Review

Begomovirus research in India: A critical appraisal and the way ahead

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Begomoviruses are a large group of whitefly-transmitted plant viruses containing single-stranded circular DNA encapsidated in geminate particles. They are responsible for significant yield losses in a wide variety of crops in India. Research on begomoviruses has focussed on the molecular characterization of the viruses, their phylogenetic analyses, infectivities on host plants, DNA replication, transgenic resistance, promoter analysis and development of virus-based gene silencing vectors. There have been a number of reports of satellite molecules associated with begomoviruses. This article aims to summarize the major developments in begomoviral research in India in the last approximately 15 years and identifies future areas that need more attention.

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1. Introduction

Begomoviruses are the largest and the most important genus within the family *Geminiviridae* (plant-infecting single-stranded DNA viruses having characteristic geminate incomplete icosahedral particles). Begomoviruses (type species: *Bean golden mosaic virus*) are transmitted by whiteflies and have either a monopartite (a single DNA) or a bipartite (with two DNA components: DNA-A and DNA-B) genome organization, infecting mostly dicotyledonous plants. Different aspects of the Family Geminiviridae have been comprehensively reviewed earlier (Gutierrez 1999, 2000; Mansoor *et al.* 2003; Jeske 2009). The DNA-A of bipartite and the single component of monopartite begomoviruses contain five (sometimes six) Open Reading Frames (ORFs), one (*AV1*) or two (*AV1* and *AV2*) in the viral sense (V-sense) strand and four (*AC1* to *AC4*) in the complementary sense (C-sense) strand. Both the DNA-A and DNA-B are approximately 2.8 kb in size. The DNA-B contains two ORFs (*BV1* and *BC1*, in V-sense and C-sense strand, respectively). In DNA-A, *AV1* codes for coat protein (CP), the *AV2* for a protein of unclear function, *AC1* for a replication-associated protein (Rep) and *AC2* for a transcriptional activator (TrAP). .The protein encoded by *AC3* is the replication enhancer (Ren) and the protein encoded by *AC4* functions as a suppressor of RNA silencing. In DNA-B, the *BV1* codes for a Nuclear shuttle protein (NSP) and the *BC1* for a Movement protein (MP), required for intracellular and intercellular movement of the viral DNA respectively. The non-coding region (called Intergenic region, IR, approximately 500 bp) contains the origin of replication, where the viral Rep protein binds for initiating Rolling Circle Replication. A part of this region is conserved between the two DNA components of bipartite begomoviruses. The IR also harbours the promoter/ regulatory elements for expression of the viral genes in both V-sense and C-sense strand.

The International Committee on the Taxonomy of Viruses (ICTV) has recommended a classification and scheme of nomenclature, based on complete nucleotide sequences of the DNA-A of bipartite and the complete single-component DNA of monopartite begomoviruses. According to the above recommendation, two sequences belong to two viruses if their

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overall DNA sequence is less than 89% identical, and are considered to be variants of the same virus, if the identity is more than 89%. The name of each virus should reflect the host plant, common symptoms and the abbreviated form should also reflect the year and location of collection of that particular virus (Fauquet *et al.* 2000, 2003).

Monopartite begomoviruses are often associated with one or more smaller DNA (sub-viral) components, about 1.4 kb in size, known as satellite DNAs. Two types of satellite DNAs are known: the alphasatellites and betasatellites, depending upon the organization of their DNA and their effects on the symptoms produced by the helper begomovirus. The alphasatellites (previously known as DNA-1) encode their own replication-associated protein and are believed to have originated from another class of single-stranded DNA viruses, the nanoviruses. The betasatellites (previously known as DNA-β), on the other hand, do not code for any replication-associated proteins but carry a single ORF (β*C1*), encoding a multifunctional protein, having, among others, RNA silencing activity. Both the alpha- and betasatellites are dependent upon the helper virus for replication and, in many cases, attenuates the symptoms produced by it (Idris *et al.* 2011).

Viral infections trigger a series of defence responses in plants, manifested by specific degradation of the invading viral RNA into small fragments (siRNA), a response known as RNA-interference (RNAi). This defence response is conserved in many organisms and the components participating in this response share a high degree of homology. It is now believed that successful viral infection results only upon suppression of this defence response by specific viral proteins, known as RNAi suppressors. RNAi suppressor activities have been discovered in several begomoviral gene products (reviewed by Voinnet 2005).

Genetic resistance against plant viruses, if available in the germplasm, is considered to be the one of the most efficient ways to control viral infections. The genes conferring such resistance phenotypes can be transferred to cultivated varieties by breeding, often assisted by molecular markers. Against begomoviruses, very few resistance genes are known, the most important of them being the *Ty* series of genes available in wild tomato (*Solanum chilense*) against *Tomato yellow leaf curl virus* (TYLCV), a begomovirus infecting cultivated tomato, mainly in the Mediterranean countries. However, although the above gene and its alleles have been introgressed into commercial tomato cultivars for TYLCV resistance, convenient molecular tools for its widespread use are not yet available (Verlaan *et al*. 2011).

Transgenic resistance against begomoviruses has been achieved in a number of plants using a variety of strategies. The strategies include expressing proteins of viral origin (CP, Rep and its derivatives and TrAP), expressing non-viral proteins having an anti-viral effect (toxic protein dianthin, antibodies raised against viral CP), DNA interference involving defective viral DNAs and RNAi against viral transcripts (reviewed by Vanderschuren *et al*. 2007). More recently, novel resistance approaches, such as expressing the GroEL chaperon protein of bacterial endosymbionts, which protects the begomoviral particle from degradation within the vector whiteflies, in the phloem tissue of tomato plants, have resulted in the viral particles getting trapped within the plants, thereby resulting in resistance (Akad *et al*. 2007).

The emerging threat of the viruses belonging to the genus *Geminivirus* has been extensively addressed earlier by Varma and Malathi (2003). The extent of yield loss caused by some geminiviruses has been estimated by Dasgupta *et al.* (2003) to be as high as 100%. Up to 96% loss in yield has been reported by *Bhendi yellow vein mosaic virus* (Pun and Doraiswamy 1999). In legumes, the yield losses have been estimated to be approximately $300 million per year taking blackgram, mungbean and soybean together (Varma and Malathi 2003).

