The Present Status of Carmoviruses Research in India

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Abstract

The genus *Carmovirus* of the family *Tombusviridae* consists of the members having isometric virion particles with positive sense ssRNA genome of 4.0–4.8 kb encoding five proteins. In India, till date four carmoviruses were reported *viz*., blackgram mottle virus (BMoV), carnation mottle virus (CarMV), melon necrotic spot virus (MNSV) and soybean yellow mottle mosaic virus (SYMMV). BMoV and SYMMV are the legume infecting carmoviruses, which differs by serology and symptomatology. The Indian isolate of CarMV is a wide spread and distinct from other isolates. The Indian isoate of SYMMV is distinct from the SYMMV isolates occurring in the other countries being highly sap transmissible to guarbean, French bean, mungbean, soybean and urdbean with the distinct symptoms. Polyclonal antiserum developed against the recombinant coat protein of the Indian isolate of SYMMV can be utilized for successful detection of SYMMV in various plant samples. However, there is a great need to exploit these carmoviruses for further understanding the process of replication, gene expression, and exploiting them as gene expression vector for the expression of heterologous proteins in plant and as virus induced gene silencing vector for studying gene functions in legume crops. This chapter summarises the research work conducted on carmoviruses occurring in India.

Keywords

Carmoviruses • Blackgram mottle virus • Carnation mottle virus • Melon necrotic spot virus • Soybean yellow mottle mosaic virus

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7.1 Introduction

Carmoviruses belongs to the family *Tombusviridae,* which is one of the largest family having genera including Aureusvirus, Avenavirus, Carmovirus, Dianthovirus, Necrovirus, Panicovirus and Tombusvirus with virions showing icosahedral symmetry of 30 nm; consists of 180 identical coat protein (CP) subunits of about 38–43 kDa. Among these viruses, significant degree of sequence similarity was observed with respect to replicase associated and CP genes. Mild or asymptomatic infections were the most common symptoms observed on relatively restricted natural host ranges. Carmoviruses contain a single-stranded (ss) positive sense RNA genome ranging in size from 4.0 to 4.8 kb. The genomic RNA has neither a 3′ poly (A) tail nor 5′ cap structure which is a most common feature for the members of genus *Carmovirus*. The genome consists of five definitive open reading frames (ORFs) from the 5′ to the 3′ end which encode proteins of about 28, 88, 8, 9 and 38 kDa respectively.

As carmoviruses possesses small genome, they are highly accessible to serve as an interesting model for understanding plant RNA virus genome structure, function and regulation. More than 30 carmoviruses have been reported from all over the world. The first carmovirus identified in India was blackgram mottle virus in 1974. Since then, three more carmovirus species *Carnation mottle virus*, *Melon necrotic spot virus* and *Soybean yellow mottle mosaic virus* were reported from India. Qu and Morris (2008) gave brief description about carmoviruses and Simon (2015) reviewed about the 3′ UTR region of carmoviruses. However, the research work conducted in India has not so far been reviewed. This chapter describes the up to date research work conducted on carmoviruses in India.

7.2 Blackgram Mottle Virus

Blackgram mottle virus (BMoV) responsible for mottling and stunting symptoms in blackgram was identified as a distinct virus based on biological and serological properties (Phatak 1974). An improved method for BMoV purification using magnesium-bentonite was described by Balasubrahmanyam et al. (1997a). BMoV was showed to be a seed borne virus (8% of seed transmission) with ssRNA, containing isometric particles with a diameter of 28 nm belongs to the genus Carmovirus, family *Tombusviridae*. BMoV had a 5×10^{-4}–1×10^{-5} dilution end point, a thermal inactivation point of 90–92 °C and longevity *in vitro* of 40–45 days (Phatak 1974). The host range study of BMoV showed that it was mainly restricted to *Leguminosae* members and induces the symptoms like mosaic, mottling and veinal necrosis in mungbean, blackgram and guarbean (Krishnareddy 1989).

Seed transmission rate of BMoV was also tested in different cultivars of blackgram which showed the highest seed transmission rate in cv. PLU-277 (15.9%), followed by cvs T-9 (11.8%), PLU-213 (7.0%) and UH-81-7 (1.3%). In addition the mean amount of the virus in various tissues was determined and found that highest in embryonic axis (48–1234 ng) followed by cotyledon (15–24 ng) and testa

(12–20 ng). Further small amount of the virus in embryonic axes was observed in the cultivars that resisted seed transmission (Varma et al. 1992).

