but also to TGB of *Potexvirus, Hordeivirus* and *Furovirus* genus (Naidu et al. 1998). In both the isolates, P7 from ORF 3 is substantially overlaps the P12 codes by ORF 2. The initiation codon of the ORF 2 and 5 are partially embedded in the termination codon of the ORF 1 and 4 respectively in both the isolates. An intergenic region separates the ORF 4 and 3 with 56 nt in CPMMV-S, and 19 nt in the case of CPMMV-M (Naidu et al. 1998).

Twenty seven cultivars of soybean were checked for CPMMV infection and all are susceptible to CPMMV (Manoj et al. 2013). The symptoms are appeared in different cultivars of soybean within 9–14 days of inoculation (DAI). CPMMV causes venial chlorosis mottling and leaf distortion in mungbean and urdbean plant, chlorotic lesions, necrotic spot with mild mottling and downward curling in cowpea it (Manoj et al. 2013). CPMMV infection on groundnut showed systemic chlorosis, mottling and leaf distortion with stunted growth where as in bean plants it produce systemic mosaic and stunting (Manoj et al. 2013). In French bean it has showed mosaic, veinal chlorosis, mottling, necrotic lesions and leaf curling (Manoj et al. 2013). *Nicotiana benthamiana* and *N. glutinosa* have shown chlorosis and systemic mosaic and stunting, whereas as in *N. tabacum*, no symptoms were found (Manoj et al. 2013). Inoculation of CPMM on fenugreek plant showed systemic chlorosis at the initial but latter it causes necrotic lesion on the leaves. After the infection, the symptoms started to appear within 15–20 DAI in mungbean, urdbean, cowpea, groundnut, French bean, asparagus bean and fenugreek, but in tobacco plants the symptoms started to appear within 8–10 DAI (Manoj et al. 2013).

Manoj et al. (2013) submitted the sequence of 1.3 kb of 3′-terminal region of CPMMV of D1 isolate containing the complete CP gene, nucleic acid binding protein (NABP) gene and 3′-UTR in NCBI GenBank with Accession number: JX524810. The CP gene of CPMMV D1 isolate has 90–94 % aa identity with other CPMMV isolates (Manoj et al. 2013). NABP gene of D1 isolate has 67–70 % nt identity with CPMMV-M and CPMMV-S and 55–59 % nt identity with other isolates. In the 3′-UTR region it has 77–81 % nt identity with CPMMVM and CPMMV-S isolates and 73–81 % nt identity with other isolates. Phylogenetic

analysis of 1300 bp of 3′-terminal region of CPMMV isolates, placed CPMMV D1 in separate position in the phylogenetic tree which is also different from other Indian isolates; CPMMV-S and CPMMV-M (Manoj et al. 2013).

First time in India, Baranwal et al. (2015), reported that CPMMV infects urdbean and mungbean and produce leaf crinkle symptom. The presence of CPMM in urdbean and mungbean was confirmed by DAS-ELISA and RT-PCR. But, by the next generation sequencing of small RNA, they identified the presence of groundnut bud necrosis virus and soybean yellow mottle mosaic virus along with CPMMV. The 3′ terminal sequence contains CP, NABP and 3′ UTR of CPMMV from mungbean (Acc. No. KJ534277) and urdbean (Acc. No. KJ534276) are available (Baranwal et al. 2015). The CP gene of CPMMV isolates from mungbean and urdbean is 867 nt in length and has low sequence identity: 77.9 % at the nucleotide and 90.9 % at amino acid level. The nucleotide length of NABP is varied between urdbean and mungbean isolates. In the urdbean isolates, nucleotide length of NABP is 327 nt whereas mungbean isolate it is 306 nt but has very low identity of 56.4% at the amino acid level (Baranwal et al. 2015). The analysis of amino acid sequences of CP and NB, clustered mungbean isolate with other Indian isolates of CpMMV and urdbean isolate with Brazil, USA and Ghana isolates. But based on 3'UTR nucleotide sequences both these isolates are clustered together with Indian isolates (Baranwal et al. 2015).