Within the last decade or so, a large number of begomoviruses have been investigated in India, encompassing fibre crops, legumes, root crops and vegetables. More recently, investigators in India are focusing on the molecular interactions between begomoviruses and their hosts, natural and experimental, to gain insights on the molecular cross-talk, which might throw light on newer and hitherto unexplored aspects of their biology and reveal novel approaches for their control and management. Considering the importance of begomoviruses to Indian agriculture, the salient research achievements related to begomoviruses, carried out in India in the last approximately 15 years, have been reviewed here and the possible directions in which future efforts could be channeled to manage these viral diseases more effectively have been pointed out.

2. Begomovirus research in India

Till date, begomoviruses have been reported from at least 16 different groups of crop in India (table 1). Extensive work has been carried out on these viruses on their sequence analysis, phylogeny, infectivity, functions of viral proteins, virus–host interactions, virus-derived transgenic resistance and associated satellites. This review collates the research work performed in India, focusing on the above aspects. They are described in the alphabetical order of their major hosts below.

2.1 *Bhendi*

Bhendi yellow vein mosaic disease was first reported from Bombay (presently Mumbai) in India (Kulkarni 1924). The causative virus, *Bhendi yellow vein mosaic virus* (BYVMV), was shown to be a begomovirus based on its morphology and serological relationship with other begomoviruses, such as *African cassava mosaic virus* (Harrison *et al.* 1991).

Table 1. Molecular studies of important begomoviruses in India

Name of the virus	Crop infected	Mono-/bipartite	Association of satellite(s), if any	Symptoms produced	Reference(s)
Bhendi yellow vein mosaic virus	Bhendi/ Okra	Monopartite	Betasatellite	Vein clearing, yellowing. Reduced size of leaves and fruits	Jose and Usha 2003; Kulkarni 1924
Bittergourd yellow mosaic disease associated virus	Bittergourd	–	–	Leaf yellowing and mosaic	Raj *et al.* 2005a
Chili leaf curl virus, *Chili leaf curl virus-India*	Chili	Monopartite	Betasatellite	Yellowing, leaf curling,, stunting,and blistering, shortening of internodes	Shih *et al.* 2006 ; Chattopadhyay *et al.* 2008
Cotton leaf curl Rajasthan virus, *Cotton leaf curl Multan virus*, *Cotton leaf curl Kokhran virus*	Cotton	Monopartite	Betasatellite, Alphasatellite (suspected)	Leaf curling, vein darkening, vein swelling, and enations on undersides of leaves	Ahuja *et al.* 2007 ; Kirthi *et al.* 2002
Croton yellow vein mosaic virus	Croton	Monopartite	Betasatellite	Yellow vein mosaic, leaf deformation, vein swelling and stunting	Snehi *et al.* 2011
Cucumis yellow mosaic disease associated virus	Cucumis	Bipartite (suspected)	–	Leaf yellowing and mosaic	Raj and Singh 1996
Indian cassava mosaic virus, Sri Lankan cassava mosaic virus	Cassava	Bipartite	–	Leaf mosaic, mottling and stunting	Hong *et al.* 1993; Calvert and Thresh 2002; Dutt *et al.* 2005
	Jatropha	–	–	Severe leaf mosaic, reduced leaf-size, and stunting	Raj *et al.* 2008
	Bittergourd	–	–	Mosaic and mottling of leaves; various degrees of chlorosis and blistering	Rajinimala and Rabindran 2007
Mesta yellow vein mosaic virus Mesta yellow vein mosaic Bahraich virus	Mesta	Monopartite	Betasatellite	Yellowing of veins and veinlets followed by complete chlorosis of leaves	Chatterjee *et al.* 2005a, 2005b; Chatterjee and Ghosh 2007b; Roy *et al.* 2009
Mungbean yellow mosaic India virus, Mungbean yellow mosaic virus, Dolichos yellow mosaic virus	Legumes (soybean, mungbean, blackgram, greengram, frenchbean, cowpea, dolichos bean)	Bipartite	–	Leaf yellowing, mosaic	Varma and Malathi 2003 ; Balaji *et al.* 2004; Girish *et al.* 2005
Papaya leaf curl virus	Papaya	Bipartite (suspected)	–	Leaf curl	Saxena *et al.* 1998
	Potato	Bipartite (suspected)	–	Apical leaf curl	Venkatasalam *et al.* 2005, 2011

Table 1 (continued)

Name of the virus	Crop infected	Mono-/bipartite	Association of satellite(s), if any	Symptoms produced	Reference(s)
Potato apical leaf curl virus					
Tobacco leaf curl Pusa virus [India: Pusa:2010]	Tobacco	Monopartite	Betasatellite, Alphasatellite	Various symtoms	Singh *et al.* 2011
Tomato leaf curl New Delhi virus, Tomato leaf curl Bangalore virus, Tomato leaf curl Gujarat virus, Tomato leaf curl Karnataka virus	Tomato	North Indian isolates bipartite, south Indian isolates monopartite	Betasatellite	Vein clearing, stunting; infection at the seedling stage can make plants sterile	Saikia and Muniyappa 1989; Srivastava *et al.* 1995; Chatchawankanphanich *et al.* 1993; Chakraborty 2003; Pratap *et al.* 2011
	Mentha	–	–	Rugosity, light yellow mosaic, upward curling, crinkling, retarded growth and drastic reduction in yield	Samad *et al.* 2008; Borah *et al.* 2010
	Eggplant	Bipartite	–	Yellow mosaic and mottling	Pratap *et al.* 2011

Inoculation of bhendi plants with cloned BYVMV DNA, a monopartite begomovirus, produced mild symptoms; typical vein yellowing symptoms were produced only in association with the cognate betasatellite (Jose and Usha, 2003), possibly due to the silencing suppression activity of the βC1, reported later (Gopal *et al.* 2007). The CP showed nuclear localization, whereas the βC1 localized to the cell periphery (Kumar *et al.* 2006).

