Sangeeta Saxena · A. K. Tiwari *Editors*

Begomoviruses: Occurrence and Management in Asia and Africa

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Preface

Begomoviruses of family *Geminiviridae* are fast-evolving plant viral pathogens with small circular single-stranded DNA as genome. They cause diseases in various crops in the tropical and subtropical regions, and with change in climatic conditions due to global warming, now temperate regions are also under the threat of these viruses. They are transmitted by the whitefly (*B. tabaci*) and enjoy a wide host range. Begomoviruses are geminate particles and can be either monopartite or bipartite based on the number of genomic components present as one (DNA-A) and two (DNA-A and DNA-B), respectively. The two genomic components (bipartite) designated as DNA-A and DNA-B are of ~2,600–2,800 nucleotides each. A number of serious diseases of commercially cultivated crops of the *Fabaceae*, *Malvaceae*, *Solanaceae*, and *Cucurbitaceae* families are caused by begomoviruses which are considered as a threat to their cultivation in India and abroad. Accurate and reliable diagnosis is important for successful disease management, since plants infected by begomoviruses do not recover and uprooting followed by burning of infected material seems to be the only solution. Infected plants besides suffering serious yield losses also are a source of inoculums in the field as the virus is further picked up and spreads to healthy plants. Reports of occurrence of new viruses and reemergence of several known viruses in new niches are pouring in from all over the world. In such a dynamic system, the production of disease-free crops with optimum yield relies on the early detection of the causal virus and better understanding of its biology to evolve appropriate management strategies. Considerable progress has been achieved in the characterization, detection, and management of the virus on different crop species in the last decade. This book covers all the latest aspects of begomoviruses including their genome organization, diagnosis, transmission, management, and occurrence and a general introduction in Unit I. In Unit II, the current status of begomoviruses from countries of Asia and the African continent has been detailed giving a comprehensive overview. Each chapter illustrates the diseases caused by begomoviruses on different crops, detection techniques, and management strategies in support of research findings by the presentation of data, graphics, figures, and tables. This book will provide a wide opportunity to the readers to have complete information of begomoviruses from one source. It will be a useful resource for researchers and extension workers involved in begomovirus disease diagnosis and molecular biology. Expert detection, accurate diagnosis, and timely management play a significant role in keeping plants free from pathogens. In this book, expert

researchers have shared their research experiences straight from the lab to the field detailing traditional as well as transgenic approaches which are vital toward the control of begomoviruses across the globe. We believe this book will enhance the existing knowledge of readers in the field of plant pathology in general and geminivirus in particular.

Lucknow, Uttar Pradesh, India Sangeeta Saxena
Gola, Khiri, Uttar Pradesh, India Ajay K. Tiwari

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About the Editors

Dr. Sangeeta Saxena was born on March 15, 1968, in Dehradun, a city in the foothills of the Himalayas in India. She did her B.Sc. (botany (Hons)), M.Sc. (microbiology), M.Phil. (biotechnology), and Ph.D. from Aligarh Muslim University, Aligarh, India. Her Ph.D. was carried out at the Plant Virology Lab, National Botanical Research Institute (CSIR), Lucknow, after being awarded a CSIR-UGC JRF-NET fellowship from the Government of India. She obtained her Ph.D. degree through her thesis entitled "Development of diagnostics against some important papaya viruses" in 1998. Her Ph.D. work led to first-time identification of the organism causing leaf curl disease in India which is a begomovirus. Further she was awarded a postdoctoral fellowship from the Swedish Council for Scientific Research and Natural Sciences (NFR) and worked at the Swedish University of Agricultural Sciences, Umea S-901 83, Sweden, during Dec. 1998–Dec. 2000. She was awarded the DST Young Scientist Award under SERB Fast Track Proposal for Young Scientists scheme from the Department of Science and Technology, Government of India (from 2002 to 2005). She joined the Department of Biotechnology of Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India, in 2005 and is currently working there as an associate professor. Her area of research is molecular virology, RNAi in general and begomoviruses causing papaya leaf curl disease in particular. She has carried out four independent research projects in the area of plant virology, diagnostics, and RNAi from various funding agencies like CST-UP, UGC, DBT, and DST of the Government of India. She has published more than 40 research and review articles in national and international journals and has authored four book chapters in edited books. Dr. Saxena is widely traveled and has visited countries like Sweden, the USA, France, Finland, and China to attend several workshops and conferences. Currently, Dr. Saxena is studying various aspects related to intra- and inter-kingdom gene regulation by plant miRNAs apart from her main research interest to develop virus-resistant plants against begomoviruses.

Dr. Ajay K. Tiwari is working as a scientific officer at the UP Council of Sugarcane Research, UP, India. He did his Ph.D. in 2011 on cucurbit viruses in the Department of Biotechnology of CCS University, Meerut, UP, India. Dr. Tiwari is a regular member of the British Society of Plant Pathology, Indian Phytopathological Society, Sugar Technologists Association of India, International Society of Sugar Cane Technologists, Society for Sugarcane Research and Promotion, Prof. H. S. Srivastava Foundation, and Society for Plant Research and Educational Promotion. He has published 70 research articles and 12 review articles in national and international journals. He has also published six book chapters in edited books and has also authored seven edited books. He has submitted more than 150 nucleotide sequences of plant pathogens in the GenBank to his credit.

He is a regular reviewer and member of the editorial board for many international journals. He has been awarded the Young Researcher Award in Italy in 2011 and the Young Scientist Award by DST-SERB and was nominated for the Narasimhan Award by the Indian Phytopathological Society. Very recently he was awarded the Young Scientist Award by the Chief Minister of the State Government of UP for his outstanding contribution in the area of plant pathology. Dr. Tiwari is the recipient of many international travel awards given by DST, DBT, and CSIR from India, Patholux from Luxembourg, and IOM from Brazil. He has visited China, Italy, Germany, and Thailand for conferences and workshops. He has been involved in research on the molecular characterization and management of agricultural plant pathogens for the last 9 years. Currently he is working on the molecular characterization of sugarcane phytoplasmas and their secondary spread in nature.

Part I

Begomovirus: Occurrence and Transmission

Begomovirus: An Introduction

1

V.G. Malathi

1.1 Introduction

The genus *Begomovirus* belonging to the family *Geminiviridae* constitutes the largest group of plant-infecting DNA viruses affecting a wide range of dicotyledonous plants. The genus is one of the nine genera of the family *Geminiviridae* which have characteristic geminate (paired) particles (20 × 38 nm) consisting of two incomplete icosahedra ($T = 1$) containing a total of 110 coat protein subunits organized as 22 pentameric capsomers, encapsidating single-stranded circular DNA genome of 2.5–2.9 kb (Harrison et al. 1977; Stanley 1985). The members of the genus *Begomovirus* are transmitted by only one vector *Bemisia tabaci* and have either monopartite (DNA A) or bipartite (DNA A and DNA B) genome. The genus derives its name from the type member, *bean golden mosaic virus* (BGMV) that causes golden mosaic disease in bean in Central America.