Naidu et al. (1998) expressed CP of CPMMV-M in *E. coli* showed same size (32 kDa) of CP purified from infected CPMMV-M infected plant. The polyclonal antiserum raised against the purified virus of CPMMV-M is reacted with recombinant CP (Naidu et al. 1998).

6.3 Chrysanthemum B Virus (CVB)

CVB is the major pathogen of chrysanthemum and it is available wherever chrysanthemum grows in India (Singh et al. 2007) and the rest of the world. CVB has filamentous particle, measuring approximately 680 nm long and 12 nm wide (Verma et al. 2003) (Fig. 6.1a, b). Electron microscopic studies of CVB infected leaf tissues showed the covering of CVB around chloroplast showed abnormalities like loss of chloroplast envelope and extensive invagination by cytoplasm (Fig. 6.1c, d). The association of the virions with the chloroplast and disruption of chloroplast envelope suggest that the chloroplast is necessary for the assembly of virions as suggested earlier in case of PVS (Garg and Hegde 2000).

Verma et al. (2003) reported that CBV infects *N. clevelandii*, *N. glutinosa*, *N. rustica*, *Petunia hybrida* and *Vicia faba* and produce symptoms like banding, mottling, clearing and mosaic in vein and during the severe infection it make the flowers get malformed.

Out of 80 cultivars of chrysanthemum tested, Regol Time and Maghi cultivars were infected by CVB, TAV, CMV, poty- and tospoviruses). The percentage of infection in 80 cultivars by CVB, CMV, TAV, tospo- and potyviruses were 48.7 %, 42.5 %, 26.2 %, 12.5 % and 6.25 % respectively. In 12 cultivars, single infection of