In vitro translation of BMoV RNA in rabbit reticulocyte lysate resulted in production of five major virus specific polypeptides with molecular weight 90,000 (p90), 82,000 (p82), 42,000 (p42), 39,000 (p39) and 32,000 (p32) respectively. The polypeptide p39 was identified as a CP based on its elctrophoretic mobility and immune-precipitation with BMoV antisera (Balasubrahmanyam et al. 1997a, b). However, natural infection of BMoV in mungbean and other legumes is still not known in India. Further due to the lack of genome sequence information, the molecular characterization of BMoV is pending and therefore, it is considered as a tentative member of the genus *Carmovirus.*

7.3 Carnation Mottle Virus

Carnation mottle virus (CarMV) is the most important and wide spread virus affects carnation cut flower crop and causing severe economic losses to the farmers. Although CarMV infection leads to mild symptoms, it causes severe infection in all types of carnations. Carnation mottle disease caused by CarMV is characterized with chlorotic spots on young apical leaves, followed by mosaic chlorosis, mottling and streaks of yellowish-white colour on infected leaves. The infected plant shows stunted growth and bushy appearance. In addition, CarMV infection results in the poor quality of cut-flower in terms of size, split calyces and reduced vigour, and also results in lesser yields by reducing number of lateral shoots, total number of flowers and fresh weight. CarMV infection not only affects the flower quality and shelf life, but also weakens the plant making it susceptible to infection by other pathogens. It can be easily transmitted by contact and cropping operations (Singh et al. 2005). Singh and Singh (1989) reported that CarMV mainly transmitted through aphid vector *Aphis gossypii* and retained within the vector for up to 8 days, indicating the persistent nature of the virus.

Bansal and Singh (1980) reported that *Chenopodium amaranticolor* and *Chenopodium quinoa* were the local lesion hosts of CarMV. The experimental host range of CarMV includes *Catharanthus roseus*, *C. amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *Lycopersicon esculentum*, *Medicago sativa*, *Nicotiana clevelandii* and *Saponaria vaccaria* (Singh et al. 2005). The presence of CarMV in different geographical regions was confirmed through screening of 93 carnation cultivars by DAS-ELISA using polyclonal IgG. Interestingly 90% of the carnation cultivars showed positive result indicating the widespread nature of CarMV in India (Singh et al. 2005).

Sequencing and sequence analysis of CarMV movement and coat protein showed that Indian isolate was distinct and belonged to a new Group PN ($p^{164}N^{331}$) (Singh et al. 2005). The complete genome of CarMV has also been sequenced, which consisted of 4005 bp in length with five ORFs. Multiplex PCR was mainly employed to detect the CarMV from annual and perennial carnations through the amplification

of CP and MP. Comparative sequence analysis of CarMV isolates from annual and perennial carnations revealed the 95–99% sequence identity for both CP and MP (Raikhy et al. 2006).

7.4 Melon Necrotic Spot Virus

A new carmovirus infecting *Cucumis melo* was identified as melon necrotic spot virus (MNSV-Hyd) in Hyderabad during 2009. The complete genome of MNSV-Hyd has been sequenced which consisted of 4274 nucleotides. The viral genome encodes five ORFS: ORF1 (p28) and ORF2 (p89) encode putative RNA dependent RNA polymerase; ORF3 (p7A) and ORF4 (p7B) encode movement protein 1 and 2 required for *in planta* movement of the virus, ORF5 (p42) encodes a coat protein (Accession No: JX879088).

7.5 Soybean Yellow Mottle Mosaic Virus

Soybean yellow mottle mosaic virus (SYMMV-Mb) has been recently reported in India from Mungbean exhibiting mosaic, mild mottling and puckering symptoms (Fig. 7.1A). SYMMV-Mb can be easily sap transmitted to French bean cv. Pusa Parvati for maintenance of pure culture and for further studies of this virus. Purified virus preparations revealed the ultraviolet light absorption spectrum with $A_{260/280}$ of about 1.75; 39 kDa protein band in SDS-PAGE analysis; single band of ~4 kb genome size on 1% formaldehyde agarose gel electrophoresis. Host range study revealed that SYMMV was mainly restricted to only *Leguminosae* members but not to *Amaranthaceae*, *Cucurbitaceae* and *Solanaceae* and induced various symptoms *viz*., veinal mottling, mild mottling, chlorotic blotching, local and systemic necrosis in soybean (*Glycine max*), mungbean (*Vigna radiata*), blackgram (*Vigna mungo*), French bean (*Phaseolus vulgaris*) and guar bean (*Cyamopsis tetragonoloba*) respectively (Fig. 7.1B-E). The local symptoms were observed at 7–10 days post inoculation (dpi) where as systemic symptoms were observed at 15–22 dpi. The progeny virions were also stable and mechanical sap inoculation resulted in 100% infection. However, the symptomatology of the Indian isolate of SYMMV-Mb was distinct from South Korean isolate as the later did not induce visible symptoms in any of the legumes other than soybean. Polyclonal antiserum was developed by over expression of coat protein gene as 39 kDa protein in *E. coli* and successfully utilized for detection of SYMMV. Samples tested for understanding the prevalence of SYMMV-Mb in India revealed that SYMMV-Mb was mainly confined to Northern India. Serological studies showed that SYMMV-Mb was serologically related to BMoV but not to CarMV (Sandra et al. 2015).