The family *Geminiviridae* comprises nine genera differentiated on the basis of host range, vector, and the genome organization (Zerbini et al. 2017; Varsani et al. 2017). Of all the nine genera, *Begomovirus* is the largest one comprising about 322 species. The species demarcation threshold value is 91% identity in DNA A (Brown et al. 2015).

1.2 Diseases Causing Economic Loss

The symptoms caused by begomoviruses are mosaic, yellow mosaic, yellow vein mosaic, leaf distortion, enation, twisting and curling of leaves, and stunting (Fig. 1.1). If infection occurs at seedling stage, yield loss is severe. The diseases

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Fig. 1.1 Plant leaves showing typical symptoms caused by begomoviruses

caused by begomoviruses were recognized as devastating problems, as early as the nineteenth century. In fact the earliest record of the virus disease is a begomoviral disease. It is the yellow vein disease of *Eupatorium chinense*, described in a poem by Empress Manyoshu in the year AD752 referring to the yellow vein symptoms. The disease outbreaks like cassava mosaic (Africa, 1894), maize streak (South Africa, 1901), curly top disease of sugar beet (United States of America and Mediterranean in the 1900s), tobacco leaf curl (Indonesia, 1912; India, 1937), and cotton leaf curl (Sudan and Angola, 1931) are some examples to cite how begomoviruses can damage crops at large scale. In recent years, cotton leaf curl disease emerged as a serious threat to cultivation in Pakistan and India. Globally, leaf curl diseases of solanaceous and cucurbitaceous vegetables are challenging. It is very evident that begomoviruses are the major pathogens to reckon with in coming years.

1.3 Historical Perspectives

Though the whitefly-transmitted diseases are well known, the etiological agents causing the disease remained elusive until the 1970s–1980s; the purification protocol standardized by Bock et al. (1974), Goodman (1977), and Sequiera and Harrison (1982) revealed the association of the characteristic geminate particles. The DNA

genome was identified, and soon the virus members were grouped as "geminiviruses" (Harrison et al. 1977). The bipartite genome was revealed by bimodal infectivity curve and restriction profile for bean golden mosaic virus (BGMV) by Haber et al. (1981, 1983). The bipartite nature of the genome was confirmed by complete nucleotide sequencing of *African cassava mosaic virus* (ACMV) (Stanley and Gay 1983), *tomato golden mosaic virus* (TGMV) (Bisaro et al. 1982), and BGMV (Goodman and Bird 1978). Genomic comparison of DNA A and phylogenetic analysis established that though there is 60% identity in DNA A, Old World and New World viruses are well separated and of diverse lineage. Further characterization of Old World begomoviruses soon revealed the monopartite nature of viruses like tomato yellow curl virus (TYLCV) (Rochester et al. 1994; Navot et al. 1991). The infectivity analysis and viral gene functions were addressed by delivery of viral genomic components into the host, through *Agrobacterium*, the technique called as agroinoculation (Grimsley et al. 1987). When the infectivity of monopartite viruses did not produce typical symptoms in the primary host, further investigations revealed the presence of alphasatellites and betasatellites contributing to viral pathogenicity (Briddon et al. 2003; Saunders et al. 1999). The difficulties in cloning the genomic components from field hosts were overcome by the rolling circle amplification protocol (Haible et al. 2006). This method has facilitated cloning of genomic components of large member of begomoviruses.

1.4 Genome Organization

Begomoviruses have either bipartite or monopartite genome. The bipartite genome consists of two circular single-stranded DNA (2.5–2.7 kb) referred to as DNA A or DNA B. Both components are independently encapsidated, and geminate particles encapsidating A and B need to be acquired by whitefly-transmitted viruses together for successful expression of disease syndrome. Examples are *Indian cassava mosaic virus* (ICMV) and *mung bean yellow mosaic India virus* (MYMIV). The monopartite genome consists of only DNA A. DNA A alone is infectious and produces typical symptoms on experimental assay hosts and on primary hosts, e.g., *tomato yellow leaf curl virus-Israel* (TYLCV-Is) and *tomato leaf curl Karnataka virus* (ToLCKV). DNA A encodes genes necessary for viral encapsidation, replication, and movement. In begomoviruses, though ssDNA is encapsidated, it is double-stranded (ds) replicative form (RF) that is template for transcription. Transcription is bidirectional, and proteins are encoded in viral and complementary strand. The putative proteins and their predicted functions are given in Table 1.1. In DNA A viral sense strand has two open reading frames (ORF AV2 and ORF AV1; V2 and V1 in monopartite) in OW begomoviruses. In complementary sense strand, there are genes important for replication (AC1 and AC3) encoding replication initiation protein (Rep, ORF AC1) and replication enhancer protein (REn, ORF AC3). One more important ORF is ORF AC2 which activates the rightward ORFs of both DNA A and DNA B and so called as transcription activator protein (TrAP). The ORF embedded within ORF AC1 is ORF AC4/C4 which is a potential PTGS suppressor. In

Table 1.1 Begomovirus genes, putative protein products, and predicted functions

Open reading frame (ORF)	Putative protein	Predicted molecular weight (kDa)	Predicted function
AV2	Pre-coat protein, movement	~13.8	Movement in monopartite/PTGS suppression
	Protein-PCP		
AV1	Coat	~29.8	Coat protein
	Protein-CP		
AC1	Replication initiation	~40.7	Replication initiation
	Protein-Rep		
AC2	Transcription activator protein (TrAP)	~17	Transcription activator of rightward ORFs
AC3	Replication enhancer protein (REn)	~15.6	Replication enhancement
AC4		~11.4	PTGS suppression
BV1	Nuclear shuttle protein (NSP)	~29.2	Nuclear export
BC1	Movement protein (MP)	~32.4	Movement across plasmodesmata

DNA B, there is one ORF in viral sense strand coding for nuclear shuttle protein (ORF BV1, NSP) and one complementary sense coding for movement protein (ORF BC1, MP). The genome organization of begomoviruses is further detailed in Chap. 2, entitled "Genome Organization of Begomoviruses" in this book.

1.5 Satellite DNA Associated with Begomoviruses

The monopartite begomoviruses in general and few of the bipartite begomoviruses of the Old World are associated with additional single-stranded circular DNA components considered as satellites (1.3 kb). There are three types of satellites; the alphasatellites which are similar to DNA-R component of nanoviruses encode only one Rep gene having similarities with Rep protein of nanovirus. The betasatellite are the 1.3 kb ss circular DNA components which share the origin of replication sequence with the helper begomoviruses and are replicated by the Rep protein encoded by DNA A of helper begomoviruses. There is one ORF (beta C1) encoded in the complementary sense DNA of betasatellite which is the pathogenicity determinant and functions as silencing suppressor. All betasatellites have an extremely conserved region referred to as satellite-conserved region (SCR), upstream of origin of replication which is essential for replication. Among monopartite begomoviruses, though DNA A alone can infect plant and systemically move, inoculation along with betasatellites leads to severe symptom production like enation, leaf malformation, twisting, and stunting. A new set of noncoding subviral molecules (633–750) designated as deltasatellites (Lozano et al. 2016) have been identified recently with begomoviruses infecting sweet potato (sweepoviruses). They are structurally similar to subgenomic betasatellite associated with tomato leaf curl virus (ToLCV)

from Australia and have the conserved stem and loop structure with nonanucleotide sequence TAATATAC and SCR similar to betasatellites. The contribution of alphasatellites and deltasatellites to viral pathogenicity is not yet understood completely.