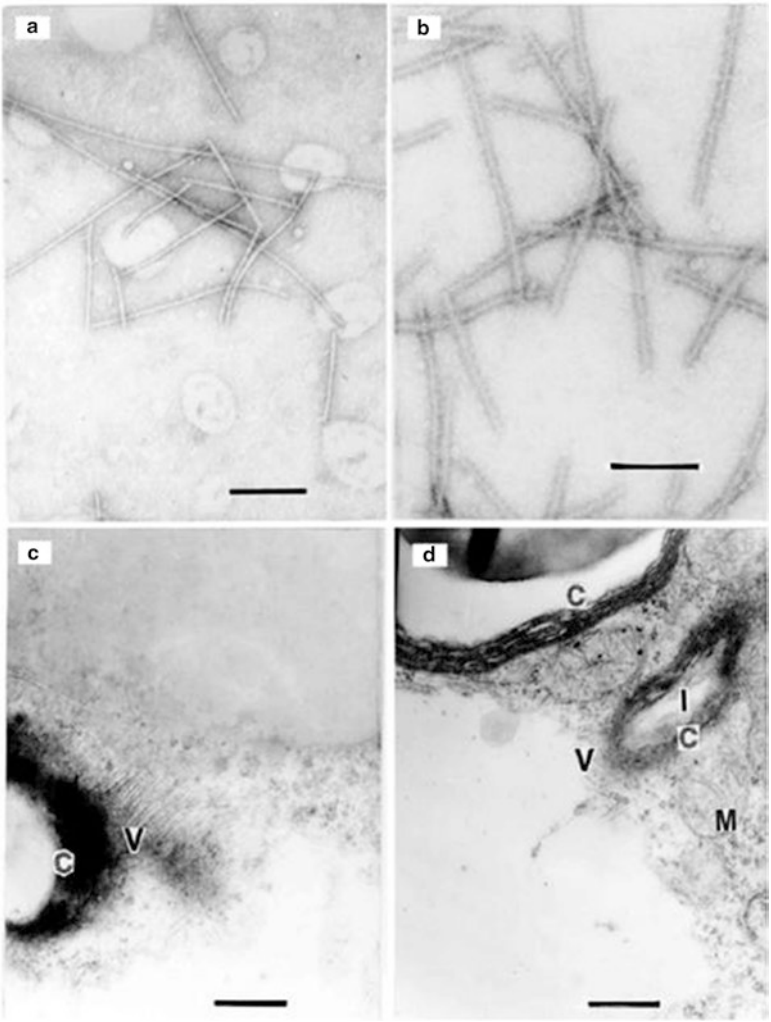

Fig. 6.1 Electron micrograph of CVB from leaf tissue. **a** Clarified virus showing negatively stained virions; **b** Virions coated with CSB carlavirus antiserum; **c** Infected leaf cell with dense chloroplast *c* and large number of virions *v*; **d** Infected leaf cell with normal mitochondria *m* and dense chloroplast *c* has cytoplasmic invagination *I* and virions *v* around it. Bar = 250 nm in (**a**, **b**, Bar = 500 nm in **c**, **d)** (Source: Verma et al. 2003)

CMV was observed with characteristic symptoms and in 9 cultivars as double infection with CVB and other viruses listed earlier. CVB is transmitted primarily by the aphid *Myzus persicae* in a non-persistent manner and secondarily through sap-transmission (Singh et al. 2012).

Singh et al. (2007) have surveyed Chrysanthemums showing symptoms of mosaic and mottling all over the India and identified 29 isolates (Table 6.1). These 29 isolates are grouped into 3 groups based on the phylogenetic analysis of CP gene.

Table 6.1 Source, size of coat protein gene and accession numbers of the various chrysanthemum B virus isolates

Abbreviation (Region)	Collected from state	Gene size (Nucleotides)	Accession number
AP (Hyderabad)	Andhra Pradesh	945	AJ580954
AR (Arunachal Pradesh)	Arunachal Pradesh	939	AJ748852
AS (Guwhati)	Assam	945	AJ812735
BR (Patna)	Bihar	945	AJ580931
CH (Chandigarh)	Chandigarh	945	AJ621814
CHH (Raipur)	Chhattisgarh	945	AM0349442
DL (Delhi)	New Delhi	945	AJ619742
GJ (Gandhinagar)	Gujrat	945	AJ871582
HP1 (Palampur)	Himachal Pradesh	969	AJ564858
HP2 (Chamba)	Himachal Pradesh	945	AJ871365
HP3 (Chail)	Himachal Pradesh	948	AJ876635
HP4 (Sangla)	Himachal Pradesh	939	AJ871583
HR (Haryana)	Haryana	945	AJ629843
HSR (Hissar)	Haryana	945	AM0349440
JK1 (Jammu)	Jammu and Kashmir	945	AJ812569
JK2 (Srinagar)	Jammu and Kashmir	945	AJ871366
JK3 (Leh)	Jammu and Kashmir	945	AJ871367
JH (Ranchi)	Jharkhand	945	AJ580930
KK (Bangalore)	Karnataka	945	AJ585240
KR(Trivandrum)	Kerala	945	AM039441
MH (Nagpur)	Maharashtra	945	AJ581993
MP (Gwalior)	Madhya Pradesh	945	AJ748853
OR (Bhubaneswar)	Orissa	945	AJ879077
PB (Ludhiana)	Punjab	945	AJ580956
RU	Russian isolate of CVB	945	S60150*
RJ (Jaipur)	Rajasthan	945	AJ619743
SK (Sikkim)	Sikkim	945	AJ585514
UA2 (Uttarkashi)	Uttaranchal	939	AJ879078
WB1 (Kolkata)	West Bengal	945	AJ619744
WB2 (Siligudi)	West Bengal	945	AJ621815
Helenium virus S (HelVS; outgroup 1)		897	D10454*
Daphne virus S (DVS; outgroup 2)		954	AJ620300*
Lily symptomless virus (LSV; outgroup 3)		876	AJ585052*

Source: Singh et al. (2007)

Group I contains seven subgroups (named A–G) representing seven lineages diverged from the common ancestral group I. The clustering of isolates was not based on geographical since diversity was observed within the same state as three (HP1, HP3 & HP4; Table 6.1) of the four isolates from Himachal Pradesh are present in group I where as HP2 in group III (Table 6.1).