The V- and C-sense promoters of BYVMV were studied in transgenic *Nicotiana benthaminana*, a common laboratory host plant to study begomoviruses, using a modified *GFP* as the reporter gene for promoter function. The C-sense promoter was found to be stronger than the V-sense promoter in the absence of AC2 protein, which was shown to be a weak suppressor of silencing. On the other hand, AC4, a strong silencing suppressor, produced abnormal phenotype when expressed in transgenic *N. benthamiana* plants (Gopal *et al.* 2007).

2.2 *Bittergourd*

Natural occurrence of yellow mosaic disease (YMD) was observed on bitter gourd (*Momordica charantia*, family: Cucurbitaceae), which lowers level of several nutrients and antioxidant (Raj *et al.* 2005a). Association of begomovirus with the disease was shown using PCR with begomovirus-specific primers and Southern hybridization. The virus was named as *Bitter gourd yellow mosaic virus* (BGYMV; Raj *et al.* 2005a). The whitefly transmissibility of BGYMV was demonstrated subsequently (Rajinimala *et al.* 2005). Later, by immunological and PCR analysis, *Indian cassava mosaic virus* (ICMV) was also found to be present in bittergourd which was whitefly- but not sap-transmissible to healthy host (Rajinimala and Rabindran 2007).

2.3 *Cassava*

Cassava mosaic disease (CMD) had been reported in India in 1966 (Alagianagalingam and Ramakrishnan 1966). CMD has subsequently become prevalent in southern India (Calvert and Thresh 2002), resulting in yield losses of between 10–15%. Two bipartite begomoviruses have been recognized to be the causative agents for CMD in India; ICMV and *Sri Lankan cassava mosaic virus* (SLCMV). The DNA-A and DNA-B of two isolates of ICMV was first cloned and sequenced by Hong *et al.* (1993). SLCMV, earlier reported from Sri Lanka (Saunders *et al.* 2002) was also reported in India (Patil *et al.* 2005) whose infectivity was demonstrated on the natural host, cassava, thus fulfilling the Koch's postulates (Dutt *et al.* 2005). Later, in a biodiversity study, while ICMV was found restricted to only certain regions, SLCMV was found to be rather widespread in southern India. In addition, based on PCR-RFLP patterns

from multiple samples, it was concluded that the isolates show high diversity (Patil *et al.* 2005; Rothenstein *et al.* 2006). Phylogenetic analysis of several CMD-affected cassava samples revealed recombination among the population of cassava infecting-begomoviruses in India (Rothenstein *et al.* 2006).

In several geminivirus-infected plants, smaller sized DNAs, referred to as defective DNA (def-DNA), often occur naturally (reviewed by Patil and Dasgupta 2006), some of which interfere with virus proliferation (Stanley *et al.* 1990; Frischmuth and Stanley 1991). Several isolates of ICMV and SLCMV produce def-DNA in bombarded *N. benthamiana*, some of which represented recombinants between DNA-A and DNA-B. Some DNA-B derived def-DNAs were associated with a decrease in levels of normal DNA-B, with a concomitant change in the symptoms (Patil *et al.* 2007). Agroinfection of cloned SLCMV DNA to *Arabidopsis thaliana* has been demonstrated, thus opening up possibilities of further studies on plant–virus interactions (Mittal *et al.* 2008). Very recently, virus-free cassava, generated by meristem-tip culture, was used to study the whitefly transmissibility of the viruses in cassava. Using cassava-adapted whiteflies, the authors have reported symptom appearance after 25th day of inoculation and the transmission to be 85% (Duraisamy *et al*. 2012).

2.4 *Chili*

The etiology of Chili leaf curl disease was reported during the 1960s (Mishra *et al.* 1963; Dhanraj and Seth 1968). Based on partial DNA-A sequences, a monopartite begomovirus was reported to be associated with the disease which was a strain of the *Chili leaf curl virus* (ChiLCV) from Pakistan (ChiLCV-PK [PK:Mul:98]; Khan *et al.* 2006; Senanayake *et al.* 2006); the complete virus was subsequently cloned and sequenced, and was found to share 95% sequence identity with ChiLCV-PK [PK:Mul:98]. Besides, its infectivity was demonstrated in the natural host (Chattopadhyay *et al.* 2008). Meanwhile, *Tomato leaf curl Joydebpur virus*, reported earlier from tomato from Joydebpur, Bangladesh, was also found to be associated with chili leaf curl disease in Punjab (Shih *et al.* 2006).

2.5 *Cotton*

The Cotton leaf curl disease is characterized by leaf curling, darkened veins, vein swelling and enations on the undersides of leaves that frequently develop into cup-shaped, leaf-like structures (Briddon and Markham 2001). The disease was observed in northwest India for the first time (Rishi and Chauhan 1994). Thereafter, it spread throughout northern India in a short span of 4 to 5 years (Monga *et al.* 2004) and has now become a potential threat in the irrigated cotton production belt of the country, especially in Haryana, Punjab and Rajasthan in north India (Rishi and Chauhan 1994; Ahuja *et al.* 2007) and in Karnataka in south India (Nateshan *et al.* 1996). At least some strains of the virus can use tomato as an alternate host (Khan and Ahmed 2005).

Cotton leaf curl disease in India is caused by several monopartite begomoviruses associated with a betasatellite (Kirthi *et al.* 2002). At least four begomoviruses are associated with this disease in India, namely, *Cotton leaf curl Rajasthan virus* (CLCuRV), *Cotton leaf curl Multan virus*, *Cotton leaf curl Kokhran virus* (CLCuKV) and *Tomato leaf curl Bangalore virus* (Ahuja *et al.* 2007). The *CP* gene sequence of another Indian isolate, *Cotton leaf curl virus-Hissar 2*, was reported from Haryana, India, which showed 97.3% amino acid sequence identity with *Pakistan cotton leaf curl virus* (Sharma *et al.* 2005). A recent work has identified two new isolates, CLCuV-SG01 and CLCuV-SG02 from Rajasthan, which are reportedly recombinants with other begomoviruses (Kumar *et al.* 2010). A recombinant CP of a cotton leaf curl virus strain was observed to have non-specific ssDNA binding activity, which demonstrates a possible role of the protein in virus assembly and nuclear transport; this property being possibly conferred by a conserved C2H2-type zinc finger motif (Priyadarshini and Savithri 2009).