1.6 Intergenic/Common Region

Between the start codons of the leftward and rightward coding regions is present a non-coding intergenic region (IR). Within this region, a short stretch of ~180 to 200 nt segment is near identical between DNA A and DNA B components. This is the only region near identical in sequence between DNA A and DNA B components and so is called the common region (CR). The nucleotide sequence of CR is highly specific for a given begomovirus. The CR/IR contains (a) the invariant stem-loop sequence, a highly conserved nine-nucleotide sequence TAATATTAC conserved in all geminiviruses. It is within these sequences that replication is initiated. (b) The 6–13 bp repeat sequence called as iteron to which Rep binds. The number of repeats and arrangement of repeat is specific for a lineage of virus. (c) The cis- regulatory (TATA and CAAT box) and promoter sequence of both leftward and rightward ORFs. The segment from the tandem repeat of iteron to the end of the stem-loop sequence is considered to represent origin of replication (*ori*).

1.7 Detection and Characterization of Begomoviruses

Earlier detection of begomoviruses was mainly by ELISA using polyclonal antibody to any begomovirus or nucleic acid spot hybridization using DNA A probe. Once sequences of viruses became available, PCR using conserved region became a useful tool. However detection and characterization of begomoviruses were always a problem due to extreme low concentration of virus and difficulty in extracting good-quality PCR compatible DNA from field-grown plants, rich in mucilage and tannin. Since the begomovirus genome evolves fast, even PCR with viruses of known sequence also fail to work. In this background, the rolling circle amplification (RCA) technique derived by Dean et al. (2001), Jeske et al. (2001), and Haible et al. (2006) came as blessing. In this technique viral replicative circular DNA is enriched by performing RCA with high-fidelity phage Phi 29 DNA polymerase with random hexamers. Using this technique, more than 1500 full-length sequences have been generated.

1.8 Life Cycle of the Virus

The deep probing mouth parts of the vector place the geminivirus in protophloem cell inside the plant cell; the assembled virion particles or ssDNA/CP complex enters the nucleus. Inside the nucleus, viral ssDNA released from the particles

replicates and becomes double-stranded DNA. The replication is facilitated by host DNA polymerase. Detection of ribonucleotides complementary to SIR (short intergenic region) in mastrevirus is suggestive of initiation at the short intergenic region site through ribonucleotides priming. The dsDNA is transcribed by the host RNA polymerase II, and the earliest gene transcribed is the C1/AC1 or replication initiation/associated protein (Rep). The replication is by a combination of rolling circle replication and recombination-dependent replication. The Rep protein initiates replication by nicking at the nononucleotide sequence TAATATT_AC (Underscore indicates the site of nicking).

The newly synthesized + strand is copied into dsDNA again by host DNA polymerase which may enter the replication cycle. Alternatively the ssDNA may get encapsidated by the coat protein. The movement of viral DNA from the infection foci is mediated by the movement protein V2 in monopartite viruses or by BV1/BC1 in bipartite viruses. The viral DNA is transported out of the nucleus into the periphery of the cell from where they are docked on to plasmodesmata and transported into adjacent cells; finally the viral DNA (either ss or ds) enters into phloem parenchyma and companion cell. It is hypothesized that geminiviruses move as ss/ds DNA/movement protein complex, spread to young unfurling leaves from where they are acquired by the vectors. Excellent reviews, such as Stanley (1985), Harrisson and Robinson (1999), Hanley-Bowdoin et al. (1999, 2013), Gutierrez (2000), and Rojas et al. (2005), are available for researchers to get an in-depth knowledge on begomoviruses and the diseases caused by them.

The begomoviruses are introduced into fully differentiated protophloem cells by the whitefly. In order to ensure that host DNA synthesis machinery is available, the viral protein Rep reprograms the cell cycle pushing it from G1 phase to S phase. The viral proteins interact with various proteins involved in host signaling pathway, cell cycle, DNA machinery, and methylation, thereby bringing about changes in host gene expression. The PTGS suppressors of the begomoviruses interfere with RNA silencing pathway of the host at key events resulting in successful viral pathogenicity.

The begomoviruses have genetic propensity to evolve and acquire genome modifications by mutation, recombination, and by a unique phenomenon of component capturing. The genetic exchange between the viruses is promoted by mixed infection in the same plant or by occurrence of closely related viruses in the same field. With emergence of new recombinants and its active spread by diverse genotypes of the whitefly, the diseases caused by begomoviruses continue to be challenging ones.

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Genome Organization of Begomoviruses

2

Poonam Roshan, Aditya Kulshreshtha, and Vipin Hallan

Abstract

Begomoviruses are a group of plant viruses with small circular single-stranded DNA as genome. These are whitefly transmitted, geographically widespread, and responsible for the considerable economic losses. The members of this genus have a wide host range and have been reported from weeds and cultivated and noncultivated (wild) plants. Weeds and wild hosts serve as viral reservoirs, acting as source of inoculum for the crops of commercial importance. On the basis of number of genomic components present, virus is designated as monopartite or bipartite *Begomovirus*. Bipartite begomoviruses have two components, DNA-A and DNA-B, whereas the genome of monopartite begomoviruses is homologous to the DNA-A of the bipartite members. Owing to their small genome size, begomoviruses utilize both sense and antisense strands for protein synthesis. Monopartite begomoviruses are often associated with alpha- and betasatellites that are approximately half the size of viral genome. Betasatellite is essential for the pathogenicity and enhancement of the titer of viral DNA. Alphasatellites are believed to evolve from nanovirus Rep-encoding components and can autonomously replicate in the host plant cells. Recently, some New World begomoviruses are also found to associate with a satellite which is one quarter the size of genome molecule, named deltasatellite. This book chapter is focused on understanding the genome organization, function of viral proteins, and the associated satellite molecules.

Poonam Roshan and Aditya Kulshreshtha contributed equally to this work.