To assess the role of recombination on CVB diversity in India, the complete genome were obtained for four isolates namely Punjab (PB), Uttarakhand (UK),

Uttar Pradesh (UP) and Tamil Nadu (TN) represents four corners in India (Singh et al. 2012). The complete genome analysis revealed that all these isolates were more similar within themselves than to other isolates from outside the India including the Japanese isolate (CVB-S). N-terminus of replicase is highly conserved among the carlaviruses but surprising replicase of the Indian isolates was 59 % identical to that of the CVB-S whereas the TGB of the Indian isolates showed 81–92 % sequence similarity to CVB-S. NABP has showed the highest degree of similarity of 89–100 % (Singh et al. 2012).

Sixteen unique recombination events were detected in the full length genome of various CVB isolates (Singh et al. 2012). In fact, CVB-S was consider to be a recombinant of UP and TN. The recombination occurred in replicase ORF between 538 and 4260 nucleotide positions (Singh et al. 2012).

Singh et al. (2011a) have observed the homotypic interaction of the CVB CP in the yeast two-hybrid system (Y2H) and co-immunoprecipitation (Co-IP). In the Y2H analysis, the complete coat protein (CP) in the activation domain (AC) interacts strongly with complete coat protein in the binding domain (BD). The complete coat protein interacts with N-terminus of CP having amino acids 1–184 aa and with middle region have 98–184 aa. There was no interaction between complete CP and N-terminus of CP having 1–102 aa. These are clearly indicate that the 86 aa from middle region spanning from 98 to 184 is extremely important for CP-CP interactions (Singh et al. 2011a). This is also confirmed with Co-IP analysis (Fig. 6.2) in which precipitation was observed between the c-Myc tagged 98–184 aa and HA-tagged 1–184 aa with c-Myc and HA antibodies. (Singh et al. 2011a).

Initially CVB was detected with immunological techniques (Raizada et al. 1989; Zaidi et al. 1990). ELISA and other forms of ELISA were extensively used to detect CVB (Verma et al. 2003). Immunosorbant electron microscopy (ISEM) was also used to identify CVB in chrysanthemum (Verma et al. 2003). The polyclonal antibodies were raised against coat protein gene of CVB with Glutathione S-Transferase (GST) tag showed specific reaction to CVB from infected plant which was used to develop ELISA based diagnostic kit for CVB and for indexing of the

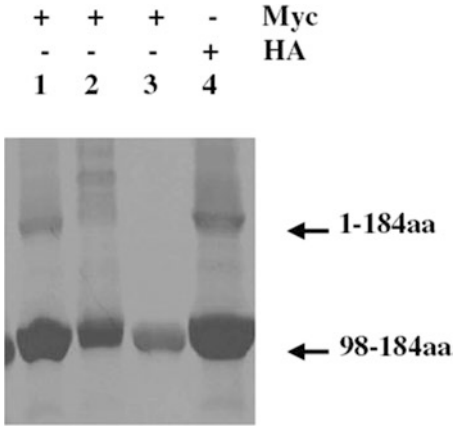

Fig. 6.2 Co-immuno precipitate of 98–184 aa (BD) with 1–184 aa (AD) in Co-IP assay of chrysanthemum B virus. Lane 1: (1–184 AD) + (98–184BD) + c-Myc antibody, Lanes 2, 3: (98–184BD) + c-Myc antibody, Lane 4: (1–184 AD) + (98–184BD) + HA-Tag antibody (Source: Singh et al. (2011a))

chrysanthemum mother stock with virus free (Singh et al. 2011b). This group is currently involved in developing the PCR based detection kit for CVB.