2.6 *Croton*

A monopartite begomovirus and a betasatellite was recently isolated and completely sequenced from symptomatic Croton (*Jatropha gossypifolia*) plants from Lucknow, India. The DNA-A molecule of the resident begomovirus shared 96% identity with *Croton yellow vein mosaic virus* (CYVMV, from public database) infecting *Croton bonplandianum* in India. The betasatellite showed the 96% sequence identity with CYVMV-associated betasatellite isolated from *Croton* sp. in Pakistan (Snehi *et al.* 2011).

2.7 *Cucumber*

Association of a geminivirus with yellow mosaic disease of *Cucumis sativus* was shown by nucleic acid-hybridization assay. The disease was transmitted experimentally onto healthy hosts by whiteflies (Raj and Singh 1996).

2.8 *Eggplant*

Recently, from central India (Nagpur) a bipartite begomovirus has been reported from eggplant showing yellow mosaic and mottling symptoms. The DNA-A and DNA-B of the virus shared high identities with the respective DNA

components of *Tomato leaf curl New Delhi virus* (ToLCNDV). Hence, this showed that the ToLCNDV has started invading a new host, eggplant. Its pathogenicity was also demonstrated in eggplant and tomato by agroinfiltration (Pratap *et al.* 2011).

2.9 *Jatropha*

Jatropha curcas is grown in India as a major bio-diesel crop. A viral disease of jatropha causing systemic mosaic and chlorosis in northern India was reported in 2006–07, which could be transmitted by whiteflies, but not mechanically. Using begomovirus-specific primers, several partial DNA-A sequences were cloned, all of which showed high homology with ICMV and SLCMV (Raj *et al.* 2008).

2.10 *Legumes*

Yellow mosaic disease (YMD) in legumes such as blackgram (*Vigna mungo*) and mungbean (*V. radiata*) was first reported by Nariani (1960), which may cause up to 85–100% yield loss (Nene 1973). YMD in soybean amounted to a yield loss of 105,000 metric tonnes (Wrather *et al.* 1997). The disease is caused by begomoviruses with bipartite genomes (Honda and Ikegami 1986; Vanitharani *et al.* 1996; Mandal *et al.* 1997; Karthikeyan *et al.* 2004). Although four species of begomoviruses have been reported to cause YMD of legumes in India (Qazi *et al.* 2007), two species, *Mungbean yellow mosaic India virus* (MYMIV) and *Mungbean yellow mosaic virus* (MYMV; Fauquet and Stanley 2003), are prevalent; the remaining two, *Dolichos yellow mosaic virus* (infecting *Lablab purpureus*; Maruthi *et al.* 2006) and *Horsegram yellow mosaic virus*, occur rarely.

Members of MYMIV infect several leguminous species like blackgram, cowpea (*V. unguiculata*), Frenchbean (*Phaseolus vulgaris*), mungbean and soybean (*Glycine max*; Varma *et al.* 1992). Molecular characterization of MYMIV isolates revealed more than 90% nucleotide sequence identity in DNA-A and DNA-B components between different isolates. Nevertheless, they show distinct host range (Varma *et al.* 1992; Mandal and Varma 1996). The DNA-A and DNA-B of MYMIV-Blackgram (MYMIV-Bg) isolate was cloned (Varma *et al.* 1991) and the infectivity was demonstrated using agroinfiltration as well as whiteflies (Mandal *et al.* 1997). A begomovirus infecting *Lablab purpureus* syn. *Dolichos lablab* was reported, showing high similarity to other MYMIV isolates (Singh *et al.* 2006). A pseudorecombinant between the DNA-A of MYMIV and DNA-B similar to that of MYMV is associated with the mosaic disease of cowpea. The cloned virus infected the natural hosts as well as several other legumes. However, the virus was not whitefly transmissible to any other but the natural host (John *et al.* 2008). A bipartite begomovirus isolate causing YMD in blackgram in southern India has been identified recently, DNA-A of which is a variant of MYMV, and DNA-B, a variant of MYMIV. The virus produced differential symptom in different leguminous hosts upon infection (Haq *et al.* 2011).

The causal organism of a mosaic disease of Velvet bean (*Mucuna pruriens*), which is used for medicinal purposes in India, was recently found to be a bipartite begomovirus and named *Velvet bean severe mosaic virus-[India: Lucknow:2009]*. The DNA-A sequence showed the highest identity (76%) with MYMIV. Koch's postulate was also validated for the combination of DNA-A and DNA-B (Zaim *et al.* 2011). A bipartite begomovirus has been recently cloned from *Rhynchosia minima*, a common weed of *Leguminoseae* family. The viral nucleotide sequence shares 84% identity to *Velvet bean severe mosaic virus* and the virus has been named as *Rhynchosia yellow mosaic India virus* (Jyothsna *et al.* 2011).

By *Agrobacterium*-mediated delivery into *N. benthamiana* and different legumes, the 5′ transcription start site of the genes in the viral sense strand and *AC1* transcripts were identified and the promoter sequences were defined including the transcription factor binding sites. The complementary-sense promoter was differentially regulated and AC2 protein was found to be not necessary for the basal expression of the virion sense promoter (Usharani *et al.* 2006).