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Keywords
Begomovirus • Helper virus • Betasatellite • Alphasatellite • Deltasatellite • Recombination

2.1 Introduction

The genus *Begomovirus* is a member of the *Geminiviridae* family, characterized by circular single-stranded DNA (ssDNA) as a genetic material enclosed in a 22 × 38 nm-sized twinned icosahedral particles (Lazarowitz 1992; Harrison and Robinson 1999). Begomoviruses are transmitted by sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in a persistent and circulative manner. It has been recognized as the largest genus with >320 species and the members infect dicotyledonous plants (Brown et al. 2015). The oldest record of virus infection dates back to 752 AD, describing the yellowing of *Eupatorium* leaves in a Japanese poem. The yellow color of that plant was due to a *Begomovirus* betasatellite infection (Saunders 2003). *Begomovirus* infection has become a serious constraint for the agricultural crop production leading to the devastating cassava mosaic disease in sub-Saharan Africa, leaf curl of cotton in Indian subcontinent, leaf curl of tomato, yellow vein disease of okra, leaf curl of papaya, and yellow mosaic disease of mung bean (Varma and Malathi 2003). Other commercially important crops affected by the members of the group are *Abelmoschus esculentus*, *Ipomoea batatas*, chillies, beans, cucurbits, papaya, cabbage, and potato (Jose and Usha 2003; Miano et al. 2006; Kumar et al. 2011b; Leke et al. 2015; Nagata et al. 2016). Ornamental and cultivated crops infected by begomoviruses include *Althea rosea*, *Hibiscus cannabinus*, and *Zinnia elegans* (Briddon et al. 2003; Das et al. 2008; Kumar et al. 2010a). Medicinal crops infected by begomoviruses are *Eclipta prostrata*, *Pedilanthus tithymaloides*, *Croton bonplandianus, Jatropha gossypifolia*, *Mucuna pruriens*, *Vernonia cinerea*, *Amaranthus hypochondriacus*, *Rumex nepalensis* (unpublished), *Datura inoxia*, and *Chrysanthemum indicum* (Haider et al. 2006; Tahir et al. 2009; Snehi et al. 2011; Hussain et al. 2011; Zaim et al. 2011; Zulfiquar et al. 2012; Srivastava et al. 2014; Marwal et al. 2012, 2013). Noncultivated wild plants and weeds have become a hot spot for the recombination events and reservoir for the virus population. Some of these hosts are *Macroptilium lathyroides*, *Ageratum conyzoides*, *Rhynchosia minima*, *Alternanthera* sp., *Malvastrum coromandelianum*, *Mimosa invisa*, *Sida acuta*, *Digera arvensis*, *Xanthium strumarium*, *Crassocephalum crepidioides*, and *Sonchus arvensis* (Idris et al. 1999; Saunders et al. 2000; Ascencio-Ibanez et al. 2002; Briddon et al. 2008; Guo et al. 2008; Ha et al. 2008; Mubin et al. 2009; Mubin et al. 2012; Kumar et al. 2011a; Mubin et al. 2010a, b; Kulshreshtha et al. 2017). Typical symptoms of the *Begomovirus* infection are leaf curling, leaf rolling, vein yellowing, mosaic, and stunting of plant (Fig. 2.1). Over the past decade, the association of Old World (OW: Asia, Europe, Africa, Australia) begomoviruses with ssDNA satellite molecules (betasatellite, defective betasatellite, alphasatellite, and deltasatellite) has led to an

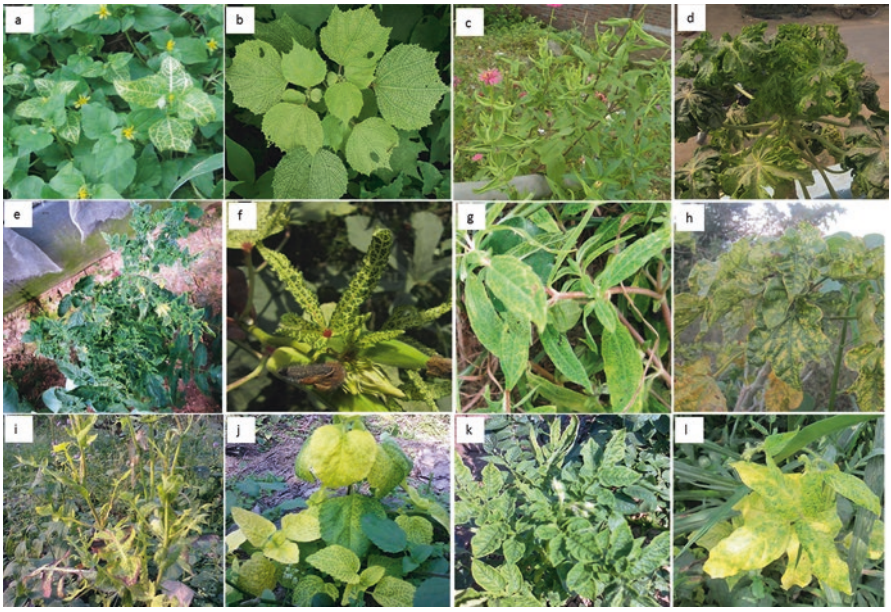

Fig. 2.1 Symptoms of *Begomovirus* on (a-l) *Synedrella* sp., *Urena* sp., *Zinnia elegans*, papaya, tomato, okra, *Eclipta* sp., *Jatropha* sp., *Sonchus asper*, *Ageratum conyzoides*, potato, and *Vigna* sp

increased incidence and severity of infection in the tropical and subtropical regions of the world (Mansoor et al. 2006; Zhou 2013).

Recombination, mutation, and reassortment introduce genetic variation in begomoviruses leading to high infection rates and an expanded host range, virulence, adaptation in changing environment; and evolution (Gutierrez et al. 2004; Seal et al. 2006; Padidam et al. 1999). It has been reported that the recombination between the virus and an associated satellite molecule plays an important role in the emergence of *Begomovirus* diversity in the OW (Nawaz-ul-Rehman and Fauquet 2009). In this chapter, we have described the genome organization, function of viral proteins, and the associated satellite molecules.

2.2 Origin and Distribution of Begomoviruses

Begomoviruses are considered to have been evolved from the primeval prokaryotic organisms as episomal DNA replicons that have adapted to the eukaryotic progenitors of modern plants. Over the time, these replicons might have developed new features as a result of recombination with the host genome (Rojas et al. 2005). These viruses have been broadly divided into two groups: Old World (OW) (Africa, Asia, Europe, Australia) and New World (NW) (Western Hemisphere, Americas) viruses on the basis of the genome organization and phylogenetic relationships (Paximadis et al. 1999). Bipartite begomoviruses are native to the NW, whereas