The CP region of SYMMV-Mb consisting of 1065 bp, showed maximum sequence identity with SYMMV Korean and USA isolates followed by cowpea mottle virus (CPMoV). The phylogenetic analysis based on CP with other carmoviruses showed that SYMMV Indian isolate formed a separate cluster with SYMMV Korean isolate and CPMoV (Fig. 7.2). The complete genome of SYMMV-Mb

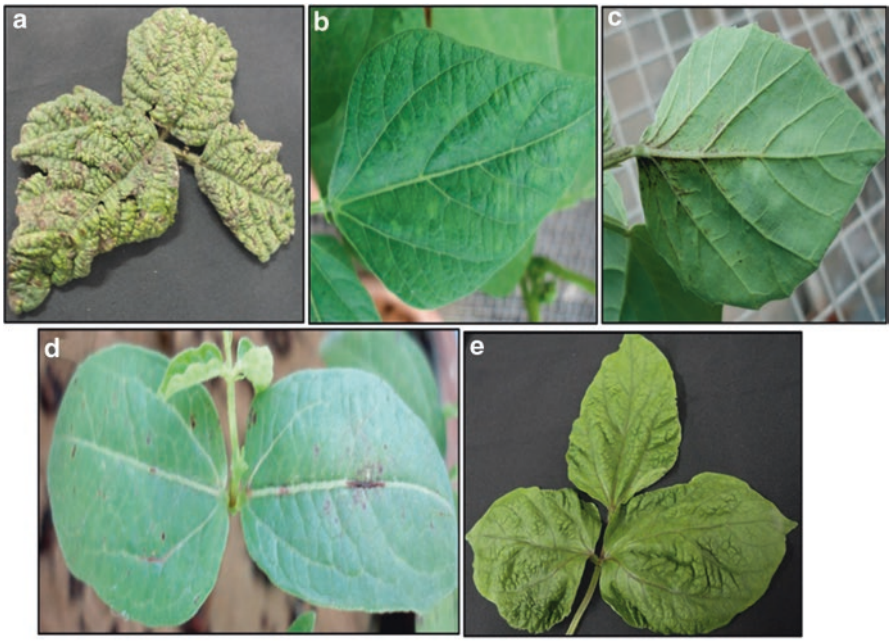

Fig. 7.1 Field symptoms of soybean yellow mottle mosaic virus on mungbean (**a**). Symptoms in leguminous plant species followed by mechanical sap inoculation of SYMMV-Mb. Systemic chlorotic blotches on frenchbean cv. Pusa Parvati (**b**). Systemic veinal necrosis on guarbean (**c**). Pinpoint necrotic spots and veinal necrosis on mungbean cv. Pusa Vishal (**d**). Mottling and puckering symptoms on blackgram cv. Bharabanki local (**e**)

isolate was sequenced which consisted of 3974 nucleotides lacking the 3′ poly (A) tail and 5′ cap structure. The viral genome encodes six ORFs: ORF1 (p25) and ORF2 (p83) encode replication associated protein; ORF3 (p8) and ORF4 (p10) encode the double gene block proteins (DGPs) required for virus movement, ORF5 (p39) encodes a coat protein (CP) and ORF6 is of unknown function. An infectious clone of SYMMV-Mb was developed, which successfully produced typical disease symptoms in various plant species similar to that caused by the native virus (Sandra et al. 2017). The infectious clone was utilized for designing a vector for expression of heterologous protein in plant system.

7.6 Concluding Remarks

Identification of new or emerging carmoviruses before they become economically important provides an opportunity to devise control measures in advance. However, the potential economic consequences of these carmoviruses are yet to realise in India, because the research pertaining to carmoviruses was confined to identification, sequencing and host responses upon mechanical inoculation. Seed transmission and infectivity studies are necessary to understand the impact of carmoviruses in India. BMoV, although, was reported as a different carmovirus, it's identity as a distinct