CVB-free chrysanthemum can be produced by chemotherapy, thermotherapy and *in vitro* meristem tip culture (Ram et al. 2005). For chemotherapy, 2-thiouracil at 40 g dm-3 concentration was effective to produce CVB-free plants when compared to other chemicals like Acyclovir, Amantadine, 5-bromouracil, 2-thiouracil and Zidovudine. Incubating the plants at 38 °C for 30 days was also effective to treat the CVB virus (Ram et al. 2005).

6.4 Lily Symptomless Virus (LSV)

LSV, is an important pathogen of Lily, is transmitted by *Myzus persisae, Aulocorthium solani Macrosiphum euphorbiae, Aphis fabae, Aphis gossypii,* or by whitefly (Suhasini et al. 2010). LSV exhibit various symptoms like chlorosis, vein clearing and deformed flowers on various lilies like *Lilium longiflorum*, *Lilium tigrinum*, Asiatic hybrid lily and Oriental hybrid lily (Singh et al. 2005). The isolates obtained from *L. Longiflorum*, *L. tigrinum*, Oriental and Asiatic hybrids and spider lily were designated as LSV-L, −T, −A, −O and -S, respectively. EMBL accession number of these isolates are AJ585052 (LSV-A), AJ748320 (LSV-L), AJ748277 (LSV-O), AJ781318 (LSV-T) and AJ780923 (LSV-S). The comparison of coat protein gene was made between these LSV isolates and isolates from other countries like China (LSV-C), the Netherlands (LSV-N), Japan (LSV-J) and Korea (LSV-K) (Singh et al. 2005). Seventy-eight to 96% homology has been observed between the Indian isolates and 83–98 % homology with isolates from other countries (Singh et al. 2005). The isolates LSV-L and LSV-A has stretches of amino acids in the core region CP protein at position between 129 and 146 in LSV-L and between 129 and 155 in LSV-O (Fig. 6.3) but this is absent in other LSV isolates. A 41 amino acids stretch is found in the C-terminal of LSV-T is unique to this isolate (Fig. 6.3) (Sing et al. 2005).

The isolate LSV-T, which is native to India, has 78–84 % homology with other isolates. Due to its less homology with other isolate, variations at C-terminal (Fig. 6.3) and phylogenetic analysis made LSV-T as a distinct isolate.

Suhashini et al. (2010) analysed the sequence of 79 LSV isolates and observed that the variations in the LSV genome are taking place at a faster rate and these variations can produce new strain which will causes widespread dispersal and damage.

The complete nucleotide sequence for the LSV obtained from the *L. Longiflorum* is available in the GenBank with Accession Number: AM422452 (Singh et al. 2008). The genome size is 8.394 Kb with six open reading frames (ORFs). This isolate is closely similar to Netherland isolate (Accession No: AJ564638) (Singh et al. 2008). This LSV isolate showed 97–98 % nucleotide sequence homology with the other isolates reported by Sing et al. (2005).

Three recombination events have been observed between Indian, Netherlands and Chinese isolates. First recombination event was observed between the Indian

Fig. 6.3 Coat protein sequence comparison between the various LSV isolates of India. Differences in the C-terminal of LSV-T and middle portion of LSV-A and LSV-L represented by shading. *LSV-A* Asiatic hybrid, *LSV-L Lilium longiflorum*, *LSV-O* Oriental hybrid, *LSV-S* spider lily, *LSV-T* Tiger lily (Source: Singh et al. 2005)

isolate (AM422452) and Chinese isolate (AM263208) which led to a formation of Korean isolate (AJ516059). In the Korean isolate, a region of 5,597–7,796 nt of the Indian Isolate (AM422452) was replaced with C-terminal of RdRp, TGB, almost complete CP region of Chinese isolate (AM263208). Second recombination event was occurred between the Indian isolate (AM422452) and the Netherlands isolate (AJ564638) and produce Korean isolate. In this recombination event, a region of RdRp (2,344–5,189 nt) of the Indian isolate was replaced with the corresponding genome sequence of the Netherlands isolate (AJ564638). Third recombinations observed between Korean isolate (AJ516059) and the Chinese isolate (AM263208) led to Korean isolate. The ending breakpoint could not be identified but detected with a low degree of confidence (Singh et al. 2008).