To understand the replication process of begomoviral DNA, properties of Rep and its interacting partners have been the focus of several studies. The Rep of blackgram-infecting MYMIV-Bg was found to bind to the intergenic region in a specific manner. The protein also undergoes ATP-regulated cleavage and conformational change, which may result in better access of Rep to the DNA-cleavage site. Site-specific topoisomerase function of Rep was revealed (Pant *et al.* 2001). Proliferative cell nuclear antigen (PCNA), an important endogenous DNA replication factor, is induced in response to MYMIV infection and interacts with Rep (Bagewadi *et al.* 2004). The Rep of MYMIV also acts as a replicative helicase in viral replication; it works as a large oligomer, needs less than six nucleotides to function and translocates in 3′-5′ direction (Choudhury *et al.* 2006). Similar helicase activity was also found in the Rep of MYMV and ICMV (Choudhury *et al.* 2006). Later, the smaller subunit of endogenous replication-associated protein, RPA, was found to interact with the C-terminus of MYMIV-Rep, enhancing its essential ATPase activity and down-regulating its nicking and closing activities (Singh *et al.* 2007). Another host factor, RAD54 (a known recombination/repair protein) has also been recently identified to be an essential interacting partner of Rep of MYMIV; the interacting domain of RAD54 was identified which enhances

the enzymatic activities of MYMIV-Rep (Kaliappan *et al.* 2012). In a recent study with mutants of AV2 of MYMIV, the ratio between open circular and supercoiled DNA was found to fluctuate (Rouhibakhsh *et al.* 2011a), indicating an important role of the protein in viral replication. This indication received a further boost from a related study where it was shown that the same protein significantly modulated the nicking activity of Rep (Rouhibaksh *et al*. 2011b).

Pseudorecombination seems to be very common among the begomoviruses infecting mungbean. A curious case of five different DNA-A molecules in combination with one DNA-B of MYMV-Vig co-infecting a single blackgram (*V. mungo*) plant was reported (Balaji *et al.* 2004). In a pseudorecombination study with DNA-A of MYMIV-Bg, MYMIV-Cowpea and MYMIV-Soybean (Sb) and DNA-B of MYMIV-Bg and MYMIV-Sb isolates, pseudorecombination between DNA-A and DNA-B was reported to occur in all legumes, except in cowpea, showing host-specificity of the viral components (Surendranath *et al.* 2005). A DNA-B swapping experiment has recently identified the *BV1/NSP* of MYMV to be the major symptom determinant (Mahajan *et al.* 2011).

There have been several efforts to achieve resistance against mungbean-infecting begomoviruses. MYMV-Vig transgenes (*CP*, *Rep*, *Rep*-antisense, truncated *Rep*, *NSP* and *MP*) were evaluated in transgenic tobacco (*N. tabacum*) by agroinoculation. Unexpectedly, the transgenic tobacco plants harbouring *CP* and *MP* ORFs accumulated even higher levels of viral DNA. However, viral DNA accumulation was inhibited in one transgenic plant harbouring the *Rep* (sense orientation) and in two plants harbouring the antisense-*Rep* ORF (Shivaprasad *et al.* 2006). To devise a specific antiviral strategy against geminiviruses, hammerhead ribozyme has been directed against the *Rep*-mRNA of MYMIV. The ribozyme showed ~33% cleavage activity on synthetic *Rep* transcript within 1 h under *in vitro* conditions. The *in vivo* efficiency of ribozyme was evaluated in yeast. The growth of the yeast cells was inhibited by 30% and generation time was increased by 2 h. A maximum of about 50% reduction in the *Rep* mRNA level and 65% decrease in the viral DNA replication in presence of the ribozyme was observed (Chilakamarthi *et al.* 2007).

A convenient transgenic system to evaluate MYMV DNA replication is tobacco leaf disc, in which the replication of the viral DNA can be easily monitored. Leaf discs of transgenic tobacco plants expressing truncated TrAP gene of MYMV at various levels showed a reduction in DNA accumulation, in proportion to the levels of transgene expression. Expressing the ssDNA binding protein VirE2 of *A. tumefaciens* in the above system also reduced viral DNA accumulation (Sunitha *et al*. 2011), which can be used as a promising tool to impart resistance against more than one geminiviurses.

To understand the molecular mechanism of natural resistance, distribution of MYMIV-derived siRNAs was analysed in a resistant variety of soybean. It was found that most of the virus-derived siRNAs were complementary to the IR in the resistant variety, while in the susceptible variety, a majority of the siRNAs corresponded to coding regions of the viral genome. Most of the IR-specific siRNA molecules produced in the resistant plants were 24 nt in size and a higher level of methylation occurred in the IR of viral DNA (Yadav and Chattopadhyay 2011).

The complete nucleotide sequences of two soybean-infecting begomoviruses were determined from central and southern India (Girish and Usha 2005). The isolate from central India was a strain of MYMIV, named as MYMIV-Sb [MP], and the southern Indian isolate was a strain of MYMV, named as MYMIV-Sb [Mad]. Multiple DNA-B components were detected with the later isolate. The sequence similarity between the DNA-A components of the two isolates was higher (82%) than the corresponding DNA-B components (71%). A considerable divergence in the origin of replication (*ori*) was also noted. Comparing these two and other isolates, the authors predicted possible recombination among all the legume-infecting begomoviruses from Southeast Asia. Subsequently, Usharani *et al.* (2004) determined the complete sequence of MYMIV-[Sb] from New Delhi. The Rep from MYMIV-Sb [MP] was overexpressed, refolded and characterized in *E. coli*, and found to form insoluble inclusion bodies (Girish *et al.* 2006). Nucleic acid recognition properties of the MP of MYMIV-Sb-[MP] were investigated by Radhakrishnan *et al.* (2008), who reported sequence non-specific binding to both ss- and dsDNA. The protein bound non-specifically to both ds- and ssDNA with higher affinity for the latter, and displayed size selection towards linear dsDNA (Radhakrishnan *et al.* 2008). In a study of four soybean cultivars differing in resistance to MYMIV, Yadav *et al.* (2009) reported more viral transcript accumulation in susceptible cultivars and enhanced production of small interfering (si)RNA much later after inoculation, than the resistant cultivars, suggesting an RNAi-mediated resistance mechanism. A candidate disease resistance gene, *cyr1* was recently isolated from *V. mungo*, which showed enhanced transcript levels upon MYMIV infection. In addition, prediction of 3-D structure of the protein product indicated a possible docking site for the MYMIV CP (Maiti *et al*. 2011).