both monopartite and bipartite begomoviruses are present in the OW (Rybicki et al. 1994). Except *Tomato leaf deformation virus* (ToLDeV), a monopartite *Begomovirus*, that has been reported from the NW possesses, PWRsMaGT motif in the coat protein (Melgarejo et al. 2013). Begomoviruses seem to have evolved more than 10 million years ago (Lefeuvre et al. 2011). It was proposed that the NW begomoviruses have originated recently in comparison to the OW begomoviruses due to the continental drift of the Americas from the Gondwana region (Rybicki 1994). Occurrence of the bipartite *Begomovirus, Corchorus yellow vein virus* (CoYVV) in the OW suggested that the ancestors of NW viruses might have been already present in the OW prior to continental drift (Ha et al. 2006). Australia, Japan, Southeast Asia, Africa, Mediterranean region, and South and Central America have been identified as the centers of *Begomovirus* diversification (Fig. 2.2), and Southeast Asia has been recognized as the "center of origin" of begomoviruses on the basis of diverse *Begomovirus* satellite complex (Nawaz-ul-Rehman and Fauquet 2009). Bipartite begomoviruses possess two genomic circles (DNA-A and DNA-B) in comparison to a single component in monopartite begomoviruses. Monopartite begomoviruses are associated with the satellite molecules, and the first evidence of defective ssDNA satellite associated with DNA-A of *Tomato leaf curl virus* (ToLCV) was reported from Australia (Dry et al. 1997). Till date, 85% of monopartite begomoviruses have been found to be associated with betasatellite, defective betasatellite, or alphasatellite (Zhou 2013). But there are several reports that also showed the association of betasatellites, alphasatellites, or deltasatellite with the bipartite *Begomovirus* (Zaidi et al. 2016; Romay et al. 2010; Fiallo-Olive et al. 2016).

2.3 Genome Organization

Bipartite begomoviruses are characterized by the presence of ~2.7 kb DNA-A and ~2.6 kb DNA-B components, whereas monopartite begomoviruses are characterized by the presence of ~2.7 kb DNA-A component. Both genomic components possess partially overlapping open reading frames (ORFs) that are present in the bidirectional manner. DNA-A possesses six ORFs: AV1/V1 and AV2/V2 in sense orientation and AC1/C1, AC2/C2, AC3/C3, and AC4/C4 in the antisense orientation. However, the presence of AC5/C5 ORF has also been reported in certain bipartite begomoviruses (Fontenelle et al. 2007; Kheyr-Pour et al. 2000). DNA-B possesses two ORFs: BV1 in sense and BC1 in the antisense orientation. Viral ORFs are separated by an intergenic region (IR) possessing a common region (CR) that consists of conserved nucleotides between the cognate DNA-A and DNA-B (Fig. 2.3). CR possesses origin of replication (ori), stem-loop like nonanucleotide sequence (TAATATT↓AC) and two bidirectional RNA polymerase II promoters. Iterons (direct repeats of five to seven nucleotides) are present upstream to the stem-loop structure. Replication initiator protein (Rep) binds to iterons and produces a nick at nonanucleotide sequence to initiate the replication of viral DNA (Hanley-Bowdoin et al. 2000).

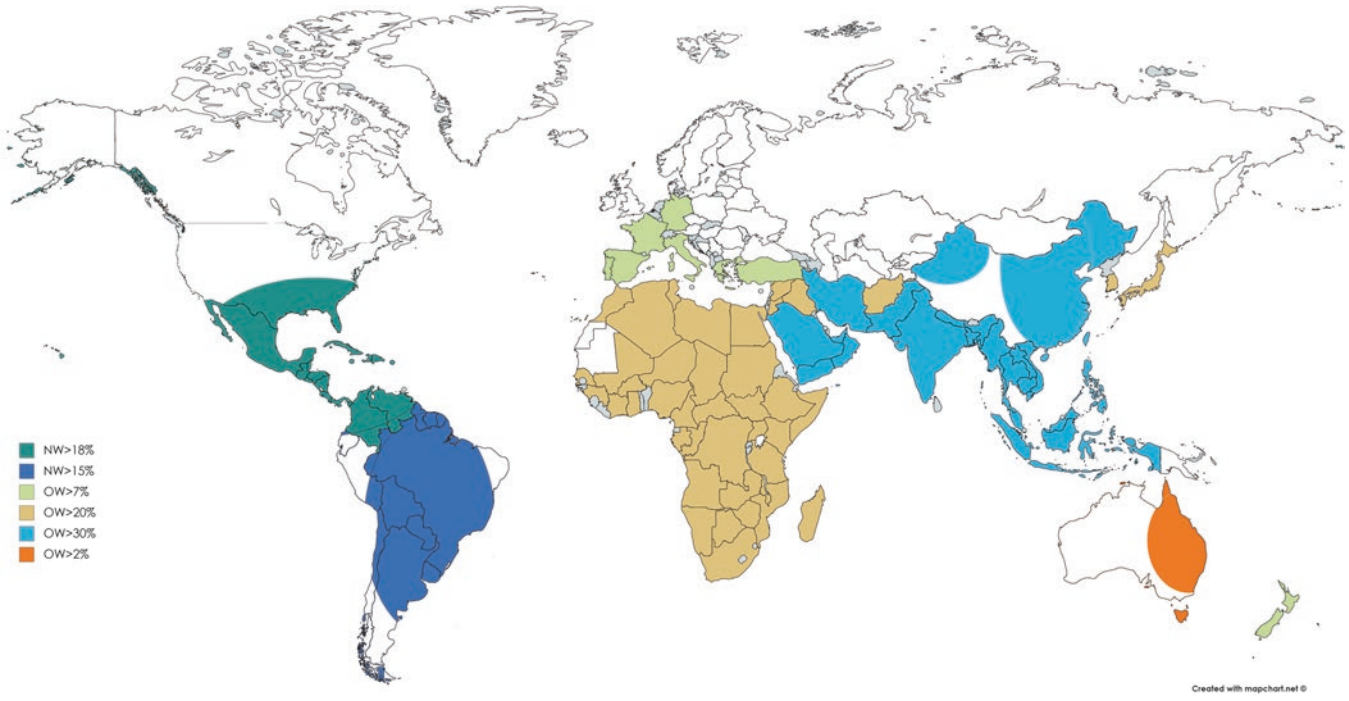

Fig. 2.2 Geographical distribution of begomoviruses indicating the highest percentage of OW begomoviruses in Southeast Asia, followed by Africa and Mediterranean region; whereas NW begomoviruses are predominantly present in America

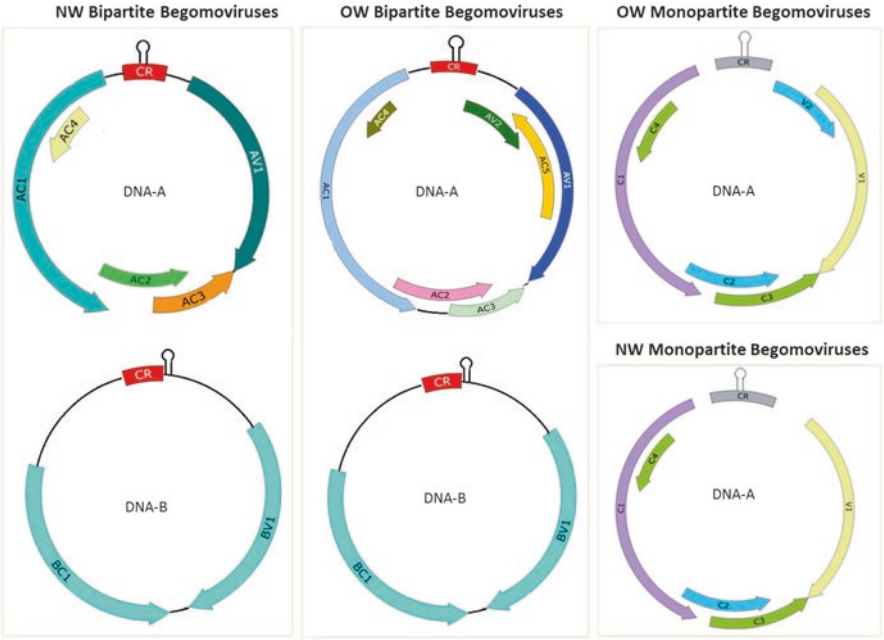

Fig. 2.3 Schematic representation of *Begomovirus* genome organization: New World bipartite, Old World bipartite, Old World monopartite *Begomovirus*, and New World monopartite *Begomovirus*