6.5 Football Lily Mosaic Virus (FLMV)

In India, football lily (*Scandoxus katharine*) is commonly grown in nurseries as ornamental flower. It is naturally occurring plant in Sikkim and Darjeeling hills. Virus-like symptoms were observed in various nurseries at Delhi, Meerut, Sikkim and Kalimpong. Electron microscopic observation revealed that flexuous particles measuring 650 × 13 nm constantly associated with the disease. Samples from naturally affected plants were collected and maintained in glass house. The disease was mechanically transmitted to football lily and *Chenopodium amaranticolor*. A filamentous virus was partially purified from the football lily plants which were grown inside the glass house. Polyclonal antibodies against the virus were developed and used for virus detection in ELISA (Das et al. 2010). Virus associated with football lily mosaic disease has single coat protein of 31 kDa. It was serologically related to

Carnation latent virus. Ultra thin sections of infected tissue revealed large number of virus particles scattered in the cytoplasm but not in nucleus. No inclusion bodies characteristic of potyvirus was observed. Particle morphology, transmission, serological relationship, size of coat protein and absence of characteristic inclusion bodies suggests that the virus may be a member of carlavirus group (Das et al. 2010).

6.6 Potato Virus S (PVS)

Potato in India is known to be infected by two carlaviruses, PVS and *potato virus* M (PVM). PVM was first recorded during late 1970s in India based on serology and transmission of the virus (Khurana and Singh 1980) and since then not much information on PVM has been generated. Recently, the complete genome sequence of one isolate from Delhi, PVM-Del-144 has been generated (GenBank No. KJ194171) and CP gene sequence of 14 isolates from the potato growing region of northern plain has been analysed that showed considerable diversity of the virus (Unpublished results, Dr. Bikash Mandal). Raigond et al. (2013) developed RT-PCR based detection kit to detect both PVY and PVS simultaneously from the potato leaf tissues and tubers. The detection is based on the amplification of coat protein gene. This detection method makes the simultaneous detection of PVY and PVS in a simple and rapid manner and also it reduces the time and cost of the consumables (Raigond et al. 2013).

6.7 Garlic Common Latent Virus (GarCLV)

The CP gene sequence is available for five Indian GarCLV isolates: Northern India (GarCLV-G1; Acc. No. JQ818259), Eastern India (GarCLV-RAU; Acc. No. JQ818256), Southern India (GarCLV-Kolar; Acc. No. JQ818257), Western India (GarCLV-JN; Acc. No. JQ818255 and Anand; Acc. No. JQ818258). Comparative nucleotide sequence analysis of CP gene with other CP sequences available in the database has revealed that there is high sequence diversity among all GarCLV isolates worldwide (11.9 %) and low sequence diversity among the Indian isolates (4.3 %) (Pramesh et al. 2013). The major variability was found at the N-terminus (1–48 aa) whereas the central and the C-terminal regions (49–319 aa) are highly conserved in all the isolates (Pramesh et al. 2013).

The phylogenetic analysis clustered the GarCLV isolates into two major clusters as subgroup I and II. Subgroup I contains isolates from USA and China and subgroup II contain isolates from Australia, Brazil, India, Japan, and South Korea. All the five isolates of India are grouped together with K2 isolate of South Korea and GCLV-BZL isolate of Brazil.

The phylogenetic analysis of CP gene five GarCLV Indian isolates with representative isolates of other 37 Carlavirus species grouped viruses of Carlavirus into two distinct phylogenetic subgroups. The subgroup 1 contains all the Indian isolates in single cluster with shallot latent virus and 10 other Carlavirus species. The subgroup 2 contains remaining 26 Carlavirus species (Pramesh et al. 2013).