2.11 *Mentha*

Mentha (mint) is a member of the genus *Mentha* (Family: Lamiaceae) and is used popularly as a flavouring agent and to extract menthol. Lately, association of a begomovirus has been reported on the basis of an approximately 800 bp begomoviral sequence cloned from symptomatic plants which showed 93% identity with *Tomato leaf curl Pakistan*

virus-Pakistan [*Rahim Yar Khan 1:2004*] (Samad *et al.* 2008). Subsequently, a complete begomoviral DNA-A sharing 94% identity with *Tomato leaf curl Karnataka virus-Bangalore* [*India:Bangalore:1993*], a betasatellite, Cotton leaf curl Multan betasatellite [India:New Delhi 2:2004] and a new satellite molecule (DNA-II) were cloned from symptomatic mentha plant. The virus was named as *Tomato leaf curl Karnataka virus-Bangalore*-[*India*:*Ludhiana*:*Mentha*:*2007*] (Borah *et al.* 2010). Transmissibility of the virus with whitefly to healthy host and of the virus and the betasatellite to *N. benthamiana* was also demonstrated.

2.12 *Mesta*

Mesta (*Hibiscus cannabinus*) is an important bast-fibre crop grown in India. The crop has medicinal values and is a raw material for paper industries (Duke 2008). In last few years, there have been several reports of occurrence of viral diseases of mesta in endemic form in different parts of India, namely the Yellow vein mosaic disease (YVMD). The disease causes yellowing of veins and chlorosis of the leaves at advanced stages of infection. Transmission electron microscopic investigation revealed association of geminate particles, 20 to 30 nm in size, with the disease (Chatterjee *et al.* 2005a). Southern blot analysis and nucleic acid spot-hybridization suggested a possible association of a begomoviral DNA-A, and a betasatellite with the disease (Chatterjee *et al.* 2005b). PCR amplification later confirmed the association of a begomovirus related to *Cotton leaf curl Rajasthan virus* with the disease (Chatterjee and Ghosh 2007a). The complete DNA-A was subsequently cloned and sequenced which showed the highest sequence identity (83.5%) with the CLCuV-[India:Bangalore:2004] isolate, hence indicating it to be a new virus. The name *Mesta yellow vein mosaic virus* (MYVMV) was proposed for the virus (Chatterjee and Ghosh 2007a). From YVMD-infected samples, *CP* gene sequences of two more isolates and a betasatellite were analysed. The nucleotide sequences of the *CP* from both the isolates showed a high sequence identity with *CP* of begomoviruses infecting tomato. The betasatellite had sequence similarity with those associated with the cotton-infecting begomoviruses from India and begomovirus infecting tomato from Pakistan (Chatterjee and Ghosh 2007a). Later, a new strain, closely related to *Cotton leaf curl Multan virus-Rajashthan [India: Sirsa:1999]* was reported to be associated with YVMD in mesta from northern India (Das *et al.* 2008a). In phylogenetic analysis of betasatellites associated with the disease, two groups were distinguished, showing close proximity with betasatellites previously reported from cucurbits and cotton respectively (Das *et al.* 2008b).

In a recent study, YVMD of mesta had been found to be associated with two different monopartite viruses, MYVMV in eastern India, and *Mesta yellow vein mosaic Bahraich virus* in northern India, together with two betasatellites, *Cotton leaf curl Multan betasatellite* and *Ludwigia leaf distortion betasatellite.* Build-up of the whitefly population was found to facilitate the spread of the disease (Roy *et al.* 2009). More recently, it has been found that virus infection leads to oxidative or nitrosative stress in mesta plants and the levels of several enzymes associated with salicylic acid pathway were significant changed. Besides, few defence-related proteins were identified using mass spectrometry in the infected plants (Sarkar *et al.* 2011).

2.13 *Papaya*

The *CP*, *Rep* and the IR of the genome of a begomovirus causing severe leaf curl in papaya plants were amplified, cloned and sequenced. The viral isolate was found to share 89.9% homology with ICMV and was named as *Papaya leaf curl virus-India* (PLCV-India). Analyses of the N-terminal 70 amino acid of the CP (and other biological features) of the virus showed its relatedness to begomoviruses from the Old World (Saxena *et al.* 1998). In a recent effort to confer resistance against geminiviruses infecting papaya, siRNAs were designed using computational tools, which could possibly be used against a wide spectrum of viral isolates and/or strains (Saxena *et al.* 2011).

2.14 *Potato*

A begomovirus had been reported to be associated with potato plants showing apical leaf curl symptoms in southern and northern India, using DAS-ELISA and nucleic acid-hybridization technique. The causal virus had been named as *Potato apical leaf curl virus* (Venkatasalam *et al.* 2005). Amplification of DNA-A component of the virus was performed from 27 samples, while DNA-B was amplified from only from two samples (Venkatasalam *et al.* 2011).

2.15 *Tobacco*

Leaf curl disease of tobacco (TbLCD) is endemic in India. A monopartite begomovirus, a betasatellite and an alphasatellite were found associated with the disease in Pusa, Bihar. The isolate was named as *Tobacco leaf curl Pusa virus [India:Pusa:2010]*. A betasatellite, associated with TbLCD, was found to be a variant of Tomato leaf curl Bangladesh betasatellite [IN:Raj:03], sharing 90.4% sequence identity. An alphasatellite, detected in the diseased plants, had 87% nucleotide sequence identity with Tomato leaf curl alphasatellite. Using sequence analysis, the

begomovirus was concluded to be a product of recombination of multiple begomovirus complexes (Singh *et al.* 2011).