2.3.1 AV1/V1

The ORF AV1/V1 encodes for a ~29 kDa coat protein (CP), present on the sense strand of the viral DNA-A. CP performs the function of ssDNA encapsidation, virus particle formation, cell to cell, systemic spread, viral DNA accumulation; and insect transmission (Briddon et al. 1990; Wartig et al. 1997; Hallan and Gafni 2001; Harrison et al. 2002). In a monopartite *Begomovirus*, CP performs the function of nuclear shuttle protein (Priyadarshini et al. 2011), and N-terminal domain of CP binds with the viral ssDNA for transport of viral DNA across the nucleus (Pitaksutheepong et al. 2007). For nuclear-cytoplasmic trafficking, CP of TYLCV and bipartite *Begomovirus, Mungbean yellow mosaic virus* (MYMV) interacts with the karyopherin-α1 and importin-α of the host, respectively (Kunik et al. 1998; Guerra-Peraza et al. 2005). A point mutation in the CP of TYLCV resulted in the loss of infectivity (Noris et al. 1998), and it interacts with the HSP70 of *B. tabaci* to mediate the virus multiplication (Gorovits et al. 2013). An interaction between TYLCV CP and cyclophilin B protein of *B. tabaci* affects the transmission of the virus (Kanakala and Ghanim 2016). The absence of CP resulted in reduction of viral ssDNA during TYLCV infection (Padidam et al. 1996). These findings support the multifunctional nature of CP that might happen as a consequence of evolution to complement the small genome of begomoviruses.

2.3.2 AV2/V2

The AV2 ORF/pre-coat ORF (homologue V2 in monopartite begomoviruses) is present in the OW bipartite begomoviruses but absent in the NW bipartite begomoviruses (Nawaz-ul-Rehman and Fauquet 2009). This ORF encodes for a ~13 kDa protein that overlaps with CP at C-terminal in the virion sense strand. In monopartite begomoviruses, V2 performs the function of movement, while in OW bipartite begomoviruses, this function is facilitated by BC1 protein of DNA-B. However, there are reports that demonstrate the role of AV2 protein in cell to cell movement in the bipartite begomoviruses also (Padidam et al. 1996; Rothenstein et al. 2007). It has been reported that AV2 mutant of *Mungbean yellow mosaic India virus* (MYMIV) resulted in attenuation of the virus symptoms (Rouhibakhsh et al. 2011). The AV2 protein of *East African cassava mosaic Cameroon virus* (EACMCV) is a pathogenicity determinant and suppressor of the RNAi (Chowda-Reddy et al. 2008). The V2 protein of monopartite begomoviruses acts as suppressor of RNA silencing and pathogenicity determinant. For instance, the V2 of TYLCV interacts with suppressor of gene silencing 3 (SGS3) to block the RNA silencing (Glick et al. 2008); V2 of *Tomato yellow leaf curl China virus* (TYLCChV) binds with 21 and 24 nt ds RNA to inhibit RNA silencing (Zhang et al. 2012); V2 of *Tomato yellow leaf curl Java virus* (TYLCJV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), and *Cotton leaf curl Kokhran virus* (CLCuKoV) suppresses the posttranscriptional gene silencing (PTGS); (Sharma and Ikegami 2010; Luna et al. 2012; Saeed et al. 2015). V2 protein of *Papaya leaf curl virus* (PaLCV), TYLCJV, and TYLCChV acts as a pathogenicity determinant during the virus infection (Mubin et al. 2009; Sharma and Ikegami 2010; Zhang et al. 2012). V2 protein of TYLCV can reverse the silencing of GFP transgene by decreasing the methylation levels of 35S promoter sequence (Wang et al. 2014).

2.3.3 AC1/C1

AC1/C1 ORF encodes for a ~41 kDa replication (Rep) protein, located in the antisense orientation of DNA-A. Rep protein is involved in the replication of viral genome (Hanley-Bowdoin et al. 2000). Rep possesses a nucleoside triphosphate-binding domain that is present in the C-terminal (Hanson et al. 1995). To initiate the replication, Rep binds to the iterons located in the conserved region, produces a nick for replication, and performs ligation after completion of replication (Fontes et al. 1992). During the virus infection, interaction between PCNA and Rep favors the assembly of replication complex (Castillo et al. 2003). Rep has been reported to interact with host retinoblastoma-related protein (RBR) to release the E2F factor, thus directing the cells into S-phase for the DNA replication (Arguello-Astorga et al. 2004; Ascencio-Ibanez et al. 2008). An interaction between the Rep and replication factor C (RFC) helps the assembly of replication factors (Luque et al. 2002). Rep of TYLCV suppresses the transcriptional gene silencing (TGS) and downregulates the expression of the DNA methyltransferases (MET1 and CMT1) (Rodriguez-Negrete et al. 2013).

2.3.4 AC2/C2

AC2/C2 protein, also known as transcriptional activator protein (TrAP), is a ~16 kDa protein encoded in the antisense orientation. It performs the function of transcriptional activation (Shivaprasad et al. 2005) and directs the transcription of AV1 by activation of the AV1 promoter in the mesophyll cell, but in the vascular tissue it represses the AV1 promoter. It has been shown that an interaction between the TrAP and PEAPOD2 (PPD2) /CP promoter complex is necessary for the expression of the CP gene (Lacatus and Sunter 2008). It disrupts the functioning of E3 ligase-mediated SCF complex by interacting with the CSN5 (COP9 signalosome) to inhibit the jasmonic acid signaling (Lozano-Duran et al. 2011). AC2 mediates the inactivation of SNF1-related protein kinase and adenosine kinase (ADK) to suppress the basal immune response in the host (Wang et al. 2005). TrAP of *Tomato golden mosaic virus* (TGMV) interacts with the kryptonite (KYP) and inhibits its histone methyltransferases activity to prevent methylation of viral genome (Castillo-Gonzalez et al. 2015). AC2 of MYMV, C2 of TYLCV, AC2 of *African cassava mosaic virus* (ACMV), and C2 protein of ToLCJV (Trinks et al. 2005; Vanitharani et al. 2005; Zrachya et al. 2007; Kon et al. 2007) have been identified as the suppressors of gene silencing. C2 protein of the monopartite TYLCSV induces the hypersensitive response (HR) in the host (Matic et al. 2016).