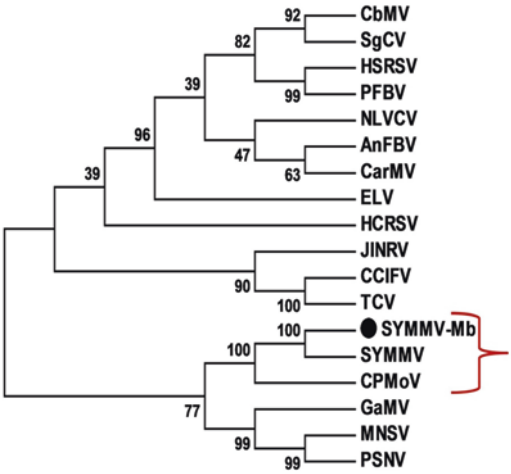

Fig. 7.2 Phylogenetic relationship of SYMMV-Mb isolate with other carmovirus species based on amino acid sequence of CP. The NJ tree was constructed in MEGA 6 programme. AnFBV angelonia flower break virus (NC_007733), CbMV calibrachoa mottle virus isolate California (GQ244431), CClFV cardamine chlorotic fleck virus (NC_001600), CarMV carnation mottle virus (NC_001265), CPMoV cowpea mottle virus (NC_003535), ELV elderberry latent virus isolate (AY038066), GaMV galinsoga mosaic virus (Y13463), HCRSV hibiscus chlorotic ringspot virus (X86448), HSRSV honeysuckle ringspot virus (NC_014967), JINRV Japanese iris necrotic ring virus (NC_002187), MNSV melon necrotic spot virus (NC_001504), NLVCV nootka lupine vein-clearing virus (NC_009017), PSNV pea stem necrosis virus (NC_004995), PFBV pelargonium flower break carmovirus (AJ514833), SgCV saguaro cactus virus (NC_001780), SYMMV SYMMV (FJ457015), SYMMVMb14 isolate of the present study (KF619242), TCV turnip crinkle virus (M22445)

virus species is not yet clear. The recent studies (Sandra et al. 2015, 2017) showed that SYMMV infected blackgram and shared serological relationships with BMoV. It may be possible that BMoV is a variant of SYMMV, however further studies are necessary to establish the correct identity of BMoV. Although, MNSV has been isolated from melon in southern India, its prevalence is not yet clear. Infectious clone of carmovirus is useful to assess the extent of yield loss due to infection of individual carmovirus as well as mixed infection with the other plant viruses. So far infectious clone has been developed for only one Indian isolate of carmovirus, SYMMV. Further, the members of the genus *Carmovirus* serve as an interesting models to deeply understand RNA plant virus genome structure, function and regulation due to their simplest genome complexity. So, there is a need to exploit the carmoviruses as gene expression and silencing vectors to understand these complex processes.

References

Balasubrahmanyam A, Kapoor HC, Varma A (1997a) Purification of blackgram mottle virus (BMoV) using magnesium-bentonite. Phytochemistry 44:1237–1240

Balasubrahmanyam A, Kapoor HC, Varma A (1997b) Blackgram mottle virus RNA and its in vitro translation. Indian J Exp Biol 35(1):37–41

Bansal PK, Singh A (1980) *Chenopodium quinoa* and *Chenopodium amaranticolor,* two local lesion hosts for carnation mottle virus. Sci Hortic 46:435–436

Krishnareddy M (1989) Studies on yellow mosaic and leaf crinkle diseases of blackgram. PhD Thesis. IARI, New Delhi, 263 pp

Phatak HC (1974) Seed-borne plant viruses-Identification and diagnosis in seed health testing. Seed Sci Technol 2:3–155

Qu F, Morris TJ (2008) Desk encyclopedia of plant and fungal virology. In: Brian W, Regenmortal MHVV (eds). Elsevier, Academic, San Diego

Raikhy G, Hallan V, Kulshrestha S, Ram R, Zaidi AA (2006) Multiplex PCR and genome analysis of Carnation mottle virus Indian isolate. Curr Sci 90(1):74–82

Sandra N, Kumar A, Sharma P, Kapoor R, Jain RK, Mandal B (2015) Diagnosis of a new variant of soybean yellow mottle mosaic virus with extended host-range in India. Virus Dis 26(4):304–314

Sandra N, Abdul Kader Jailani A, Jain RK, Mandal B (2017) Genome characterization, infectivity assays of *in vitro* and *in vivo* infectious transcripts of soybean yellow mottle mosaic virus from India reveals a novel short mild genotype. Virus Res 232:96–105

Simon AE (2015) 3′ UTR of carmoviruses. Virus Res 206:27–36

Singh R, Singh A (1989) *Aphis gossypii* Glover, an insect vector of carnation (*Dianthus caryophyllus* L.) mottle virus- a new record. Curr Sci 58:931

Singh HP, Hallan V, Raikhy G, Kulshreshtha S, Sharma ML, Ram R, Garg ID, Zaidi AA (2005) Characterization of an Indian isolate of Carnation mottle virus infecting carnations. Curr Sci 88(4):594–601

Varma A, Krishnareddy M, Malathi VG (1992) Influence of the amount of blackgram mottle virus in different tissues on transmission through the seeds of *Vigna mungo*. Plant Pathol 41(3):274–281