2.16 *Tomato*

Tomato leaf curl disease (ToLCD) is a common disease of tomato all over India. ToLCD was first reported in northern India by Vasudeva and Sam Raj (1948), and subsequently from central India (Varma 1959) and Southern India (Govindu 1964; Sastry and Singh 1973). Symptoms of ToLCD include leaf curling, vein clearing and stunting, which can often lead to sterility (Saikia and Muniyappa 1989). Several closely related begomoviruses associated with this disease have been cloned and sequenced from India. Two isolates from New Delhi, *Tomato leaf curl New Delhi virus-Severe* (ToLCNDV-Severe) and *Tomato leaf curl New Delhi virus-Mild* (TOLCNDV-Mild), with bipartite genomes, sharing 94% identity in the DNA-A component (with identical DNA-B components), were reported by Padidam *et al.* (1995), both of which contained an extra ORF (AV3). Four additional isolates, three from Bangalore (Chatchawankanphanich *et al.* 1993; Hong and Harrison 1995) and one from Lucknow (Srivastava *et al.* 1995) were also reported, which indicated that those from north India had a bipartite genome while those from south, monopartite genome (like the ones reported from Australia and Taiwan; Muniyappa *et al.* 2000). DNA sequences of two more isolates, ToLCV-Ban-2 and ToLCV-Ban-4 were reported from Bangalore, sharing 91% identity with each other. In addition, genomic sequences of two additional isolates, which were named *Tomato leaf curl Bangalore virus*, ToLCBV [ToLCBV-Ban-5] and [ToLCBV-Kolar] were reported subsequently (Kirthi *et al.* 2002). Later, a new begomovirus, *Tomato leaf curl Gujarat virus* (ToLCGV-[India: Varanasi:2001]), was reported from Varanasi, north India, and was shown to be infectious on the natural host (Chakraborty *et al*. 2003). In general, the population of tomato leaf curl viruses (ToLCVs) in India is highly diverse, which was shown after analysis of the *CP* sequence from 29 infected tomato samples across India. Five clusters (with less than 88% similarity among them) were observed among the population; while four of them represented the known tomato leaf curl viruses, one cluster showed more similarity (89%) with *Croton yellow vein mosaic virus* (Reddy *et al.* 2005).

Potential recombination sites among the DNA-A components of the strains/species of ToLCVs from Bangalore were mapped in an early study (Kirthi *et al.* 2002). There has been report of a distinct bipartite begomovirus from a temperate region (Palampur), which is possibly a natural pseudorecombinant (Kumar *et al.* 2008). Possible recombination has also been reported in two monopartite begomoviruses, one from New Delhi (ToLCV-CTM) and another from Kerala (ToLCV-K3/K5, Pandey *et al.* 2010); and *Tomato leaf curl Ranchi virus* (ToLCRnV), which is a putative recombinant of *Tomato leaf curl Bangladesh virus*, *Tobacco curly shoot virus* and ToLCGV. ToLCRnV is also associated with Tomato leaf curl Ranchi betasatellite (ToLCRnB) (which shares 74.5% sequence identity with Tomato leaf curl Bangladesh betasatellite; Kumari *et al.* 2011). ToLCNDV-Severe and ToLCGV-[India:Varanasi:2001] were reported to act synergistically; the DNA-A of the former virus enhancing the replication of DNA-B of the latter, and *vice versa*. This resulted in enhanced pathogenicity when DNA-A of ToLCNDV-Severe was trans-complemented with ToLCGV-[India:Varanasi:2001] DNA-B (Chakraborty *et al.* 2008).

Sivalingam *et al.* (2010) have investigated the diversity of betasatellites associated with ToLCD in various parts of India. They report two new betasatellites associated with the disease and found that the reported betasatellites formed four major clusters according to geographical distribution. The betasatellites from central and southern India were found to be more closely related than those of northern India. Depending on sequence information, they also concluded that betasatellites move across plant species. As in cotton, betasatellites are also major contributors of symptom production and viral DNA accumulation, as reported in the case of ToLCBV; besides, ToLCBV was also reported to be capable of transreplicating non-cognate betasatellites (Tiwari *et al.* 2010).

In an early study, the amino acids #65 and #172 of the CP of ToLCNDV were shown to be essential for symptom production (Padidam *et al.* 1995). Besides, AV2 was shown to be required for viral ssDNA accumulation. AV2, along with BV1 and BC1, are also involved in efficient viral movement. It was demonstrated that changes in DNA-A virion-sense mRNA structure or translation affect viral replication (Padidam *et al.* 1996). In another gene-function study, the replicase function was reported to be determined by the amino acid residue Asn^{10} in the N-terminus of Rep protein (Chatterji *et al.* 1999). The 134-amino-acid-long AC3 protein of *Tomato leaf curl Kerala virus* (ToLCKeV) was seen to form 12 to 14 mers and to interact with the AC1 protein *in vitro*. This interaction resulted in an increase in the ATPase activity of AC1 up to 200%, resulting in enhancement of viral replication (Pasumarthy *et al.* 2010).

There have been several efforts to confer resistance against the tomato leaf curl viruses in India using different strategies. Transgenic tomato lines harbouring the *CP* of ToLCNDV-[India:Lucknow] were generated, which showed durable resistance against the virus (Raj *et al.* 2005b). Transgenics carrying antisense sequence of *Rep* gene was shown to recover from ToLCD (Praveen *et al.* 2005a, b). In a biosafety analysis, the above transgenics were shown to be non-toxic to mice (Singh *et al.* 2009), thereby making the product easily acceptable to consumers. In another effort for

engineering resistance, targeting the conserved regions in *AC1*/*Rep* (overlapping sequences of the *AC4* ORF) with hairpin-mediated strategies was shown to be a promising means to suppress a wide spectrum of ToLCVs infection in tomato (Ramesh *et al.* 2007). In a recent study, multiple siRNAs were designed using computational tools, which could possibly be used against a wide spectrum of ToLCVs (Saxena *et al.* 2011).

Virus-induced gene silencing (VIGS) vector are useful tools for the study of gene functions in plants. A VIGS vector, spanning the intergenic region and the *AC3* had been constructed from ToLCV, which was shown to successfully silence endogenous plant gene, PCNA. It was also shown that a mutation in the *AC3* (a putative silencing suppressor) can increase the silencing efficiency several folds (Pandey *et al.* 2009).