2.3.5 AC3/C3

AC3, also known as replication enhancer protein (REn), is a ~16 kDa oligomeric protein encoded in antisense orientation and interacts with Rep for the accumulation of viral DNA up to 50-folds (Settlage et al. 1996). An interaction network of REn, Rep, DNA sliding clamp protein (PCNA), and retinoblastoma-related protein (pRBR) favors the cellular environment for the viral DNA replication (Castillo et al. 2003). C3 protein of *Tomato leaf curl virus* (ToLCV) associates with another protein, NAC domain protein (NAC1), to enhance the virus replication (Selth et al. 2005). It has been demonstrated that the AC2 protein of *Tomato leaf curl Kerala virus* (ToLCKeV) associates with the Rep and enhances its ATPase activity, for the efficient viral replication (Pasumarthy et al. 2010).

2.3.6 AC4/C4

AC4/C4 is least conserved among all begomoviral ORFs and nested within the AC1/C1 ORF, but in a different reading frame. It has diverse role in the disease development, pathogenicity, and suppression of the host defense. The abolishment of the C4 ORF of TYLCV resulted in the loss of the symptoms and reduced viral DNA accumulation, suggesting its role in the disease development (Jupin et al. 1994). Contrarily, disruption of AC4 ORF in the two bipartite begomoviruses, ACMV and *East African cassava mosaic Zanzibar virus* (EACMZV), failed to

produce an effect on the virus infection (Bull et al. 2007; Etessami et al. 1991). Overexpression of C4 protein under the 35S promoter leads to the developmental abnormalities that mimic the virus-like symptoms in the host (Luna et al. 2012; Saeed et al. 2015). In the case of TGMV infection, AC4 has been shown to participate in the virus movement (Pooma and Petty 1996). AC4 protein of ACMV, EACMZV, and MYMV suppresses the RNA silencing by binding to miRNA and siRNA (Vanitharani et al. 2004; Chellappan et al. 2004; Sunitha et al. 2013). N-Myristoylation motif at glycine-2 (glycine-2) has been mapped in AC4 protein that is involved in membrane binding and suppression of RNA silencing (Fondong et al. 2007). C4 protein of monopartite viruses suppresses the local PTGS (Luna et al. 2012). C4 protein of *Tomato leaf curl Australia virus* (ToLCV-Au) is a pathogenicity determinant protein that interacts with the Shaggy-like protein kinase of the brassinosteroid signaling pathway (Dogra et al. 2009).

2.3.7 AC5/C5

The AC5/C5 ORF is present downstream of the AC3/C3 ORF in antisense orientation of DNA-A. AC5 ORF is conserved and involved in the DNA replication of MYMIV (Raghvan et al. 2004). It was found that the null mutants of AC5 ORF did not affect the infection of *Tomato chlorotic mottle virus* (ToCMoV) and *Watermelon chlorotic stunt virus* (WmCSV) (Kheyr-Pour et al. 2000; Fontenelle et al. 2007). However, in the case of *Tomato leaf curl deformation virus* (ToLDeV), null mutant of C5 ORF in two isolates produced no effect on the virus infection, whereas the C5-null mutant of the third isolate (PA10-3) resulted in the reduction of symptom severity (Melgarejo et al. 2013). Recently, the AC5 ORF of MYMIV was shown to play a key role in the virus infection, inducing hypersensitive response and reversing the established transcriptional gene silencing (TGS) by inhibiting the transcription of DNA methyltransferases (RdDM), and its C-terminal domain was involved in the suppression of the TGS activity. AC5 that also suppressed the PTGS and N-terminal region of the AC5 protein was found to be indispensable for the suppression of the PTGS (Li et al. 2015).

2.3.8 BV1

BV1 ORF encodes for a ~29 kDa nuclear shuttle protein (NSP) in antisense orientation on DNA-B of bipartite begomoviruses. NSP is localized in the nucleus, nucleolus, and the cell periphery. It facilitates the shuttling of viral DNA between the nucleus and cytoplasm, and in the case of *Cauliflower leaf curl virus* (CaLCV) infection, the mechanism of transport was explained on the basis of association of the BV1 and host nuclear shuttle protein interactor (AtNSI), leading to the acetylation of BV1 (McGarry et al. 2003). The NSP of ToLCNDV has been identified as pathogenicity determinant in *Nicotiana tabacum* and *Solanum lycopersicum* (Hussain et al. 2005). The NSP interacts with the NSP-interacting kinase (NIK) and

the proline-rich extensin-like receptor protein kinase (PERK), involved in signal transduction pathways and phosphorylation, respectively (Florentino et al. 2006). The BV1 protein of CaLCuV weakens the host defense system by promoting the export of a negative regulator, ASYMMETRIC LEAVES2 (AS2), that reduces the siRNA levels in the infected host (Ye et al. 2015).

2.3.9 BC1

BC1 ORF encodes for a ~29 kDa movement protein in antisense orientation on the DNA-B of bipartite begomoviruses. BC1 is involved in the local and systemic movement of virus via phloem, and it interacts with NSP to export the nascent viral DNA from the nucleus (Noueiry et al. 1994; Hehnle et al. 2004). In the BC1 protein of bipartite *Begomovirus*, MYIMV binds to both ssDNA and dsDNA with high affinity for ssDNA which indicated the role of BC1 in transport of viral DNA (Radhakrishnan et al. 2008). NSP and MP form complex with the histone H3 protein in the nucleus to facilitate the export of viral DNA from nucleus to the cell periphery during *Bean dwarf mosaic virus* (BDMV) infection (Zhou et al. 2011).

2.4 ssDNA Satellites Associated with Begomoviruses

The presence of additional satellite molecule with begomoviruses was suspected when an agro-infectious clone of monopartite *Ageratum yellow vein virus* (AYVV) produced systemic infection in *Nicotiana benthamiana*, *Phaseolus vulgaris*, and *Lycopersicum esculentum* but failed to re-establish yellow vein symptoms on its natural host, *A. conyzoides*. These results suggested the presence of some additional components that are essential for the disease development (Tan et al. 1995; Saunders and Stanley 1999). ToLCV-sat, the first DNA satellite molecule associated with *Tomato leaf curl virus* was identified in the Northern Australia. It was 682 nucleotides long, noncoding DNA satellite that share no significant sequence homology with the helper virus. ToLCV-sat is not required for the viral infectivity but depends on the helper begomovirus for the replication and encapsidation by the viral CP (Dry et al. 1997). In search of the potential viral components, some defective circular recombinant molecules, half of the sizes of AYVV genome were identified. These defective DNAs retain the viral intergenic region, 5′ sequence of Rep gene and sequence of unknown origin. When co-inoculated with AYVV, these defective DNAs ameliorate disease symptoms and reduce the viral DNA accumulation (Stanley et al. 1997). Later on, similar defective molecules were also identified in the cotton infected with *Cotton leaf curl virus* (CLCV); (Liu et al. 1998). To investigate the potential viral component required for cotton leaf curl disease, a pair of abutting primer was designed to the sequence of unknown origin of defective molecule associated with CLCV. PCR amplified a new component, named as DNA-β (now called as betasatellite). DNA-β was shown to be essential for the typical diseased phenotype in their natural hosts, ageratum and cotton, respectively