During the intra species analysis of CP gene of Indian isolates, Pramesh et al. (2013) observed the potential recombination in the CP gene of the Anand isolate from JN and Kolar isolates and also observed the absence of genetic exchange from Carlavirus species to GarCLV (Pramesh et al. 2013).

6.8 Microsatellites in Carlvirus Genome

The genome wide screening of 32 carlavirus for the presence, abundance, and composition of simple sequence repeat (SSR) has revealed that carlavirus has 18–42 SSRs (Chaudhary et al. 2014) as compared to similar genomes of potyviruses (23–45 SSRs) (Xiangyan et al. 2011) or *Human immunodeficiency virus* isolates (22–48 SSRs) (Chen et al. 2009) but having more SSRs than that of geminivirus (4–19) with a smaller genome. A single nucleotide repeats were observed in all the carlavirus genomes. Among polyrepeats, poly (A/T) repeats being more prevalent than poly (G/C) repeats. According to Karaoglu et al. (2005), the percentage of poly (A/T) repeats (78 %) are significantly higher than poly (G/C) repeats in each complete carlavirus genome. Chaudhary et al. (2014) based on the analysis of 32 carlavirus genome reported that the (A/T) content is slightly higher than G/C content.

Di-nucleotide repeats (GT/TG. AG/GA, AC/CA, AT/TA, CT/TC and CG/GC are found across the carlavirus genomes. GT/TG repeats predominately present in carlaviruses genome whereas GC/CG is rarely available (Chaudhary et al. 2014). Among 64 triplet repeat types, AAG/GAA type repeats codes for lysine/glutamic acid was most abundant followed by GAG coding for glutamic acid (Chaudhary et al. 2014). Di-nucleotide repeats are more prevalent because of higher slippage rate (Katti et al. 2001). Tetra-nucleotide repeats like AAGA, GTAC, TAAA and CAAG are present in many carlavirus genomes and pentanucleotide CCATA repeat is present in one genome. The occurrence of diverse types of repeats in carlavirus genome facilitates genome evolution (Chaudhary et al. 2014).

The search for compound simple sequence repeat (cSSR) in carlavirus genomes yielded a total of 34 compound microsatellites (Chaudhary et al. 2014). In general, cSSR are involved in regulating the gene expression in several species (Kashi and King 2006; Chen et al. 2011) but the function of these cSSR in carlavirus is not clear but possibly of complex regulation at the functional level. In general, the largest compound microsatellite in carlavirus is composed of three cSSRs. Interestingly, cSSRs% in carlavirus genome is varied between 0 % and 11.42 % (Chaudhary et al. 2014). Among 32, 11 of the carlavirus species does not possess even a single compound microsatellite, this may be due to less number of strains present in these species where in non availability of cSSR might restrict their variation and evolution (Chaudhary et al. 2014).

According to Chaudhary et al. (2014), 66.4 % and 67.52 % of SSRs and compound simple sequence repeats (cSSRs) are found in RDRP gene respectively followed by 8.6 % and 8.8 % of SSRs and cSSRs in ORF-6 respectively. The intergenic region of TGB1/TGB2 and TGB2/TGB3 contains very low SSRs and cSSR and RDRP gene contains very high mono-, di- and tri-nucleotide repeats.

6.9 Concluding Remarks

Although, six different carlaviruses has been identified in India, their prevalence in the different crops and regions has not yet been adequately investigated. The reason for this may be due to the fact that these viruses are not known to cause as severe disease that is crop yield limiting. The complete genome information is available from India only for CVB and LSV. The study on the genetic diversity between the isolates of CVB and LSV revealed the occurrence of recombination between the isolates of each species. The polyclonal antisera raised against the coat protein of CPMMV, CVB and FLMV have the potential application in determining their prevalence and also for indexing the plants for the freedom from carlavirus.

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