Some of the factors important in the symptom development of geminivirus-infected host have been investigated in relation to host small RNA pathway. Naqvi *et al.* (2010) reported that, following infection of ToLCNDV, several miRNAs (miR159/319, miR172) are significantly deregulated in leaves, but not in flowers of tomato plants, leading to a corresponding change in the expression levels of their target genes. These may form, at least partly, the basis of symptom development seen in ToLCNDV-infected tomato (and other geminivirus infections). In a subsequent study, the same group, using *in silico* analysis, predicted a number of tomato miRNAs to have potential to bind to ToLCNDV proteins resulting in a dynamic interaction between the host and ToLCNDV (Naqvi *et al.* 2011).

Genetic resistance against geminiviruses is known in some crops which can act as sources of resistance, and as subjects for study of plant–pathogen interaction. ToLCNDV-resistant cultivar H-88-78-1 has been found to differentially express 106 transcripts in response to viral infection, eight of which were induced more than fourfold compared to an un-infected control. They represented proteins participating in defence response, transcription, proteolysis and hormone signalling (Sahu *et al.* 2010). Similarly, about 20 ESTs in ToLCNDV-infected plants were induced that play crucial roles in innate immunity, metabolism and ethylene signalling. Expression patterns of few of them were also validated by RNA-blot analysis. While the regulation was found to be positive in leaves, flowers and fruits, it was negative in the stems and roots (Naqvi *et al.* 2011). Such studies will help in the deployment of genes in developing virus resistance using transgenics and marker-assisted selection.

3. Other advances

There are several other promising advances established in various Indian laboratories that might have important influence on research on begomoviruses. A simple method of concentrating the supercoiled replicative form of geminiviral DNA using alkaline denaturation procedure, identical to plasmid isolation, was developed for cloning of begomoviral genomes associated with ToLCD (Srivastava *et al.* 1995). The importance of ToLCD has prompted a need for rapid identification of the causative viruses in its hosts and vector; therefore, a PCR methodology to identify the viral DNA inside the vector *Bemisia tabaci* was developed (Khan 2000). A yeast model system to support MYMIV replication was developed by Raghavan *et al.* (2004), which could be used to understand the factors required for viral replication and which could possibly be extended to other viruses. For legumes, an inexpensive protocol for the detection of whitefly-transmitted begomoviruses was reported (Rouhibakhsh *et al.* 2008). It involves extraction of DNA with a modified CTAB buffer containing up to 5% β-mercaptoethanol and 1.4 to 2.0 M sodium chloride. Using this method, PCR amplifiable DNA could be extracted from mature leaves of legume hosts rich in polyphenols, tannins and polysaccharides. Karjee *et al.* (2008) described two assay techniques to screen viral-encoded RNAi suppressor(s) of begomoviruses, one based on reversal of silencing and another on enhancement of replication of the virus. *Agrobacterium* cells harbouring tandem partial (or complete) dimers of the begomoviral genome(s), cloned in a binary vector, is a preferred method for infectivity to the plant, a process known as agroinfection. In case of bipartite begomoviruses, inoculation is generally performed with a mixture of two *Agrobacterium* cultures, containing the partial dimers of DNA-A and DNA-B. However, successful infection of soybean with a single binary plasmid containing tandem dimers of both DNA-A and DNA-B of MYMIV was demonstrated (Yadav *et al.* 2009).

4. Conclusion and future prospects

A large number of begomoviruses have been reported from India, probably in part due to its warm tropical climate supporting the year-round survival of the whitefly vector and intensive crop cultivation. Begomoviruses cause serious diseases in many crop plants, which is undoubtedly a primary reason for the strong research interest they have generated. An interesting aspect of Indian begomoviruses is their overlapping host range (for example, tomato-infecting begomoviruses have been reported from plants as diverse as chili, cotton and mentha and cassava-infecting begomoviruses have been reported from bittergourd and jatropha). One of the major factors responsible for this overlapping host range could be the polyphagous nature of the vector whitefly and the mixed cropping systems prevalent in the country. An expected consequence of this scenario would be recombination and one would assume that there would

be many more reports of such recombination events between begomoviruses than what has actually been reported, once bioinformatic tools for their detection are widely applied by the researchers. This also requires extra vigilance on the part of researchers to anticipate and deal with synergistic effects of multiple infections and/or recombinations, resulting in the emergence of unexpectedly virulent forms of begomoviruses, as experienced in the case of cassava begomoviruses in the African continent in the last decade. Since RNAi suppression by the begomoviruses is likely to play a major role in such synergisms, it necessitates a careful look into the mechanisms of RNAi suppression by begomoviruses prevalent in India.

The emergence of a large number of betasatellites and more recently, alphasatellites associated with begomoviruses in India is also remarkable. The interdependence of the satellites and their helper begomoviruses is thus an area of immense importance for investigation and promises to be both scientifically fascinating as well as amply rewarding by opening up new methods of disease control, a challenge for begomoviral diseases in India.

The methods of controlling begomovirus infections in crop plants are expanding. The reports of success in controlling begomoviruses with virus-derived and other transgenes are encouraging. Well-characterized resistance genes hold a lot of promise in controlling begomoviruses. However, as mentioned earlier, only a few such genes have been characterized to a level where they can be used for breeding begomovirus resistance and can be used to introgress into popular crop varieties. Hence, more work needs to be undertaken to search for natural begomovirus-resistant wild varieties of crop plants, against begomoviruses, and, when found, to characterize the resistance traits. One recent example is the characterization of a new resistance trait in pepper against the two begomoviruses, *Pepper golden mosaic virus* and *Pepper huasteco yellow vein virus* in Mexico (Garcia-Neria and Rivera-Bustamante 2011).

The interaction of begomoviruses with the vector whiteflies, a crucial step in the spread of begomoviruses in the field, also needs to be carefully looked at. These, as well as the exciting developments on plant–virus interactions, promise many more avenues of begomovirus control opening up in the near future. These need to be urgently deployed to assure crop protection against the huge losses incurred due to begomoviral infections in India.

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