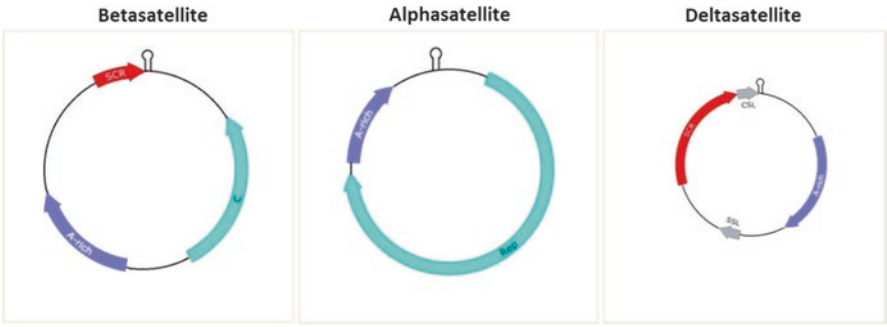

Fig. 2.4 Schematic representation of ssDNA satellites associated with begomoviruses: betasatellites, alphasatellites, and deltasatellites

(Briddon et al. 2001; Saunders et al. 2000). On the basis of two DNA-β sequences (characterized at that time), a pair of universal primer was designed for their full-length amplification (Briddon et al. 2002). Later, many betasatellites were reported from several plant species and shown to be essential for increased virulence (Briddon et al. 2003; Chen et al. 2009; Mansoor et al. 2003; Saunders et al. 2004; Shih et al. 2003). Another satellite molecule, the alphasatellite (formerly known as DNA 1) was reported in association with *Ageratum yellow vein virus* (AYVV) infection (Saunders and Stanley 1999)**.** However, AYVV- and alphasatellite**-**inoculated plants remained asymptomatic suggesting that alphasatellite did not contribute toward the disease development (Saunders and Stanley 1999; Saunders et al. 2000). Satellite molecules are usually associated with monopartite begomoviruses, but recently some reports have shown their presence with the bipartite begomoviruses also (Jyothsna et al. 2013). Kumar et al. (2014) showed that satellite (both alpha and beta) association is not limited to begomoviruses. They have been also associated with the *Wheat dwarf India virus* (WDIV), a *Mastrevirus*, and enhance the level of WDIV DNA in host.

2.4.1 Genome Organization of Betasatellite

The genome of betasatellite is half (~1350 nt) of the size of helper begomovirus that share no significant sequence homology with the helper begomovirus except for the ubiquitous nonanucleotide TAATATTAC, required for the rolling circle replication. To date, >450 full-length betasatellite sequences are available at NCBI database. All betasatellite sequences show conserved organization, encode a single-multifunctional protein (βC1), and have a highly conserved region known as satellite conserved region (SCR) and an adenine-rich region (adenine content of 57–65%) of approximately 160–280 nt (Fig. 2.4). SCR encompasses a potential hairpin stem-loop structure with the loop sequence TAATATTAC, similar to begomovirus origin of replication (Briddon et al. 2003, 2008). Betasatellites depend on the helper begomovirus for replication, encapsidation by viral coat protein, cell to cell movement, and

systemic spread throughout the plant (Briddon et al. 2003; Zhou 2013). Rolling circle replication of begomoviruses requires the recognition and binding of the viral replication-associated protein (Rep) with the repeated sequence motifs called iterons, located in the viral origin of replication (Arguello-Astorga and Ruiz-Medrano 2001; Fontes et al. 1994). Betasatellite lack the iterons and are capable to interact with diverse begomoviruses for their *trans*-replication (Ito et al. 2009). For example, cotton leaf curl Multan betasatellite (CLCuMuB) can be *trans*-replicated by distinct monopartite begomoviruses (Mansoor et al. 2003). In a similar way, AYVV and *Eupatorium yellow vein virus* (EpYVV) can *trans*-replicate the betasatellites associated with AYVV, EpYVV, *Cotton leaf curl Multan virus* (CLCuMV), and *Honeysuckle yellow vein virus* (HYVV), while HYVV can *trans*-replicate only its own satellite. This showed that some *trans*-replication specificity exists between the begomoviruses and betasatellites (Saunders et al. 2008). Betasatellite deletion analysis identified a region between SCR and A-rich region which might be involved in the Rep binding (Saunders et al. 2008). Although the begomovirus can *trans*-replicate non-cognate betasatellite, its accumulation is lower as compared to the cognate betasatellite (Qing and Zhou 2009). Recently, Zhang et al. (2016) reported a Rep-binding motif (RBM) in the SCR upstream of betasatellite origin of replication that is required for the Rep binding. It has been shown that RBM binds with a higher affinity to the cognate Rep in comparison to the non-cognate Rep. Some experimental evidences indicate that the betasatellite can *trans*-replicate with NW begomoviruses (Nawaz-ul-Rehman et al. 2009), but till date there is no report of association of the betasatellite with NW begomoviruses in natural infection. The actual mechanism of betasatellite *trans*-replication at molecular level is not yet fully understood. The betasatellite encoded βC1 protein is ~13.5 kDa, which has several activities including pathogenicity protein, a possible movement protein, and, most importantly, a suppressor of posttranscription and transcription gene silencing (Cui et al. 2004; Sharma et al. 2010). βC1 protein of tomato yellow leaf curl China betasatellite (TYLCCNB) suppresses the methylation-mediated transcriptional gene silencing (TGS) and interacts with the S-adenosyl homocysteine hydrolase (SAHH), a methyl cycle enzyme required for TGS to inhibit SAHH activity (Yang et al. 2011). Furthermore, βC1 of cotton leaf curl Multan betasatellite impairs the plant ubiquitination pathway and upregulates the viral genomic DNA levels (Jia et al. 2016). Additionally, radish leaf curl betasatellite (RLCB), βC1 protein is localized in the plant chloroplast and damages its integrity, resulting in obstruction of the photosynthesis (Bhattacharyya et al. 2015). These results showed that βC1 is a multifunctional protein, and more experimentation is needed to better understand the host-betasatellite interaction.

2.4.2 Alphasatellites

The alphasatellite (~1370 nucleotides) is a single-stranded DNA molecule, approximately half of the size of *Begomovirus* genome, and usually associated with begomovirus-betasatellite complexes (Akhtar et al. 2014; Kumar et al. 2010b). Although alphasatellites are frequently identified with the OW begomoviruses, there